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Systematic Review of Toxicity Removal by Advanced Wastewater Treatment Technologies via Ozonation and Activated Carbon

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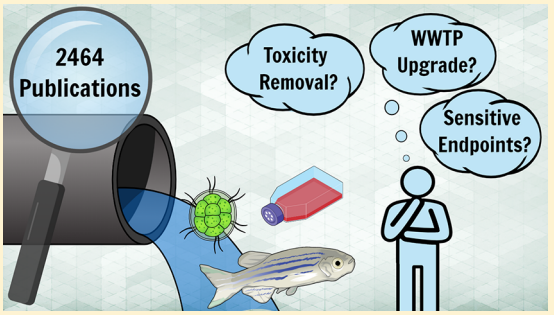
Supporting Information

ABSTRACT: Upgrading wastewater treatment plants (WWTPs) with advanced technologies is one key strategy to reduce micropollutant emissions. Given the complex chemical composition of wastewater, toxicity removal is an integral parameter to assess the performance of WWTPs. Thus, the goal of this systematic review is to evaluate how effectively ozonation and activated carbon remove in vitro and in vivo toxicity. Out of 2464 publications, we extracted 46 relevant studies conducted at 22 pilot or full-scale WWTPs. We performed a quantitative and qualitative evaluation of in vitro (100 assays) and in vivo data (20 species), respectively. Data is more abundant on ozonation (573 data points) than on an activated carbon treatment (162 data points), and certain in vitro end points (especially estrogenicity) and in vivo models (e.g., daphnids) dominate. The literature shows that while a conventional treatment effectively reduces toxicity, residual effects in the effluents may represent a risk to the receiving ecosystem on the basis of effect-based trigger values. In general, an upgrade to ozonation or activated carbon treatment will significantly increase toxicity removal with similar performance. Nevertheless, ozonation generates toxic transformation products that can be removed by a post-treatment. By assessing the growing body of effect-based studies, we identify sensitive and underrepresented end points and species and provide guidance for future research.



1. INTRODUCTION

Micropollutants affect the ecological status of freshwater ecosystems.[1,2] In addition to significant diffuse sources, such as runoff from urban and agricultural areas,[3,4] the discharge of conventionally treated wastewater represents a major point source of pollutants entering aquatic ecosystems.[5] Accordingly, numerous studies demonstrate a negative impact of wastewater discharge on the receiving ecosystem, such as a decline of biodiversity and essential ecosystem functions (e.g., leaf litter decomposition).[6−8] Moreover, the continuous discharge of pollutants by wastewater treatment plants (WWTPs) may also affect drinking water quality, in particular in densely populated regions, where groundwater is replenished by bank filtration.[9] For instance, in the Berlin metropolitan region, several wastewater-borne compounds have been detected in ground and tap water.[10,11]

To improve the water quality of receiving aquatic ecosystems and at the same time protect drinking water resources, conventional WWTPs based on an activated sludge treatment can be upgraded with oxidative and sorptive technologies.[12] Full-scale trials at WWTPs demonstrate that both ozonation and activated carbon treatment reduce the load of a broad range of micropollutants by over 80%.[13,14] Consequently and following a precautionary approach, several countries either are considering or have already started to upgrade their WWTPs. Switzerland has taken a pioneering role by implementing a national policy to upgrade 123 of their 750 WWTPs, which enjoys, despite the high implementation and maintenance cost, widespread public acceptance.[15]

Chemical analysis is the norm for water quality assessment. Nevertheless, an evaluation of wastewater treatment technologies on a per-chemical basis covers only a small fraction of known micropollutants and may, thus, not represent the actual removal performance. In addition, gaps in knowledge regarding the occurrence of unknown compounds, transformation products (TPs),[16] and potential mixture effects exist.[17] To address these limitations, complementary effect-based measurements are increasingly integrated into the evaluation of advanced wastewater treatment technologies. In contrast to chemical analysis, bioassays determine the actual toxicity, integrating the joint effects of all chemicals, including the unknowns and TPs.

While for some specific bioassays (e.g., estrogenic activity), the effect is caused by a few potent chemicals, for most bioassays, only a minor fraction can be explained by the detected chemicals.[4,18] Accordingly, several studies on wastewater or surface waters reported a marked discrepancy

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between the toxicity observed in bioassays and the toxicity predicted based on chemical analysis, even when a broad set of target micropollutants (>400) was included.[4,18,19] Hence, the integration of effect-based measurements into the assessment of wastewater treatment technologies is crucial to obtain a comprehensive picture of their performance in removing toxicity rather than single chemicals. Accordingly, various in vitro and in vivo bioassays have been included in monitoring studies at advanced wastewater treatment pilot or full-scale plants.

While some reviews of targeting micropollutant removal exist,[20−22] a review addressing effect-based studies is lacking. Therefore, the aim of this systematic review is (1) to evaluate the toxicity removal by advanced wastewater treatment and (2) to provide an inventory of the in vitro and in vivo bioassays with the aim of (3) identifying underrepresented and sensitive end points and species. On this basis, we highlight (4) knowledge gaps to guide future research. We, thus, performed a systematic review of effect-based studies and focused on technologies that have already been applied to reduce the emission of chemicals to aquatic ecosystems. Here, ozonation and activated carbon treatment represent the most common and mature technologies that are efficient, technically feasible, and sufficiently cost-effective.[12] We excluded membrane technologies (e.g., ultrafiltration or reverse osmosis) and advanced oxidation processes (e.g., UV/H_2O_2) because these are mainly applied for water reuse or have not been tested in full-scale for wastewater treatment.[23] The lessons learned are useful to benchmark existing technologies, guide future research, and evaluate other advanced wastewater treatment technologies.

2. MATERIAL AND METHODS

2.1. Literature Search. We searched Web of Science (Core Collection) for effect-based studies investigating ozonation or activated carbon treatment using the following search strings: (ozon* OR activ* carbon*) AND (wastew* OR sewage*) AND (ecotox* OR tox* OR in vitro OR in vivo) (accessed July 12, 2018). This search returned 2456 publications, from which we removed studies published before 2000, reviews, and duplicates (Figure 1). To address the goals of the EU Water Framework Directive and as part of the implementation of the Swiss policy to upgrade their WWTPs, several projects have assessed advanced wastewater treatment. Thus, we complemented the peer-reviewed literature with nine relevant project reports.[24−32]

2.2. Eligibility Criteria. To ensure that the data reflects realistic scenarios and is comparable, we exclusively focused on studies that investigated an ozonation or activated carbon treatment at pilot or full-scale implemented at municipal WWTPs. Accordingly, we excluded all studies that (1) did not use bioassays, (2) investigated other treatment technologies (e.g., reverse osmosis), (3) were performed at lab-scale, or (4) investigated hospital or industrial wastewaters.

2.3. Selection of Studies. Two authors (J.V., M.W.) conducted an independent and blinded screening of the literature according to the criteria provided above. For this, we used Rayyan for systematic reviews (https://rayyan.qcri.org).[33] After the initial screening of titles and abstracts, we selected 164 studies for full-text review (Figure 1). In case of conflicting decisions during initial screening, the respective study was included in the full text screening. After the full-text screening, we excluded 118 publications on the basis of the above criteria

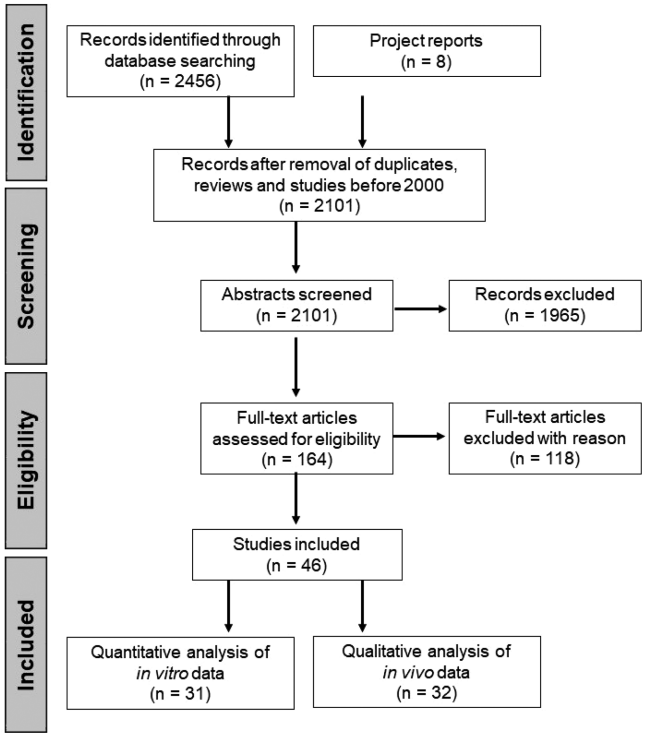

Figure 1. PRISMA flow diagram[35] of the study selection process.

and categorized the remaining 46 studies in two groups (in vitro and in vivo data). While the majority of studies included both into their assessment (46%), 24% and 30% of the studies focused exclusively on in vitro and in vivo bioassays, respectively. We decided not to perform a quality assessment of the selected studies to keep the database broad. However, all in vitro studies included reference compounds used for bioanalytical equivalent concentrations (BEQ) estimation, and all in vivo studies included negative controls and were mostly performed in accordance with international guidelines.

2.4. Data Extraction. Bioassay data from the final set of studies were extracted into an Excel database (see Excel file in Supporting Information (SI)) and categorized according to the location of the pilot plant, the respective in vitro or in vivo end point, as well as to the different treatment types: (1) WWTP influent (INF), (2) conventional activated sludge treatment (CT) and subsequent (3) ozonation (ozone), (4) combination of ozonation and post-treatment (ozone + PT), or (5) activated carbon treatment (AC). If AC was performed subsequent to ozonation, we treated this data as ozone + PT. To provide a general overview, we pooled the data for each treatment type regardless of location-specific differences (e.g., operation parameters, ozone doses). Furthermore, we pooled the data from the different PTs after ozonation (e.g., sand filtration). Nevertheless, we collected all technological specifications of the WWTPs and the standard wastewater parameters (Tables S2−S6). Moreover, because parts of the data from the project reports have also been published in research articles, we compared all bioassay data from the same WWTP and removed duplicates.

2.5. Data Analysis. We performed a quantitative evaluation of the in vitro data by calculating removal effectiveness for the different treatments and end points. The majority of studies reported BEQs (e.g., 17β-estradiol equivalent concentration in ng/L (EEQ)). Furthermore,

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Environ. Sci. Technol. 2019, 53, 7215−7233

some studies reported their results as effect concentrations (EC) in units of relative enrichment factor (REF)[34] or as receptor activation and inhibition. We calculated the removal effectiveness (% toxicity reduction) by comparing the corresponding data points for CT to INF and the advanced wastewater treatment to CT (see equations in SI section 3.1) using the mean toxicity level reported as BEQ or EC for individual samples (e.g., one-week mixture sample). Otherwise, we either used the reported overall removal or performed the calculation based on the given overall mean values. For values below the limit of detection (LOD), the removal effectiveness was calculated using the LOD. The individual calculation for each study is presented in the Excel file (SI). To test for significant differences between the treatments, we applied Kruskal–Wallis with Dunn's post hoc tests using GraphPad Prism 7.0 (GraphPad Software, San Diego, CA). A $p < 0.05$ was considered significant.

For in vitro mutagenicity and all in vivo end points, a quantitative evaluation was hampered by differences in the data presentation and reporting (e.g., lack of raw data, EC_{20} or LOEC). Thus, we were unable to calculate removal effectiveness and performed a qualitative evaluation instead. We recorded the number of experiments in each study reporting either null or adverse effects according to treatment and end points.

3. REMOVAL OF IN VITRO TOXICITY

In vitro bioassays provide mechanistic insights, are ethically sound and economically favorable and offer a high-throughput capability.[36] They are, therefore, increasingly applied to assess water quality and wastewater treatment technologies. However, several challenges and limitations of an effect-based assessment by in vitro bioassays exist. The sample preparation can significantly affect the outcomes of a bioassay.[23,37] For instance, solid-phase extraction (SPE) inevitability leads to a loss of chemicals present in the original sample, in particular of substances with high polarity.[38] Because the causative compounds often remain unknown, an optimization of SPE to extract the toxicity remains challenging. Hence, even though several studies demonstrated an adequate effect recovery by SPE,[39,40] a complete recovery of active compounds can never be guaranteed.[41] However, an extraction of water samples is often necessary (1) to increase the probability of detecting toxicity with regard to the sensitivity of an assay, (2) to remove interfering matrix components (nutrients, salts, pathogens), and (3) to conserve the sample for later analysis. Accordingly, 30/31 studies containing in vitro data analyzed extracted wastewater. Thus, for the review, we focused on the results of SPE-extracted wastewater samples.

Moreover, the assessment by in vitro bioassays can result in false negative and positive effects.[42] Thus, to detect or exclude potential artifacts, quality controls (e.g., adequate blanks and reference compounds), as well as the determination of confounding factors (e.g., DOC or slight cytotoxicity) in the samples, should be included. The latter is particularly crucial for the investigation of hormone receptor antagonism.[43] However, a coherent approach is lacking, and only a few in vitro bioassays have been standardized by OECD or ISO. In addition, a consensus of a uniform data processing and reporting does not exist, which complicates the comparability of results.[44] This can be improved by agreeing on standardized data evaluation processes.[44,45]

Finally, the most significant challenge is to predict the impacts on whole organisms and, a fortifori, on whole ecosystems on the basis of in vitro data. Sample enrichment and highly sensitive cell lines enable the detection of effects even in samples with a low chemical burden (e.g., drinking water).[34] Hence, the detection of an effect does not necessarily translate to an adverse effect in wildlife or human.[46] In addition, several other factors hamper the interpretation of in vitro effects. For instance, in vitro bioassays usually do not cover toxicokinetic processes (i.e., detoxification, metabolic activation), as well as tissue- or organ-specific effects.[23] Accordingly, although they represent fast and sensitive screening tools, they cannot readily replace in vivo experiments.[47] Nevertheless, several studies successfully linked in vitro and in vivo effects, such as the prediction of in vivo vitellogenin induction from in vitro estrogenicity data.[48−50] Accordingly, effect-based trigger values (EBT), which facilitate a decision regarding whether an observed in vitro effect is acceptable or not, have been proposed for water quality monitoring on the basis of read-across approaches from existing guideline values and effect data for single chemicals.[46,51,52]

3.1. Data Availability. Wastewater triggers various mechanisms of action in a battery of in vitro bioassays.[4] Thus, a broad spectrum of assays for multiple relevant end points should be applied to evaluate wastewater treatment technologies. On the basis of our selection criteria, we identified and analyzed 31 studies containing data from 100 in vitro assays. We excluded cytotoxicity assays because the results vary with the cell line and are hard to compare, and the Microtox assay is often more sensitive.[53] These studies cover 28 end points, including several endocrine end points, induction of xenobiotic metabolism, neurotoxicity, phytotoxicity, oxidative stress response, baseline toxicity, as well as genotoxicity and mutagenicity (Tables S7 and S8).[13,24,25,27−32,34,54−74] By far the best-studied end point is estrogenicity (22 out of 31 studies), followed by genotoxicity (18), mutagenicity (12), phytotoxicity(11), bacterial toxicity (11), androgenicity (11), antiandrogenicity (9), aryl-hydrocarbon receptor (AhR) activity (8), antiestrogenicity (7), as well as acetylcholinesterase (AchE) inhibition (4) and glucocorticoid and thyroid activity (4). For all other end points, only data from less than four studies was available.

All studies reported toxicity in at least one assay, while 41 assays were negative throughout all studies (Tables S7 and S8). For the remaining 59 assays, we extracted 647 data points for the calculation of removal effectiveness of the advanced treatment technologies (ozone, 243; ozone + PT, 325; AC, 79). Moreover, many studies did not analyze the influent of the conventional treatment (CT), reducing the data for the calculation of the removal by the CT (138 data points) compared to the advanced wastewater treatment technologies.

3.2. Endocrine End Points. In total, 54 in vitro bioassays for 13 endocrine end points were applied to investigate the removal of endocrine disrupting chemicals (EDCs), which "can interfere with any aspect of hormone action"[75] and include a vast and diverse group of anthropogenic chemicals.[76] There is increasing evidence that exposure to EDCs negatively affects wildlife at comparatively low concentrations.[36,77] Here, wastewater discharge is an important point source. As a prominent example, wild fish populations downstream of WWTPs have been feminized.[78,79] This intersex phenomenon in male fish is often associated with estrogens or estrogen-like

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Environ. Sci. Technol. 2019, 53, 7215−7233

chemicals in the treated effluents.[79] Nonetheless, a range of studies suggests that several other factors contribute, such as the exposure to antiandrogens[80] and to chemicals acting through other mechanisms than classical steroid receptors.[81]

3.2.1. Estrogenicity. Twenty-two studies apply 16 assays for estrogenicity.[13,25,28−32,34,54−59,61,62,65,67,70−72,74] On the basis of these studies, the CT already eliminates estrogenicity effectively with a median removal of 91.8% (Table 1), which is in line with previously reported values for a CT.[82−85] Despite this effective removal, the remaining estrogenicity of the effluents (median of 1.77 ng EEQ/L) may still induce adverse effects on organisms in the receiving water with regard to the low predicted no effect concentrations of 0.1 and 2 ng/L for 17α-ethinylestradiol (EE2) and 17β-estradiol (E2), respectively.[86] Both advanced wastewater treatment technologies eliminate the residual estrogenicity (Figure 2). While ozonation (91.7%) is more effective in removing estrogenicity than AC (75.0%), this difference is not significant (p = 0.08, Table S9).

Table 1. Median Removal [%] of in Vitro Toxicity by a Conventional Treatment (CT) Compared to the Activities Present in the Influent (INF)[a]

end point	median removal ΔINF (%)	95% CI	n	ref
estrogenicity	91.8	82.9−93.3	35	13, 29−32, 54, 57, 59, 61, 67, 72
androgenicity	98.6	93.7−98.9	10	31, 54, 67
progestogenic activity	−273	−657−31.5	4	31
glucocorticoid activity	16.4	−70.6−63.3	4	31
AhR activity	74.5	57.7−83.8	8	60, 67
PPARγ activity	79.0	65.6−90.9	3	31
oxidative stress response	87.1	68.2−103	2	66
AchE inhibition	71.2	59.0−83.1	10	32, 72
algae PSII inhibition	38.8	25.2−53.6	23	24, 29−32, 72
algae growth inhibition	81.3	75.1−86.3	23	24, 29−32, 72
bacterial toxicity	92.1	74.1−97.7	14	24, 32, 66, 72

[a]INF = influent, CI = confidence interval, ref = references, AhR = aryl hydrocarbon receptor, PPAR = peroxisome proliferator-activated receptor, AchE = acetylcholinesterase, PSII = photosystem II.

3.2.2. Androgenicity. Nine different assays for androgenicity were applied in 11 studies.[25,31,34,54−57,59,62,67,74] Here, the CT almost completely removes the androgenicity (median removal of 98.6%, tab. 1), which is in line with previously reported values.[83] Thus, in many cases, a further reduction of androgenicity by an advanced wastewater treatment technology is not detectable.[34,54,59,62,67] If low residual activity exists, both technologies further reduce the androgenicity with a median removal of 43.2% and 54.6% for ozonation and AC, respectively (Figure 2).

3.2.3. Progestogenic and Glucocorticoid Activity. Since they are widely used as pharmaceuticals, various glucocorticoids, mineralocorticoids, and progestogens have been frequently detected in WWTP effluents and surface waters.[87,88] Accordingly, glucocorticoid and progestogenic activity can be detected in conventionally treated wastewater and surface waters.[83,89−93] Hence, these end points should also be considered when evaluating advanced wastewater treatment technologies because of their known effects on fish health[94,95] and increased potency when co-occurring with estrogens and androgens.[96,97]

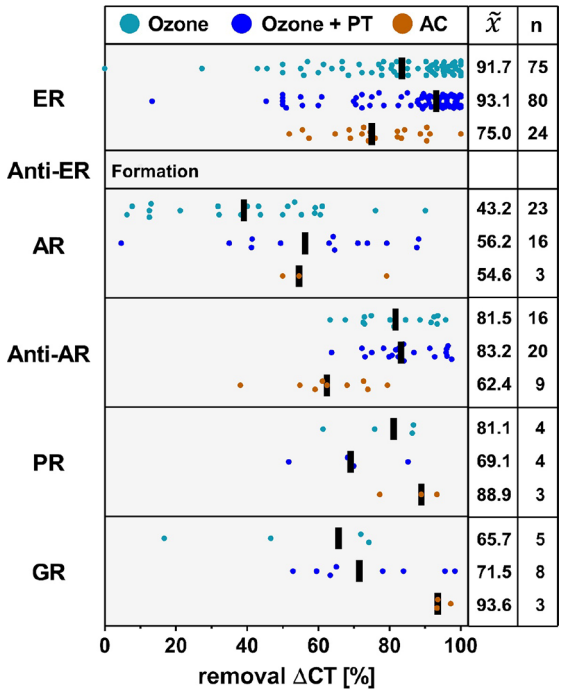

Figure 2. Removal of endocrine activities [%] by advanced wastewater treatment technologies compared to the activities present in the conventional treatment (CT). ER = estrogenicity, anti-ER= antiestrogenicity, AR = androgenicity, anti-AR = antiandrogenicity, PR = progestogenic activity, and GR = glucocorticoid activity. Four data points for AR indicating a toxicity formation (removal < 0%) are not shown here but are included in the Excel file (SI).

However, only three studies investigated progestogenic activity (2 assays)[31,34,62] and four studies glucocorticoid activity (5 assays).[31,34,59,62] Influent samples were only analyzed in one pilot plant.[31] On the basis of this study, CT leads to a formation of progestogenic activity (−273%) and is ineffective to remove glucocorticoid activity (16.4%, Table 1), which is in accordance with previous observations for a CT.[90] While the values between the sampling campaigns vary widely, an increase in toxicity rather than elimination of toxicity is not uncommon and can be, for instance, caused by a deconjugation of phase II human metabolites.[98]

Out of the three studies investigating progestogenic activity, only one study reported activities above the limit of detection (LOD).[31] Here, both advanced technologies are equally effective in reducing the residual progestogenic activity with a median removal of 88.9% and 81.1%, respectively (Figure 2). In contrast, all four studies reported residual glucorticoid activity in conventionally treated wastewater above the LOD. On the basis of these studies, AC is more effective in reducing the glucocorticoid activity than ozonation with a median removal of 93.6% and 65.7%, respectively. However, because of the low sample size, this difference is not statistically significant (p = 0.131, Table S9).

3.2.4. Retinoid-like Activity. In addition to steroid receptors, environmental chemicals can disrupt retinoid signaling.[99] Retinoids control vertebrate morphogenesis, growth, cellular differentiation, and tissue homeostasis,[100]

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Environ. Sci. Technol. 2019, 53, 7215−7233

and an imbalance of retinoids and related substances can induce teratogenic effects in amphibians[101] and fish.[102] Retinoid acid receptor α (RARα) activity was frequently detected in (un)treated wastewater and surface waters.[82,103−105] Compared to that, retinoid X receptor (RXR) activity is less common.[106,107] Nevertheless, RARα activity in conventionally treated wastewater is mostly low or below the LOD due to the effective removal by CT.[82,103,104] Three studies investigated retinoid-like activity during advanced wastewater treatment in five assays.[25,34,73] The first study reported no activity above the LOD for the RXR and RARα.[25] In contrast, Cao et al. observed a very effective reduction of RARα activity by ozonation, even at a low ozone dose of 2 mg L^{-1}. This observation is in line with the third study, which analyzed the performance of an ozone treatment in combination with a granulated activated carbon (GAC) post-treatment and reported a removal of RARα activity by 73.3% (SI Excel file).

3.2.5. Thyroid Signaling. Thyroid hormone signaling is essential for metabolism, growth, and organ development, including the brain.[108,109] While some studies reported thyroid receptor α (TRα)activation by (un)treated wastewater,[107,110] only four studies (5 assays) included advanced wastewater treatment technologies and detected no thyroid activity in the effluents of the CT or the advanced treatment.[25,31,34,62] In contrast, in vivo studies with the *Xenopus* embryonic thyroid assay (XETA) suggest the occurrence of thyroid disruptors in conventionally treated wastewater,[111−113] which is not surprising, given that these compounds often act via non-receptor-mediated mechanisms.[114] Thus, the XETA assay or in vitro bioassays for thyroid hormone biosynthesis (e.g., inhibition of the sodium-iodide symporter)[115] are more relevant than TRα activation to investigate the removal of thyroid disruptors by (advanced) wastewater treatment.

3.2.6. Hormone Receptor Antagonists. While most of the studies focus on agonistic activities of EDCs, a range of environmental contaminants including several herbicides (e.g., prochloraz), flame retardants (e.g., PBDEs), or nonsteroidal anti-inflammatory drugs (e.g., diclofenac, ibuprofen) are known to act as hormone receptor antagonists.[77,116] Thus, antagonistic effects are relevant for the assessment of advanced wastewater treatment but considered in only nine studies.[25,31,34,54−56,59,62,67] Even though antagonistic effects can occur at any hormone receptor, only three studies included antagonistic effects at hormone receptors other than the estrogen and androgen receptors and observed no effects in all investigated effluents.[31,34,62]

3.2.7. Antiandrogenicity. Nine studies covered antiandrogenicity in three assays.[25,31,34,54−56,59,62,67] Because the high agonistic activities in the influent usually mask antagonistic activities, no removal for the CT can be derived based on the available studies. Following an effective elimination of androgenicity by CT (Table 1), four studies reported significant antiandrogenicity in the effluents,[25,34,55,67] which is in line with several studies describing the presence of antiandrogenicity in conventionally treated wastewater[82,117] and in the receiving river.[118,119] Both advanced technologies reduce the antiandrogenic activities (Figure 2). Ozonation (81.5%) is more effective than AC (62.4%), however, not significantly (p = 0.51, Table S9).

3.2.8. Antiestrogenicity. Seven studies included the end point antiestrogenicity (2 assays).[25,31,34,54,55,62,67] While three studies reported activities below the LOD,[31,34,62] an increase of antiestrogenicity in the course of advanced treatment was observed at four WWTPs (Figure S1),[25,54,55,67] which contradicts lab-scale experiments suggesting a good removal of antiestrogenicity by ozonation or AC.[120,121] While bioassays detect the net effect of mixtures of agonists and antagonists,[122] the increase of antiestrogenicity may be explained by the improved removal of estrogenicity as previously observed for the opposite case.[49] However, in the case of ozonation, the antiestrogenicity appears to increase with elevated ozone doses,[25] which suggest the formation of antiestrogenic TPs, as recently described for tamoxifen.[123]

No general conclusion about the effectiveness of the PTs in reducing antiestrogenicity can be drawn based on the available data. Sand filtration or GAC seems to reduce the antiestrogenicity of the effluents (Figure S1). However, at one pilot plant (Neuss), a sand filtration led to a further increase in antiestrogenicity,[67] and at another pilot plant (Eriskirch), the GAC treatment reduced the antiestrogenicity only marginally.[54] Thus, further studies are required to understand the potential formation of antiestrogenicity and identify an appropriate PT.

3.2.9. Disruption of Hormone Biosynthesis. In addition to direct interactions with hormone receptors, several environmental contaminants are known to disrupt endocrine signaling via other than receptor-mediated mechanisms, for example, by binding to transport proteins or blocking enzymes involved in hormone synthesis.[124] However, in vitro assays for hormone biosynthesis were only performed at two advanced WWTPs,[31,34] and only one study provides effect data. On the basis of this study, ozonation in combination with GAC further eliminates effects on steroidogenesis with a median removal of ≥80% (SI Excel file).[34]

3.3. Beyond Endocrine End Points. Since EDCs represent only one group of micropollutants and several other mechanisms of action exist,[125] 38 additional in vitro bioassays for 13 end points were applied to evaluate advanced wastewater treatment (Tables S7 and S8).

3.3.1. Aryl Hydrocarbon Receptor (AhR) Activity. Because AhR activity is frequently detected in municipal wastewater, the end point was also included in eight studies (5 assays) on advanced wastewater treatment.[25,34,55,60,62,67,70,71] The AhR is a ligand-activated factor involved in the regulation of xenobiotic metabolism, liver development, and female reproduction.[126] Because polycyclic aromatic hydrocarbons, polychlorinated biphenyls, furans, and dioxins are well-described AhR ligands, the activation of AhR is often referred to as dioxin-like activity. On the basis of the selected studies, the CT removes the AhR activity with a median removal of 74.5% (tab. 2). Nevertheless, residual AhR activity is still detectable in effluents. Given that the known AhR ligands are highly hydrophobic and, thus, should be well removed by sorption to the sludge particles, it remains unclear, which compounds are responsible for this effect. However, the promiscuous AhR can be activated by a wide range of structurally diverse compounds including hydrophilic substances.[127,128] This is also reflected by ToxCast data with 13.8% of 3860 compounds activating the AhR.[18] Examples for wastewater-borne compounds are biocides, such as the fungicide propiconazole and the herbicide terbuthylazine.[4] Regardless of the unknown causative compounds, ozonation and AC eliminate the residual AhR activity equally effective with a median removal of 84.1% and 78.6%, respectively (Figure 3).

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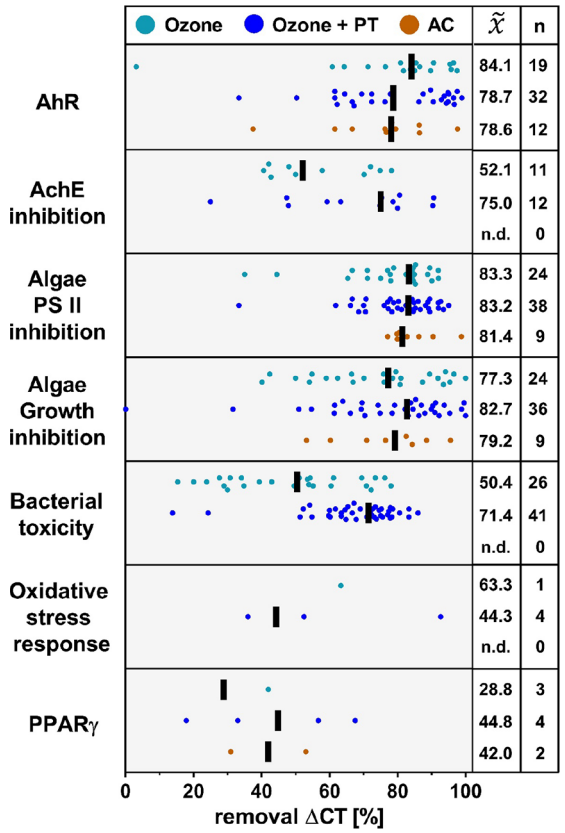

Figure 3. Removal of in vitro toxicity [%] by advanced wastewater treatment technologies compared to the activities present in the conventional treatment (CT). AhR = aryl hydrocarbon receptor activity, PPARγ = peroxisome proliferator-activated receptor γ activity, AchE = acetylcholinesterase, and nd = no data. Six data points indicating an increase in toxicity (reduction < 0%) and are not shown here but are in the Excel file (SI).

3.3.2. Acetylcholinesterase (AchE) Inhibition. According to monitoring data from European rivers, neuroactive chemicals represent the largest group of target micropollutants with a known mechanism of action.[129] However, the AchE inhibition bioassay is the only neurotoxicity assay applied to advanced wastewater treatment. Given that AchE inhibition is the mechanism of action of several insecticides, such as organophosphates or carbamates, the assay represents a crucial insecticide marker, and four studies included this end point.[32,65,71,72] On the basis of the selected studies, the CT reduces AchE inhibition with a median removal of 71.2% (Table 1), which is in line with previously reported values.[85] However, the usefulness of applying this assay to wastewater is limited because a DOC > 2 mg L^{-1}, which is common in treated wastewater, can lead to false-positive results.[130] Ozonation reduces the effect by 52.1% (Figure 3). Moreover, a PT further increases the removal to 75.0%. No data is available for AC.

3.3.3. Combined Algae Assay. For the assessment of the occurrence and elimination of herbicides, the combined algae assay was applied in 11 studies.[13,24,29−32,34,62,70−72] Compared to the classic algae growth inhibition test (4.2), the combined algae assay is modified in a 96-well approach and includes the photosystem II (PSII) inhibition as additional end point. Since this is the mechanism of action of many herbicides (e.g., atrazine, diuron, terbutryn, simazine), the PSII inhibition correlates well with the herbicide content of wastewater.[13] The growth inhibition after 24 h can rather serve as a marker for unspecific toxicity because numerous other substances may cause this effect.

The experiments with the combined algae assay showed an effective removal of growth inhibition by CT (81.3%), while PSII inhibition is insufficiently eliminated (38.8%, Table 1), which is in accordance with previous observations for a CT.[85] Both advanced technologies further decrease the effects with a median removal of ≥77.3% (Figure 3) suggesting a good removal of herbicides. Moreover, a PT after ozonation does only marginally affect the removal of both end points.

3.3.4. Baseline Toxicity. In addition to specific mechanisms of action, many in vitro bioassays cover the baseline or nonspecific toxicity of wastewater. For this purpose, several studies analyzed the cytotoxicity of wastewater on different vertebrate cell lines (e.g., Hep-G2, CHO-9, or GH3).[57,67] The Microtox assay in a 96-well format is an alternative approach.[131] In addition to cytotoxic effects on the bacteria, it also responds to a disruption of the energy budget and is often more sensitive than vertebrate cell lines.[53] Thus, we focused on the Microtox assay, which was applied in 11 studies on advanced WWTPs.[24,32,34,62,64−66,68,70−72] On the basis of these studies, the CT nearly eliminates the bacterial toxicity (92.1%, Table 1), which is in line with previously reported values.[85,132] Ozonation further reduces the toxic effects by 50.4%. A subsequent PT further improved the reduction of bacterial toxicity with a median removal of 71.4% (Figure 3). No data is available for AC.

3.3.5. Induction of Oxidative Stress Response. A broad spectrum of micropollutants induces an oxidative stress response;[99] thus, in vitro bioassays for this end point are increasingly applied to evaluate water quality and water treatment effectiveness.[4,53,132] However, only three studies investigate the reduction of oxidative stress responses by an advanced wastewater treatment in five assays.[34,62,66] Influents were only analyzed in one study.[66] On the basis of this study, the CT already eliminates the oxidative stress response effectively with a median removal of 87.1% (Table 1), which is in accordance with previous values for CT.[132] With regard to the advanced WWTPs, one value is reported for the removal by ozonation alone (63.3%), and four values are reported for ozonation in combination with GAC, which vary widely, resulting in a median removal of 44.3% (Figure 3). However, this variation is the result of the different detection limits of the assays (SI Excel file), and the majority of detected values (4/5) are in range or below the LOD, suggesting a good removal of compounds causing oxidative stress by an ozone treatment. Again, no data for AC is available.

3.3.6. Peroxisome Proliferator-Activated Receptors (PPARs). PPARs play essential roles in the regulation of cellular differentiation, development, and in particular, metabolism.[133] For instance, PPARγ is a key player in adipogenesis and lipid metabolism.[134,135] Agonists of PPARγ (e.g., rosiglitazone or pioglitazone) are commonly used to treat type 2 diabetes.[136] In addition to specific pharmaceuticals, a broad spectrum of micropollutants (e.g., organotins or phthalates) activate PPARγ.[137] For the evaluation of advanced WWTPs, three studies included PPARγ activity.[31,34,62] However, only one of this study analyzed influent samples.[31] On this basis, CT already reduces most of the PPARγ activity (79.0%, Table 1). Regarding the advanced wastewater treatment technologies, only two studies detected PPARγ

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Environ. Sci. Technol. 2019, 53, 7215−7233

activity above the LOD.[31,34] On the basis of these studies, AC (42%) is slightly more effective than ozonation (28.8%, Figure 3), and a GAC treatment after ozonation leads to a slightly improved elimination (44.8%).

3.3.7. Pregnane X Receptor (PXR) and the Constitutive Androstane Receptor (CAR). PXR and CAR are both essential mediators of xenobiotic responses, such as the upregulation of genes that encode metabolizing enzymes (e.g., CYP3A).[138] Thus, the activation of both receptors represent an end point for xenobiotic metabolism. To date, the ecological implication of these end points is unknown. For the evaluation of advanced wastewater treatment technologies, only one study investigated PXR and CAR activation.[34] According to this study, ozonation combined with GAC reduce the PXR and CAR activity of the conventionally treated wastewater with a median removal of ≥77.8% (SI Excel file).

3.4. Genotoxicity and Mutagenicity. The majority of studies included bioassays targeting genotoxicity or mutagenicity in their assessment, which are often performed in the absence and presence of a liver enzyme mix (S9) to address metabolic activation. Especially for oxidative processes, both end points are highly relevant because inactive compounds can be transformed to genotoxic TPs, such as shown for the nontoxic metabolite of the fungicide tolylfluanid to *N*-nitrosodimethylamine (NDMA) during ozonation.[139,140] Furthermore, most of the oxidants are rather consumed by the dissolved organic matter (DOM) than the micropollutants in wastewater,[141] which can lead to the formation of toxic oxygen-rich byproducts (e.g., aldehydes or ketones).[142,143] In addition, if the wastewater contains comparatively high bromide concentrations (e.g., high industrial wastewater), an oxidative process will trigger the formation of bromate, which is a possible human carcinogen.[144−146]

3.4.1. Genotoxicity. Twelve studies used the UmuC assay, which determines the induction of DNA repair mechanisms, to assess genotoxicity of the wastewater.[27,31,32,34,56,58,62,63,70−73] The extracts of conventionally treated wastewater were genotoxic in the majority of studies. Both advanced wastewater treatment processes lead to a considerable reduction of the genotoxicity. AC is more effective than ozonation with a median removal of 62.5% (−S9) and 86.8% (+S9) compared to 34.4% (−S9) and 64.7% (+S9), respectively (Figure 4 A). A PT after ozonation further increases the removal of genotoxicity to 94.3% (−S9) and 80.5% (+S9).

Another option to study DNA damage is the comet assay, which has also been used to assess advanced wastewater treatment.[28,57,59,63,69,147] After exposure to conventionally treated wastewater, no or only moderate effects were reported. With regard to the advanced technologies, the outcomes of the studies differ considerably. At two plants, a significant increase in DNA damage was found after ozonation,[63,147] which indicates a formation of genotoxic TPs and contradicts the outcomes of the UmuC assay. In contrast, two studies reported no effects,[28,57] one study reported an increased tail intensity after exposure to conventionally treated and ozonated wastewater,[59] and yet, another study a decrease of tail intensity in the course of ozonation.[69] In addition to variations in the wastewater composition, the main difference is that all studies that observed an effect in ozonated wastewater performed the comet assay with cells isolated from exposed organisms[59,63,147] instead of laboratory cell cultures.[28,57,69] Thus, DNA damage may manifest in vivo only, and future studies should perform the comet assay with isolated cells from intact organisms, preferably after chronic exposure.

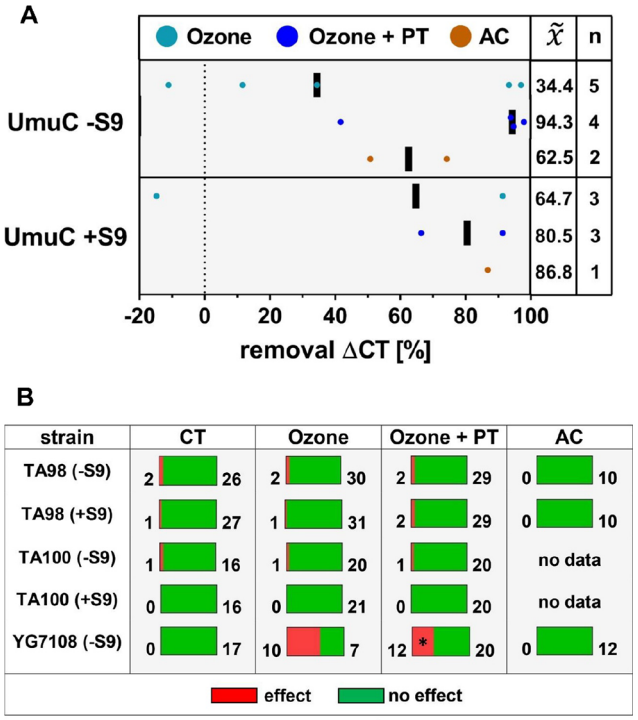

Figure 4. Removal of genotoxicity in the UmuC assay by advanced wastewater treatment technologies compared to the activities present in the conventional treatment (A) and qualitative analysis of the mutagenicity in the Ames assay (B). A significant effect compared to the control is indicated in red including the number of experiments (green = no effect). The asterisk (*) indicates a reduction of mutagenicity that is still significantly increased compared to the control. AC = activated carbon treatment, CT = conventional treatment, PT = post-treatment, and ±S9 = with/without metabolic activation.

A third option to assess genotoxicity is the micronucleus assay, which has been used by six studies to assess advanced WWTPs.[27,28,30,34,57,69] Four studies reported a sporadic micronucleus formation,[27,30,34,57] while in the majority of cases, the effluents of both CT and advanced technologies were not genotoxic (see SI section 3.3 for further information).

3.4.2. Mutagenicity. The Ames fluctuation test with the bacterium *Salmonella typhimurium* is widely applied to analyze mutagenicity and was included by 11 studies to evaluate advanced WWTPs.[24,25,27,28,30,34,54,55,62,63,69] Several strains responsive to different types of mutation exist. To assess wastewater, the standard strains TA98 (frameshift mutation) and TA100 (base pair mutation) are most commonly used. In addition, four studies also included the strain YG7108[25,54,55,63] because of its high sensitivity to alkylating agents and nitrosamines.[148,149] For the summary, we focused on the outcomes of these three frequently used strains, even though three studies used additional Ames strains (SI Excel file).

Mutagenic effects in conventionally treated wastewater and after advanced treatment were only sporadically detected in the standard strains TA98/100 (Figure 4B). In contrast, the strain YG7108 shows an increase of mutagenicity in the course of the ozone treatment in most of the samples,[25,54,55,63] whereas no effects are detectable after AC.[63] These findings are further supported by a lab-scale study, which also observed a marked

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Environ. Sci. Technol. 2019, 53, 7215−7233

increase in mutagenicity after ozonation.[150] The mutagenic effect increased with an elevated ozone dose suggesting that the formation of mutagenic TPs causes this effect.[63] While nitrosamines are formed during ozonation,[139,140] they can be ruled out as causative agents since the mutagenicity occurs without S9 (Figure 4B), and nitrosamines require metabolic activation to be mutagenic.[151] Thus, the mutagenic compounds remain unknown and deserve further research. A subsequent PT reduces the mutagenicity in all cases. Nevertheless, it is still significantly increased compared to the control, as well as to CT. Furthermore, no general conclusion about the effectiveness of the different PTs can be drawn based on the available data. While a biofilter with an expanded clay layer seems to be ineffective,[25,55] sand filtration and a GAC treatment significantly reduce the mutagenicity. However, the effectiveness varies between these technologies,[54,55,63] and further investigations are required to identify an appropriate PT.

3.5. Comparison with Proposed Effect-Based Trigger Values (EBTs). While it remains challenging to extrapolate ecological impacts of wastewater discharge based on in vitro data (see section 3), EBTs represent one way forward for including in vitro data in an assessment of water quality. However, these come with certain limitations: First, the link between in vitro end points and in vivo toxicity is weak in many cases (e.g., for xenobiotic metabolism). Second, the compounds triggering in vitro effects are often unknown (e.g., oxidative stress response). Thus, EBTs are based on the few known compounds for which in vivo data exist. Third, there is currently no consensus on how to derive EBTs (e.g., which type of in vivo data should be included), limiting their acceptance. Finally, defining a single threshold level for complex chemical−biological interactions will always be reductionist.

Taking a pragmatic approach, experts from an international network proposed a set of tentative EBTs to integrate in vitro data into a water quality assessment. As these are the only EBTs available for surface waters, we compared those to the extracted in vitro data.[46,51] We identified seven EBTs for which sufficient data was available for advanced wastewater treatment: estrogenicity (0.4 ng EEQ/L), glucocorticoid activity (100 ng dexamethasone-EQ/L, GR-CALUX), AhR activity (50 pg TCDD-EQ/L, AhR-CALUX), PPARγ activity (10 ng rosiglitazone-EQ/L, PPARγ-CALUX), PSII-inhibition (0.07 μg diuron-EQ/L, combined algae assay), bacterial toxicity (1246 μg TEQ/L, Microtox), and oxidative stress response (156 μg dichlorvos-EQ/L, AREc32 assay). To take all estrogenicity data into account, we used the bioassay-independent value of 0.4 ng EEQ/L,[152] which is in the range of bioassay-specific EBTs.[46,153] Nevertheless, we also performed the comparison with the assay-specific EBTs for estrogenicity (Figure S2). For all other end points, we used assay-specific EBTs exclusively and compared these to the empirical data from the corresponding assay, only.

The bioassay equivalent concentrations for the CT exceed the proposed EBTs in the majority of cases: 100% of the PPARγ activity data (n = 3), followed by estrogenicity (89.7%, n = 68), AhR activity (87.5%, n = 8), PSII inhibition (84%, n = 25), oxidative stress response (66.7%, n = 3), bacterial toxicity (52%, n = 25), and glucocorticoid activity (40%, n = 5). Depending on the dilution, the highest risk to exceed the EBT in the receiving system exists for PPARγ activity with a 10-fold higher median BEQ in CT. This is followed by estrogenicity (3.9-fold), oxidative stress response (3.3-fold), AhR activity (2.8-fold), PSII inhibition (1.3-fold), bacterial toxicity (1.0-fold), and glucocorticoid activity (0.6-fold). To investigate further, we used the data set for estrogenicity because of its wealth of information to derive critical wastewater fractions in the receiving system (Figure 5). To simplify, we assumed a linear dilution of the effect and neglected upstream contaminations.

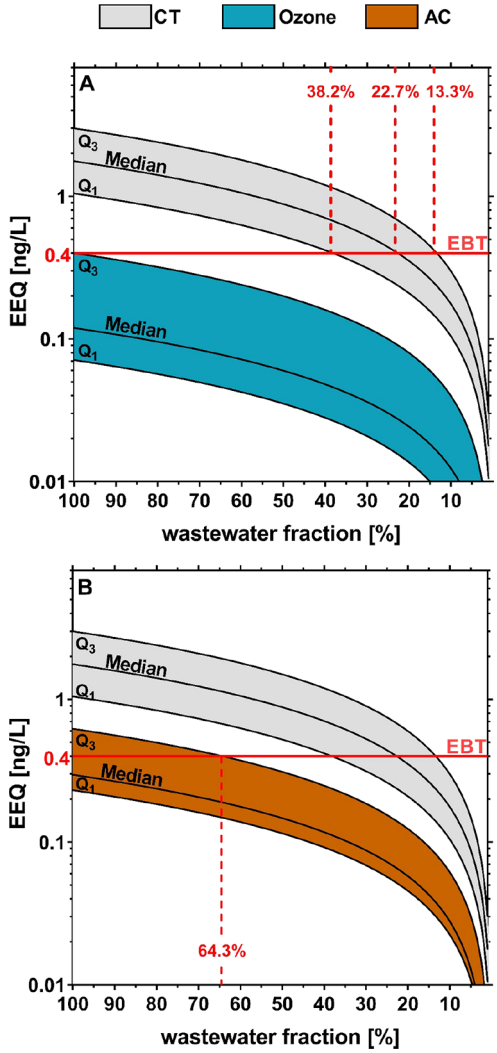

Figure 5. Comparison of 17β-estradiol equivalents (EEQ) at different wastewater fractions in the receiving stream between (A) the conventional treatment (CT, n = 68) and ozonation (ozone, n = 63) and (B) CT and activated carbon treatment (AC, n = 22). Data is presented as 25th (Q_1), 50th (median), and 75th (Q_3) percentiles. The red line indicates the effect-based trigger value (EBT) of 0.4 ng/L. The dotted red lines illustrate the estimated critical wastewater fractions.

On the basis of the EEQs in CT, the critical wastewater fractions for the 25th, 50th, and 75th percentiles are 38.2%, 22.7%, and 13.3%, respectively (Figure 5A). Especially under low flow conditions, these values are exceeded in a large number of European and U.S. surface waters,[154,155] which is in line with studies modeling critical concentrations of individual wastewater-borne estrogenic substances in rivers.[154,156] Both advanced wastewater treatment technologies reduce the median EEQ below the EBT (Figure 5). Accordingly, both

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Environ. Sci. Technol. 2019, 53, 7215−7233

treatment options will enable compliance with the proposed environmental quality criteria. Nevertheless, the lower reduction of estrogenicity by AC (see section 3.2) is also reflected in the critical wastewater fractions. Taking the 75th percentile of the EEQs in AC, a risk of exceeding the EBT still exists in surface waters receiving a wastewater fraction >64.3% (Figure 5B), which are rarely observed.[154,155]

Regarding the other end points, both advanced technologies reduce the median toxicities of the effluents far below the EBT for the end points PSII inhibition, glucocorticoid activity and for bacterial toxicity (Figure S3). For the AhR-CALUX assay, the proposed EBT coincides with the LOD. Thus, most effects of the effluents of the advanced WWTPs are also in this area. While data on the removal of the oxidative stress response during advanced wastewater treatment is scarce, there is a general trend that it is effectively reduced (see section 3.3). The only available value for ozone is far below the EBT, whereas one of the two values for ozonation in combination with a PT is above (Figure S3). In contrast, all PPARγ-CALUX BEQs of the effluents of the advanced technologies exceed the proposed EBT by more than 5-fold (Figure S3). This suggests a remaining risk even after an upgrade. Nevertheless, because the EBT derivation by van der Oost et al. is based on the background BEQ, the proposed EBT can be rather used as an indicator of overall chemical stress than for a micropollutant risk.[51] Since in vitro data for (advanced) WWTPs is scarce (see section 3.3) and the causative PPARγ agonists remain largely unknown,[46] further knowledge is required to clarify this finding.

Overall, the available EBTs were crucial to distinguish between CT and advanced WWTPs. While the BEQs in the CT exceeded the EBTs in the majority of cases, advanced wastewater reduced the toxicity to a level largely below. Thus, as long other contamination sources contribute to a minor extent, the implementation of advanced wastewater treatment ensures compliance with the proposed environmental quality criteria for in vitro toxicity even at high wastewater fractions in the receiving ecosystem.

4. REDUCTION OF IN VIVO TOXICITY

An in vivo assessment aims at characterizing integrative, apical effects on end points like mortality, development, growth, reproduction, or behavior in aquatic key species representing the different trophic levels.[23] Initially developed for the risk assessment of single chemicals, in vivo bioassays are now widely used for investigating the toxicity of environmental samples, including wastewater either in laboratory or on-site flow-through systems. The latter integrates changes in chemical composition over time and avoids sample storage, transport, and treatment[157] but also increases the complexity and costs.

While in vivo bioassays integrate toxicokinetic and toxicodynamic processes, they provide only limited information about the underlying mechanisms.[23] This is particularly critical when evaluating wastewater because the organisms used in standardized in vivo studies are quite sensitive to the wastewater matrix (e.g., salinity, nutrients, or suspended organic matter). Thus, beneficial effects resulting from an additional nutrient or food supply may mask toxic effects caused by micropollutants,[158,159] making it is impossible to differentiate between the two.

For the majority of model organisms, standardized test guidelines for determining acute and chronic toxicity exist. The latter is particularly relevant since the low micropollutant concentrations in wastewater from state-of-the-art WWTPs usually do not induce acute effects.

4.1. Data Availability. On the basis of our selection criteria, we identified 32 studies containing data from in vivo bioassays with 20 species (Table S10).[13,24−26,28−32,34,54−57,59,63,73,74,147,157,160−171] Since 29 out of the 32 studies exclusively analyzed aqueous samples, we focused on these and the most frequently used model organisms (see SI section 4.3 for less common species). Daphnids (*Daphnia magna* or *Ceriodaphnia dubia*) were most common (11 studies), followed by *Danio rerio* (8), *Lemna minor* (8), microalgae (8, see Table 2 for full list). For these 12 species, we extracted, in total, 219 data points for the CT, 201 for ozone, 224 for ozone + PT, and 51 for AC.

4.2. Phytotoxicity. Twelve studies used the classic growth inhibition test with microalgae or duckweed, *L. minor*, to analyze the phytotoxicity of wastewater.[26,28,31,32,55,57,59,74,147,157,163,169] Both test systems include a chronic exposure and are standardized by ISO and OECD.[172−174]

4.2.1. Algae. The algae growth inhibition experiments provide a heterogeneous picture. The majority of studies (6/8) did not observe an effect on the growth of *Pseudokirchneriella subcapitata* after exposure to wastewater from conventional and advanced treatment (Table 2), which is in line with the outcomes of the combined algae assay (see section 3.3). In contrast, two studies with *Desmodesmus subspicatus* observed a persistent low growth inhibition after exposure to CT (<30%). While the first study observed no removal of the effect by the advanced treatments,[28] the second study reported an algae growth on control level after ozonation in combination with a fluid bed reactor (2/3 cases).[57]

4.2.2. Higher Plants. In more than half of the laboratory experiments with *L. minor* (54%), a low growth inhibition (<30%) was reported after exposure to CT, which was not reduced by an additional ozone treatment (Table 2). Moreover, three on-site experiments with *L. minor* indicate a slight increase of toxicity in the course of ozonation compared to the CT. In contrast, exposure to effluents of AC did not result in growth inhibition (Table 2).

4.3. Invertebrate Toxicity. Twenty-two studies investigated the effects of wastewater on aquatic invertebrates.[24−26,28−32,54,55,59,73,74,147,157,163,165−167,169−171] In addition to acute toxicity in *D. magna*, all other methods use chronic exposure regimes. In addition to the classic reproduction test with *D. magna* and *C. dubia*, studies were carried out with the nonbiting midge *Chironomus riparius*, the benthic organism *Lumbriculus variegatus*, as well as the mud snail *Potamopyrgus antipodarum*. The majority of bioassays are standardized[175−179] but sometimes slightly modified for testing wastewater. Moreover, *Gammarus fossarum* as a key decomposer[180] was used in seven studies.[25,29,55,165−167,170]

4.3.1. Daphnids. With one exception,[73] no acute toxicity was reported for *D. magna* exposed to wastewater from conventional and advanced treatment (Table 2). Likewise, chronic exposure did not induce reproductive toxicity.[26,28,157,169] Similarly, in the majority of experiments with *C. dubia*, no adverse effects were reported. However, in 19% of the experiments, the reproduction was negatively affected after exposure to CT. The occurrence of reproductive effect was lowest in the effluents of AC (11%) compared to ozonation (17%) and ozone + PT (27%).[24,29−32]

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Environ. Sci. Technol. 2019, 53, 7215−7233

Table 2. Qualitative Analysis of the in Vivo Experiments[a]

model organism	endpoint	CT	Ozone	Ozone + PT	AC	references
Algae and higher plants						
P. subcapitata (lab.)	growth inhibition	1 18	0 15	1 17	0 3	31, 32, 55, 59, 74, 163
D. subspicatus (lab.)	growth inhibition	20 0	18 3	10 2	1 0	28, 57
L. minor (lab.)	growth inhibition	12 13	11 10	9 7	0 4	28, 31, 32
L. minor (on-site)	growth inhibition	0 3	1 2	4 2	0 1	26, 147, 157, 169
Invertebrates						
D. magna (lab.)	acute toxicity	1 20	1 20	0 7	0 1	28, 73, 74
D. magna (lab.)	reproduction	0 12	0 12	0 7	0 1	28
D. magna (on-site)	reproduction	0 2	0 2	0 2	no data	26, 157, 169
C. dubia (lab.)	reproduction	4 17	3 15	9 24	1 8	24, 29-32
C. riparius (on-site)	reproduction	0 2	0 2	0 2	0 1	147, 157
L. variegatus (on-site)	reproduction	2 10	2 10	0 19	0 2	24, 26, 28, 31, 147, 157, 169
L. variegatus (on-site)	biomass	0 12	6 6	0 19	1 1	24, 26, 28, 31, 147, 157, 169
P. antipodarum (lab.)	reproduction ↓	4 0	3 0	10 0	2 0	54
P. antipodarum (on-site)	reproduction ↑	7 1	0 8	0 8	0 1	25, 28, 55, 147, 157
G. fossarum (lab.)	feeding activity	6 0	no data	0 6	4 * 0	166, 167
G. fossarum (on-site)	feeding activity	1 1	0 1	0 2	0 1	29, 165
Fish						
D. rerio (lab)	embryo toxicity	2 30	1 30	1 20	0 4	28, 31, 32, 55, 59, 74, 163
O. latipes (lab)	embryo toxicity	2 0	1 0	no data	1 1	73, 160
O. mykiss (on-site)	mortality	2 3	2 3	1 8	0 3	13, 24, 29, 31, 63, 164
O. mykiss (on-site)	hatching success	2 2	1 3	1 7	0 2	13, 24, 29, 31, 63, 164
O. mykiss (on-site)	swim-up	2 1	3 0	2 1	0 2	13, 24, 29, 31, 63, 164
O. mykiss (on-site)	weight & length	3 2	2 3	2 7	0 3	13, 24, 29, 31, 63, 164

[a]A significant effect compared to the control is indicated in red including the number of experiments (no effect = green). * No effect after nutrients were spiked. AC = activated carbon treatment, CT = conventional treatment, and PT = post-treatment.

4.3.2. Chironomus riparius. On-site experiments with the nonbiting midge *C. riparius* were conducted at two WWTPs.[147,157] In both cases, no toxicity was detected after exposure to wastewater from CT, ozone, or AC.

4.3.3. Lumbriculus variegatus. Seven studies included on-site experiments with the sediment-dwelling oligochaete *Lumbriculus variegatus*.[24,26,28,31,147,157,169] Effects on reproduction occurred in 17% of the experiments in the CT and ozonated effluents, while no effects were observed after a PT or AC (Table 2). Moreover, oligochaetes exposed to ozonated wastewater had a significantly reduced biomass compare to the CT in 50% of the experiments. This effect is associated with the formation of toxic oxidation byproducts. In all cases, a PT reduced this effect, underlining its importance as a barrier for toxic TPs. A significant reduction of biomass was also reported in one experiment after AC. Here, toxic TPs as a causal factor can be ruled out.[31]

4.3.4. Potamopyrgus antipodarum. The chronic reproduction test with *P. antipodarum* was performed by one laboratory study[54] and by six studies in on-site, flow-through experiments.[25,28,54,55,147,157] The laboratory experiments resulted in persistent reproductive toxicity after the CT, which was not reduced by advanced treatment (Table 2). In contrast, exposure to CT in seven on-site experiments resulted in a significant increase of fecundity,[25,28,147,157] an effect that has been associated with exposure to residual estrogenic compounds.[181] While this is supported by parallel exposure to estrogenic compounds increasing reproduction,[28,157] a mechanistic link is missing because the molluskan steroid receptor orthologs are ligand-independent.[182] Both advanced wastewater treatment technologies reduced this effect in all cases (Table 2).

4.3.5. Gammarus fossarum. A series of laboratory experiments with *G. fossarum* reported a significant reduction of feeding activity after exposure to CT.[166,167] An ozone treatment coupled to sand filtration increased the feeding activity to control level suggesting an effective removal of this effect. In contrast, the feeding activity remained low in all

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Environ. Sci. Technol. 2019, 53, 7215−7233

effluents of AC.[167] The negative effect was recovered when spiking the respective effluents with nutrients. This suggests that nutrient limitation rather than micropollutants reduced the feeding activity. The findings are further supported by an on-site experiment in which a higher feeding activity and population size in ozonated wastewater compared to the CT was observed.[165] However, in a second on-site experiment at another WWTP, no alteration of the feeding activity was detected.[29] Furthermore, Wigh et al. reported a delayed molt cycle, reduced fecundity, and fertility of female gammarids exposed to CT, which were not reduced by an ozone treatment.[170] In addition, >90% of embryos exhibited developmental malformations.[170]

4.4. Fish Toxicity. To assess adverse effects on fish, nine studies conducted the acute fish embryo toxicity test (FET) with zebrafish (*Danio rerio*) or Japanese medaka (*Oryzias latipes*),[28,31,32,55,59,73,74,160,163] which is standardized by the OECD.[183] One study each performed chronic, long-term experiments with *D. rerio*[56] and *O. latipes*.[74] Furthermore, to examine effects on the early stages of the development of rainbow trout (*Oncorhynchus mykiss*), the fish early life stage test (FELST)[184] was performed in on-site flow-through experiments.[13,24,29,31,63,164] Moreover, four studies investigated changes in gene expression of chronically exposed *O. mykiss*.[24,161,162,168]

4.4.1. Danio rerio. Most data is available for the FET with *D. rerio*.[28,31,32,55,59,74,163] Here, the exposure to wastewater from conventional and advanced treatment did not induce mortality in most cases (Table 2). Hence, this test is not sensitive enough to detect effects of micropollutants. Moreover, one study assessed chronic toxicity on *D. rerio*[56] and analyzed vitellogenin (VTG) as a biomarker of exposure to estrogenic substances. Exposure to CT increased mortality, as well as VTG levels. Both advanced treatment technologies reduced these effects.

4.4.2. Oryzias latipes. Two studies performed the FET with *O. latipes*[73,160] and reported an increased embryo mortality after exposure to CT. While in one study AC reduced this effect,[160] neither ozonation nor AC reduced the embryo toxicity in a second study.[73] Here, the ozone treatment further increased the mortality and induced a higher incidence of morphological abnormalities with an increasing ozone doses.[73] This observation contradicts the outcomes of a third study,[74] which performed a 21-day medaka screening assay for estrogenic and androgenic activities, and aromatase inhibition.[185] Here, the authors reported no mortality or any signs of diseases, as well as no estrogenic or androgenic effects after exposure to CT and ozone.

4.4.3. Oncorhynchus mykiss. The FELST test with *O. mykiss* was the most frequently applied assay to assess chronic fish toxicity.[13,24,29,31,63,164] These investigations provide a heterogeneous picture. At the WWTP Regensdorf, the hatching success, swim-up behavior, and length and weight of the fish were impaired after the exposure to CT.[164] Here, the ozone treatment led to a significant increase in toxicity rather than a removal. These effects were again associated with the formation of toxic oxidation byproducts. A subsequent sand filtration reduced the effects to the level of the CT, and, thus, seems to be an effective barrier for the formed TPs. These findings are further support by investigations at the pilot plant Neuss. Here, ozonation significantly increased mortality and delayed the swim-up behavior compared to the CT, whereas the weight and the length were not affected.[63] In contrast, three studies conducted at the WWTP Lausanne, Basel, and Neugut reported positive effects of an ozone treatment on the early development of *O. mykiss*.[13,24,29,31] At the WWTP Lausanne, all investigated end points were affected after exposure to CT, whereas, in Neugut, only a significantly increased mortality, and in Basel, a reduction of the weights and lengths of the fishes were observed. In all cases, an ozone treatment reduced these effects considerably. Importantly, no adverse effects were reported for AC (Table 2).

Three out of the five studies also analyzed VGT.[29,31,164] In accordance with the findings in *D. rerio*,[56] VGT concentrations were significantly increased in fish exposed to CT, indicating the presence of estrogenic substances. Both advanced technologies reduced this effect in all cases, which is in line with the improved reduction of in vitro estrogenicity (section 3.2).

In addition to the FELST, gene expression analyses of chronically exposed *O. mykiss* were performed by four studies.[24,161,162,168] At the WWTP Neugut, a broad spectrum of genes involved in estrogen response, xenobiotic metabolism, immune regulation, cell cycle control, as well as metal, oxidative, and general stress responses were investigated.[24] The majority of these genes was significantly upregulated after exposure to CT. Except for some genes for metal and oxidative stress, ozonation reduced the upregulation considerably, which is in line with the respective in vitro end points (sections 3.2 and 3.3). These findings are further supported by three studies at the pilot plant Stockholm, which reported similar outcomes for an ozonation.[161,162,168] In contrast, Cuklev et al. observed an induction of *hsp70* as biomarker for a general stress in all fish exposed to ozonated effluents, which might be related to TP formation.[168] Importantly, none of the analyzed genes were differentially expressed in fish exposed to effluents of AC, which is in line with the absence of effects on apical end points in the FELST.[168]

5. KEY FINDINGS, RESEARCH GAPS, AND RECOMMENDATIONS

5.1. Conventional Treatment Effectively Reduces the Toxicity. So, Is There Still an Environmental Risk? On the basis of the available influent data, conventional activated sludge treatment already removes a large part of the in vitro toxicity: AR > bacterial toxicity > ER > algae growth inhibition > PPARγ > AhR > AchE inhibition are all reduced by $\geq$70%. This finding is in line with the absence of toxicity in a range of in vivo bioassays (Table 2), even though exposure to an undiluted CT effluent represents a worst-case scenario. In contrast, PR < GR < algae PSII inhibition are insufficiently reduced (<40%, Table 1), and significant in vitro activities at multiple end points can still be detected in the effluent of a CT. These residual toxicities exceed available EBTs for surface waters in the majority of cases and in accordance with previous studies.[46,89,186] This suggests that the discharge of conventionally treated wastewater can represent an environmental risk. The low critical wastewater fractions for estrogenicity further support this. Especially in surface waters in (semi)arid and densely populated regions,[154,155] it will be difficult to comply with the proposed EBTs without implementing additional reduction measures. Although threshold values are absent, a residual environmental risk may also exist in vivo. Here, 92% of the reproduction experiments with *P. antipodarum* (n = 12), followed by the feeding activity test with *G. fossarum* (88%, n = 8), FELST with *O. mykiss* (60%, n

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Environ. Sci. Technol. 2019, 53, 7215−7233

= 5), growth inhibition test with *L. minor* (48%, *n* = 27), *C. dubia* (19%, *n* = 21) and *L. variegatus* (15%, *n* = 13) reproduction tests reported negative impacts after exposure to CT effluents.

5.2. An Upgrade to Advanced Treatments Will Significantly Increase the Toxicity Removal. Both advanced wastewater treatment technologies considerably reduce the in vitro toxicity (Figures 2 and 3). Here, the performance of AC and ozone treatment do not differ significantly for all studied end points (Table S9). Moreover, the effluent toxicities of the advanced treatment were mainly below the EBTs with the exception of PPARγ activity. Thus, an upgrade leads to a significant, additional detoxification and enables compliance with proposed environmental quality criteria even at high wastewater fractions in the receiving surface water. Similar to the effective removal of in vitro toxicity, advanced wastewater treatment also significantly reduces in vivo toxicity. These included the experiments with *G. fossarum* and *O. mykiss* FELST at the WWTPs in Lausanne, Basel, and Neugut. Moreover, the VGT analyses in exposed fish, as well as the on-site experiments with *P. antipodarum*, suggest that the effective removal of in vitro estrogenicity by both technologies (Figure 2) also results in a reduction of the corresponding in vivo effects. These positive findings are in line with field studies reporting an improvement of biodiversity,[187] ecosystem functions,[188] and fish health[60,189] after implementing an advanced treatment technology.

5.3. Ozonation Generates Toxicity That Can Be Removed by a Post-Treatment. Notably, several studies reported increased in vitro and in vivo toxicity in the effluent of an ozone treatment. These include genotoxicity and mutagenicity (section 3.4), the growth of *L. minor* and biomass of *L. variegatus*, as well as embryo toxicity in *O. latipes* and effects on the early development of *O. mykiss* (Table 2). These observations are associated with the formation of toxic TPs. However, the specifics of this formation, as well as the causative compounds, remain to be elucidated. Thus, from an ecotoxicological perspective, AC is preferable to ozonation because it is comparably effective in removing toxicity without generating toxic TPs. Nevertheless, an ozone treatment has additional benefits, including disinfection[190−192] and lower implementation and maintenance costs,[193,194] which is also reflected by the larger number of studies (573 data points) compared to the number of studies for AC treatment (162 data points).

The literature suggests that an ozone treatment should only be implemented with a subsequent PT to remove the generated TPs. While various PTs were assessed (Tables S4 and S5), comparison of their relative performance is difficult because data for some options is scarce (e.g., fix and fluid bed reactor) and only a few studies compare different PTs in parallel.[24−26,54,55,169] The combination with a GAC filter can lead to significant, additional micropollutant removal via sorption,[195] while all other PTs are solely based on biological degradation. Accordingly, GAC filters outperformed conventional filters, as well as fix and fluid bed reactors, in removing micropollutants, TPs, and mutagenicity.[196,55,197] However, considering the limited data and given that the performance in reducing toxicity differs significantly between studies, further investigations are required to identify an optimal PT.

5.4. What are the Sensitive End Points and Species? Certain end points, especially estrogenicity (27% of all data), dominate the in vitro assessment, whereas certain animal models, such as daphnids (24% of all data) are overrepresented in the in vivo data. Conventionally treated wastewater triggers multiple in vitro end points, including estrogenicity, glucocorticoid activity, AhR activity, neuro- and phytotoxicity, oxidative stress response, as well as baseline toxicity. In terms of chronic in vivo effects, *P. antipodarum*, *G. fossarum*, and *O. mykiss* are the most sensitive species. Thus, these end points and species are suitable to investigate an additional removal of toxicity by advanced technologies. In addition, mutagenicity with the Ames strain YG7108, genotoxicity in the comet assay with cells from exposed organisms, and biomass of *L. variegatus* after chronic exposure represent sensitive end points to investigate the formation of toxic TPs during ozonation.

In contrast, progestogenic and thyroid receptor activity are rarely observed. Moreover, short-term in vivo bioassays and certain species (daphnids, *C. riparius*) are not sensitive enough to detect toxic effects of conventionally treated wastewater.

5.5. Future Research Needs. On the basis of this systematic review, we recommend the following:

- Research should focus on toxicity reduction by an AC treatment, as well as by multiple ozonation PTs, to balance current biases in knowledge.
- Given the wealth of estrogenicity data, future research should focus on underrepresented end points, including unspecific xenobiotic sensors (PPAR, PXR, CAR) and specific end points (retinoid-like, steroidogenesis, and neurotoxicity) with established links to in vivo impacts.
- A well-designed battery of in vitro bioassays is needed to assess advanced wastewater treatment, considering commonly detected end points, as well as cellular toxicity and adverse outcome pathways, to link effects to higher levels of biological organization.[198]
- Given their limited sensitivity, short-term in vivo assays are not suitable for evaluating toxicity reduction during advanced wastewater treatment when aqueous samples are analyzed. Here, testing extracted water samples might represent an alternative.
- Model organisms for an in vivo assessment should be selected on the basis of their sensitivity to the toxicity present in the CT. This excludes daphnids and chironomids and includes *P. antipodarum*, *G. fossarum*, and *O. mykiss*.
- The toxicity removal by the CT should serve as one critical parameter to determine if an upgrade to WWTPs is needed.
- EBTs are useful to benchmark the toxicity removal. Here, some tentative EBTs need to be refined (e.g., PPARγ activity) and missing EBTs established (e.g., antiestrogenicity).
- The PPARγ activity in effluents of an advanced treatment exceeds the EBT by 5-fold. Because this is based on limited data, the PPARγ activity deserves more attention.
- If toxicity elimination is a major aim of wastewater treatment, advanced technologies based on ozonation or AC represent suitable options to achieve that goal.
- Because ozonation generates toxic byproducts, a PT needs to be implemented. Here, future research should focus on a comparative assessment of the available technologies.

To conclude, the literature highlights that toxicity removal is a crucial aspect of benchmarking the performance of

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Environ. Sci. Technol. 2019, 53, 7215−7233

conventional and advanced treatment technologies. Depending on the goal of an upgrade, in vitro and in vivo bioassays are suitable tools to assess if the water quality has improved. To understand if toxicity reduction indeed translates to an improved ecological status, more field studies are needed. The key question that remains is which parameters to consider when deciding whether or not to upgrade WWTPs. Toxicity removal will be a major factor, but it is not the only aspect. It needs to be balanced with other factors, such as disinfection and nutrient and target micropollutant removal, as well as economic and environmental costs.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.9b00570.

Additional information on the technological specifications of the (advanced) WWTPs, data evaluation, in vitro and in vivo bioassays, comparison with EBTs, and statistics (PDF)

All extracted in vitro and in vivo data (XLSX)

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Notes

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■ REFERENCES

(1) Schäfer, R. B.; Kühn, B.; Malaj, E.; König, A.; Gergs, R. Contribution of organic toxicants to multiple stress in river ecosystems. *Freshwater Biol.* **2016**, *61*, 2116−2128.

(2) Malaj, E.; von der Ohe, P. C.; Grote, M.; Kuhne, R.; Mondy, C. P.; Usseglio-Polatera, P.; Brack, W.; Schafer, R. B. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111* (26), 9549−9554.

(3) Carpenter, C. M. G.; Helbling, D. E. Widespread Micropollutant Monitoring in the Hudson River Estuary Reveals Spatiotemporal Micropollutant Clusters and Their Sources. *Environ. Sci. Technol.* **2018**, *52* (11), 6187−6196.

(4) Neale, P. A.; Munz, N. A.; Aït-Aïssa, S.; Altenburger, R.; Brion, F.; Busch, W.; Escher, B. I.; Hilscherová, K.; Kienle, C.; Novák, J.; Seiler, T.-B.; Shao, Y.; Stamm, C.; Hollender, J. Integrating chemical analysis and bioanalysis to evaluate the contribution of wastewater effluent on the micropollutant burden in small streams. *Sci. Total Environ.* **2017**, *576*, 785−795.

(5) Loos, R.; Carvalho, R.; Antonio, D. C.; Comero, S.; Locoro, G.; Tavazzi, S.; Paracchini, B.; Ghiani, M.; Lettieri, T.; Blaha, L.; Jarosova, B.; Voorspoels, S.; Servaes, K.; Haglund, P.; Fick, J.; Lindberg, R. H.; Schwesig, D.; Gawlik, B. M. EU-wide monitoring survey on emerging polar organic contaminants in wastewater treatment plant effluents. *Water Res.* **2013**, *47* (17), 6475−6487.

(6) Stalter, D.; Magdeburg, A.; Quednow, K.; Botzat, A.; Oehlmann, J. Do contaminants originating from state-of-the-art treated wastewater impact the ecological quality of surface waters? *PLoS One* **2013**, *8* (4), No. e60616.

(7) Englert, D.; Zubrod, J. P.; Schulz, R.; Bundschuh, M. Effects of municipal wastewater on aquatic ecosystem structure and function in the receiving stream. *Sci. Total Environ.* **2013**, *454*, 401−410.

(8) Bunzel, K.; Kattwinkel, M.; Liess, M. Effects of organic pollutants from wastewater treatment plants on aquatic invertebrate communities. *Water Res.* **2013**, *47* (2), 597−606.

(9) Maeng, S. K.; Sharma, S. K.; Lekkerkerker-Teunissen, K.; Amy, G. L. Occurrence and fate of bulk organic matter and pharmaceutically active compounds in managed aquifer recharge: A review. *Water Res.* **2011**, *45* (10), 3015−3033.

(10) Nodler, K.; Hillebrand, O.; Idzik, K.; Strathmann, M.; Schiperski, F.; Zirlewagen, J.; Licha, T. Occurrence and fate of the angiotensin II receptor antagonist transformation product valsartan acid in the water cycle - A comparative study with selected beta-blockers and the persistent anthropogenic wastewater indicators carbamazepine and acesulfame. *Water Res.* **2013**, *47* (17), 6650−6659.

(11) Kulaksız, S.; Bau, M. Anthropogenic gadolinium as a microcontaminant in tap water used as drinking water in urban areas and megacities. *Appl. Geochem.* **2011**, *26* (11), 1877−1885.

(12) Eggen, R. I. L.; Hollender, J.; Joss, A.; Scharer, M.; Stamm, C. Reducing the Discharge of Micropollutants in the Aquatic Environment: The Benefits of Upgrading Wastewater Treatment Plants. *Environ. Sci. Technol.* **2014**, *48* (14), 7683−7689.

(13) Margot, J.; Kienle, C.; Magnet, A.; Weil, M.; Rossi, L.; de Alencastro, L. F.; Abegglen, C.; Thonney, D.; Chevre, N.; Scharer, M.; Barry, D. A. Treatment of micropollutants in municipal wastewater: ozone or powdered activated carbon? *Sci. Total Environ.* **2013**, *461*−*462*, 480−98.

(14) Hollender, J.; Zimmermann, S. G.; Koepke, S.; Krauss, M.; McArdell, C. S.; Ort, C.; Singer, H.; von Gunten, U.; Siegrist, H. Elimination of Organic Micropollutants in a Municipal Wastewater Treatment Plant Upgraded with a Full-Scale Post-Ozonation Followed by Sand Filtration. *Environ. Sci. Technol.* **2009**, *43* (20), 7862−7869.

(15) Logar, I.; Brouwer, R.; Maurer, M.; Ort, C. Cost-Benefit Analysis of the Swiss National Policy on Reducing Micropollutants in Treated Wastewater. *Environ. Sci. Technol.* **2014**, *48* (21), 12500−12508.

(16) Escher, B. I.; Fenner, K. Recent advances in environmental risk assessment of transformation products. *Environ. Sci. Technol.* **2011**, *45* (9), 3835−47.

(17) Backhaus, T.; Karlsson, M. Screening level mixture risk assessment of pharmaceuticals in STP effluents. *Water Res.* **2014**, *49*, 157−165.

(18) Blackwell, B. R.; Ankley, G. T.; Bradley, P. M.; Houck, K. A.; Makarov, S. S.; Medvedev, A. V.; Swintek, J.; Villeneuve, D. L. Potential Toxicity of Complex Mixtures in Surface Waters from a Nationwide Survey of United States Streams: Identifying in Vitro Bioactivities and Causative Chemicals. *Environ. Sci. Technol.* **2019**, *53* (2), 973−983.

(19) Tang, J. Y. M.; Busetti, F.; Charrois, J. W. A.; Escher, B. I. Which chemicals drive biological effects in wastewater and recycled water? *Water Res.* **2014**, *60*, 289−299.

(20) Gomes, J.; Costa, R.; Quinta-Ferreira, R. M.; Martins, R. C. Application of ozonation for pharmaceuticals and personal care products removal from water. *Sci. Total Environ.* **2017**, *586*, 265−283.

(21) Ahmed, M. B.; Zhou, J. L.; Ngo, H. H.; Guo, W. S.; Thomaidis, N. S.; Xu, J. Progress in the biological and chemical treatment technologies for emerging contaminant removal from wastewater: A critical review. *J. Hazard. Mater.* **2017**, *323*, 274−298.

(22) Rizzo, L.; Malato, S.; Antakyali, D.; Beretsou, V. G.; Dolic, M. B.; Gernjak, W.; Heath, E.; Ivancev-Tumbas, I.; Karaolia, P.; Lado Ribeiro, A. R.; Mascolo, G.; McArdell, C. S.; Schaar, H.; Silva, A. M. T.; Fatta-Kassinos, D. Consolidated vs new advanced treatment methods for the removal of contaminants of emerging concern from urban wastewater. *Sci. Total Environ.* **2019**, *655*, 986−1008.

(23) Prasse, C.; Stalter, D.; Schulte-Oehlmann, U.; Oehlmann, J.; Ternes, T. A. Spoilt for choice: A critical review on the chemical and

DOI: 10.1021/acs.est.9b00570
Environ. Sci. Technol. 2019, 53, 7215−7233

biological assessment of current wastewater treatment technologies. *Water Res.* **2015**, *87*, 237−270.
(24) Kienle, C.; Langer, M.; Ganser, B.; Gut, S.; Schifferli, A.; Vermeirssen, E.; Werner, I. *Biologische Nachbehandlung von kommunalem Abwasser nach Ozonung—ReTREAT: Teilprojekt Biotests. Studie im Auftrag des Bundesamtes für Umwelt (BAFU)*; Swiss Centre for Applied Ecotoxicology, Eawag-EPFL: Dübendorf, 2017. https://www.oekotoxzentrum.ch/media/168206/2017_kienle_retreat_oekotox.pdf (accessed 25 January 2015).
(25) Schneider, I.; Abbas, A.; Wagner, M.; Schulte-Oehlmann, U.; Oehlmann, J. *Trans Risk—Abschlussbericht-Teilprojekt 2—Ökotoxikologie*; Goethe University Frankfurt, Institute for Ecology, Diversity and Evolution, Department Aquatic Ecotoxicology, 2015. DOI: DOI: 10.2314/GBV:863767060.
(26) Schlüter-Vorberg, L.; Coors, A. *Chrakterisierung, Kommunikation und Minimierung von Risiken durchneue Schadstoffe und Krankheitserrefer im Wasserkreislauf: Arbeitspaket 2: Risikocharakterisierung—Ökotoxikologie: Schlussbericht.*; ECT Oekotoxikologie GmbH: Flörsheim, 2015. DOI: DOI: 10.2314/GBV:863617018.
(27) Jekel, M.; Baur, N.; Böckelmann, U.; Dünnbier, U.; Eckhardt, A.; Gnirß, R.; Grummt, T.; Hummelt, D.; Lucke, T.; Meinel, F.; Miehe, U.; Mutz, D.; Pflugmacher Lima, S.; Reemtsma, T.; Remy, C.; Schlittenbauer, L.; Schulz, W.; Seiwert, B.; Sperlich, A.; Stapf, M.; Zerball-Van Baar, P.; Wenzel, M.; Zietzschmann, F.; Ruhl, A. S. *Anthropogene Spurenstoffe und Krankheitserreger imurbanen Wasserkreislauf: Bewertung, Barrieren und Risikokommunikation.*; Universitätsverlag der TU Berlin, 2015. DOI: DOI: 10.14279/depositonce-4979.
(28) Schmidt, T. C.; Kowal, S.; Boergers, A.; Dopp, E.; Erger, C.; Gebhardt, W.; Gehrmann, L.; Hammers-Wirtz, M.; Herbst, H.; Kasper-Sonnenberg, M.; Linnemann, V.; Lutze, H.; Lyko, S.; Magdeburg, A.; Maus, C.; Portner, C.; Richard, J.; Tuerk, J. *Abschlussbericht zum Forschungsvorhaben "Metabolitenbildung beim Einsatz von Ozon—Phase 2"*; IWW Rheinisch-Westfälisches Institut für Wasserforschung Gemeinnützige, GmbH: Mülheim, 2014. https://www.lanuv.nrw.de/fileadmin/lanuv/wasser/abwasser/forschung/pdf/Abschlussbericht_TP10-Phase2_29.12.2014.pdf (accessed 25 January 2019).
(29) Kienle, C.; Baumberger, D.; Schifferli, A.; Werner, I.; Santiago, S.; Weil, M. *Evaluation der Ökotoxizität von Kläranlagenabwasser der ARA Basel mit Biotests vor und nach der Anwendung erweiterter Abwasserbehandlungsmethoden—Abschlussbericht März 2013.*; Swiss Centre for Applied Ecotoxicology, Eawag-EPFL: Dübendorf, 2013. https://www.dora.lib4ri.ch/eawag/islandora/object/eawag%3A14939/datastream/PDF/view (accessed 25 January 2019).
(30) Kienle, C.; Baumberger, D.; Läuppi, B.; Schifferli, A.; Werner, I. *Evaluation der Ökotoxizität von Kläaranlagenabwasser der ARA Basel mit Biotests vor und nach der Anwendung erweiterter Abwasserbehandlungsmethoden—Abschlussbericht Dezember 2013*; Swiss Centre for Applied Ecotoxicology, Eawag-EPFL: Dübendorf, 2013.
(31) Kienle, C.; Kase, R.; Werner, I. *Evaluation of bioassays and wastewater quality—In vitro and in vivo bioassays for the performance review in the project Strategy Micropoll*; Swiss Centre for Applied Ecotoxicology, Eawag-EPFL: Dübendorf, 2011. https://www.oekotoxzentrum.ch/media/2229/2011_kienle_bioassays_micropoll.pdf (accessed 25 January 2019).
(32) Abegglen, C.; Escher, B.; Hollender, J.; Koepke, S.; Ort, C.; Peter, A.; Siegrist, H.; von Gunten, U.; Zimmermann, S. *Ozonung von gereinigtem Abwasser—Schlussbericht Pilotversuch Regensdorf*; Eawag: Dübendorf, 2009. http://spurenstoffelimination.de/files/Ozonung_Abwasser_Schlussbericht_Regensdorf.pdf (accessed 25 January 2019).
(33) Ouzzani, M.; Hammady, H.; Fedorowicz, Z.; Elmagarmid, A. Rayyan-a web and mobile app for systematic reviews. *Systematic Reviews* **2016**, *5* (1), 210.
(34) 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.; Mehinto, A. C.; Mendez, J. E.; Poulsen, A.; Prochazka, E.; Richard, J.; Schifferli, A.; Schlenk, D.; Scholz, S.; Shiraishi, F.; Snyder, S.; Su, G.; Tang, J. Y.; van der Burg, B.; van der Linden, S. C.; Werner, I.; Westerheide, S. D.; Wong, C. K.; Yang, M.; Yeung, B. H.; Zhang, X.; Leusch, F. D. Benchmarking organic micropollutants in wastewater, recycled water and drinking water with in vitro bioassays. *Environ. Sci. Technol.* **2014**, *48* (3), 1940−56.
(35) Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D. G. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med.* **2009**, *6* (7), No. e1000097.
(36) Wagner, M.; Kienle, C.; Vermeirssen, E. L.; Oehlmann, J. Endocrine Disruption and In Vitro Ecotoxicology: Recent Advances and Approaches. *Adv. Biochem. Eng./Biotechnol.* **2017**, *157*, 1−58.
(37) Abbas, A.; Schneider, I.; Bollmann, A.; Funke, J.; Oehlmann, J.; Prasse, C.; Schulte-Oehlmann, U.; Seitz, W.; Ternes, T.; Weber, M.; Wesely, H.; Wagner, M. What you extract is what you see: Optimising the preparation of water and wastewater samples for in vitro bioassays. *Water Res.* **2019**, *152*, 47−60.
(38) Benner, J.; Ternes, T. A. Ozonation of Propranolol: Formation of Oxidation Products. *Environ. Sci. Technol.* **2009**, *43* (13), 5086−5093.
(39) Simon, E.; Schifferli, A.; Bucher, T. B.; Olbrich, D.; Werner, I.; Vermeirssen, E. L. M. Solid-phase extraction of estrogens and herbicides from environmental waters for bioassay analysis-effects of sample volume on recoveries. *Anal. Bioanal. Chem.* **2019**, *411* (10), 2057−2069.
(40) Neale, P. A.; Brack, W.; Ait-Aissa, S.; Busch, W.; Hollender, J.; Krauss, M.; Maillot-Marechal, E.; Munz, N. A.; Schlichting, R.; Schulze, T.; Vogler, B.; Escher, B. I. Solid-phase extraction as sample preparation of water samples for cell-based and other in vitro bioassays. *Environmental Science: Processes & Impacts* **2018**, *20*, 493−504.
(41) Wagner, M.; Oehlmann, J. Endocrine disruptors in bottled mineral water: Estrogenic activity in the E-Screen. *J. Steroid Biochem. Mol. Biol.* **2011**, *127* (1−2), 128−135.
(42) Neale, P. A.; Escher, B. I. Does co-extracted dissolved organic carbon cause artefacts in cell-based bioassays? *Chemosphere* **2014**, *108*, 281−288.
(43) Neale, P. A.; Leusch, F. D. Considerations when assessing antagonism in vitro: Why standardizing the agonist concentration matters. *Chemosphere* **2015**, *135*, 20−23.
(44) Wagner, M.; Vermeirssen, E. L. M.; Buchinger, S.; Behr, M.; Magdeburg, A.; Oehlmann, J. Deriving bio-equivalents from in vitro bioassays: Assessment of existing uncertainties and strategies to improve accuracy and reporting. *Environ. Toxicol. Chem.* **2013**, *32* (8), 1906−1917.
(45) Escher, B. I.; Neale, P. A.; Villeneuve, D. L. The advantages of linear concentration-response curves for in vitro bioassays with environmental samples. *Environ. Toxicol. Chem.* **2018**, *37* (9), 2273−2280.
(46) Escher, B. I.; Aït-Aïssa, S.; Behnisch, P. A.; Brack, W.; Brion, F.; Brouwer, A.; Buchinger, S.; Crawford, S. E.; Du Pasquier, D.; Hamers, T.; Hettwer, K.; Hilscherova, K.; Hollert, H.; Kase, R.; Kienle, C.; Tindall, A. J.; Tuerk, J.; van der Oost, R.; Vermeirssen, E.; Neale, P. A. Effect-based trigger values for in vitro and in vivo bioassays performed on surface water extracts supporting the environmental quality standards (EQS) of the European Water Framework Directive. *Sci. Total Environ.* **2018**, *628−629*, 748−765.
(47) Judson, R.; Kavlock, R.; Martin, M.; Reif, D.; Houck, K.; Knudsen, T.; Richard, A.; Tice, R. R.; Whelan, M.; Xia, M. H.; Huang, R. L.; Austin, C.; Daston, G.; Hartung, T.; Fowle, J. R.; Wooge, W.; Tong, W. D.; Dix, D. Perspectives on Validation of High-Throughput Assays Supporting 21st Century Toxicity Testing. *Altex-Altern Anim Ex* **2013**, *30* (1), 51−66.
(48) Stadnicka-Michalak, J.; Schirmer, K.; Ashauer, R. Toxicology across scales: Cell population growth in vitro predicts reduced fish growth. *Science Advances* **2015**, *1* (7), No. e1500302.
(49) Ihara, M.; Kitamura, T.; Kumar, V.; Park, C. B.; Ihara, M. O.; Lee, S. J.; Yamashita, N.; Miyagawa, S.; Iguchi, T.; Okamoto, S.; Suzuki, Y.; Tanaka, H. Evaluation of Estrogenic Activity of

DOI: 10.1021/acs.est.9b00570
Environ. Sci. Technol. 2019, 53, 7215−7233

Wastewater: Comparison Among In Vitro ERalpha Reporter Gene Assay, In Vivo Vitellogenin Induction, and Chemical Analysis. *Environ. Sci. Technol.* **2015**, *49* (10), 6319−26.
(50) Brinkmann, M.; Eichbaum, K.; Buchinger, S.; Reifferscheid, G.; Bui, T.; Schaffer, A.; Hollert, H.; Preuss, T. G. Understanding receptor-mediated effects in rainbow trout: in vitro-in vivo extrapolation using physiologically based toxicokinetic models. *Environ. Sci. Technol.* **2014**, *48* (6), 3303−9.
(51) van der Oost, R.; Sileno, G.; Suarez-Munoz, M.; Nguyen, M. T.; Besselink, H.; Brouwer, A. SIMONI (smart integrated monitoring) as a novel bioanalytical strategy for water quality assessment: Part i-model design and effect-based trigger values. *Environ. Toxicol. Chem.* **2017**, *36* (9), 2385−2399.
(52) Escher, B. I.; Neale, P. A.; Leusch, F. D. Effect-based trigger values for in vitro bioassays: Reading across from existing water quality guideline values. *Water Res.* **2015**, *81*, 137−48.
(53) Neale, P. A.; Antony, A.; Bartkow, M. E.; Farre, M. J.; Heitz, A.; Kristiana, I.; Tang, J. Y. M.; Escher, B. I. Bioanalytical Assessment of the Formation of Disinfection Byproducts in a Drinking Water Treatment Plant. *Environ. Sci. Technol.* **2012**, *46* (18), 10317−10325.
(54) Giebner, S.; Ostermann, S.; Straskraba, S.; Oetken, M.; Oehlmann, J.; Wagner, M. Effectivity of advanced wastewater treatment: reduction of in vitro endocrine activity and mutagenicity but not of in vivo reproductive toxicity. *Environ. Sci. Pollut. Res.* **2018**, *25* (5), 3965−3976.
(55) Ternes, T. A.; Prasse, C.; Eversloh, C. L.; Knopp, G.; Cornel, P.; Schulte-Oehlmann, U.; Schwartz, T.; Alexander, J.; Seitz, W.; Coors, A.; Oehlmann, J. Integrated Evaluation Concept to Assess the Efficacy of Advanced Wastewater Treatment Processes for the Elimination of Micropollutants and Pathogens. *Environ. Sci. Technol.* **2017**, *51* (1), 308−319.
(56) Sun, J.; Wang, J.; Zhang, R.; Wei, D.; Long, Q.; Huang, Y.; Xie, X.; Li, A. Comparison of different advanced treatment processes in removing endocrine disruption effects from municipal wastewater secondary effluent. *Chemosphere* **2017**, *168*, 1−9.
(57) Itzel, F.; Gehrmann, L.; Bielak, H.; Ebersbach, P.; Boergers, A.; Herbst, H.; Maus, C.; Simon, A.; Dopp, E.; Hammers-Wirtz, M.; Schmidt, T. C.; Tuerk, J. Investigation of full-scale ozonation at a municipal wastewater treatment plant using a toxicity-based evaluation concept. *J. Toxicol. Environ. Health, Part A* **2017**, *80* (23−24), 1242−1258.
(58) Zeng, S.; Huang, Y.; Sun, F.; Li, D.; He, M. Probabilistic ecological risk assessment of effluent toxicity of a wastewater reclamation plant based on process modeling. *Water Res.* **2016**, *100*, 367−376.
(59) Wigh, A.; Devaux, A.; Brosselin, V.; Gonzalez-Ospina, A.; Domenjoud, B.; Ait-Aissa, S.; Creusot, N.; Gosset, A.; Bazin, C.; Bony, S. Proposal to optimize ecotoxicological evaluation of wastewater treated by conventional biological and ozonation processes. *Environ. Sci. Pollut. Res.* **2016**, *23* (4), 3008−17.
(60) Maier, D.; Benisek, M.; Blaha, L.; Dondero, F.; Giesy, J. P.; Kohler, H. R.; Richter, D.; Scheurer, M.; Triebskorn, R. Reduction of dioxin-like toxicity in effluents by additional wastewater treatment and related effects in fish. *Ecotoxicol. Environ. Saf.* **2016**, *132*, 47−58.
(61) Hamilton, L. A.; Tremblay, L. A.; Northcott, G. L.; Boake, M.; Lim, R. P. The impact of variations of influent loading on the efficacy of an advanced tertiary sewage treatment plant to remove endocrine disrupting chemicals. *Sci. Total Environ.* **2016**, *560−561*, 101−9.
(62) Jia, A.; Escher, B. I.; Leusch, F. D.; Tang, J. Y.; Prochazka, E.; Dong, B.; Snyder, E. M.; Snyder, S. A. In vitro bioassays to evaluate complex chemical mixtures in recycled water. *Water Res.* **2015**, *80*, 1−11.
(63) Magdeburg, A.; Stalter, D.; Schlusener, M.; Ternes, T.; Oehlmann, J. Evaluating the efficiency of advanced wastewater treatment: target analysis of organic contaminants and (geno-)toxicity assessment tell a different story. *Water Res.* **2014**, *50*, 35−47.
(64) Tang, J. Y.; McCarty, S.; Glenn, E.; Neale, P. A.; Warne, M. S.; Escher, B. I. Mixture effects of organic micropollutants present in water: towards the development of effect-based water quality trigger values for baseline toxicity. *Water Res.* **2013**, *47* (10), 3300−14.
(65) Reungoat, J.; Escher, B. I.; Macova, M.; Argaud, F. X.; Gernjak, W.; Keller, J. Ozonation and biological activated carbon filtration of wastewater treatment plant effluents. *Water Res.* **2012**, *46* (3), 863−72.
(66) Escher, B. I.; Dutt, M.; Maylin, E.; Tang, J. Y. M.; Toze, S.; Wolf, C. R.; Lang, M. Water quality assessment using the AREc32 reporter gene assay indicative of the oxidative stress response pathway. *J. Environ. Monit.* **2012**, *14* (11), 2877−2885.
(67) Stalter, D.; Magdeburg, A.; Wagner, M.; Oehlmann, J. Ozonation and activated carbon treatment of sewage effluents: removal of endocrine activity and cytotoxicity. *Water Res.* **2011**, *45* (3), 1015−24.
(68) Reungoat, J.; Escher, B. I.; Macova, M.; Keller, J. Biofiltration of wastewater treatment plant effluent: effective removal of pharmaceuticals and personal care products and reduction of toxicity. *Water Res.* **2011**, *45* (9), 2751−62.
(69) Misik, M.; Knasmueller, S.; Ferk, F.; Cichna-Markl, M.; Grummt, T.; Schaar, H.; Kreuzinger, N. Impact of ozonation on the genotoxic activity of tertiary treated municipal wastewater. *Water Res.* **2011**, *45* (12), 3681−91.
(70) Reungoat, J.; Macova, M.; Escher, B. I.; Carswell, S.; Mueller, J. F.; Keller, J. Removal of micropollutants and reduction of biological activity in a full scale reclamation plant using ozonation and activated carbon filtration. *Water Res.* **2010**, *44* (2), 625−637.
(71) Macova, M.; Escher, B. I.; Reungoat, J.; Carswell, S.; Chue, K. L.; Keller, J.; Mueller, J. F. Monitoring the biological activity of micropollutants during advanced wastewater treatment with ozonation and activated carbon filtration. *Water Res.* **2010**, *44* (2), 477−92.
(72) Escher, B. I.; Bramaz, N.; Ort, C. JEM spotlight: Monitoring the treatment efficiency of a full scale ozonation on a sewage treatment plant with a mode-of-action based test battery. *J. Environ. Monit.* **2009**, *11* (10), 1836−46.
(73) Cao, N.; Yang, M.; Zhang, Y.; Hu, J.; Ike, M.; Hirotsuji, J.; Matsui, H.; Inoue, D.; Sei, K. Evaluation of wastewater reclamation technologies based on in vitro and in vivo bioassays. *Sci. Total Environ.* **2009**, *407* (5), 1588−97.
(74) Altmann, D.; Schaar, H.; Bartel, C.; Schorkopf, D. L. P.; Miller, I.; Kreuzinger, N.; Mostl, E.; Grillitsch, B. Impact of ozonation on ecotoxicity and endocrine activity of tertiary treated wastewater effluent. *Water Res.* **2012**, *46* (11), 3693−3702.
(75) Zoeller, R. T.; Brown, T. R.; Doan, L. L.; Gore, A. C.; Skakkebaek, N. E.; Soto, A. M.; Woodruff, T. J.; Vom Saal, F. S. Endocrine-Disrupting Chemicals and Public Health Protection: A Statement of Principles from The Endocrine Society. *Endocrinology* **2012**, *153* (9), 4097−4110.
(76) Bergman, A.; Heindel, J. J.; Jobling, S.; Kidd, K. A.; Zoeller, R. T. *State of the Science of Endocrine Disrupting Chemicals 2012*; World Health Organization, United Nations Environment Programme, 2012. https://www.who.int/ceh/publications/endocrine/en/ (accessed 25 January 2019).
(77) Hotchkiss, A. K.; Rider, C. V.; Blystone, C. R.; Wilson, V. S.; Hartig, P. C.; Ankley, G. T.; Foster, P. M.; Gray, C. L.; Gray, L. E. Fifteen years after "Wingspread"—Environmental endocrine disrupters and human and wildlife health: Where we are today and where we need to go. *Toxicol. Sci.* **2008**, *105* (2), 235−259.
(78) Tetreault, G. R.; Bennett, C. J.; Shires, K.; Knight, B.; Servos, M. R.; McMaster, M. E. Intersex and reproductive impairment of wild fish exposed to multiple municipal wastewater discharges. *Aquat. Toxicol.* **2011**, *104* (3−4), 278−290.
(79) Jobling, S.; Nolan, M.; Tyler, C. R.; Brighty, G.; Sumpter, J. P. Widespread sexual disruption in wild fish. *Environ. Sci. Technol.* **1998**, *32* (17), 2498−2506.
(80) Jobling, S.; Burn, R. W.; Thorpe, K.; Williams, R.; Tyler, C. Statistical modeling suggests that antiandrogens in effluents from wastewater treatment works contribute to widespread sexual disruption in fish living in English rivers. *Environ. Health Perspect.* **2009**, *117* (5), 797−802.

DOI: 10.1021/acs.est.9b00570
Environ. Sci. Technol. 2019, 53, 7215−7233

(81) Niemuth, N. J.; Klaper, R. D. Emerging wastewater contaminant metformin causes intersex and reduced fecundity in fish. *Chemosphere* **2015**, *135*, 38−45.
(82) Völker, J.; Castronovo, S.; Wick, A.; Ternes, T. A.; Joss, A.; Oehlmann, J.; Wagner, M. Advancing Biological Wastewater Treatment: Extended Anaerobic Conditions Enhance the Removal of Endocrine and Dioxin-like Activities. *Environ. Sci. Technol.* **2016**, *50* (19), 10606−10615.
(83) Leusch, F. D.; Khan, S. J.; Gagnon, M. M.; Quayle, P.; Trinh, T.; Coleman, H.; Rawson, C.; Chapman, H. F.; Blair, P.; Nice, H.; Reitsema, T. Assessment of wastewater and recycled water quality: A comparison of lines of evidence from in vitro, in vivo and chemical analyses. *Water Res.* **2014**, *50*, 420−31.
(84) Liu, Z. H.; Kanjo, Y.; Mizutani, S. Removal mechanisms for endocrine disrupting compounds (EDCs) in wastewater treatment - physical means, biodegradation, and chemical advanced oxidation: A review. *Sci. Total Environ.* **2009**, *407* (2), 731−748.
(85) Escher, B. I.; Bramaz, N.; Quayle, P.; Rutishauser, S.; Vermeirssen, E. L. Monitoring of the ecotoxicological hazard potential by polar organic micropollutants in sewage treatment plants and surface waters using a mode-of-action based test battery. *J. Environ. Monit.* **2008**, *10* (5), 622−31.
(86) Caldwell, D. J.; Mastrocco, F.; Anderson, P. D.; Lange, R.; Sumpter, J. P. Predicted-no-effect concentrations for the steroid estrogens estrone, 17 beta-estradiol, estriol, and 17 alpha-ethinylestradiol. *Environ. Toxicol. Chem.* **2012**, *31* (6), 1396−1406.
(87) Weizel, A.; Schluesener, M. P.; Dierkes, G.; Ternes, T. A. Occurrence of Glucocorticoids, Mineralocorticoids and Progestogens in Various Treated Wastewater, Rivers and Streams. *Environ. Sci. Technol.* **2018**, *52* (9), 5296−5307.
(88) Schriks, M.; van Leerdam, J. A.; van der Linden, S. C.; van der Burg, B.; van Wezel, A. P.; de Voogt, P. High-Resolution Mass Spectrometric Identification and Quantification of Glucocorticoid Compounds in Various Wastewaters in The Netherlands. *Environ. Sci. Technol.* **2010**, *44* (12), 4766−4774.
(89) Leusch, F. D. L.; Neale, P. A.; Arnal, C.; Aneck-Hahn, N. H.; Balaguer, P.; Bruchet, A.; Escher, B. I.; Esperanza, M.; Grimaldi, M.; Leroy, G.; Scheurer, M.; Schlichting, R.; Schriks, M.; Hebert, A. Analysis of endocrine activity in drinking water, surface water and treated wastewater from six countries. *Water Res.* **2018**, *139*, 10−18.
(90) Roberts, J.; Bain, P. A.; Kumar, A.; Hepplewhite, C.; Ellis, D. J.; Christy, A. G.; Beavis, S. G. Tracking multiple modes of endocrine activity in Australia's largest inland sewage treatment plant and effluent- receiving environment using a panel of in vitro bioassays. *Environ. Toxicol. Chem.* **2015**, *34* (10), 2271−2281.
(91) Macikova, P.; Groh, K. J.; Ammann, A. A.; Schirmer, K.; Suter, M. J. Endocrine Disrupting Compounds Affecting Corticosteroid Signaling Pathways in Czech and Swiss Waters: Potential Impact on Fish. *Environ. Sci. Technol.* **2014**, *48* (21), 12902−12911.
(92) Stavreva, D. A.; George, A. A.; Klausmeyer, P.; Varticovski, L.; Sack, D.; Voss, T. C.; Schiltz, R. L.; Blazer, V. S.; Iwanowicz, L. R.; Hager, G. L. Prevalent Glucocorticoid and Androgen Activity in US Water Sources. *Sci. Rep.* **2012**, *2*, 937.
(93) Van der Linden, S. C.; Heringa, M. B.; Man, H. Y.; Sonneveld, E.; Puijker, L. M.; Brouwer, A.; Van der Burg, B. Detection of multiple hormonal activities in wastewater effluents and surface water, using a panel of steroid receptor CALUX bioassays. *Environ. Sci. Technol.* **2008**, *42* (15), 5814−5820.
(94) Kugathas, S.; Runnalls, T. J.; Sumpter, J. P. Metabolic and Reproductive Effects of Relatively Low Concentrations of Beclomethasone Dipropionate, a Synthetic Glucocorticoid, on Fathead Minnows. *Environ. Sci. Technol.* **2013**, *47* (16), 9487−9495.
(95) Kugathas, S.; Runnalls, T.; Sumpter, J. Synthetic progestins and glucocorticoids affect fish reproduction and physiology. *Toxicol. Lett.* **2012**, *211*, S30−S30.
(96) Thrupp, T. J.; Runnalls, T. J.; Scholze, M.; Kugathas, S.; Kortenkamp, A.; Sumpter, J. P. The consequences of exposure to mixtures of chemicals: Something from 'nothing' and 'a lot from a little' when fish are exposed to steroid hormones. *Sci. Total Environ.* **2018**, *619−620*, 1482−1492.
(97) Runnalls, T. J.; Beresford, N.; Kugathas, S.; Margiotta-Casaluci, L.; Scholze, M.; Scott, A. P.; Sumpter, J. P. From single chemicals to mixtures-Reproductive effects of levonorgestrel and ethinylestradiol on the fathead minnow. *Aquat. Toxicol.* **2015**, *169*, 152−167.
(98) Cwiertny, D. M.; Snyder, S. A.; Schlenk, D.; Kolodziej, E. P. Environmental Designer Drugs: When Transformation May Not Eliminate Risk. *Environ. Sci. Technol.* **2014**, *48* (20), 11737−11745.
(99) Martin, M. T.; Dix, D. J.; Judson, R. S.; Kavlock, R. J.; Reif, D. M.; Richard, A. M.; Rotroff, D. M.; Romanov, S.; Medvedev, A.; Poltoratskaya, N.; Gambarian, M.; Moeser, M.; Makarov, S. S.; Houck, K. A. Impact of Environmental Chemicals on Key Transcription Regulators and Correlation to Toxicity End Points within EPA's ToxCast Program. *Chem. Res. Toxicol.* **2010**, *23* (3), 578−590.
(100) Chambon, P. A decade of molecular biology of retinoic acid receptors. *FASEB J.* **1996**, *10* (9), 940−954.
(101) Alsop, D. H.; Brown, S. B.; Van Der Kraak, G. J. Dietary retinoic acid induces hindlimb and eye deformities in Xenopus laevis. *Environ. Sci. Technol.* **2004**, *38* (23), 6290−6299.
(102) Haga, Y.; Suzuki, T.; Takeuchi, T. Retinoic acid isomers produce malformations in postembryonic development of the Japanese flounder, Paralichthys olivaceus. *Zool. Sci.* **2002**, *19* (10), 1105−12.
(103) Sawada, K.; Inoue, D.; Wada, Y.; Sei, K.; Nakanishi, T.; Ike, M. Detection of retinoic acid receptor agonistic activity and identification of causative compounds in municipal wastewater treatment plants in Japan. *Environ. Toxicol. Chem.* **2012**, *31* (2), 307−315.
(104) Zhen, H.; Wu, X.; Hu, J.; Xiao, Y.; Yang, M.; Hirotsuji, J.; Nishikawa, J.; Nakanishi, T.; Ike, M. Identification of retinoic acid receptor agonists in sewage treatment plants. *Environ. Sci. Technol.* **2009**, *43* (17), 6611−6.
(105) Inoue, D.; Nakama, K.; Matsui, H.; Sei, K.; Ike, M. Detection of agonistic activities against five human nuclear receptors in river environments of Japan using a yeast two-hybrid assay. *Bull. Environ. Contam. Toxicol.* **2009**, *82* (4), 399−404.
(106) König, M.; Escher, B. I.; Neale, P. A.; Krauss, M.; Hilscherova, K.; Novak, J.; Teodorovic, I.; Schulze, T.; Seidensticker, S.; Kamal Hashmi, M. A.; Ahlheim, J.; Brack, W. Impact of untreated wastewater on a major European river evaluated with a combination of in vitro bioassays and chemical analysis. *Environ. Pollut.* **2017**, *220*, 1220−1230.
(107) Inoue, D.; Nakama, K.; Sawada, K.; Watanabe, T.; Matsui, H.; Sei, K.; Nakanishi, T.; Ike, M. Screening of agonistic activities against four nuclear receptors in wastewater treatment plants in Japan using a yeast two-hybrid assay. *J. Environ. Sci.* **2011**, *23* (1), 125−132.
(108) Taylor, P. N.; Razvi, S.; Pearce, S. H.; Dayan, C. M. A Review of the Clinical Consequences of Variation in Thyroid Function Within the Reference Range. *J. Clin. Endocrinol. Metab.* **2013**, *98* (9), 3562−3571.
(109) Moog, N. K.; Entringer, S.; Heim, C.; Wadhwa, P. D.; Kathmann, N.; Buss, C. Influence of Maternal Thyroid Hormones during Gestation on Fetal Brain Development. *Neuroscience* **2017**, *342*, 68−100.
(110) Jugan, M. L.; Oziol, L.; Bimbot, M.; Huteau, V.; Tamisier-Karolak, S.; Blondeau, J. P.; Levi, Y. In vitro assessment of thyroid and estrogenic endocrine disruptors in wastewater treatment plants, rivers and drinking water supplies in the greater Paris area (France). *Sci. Total Environ.* **2009**, *407* (11), 3579−87.
(111) Leusch, F. D. L.; Aneck-Hahn, N. H.; Cavanagh, J. E.; Du Pasquier, D.; Hamers, T.; Hebert, A.; Neale, P. A.; Scheurer, M.; Simmons, S. O.; Schriks, M. Comparison of in vitro and in vivo bioassays to measure thyroid hormone disrupting activity in water extracts. *Chemosphere* **2018**, *191*, 868−875.
(112) Valitalo, P.; Massei, R.; Heiskanen, I.; Behnisch, P.; Brack, W.; Tindall, A. J.; Du Pasquier, D.; Kuster, E.; Mikola, A.; Schulze, T.;

DOI: 10.1021/acs.est.9b00570
Environ. Sci. Technol. 2019, 53, 7215−7233

Sillanpaa, M. Effect-based assessment of toxicity removal during wastewater treatment. *Water Res.* **2017**, *126*, 153−163.
(113) Castillo, L.; Seriki, K.; Mateos, S.; Loire, N.; Guedon, N.; Lemkine, G. F.; Demeneix, B. A.; Tindall, A. J. In vivo endocrine disruption assessment of wastewater treatment plant effluents with small organisms. *Water Sci. Technol.* **2013**, *68* (1), 261−268.
(114) Murk, A. J.; Rijntjes, E.; Blaauboer, B. J.; Clewell, R.; Crofton, K. M.; Dingemans, M. M. L.; Furlow, J. D.; Kavlock, R.; Kohrle, J.; Opitz, R.; Traas, T.; Visser, T. J.; Xia, M. H.; Gutleb, A. C. Mechanism-based testing strategy using in vitro approaches for identification of thyroid hormone disrupting chemicals. *Toxicol. In Vitro* **2013**, *27* (4), 1320−1346.
(115) Wang, J.; Hallinger, D. R.; Murr, A. S.; Buckalew, A. R.; Simmons, S. O.; Laws, S. C.; Stoker, T. E. High-Throughput Screening and Quantitative Chemical Ranking for Sodium-Iodide Symporter Inhibitors in ToxCast Phase I Chemical Library. *Environ. Sci. Technol.* **2018**, *52* (9), 5417−5426.
(116) Ezechiáš, M.; Janochová, J.; Filipová, A.; Křesinová, Z.; Cajthaml, T. Widely used pharmaceuticals present in the environment revealed as invitro antagonists for human estrogen and androgen receptors. *Chemosphere* **2016**, *152*, 284−291.
(117) Johnson, I.; Hetheridge, M.; Tyler, C. R. *Assessment of (anti-) oestrogenic and (anti-) androgenic activities of final effluents from sewage treatment works*; SC020118/SR; Environmental Agency, UK, 2007. [https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/291074/scho0207bmax-e-e.pdf, last accessed 25.01.2019].
(118) Liscio, C.; Abdul-Sada, A.; Al-Salhi, R.; Ramsey, M. H.; Hill, E. M. Methodology for profiling anti-androgen mixtures in river water using multiple passive samplers and bioassay-directed analyses. *Water Res.* **2014**, *57*, 258−269.
(119) Jalova, V.; Jarosova, B.; Blaha, L.; Giesy, J. P.; Ocelka, T.; Grabic, R.; Jurcikova, J.; Vrana, B.; Hilscherova, K. Estrogen-, androgen- and aryl hydrocarbon receptor mediated activities in passive and composite samples from municipal waste and surface waters. *Environ. Int.* **2013**, *59*, 372−383.
(120) Ma, D. H.; Chen, L. J.; Wu, Y. C.; Liu, R. Evaluation of the removal of antiestrogens and antiandrogens via ozone and granular activated carbon using bioassay and fluorescent spectroscopy. *Chemosphere* **2016**, *153*, 346−355.
(121) Tang, X.; Wu, Q. Y.; Zhao, X.; Du, Y.; Huang, H.; Shi, X. L.; Hu, H. Y. Transformation of anti-estrogenic-activity related dissolved organic matter in secondary effluents during zonation. *Water Res.* **2014**, *48*, 605−612.
(122) Ihara, M.; Ihara, M. O.; Kumar, V.; Narumiya, M.; Hanamoto, S.; Nakada, N.; Yamashita, N.; Miyagawa, S.; Iguchi, T.; Tanaka, H. Co-occurrence of estrogenic and antiestrogenic activities in wastewater: quantitative evaluation of balance by in vitro ERalpha reporter gene assay and chemical analysis. *Environ. Sci. Technol.* **2014**, *48* (11), 6366−73.
(123) Knoop, O.; Itzel, F.; Tuerk, J.; Lutze, H. V.; Schmidt, T. C. Endocrine effects after ozonation of tamoxifen. *Sci. Total Environ.* **2018**, *622*, 71−78.
(124) Sharma, R. P.; Schuhmacher, M.; Kumar, V. Review on crosstalk and common mechanisms of endocrine disruptors: Scaffolding to improve PBPK/PD model of EDC mixture. *Environ. Int.* **2017**, *99*, 1−14.
(125) Stamm, C.; Räsänen, K.; Burdon, F. J.; Altermatt, F.; Jokela, J.; Joss, A.; Ackermann, M.; Eggen, R. I. L. *Adv. Ecol. Res.* **2016**, *55*, 183−223.
(126) Matthews, J.; Gustafsson, J. A. Estrogen receptor and aryl hydrocarbon receptor signaling pathways. *Nucl. Recept. Signaling* **2006**, *4*, No. nrs.04016.
(127) Zhao, B.; Bohonowych, J. E. S.; Timme-Laragy, A.; Jung, D.; Affatato, A. A.; Rice, R. H.; Di Giulio, R. T.; Denison, M. S. Common Commercial and Consumer Products Contain Activators of the Aryl Hydrocarbon (Dioxin) Receptor. *PLoS One* **2013**, *8* (2), No. e56860.
(128) Denison, M. S.; Nagy, S. R. Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. *Annu. Rev. Pharmacol. Toxicol.* **2003**, *43*, 309−334.
(129) Busch, W.; Schmidt, S.; Kuhne, R.; Schulze, T.; Krauss, M.; Altenburger, R. Micropollutants in European rivers: A mode of action survey to support the development of effect-based tools for water monitoring. *Environ. Toxicol. Chem.* **2016**, *35* (8), 1887−99.
(130) Neale, P. A.; Escher, B. I. Coextracted dissolved organic carbon has a suppressive effect on the acetylcholinesterase inhibition assay. *Environ. Toxicol. Chem.* **2013**, *32* (7), 1526−34.
(131) Escher, B. I.; Bramaz, N.; Mueller, J. F.; Quayle, P.; Rutishauser, S.; Vermeirssen, E. L. M. Toxic equivalent concentrations (TEQs) for baseline toxicity and specific modes of action as a tool to improve interpretation of ecotoxicity testing of environmental samples. *J. Environ. Monit.* **2008**, *10* (5), 612−621.
(132) Völker, J.; Vogt, T.; Castronovo, S.; Wick, A.; Ternes, T. A.; Joss, A.; Oehlmann, J.; Wagner, M. Extended anaerobic conditions in the biological wastewater treatment: Higher reduction of toxicity compared to target organic micropollutants. *Water Res.* **2017**, *116*, 220−230.
(133) Wahli, W.; Michalik, L. PPARs at the crossroads of lipid signaling and inflammation. *Trends Endocrinol. Metab.* **2012**, *23* (7), 351−363.
(134) Kersten, S.; Desvergne, B.; Wahli, W. Roles of PPARs in health and disease. *Nature* **2000**, *405* (6785), 421−4.
(135) Barak, Y.; Nelson, M. C.; Ong, E. S.; Jones, Y. Z.; Ruiz-Lozano, P.; Chien, K. R.; Koder, A.; Evans, R. M. PPAR gamma is required for placental, cardiac, and adipose tissue development. *Mol. Cell* **1999**, *4* (4), 585−595.
(136) O'Moore-Sullivan, T. M.; Prins, J. B. Thiazolidinediones and type 2 diabetes: new drugs for an old disease (vol 176, pg 381, 2002). *Medical Journal of Australia* **2002**, *177* (7), 396−396.
(137) Janesick, A.; Blumberg, B. Minireview: PPAR gamma as the target of obesogens. *J. Steroid Biochem. Mol. Biol.* **2011**, *127* (1−2), 4−8.
(138) Omiecinski, C. J.; Vanden Heuvel, J. P.; Perdew, G. H.; Peters, J. M. Xenobiotic Metabolism, Disposition, and Regulation by Receptors: From Biochemical Phenomenon to Predictors of Major Toxicities. *Toxicol. Sci.* **2011**, *120*, S49−S75.
(139) Von Gunten, U.; Salhi, E.; Schmidt, C. K.; Arnold, W. A. Kinetics and Mechanisms of N-Nitrosodimethylamine Formation upon Ozonation of N,N-Dimethylsulfamide-Containing Waters: Bromide Catalysis. *Environ. Sci. Technol.* **2010**, *44* (15), 5762−5768.
(140) Schmidt, C. K.; Brauch, H. J. N,N-dimethosulfamide as precursor for N-nitrosodimethylamine (NDMA) formation upon ozonation and its fate during drinking water treatment. *Environ. Sci. Technol.* **2008**, *42* (17), 6340−6346.
(141) von Gunten, U. Oxidation Processes in Water Treatment: Are We on Track? *Environ. Sci. Technol.* **2018**, *52* (9), 5062−5075.
(142) Wert, E. C.; Rosario-Ortiz, F. L.; Drury, D. D.; Snyder, S. A. Formation of oxidation byproducts from ozonation of wastewater. *Water Res.* **2007**, *41* (7), 1481−1490.
(143) Hammes, F.; Salhi, E.; Koster, O.; Kaiser, H. P.; Egli, T.; von Gunten, U. Mechanistic and kinetic evaluation of organic disinfection by-product and assimilable organic carbon (AOC) formation during the ozonation of drinking water. *Water Res.* **2006**, *40* (12), 2275−86.
(144) Soltermann, F.; Abegglen, C.; Tschui, M.; Stahel, S.; von Gunten, U. Options and limitations for bromate control during ozonation of wastewater. *Water Res.* **2017**, *116*, 76−85.
(145) Pinkernell, U.; von Gunten, U. Bromate minimization during ozonation: Mechanistic considerations. *Environ. Sci. Technol.* **2001**, *35* (12), 2525−2531.
(146) Von Gunten, U.; Oliveras, Y. Advanced oxidation of bromide-containing waters: Bromate formation mechanisms. *Environ. Sci. Technol.* **1998**, *32* (1), 63−70.
(147) Stalter, D.; Magdeburg, A.; Oehlmann, J. Comparative toxicity assessment of ozone and activated carbon treated sewage effluents using an in vivo test battery. *Water Res.* **2010**, *44* (8), 2610−2620.

DOI: 10.1021/acs.est.9b00570
Environ. Sci. Technol. 2019, 53, 7215−7233

(148) Emmert, B.; Bunger, J.; Keuch, K.; Muller, M.; Emmert, S.; Hallier, E.; Westphal, G. A. Mutagenicity of cytochrome P450 2E1 substrates in the Ames test with the metabolic competent S-typhimurium strain YG7108pin3ERb(5). *Toxicology* **2006**, *228* (1), 66−76.
(149) Yamada, M.; Matsui, K.; Sofuni, T.; Nohmi, T. New tester strains of Salmonella typhimurium lacking O-6-methylguanine DNA methyltransferases and highly sensitive to mutagenic alkylating agents. *Mutat. Res., Fundam. Mol. Mech. Mutagen.* **1997**, *381* (1), 15−24.
(150) Schindler Wildhaber, Y.; Mestankova, H.; Scharer, M.; Schirmer, K.; Salhi, E.; von Gunten, U. Novel test procedure to evaluate the treatability of wastewater with ozone. *Water Res.* **2015**, *75*, 324−335.
(151) Wagner, E. D.; Hsu, K. M.; Lagunas, A.; Mitch, W. A.; Plewa, M. J. Comparative genotoxicity of nitrosamine drinking water disinfection byproducts in Salmonella and mammalian cells. *Mutat. Res., Genet. Toxicol. Environ. Mutagen.* **2012**, *741* (1−2), 109−115.
(152) Kunz, P. Y.; Simon, E.; Creusot, N.; Jayasinghe, B. S.; Kienle, C.; Maletz, S.; Schifferli, A.; Schonlau, C.; Ait-Aissa, S.; Denslow, N. D.; Hollert, H.; Werner, I.; Vermeirssen, E. L. M. Effect-based tools for monitoring estrogenic mixtures: Evaluation of five in vitro bioassays. *Water Res.* **2017**, *110*, 378−388.
(153) Jarosova, B.; Blaha, L.; Giesy, J. P.; Hilscherova, K. What level of estrogenic activity determined by in vitro assays in municipal waste waters can be considered as safe? *Environ. Int.* **2014**, *64*, 98−109.
(154) Rice, J.; Westerhoff, P. High levels of endocrine pollutants in US streams during low flow due to insufficient wastewaterdilution. *Nat. Geosci.* **2017**, *10* (8), 587−591.
(155) Link, M.; von der Ohe, P. C.; Voss, K.; Schafer, R. B. Comparison of dilution factors for German wastewater treatment plant effluents in receiving streams to the fixed dilution factor from chemical risk assessment. *Sci. Total Environ.* **2017**, *598*, 805−813.
(156) Johnson, A. C.; Dumont, E.; Williams, R. J.; Oldenkamp, R.; Cisowska, I.; Sumpter, J. P. Do Concentrations of Ethinylestradiol, Estradiol, and Diclofenac in European Rivers Exceed Proposed EU Environmental Quality Standards? *Environ. Sci. Technol.* **2013**, *47* (21), 12297−12304.
(157) Magdeburg, A.; Stalter, D.; Oehlmann, J. Whole effluent toxicity assessment at a wastewater treatment plant upgraded with a full-scale post-ozonation using aquatic key species. *Chemosphere* **2012**, *88* (8), 1008−1014.
(158) Aristi, I.; Casellas, M.; Elosegi, A.; Insa, S.; Petrovic, M.; Sabater, S.; Acuna, V. Nutrients versus emerging contaminants-Or a dynamic match between subsidy and stress effects on stream biofilms. *Environ. Pollut.* **2016**, *212*, 208−215.
(159) Aristi, I.; von Schiller, D.; Arroita, M.; Barcelo, D.; Ponsati, L.; Garcia-Galan, M. J.; Sabater, S.; Elosegi, A.; Acuna, V. Mixed effects of effluents from a wastewater treatment plant on river ecosystem metabolism: subsidy or stress? *Freshwater Biol.* **2015**, *60* (7), 1398−1410.
(160) Zha, J. M.; Wang, Z. J. Assessing technological feasibility for wastewater reclamation based on early life stage toxicity of Japanese medaka (*Oryzias latipes*). *Agric., Ecosyst. Environ.* **2005**, *107* (2−3), 187−198.
(161) Gunnarsson, L.; Adolfsson-Erici, M.; Bjorlenius, B.; Rutgersson, C.; Forlin, L.; Larsson, D. G. J. Comparison of six different sewage treatment processes-Reduction of estrogenic substances and effects on gene expression in exposed male fish. *Sci. Total Environ.* **2009**, *407* (19), 5235−5242.
(162) Albertsson, E.; Larsson, D. G. J.; Forlin, L. Induction of hepatic carbonyl reductase/20 beta-hydroxysteroid dehydrogenase mRNA in rainbow trout downstream from sewage treatment works-Possible roles of aryl hydrocarbon receptor agonists and oxidative stress. *Aquat. Toxicol.* **2010**, *97* (3), 243−249.
(163) Lundstrom, E.; Adolfsson-Erici, M.; Alsberg, T.; Bjorlenius, B.; Eklund, B.; Laven, M.; Breitholtz, M. Characterization of additional sewage treatment technologies: ecotoxicological effects and levels of selected pharmaceuticals, hormones and endocrine disruptors. *Ecotoxicol. Environ. Saf.* **2010**, *73* (7), 1612−9.
(164) Stalter, D.; Magdeburg, A.; Weil, M.; Knacker, T.; Oehlmann, J. Toxication or detoxication? In vivo toxicity assessment of ozonation as advanced wastewater treatment with the rainbow trout. *Water Res.* **2010**, *44* (2), 439−448.
(165) Bundschuh, M.; Schulz, R. Population response to ozone application in wastewater: an on-site microcosm study with Gammarus fossarum (Crustacea: Amphipoda). *Ecotoxicology* **2011**, *20* (2), 466−73.
(166) Bundschuh, M.; Schulz, R. Ozonation of secondary treated wastewater reduces ecotoxicity to Gammarus fossarum (Crustacea; Amphipoda): Are loads of (micro)pollutants responsible? *Water Res.* **2011**, *45* (13), 3999−4007.
(167) Bundschuh, M.; Zubrod, J. P.; Seitz, F.; Stang, C.; Schulz, R. Ecotoxicological evaluation of three tertiary wastewater treatment techniques via meta-analysis and feeding bioassays using Gammarus fossarum. *J. Hazard. Mater.* **2011**, *192* (2), 772−778.
(168) Cuklev, F.; Gunnarsson, L.; Cvijovic, M.; Kristiansson, E.; Rutgersson, C.; Bjorlenius, B.; Larsson, D. G. J. Global hepatic gene expression in rainbow trout exposed to sewage effluents: A comparison of different sewage treatment technologies. *Sci. Total Environ.* **2012**, *427*, 106−114.
(169) Schluter-Vorberg, L.; Knopp, G.; Cornel, P.; Ternes, T.; Coors, A. Survival, reproduction, growth, and parasite resistance of aquatic organisms exposed on-site to wastewater treated by advanced treatment processes. *Aquat. Toxicol.* **2017**, *186*, 171−179.
(170) Wigh, A.; Geffard, O.; Abbaci, K.; Francois, A.; Noury, P.; Berge, A.; Vulliet, E.; Domenjoud, B.; Gonzalez-Ospina, A.; Bony, S.; Devaux, A. Gammarus fossarum as a sensitive tool to reveal residual toxicity of treated wastewater effluents. *Sci. Total Environ.* **2017**, *584*, 1012−1021.
(171) Abbas, A.; Valek, L.; Schneider, I.; Bollmann, A.; Knopp, G.; Seitz, W.; Schulte-Oehlmann, U.; Oehlmann, J.; Wagner, M. Ecotoxicological impacts of surface water and wastewater from conventional and advanced treatment technologies on brood size, larval length, and cytochrome P450 (35A3) expression in Caenorhabditis elegans. *Environ. Sci. Pollut. Res.* **2018**, *25* (14), 13868−13880.
(172) OECD. *Freshwater Alga and Cyanobacteria, Growth Inhibition Test*, OECD Guidelines for the Testing of Chemicals No. 201; Organisation for Economic Cooperation and Development: Paris, 2006.
(173) OECD. *Lemna sp. Growth Inhibition Test*, OECD Guidelines for the Testing of Chemicals No. 221; Organisation for Economic Cooperation and Development: Paris, 2006.
(174) *ISO 8692, Water quality—Fresh water algal growth inhibition test with unicellular green algae*; International Organization for Standardization (ISO): Geneva, Switzerland, 2012.
(175) OECD. *Daphnia magna Reproduction Test*, OECD Guidelines for the Testing of Chemicals No. 211; Organisation for Economic Cooperation and Development: Paris, 2012.
(176) OECD. *Daphnia sp., Acute Immobilisation Test*, OECD Guideline for the Testing of Chemicals No. 202; Organisation for Economic Cooperation and Development: Paris, 2004.
(177) OECD. *Potamopyrgus antipodarum Reproduction Test*, OECD Guidelines for the Testing of Chemicals No 242; Organisation for Economic Cooperation and Development: Paris, 2016.
(178) OECD. *Sediment−Water Chironomid Toxicity Test Using Spiked Water*, OECD Guidelines for the Testing of Chemicals No. 219; Organisation for Economic Cooperation and Development: Paris, 2004.
(179) OECD. *Sediment−Water Lumbriculus Toxicity Test Using Spiked Sediment*, OECD Guidelines for the Testing of Chemicals No. 225; Organisation for Economic Cooperation and Development: Paris, 2007.
(180) Maltby, L.; Clayton, S. A.; Wood, R. M.; McLoughlin, N. Evaluation of the Gammarus pulex in situ feeding assay as a biomonitor of water quality: robustness, responsiveness, and relevance. *Environ. Toxicol. Chem.* **2002**, *21* (2), 361−8.

DOI: 10.1021/acs.est.9b00570
Environ. Sci. Technol. 2019, 53, 7215−7233

(181) Jobling, S.; Casey, D.; Rodgers-Gray, T.; Oehlmann, J.; Schulte-Oehlmann, U.; Pawlowski, S.; Baunbeck, T.; Turner, A. P.; Tyler, C. R. Comparative responses of molluscs and fish to environmental estrogens and an estrogenic effluent (vol 65, pg 205, 2003). *Aquat. Toxicol.* **2004**, *66* (2), 207−222.
(182) Stange, D.; Sieratowicz, A.; Horres, R.; Oehlmann, J. Freshwater mudsnail (Potamopyrgus antipodarum) estrogen receptor: Identification and expression analysis under exposure to (xeno-)hormones. *Ecotoxicol. Environ. Saf.* **2012**, *75*, 94−101.
(183) OECD. *Fish Embryo Acute Toxicity (FET) Test*, OECD Guidelines for the Testing of Chemicals No. 236; Organisation for Economic Cooperation and Development: Paris, 2013.
(184) OECD. *Fish, Early-life Stage Toxicity Test*, OECD Guidelines for the Testing of Chemicals No. 210; Organisation for Economic Cooperation and Development: Paris, 2013.
(185) OECD. *21-day Fish Assay. A Short-Term Screening for Oestrogenic and Androgenic Activity, and Aromatase Inhibition*, OECD Guidelines for the Testing of Chemicals No. 230; Organisation for Economic Cooperation and Development: Paris, 2009.
(186) Daniels, K. D.; VanDervort, D.; Wu, S.; Leusch, F. D. L.; van de Merwe, J. P.; Jia, A.; Snyder, S. A. Downstream trends of in vitro bioassay responses in a wastewater effluent-dominated river. *Chemosphere* **2018**, *212*, 182−192.
(187) Ashauer, R. Post-ozonation in a municipal wastewater treatment plant improves water quality in the receiving stream. *Environ. Sci. Eur.* **2016**, *28*, 1.
(188) Bundschuh, M.; Pierstorf, R.; Schreiber, W. H.; Schulz, R. Positive Effects of Wastewater Ozonation Displayed by in Situ Bioassays in the Receiving Stream. *Environ. Sci. Technol.* **2011**, *45* (8), 3774−3780.
(189) Wilhelm, S.; Henneberg, A.; Kohler, H. R.; Rault, M.; Richter, D.; Scheurer, M.; Suchail, S.; Triebskorn, R. Does wastewater treatment plant upgrading with activated carbon result in an improvement of fish health? *Aquat. Toxicol.* **2017**, *192*, 184−197.
(190) Sousa, J. M.; Macedo, G.; Pedrosa, M.; Becerra-Castro, C.; Castro-Silva, S. C.; Pereira, M. F. R.; Silva, A. M. T.; Nunes, O. C.; Manaia, C. M. Ozonation and UV254 nm radiation for the removal of microorganisms and antibiotic resistance genes from urban wastewater. *J. Hazard. Mater.* **2017**, *323*, 434−441.
(191) Alexander, J.; Knopp, G.; Dotsch, A.; Wieland, A.; Schwartz, T. Ozone treatment of conditioned wastewater selects antibiotic resistance genes, opportunistic bacteria, and induce strong population shifts. *Sci. Total Environ.* **2016**, *559*, 103−112.
(192) Luddeke, F.; Hess, S.; Gallert, C.; Winter, J.; Gude, H.; Loffler, H. Removal of total and antibiotic resistant bacteria in advanced wastewater treatment by ozonation in combination with different filtering techniques. *Water Res.* **2015**, *69*, 243−251.
(193) Joss, A.; Siegrist, H.; Ternes, T. A. Are we about to upgrade wastewater treatment for removing organic micropollutants? *Water Sci. Technol.* **2008**, *57* (2), 251−255.
(194) Mousel, D.; Palmowski, L.; Pinnekamp, J. Energy demand for elimination of organic micropollutants in municipal wastewater treatment plants. *Sci. Total Environ.* **2017**, *575*, 1139−1149.
(195) Bourgin, M.; Beck, B.; Boehler, M.; Borowska, E.; Fleiner, J.; Salhi, E.; Teichler, R.; von Gunten, U.; Siegrist, H.; McArdell, C. S. Evaluation of a full-scale wastewater, treatment plant upgraded with ozonation and biological post-treatments: Abatement of micro-pollutants, formation of transformation products and oxidation by-products. *Water Res.* **2018**, *129*, 486−498.
(196) Schollee, J. E.; Bourgin, M.; von Gunten, U.; McArdell, C. S.; Hollender, J. Non-target screening to trace ozonation transformation products in a wastewater treatment train including different post-treatments. *Water Res.* **2018**, *142*, 267−278.
(197) Knopp, G.; Prasse, C.; Ternes, T. A.; Cornel, P. Elimination of micropollutants and transformation products from a wastewater treatment plant effluent through pilot scale ozonation followed by various activated carbon and biological filters. *Water Res.* **2016**, *100*, 580−592.
(198) Neale, P. A.; Altenburger, R.; Aït-Aïssa, S.; Brion, F.; Busch, W.; de Aragão Umbuzeiro, G. D.; Denison, M. S.; Du Pasquier, D.; Hilscherova, K.; Hollert, H.; Morales, D. A.; Novak, J.; Schlichting, R.; Seiler, T. B.; Serra, H.; Shao, Y.; Tindall, A. J.; Tollefsen, K. E.; Williams, T. D.; Escher, B. I. Development of a bioanalytical test battery for water quality monitoring: Fingerprinting identified micropollutants and their Contribution to effects in surface water. *Water Res.* **2017**, *123*, 734−750.

DOI: 10.1021/acs.est.9b00570
Environ. Sci. Technol. 2019, 53, 7215−7233