Peta Neale ORCID iD: 0000-0002-4418-1654
Beate Escher ORCID iD: 0000-0002-5304-706X

Jérôme Enault ORCID iD: 0000-0003-2440-4344

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Effect-based trigger values are key for uptake of bioassays

Effect-Based Trigger Values are Essential for the Uptake of Effect-Based Methods in Water Safety Planning

Peta A. Neale[a*], Beate I. Escher[a,b,c], Milo L. de Baat[d], Jérôme Enault[e], and Frederic D.L. Leusch[a]

[a]Australian Rivers Institute, School of Environment and Science, Griffith University, Southport, Queensland, Australia

[b]Department of Cell Toxicology, UFZ – Helmholtz Centre for Environmental Research, Leipzig, Germany

[c]Environmental Toxicology, Department of Geosciences, Eberhard Karls University Tübingen, Tübingen, Germany

[d]KWR Water Research Institute, Nieuwegein, The Netherlands

[e]SUEZ CIRSEE, Le Pecq, France

Abstract: Effect-based methods (EBM) using *in vitro* bioassays and well plate-based *in vivo* assays are recommended for water quality monitoring as they can capture the mixture effects of the many chemicals present in water. Many *in vitro* bioassays are highly sensitive, so an effect in a bioassay does not necessarily indicate poor chemical water quality. Consequently, effect-based trigger values (EBTs) have been introduced to

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differentiate between acceptable and unacceptable chemical water quality and are required for the wider acceptance of EBM by the water sector and regulatory bodies. EBTs have been derived for both drinking water and surface water to protect human- and ecological health, respectively, and are available for assays indicative of specific receptor-mediated effects, as well as assays indicative of adaptive stress responses, apical effects and receptor-mediated effects triggered by many chemicals. An overview of currently available EBTs is provided, and a simple approach is proposed to predict interim EBTs for assays currently without an EBT based on the effect concentration of the assay reference compound. There was good agreement between EBTs predicted using this simplistic approach and EBTs from the literature derived using more robust methods. Finally, an interpretation framework that outlines the steps to take if the effect of a sample exceeds the EBT was developed to help facilitate the uptake of EBM in routine water quality monitoring and water safety planning for drinking water production.

KEYWORDS: Chemical water quality; Drinking water; In vitro bioassays; Source water; Well plate-based in vivo assays

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*Correspondence p.neale@griffith.edu.au

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INTRODUCTION

Treated wastewater and surface water contain a complex cocktail of pesticides, pharmaceuticals and industrial compounds, as well as transformation products (Malaj et

al., 2014; Gago-Ferrero et al., 2020; Wilkinson et al., 2022). After treatment, drinking water may also contain residual chemicals and – if disinfected – may contain disinfection by-products (Hebert et al., 2018; Troger et al., 2018). Chemical water quality is typically assessed by targeted analysis of a few hundred chemicals at most. However, the countless number of chemicals present in water means that targeted chemical analysis alone cannot assess the total chemical burden. While non-target analysis can be applied to identify unknown chemicals, neither targeted nor non-targeted chemical analysis can detect the mixture effects of all active chemicals in a sample. As a result, effect-based methods (EBM) using *in vitro* bioassays and well plate-based *in vivo* assays are recommended for water quality monitoring (Brack et al., 2019). EBM can detect the effect of all active chemicals in a sample extract, including both known and unknown chemicals, and can account for mixture effects. Effect-based recovery experiments have demonstrated that common solid-phase extraction methods can extract a diverse set of organic compounds with high yield and have excellent effect recovery (Neale et al., 2018). EBM are ideally applied in parallel with targeted chemical analysis to provide a better understanding of the chemical burden in water and account for chemicals acting together in mixtures.

The primary applications of EBM include the assessment of treatment efficacy of a certain engineered or natural process (e.g., Bain et al., 2014; Sossalla et al., 2021), evaluation of time trends in natural and engineered systems (e.g., Cavallin et al., 2021), and to benchmark the quality of water from different origins (e.g., Escher et al., 2014; Leusch et al., 2018b). Therefore, the effects are typically compared within a process, along a time axis or across different locations. To use EBM for absolute assessment of water quality necessitates information on acceptable effect levels.

Many *in vitro* bioassays, particularly mammalian reporter gene assays, are highly sensitive by design and can detect effects in relatively clean waters, such as drinking water and recycled water, after sufficient enrichment (e.g., Jia et al., 2015; Conley et al., 2017; Neale et al., 2020b). However, detecting an effect does not necessarily mean that the chemical water quality is unacceptable.

Acceptable concentrations of individual chemicals in diverse water types are already available, including environmental quality standards (EQS) for surface water in the European Union (EP&EC, 2013) and Australia (Australian Government, 2018) or drinking water guidelines such as those proposed by the World Health Organization (WHO) (WHO, 2022) or the US EPA (US EPA, 2018). To date, there has been limited uptake of EBM in regulatory applications, though one prominent example is the use of EBM to monitor recycled water in California (State Water Resources Control Board, 2019). Researchers have proposed diverse approaches to differentiate between an acceptable and unacceptable response by defining effect-based trigger values (EBTs) (e.g., Brand et al., 2013; van der Oost et al., 2017; Escher et al., 2018; Been et al., 2021). What is acceptable or not depends on the water type and its usage, which means that there should be specific EBTs for different water types. EBTs should be related to safe concentrations of regulated chemicals and align with the water protection goals, i.e., integrity of ecological health in the case of surface water (e.g., ecological EBTs) and human health in the case of drinking water (e.g., human EBTs).

EBTs are bioassay- and endpoint-specific and protective only for the endpoint targeted by the bioassay. Hence for an overall water quality assessment, a battery of bioassays should be used with associated EBTs. This is analogous to chemical guideline

values, where the measured concentrations of many individual chemicals should be compared to their associated guideline value for reliable water quality assessment. EBTs have been derived for a wide range of endpoints and cover all stages of cellular toxicity pathways (e.g., induction of xenobiotic metabolism, receptor-mediated effects, adaptive stress responses and cytotoxicity), as well as apical (whole-organism) effects in well plate-based *in vivo* assays (e.g., van der Oost et al., 2017; Escher et al., 2018).

EBM have great potential to be applied in regulatory water quality monitoring and in Water Safety Plans to assess the risks associated with chemical hazards in drinking water (Neale et al., 2022), but EBTs are required for the wider acceptance of EBM by regulators and the water industry. In this article, we provide an overview of currently available EBTs and propose an interim approach to derive EBTs for bioassays currently without an EBT. We also provide operational guidance on the steps to take if the effect in a water sample exceeds the EBT.

CURRENTLY AVAILABLE EFFECT-BASED TRIGGER VALUES FOR SPECIFIC RECEPTOR-MEDIATED EFFECTS

Assays indicative of receptor-mediated effects, such as activation of the estrogen receptor (ER) (Leusch et al., 2010; Serra et al., 2020), activation of the glucocorticoid receptor (GR) (Jia et al., 2016) and photosynthesis inhibition (Bengtson Nash et al., 2006; Tang and Escher, 2014), are highly specific bioassays and almost all of the detected effects can typically be explained by a small number of known and potent chemicals. For example, >90% of the estrogenic activity in most water samples is caused by natural hormones (such as 17β-estradiol and estrone) and synthetic hormones (such as 17α-ethinylestradiol), with industrial xenoestrogens, such as alkylphenols, only having a

minor contribution (Korner et al., 2001; Ra et al., 2011). For those assays it is straightforward to derive an EBT because the causative chemicals are well characterised and they are highly potent, i.e., typically a small number of highly potent chemicals dominate the mixture effect.

Various approaches have been proposed to develop EBTs for specific receptor-mediated effects, including simple translation from acceptable daily intake (ADI) and guideline values (GV) of single highly potent reference chemicals (Kunz et al., 2015), incorporation of chemical potency (Brand et al., 2013; Escher et al., 2015; Escher et al., 2018), using multiple lines of evidence (van der Oost et al., 2017) and comparison of *in vitro* and *in vivo* responses to determine maximum sensitivity and specificity cut-offs (Brion et al., 2019). Recently, Finckh et al. (2022) used predicted no-effect concentrations (PNEC) as proxies for EQS and derived EBTs by comparing differences in potency *in vitro* and *in vivo*.

A summary of currently available EBTs for these bioassays is provided in Table 1. All EBTs in Table 1 are expressed in units of bioanalytical equivalent concentrations (BEQ), which relate the effect of a water sample to the effect of the assay reference compound (Escher et al., 2021; ISO 23196, 2022). Many EBTs are available for some endpoints, such as estrogenic activity, while fewer EBTs are available for other specific receptor-mediated endpoints, including only preliminary ecological EBTs for androgenic activity and progestagenic activity.

CURRENTLY AVAILABLE EFFECT-BASED TRIGGER VALUES FOR ASSAYS INDICATIVE OF ADAPTIVE STRESS RESPONSES, APICAL EFFECTS AND RECEPTOR-MEDIATED EFFECTS THAT ARE TRIGGERED BY MANY CHEMICALS

Some assays respond to many chemicals and consequently, only a small fraction of the effect can typically be explained by known chemicals. This includes assays indicative of adaptive stress responses, such as oxidative stress (Neale et al., 2017b), apical effects, such as mortality in fish embryos (Neale et al., 2015) and xenobiotic metabolism, such as the pregnane X receptor (PXR) (Creusot et al., 2014). For example, only between 0.0004% and 0.20% of the oxidative stress response could be explained in wastewater, despite effect data being available for 46 of the detected chemicals (Neale et al., 2020c).

Currently available EBTs for these more general endpoints are also provided in Table 1. While there are multiple ecological EBTs available for some assays indicative of xenobiotic metabolism, oxidative stress and apical effects, there are very few human EBTs available for these endpoints. The derivation of EBTs for such endpoints is much less straightforward than for specific receptor-mediated effects. Sometimes, the same approach for assays indicative of specific receptor-mediated effects was applied, though it was necessary to remove low-potency chemicals to prevent them from skewing the distribution used to derive the EBT (Escher et al., 2018; Been et al., 2021) or to include a mixture assessment factor to account for the many unknown low potency chemicals contributing to the effect (Escher et al., 2018). An alternative approach derived EBTs using a distribution of specificity ratios of all active chemicals in a particular bioassay

and used acceptable negligible cytotoxicity as the point of departure (Escher and Neale, 2021). The specificity ratio is the ratio between the predicted baseline toxicity and specific toxicity in the same assay, with a high specificity ratio indicating that a chemical has a specific effect in the assay. This approach avoids the need for mixture assessment factors but requires a lot of experimental effect data for the specificity ratio distribution. The amount of effect data for individual chemicals has increased in recent years, with experimental values available in the peer reviewed literature (Neale et al., 2017a; Neale et al., 2020a) and the US EPA CompTox Chemicals Dashboard, which includes ToxCast and Tox21 data (Williams et al., 2017).

OPPORTUNITIES AND CHALLENGES OF EXISTING EBTS

EBTs are increasingly applied in a research context to help interpret EBM results and understand whether the chemical water quality is acceptable or unacceptable (e.g., Kienle et al., 2019; De Baat et al., 2020; Bain et al., 2021). To date, there has been less uptake of EBM and consequently EBTs in a regulatory context. However, bioassays indicative of estrogenic activity and aryl hydrocarbon (AhR) activity are applied to monitor recycled water quality in California (State Water Resources Control Board, 2019). Reported effects are compared to monitoring trigger levels of 3.5 ng/L estradiol equivalent concentrations (EEQ) and 0.5 ng/L 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) equivalent concentrations. Monitoring trigger levels are analogous to EBTs and certain response actions are triggered if the reported effect exceeds the monitoring trigger level. Further, while not legally required, EBM are recommended for water quality monitoring by water authorities and drinking water utilities in the Netherlands, with EBTs used to interpret the results (KIWK, 2022).

Despite the many different derivation approaches with differing requirements for expert opinion being applied, it is remarkable that most of the current EBTs for the same endpoint generally ended up within a log unit of each other. As an example, available EBTs for estrogenic activity are shown in Figure 1, with ecological EBTs often lower than human EBTs. Similar to EQS, EBTs derived for surface water to protect ecosystem health must be protective of all species of the ecosystem, including those that live their entire lifetime in the aquatic environment and can take up chemicals via diverse pathways, while drinking water has a different uptake route in humans, mainly via ingestion of on average 2 L of water per day (Escher et al., 2018). In theory, WWTP effluent should have a higher EBT than surface water due to dilution of effluent in the receiving river but existing EBTs for WWTP effluent are in fact often lower (Jarošová et al., 2014).

In addition to differences between ecological and human EBTs, differences between EBTs indicative of the same endpoint exist due to inherent sensitivity differences of the bioassays for the same endpoint. Moreover, even for the same assay and the same water type, there are still variations within an order of magnitude (e.g., EBTs for ERα CALUX for drinking water) due to differences in the derivation approaches applied. From a precautionary point of view, the lowest available EBT should be used, excluding preliminary EBTs derived from limited databases.

Thus, despite true biological differences, a bigger challenge seems to be the diversity of derivation methods leading to EBTs that have limited comparability. As EBTs are essential for the uptake of bioassays for routine water quality monitoring, this is

a severe handicap to the broader application of EBM. Another challenge is the uneven coverage of EBTs for different endpoints, as discussed above.

A SIMPLE METHOD TO DERIVE INTERIM EFFECT-BASED TRIGGER VALUES

A simple method is proposed here to derive an interim EBT for bioassays that currently lack an EBT or whose EBT is based on a limited database. This simple approach uses the effect concentrations (EC) of the assay reference compound (i.e., a potent chemical in the assay and preferably an environmentally relevant chemical), which is readily available.

The concentration causing a 10% effect (EC_{10}) was used for most assays, with the concentration causing an induction ratio of 1.5 ($EC_{IR1.5}$) used for assays indicative of adaptive stress responses and the concentration causing a suppression ratio of 20% (EC_{SPR20}) used for assays indicative of antagonism (Table 1). These effect levels were selected as they are typically close to the assay limit of detection (Escher et al., 2014). For assays where only the EC_{50} was available, the EC_{50} was converted to EC_{10} assuming the slope of the log-logistic concentration-response curve was 1. Low level effect concentrations (e.g., EC_{10}, $EC_{IR1.5}$) were used to derive interim EBTs as these are a measure of molecular initiating events or key events. Effects at the cellular level can potentially lead to effects in organisms and populations according to the adverse outcome pathway concept (Ankley et al., 2010). It should be noted that cellular responses will not necessarily result in effects in whole organisms, but they are required for higher-level effects. The majority of EC_{10}, $EC_{IR1.5}$ and EC_{SPR20} values were collected from Escher et

al. (2015) and Escher et al. (2018), with the literature source for each assay provided in those studies.

For each assay in Table 1, the EC value to (existing) EBT ratio was calculated, with the EC value to EBT ratio provided in Tables S1 and S2 of the Supplemental Data. Several of the EBTs in Table 1 were deemed too preliminary in the cited studies (indicated in italics) and this was often due to the EBT being derived using a limited number of chemicals (e.g., Escher et al., 2018; Been et al., 2021). Removing the preliminary EBTs narrowed the log-normal distributions of the log EC to EBT ratios, particularly for the ecological EBTs (Figure S1). The log EC to EBT ratios were plotted against rank, which was expressed in probit units, and the linearity of the probit plots increased after removing the preliminary values for the ecological EBTs (Figure S2). This indicates that the preliminary EBTs were indeed very limited as discussed when they were published. Without the preliminary EBTs, the median EC value to EBT ratio was 5.8 ($n = 55$, $\sigma = 52$) for ecological EBTs and 0.7 ($n=18$, $\sigma = 4.3$) for human EBTs. The data analysis was conducted using Microsoft Excel and GraphPad Prism (version 9.4.0).

The predicted (i.e., interim) assay-specific EBTs were then estimated by dividing the EC value of the assay reference compound by the median EC to EBT ratio (5.8 and 0.7 for ecological EBTs and human EBTs, respectively). The interim EBTs are provided in Tables S1 and S2 and are reported to one significant figure. This simple calculation yielded a reasonably good estimate of the EBT, as evidenced by the relationship between the current and interim EBTs shown in Figure 2, with most interim EBTs within a log unit of the current EBT. There were some outliers. For example, the interim human EBT

for PR CALUX, 9 ng/L Levonorgestrel EQ, underestimated the current EBT of 724 ng/L Levonorgestrel EQ (Brand et al., 2013). The interim ecological EBT for PPARγ-GeneBLAzer of 30 ng/L Rosiglitazone EQ was close to some of the available EBTs (e.g., 36 ng/L Rosiglitazone EQ (Escher et al., 2018) and 19 ng/L Rosiglitazone EQ (Neale et al., 2020a)), but underestimated the current EBT of 1200 ng/L Rosiglitazone EQ from Escher and Neale (2021). Further, the interim ecological EBT for PPARγ-CALUX, 600 ng/L Rosiglitazone EQ, overestimated the current EBT of 10 ng/L Rosiglitazone EQ (van der Oost et al., 2017). The large range of current ecological EBTs for assays indicative of peroxisome proliferator-activated receptor gamma (PPARγ) activity reflects the different derivation approaches applied, as well as increased availability of single chemical effect data over time.

The log-linear regression yielded a slope of 1.01 for ecological EBTs (R^2 0.92) and a slope of 1.00 for human EBTs (R^2 0.91). This indicates a good agreement between the simple interim calculation and the more robust EBT derivations. The interim approach proposed here is not anchored in a biological understanding of the assay, but it is simple, practical and yields reasonable interim EBTs. Therefore, this approach can be applied to generate preliminary EBTs for assays currently without EBTs to assess the risk associated with a bioassay response. While most interim EBTs were similar to current EBTs, some overestimated or underestimated the current EBT. For assays without an existing EBT, it is not possible to make such a comparison, but the appropriateness of the interim EBT can be assessed by comparing it with bioanalytical responses detected in water samples.

As an example, androgenic, glucocorticoid and progestagenic activity is often detected in surface water (Leusch et al., 2017), but few ecological EBTs are currently available for these endpoints. Using the proposed approach, we estimated interim ecological EBTs for the commonly used CALUX and GeneBLAzer assays for androgenic, glucocorticoid and progestagenic activity and compared the predicted EBTs with effects detected in wastewater effluent and surface water from the literature. The interim EBT for AR CALUX is 0.6 ng/L 5α-dihydrotestosterone (DHT) equivalent concentrations (DHT EQ), with the majority of reported DHT EQ in surface water below the predicted EBT (Figure 3 A). Similarly, all reported surface water DHT EQ values were below the interim EBT of 7 ng/L DHT EQ for AR GeneBLAzer (Figure 3 B). The predicted EBT of 8 ng/L dexamethasone EQ for GR CALUX showed good separation between wastewater and surface water samples, with all but three of the surface water samples below the interim EBT and all wastewater effluent samples above the EBT (Figure 3 C). In contrast, the majority of surface water samples exceeded the interim EBT of 10 ng/L dexamethasone EQ for GR GeneBLAzer (Figure 3 D). There were fewer effect data available in the literature for PR CALUX and PR GeneBLAzer, though three out of four surface water samples were below the interim EBT of 4 ng/L levonorgestrel for PR GeneBLAzer (Figure 3 F).

In Table 1 there are very few human EBTs currently available for assays indicative of xenobiotic metabolism, with the available EBTs all considered too preliminary. Using the proposed approach, we estimated interim EBTs of 70 ng/L benzo[a]pyrene (B[a]P) EQ for PAH CALUX, which is around 3 times higher than the preliminary EBT for PAH CALUX in Been et al. (2021), and 300 ng/L B[a]P EQ for the

H4L1.1c4 AhR assay. Currently, there is limited effect data for drinking water for these assays, but the interim EBT for the H4L1.1c4 AhR assay was higher than the reported B[a]P EQ in riverbank filtrate used for drinking water treatment (Albergamo et al., 2020). Similarly, the interim EBT of 200 µg/L di (2-ethylhexyl) phthalate (DEHP) EQ for the HG5LN-hPXR assay was higher than the effect reported in Australian drinking water (Escher et al., 2014).

WHAT TO DO IF THE EFFECT OF A SAMPLE EXCEEDS ITS EFFECT-BASED TRIGGER VALUE

EBM can be applied in different monitoring categories within the Water Safety Plan framework, including system assessment, validation, operational, and verification monitoring (Neale et al., 2022). For most practical applications, it is necessary to compare the reported effect with an EBT. Therefore, it is important to guide bioassay users through the steps to take if the effect in a sample exceeds the EBT, with an interpretation framework presented in Figure 4. This framework focuses on drinking water treatment, with the effect of the treated drinking water compared to the corresponding human EBT. This framework is based on the framework developed by Leusch and Snyder (2015), but also includes guidance for endpoints where many chemicals contribute to the observed effect (e.g., assays indicative of apical effects and adaptive stress responses). The response in a bioassay, expressed as BEQ_{bio}, can be compared to the EBT-BEQ, with no further action required if BEQ_{bio} is lower than the EBT-BEQ. If the measured BEQ_{bio} value exceeds the EBT-BEQ, the first step is to check the bioassay quality control (QC) and collect another water sample from the same site and re-test. This is comparable to what is currently done for targeted chemical analytes

(e.g., US EPA, 2005) and is to confirm that the observed effect is not an isolated occurrence (Leusch and Snyder, 2015). If the BEQ_{bio} of the second sample is below the EBT-BEQ (in other words, if the re-test does not confirm the initial positive result), then no further action is required. If, however, the second test confirms the initial positive result and both samples report a BEQ_{bio} > EBT-BEQ, then further action is needed. This is a similar approach as prescribed by the Californian State Water Resources Control Board in their "*Water quality control policy for recycled water"* document where exceedance of the monitoring trigger level (which is equivalent to the EBT) prompts a resampling within 72 h (State Water Resources Control Board, 2019).

If the assay is responsive to few known and potent chemicals (e.g., assays listed under "receptor-mediated effects" in Table 1) it is usually possible to target a relatively short list of chemicals for each assay for chemical analysis. For example, photosystem II herbicides, such as diuron, terbuthylazine, terbutryn and atrazine, should be targeted for photosynthesis inhibition assays as these chemicals explain most of the observed effect (Kienle et al., 2019). The chemicals contributing to the observed effect may be different at different locations depending on local regulations and agricultural practices, but typically three to five photosystem II herbicides explain most of the effect (Tang and Escher, 2014; Neale et al., 2017b; Kienle et al., 2019). The concentration of each compound detected (C_i) is then multiplied by the potency of each compound in the bioassay to produce a calculated bioassay response $BEQ_{chem,i}$. Potency data for each chemical can be calculated from available single chemical effect data. $BEQ_{chem,i}$ is then summed up for all chemicals to obtain the bioanalytical equivalent concentration from chemical analysis BEQ_{chem} ($BEQ_{chem}=\sum BEQ_{chem,i}$), which can be compared to the actual

bioassay response BEQ_{bio}. If the two values agree (*i.e.*, are within 30%), then it is concluded that the identified chemicals are indeed driving most of the bioassay response, and the concentrations of chemicals detected (C_i) can be compared to available conventional chemical guideline values (GV_i). While known potent chemicals should explain most of the effect in assays indicative of specific receptor-mediated effects, the threshold of 30% was selected to account for the uncertainties associated with chemical analysis of trace chemical concentrations. If the concentrations exceed the guideline values, then the usual process is followed for the exceedance of regulatory standards. Otherwise, if the chemical concentrations do not exceed individual guideline values, the water is technically compliant with regulatory expectations. While no further immediate action is required from a regulatory perspective, the bioassay response may indicate a potential risk caused by unregulated chemicals.

However, if BEQ_{chem} is less than 70% of BEQ_{bio} ($BEQ_{chem}<0.7\times BEQ_{bio}$), then this indicates that other unidentified chemicals are contributing to the bioassay response. The next steps will depend on the magnitude of the exceedance. If BEQ_{bio} is less than 10 times the EBT-BEQ, more frequent monitoring is recommended until BEQ_{bio} is less than EBT-BEQ. Further action may be required if BEQ_{bio} is between 1 to 10 times the EBT-BEQ for a long period of time (e.g., 6 to 12 months). This is in line with previous recommendations (Leusch and Snyder, 2015; NORMAN Network, 2019; State Water Resources Control Board, 2019).

If BEQ_{bio} is greater than 10 times the EBT-BEQ, further action is required. Firstly, an effort should be made to identify those unknown mixture risk drivers. Effect-directed analysis (EDA) has been applied successfully to identify unknown contributors

to the mixture effects in water samples for assays indicative of hormone receptor-mediated effects (Sonavane et al., 2018; Hashmi et al., 2020). If additional causative chemicals are identified, then they can be included in the BEQ_{chem} calculation, which can again be compared to BEQ_{bio}. If the additional chemicals have now improved the agreement between BEQ_{chem} and BEQ_{bio}, then the conventional approach can be used, as described above.

If BEQ_{bio} remains significantly different from BEQ_{chem} (e.g., $BEQ_{chem}<0.7\times BEQ_{bio}$), then even EDA has not been able to identify all significant bioactive chemicals. In consultation with the relevant regulatory body, additional steps may be needed. Conferring with regulatory authorities about corrective actions is also recommended in other trigger value guidance documents (Leusch and Snyder, 2015; State Water Resources Control Board, 2019), as well as drinking water quality guidelines (NHMRC/NRMMC, 2011). It may be possible to optimize the treatment process to remove the bioassay response. This could be first tested at the bench-scale to fine-tune the treatment process. Further, recent reviews on the removal of biological effects by different wastewater and drinking water treatment processes may help identify suitable treatment processes (Völker et al., 2019).

If the response in the assay is triggered by many low potency chemicals (e.g., assays indicative of xenobiotic metabolism, adaptive stress responses and apical effects), it makes little sense to try to identify causative chemicals as BEQ_{chem} is usually much lower than BEQ_{bio} (e.g., BEQchem is often less than 10% of BEQbio ($BEQchem<0.1\times BEQbio$)). In this case, the cytotoxicity response (expressed here as the concentration causing 10% inhibition (IC_{10})) is compared to the cytotoxicity EBT

(referred to here as EBT-IC_{10}). Escher and Neale (2021) assumed that 1% cytotoxicity in a water sample was acceptable, which corresponds to an EBT-IC_{10} of relative enrichment factor 10 (i.e., a water sample would need to be enriched 10 times to induce 10% cytotoxicity). If the IC_{10} in the assay is lower than the EBT-IC_{10}, the sample is additionally cytotoxic and risk mitigation is required. Note that while a larger BEQ indicates a greater effect in the assay, the opposite is the case for IC values, with a lower IC value indicating a greater effect as less enrichment is required to cause cytotoxicity. As above, optimisation of the treatment process should be investigated to reduce the bioassay response if the bioassay response exceeds the specific or cytotoxicity EBT by more than 10 times.

If the sample is not significantly cytotoxic (IC_{10} > EBT-IC_{10}), then this suggests that while there are bioactive compounds present (which explain the BEQ_{bio} being greater than the EBT-BEQ), those compounds may not pose an acute risk. However, the EBT exceedance still indicates a potential risk, so it is suggested that the water quality be monitored with other bioassays that cover other endpoints, including assays indicative of specific receptor-mediated effects, particularly if BEQ_{bio} is greater than 10 times the EBT-BEQ. A broad characterisation of the chemical water quality should also be conducted.

Compliance or verification monitoring of chemical hazards using chemical analysis within the Water Safety Plan framework is recommended on a quarterly to biannual basis as chemical hazards are not likely to be present at acute concentrations (Bartram et al., 2009). Therefore, a similar frequency is recommended for routine monitoring using EBMs.

This framework can also be adapted to surface water monitoring, with the BEQ_{bio} compared to the corresponding ecological EBT. The quality of the source water or the health of the receiving ecosystem should be investigated (using conventional ecosystem assessment methods) and the source of the pollution identified, where possible, if BEQ_{bio} remains significantly different from BEQ_{chem}. Further, optimising wastewater treatment processes may be an appropriate response if discharged effluent is causing the EBT exceedance in surface water. The above actions should be considered if the observed effect is more than 10 times the ecological EBT.

CONCLUSIONS

The complex mixture of chemicals present in water necessitates the use of EBM in addition to traditional targeted chemical analysis for water quality monitoring. EBTs, which help users differentiate between acceptable and unacceptable chemical water quality, are required for the wider use and acceptance of EBM, including their integration into Water Safety Plans. EBTs have been previously developed using a number of different approaches for assays indicative of specific receptor-mediated effects, as well as assays that are responsive to many chemicals, for both human health and ecological health. As the availability of EBTs is limited for some endpoints, a simple approach using the effect concentration of the assay reference compound was proposed to predict interim EBTs. While this approach is not anchored in a biological understanding of the assay, it yielded reasonable interim EBTs that were in good agreement with currently available EBTs. An interpretation framework was developed to guide bioassay users in the steps to take if the effect in a water sample exceeds the EBT, with the magnitude of the response actions moderated by the magnitude of the EBT exceedance.

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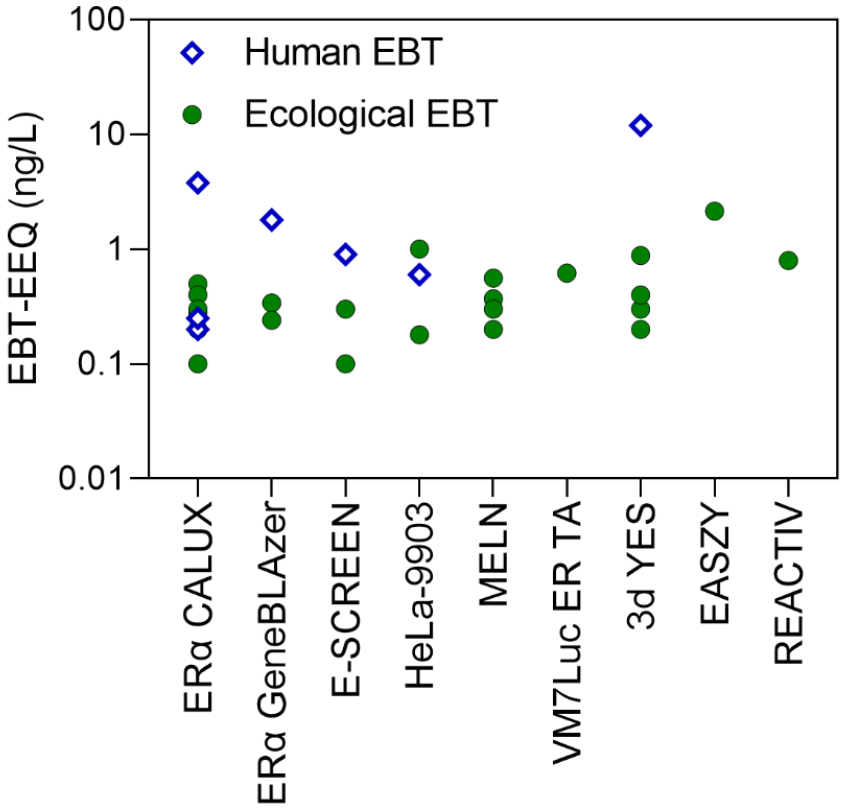

Figure 1: Overview of published effect-based trigger values (EBTs) for estrogenic activity in units of 17β-estradiol equivalent concentration (EBT-EEQ) for human health (drinking water) and ecological health (surface water and wastewater effluent). See Table 1 for further details.

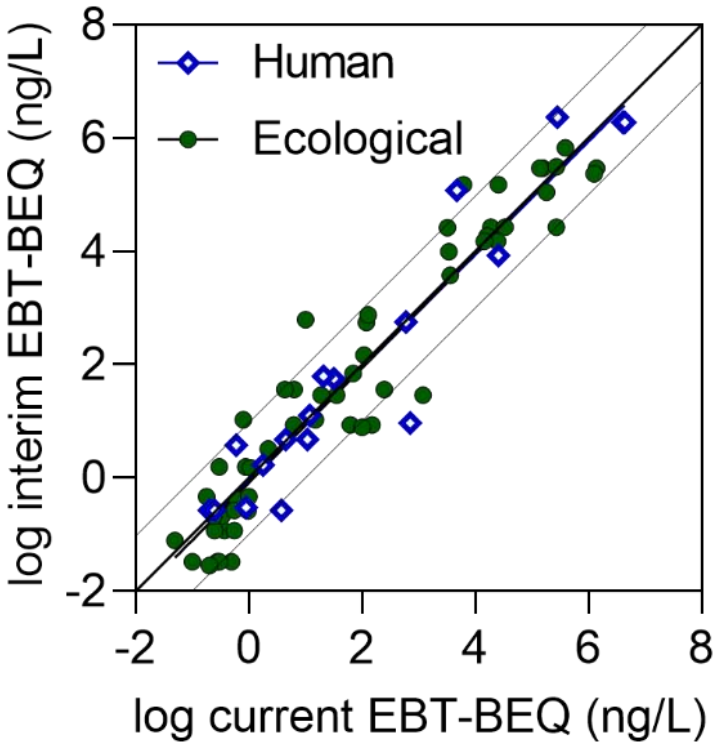

Figure 2: Currently available ecological and human EBTs compared to ecological and human EBTs predicted using the interim approach proposed in the current study. Ecological EBTs are derived for surface water, while human EBTs are derived for drinking water.

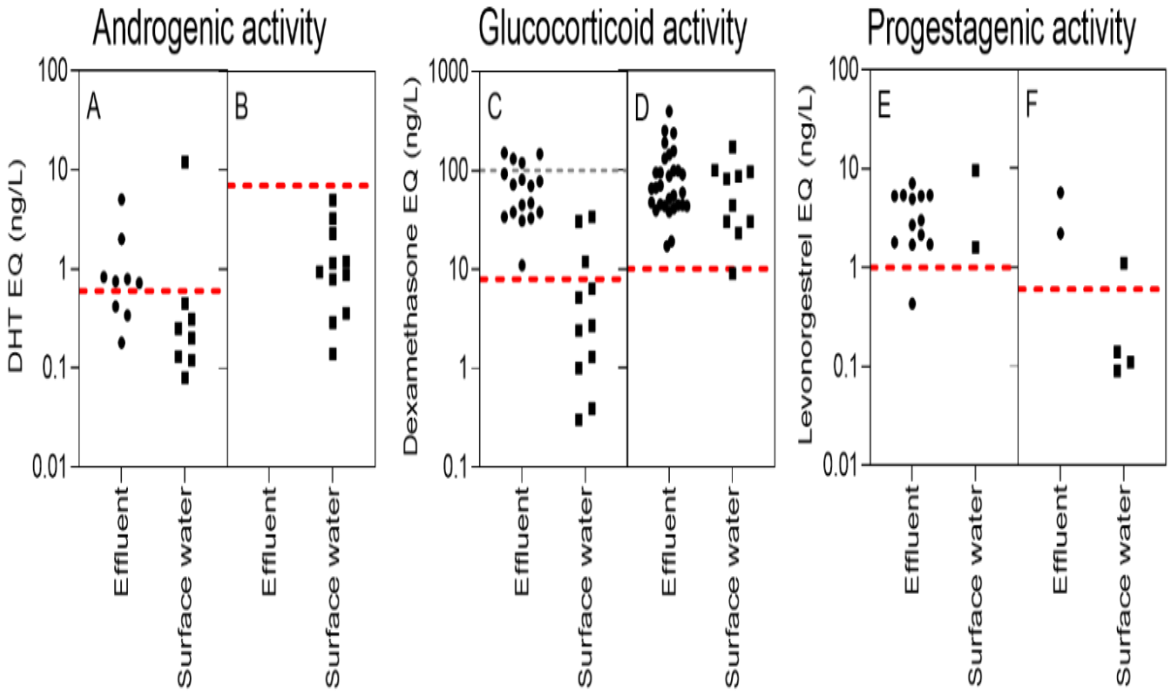

Figure 3: Comparison of interim ecological EBTs (red dashed line) with effect data in wastewater effluent and surface water for A) AR CALUX, B) AR GeneBLAzer, C) GR CALUX, D) GR GeneBLAzer, E) PR CALUX and F) PR GeneBLAzer. The grey dashed line in C) is the current EBT from van der Oost et al. (2017). The effect data was collected from Van der Linden et al. (2008); Brand et al. (2013); Schriks et al. (2013); Bain et al. (2014); Leusch et al. (2014); Mehinto et al. (2015); Roberts et al. (2015); Chen et al. (2016); Jia et al. (2016); Mehinto et al. (2016); Konig et al. (2017); Mehinto et al. (2017); Tousova et al. (2017); Daniels et al. (2018); Hashmi et al. (2018); Houtman et al. (2018); Leusch et al. (2018b); Muller et al. (2018); Nivala et al. (2018); Sauer et al. (2018); Scott et al. (2018); Alygizakis et al. (2019); Neale et al. (2020c); Zwart et al. (2020); Mehinto et al. (2021); Kienle et al. (2022); Maruya et al. (2022) DHT: 5α-Dihydrotestosterone

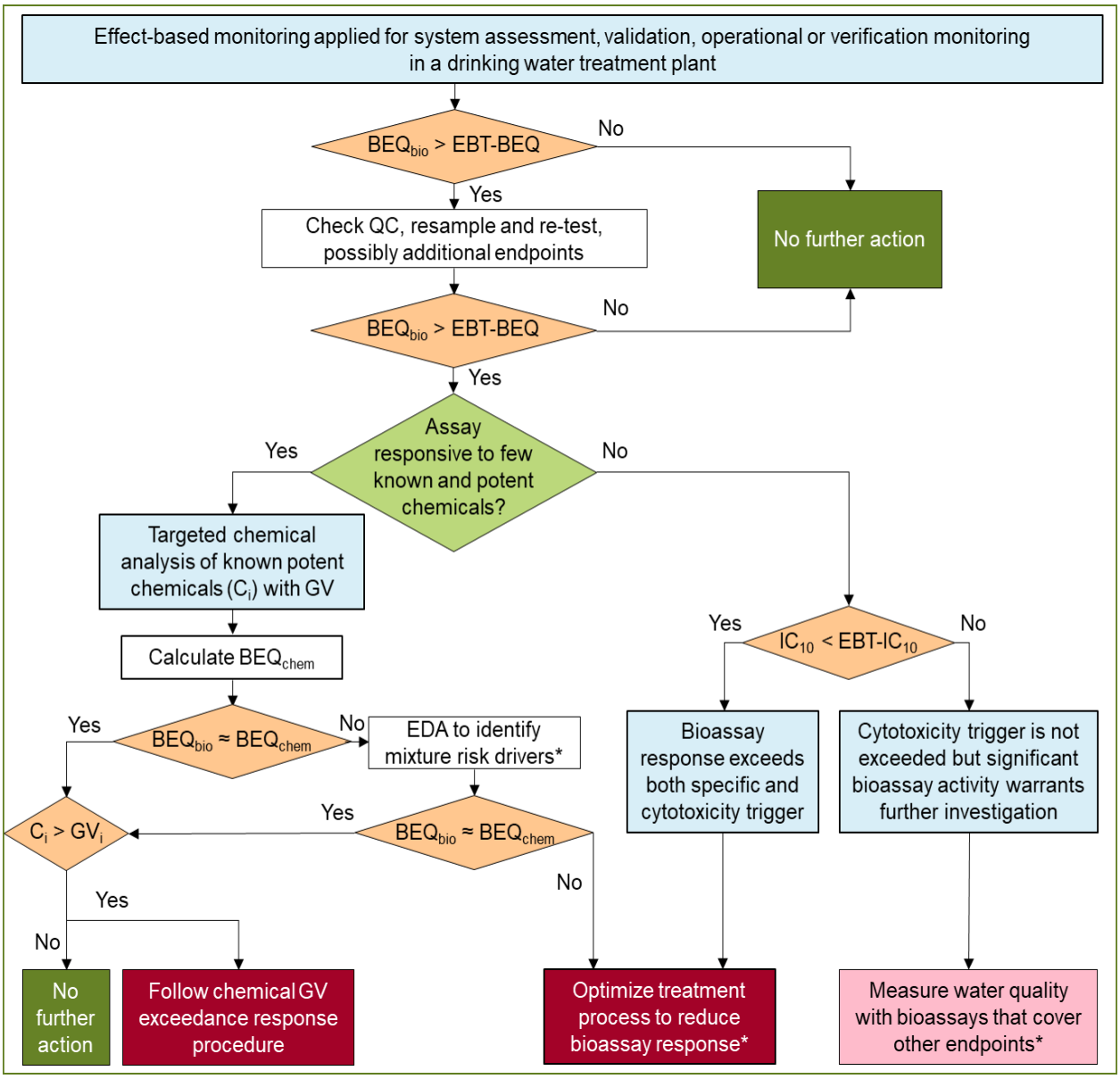

Figure 4: Interpretation framework for EBT exceedance in a drinking water treatment plant.

*Steps to be taken if BEQ_{bio} is more than 10 times the EBT-BEQ.

BEQ_{bio}: bioanalytical equivalent concentration from bioanalysis; BEQ_{chem}: bioanalytical equivalent concentration from chemical analysis; EBT-BEQ: effect-based trigger value expressed as a bioanalytical equivalent concentration; EBT-IC_{10}: effect-based trigger value expressed as a concentration causing 10% inhibition; EDA: effect-directed

analysis; GV: guideline value; IC_{10}: concentration causing 10% inhibition; QC: quality control.

Table 1: Summary of proposed effect-based trigger values (EBTs) for both human health and ecological health expressed as bioanalytical equivalent concentrations (EBT-BEQ) that are currently available in the literature, along with the effect concentration (EC) value for each assay. EBTs calculated specifically for wastewater effluent are shown in bold, while EBTs deemed as preliminary in the cited studies are shown in italics.

Endpoint	Assay name	Reference compound	Effect concentration EC of reference compound	Human EBT-BEQ (Drinking and recycled water for indirect potable reuse)	Ecological EBT-BEQ (Surface water)
Receptor-mediated effects					
Estrogenic activity	ERα CALUX	17β-Estradiol	EC_{10} 0.19 ng/L [1]	0.2 ng/L EEQ [2] 0.25 ng/L EEQ [3] 3.8 ng/L EEQ [5]	0.10 ng/L EEQ [1] 0.5 ng/L EEQ [4] 0.28 ng/L EEQ [6] **0.2 – 0.4 ng/L EEQ [7]**
	ERα GeneBLAzer	17β-Estradiol	EC_{10} 1.23 ng/L [8]	1.8 ng/L EEQ [2]	0.34 ng/L EEQ [1] 0.24 ng/L EEQ [6] *0.01 – 0.20 ng/L EEQ [9]*
	E-SCREEN	17β-Estradiol	EC_{10} 0.21 ng/L* [2]	0.9 ng/L EEQ [2]	**0.1 – 0.3 ng/L EEQ [7]**
	HeLa-9903	17β-Estradiol	EC_{10} 2.7 ng/L [1]	0.6 ng/L EEQ [2]	1.01 ng/L EEQ [1] 0.18 ng/L EEQ [6]
	MELN	17β-Estradiol	EC_{10} 0.68 ng/L [1]		0.37 ng/L EEQ [1] 0.56 ng/L EEQ [6] **0.2 – 0.3 ng/L EEQ [7]**
	VM7Luc ER TA	17β-Estradiol	EC_{10} 2.4 ng/L [1]		0.62 ng/L EEQ [1]
	A-YES	17β-	EC_{10} 1.5		0.56 ng/L EEQ

Endpoint	Assay name	Reference compound	Effect concentration EC of reference compound	Human EBT-BEQ (Drinking and recycled water for indirect potable reuse)	Ecological EBT-BEQ (Surface water)
		Estradiol	ng/L* [1]		[1]
	3d YES	17β-Estradiol	EC_{10} 9.1 ng/L* [1]	12 ng/L EEQ [2]	0.88 ng/L EEQ [1]
					0.2 – 0.4 ng/L EEQ [7]
	ISO-LYES (Routledge & Sumpter strain)	17β-Estradiol	EC_{10} 1.5 ng/L* [1]		0.97 ng/L EEQ [1]
	ISO-LYES (McDonnell strain)	17β-Estradiol	EC_{10} 8.7 ng/L* [1]		1.07 ng/L EEQ [1]
	pYES	17β-Estradiol	N/A		0.50 ng/L EEQ [6]
	EASZY (Cyp19a1b-GFP)	17β-Estradiol	EC_{10} 19 ng/L* [1]		2.15 ng/L EEQ [1]
	REACTIV (unspiked)	17β-Estradiol	EC_{10} 62 ng/L [1]		0.80 ng/L EEQ [1]
Androgenic activity	AR CALUX	5α-Dihydro-testosterone (DHT)	EC_{10} 3.4 ng/L [10]	4.5 ng/L DHT EQ [3] 11 ng/L DHT EQ [5]	
	AR GeneBLAzer	DHT	EC_{10} 41 ng/L [10]	32 ng/L DHT EQ• [2]	*15 – 41 ng/L DHT EQ† [9]*
Anti-androgenic activity	Anti-AR CALUX	Flutamide	EC_{SPR20} 87 µg/L [1]	4.8 µg/L Flutamide EQ [3]	14.4 µg/L Flutamide EQ [1] 25 µg/L Flutamide EQ [4]
	Anti-AR GeneBLAzer	Flutamide	EC_{SPR20} 152 µg/L [1]		3.28 µg/L Flutamide EQ [1]
	Anti-MDA-kb2	Flutamide	EC_{SPR20} 57 µg/L [1]		3.46 µg/L Flutamide EQ [1]
	Anti-AR RADAR (spiked)	Flutamide	EC_{SPR20} 22 µg/L [1]		3.63 µg/L Flutamide EQ [1]
Glucocorticoid activity	GR CALUX	Dexamethasone	EC_{10} 45 ng/L [10]	*150 ng/L Dexamethasone EQ [2]* *47.9 ng/L Dexamethasone EQ [3]* 21 ng/L Dexamethasone	100 ng/L Dexamethasone EQ [4]

Endpoint	Assay name	Reference compound	Effect concentration EC of reference compound	Human EBT-BEQ (Drinking and recycled water for indirect potable reuse)	Ecological EBT-BEQ (Surface water)
				EQ [5]	
	GR GeneBLAzer	Dexamethasone	EC_{10} 152 ng/L [11]		*0.1 – 7.3 ng/L Dexamethasone EQ [9]*
Progestagenic activity	PR CALUX	Levonorgestrel	EC_{10} 6.7 ng/L [10]	724 ng/L Levonorgestrel EQ‡ [5] *2.5 ng/L Levonorgestrel EQ‡ [3]*	
	PR GeneBLAzer	Levonorgestrel	EC_{10} 3.0 ng/L [11]		*286 – 407 ng/L Levonorgestrel EQ# [9]*
Anti-progestagenic activity	Anti-PR CALUX	Endosulfan	EC_{SPR20} 64500 µg/L [1]		*1967 ng/L Endosulfan EQ [1]*
Thyroid activity	TTR RLBA	Thyroxine	EC_{10} 4.8 µg/L* [1]		*0.06 µg/L Thyroxine EQ [1]*
	TTR FITC-T4	Thyroxine	EC_{10} 8.7 µg/L* [1]		*0.49 µg/L Thyroxine EQ [1]*
	XETA (unspiked)	Thyroxine	EC_{10} 10,400 ng/L* [12]		*17 ng/L Thyroxine EQ▼ [1]*
Anti-thyroid activity	Anti-TR-LUC-GH3	Bisphenol A	EC_{SPR20} 3173 µg/L [1]		*0.60 µg/L Bisphenol A EQ [1]*
Photosynthesis inhibition	Combined algae assay (2 h PSII inhibition)	Diuron	EC_{10} 0.40 µg/L* [2]	0.6 µg/L Diuron EQ [2]	0.07 µg/L Diuron EQ [1]
Acetylcholinesterase inhibition	AChE assay	Parathion	EC_{10} 6.2 µg/L* [2]	26 µg/L Parathion EQ [2]	
Xenobiotic metabolism					
Aryl hydrocarbon (AhR) activity	AhR-cisFACTORIAL	Carbaryl	$EC_{IR1.5}$ 241 µg/L [2]	*18 µg/L Carbaryl EQ [2]*	
	PAH CALUX	Benzo[a]pyrene (B[a]P)	EC_{10} 50 ng/L [1]	*24.4 ng/L B[a]P EQ [3]*	6.21 ng/L B[a]P EQ [1] 150 ng/L B[a]P EQ [4] 62.1 ng/L B[a]P EQ [13]
	DR CALUX	TCDD	EC_{10} 0.45		0.05 ng/L TCDD

Endpoint	Assay name	Reference compound	Effect concentration EC of reference compound	Human EBT-BEQ (Drinking and recycled water for indirect potable reuse)	Ecological EBT-BEQ (Surface water)
			ng/L* [14]		EQ [4]
	H4L1.1c4 AhR assay	B[a]P	EC_{10} 211 ng/L [1]		6.36 ng/L B[a]P EQ [1] 4.3 ng/L B[a]P EQ [15] 250 ng/L B[a]P EQ [16]
Peroxisome proliferator-activated receptor gamma (PPARγ) activity	PPARγ CALUX	Rosiglitazone	EC_{10} 3574 ng/L [1]		10 ng/L Rosiglitazone EQ [4]
	PPARγ-GeneBLAzer	Rosiglitazone	EC_{10} 166 ng/L [15]		36 ng/L Rosiglitazone EQ [1] 19 ng/L Rosiglitazone EQ [15] 1200 ng/L Rosiglitazone EQ [16]
Pregnane X receptor (PXR) activity	PXR-cisFACTORIAL	Metolachlor	$EC_{IR1.5}$ 681 µg/L [2]	*59 µg/L Metolachlor EQ [2]*	
	PXR CALUX	Di (2-ethylhexyl)-phthalate (DEHP)	EC_{10} 155 µg/L [1]		272 µg/L DEHP EQ [1] 19 µg/L DEHP EQ° [4] 34 µg/L DEHP EQ° [13]
	HG5LN-hPXR	DEHP	EC_{10} 108 µg/L [1]		16.3 µg/L DEHP EQ [1]
Adaptive stress response					
Oxidative stress response	AREc32	Dichlorvos	$EC_{IR1.5}$ 1700 µg/L [17]	284 µg/L Dichlorvos EQ§ [17]	156 µg/L Dichlorvos EQ [1] 140 µg/L Dichlorvos EQ [15] 1400 µg/L Dichlorvos EQ [16]
	Nrf2 CALUX	Dichlorvos	$EC_{IR1.5}$ 880 µg/L [1]		26 µg/L Dichlorvos EQ [1] 6.2 µg/L Dichlorvos EQ˜ [4]

Endpoint	Assay name	Reference compound	Effect concentration EC of reference compound	Human EBT-BEQ (Drinking and recycled water for indirect potable reuse)	Ecological EBT-BEQ (Surface water)
	ARE GeneBLAzer	Dichlorvos	$EC_{IR1.5}$ 3867 µg/L (1)		392 µg/L Dichlorvos EQ (1)
Apical effects in well plate-based* in vivo *assays					
Bacterial toxicity	Microtox	Virtual baseline toxicant	EC_{10} 1370 µg/L* (1)	4100-4392 µg/L Baseline TEQ$^{\perp}$ (18)	1264 µg/L Baseline TEQ (1)
Algal growth	72 h algal growth inhibition	Diuron	EC_{10} 3.2 µg/L* (1)		0.12 µg/L Diuron EQ (1)
	24 h synchronous algae reproduction	Diuron	EC_{10} 0.86 µg/L* (1)		0.11 µg/L Diuron EQ (1)
	Combined algae assay (24 h growth)	Diuron	EC_{10} 4.3 µg/L* (1)		0.13 µg/L Diuron EQ (1)
Immobilization	48 h daphnia immobilization test	Chlorpyrifos	EC_{10} 61 ng/L* (1)		15 ng/L Chlorpyrifos EQ (1)
Mortality	Fish embryo toxicity (48 h)	Bisphenol A	EC_{10} 1820 µg/L* (1)		276 µg/L Bisphenol A EQ (1)
	Fish embryo toxicity (96 – 120 h)	Bisphenol A	EC_{10} 637 µg/L* (1)		183 µg/L Bisphenol A EQ (1)

EC_{10}: effect concentration causing 10% effect; EC_{SPR20}: effect concentration causing a suppression ratio of 20%; $EC_{IR1.5}$: effect concentration causing an induction ratio of 1.5.

*Presented EC_{10} value converted from EC_{50} value assuming a slope of the log-logistic concentration-response curve of 1; •Converted from testosterone equivalent concentration to DHT equivalent concentration using effect concentration data in Lynch et al. (2017); †Converted from methyltrienolone (R1881) equivalent concentration to DHT equivalent concentration using effect concentration data in Hashmi et al. (2018); ‡Converted from Org2058 equivalent concentration and progesterone equivalent concentration, respectively, to levonorgestrel equivalent concentration using effect concentration data in Houtman et al. (2009); #Converted from progesterone equivalent concentration to levonorgestrel equivalent concentration using effect concentration data in Hashmi et al. (2020); ▼Converted from triiodothyronine equivalent concentration to thyroxine equivalent concentration using effect concentration data in Leusch et

al. (2018a); °Converted from nicardipine equivalent concentration to DEHP equivalent concentration using effect concentration data in Escher et al. (2018); §Converted to dichlorvos equivalent concentration using dichlorvos EC value in Escher et al. (2013); ˜Converted to dichlorvos equivalent concentration using dichlorvos EC value in Escher et al. (2018); ⊥Converted to baseline toxic equivalent concentration (TEQ) using the virtual baseline toxicant EC value in Escher et al. (2018).

(1) Escher et al. (2018); (2) Escher et al. (2015); (3) Been et al. (2021); (4) van der Oost et al. (2017); (5) Brand et al. (2013); (6) Brion et al. (2019); (7) Jarošová et al. (2014); (8) Hashmi et al. (2018); (9) Finckh et al. (2022); (10) Leusch et al. (2017); (11) Hashmi et al. (2020); (12) Leusch et al. (2018a); (13) De Baat et al. (2020); (14) Hinger et al. (2011); (15) Neale et al. (2020a); (16) Escher and Neale (2021); (17) Escher et al. (2013); (18) Tang et al. (2013)