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How effective are water treatment processes in removing toxic effects of micropollutants? A literature review of effect-based monitoring data

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ABSTRACT

Over the past decade, effect-based monitoring (EBM) has been increasingly applied for water quality monitoring. Despite being recommended as a monitoring tool in several guideline documents, the use of EBM remains limited to research projects. This study aimed to review the bioanalytical data published from studies conducted on wastewater, drinking water or reuse and to identify knowledge gaps and priorities for action. The results provide an overview of the biological effects associated with raw and treated waters, the reduction of these effects by treatment and a comparison of the detected response with effect-based trigger values. This review highlights a lack of data for many biological effects and the need to more thoroughly investigate effects such as aryl hydrocarbon receptor agonism, genotoxicity and oxidative stress. The results show that most drinking water schemes effectively eliminate the biological effects associated with environmental micropollutants. However, the oxidative stress response and genotoxicity, likely related to formed disinfection by-products, deserve closer attention since they seem to represent a higher concern in drinking water than any other effect. Overall, existing wastewater treatment schemes are less effective in removing biological effects, and consequently, priority should be given to the improvement of wastewater treatment for the better protection of the environment.

Key words: bioassays, drinking water, reuse, wastewater, water safety planning

HIGHLIGHTS

- This study performs a wide review of effect-based monitoring in water contexts.
- It includes the latest limits (trigger values) for health and environmental risk assessments using effect-based monitoring.
- Results are put into context of water treatment performance for both drinking water and wastewater.

ABBREVIATIONS

Ache	Acetylcholinesterase inhibition
Ago.	Agonist
AH	Amiodarone hydrochloride
AhR	Aryl hydrocarbon receptor agonism
Anta.	Antagonist
AR	Androgen receptor
BEQ	Bioanalytical equivalent concentration
Dexa.	Dexamethasone
DHT	Dihydrotestosterone
DW	Drinking water
DWTP	Drinking water treatment plant

E2	17β-estradiol
EBM	Effect-based monitoring
EBT	Effect-based trigger value
ER	Estrogen receptor
GR	Glucocorticoid receptor
GWRC	Global Water Research Coalition
LOD	Limit of detection
Levo.	Levonorgestrel
MBR	Membrane bioreactor
Mife.	Mifepristone
NQO	4-Nitroquinoline 1-oxide
Ox. stress	Oxidative stress
Photo. inhib.	Photosynthesis inhibition
PPAR	Peroxisome proliferator-activated receptor
PR	Progesterone receptor
REF	Relative enrichment factor
Rosi.	Rosiglitazone
T3	Triiodothyronine
Tamox.	Tamoxifen
tBHQ	tert-Butylhydroquinone
TCDD	2,3,7,8-Tetrachlorodibenzodioxin
TR	Thyroid hormone receptor
WHO	World Health Organization
WW	Wastewater
WWTP	Wastewater treatment plant

INTRODUCTION

Domestic and industrial wastewater contains a complex mixture of micropollutants, including pharmaceuticals and personal care products, pesticides and industrial compounds (e.g., Gago-Ferrero *et al.* 2020; McLachlan *et al.* 2022). Wastewater effluent is typically discharged into surface waters where it can potentially affect ecological health due to the presence of micropollutants that are not completely removed by wastewater treatment (Stalter *et al.* 2013). Surface water can also act as source water for drinking water treatment plants (DWTPs), with micropollutants detected in both source and treated drinking water (e.g., Benner *et al.* 2013; Tröger *et al.* 2018). Furthermore, disinfection by-products can be formed during drinking water treatment processes (Neale *et al.* 2012).

Effective treatment processes are required to ensure safe water for both human consumption and ecological health. The efficiency of wastewater and DWTPs is typically assessed for individual chemicals using chemical analysis (e.g., Luo *et al.* 2014; Borrull *et al.* 2021). However, this approach fails to account for the mixture effects of the many chemicals present or the removal of unknown chemicals. Consequently, effect-based monitoring (EBM) using *in vitro* bioassays and well plate-based *in vivo* assays can be applied in parallel to chemical analysis as it can detect the mixture effects of all active chemicals in a sample, including both known and unknown chemicals (Escher *et al.* 2021). Recently, the potential for the application of EBM in water quality assessment has been recognized by the World Health Organization and in the Australian guidelines for water reuse (NRMMC-EPHC-NHMRC 2008; WHO 2017). A number of studies have applied EBM to assess the treatment efficacy of both DWTPs and wastewater treatment plants (WWTPs) by comparing the effect in the inlet and outlet of the plants (e.g., Bain *et al.* 2014; Conley *et al.* 2017; Houtman *et al.* 2018; Neale *et al.* 2020).

Assays indicative of different stages of cellular toxicity pathways, including induction of xenobiotic metabolism, receptor-mediated effect, adaptive stress responses and apical effects, have been applied to drinking water, surface water and wastewater (e.g., Escher *et al.* 2014; Rosenmai *et al.* 2018; De Baat *et al.* 2020). The effect in a bioassay can be expressed as a bioanalytical equivalent concentration (BEQ), which relates the effect of a water sample to the effect elicited by the assay reference compound. To determine if the chemical water quality is acceptable or not, the response in the bioassay can be compared with an effect-based trigger (EBT) value. EBTs have been developed for both drinking water for the protection of human health (e.g., Brand *et al.* 2013, Escher *et al.* 2015) and surface water for the protection of ecosystem health (e.g., van der Oost *et al.* 2017; Escher *et al.* 2018).

Given the increasing attention EBM is gaining, it is important to look back at the published literature and build upon the existing scientific knowledge. To date, only a few studies have reviewed the efficiency of conventional and advanced WWTPs

to remove biological effects (e.g., Prasse *et al.* 2015; Völker *et al.* 2019), with no reviews considering effect removal in DWTPs. Therefore, the objective of this literature review was to characterize the range of BEQs observed in different water matrices and the ability of different water treatment schemes to reduce a large variety of biological effects.

METHODS

Selection of the publications

To select the relevant publications for the review, four selection criteria were applied. Based on previous reports (GWRC 2020a, 2020b), they consisted of:

(1) *Sampling*: At least two points of the treatment scheme (typically inlet and outlet of the WWTP, DWTP or reuse plant) must have been sampled.
(2) *Sample preparation*: The water samples must have been prepared with solid-phase extraction, which is the most commonly used extraction method for water combined with bioanalysis (GWRC 2020a). In addition to micropollutants, unextracted samples also contain metals, salts and other inorganics, so the effect in unextracted samples cannot be attributed to micropollutants alone.
(3) *Analysis*: At least one *in vitro* bioassay must have been applied to the sample extracts.
(4) *Result expression*: Bioassay results must be expressed quantitatively (e.g., equivalent concentration). This is essential for comparison between studies that applied the same assay.

A literature search was conducted on 14th January 2020 using both the Web of Science and Scopus. We searched for water AND '*in vitro* bioassay*' OR 'bioanalytical tool*' OR 'effect-based method*' OR 'cell-based bioassay' OR 'effect-based monitor*' as the 'topic' in the Web of Science and 'title, abstract, keyword' in Scopus. This identified 623 papers. Furthermore, the terms '*in vitro* assay' and 'wastewater' OR 'sewage' OR 'drinking water' OR 'recycled water' or 'surface water' were also searched in the Web of Science and Scopus. This brought the total to 760 papers. An additional 24 papers missed in the Web of Science and Scopus searches were also added, bringing the total to 784 papers. Out of these 784 papers, 49 were identified by the authors as related to wastewater treatment, reuse or drinking water production (first criteria). At this stage, one important publication that was just issued was also added (Petosa *et al.* 2022). In total, 37 publications meeting the three other criteria above were selected and included in the review.

Data collection

These 37 publications were analysed in depth to extract the bioanalytical results and associated information. The following information was collected: (1) study site location, (2) water treatment in place at the study site, (3) use of the treated water, (4) sampling dates, (5) water matrices analysed, and (6) description of the bioassays performed (endpoint, name of the assay, reference compound, expressed results and limit of detection). The analytical results were collected from the main text, tables, figures and/or supporting information. When presented graphically, the online tool WebPlotDigitizer© (Rohtagi 2021) was used to retrieve numerical results.

Data clustering and definitions

To ensure a proper interpretation, the analytical results were clustered according to the type of water treatment scheme and according to the bioanalytical endpoint.

Four categories of treatment schemes were defined (Table 1).

The bioanalytical endpoints described in the different publications were clustered into 16 categories (see Table 2). These categories included the 14 categories with proposed EBT values defined by Escher *et al.* (2021). Two additional biological endpoints were added, given the important number of bioanalytical results related to these assays: genotoxicity with the UmuC assay and assays indicative of the NF-κB-mediated response to inflammation. These categories were defined based on the bioassays used, as presented in Table 2.

Result expression

The bioanalytical data were expressed as BEQs and paired for the inlet and the outlet of a specific treatment scheme on the same date. This means that two different pairs of BEQs (BEQ_{inlet} and BEQ_{outlet}) correspond either to different treatment schemes or different sampling moments within the same treatment scheme. If water sampled at the inlet or the outlet was cytotoxic, the bioanalytical pair was not considered for this specific endpoint.

Table 1 | Categories of water treatment schemes

	Conventional drinking water treatment plant (Conv. DWTP)	Advanced drinking water treatment plant (Adv. DWTP)	Secondary wastewater treatment plant (Sec. WWTP)	Advanced wastewater treatment plant (Adv. WWTP)
Water type and usage	Drinking water produced from ground or surface waters	Drinking water produced from ground or surface waters	Wastewater treated and released into the environment	Wastewater treated and reused or released into the environment
Typical treatment steps	Sedimentation, flotation and/or granular filtration; chlorination or chloramination	Any additional treatment specifically designed for micropollutants removal (oxidation, adsorption and/or membrane filtration)	Any physical treatment based on sedimentation or filtration and biological treatment (activated sludge, membrane bioreactor (MBR), wetlands, anaerobic/aerobic reactors…)	Any additional treatment specifically designed for micropollutants removal (oxidation, adsorption and/or membrane filtration)

Table 2 | Endpoint categories and related assays

Biological endpoint	Assays
Acetylcholinesterase inhibition (AChE inhib.)	AChE inhibition assay
Androgen receptor agonism (AR ago.)	AR-CALUX; YAS; AR rainbow trout; AR-Geneblazer; MDA-kb2; AR CHO cells
Androgen receptor antagonism (AR anta.)	AR-CALUX; YAS
Aryl hydrocarbon receptor agonism (AhR ago.)	AhR CAFLUX; H4IIE-luc; EROD; YDS; Hepa1.12cR cells; AhR CALUX
NF-κB response	NF-κB Geneblazer; NF-κB-bla
Estrogen receptor agonism (ER ago.)	YES; ER-CALUX; E-Screen; ERSheep uteri; T47D-KBluc; MELN; ERBA; hER; MCF-7
Estrogen receptor antagonism (ER anta.)	ER-CALUX; YES, ER-Geneblazer
Genotoxicity (UmuC + S9; UmuC − S9)	UmuC + S9; UmuC − S9
Glucocorticoid receptor agonism (GR ago.)	GR-CALUX; GR-Geneblazer; GR-Switchgear; CV1 cell line
Oxidative stress (Ox. stress)	AREc32; Nrf2
Peroxisome proliferator-activated receptor gamma (PPARγ)	PPARγ-CALUX; PPARγ-GeneBLAzer; PPARγ-bla
Photosynthesis inhibition (photo. inhib.)	I-PAM; Max-I-PAM
Progesterone receptor agonism (PR ago.)	PR-CALUX; PR-Geneblazer
Progesterone receptor antagonism (PR anta.)	PR-CALUX; PR-Geneblazer
Thyroid hormone receptor agonism (TR ago.)	PC-DR-LUC; TR-CALUX; T-Screen; HTR-GRIP1
Thyroid hormone receptor antagonism (TR anta.)	hTR-GRIP1

The results were expressed in three different ways (Figure 1).

Results expressed in any other form than BEQ were excluded from the review. Overall, this represented an exclusion of 22% of the individual analytical results, which were mainly expressed as effect concentrations in units of relative enrichment factor. Given that the limits of detection (LOD) were inconsistently reported, results below the LOD were presented as 0.

For three endpoints, results were expressed with different reference compounds. To enable an accurate comparison of the results, all data for each assay were converted to a common reference compound, using conversion factors based on previously published relative effect potencies (Table 3).

The EBTs used in this study were taken from Escher *et al.* (2021). In cases where multiple EBTs were available (e.g., estrogen receptor (ER) agonism), the minimum and maximum are presented. For a few endpoints, such as aryl hydrocarbon

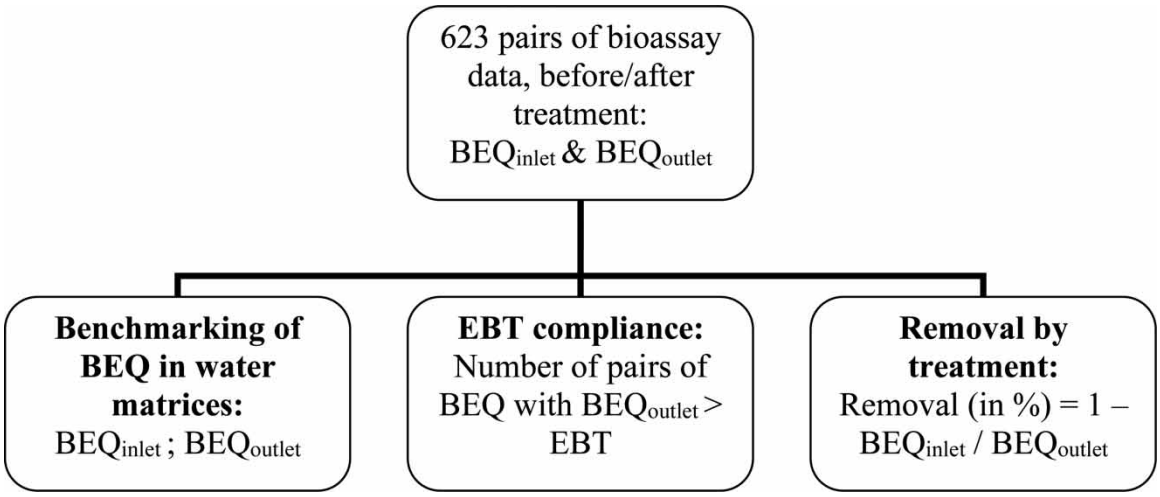

Figure 1 | Result expression for the bioanalytical pairs.

Table 3 | Conversion factors for the three biological endpoints expressed with various reference compounds

Biological endpoint	Conversion applied (reference compound used for comparison in bold)	Reference
AR ago.	AR rainbow trout-binding assay: BEQ in **DHT** eq. = BEQ in testosterone eq. × 6.8	Leusch *et al.* (2014b)
	YAS: BEQ in **DHT** eq. = BEQ in testosterone eq./1.35	Gaido *et al.* (1997)
AR anta.	AR-CALUX: BEQ in **flutamide** eq. = BEQ in vinclozolin eq. = BEQ in bicalutamide eq.	Comptox chemistry dashboard (EPA 2020)
PR ago.	PR-CALUX: BEQ in **levonorgestrel** eq. = BEQ in 19-norprogesterone eq./1.5 = BEQ in progesterone eq./6.3	Sonneveld *et al.* (2011)

receptor agonism (AhR) agonism, oxidative stress and PR antagonism, the reference compound of the EBT was different from the reference compound used for the bioassay quality control. In these cases, the EBTs were converted (Table 4).

BEQs in drinking water were compared with human EBTs, whereas BEQs in WWTPs were compared with ecological EBTs (Table 4). To assess compliance with EBTs at the WWTP outlet, the dilution factor in the receiving water body was not considered. This can result in an overestimation of the ecological risk, which might be lower once dilution in the receiving environment is considered. Where multiple EBTs were available for a specific endpoint, the number of exceedances was determined for the minimum and maximum EBTs.

Toxicity removal was calculated for each individual pair of bioanalytical results.

For BEQ values below the detection limit, the following rules were applied:

If $BEQ_{inlet} > LOD$ and $BEQ_{outlet} < LOD$, removal was set to 100%;
If $BEQ_{inlet} < LOD$ and $BEQ_{outlet} > LOD$, removal was set to 0%;
If $BEQ_{inlet} < LOD$ and $BEQ_{outlet} < LOD$, removal was not determined.

RESULTS

Data collection

The final bioanalytical dataset represented measurements from 12 countries on four continents (Figure 2). More than half of the results from this review originate from two countries, China and Australia. Except for China (middle-income), all countries in the dataset are high-income.

Table 4 | Biological endpoints and associated reference compounds, BEQ units and human and ecological EBTs

Biological endpoints	BEQ reference compounds and units	Human EBT	Ecological EBT
AChE inhib.	µg/L parathion eq.	26	No EBT
AhR ago.	µg/L carbaryl eq.	18	
	Converted in EBT for 2,3,7,8-tetrachlorodibenzodioxin (TCDD) (ng/L) = EBT in carbaryl (ng/L) /303,000 (Neale *et al.* 2015)	0.06	
	ng/L bisphenol A eq.		4.3
	Converted in EBT for TCDD (ng/L) = EBT in B(a)P (ng/L)/1416 (Neale *et al.* 2017; Nivala *et al.* 2018)		0.003
AR ago.	ng/L dihydrotestosterone (DHT) eq.	4.5–11	No EBT
AR anta.	µg/L flutamide eq.	4.8	14
NF-κB response	ng/L TNFα eq.	No EBT	
ER ago.	ng/L 17β-estradiol (E2) eq.	0.2–2	0.1–2.2
ER anta.	µg/L tamoxifen (Tamox.) eq.	No EBT	
GR ago.	ng/L dexamethasone (Dexa.) eq.	21–150	100
Ox. stress	µg/L dichlorvos eq.	284	26–140
	Converted in EBT for tBHQ (tert-Butylhydroquinone) (µg/L) = EBT in dichlorvos (µg/L)/3.9 (Escher *et al.* 2013)	72.8	6.7–35.9
Photo. inhib.	µg/L diuron eq.	0.6	0.07
PPARγ	ng/L rosiglitazone (Rosi.) eq.	No EBT	10–36
PR ago.	ng/L levonorgestrel (Levo.) eq.	724	No EBT
PR anta.	ng/L endosulfan eq.	No EBT	1967
	Converted in EBT for mifepristone (Mife.) (ng/L) = EBT in endosulfan (ng/L)/1585 (Escher *et al.* 2018)	No EBT	1.24
TR ago.	ng/L triiodothyronine (T3) eq.	No EBT	0.62
TR anta.	ng/L amiodarone hydrochloride (AH) eq.	No EBT	No EBT
UmuC + S9	µg/L 2-aminoanthracene eq.	No EBT	
UmuC − S9	µg/L 4-nitroquinoline 1-oxide (4-NQO) eq.	No EBT	

The number of results and related publications per biological endpoints and treatment schemes was not evenly distributed among the study sites (Table 5). The most studied biological endpoint was ER agonism, well above any other. ER agonism and oxidative stress were the only two endpoints with study sites covering the four types of treatment schemes. Furthermore, the number of studies dedicated to drinking water is limited (187 pairs of results) when compared to the number of studies on wastewater (437 pairs of results). The corresponding references are presented in Supplementary Material, Table S1.

Drinking water treatment

The results for drinking water treatment schemes are summarized separately for conventional treatment plants (Table 6) and advanced treatment plants (Table 7).

These results show that for ER ago., the removal of DWTPs is high or very high (median: 97% for conventional DWTP and 100% for advanced DWTP). This leads to very low concentrations in drinking water, typically below 1 ng/L E2 eq.. The risk to human health associated with this biological endpoint is negligible for most of the bioanalytical pairs, with only a few sites (seven conventional DWTPs and two advanced DWTPs) where the risk could be elevated, depending on the EBT value selected for the interpretation of results and the duration of exposure of the populations to such waters.

For the other endpoints, there are fewer bioanalytical pairs; therefore, the results are less robust. For AhR ago. and the NF-κB response, the removal was also high, with medians at 100 and 88%, respectively. These two endpoints are associated with contaminants that are usually effectively removed via conventional drinking water treatment, such as dioxins and endotoxins, respectively. For oxidative stress and especially genotoxicity, the removal was very low or negligible and for some of

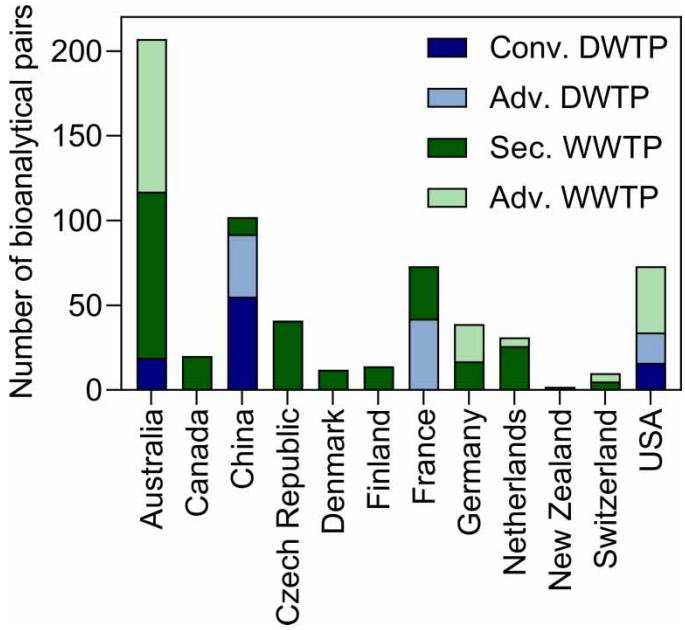

Figure 2 | Number of bioanalytical pairs per country and water treatment scheme.

Table 5 | Number of bioanalytical pairs, number of associated publications, and availability of EBTs for each endpoint

	Type of treatment scheme				
Biological endpoint	**Conv. DWTP**	**Adv. DWTP**	**Secondary WWTP**	**Adv. WWTP**	**Availability of EBT human/ecological**
AChE inhib.	1/1	(0/0)	1/1	2/2	Y/N
AhR ago.	7/2	(1/1)	16/4	7/3	Y/Y
AR ago.	(0/0)	(3/1)	57/10	20/9	Y/N
AR anta.	(0/0)	(2/1)	16/6	15/7	Y/Y
NF-κB response	(0/0)	11/1	2/1	(0/0)	N/N
ER ago.	56/7	67/9	104/18	24/11	Y/Y
ER anta.	(0/0)	(1/1)	2/1	13/4	N/N
GR ago.	(3/1)	(4/1)	9/5	17/7	Y/Y
Ox. stress	3/1	3/1	13/3	6/2	Y/Y
Photo. inhib.	1/1	(0/0)	18/3	7/4	Y/Y
PPARγ	(0/0)	(0/0)	5/2	8/2	N/Y
PR ago.	(0/0)	(6/1)	9/3	14/6	Y/N
PR anta.	(0/0)	(4/1)	(0/0)	(5/1)	N/Y
TR ago.	(0/0)	(4/1)	18/3	(9/2)	N/Y
TR anta.	(0/0)	(0/0)	3/1	(0/0)	N/Y
UmuC + S9	5/2	(0/0)	2/2	7/3	N/N
UmuC − S9	5/2	(0/0)	2/2	7/3	N/N

Results expression: Number of bioanalytical pairs/Number of publications.
Results in brackets: BEQ_{inlet} and $BEQ_{outlet} <$ LD for all bioanalytical pairs.

the bioanalytical pairs, the concentrations at the outlet were higher than those at the inlet. These two endpoints could be indicators of the toxicity associated with disinfection by-products, which can be formed after disinfection with chlorine, chloramine, chlorine dioxide or ozone.

Table 6 | Summary of the bioanalytical results for conventional DWTPs

Biological endpoint	N > LD/N total	Raw water (inlet) [min; median; max]	Treated water (outlet) [min; median; max]	Removal (%) [min; median; max]	N > humEBT
AChE inhib. Parathion eq. (μg/L)	1/1	0.21	0.28	0	0
AhR ago. TCDD eq. (ng/L)	7/8	[0.053; 0.086; 0.18]	[0; 0; 0.17]	[0; 100; 100]	1
ER ago. E2 eq. (ng/L)	49/56	[0; 0.58; 129]	[0; 0.03; 5.3]	[0; 97; 100]	0–7
Ox. stress TBHQ eq. (μg/L)	3/3	[10; 10; 70]	[28; 108; 108]	[0; 0; 60]	2
Photo. inhib. Diuron eq. (μg/L)	1/1	0.01	0.05	0	0
UmuC + S9 2-AA eq. (μg/L)	4/5	[0; 0; 0.345]	[0; 0.95; 1.29]	[0; 0; 0]	No EBT
UmuC – S9 4-NQO eq. (μg/L)	4/5	[0; 0.07; 0.15]	[0; 0.54; 0.78]	[0; 0; 0]	No EBT

N > LOD: number of bioanalytical pairs with inlet and outlet concentrations above the detection limit.
N total: total number of bioanalytical pairs.
N > humEBT: number of bioanalytical pairs with the outlet concentration above proposed human-relevant EBT.

Table 7 | Summary of the bioanalytical results for advanced DWTPs

Biological endpoint	N > LD/N total	Raw water (inlet) [min; median; max]	Treated water (outlet) [min; median; max]	Removal (%) [min; median; max]	N > humEBT
NF-κB response TNFα eq. (ng/L)	10/11	[0.33; 1.4; 2.5]	[0; 0.19; 0.82]	[34; 88; 100]	No EBT
ER ago. E2 eq. (ng/L)	47/67	[0; 0.36; 16.9]	[0; 0; 1,41]	[0; 100; 100]	0–2
Ox. stress TBHQ eq. (μg/L)	3/3	[0; 8.35; 11.9]	[5; 5.96; 5.98]	[0; 29; 50]	0

N > LOD: number of bioanalytical pairs with inlet and outlet concentrations above the detection limit.
N total: total number of bioanalytical pairs.
N > humEBT: number of bioanalytical pairs with the outlet concentration above proposed human-relevant EBT.

Secondary wastewater treatment

There was a high number of studies and bioanalytical pairs (>50) for ER ago. and androgen receptor (AR) ago (Table 8), enabling a robust interpretation of the results. For these two endpoints, the removal in conventional wastewater treatment was high: the median was 100% for AR ago. and 95% for ER ago. As a result, these effects were much lower in outlet water for AR ago. (median = undetected) and ER ago. (median = 2.4 ng/L E2 eq.) compared to the inlet. There are no EBTs for AR ago., but there are several EBTs for ER ago. Despite the significant removal during treatment, the remaining ER ago. activity in outlet water exceeded the relevant EBTs for 50–76 bioanalytical results out of 99, depending on the selected EBT. This means that, without considering the dilution factor in the environment, the environmental risks associated with ER activity were significant.

The other endpoints had fewer bioanalytical pairs and therefore were less robust (Table 8). It seems that the removal was highly variable for most of the other endpoints, especially AhR, AR anta., glucocorticoid receptor (GR) ago., oxidative stress, photosynthesis inhibition, PPARγ, PR ago. and thyroid hormone receptor (TR) anta. For these endpoints, in most cases, an effect was detected at the outlet. For the endpoints with an EBT available, most of the results at the outlet caused an EBT exceedance, except for GR ago. and photosynthesis inhibition.

Table 8 | Summary of the bioanalytical results for secondary WWTPs

Biological endpoint	N > LD/N total	Raw WW (inlet) [min; median; max]	Treated WW (outlet) [min; median; max]	Removal (%) [min; median; max]	N > ecoEBT
Ache inhib. Parathion eq. (μg/L)	1/1	4.4	1	77	No EBT
AhR ago. TCDD eq. (ng/L)	16/16	[0.1; 1; 7.3]	[0.12; 0.4; 2.4]	[0; 53; 89]	16
AR ago. DHT eq. (ng/L)	50/57	[0; 54; 47,000]	[0; 0; 15,600]	[57; 100; 100]	No EBT
AR anta. Flutamide eq. (μg/L)	15/16	[0; 0.01; 26]	[0; 2.1; 510]	[0; 15; 100]	2
NF-κB response TNFα eq. (ng/L)	2/2	[31; 222]	[34; 120]	[0; 85]	No EBT
ER ago. E2 eq. (ng/L)	99/104	[0.55; 42; 2221]	[0; 2.4; 143]	[0; 95; 100]	50–76
ER anta. Tamox. eq. (ng/L)	2/2	[0; 0]	[6; 19]	[0; 0]	No EBT
GR ago. Dexa eq. (ng/L)	9/9	[37; 66; 121]	[0; 45; 163]	[0; 27; 100]	2
Ox. stress TBHQ eq. (μg/L)	13/13	[32; 410; 920]	[3.5; 137; 240]	[40; 64; 99]	11–12
Photo. inhib. Diuron eq. (μg/L)	18/18	[0; 0.17; 2.2]	[0; 0.056; 1.4]	[0; 55; 100]	6
PPARγ Rosi. eq. (ng/L)	5/5	[517; 803; 936]	[83; 249; 309]	[40; 70; 88]	5
PR ago. Levo. eq. (ng/L)	9/9	[0.015; 0.87; 3.6]	[0; 1.7; 4.6]	[0; 0; 100]	No EBT
TR ago. T3 eq. (ng/L)	3/18	197	0	100	0
TR anta. AH eq. (ng/L)	3/3	[17; 57; 403]	[13; 37; 91]	[23; 36; 77]	No EBT
UmuC – S9 4-NQO eq. (μg/L)	2/2	[0.56; 1.52]	[0.19; 0.24]	[57; 88]	No EBT

N > LOD: number of bioanalytical pairs with inlet and outlet concentrations above the detection limit.
N total: total number of bioanalytical pairs.
N > ecoEBT: number of bioanalytical pairs with the outlet concentration above proposed ecologically relevant EBT.

Advanced wastewater treatment

For advanced wastewater treatment schemes, the number of bioanalytical pairs was lower than that of DWTPs and secondary WWTPs (Table 9). ER ago. was still the most common biological endpoint, followed by AR ago., GR ago. and PR ago. For these four endpoints, the median removal was above 50%. This was also the case for most of the biological endpoints with enough bioanalytical pairs for robust interpretation (>10). The median removal was zero for two endpoints: genotoxicity and ER anta. For genotoxicity, this result comes from only one study site where the effect of advanced oxidation was investigated at a pilot scale with various treatments (Jia *et al.* 2015), where the formation of disinfection by-products could explain the observed response. Regarding ER anta., the cause of this result was not identified.

To better describe the results presented in Tables 6–8 for the most studied endpoint, i.e., ER ago., these results are presented graphically in Figure 3.

DISCUSSION

Chemical risk assessment approaches used to establish chemical water quality standards rely on toxicity data generated for individual chemicals, without considering the combined effects of mixtures. Most health agencies worldwide are currently

Table 9 | Summary of the bioanalytical results for advanced WWTPs

Biological endpoint	N > LD/N total	Secondary-treated WW (inlet) [min; median; max]	Tertiary-treated WW (outlet) [min; median; max]	Removal (%) [min; median; max]	N > ecoEBT
Ache inhib. Parathion eq. (μg/L)	1/2	3.2	0.12	96	No EBT
AhR ago. TCDD eq. (ng/L)	7/7	[0.007; 0.007; 1.2]	[0.0041; 0.013; 0.082]	[0; 4; 93]	7
AR ago. DHT eq. (ng/L)	5/20	[0; 0; 1.04]	[0; 0; 5.3]	[0; 64; 90]	No EBT
AR anta. Flutamide eq. (μg/L)	5/15	[0; 0; 510]	[0; 0; 53]	[0; 81; 100]	2
ER ago. E2 eq. (ng/L)	19/24	[0.08; 1.8; 9.1]	[0; 0; 5]	[0; 100; 100]	2–8
ER anta. Tamox. eq. (ng/L)	6/13	[0; 3.0; 19]	[0.14; 5.7; 84]	[0; 0; 0]	No EBT
GR ago. Dexa eq. (ng/L)	15/18	[0; 19; 163]	[0; 0; 151]	[0; 53; 100]	1
Ox. stress TBHQ eq. (μg/L)	6/6	[32.4; 410; 923]	[3.5; 137; 240]	[40; 64; 99]	5
Photo. inhib. Diuron eq. (μg/L)	7/7	[0.033; 0.093; 0.26]	[0; 0; 0.05]	[78; 100; 100]	0
PPARγ Rosi. eq. (ng/L)	3/8	[242; 302; 309]	[0; 139; 261]	[14; 43; 100]	2
PR agonism Levo. eq. (ng/L)	8/14	[0; 0.13; 5.7]	[0; 0; 7.6]	[0; 100; 100]	No EBT
UmuC + S9 2-AA eq. (μg/L)	6/7	[0; 1.8; 1.8]	[0; 2.1; 4.3]	[0; 0; 28]	No EBT
UmuC – S9 4-NQO eq. (μg/L)	7/7	[0.12; 0.12; 0.24]	[0; 0.13; 0.29]	[0; 0; 100]	No EBT

N > LOD: number of bioanalytical pairs with inlet and outlet concentrations above the detection limit.
N total: total number of bioanalytical pairs.
N > ecoEBT: number of bioanalytical pairs with the outlet concentration above proposed ecologically relevant EBT.

developing approaches for the risk assessment of combined exposure to multiple chemicals, and in the latest version of the Guidelines for Drinking Water Quality, the WHO proposed to assume that the combined effect of chemicals present in drinking water at levels near their respective guideline value is additive (WHO 2022). Such an approach is, however, limited to the number of chemicals that can be quantified by analytical techniques and the lack of knowledge on their individual toxicities. This is a serious limitation to a chemical risk assessment, since the application of non-targeted screening techniques has demonstrated that most organic molecules present in drinking waters remain unidentified, although they can be responsible in some cases for the majority of toxic effects (Brunner *et al.* 2020). Consequently, chemical analysis alone is generally insufficient to assess the toxicity of a water sample.

In epidemiological studies, associations are sought between a health condition among a population and their exposure to a micropollutant or a group of pollutants. This approach has been particularly useful to demonstrate the health impact of disinfection by-products in drinking water (Villanueva *et al.* 2004; Mashau *et al.* 2018; Diana *et al.* 2019). Such studies, however, require studies on large populations over long periods and cannot provide a rapid assessment of the toxicity of a water sample.

Compared with these traditional approaches, effect-based methods present the advantage of being able to rapidly provide a more accurate assessment of the toxic effects of a mixture of micropollutants in a water sample. Although not yet included in water regulations, their use was deemed promising especially for prioritization before chemical analyses or for process evaluation by the Australian Guidelines for Water Recycling (NRMMC-EPHC-NHMRC 2008), the WHO guidelines for potable reuse (WHO 2017) and the European Water Framework Directive (Wernersson *et al.* 2015). This usage is of particular

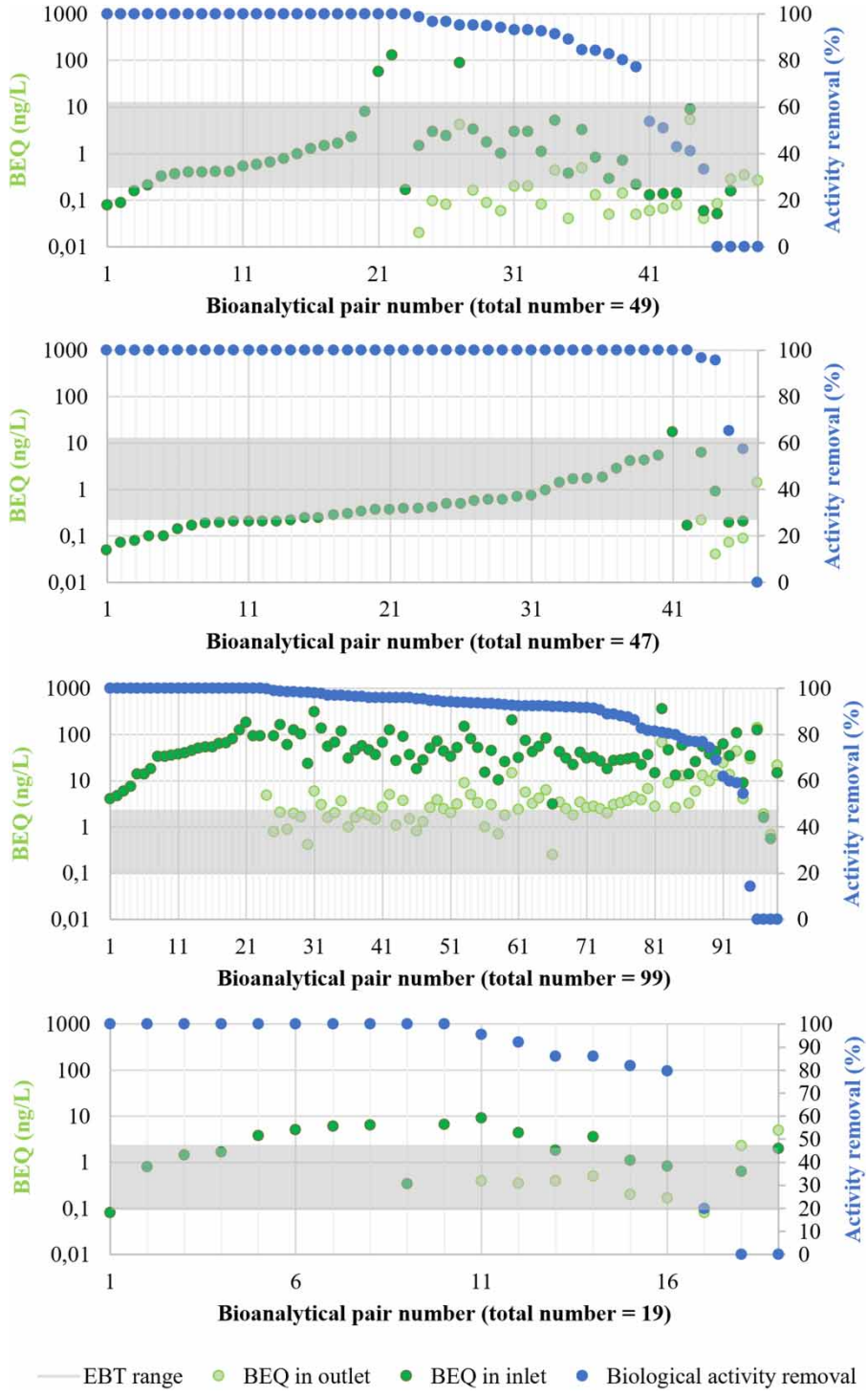

Figure 3 | Results for ER ago. for conventional DWTPs (1), advanced DWTPs (2), secondary WWTPs (3), and advanced WWTPs (4). Human EBTs are presented for graphs 1 and 2, and ecological EBTs for 3 and 4.

interest in the framework of Water Safety Plans, especially to validate control measures for chemical pollutants (Neale *et al.* 2022). Therefore, the present review aimed to collect published *in vitro* bioassay data and quantify the toxicity reduction of the major steps usually applied for wastewater or drinking water treatment.

The 37 publications included in this review yielded 624 bioanalytical pairs with inlet and outlet concentrations above the detection limit. These data spanned 16 different endpoints in wastewater (approximately 70% of the data, see Table 5) and drinking water (approximately 30% of the data). In most publications, the reduction in toxicity was considered globally for a whole treatment chain and did not provide details on the efficacy of individual treatment steps. As a consequence, the available data were insufficient to draw solid conclusions on the efficacy of individual treatment steps, but a global assessment of treatment efficacy for conventional or advanced water treatment schemes was achieved.

This review also pointed out an important heterogeneity in the origin of the data, with EBM as of yet applied primarily in a limited number of countries, i.e., Australia and New Zealand, China, USA and Canada, and several European countries. The exact location of the study sites and the number of people served by the water systems were not consistently reported in the publications. However, the available information suggested that most of these sites were major treatment plants properly operated and monitored and located in large urban areas. As a consequence, this review is probably not representative of low- to middle-income countries, although these countries seem to be suffering higher levels of pollution of their water resources, as highlighted by a recent compilation of data on pharmaceutical pollution of water resources worldwide (Wilkinson *et al.* 2022). Treatment schemes are also often shorter and lack proper maintenance in low- to middle-income countries, thus likely leading to higher levels of exposure and higher resulting chemical risks for aquatic wildlife and human populations. Consequently, this review rather reflects the level of toxic effects that can be detected in a subset of geographical situations and provides insights into the potential efficacy that can be expected from well-operated and well-maintained water treatment schemes in affluent countries.

This review illustrated that only a few endpoints are sufficiently well documented and primarily in wastewater, as drinking water appears to be under-represented in EBM studies (see Table 5). The four most responsive endpoints identified in previous studies by Escher *et al.* (2014, 2021) for the water cycle are activation of AhR, activation of the ER, genotoxicity and oxidative stress response. While ER activation was well represented in this review, the three others were poorly documented in wastewater and even less in drinking water. Except AR ago. and AR anta., all other effects are still poorly documented in wastewater and even more in drinking water and consequently would need further studies.

Among these four major endpoints, toxicity removal in DWTPs was found to be very high for ER ago. (see Figure 3) and AhR (median: 97 and 100%, respectively, see Table 6). On the other hand, it was lower or even null for oxidative stress and genotoxicity, with higher levels in some cases in treated water compared to raw water, thus suggesting that this effect was generated by the treatment itself. This highlights that, for DWTP, these four endpoints are complementary, with responsiveness most probably linked with environmental contaminants for ER and AhR, and disinfection by-products for oxidative stress and genotoxicity.

For the most often applied bioassay, i.e., ER ago., it is worth noting that for conventional DWTPs, 42 results out of a total of 49 (86%) presented values below the lowest trigger value of 0.2 ng/L E2 eq. in drinking water. Even the site presenting the highest ER response in the raw water (Huaihe River in China), with a level of 129 ng/L E2 eq., was able to achieve non-detection in the treated water. This demonstrates that well-designed and operated conventional treatment plants can effectively protect consumers from pollutants with estrogenic effects. Only seven responses in drinking water, all from Chinese sites treating surface waters, exceeded the lowest trigger value of 0.2 ng/L E2 eq. However, these sites did not systematically present high ER responses in the corresponding raw waters. Hence, inadequate design or operation of the plants may explain these exceedances. As expected, treatment lines equipped with advanced treatment (consisting here of granular activated carbon filtration and/or ozonation) showed (except for one Chinese site) ER responses below the lowest trigger value of 0.2 ng/L E2 eq. in drinking water.

Among the seven exploitable results for AhR ago., in drinking water, only one was above the trigger value of 0.06 ng/L TCDD eq. This result comes from an indirect potable reuse scheme in Australia, treating surface water influenced by treated wastewater with a short line composed of clarification, rapid sand filtration and chlorination. In such a case, process optimization or enhancement could be envisaged.

Although not extensively documented, the detection of oxidative stress and genotoxic effects in the majority of the treated waters considered in this review highlights the need for further studies. While the link between chlorine DBPs and oxidative stress has been clearly identified (Farré *et al.* 2013; Hebert *et al.* 2018) and some of the responsible chemicals identified (Stalter *et al.* 2016), most of the DBPs and their conditions of formation remain to be investigated. This review indicates that disinfection by-products could potentially generate more toxic effects in drinking water than the traces of micropollutants of environmental origin still present in the water after treatment but before disinfection, as already pointed out by a recent

study coupling *in vitro* with *in vivo* assays (Lévi *et al.* 2018). Recent European studies on the health impact of disinfection by-products in drinking water emphasize the need to better monitor these by-products and their biological effects (Evlampidou *et al.* 2020).

For the endpoints overall, wastewater treatments do not achieve high removal rates. This can be explained by the low efficiency of the physical and biological steps used for wastewater treatment, which are not especially designed to remove micropollutants. This leads to a high number of EBT exceedances in WWTP effluents. For AhR ago., all the values observed at WWTP outlets are above the ecological EBT of 0.003 ng/L TBHQ eq. For ER ago., 76 values out of 104 (73%) were above the lowest EBT of 0.1 ng/L E2 eq. at the outlet of the secondary treatment, and 8 out of 24 (33%) for advanced WWTPs. For oxidative stress, a majority of samples (12 out of 13, i.e., 92%) were above the EBT of 6.7 ng/L TBHQ eq. after secondary treatment and also after advanced treatment (5 out of 6, i.e., 83%), and genotoxic effects were detectable in the majority of treated wastewaters after secondary and advanced treatment as well.

BEQs in WWTP outlets were compared with ecological EBT without considering the dilution in the receiving water body. For ER ago., in secondary WWTPs, taking into account a 10% dilution would lead to a reduction of EBT exceedance from 73% (no dilution) to 64%. A 1% dilution would still lead to 11% of exceedances.

Overall, except for AR ago., all the toxic effects investigated in these studies were poorly eliminated by the wastewater treatments and all WWTPs present measurable biological effects in their effluents, representing a potential risk for the aquatic wildlife if the dilution is insufficient in the receiving body. This supports previous studies that indicated a higher risk to ecosystems than to human consumers from exposure to water (Leusch *et al.* 2018).

CONCLUSIONS

This review provides the first mapping of toxic effects in the water cycle which can help define priorities of action to better control micropollutants and their biological effects. It also highlights the potential of bioassays to contribute to the assessment of water safety and water treatment performance, making them a useful tool in the framework of Water Safety Plans.

Overall, the available data showed that toxic effects resulting from environmental pollutants seem to be well eliminated by drinking water treatments, even with conventional schemes. This indicates that presently, well-operated DWTPs offer a sufficient level of chemical safety. On the other hand, oxidative stress and genotoxicity generated by chemical disinfection of drinking water deserve closer attention. Disinfection by-products in some drinking waters seem to be generating more toxic effects than the pollutants of environmental origin, illustrating an important trade-off to consider when designing water treatment processes.

Wastewater treatment seems to be poorly effective in removing toxic effects, compared with drinking water treatments. The majority of responses in wastewater effluents present values above the existing ecological trigger values, and toxic effects are detectable in all these effluents. Consequently, priority should be given to the better treatment of wastewater to better protect the environment and water resources, especially in cases where the dilution effect in the receiving body is not sufficient to protect aquatic wildlife.

The application of bioassays is still limited today in the water domain, and bioassays have been primarily applied to wastewaters so far, with an important focus on estrogenic effects. Therefore, further exploration of other important effects such as AhR, genotoxicity and oxidative stress response in wastewaters and especially drinking waters is warranted.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

CONFLICT OF INTEREST

The authors declare there is no conflict.

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