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Transformation of endocrine disrupting chemicals, pharmaceutical and personal care products during drinking water disinfection

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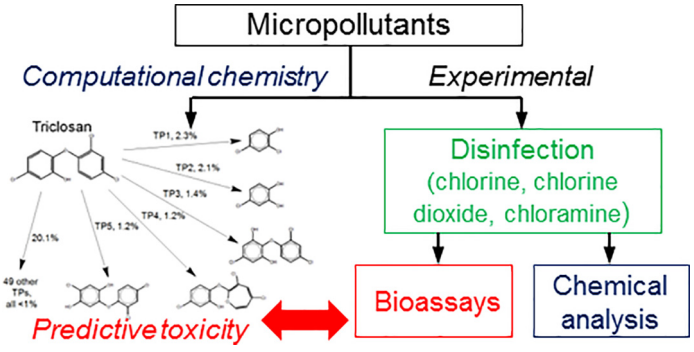
HIGHLIGHTS

- Endocrine disrupting compounds (EDCs) frequently detected in drinking water sources
- Raises concern that disinfection of drinking water could produce more potent EDCs
- This study applied a combination of computational and experimental methods.
- Chlorination decreased specific, but increased reactive & non-specific toxicity.
- Toxicity less than that produced from reaction of chlorine with organic matter

GRAPHICAL ABSTRACT



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ABSTRACT

Pharmaceuticals and personal care products (PPCPs) and endocrine disrupting compounds (EDCs) are frequently detected in drinking water sources. This raises concerns about the formation of potentially more toxic transformation products (TPs) after drinking water disinfection. This study applied a combination of computational and experimental methods to investigate the biological activity of eight EDCs and PPCPs commonly detected in source waters (acetaminophen, bisphenol A, carbamazepine, estrone, 17α-ethinylestradiol, gemfibrozil, naproxen and triclosan) before and after disinfection. Using a Stepped Forced Molecular Dynamics (SFMD) method, we detected 911 unique TPs, 36% of which have been previously reported in the scientific literature. We calculated

Abbreviations: AhR, aryl hydrocarbon receptor; AOP, advanced oxidation processes; AR, androgen receptor; CypAcEQ, cyproterone acetate equivalent; DBP, disinfection by-product; DexaEQ, dexamethasone equivalent; DHTEQ, dihydrotestosterone equivalent; D_{lipw}, liposome-water distribution ratio; EC, effect concentration; ED, Endocrine Disruptome; EDCs, endocrine disrupting chemicals; EEQ, estradiol equivalent; ER, estrogen receptor; GC–MS, gas chromatography–mass spectrometry; GR, glucocorticoid receptor; K_{ow}, octanol-water partition coefficient; LC-HRMS, liquid chromatography-high resolution mass spectrometer; LevoEQ, levonorgestrel equivalent; MifEQ, mifepristone equivalent; PPCP, pharmaceutical and personal care product; PXR, pregnane X receptor; QM/MM, quantum mechanics/molecular mechanics; QSAR, Quantitative Structure-Activity Relationship; REF, relative enrichment factor; SA, structural alert; SFMD, Stepped Forced Molecular Dynamics; SPE, solid-phase extraction; TD, toxicodynamics; TK, toxicokinetics; TMXEQ, tamoxifen equivalent; TP, transformation product; TR, toxic ratio; TU, toxic units.

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the likelihood that TPs would cause damage to biomolecules or DNA relative to the parent compound based on lipophilicity and the occurrence of structural alerts, and applied two Quantitative Structure-Activity Relationship (QSAR) tools to predict toxicity via receptor-mediated effects. In parallel, batch experiments were performed with three disinfectants, chlorine, chlorine dioxide and chloramine. After solid-phase extraction, the resulting TP mixtures were analyzed by chemical analysis and a battery of eleven *in vitro* bioassays covering a variety of endpoints. The laboratory results were in good agreement with the predictions. Overall, the combination of computational and experimental chemistry and toxicity methods used in this study suggest that disinfection of the studied EDCs and PPCPs will produce a large number of TPs, which are unlikely to increase specific toxicity (e.g., endocrine activity), but may result in increased reactive and non-specific toxicity.

1. Introduction

Access to safe drinking water is critical for public health, with disinfection using oxidants such as chlorine and chloramine commonly applied to ensure microbiologically safe water (WHO, 2011). However, these disinfectants can react with naturally occurring organic and inorganic material in water, such as humic acids and bromide, to form disinfection by-products (DBPs), which can have implications for human health (Richardson and Postigo, 2012). In addition to natural organic and inorganic matter, several studies have shown that source water used for drinking water treatment can contain low levels of anthropogenic micropollutants, including pharmaceuticals and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) (Benotti et al., 2009; Focazio et al., 2008; Glassmeyer et al., 2017; Simazaki et al., 2015). While micropollutants are typically found in the ng/L range in source water, some compounds, such as acetaminophen (Fram and Belitz, 2011) and bisphenol A (Focazio et al., 2008), have been detected at low μg/L concentrations. Disinfection with chlorine, chloramine and chlorine dioxide can reduce micropollutant concentrations in drinking water, but will often not completely mineralize them (Boyd et al., 2005; Rigobello et al., 2013). Instead, halogenated or partly oxidized transformation products (TPs) can be formed as some PPCPs and EDCs contain chemical moieties that are reactive with disinfectants (Snyder et al., 2003).

A range of TPs formed after disinfection with chlorine have been reported for micropollutants including acetaminophen (Bedner and MacCrehan, 2006; Cao et al., 2016), bisphenol A (Bourgin et al., 2013; Fukazawa et al., 2001; Gallard et al., 2004), carbamazepine (Han et al., 2018; Soufan et al., 2013), estrone (Nakamura et al., 2007), 17α-ethinylestradiol (Moriyama et al., 2004; Nakamura et al., 2006), gemfibrozil (Bulloch et al., 2012; Krkošek et al., 2011) and triclosan (Ben et al., 2016; Buth et al., 2011; Canosa et al., 2005). However, less is known about the toxicity of the formed TPs and, as many PPCPs and EDCs are biologically active, the question remains whether disinfection will form more or less potent TPs. In most cases, TPs formed from organic micropollutants via wastewater treatment and disinfection processes or in the environment (e.g. biodegradation) are less toxic towards aquatic organisms than the parent micropollutants, although there are exceptions where TPs are more toxic (Boxall et al., 2004). A further hindrance to assessing the risk of EDC or PPCP TPs is that the parent compounds are often found in source waters at low concentrations making the detection of the formed TPs particularly challenging with current analytical techniques, even though the TPs may still contribute to potential adverse effects due to mixture toxicity (Escher and Fenner, 2011).

An alternative approach to identify potential micropollutant TPs and characterize their risk is through computational chemistry benchmarked to toxicology. Reaction algorithms based on canonical reaction rules combined with group contribution theory or quantitative structure-property relationship analysis have been applied to advanced oxidation processes (AOP) (Li and Crittenden, 2009; Minakata et al., 2009). While such tools have been successfully applied in AOP treatment systems, they may not consider every reaction pathway or outcome, especially if a chemical species falls outside established reaction rules. Therefore, there is a need for a more fundamental non-stochastic approach that has the ability to predict a greater diversity of reaction products, including transient and minor constituents that could be significant from a mechanistic or toxicological perspective. One such approach that has recently been developed and implemented is referred to as "Stepped Forced Molecular Dynamics" (SFMD) (Ridgway et al., 2017). This involves a hybrid quantum mechanical/molecular mechanics (QM/MM) method whereby optimized reactant species (e.g. parent compounds) are forced to collide with the oxidant molecule (e.g. hypochlorite) in a series of quantum mechanical steps that are alternated with a molecular mechanic adjustment of the reactants' water environment. This method allows reaction products and their relative proportions to be identified.

Several different predictive toxicity approaches that consider different modes of toxic action, including non-specific, specific and reactive toxicity, can be applied to assess the toxicity of TPs compared to their parent compound. A first approximation of potential toxicity can be achieved through analysis of structural alerts (SAs, also called toxicophores) formed or destroyed during transformation processes. A SA is a chemical functional group or unit within the chemical structure that contributes to the toxic properties of the compound. If, during a transformation process, the SA of a chemical is lost, the resulting TP will most likely exhibit baseline toxicity (Escher and Fenner, 2011). Transformations produced by disinfectants may form new SAs, resulting in TPs with intrinsic toxicity that may be different to the parent. Thus, a prioritization scheme was developed to qualitatively select compounds that are likely to form TPs with baseline, reactive or specific toxicity that is greater than or equal to the parent effects (Escher and Fenner, 2011). Quantitative Structure-Activity Relationships (QSARs) are suitable for predicting baseline (non-specific) toxicity (Escher et al., 2009), which constitutes an anchor for the toxicity on top of which specific and reactive mechanisms enhance the potency of a chemical or TP. In principle it is possible to derive QSARs specific for each reactive mechanism, but in practice the applicability domain of each QSAR is fairly limited (Harder et al., 2003); therefore, our approach was to apply SAs as semi-quantitative indicators for enhanced activity relative to baseline toxicity. In the current study, this approach was applied to reactive modes of toxic action because these effects are most likely to arise *de novo* through transformations with AOPs and other biotic or abiotic transformation processes. Prediction of specific modes of action, such as endocrine activity, is more difficult to predict by QSARs, but molecular docking was used to assess the potential of a chemical to bind to a particular receptor, such as the estrogen receptor (ER) (Kolšek et al., 2014).

The aim of the current study was to predict the likely TPs of EDCs and PPCPs formed from the reaction of commonly used drinking water disinfectants, namely chlorine, chloramine and chlorine dioxide, and to determine their likely toxicity using both computational and experimental toxicity assessment. The experimental approach is outlined in Fig. 1. Eight EDCs and PPCPs, including acetaminophen, bisphenol A, carbamazepine, estrone, 17α-ethinylestradiol, gemfibrozil, naproxen and triclosan, were selected for study. A computational chemistry SFMD simulation was applied to predict potential TPs, while the toxicity of

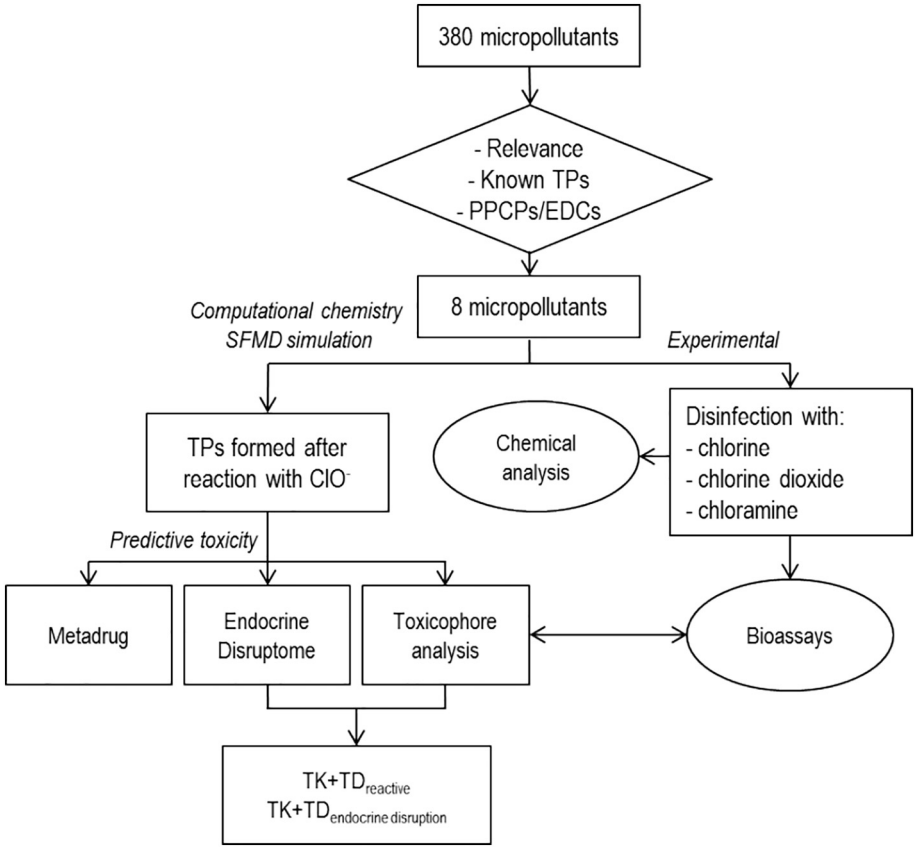

Fig. 1. Overview of current study.

the parent compound and predicted TPs were assessed using SA analysis, QSARs and molecular docking methods. The computational methods were complemented with bench-top disinfection experiments, with the parent compounds spiked at environmentally relevant concentrations (1 μg/L) and left to incubate for 7 d to simulate a long residence time in a distribution pipe. The disinfected samples, along with a control sample with no disinfection, were assessed using both targeted chemical analysis and bioassays indicative of specific toxicity, reactive toxicity, non-specific toxicity, xenobiotic metabolism and adaptive stress responses. The bioassays were selected to determine if the mixture effect was decreasing proportionally to the effect of the parent compound (i.e., dominant toxicity of the parent compound), increasing after disinfection (i.e., dominant toxicity of the TPs) or somewhere in between.

2. Materials and methods

2.1. Chemical selection

Eight compounds, acetaminophen, bisphenol A, carbamazepine, estrone, 17α-ethinylestradiol, gemfibrozil, naproxen and triclosan, were selected from a list of 380 previously prioritized chemicals representing pesticides, hormones, PPCPs and industrial compounds (Chapman et al., 2011; Institute of Environment and Health (IEH), 2012; Snyder et al., 2010; Snyder et al., 2008a; Snyder et al., 2008b; Snyder et al., 2007). The 380 compounds were ranked based on criteria including occurrence in water, availability of chemical analysis methods and inclusion in industry gray and white literature. Further, chemicals that were either EDCs or PPCPs were prioritized. The overall score of the eight prioritized chemicals can be found in Table S1 of the Supplementary Material, with physiochemical properties of the chemicals provided in Table S2.

2.2. Stepped Forced Molecular Dynamics (SFMD)

A detailed schematic representation and description of the SFMD method (Ridgway et al., 2017) is provided in Fig. S1 and Section S1 of the Supplementary Material. This method combines classical molecular mechanics and quantum mechanics using Hyperchem Version 8.10 in order to predict collision fragmentation products in an aqueous environment. The collision event for each parent compound–oxidant combination was simulated 1000 times, with the relative number of products obtained analyzed.

2.3. Predictive toxicity

2.3.1. Structural alert analysis

SA analysis was used to predict the effect of a transformation product i (TP_i) in relation to its parent compound based on the assumption that the effect has two dimensions, a toxicokinetic (TK) dimension and a toxicodynamic (TD) dimension (Escher et al., 2009; Escher and Fenner, 2011) (Fig. 2). Whether the TP_i will have more or less effect than its parent will depend on whether the TP_i is more or less lipophilic than the parent (change in TK dimension) or whether a specific effect is gained or lost (change in TD dimension). Further, the fraction of TP_i formed has an influence on predicting the mixture effect. Compounds that constitute a very small fraction of the mixture will not contribute significantly to the mixture toxicity unless their toxic effect is orders of magnitude more potent than other compounds in the mixture. Therefore, only TPs in the top 1% of successful SFMD reactions were considered. The TPs in the top 1% were re-scaled so that the sum fraction of all of the included TPs was 100%. By this approach, the weight of the TPs is exaggerated.

Baseline toxicity, which is defined by the TK of a compound, is directly related to the uptake of a compound, as measured by the liposome-water

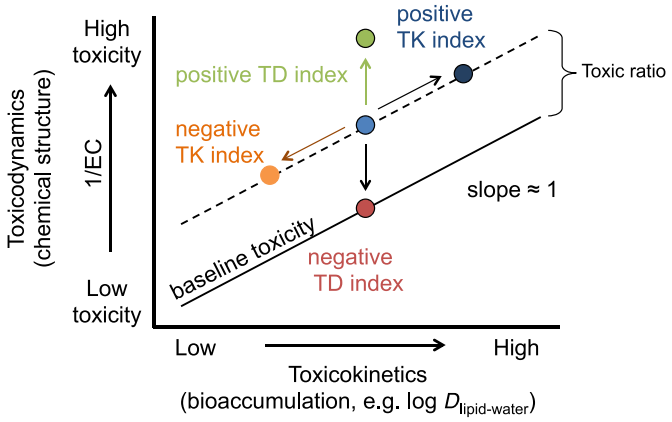

Fig. 2. Conceptual framework for the toxicokinetic (TK) and toxicodynamic (TD) analysis of the effects of transformation products in relation to the parent. The solid line represents the minimum toxicity that every compound has (baseline toxicity) and the dashed line the effect concentration (EC) of reactive toxic chemicals, which are more toxic than baseline by the toxic ratio.
Adapted with permission from Escher, B.I., Fenner, K., 2011. Recent advances in the environmental risk assessment of transformation products. Environ. Sci. Technol. 45, 3835–3847. Copyright (2011) American Chemical Society.

distribution ratio (log D_{lipw}) (Escher and Schwarzenbach, 2002). It is known that one order of magnitude increase in log D_{lipw} causes approximately one order of magnitude decrease in the effect concentration causing 50% effect (EC_{50}) for baseline toxicity (i.e., one order of magnitude increase in toxicity) due to a slope close to 1 of any baseline toxicity QSAR. Thus, each order of magnitude increase or decrease of the D_{lipw} of the TP_i from the D_{lipw} of the parent is proportional to an order of magnitude change in baseline toxicity. Therefore, the TK index basically sums up the contributions of the change in hydrophobicity of each TP_i in relation to the parent (P) using Eq. (1), where f_{TPi} is the re-scaled fraction of TP_i in the mixture.

$$\text{TK index} = \sum_{i=1}^{n}\left[f_{TP_i}\cdot\left(\log D_{lipw,TP_i} - \log D_{lipw,P}\right)\right] \quad (1)$$

Log D_{lipw} was calculated from estimated octanol-water partition coefficients (log K_{ow}), which were collected from both the US EPA EPI Suite software (US EPA, 2008) and SPARC (Hilal et al., 2005) according to Lienert et al. (2007). When the difference between log K_{ow} values calculated by EPI Suite and SPARC was <1.0, the EPI Suite value was used. If the difference between the two programs was >1.0, the average log K_{ow} among several predictive programs was calculated using the Virtual Computational Chemistry (VCC) Laboratory ALOGPS program (Virtual Computational Chemistry Laboratory, 2009) and the EPI Suite or SPARC value that was closer to the VCC Labs average log K_{ow} was used.

The TD of a compound is less straightforward to predict. In principle, the toxic ratio (TR) is a measure of how much more toxic a chemical is in relation to baseline toxicity (Fig. 2), but apart from empirical assessment, there are no good models to predict the TR. For the purposes of this prioritization scheme, it is assumed that each SA lost decreases the reactive toxicity EC_{50} value by one order of magnitude. Thus, the difference in the number of SAs between the parent and the TP is assumed to be related to a logarithmic increase or decrease in the EC_{50} value relative to the EC_{50} value of the parent. Therefore, the TD index for reactive toxicity ($TD_{reactive}$ index) was predicted using Eq. (2), where #SA is the number of SAs for the parent ($\#SA_P$) and transformation product i ($\#SA_{TPi}$). A library of relevant reactive SAs is provided in Table S3.

$$TD_{reactive}\,\text{index} = \sum_{i=1}^{n}\left[f_{TP_i}\cdot\left(\#SA_{TP_i} - \#SA_P\right)\right] \quad (2)$$

Although both indices are effectively perpendicular to each other, since the slope of a typical QSAR is close to 1, we can sum up the TK and $TD_{reactive}$ indices to the combined index of reactive toxicity (TK + $TD_{reactive}$). The baseline toxicity of a compound is dependent only on its TK properties, thus the predicted changes in the baseline toxicity between parent and TPs are related to the TK index. The TR of a compound is related to its TK properties and TD effects, so the change in reactive toxicity is related to the combined TK + $TD_{reactive}$ index.

2.3.2. Endocrine Disruptome

Endocrine Disruptome (ED), a freely available online tool, was used to predict the binding of the parent and TPs to the androgen receptor (AR), ERα and glucocorticoid receptor (GR) in both agonist and antagonist mode (Kolšek et al., 2014). The predicted binding affinity was grouped into four ED classes based on expected potency, namely Class 3, which was 1000 times more potent than baseline toxicity, Class 2, which was 100 times more potent, Class 1, which was 10 times more potent, and Class 0, which had no effect. Information about the predicted binding affinity for the different receptors can be found in Table S4. The TD index for endocrine disruption ($TD_{endocrine\ disruption}$ index) was calculated using Eq. (3) with the ED class of the TP_i and the parent, with the combined index for endocrine disruption (TK + $TD_{endocrine\ disruption}$ index) calculated as the sum of the derived $TD_{endocrine\ disruption}$ index and the TK index.

$$TD_{endocrine\ disruption}\,\text{index} = \sum_{i=1}^{n}\left[f_{TP_i}\cdot\left(\text{ED Class}_{TP_i} - \text{ED Class}_P\right)\right] \quad (3)$$

2.3.3. MetaDrug

Reactive and non-specific toxicity of the parent compounds and TPs were predicted using the commercial QSAR tool MetaDrug. Only predicted TPs in the top 1% of successful reactions were considered. The MetaDrug QSARs utilized in this study include the potential to be mutagenic (Ames bacterial assay), carcinogenic (rats and mice), cytotoxic (MCF7 cell line), genotoxic (rats and mice), hepatotoxic (rats, mice and humans) and toxic to bacteria. Likelihood of activation of the pregnane X receptor (PXR) was also included. The QSAR predictions generated values from 0 to 1, with values <0.5 considered negative, 0.5–0.7 considered low likelihood, 0.71–0.85 considered as moderate likelihood and >0.85 considered as high likelihood. Tanimoto Prioritization values ranging from 0 to 100 were provided to give an indication of the similarity of the compound structure to the structures included in the QSAR model training set, with the higher the value, the greater the similarity of the structure to the training set.

2.4. Disinfection experiments

Each studied compound was spiked individually at 1 μg/L into 1 L of phosphate buffered ultrapure water at pH 7. Each compound was exposed to three different disinfection reactions in duplicate, 3 mg/L chlorine, 2 mg/L pre-formed chloramine and 1 mg/L chlorine dioxide, as well as a spiked disinfectant-free control. The disinfectant concentrations were selected to represent typical concentrations used for primary disinfection of drinking water. A laboratory blank (ultrapure water) and a surface water sample collected from a drinking water reservoir in Southeast Queensland, both phosphate buffered to pH 7, were also included without chemical spiking. The surface water sample had a total organic carbon concentration of 8.5 mg/L and was not further treated prior to the disinfection experiments. To simulate a long residence time in a distribution pipe, the disinfection reactions were allowed to incubate for 7 d at 25 °C in the dark with gentle shaking (80 rpm). In the case of the surface water sample only, the disinfectant demand was determined prior to the 7 d experiment by dosing the surface water

sample with 100 mg/L disinfectant for 24 h and measuring the residual using a Hach colorimeter. The surface water was then spiked with sufficient disinfectant to give a residual of 3 mg/L for chlorine, 2 mg/L for chloramine and 1 mg/L for chlorine dioxide. The disinfectant residuals after the 7 d exposure for all samples were 2.8 to 3.9 mg/L for chlorine, 2.0 to 3.1 mg/L for chloramine and 1.0 to 1.3 mg/L for chlorine dioxide.

After the 7 d disinfection experiments the samples were filtered with glass fiber filters and extracted using Oasis HLB 6 mL solid-phase extraction (SPE) cartridges. Briefly, the cartridges were conditioned using 2 × 5 mL acetone:hexane (1:1, v/v), 2 × 5 mL methanol and 2 × 5 mL ultrapure water. After enriching 1 L of sample per cartridge, the cartridges were dried under vacuum and then eluted with 2 × 5 mL methanol and 2 × 5 mL acetone:hexane (1:1, v/v). The extracts were blown to dryness using a gentle nitrogen stream and then reconstituted in methanol to give an enrichment factor of 700. The SPE extracts were used for both chemical analysis and bioanalysis.

2.5. Chemical analysis

Detection of the majority of parent compounds and TPs was performed by liquid chromatography-high resolution mass spectrometry (LC-HRMS) using a Thermo Accela 600 LC system coupled to a LTQ Orbitrap XL MS. Bisphenol A and its TPs were analyzed by gas chromatography–mass spectrometry (GC–MS) based on Yamamoto and Yasuhara (2002) using a HP 6890 series GC with a single quadrupole MS. In addition to TPs predicted by the SFMD approach, TPs previously detected in the literature were also targeted. Further details can be found in Section S2 and Table S5 of the Supplementary Material, with the targeted TPs provided in Tables S6 to S13.

2.6. Bioanalysis

Eleven *in vitro* bioassays covering 15 different endpoints were applied to evaluate the effect of the studied compounds and their TPs. The studied bioassays are summarized in Table 1, with further information about the applied bioassays available in Leusch et al. (2014) and Escher et al. (2014). Each sample was run at least twice in each assay, with positive reference compounds and negative controls included on every plate. Linear or log-logistic concentration-effect curves were applied to determine effect concentration (EC) values in units of relative enrichment factor (REF), which takes into account sample enrichment by SPE and dilution in the assay. For the specific toxicity assays, the effect was reported in bioanalytical equivalent concentrations (BEQ) in units of ng/L using the EC value of the assay reference compound and the EC value of the sample (Equation 4) (Escher and Leusch, 2012). The effect was converted to toxic units (TU) for the assays indicative of xenobiotic metabolism, reactivity toxicity, non-specific toxicity and adaptive stress responses (Equation 5), where a higher TU indicates a greater effect.

$$\mathrm{BEQ} = \frac{\mathrm{EC(reference\ compound)}}{\mathrm{EC(sample)}} \tag{4}$$

$$\mathrm{TU} = \frac{1}{\mathrm{EC(sample)}} \tag{5}$$

3. Results

3.1. Computational chemistry

The results of the SFMD simulations, including most common simulated TP, are summarized in Table 2, with all results shown in Tables S14 to S21. Not all reactions resulted in altered products, with the reactants often bouncing off each other without any configuration changes. The collisions that resulted in products different from the parent were analyzed for chemical identity and stability, with the percent successful reaction rate ranging from 24.9% (gemfibrozil) to 52.6% (carbamazepine). The SFMD approach has recently shown encouraging results for predicting TP formation, especially for the prediction of volatile organic compound oxidation products (Ridgway et al., 2017). A number of known DBPs, including formaldehyde, acetaldehyde and chloromethane (Krasner et al., 2006; Richardson et al., 2007), were also reported by SFMD in the current study, which lends further support to the method. It should be noted that the approach only simulates the first product of a reaction, though in reality TPs may further react with the disinfectant to form other TPs. Simulated TPs in the top 1% of successful reactions were considered further for predictive toxicity.

Table 1
Battery of bioassays applied in the current study.

Endpoint	Bioassay	Assay positive reference compound	Bioanalytical equivalent concentration/EC value	Reference
Specific toxicity				
Estrogenic activity (+/−)	ER-GeneBLAzer	17β-Estradiol (+), tamoxifen (−)	EEQ (+), TMXEQ (−)	Neale and Leusch (2015)
Androgenic activity (+/−)	AR-GeneBLAzer	Dihydrotestosterone (+), cyproterone acetate (−)	DHTEQ (+), CypAcEQ (−)	König et al. (2017)
Glucocorticoid activity (+/−)	GR-GeneBLAzer	Dexamethasone (+), mifepristone (−)	DexaEQ (+), MifEQ (−)	Neale and Leusch (2015)
Progestagenic activity (+/−)	PR-GeneBLAzer	Levonorgestrel (+), mifepristone (−)	LevoEQ (+), MifEQ (−)	Neale and Leusch (2015)
Reactive toxicity				
Genotoxicity (human cells)	Micronucleus assay	Methyl methanesulfonate	EC_{05}	Laingam et al. (2008)
Genotoxicity (bacteria)	umuC	4-Nitroquinolone-*N*-oxide (4-NQO)	$EC_{IR1.5}$	EN ISO 13829 (2000)
Non-specific toxicity				
Bacterial toxicity	Microtox	Phenol	EC_{10}	Escher et al. (2008)
Toxicity to human cells	WIL2NS TOX	Methyl methanesulfonate	EC_{12}	Leusch et al. (2014)
Xenobiotic metabolism				
Liver enzyme induction	CYP1A2 induction assay	Benzo(*a*)pyrene	$EC_{IR1.7}$	Leusch et al. (2014)
Aryl hydrocarbon receptor	AhR CAFLUX	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD)	EC_{10}	Nagy et al. (2002)
Adaptive stress response				
Oxidative stress response	AREc32	*tert*-Butylhydroquinone (tBHQ)	$EC_{IR1.5}$	Escher et al. (2012)

+: agonist; −: antagonist; EEQ = estradiol equivalents; TMXEQ = tamoxifen equivalents; DHTEQ = dihydrotestosterone equivalents; CypAcEQ = cyproterone acetate equivalents; DexaEQ = dexamethasone equivalents; MifEQ = mifepristone equivalents; LevoEQ = levonorgestrel equivalents.
EC_{05}: effect concentration causing 5% micronucleus incidence; $EC_{IR1.5}$: effect concentration causing an induction ratio of 1.5; EC_{10}: effect concentration causing 10% effect, EC_{12}: effect concentration causing 12% effect, $EC_{IR1.5}$: effect concentration causing an induction ratio of 1.7.

Table 2
Summary of the SFMD results with the most common simulated transformation product (TP) and the percent reaction success.

Parent	Unreactive in % of simulations	Most common simulated TP	% Reaction success of TP
Acetaminophen	73.5%	5-Acetamido-7-oxabicyclo[4.1.0]hepta-2,4-dien-2-olate	4.5%
Bisphenol A	65.7%	4-[1-Hydroxy-2-(4-hydroxyphenyl)propan-2-yl]phenol	4.7%
Carbamazepine	47.4%	Acetylene	4.4%
Estrone	56.7%	5,16-Dihydroxy-15-methyltetracyclo[8.7.0.0^{2,7}.0^{11,15}] heptadeca-2(7),3,5-trien-14-one	2.8%
17α-Ethinylestradiol	61.5%	(1*S*,11*R*,12*S*,15*R*,16*S*)-15-ethynyl-15-hydroxy-16-methyl-4-oxapenta cyclo[9.7.0.0^{2,8}.0^{3,5}.0^{12,16}]octadeca-2(8),6-dien-6-olate	1.0%
		(2*S*,5*S*,6*R*,9*S*,10*S*)-6-ethynyl-5-methyl-18-oxatetracyclo [11.4.1.0^{2,10}.0^{5,9}]octadeca-1(17),13,15-triene-6,15-diol	1.0%
		(1*S*,10*R*,11*S*,14*R*,15*S*)-14-ethynyl-15-methyltetracyclo [8.7.0.0^{2,7}.0^{11,15}] heptadeca-2,4,6-triene-5,9,14-triol	1.0%
		(1*S*,10*S*,11*S*,14*R*,15*S*)-14-ethynyl-15-methyltetracyclo [8.7.0.0^{2,7}.0^{11,15}]heptadeca-2(7),3,5-triene-5,14,17-triol	1.0%
Gemfibrozil	75.1%	2,5-Dimethylphenol	8.4%
Naproxen	62.8%	Methanedione	9.4%
Triclosan	56.8%	2,4-Dichlorobenzen-1-olate	2.3%

3.2. Predictive toxicity

The predictive toxicity approaches for reactive toxicity and endocrine disruption were applied to assess whether the TPs predicted using the SFMD approach were more toxic than the studied parent compounds. The identified SAs for the parent compounds and simulated TPs in the top 1% of reactions are shown in Figs. S2 to S8, with carbamazepine shown in Fig. 3. Due to the large number of predicted TPs in Tables S14 to S21, simulated TPs formed at <1% were excluded from the predictive toxicity assessment. While some of these TPs may potentially be more potent than the simulated TPs in the top 1%, they would need to be over 100 times more potent that the parent compound to elicit a greater effect. The TK and $TD_{reactive}$ indices for the reactive mixture toxicity of the predicted TPs for the studied compounds are shown in Table 3. Positive index values predict an increase in toxicity among the TPs compared to the parent after disinfection, values near zero indicate a similar toxicity of the mixture of formed TPs and the parent and negative values predict a decrease in toxicity of the mixture of formed TPs compared to the parent. The TK index decreased for all target chemicals, indicating that the uptake potential in cell-based bioassays of most of the putative TPs was lower than for the parent compounds. This finding was expected because oxidative transformation processes generally lead to more polar and therefore less lipophilic TPs. In contrast, the $TD_{reactive}$ index increased for all studied chemicals, with the exception of carbamazepine, which indicates both the retention of the parent SAs among the TPs and the formation of new SAs on

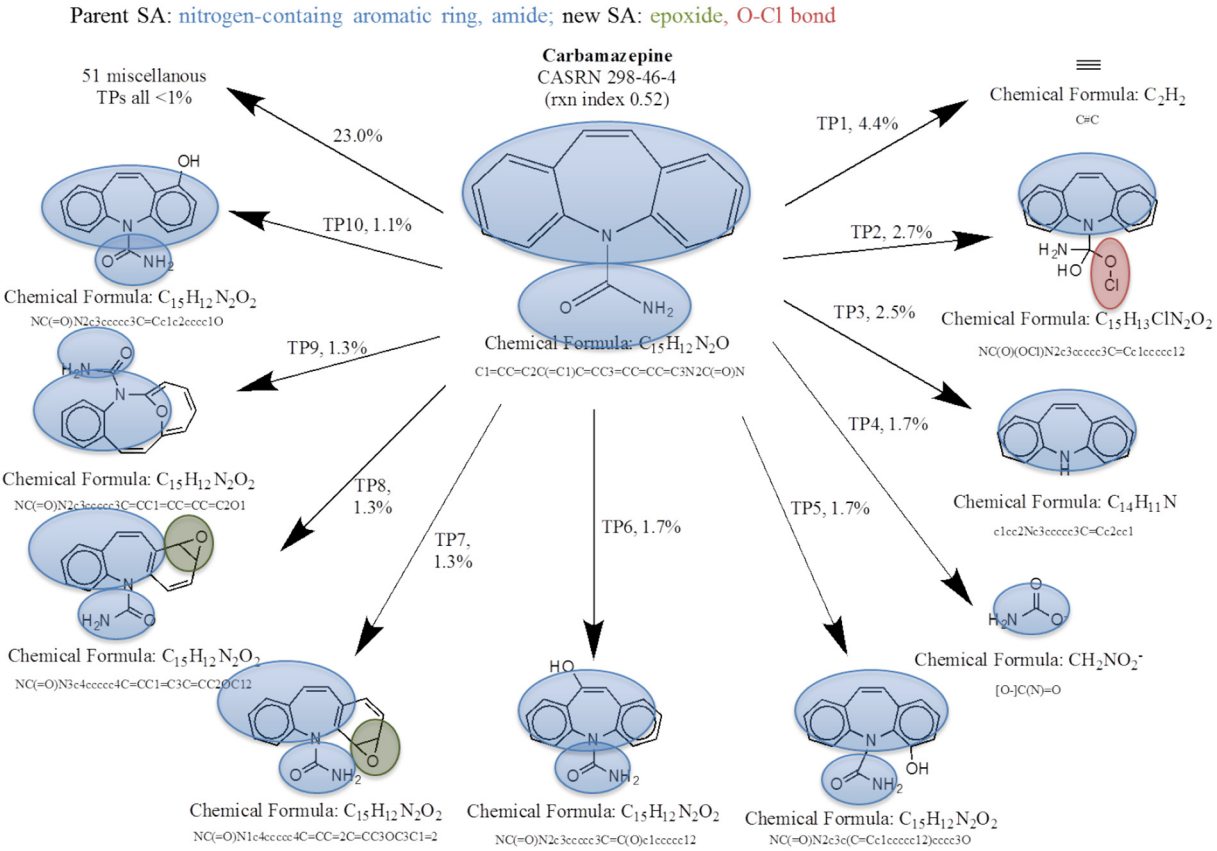

Fig. 3. Transformation products predicted by the SFMD approach for carbamazepine with structural alerts (SA) highlighted.

Table 3
TK index, $TD_{reactive}$ index and $TD_{endocrine\ disruptor}$ index and the combined TK + $TD_{reactive}$ and TK + $TD_{endocrine\ disruptor}$ indices for all compounds. Positive values are indicated in bold.

Chemical	Log D_{lipw} pH 7.6	#SA_P	TK index	$TD_{reactive}$ index	$TD_{endocrine\ disruptor}$ index						Combined TK + $TD_{reactive}$ index	Combined TK + $TD_{endocrine\ disruptor}$ index					
					AR+	*AR−*	*ER+*	*ER−*	*GR+*	*GR−*		*AR+*	*AR−*	*ER+*	*ER−*	*GR+*	*GR−*
Acetaminophen	0.93	1	−0.53	**0.79**	–	−0.07	–	–	–	–	**0.27**	–	−0.60	–	–	–	–
Bisphenol A	3.52	0	−0.59	**0.69**	−0.14	−0.08	−0.53	−0.29	−0.08	−0.39	**0.09**	−0.73	−0.67	−1.12	−0.88	−0.67	−0.98
Carbamazepine	2.73	2	−1.11	−0.52	−0.19	−0.28	−0.36	−0.25	−0.06	−0.06	−1.63	−1.30	−1.39	−1.47	−1.36	−1.17	−1.17
Estrone	3.35	0	−1.04	**0.08**	−0.53	−0.42	−0.22	−0.59	–	–	−0.95	−1.57	−1.46	−1.26	−1.63	–	–
17α-Ethinylestradiol	3.84	1	−0.12	**0.25**	−0.50	−0.75	−2.00	−2.00	–	–	**0.13**	−0.62	−0.87	−2.12	−2.12	–	–
Gemfibrozil	3.83	0	−1.75	**0.04**	**0.17**	−0.24	–	–	–	–	−1.71	−1.58	−1.99	–	–	–	–
Naproxen	2.40	0	−1.62	**0.31**	−1.23	−0.53	–	–	−0.53	–	−1.31	−2.85	−2.15	–	–	−2.15	–
Triclosan	4.82	1	−1.62	**0.26**	**0.31**	–	–	–	−0.54	–	−1.36	−1.31	–	–	–	−2.16	–

+: agonist; −: antagonist; AR: androgen receptor; ER: estrogen receptor α; GR: glucocorticoid receptor.

the TPs. However, when considering the combined TK and $TD_{reactive}$ index, only acetaminophen (0.27), bisphenol A (0.09) and 17α-ethinylestradiol (0.13) had a positive combined TK + $TD_{reactive}$ index, suggesting these chemicals may be of potential further interest. Further information about the SA analysis results can be found in Section S3.

ED was used to predict molecular binding to different receptors, with the ED classes for the parent and TPs shown in Table S22. The $TD_{endocrine\ disruptor}$ indices for the studied compounds are shown in Table 3, with positive values obtained only for gemfibrozil and triclosan for binding to AR. Binding to ERα and GR were negative for all compounds, suggesting that the binding capacity was reduced compared to the parent compound. For all compounds, the combined TK + $TD_{endocrine\ disruptor}$ index was negative, indicating that disinfection is unlikely to produce TPs with higher ER, AR or GR activity.

The results from the MetaDrug QSARs for mutagenicity (Ames), carcinogenicity, genotoxicity, hepatotoxicity, bacterial toxicity and activation of PXR are provided in Table S23. The QSAR predicted toxicity of the TPs varied compared to the parent compounds. For example, many of the acetaminophen TPs had reduced genotoxicity compared to the parent and all 17α-ethinylestradiol TPs had lower carcinogenicity and hepatotoxicity compared to 17α-ethinylestradiol. In contrast, the top three predicted gemfibrozil TPs had higher carcinogenicity, genotoxicity, hepatotoxicity and bacterial toxicity compared to the parent compound.

3.3. Chemical analysis

Targeted chemical analysis of known and predicted TPs was conducted on the spiked disinfected samples and the spiked disinfectant-free control. While each parent compound was detected in the disinfectant-free control, in most cases neither the parent compound nor the targeted TPs were detected after disinfection. The exceptions were carbamazepine and estrone, with several TPs detected after disinfection. Acridine and acridine-9-carbaldehyde were detected after disinfection of carbamazepine with chlorine. Predicted TPs 5–10 were also detected, but could not be identified to the individual TP level as they all had the same elemental formula and theoretical accurate mass (Table S24). Furthermore, due to the relatively low concentration of TPs 5–10 in the sample, diagnostic fragmentation spectra could not be acquired over the chromatographic run. Acridine-9-carbaldehyde, TPs 5–10 and the parent compound were also detected after disinfection with chlorine dioxide (Table S24). Previous studies have also found that acridine and acridine-9-carbaldehyde are the primary TPs formed from the reaction of chlorine and chlorine dioxide with carbamazepine (Furst and Uetrecht, 1993; Han et al., 2018; Kosjek et al., 2009). Predicted TPs 1–8, which all had the same formula and theoretical accurate mass, were detected after disinfection of estrone with chlorine dioxide (Table S25). The fact that few TPs were detected after disinfection can be explained by the low spiked chemical concentration (1 μg/L), which was selected to represent an environmentally relevant concentration. Enrichment using SPE was conducted, but the recovery of the TPs by SPE is unknown. Assuming an estimated average recovery of 20–60% and a detection limit of 10 ng/L after SPE, the inability to detect TPs for the majority of parent compounds may indicate that individual TPs were not present at concentrations > 5% of the parent compound. Additional experiments at higher parent compound concentrations are required to test this assumption.

3.4. Bioanalysis

A suite of bioassays covering non-specific toxicity, specific toxicity, reactive toxicity, xenobiotic metabolism and adaptive stress responses were applied to assess the effect of the disinfected samples, as well as the spiked disinfectant-free control samples. Both the spiked disinfectant-free control and disinfected samples were inactive in the GR-GeneBLAzer, PR-GeneBLAzer, Micronucleus, umuC, WIL2NS TOX and CYP1A2 induction assays (Tables S26 to S31), with only the disinfected surface water samples active in AREc32 (Table 4). The fact that the spiked disinfectant-free controls did not have an effect in some of the assays is not surprising given that bioassays only detect chemicals that are active in the studied assay endpoint. Several parent compounds were active in the ER-GeneBLAzer, including estrone, 17α-ethinylestradiol, gemfibrozil and naproxen, while 17α-ethinylestradiol was also active in the anti-AR-GeneBLAzer assay (Table 4). The observed effect decreased for the majority of active compounds after disinfection, though estrogenic activity did increase slightly for gemfibrozil after chlorination. While many of the samples disinfected with chlorine and chlorine dioxide had an effect in the Microtox assay (Table 4), this was within the same range as the disinfected ultrapure water, suggesting that the observed effect was not due formed TPs, but some other contamination. While chlorine is not expected to be retained by SPE, the effect in the assay could be due to potential organic contamination in the disinfectant as the Microtox assay is very sensitive to organic compounds.

Both the disinfectant-free gemfibrozil and 17α-ethinylestradiol samples had an effect in the AhR-CAFLUX, while disinfection with chloramine resulted in an increased effect for acetaminophen, bisphenol A, carbamazepine and estrone in the AhR-CAFLUX assay. There was also an increased effect for carbamazepine, 17α-ethinylestradiol and gemfibrozil after disinfection with chlorine dioxide in the AhR-CAFLUX assay, but this was in the same range as the effect observed in the ultrapure water after chlorine dioxide disinfection. Surface water, which was collected from a drinking water reservoir in a protected catchment in Southeast Queensland, proved to be the most responsive sample after disinfection, with increased effect observed in anti-AR-GeneBLAzer, Microtox, AhR-CAFLUX and AREc32 after disinfection with chlorine, chloramine and chlorine dioxide.

4. Discussion

4.1. Transformation product formation

The current study applied a novel combination of computational and experimental methods to assess TP formation and toxicity after

Table 4
Summary of activity in the bioassay test battery, with parent compounds in black font and disinfected samples in gray font. Compounds where a change in effect with disinfection was observed are indicated in bold. The results are expressed in bioanalytical equivalent concentrations (ng/L) for ER-GeneBLAzer and AR-GeneBLAzer, while the results in the Microtox, AhR CALUX and AREc32 assays are expressed in toxic units. None of the samples had an effect in the GR-GeneBLAzer, PR-GeneBLAzer, Micronucleus, umuC, WIL2NS TOX or CYP1A2 induction assays.

Compound	ER-GeneBLAzer (+) (ng/L EEQ)	ER-GeneBLAzer (−) (ng/L TMXEQ)	AR-GeneBLAzer (+) (ng/L DHTEQ)	AR-GeneBLAzer (−) (ng/L CypAcEQ)	Microtox (TU)	AhR CAFLUX (TU)	AREc32 (TU)
Acetaminophen	<0.1	<6000	<10	<10000	<0.03	<0.10	<0.03
+Chlorine	<0.1	<6000	<10	<10000	**0.05**	<0.10	<0.03
+Chlorine dioxide	<0.1	<6000	<10	<10000	**0.05**	<0.10	<0.03
+Chloramine	<0.1	<6000	<10	<10000	<0.03	**0.27**	<0.03
Bisphenol A	<0.1	<6000	<10	<10000	<0.03	<0.10	<0.03
+Chlorine	<0.1	<6000	<10	<10000	**0.06**	<0.10	<0.03
+Chlorine dioxide	<0.1	<6000	<10	<10000	**0.05**	<0.10	<0.03
+Chloramine	<0.1	<6000	<10	<10000	<0.03	**0.15**	<0.03
Carbamazepine	<0.1	<6000	<10	<10000	<0.03	<0.10	<0.03
+Chlorine	<0.1	<6000	<10	<10000	**0.06**	<0.10	<0.03
+Chlorine dioxide	<0.1	<6000	<10	<10000	**0.06**	**0.16**	<0.03
+Chloramine	<0.1	<6000	<10	<10000	<0.03	**0.18**	<0.03
Estrone	**12**	<6000	<10	<10000	<0.03	<0.10	<0.03
+Chlorine	**0.16**	<6000	<10	<10000	**0.05**	<0.10	<0.03
+Chlorine dioxide	**<0.1**	<6000	<10	<10000	**0.05**	<0.10	<0.03
+Chloramine	**<0.1**	<6000	<10	<10000	<0.03	**0.26**	<0.03
17α-Ethinylestradiol	**2000**	<6000	<10	**13000**	<0.03	**0.13**	<0.03
+Chlorine	**0.40**	<6000	<10 2	**<10000**	**0.04**	<0.10	<0.03
+Chlorine dioxide	**<0.1**	<6000	<10	**<10000**	**0.05**	**0.27**	<0.03
+Chloramine	**<0.1**	<6000	<10	**<10000**	<0.03	<0.10	<0.03
Gemfibrozil	**0.23**	<6000	<10	<10	<0.03	**0.13**	<0.03
+Chlorine	**0.32**	<6000	<10	<10000	**0.05**	<0.10	<0.03
+Chlorine dioxide	**<0.1**	<6000	<10	<10000	**0.06**	**0.14**	<0.03
+Chloramine	**<0.1**	<6000	<10	<10000	<0.03	<0.10	<0.03
Naproxen	**1.5**	<6000	<10	<10000	<0.03	<0.10	<0.03
+Chlorine	**<0.1**	<6000	<10	<10000	**0.06**	<0.10	<0.03
+Chlorine dioxide	**<0.1**	<6000	<10	<10000	**0.04**	<0.10	<0.03
+Chloramine	**<0.1**	<6000	<10	<10000	<0.03	<0.10	<0.03
Triclosan	<0.1	<6000	<10	<10000	<0.03	<0.10	<0.03
+Chlorine	<0.1	<6000	<10	<10000	**0.05**	<0.10	<0.03
+Chlorine dioxide	<0.1	<6000	<10	<10000	**0.04**	<0.10	<0.03
+Chloramine	<0.1	<6000	<10	<10000	<0.03	<0.10	<0.03
Ultrapure water	<0.1	<6000	<10	<10000	<0.03	<0.10	<0.03
+Chlorine	<0.1	<6000	<10	<10000	**0.05**	<0.10	<0.03
+Chlorine dioxide	<0.1	<6000	<10	<10000	**0.05**	**0.20**	<0.03
+Chloramine	<0.1	<6000	<10	<10000	<0.03	<0.10	<0.03
Surface water	<0.1	<6000	<10	<10000	**0.14**	**0.28**	<0.03
+Chlorine	<0.1	<6000	<10	**13000**	**0.59**	**0.22**	**0.09**
+Chlorine dioxide	<0.1	<6000	<10	**40000**	**0.63**	**0.56**	**0.09**
+Chloramine	<0.1	<6000	<10	**35000**	**0.24**	**0.53**	**0.05**

EEQ = estradiol equivalents; TMXEQ = tamoxifen equivalents; DHTEQ = dihydrotestosterone equivalents; CypAcEQ = cyproterone acetate equivalents; TU = toxic units.

disinfection with chlorine, chloride dioxide and chloramine. While all parent compounds were detected in the disinfectant-free control sample, TPs were only detected for estrone and carbamazepine after disinfection. With the possible exception of carbamazepine (Table S24), it appears that most reactions did not form a dominant TP (e.g. >5% of the parent compound). This is in agreement with the SFMD simulations, where the average reaction success of the most common TP was 4.7% (ranged from 1.0% for 17α-ethinylestradiol to 9.4% for naproxen) (Table 2).

The majority of TPs predicted to form from the reaction with hypochlorite in the SFMD approach, as well as the detected carbamazepine and estrone TPs, were non-chlorinated compounds, with mainly oxidized products predicted to be formed with a yield above 1%. To date, much of the research on micropollutant TPs formed after chlorination has focused on chlorinated TPs, but our findings suggest that the current research may be missing an important component of the formed TPs.

4.2. Does disinfection form more toxic transformation products?

Both the predictive toxicity results and the bioassays suggest that specific effects, such as binding to hormone receptors, will generally decrease after disinfection, while the predictive toxicity results indicate that reactive toxicity may increase. This seems to support the hypothesis that because specific toxicity requires a very particular chemical structure and size, transformation during disinfection reduces the compound's ability to induce the specific response while occasionally creating reactive SAs. Our observations fit with previous findings for other water treatment processes, such as ozonation, where disinfection tends to decrease specific effects, including estrogenic activity (Huber et al., 2004) and anti-bacterial activity (Dodd et al., 2009), but can increase reactive toxicity (Magdeburg et al., 2014).

The one exception was gemfibrozil, where a 39% increase in estrogenic activity after disinfection with chlorine compared to the disinfectant-free sample was observed (Table 3). Disinfection by chlorine dioxide and chloramine reduced the estrogenic activity of gemfibrozil to below the detection limit. The predicted gemfibrozil TP mixture also had a positive AR $TD_{endocrine\ disruptor}$ index (Table 3). Chlorinated TP 4′-chlorogemfibrozil has been previously shown to be a more potent antiandrogenic compound than gemfibrozil in fish (Bulloch et al., 2012). 4′-Chlorogemfibrozil was analyzed in the current study but was not detected after disinfection.

Based on the SA analysis, the $TK + TD_{reactive}$ index yielded positive values for acetaminophen, bisphenol A and 17α-ethinylestradiol, indicating that they have the potential to form more reactive TPs than the parent compound after disinfection. None of the samples were active in the umuC assay, which is indicative of genotoxicity, or in the AREc32 assay, which is indicative of the oxidative stress response, but both acetaminophen and bisphenol A produced TPs that were active in the AhR CAFLUX assay after disinfection with chloramine. Han et al. (2018) observed increased genotoxicity of carbamazepine after chlorination and chloramination compared to the parent compound in the umuC assay; however, the spiked concentration of carbamazepine was over 20,000 times higher than spiked in the current study. Similar to acetaminophen and bisphenol A, carbamazepine was active in the AhR CAFLUX assay after chloramination. The AhR CAFLUX assay assesses activation of the aryl hydrocarbon receptor (AhR), which is indicative of xenobiotic metabolism. This assay is typically used to detect dioxin-like chemicals, but recent studies have shown that a wide range of environmental chemicals can activate this endpoint (Ghisari et al., 2015; Long et al., 2012; Martin et al., 2010). While none of the causative compounds could be identified and bisphenol A was previously found to be inactive in AhR CALUX (Neale et al., 2017), Jia et al. (2015) observed a slight increase in AhR activation after chlorination and UV treatment during advanced water treatment processes, and this was attributed to the formation of TPs.

Overall, the strongest response in the bioassays after disinfection was not induced by any of the studied EDCs or PPCPs, but by the surface water sample from a drinking water reservoir, where disinfection with chlorine, chloride dioxide and chloramine resulted in an increased effect in antagonist mode in the AR-GeneBLAzer, Microtox and AREc32 assays and disinfection by chloride dioxide and chloramine resulted in an increased effect in the AhR CALFUX assay. The disinfectant-free surface water sample also had a response in the Microtox and AhR CALUX assays, though the toxic units were typically lower before disinfection. The surface water had a total organic carbon concentration of 8.5 mg/L, thus the increased effect was most likely due to the formation of DBPs from natural organic matter. This has been previously observed during drinking water treatment, where non-specific and reactive toxicity increased at the outlet of the plant after disinfection with chlorine and chloramine (Neale et al., 2012). This suggests that DBPs formed from the reaction of naturally occurring organic and inorganic matter are likely to be of greater toxicological concern compared to micropollutant TPs, given that natural organic matter is found at concentrations several orders of magnitude higher than micropollutants in source water. While the potential contribution of micropollutants and their TPs to the observed effect in surface water cannot be ruled out as they were not quantified, the mixture effect is related to both chemical concentration and potency and the concentration of formed DBPs is expected to be much higher than any formed TPs.

4.3. Limitations and future research

The current study applied a number of novel approaches to evaluate TP formation and toxicity, but these are not without their limitations. The SFMD approach was applied for the first time to evaluate EDC and PPCP TPs and, while it represents an improvement compared to other available models, there are some limitations. One is that the method in its current form can only predict the first product of transformation from the reaction of hypochlorite with the parent compound due to the exponential expansion of reactions required to be modelled in subsequent steps (i.e. for a second transformation step, each TP would in turn be the target compound for the pursuant 1000 reactions). However, in actuality the formed TPs may continue to react with the disinfectant, forming additional TPs. As a result, the method does not provide complete information about the final TPs that may be present in water. The localized nature of the SFMD approach also cannot take into consideration the concentration of the reactant or the contact time. Further, while computational chemistry can provide an indication of potential TPs, it cannot currently provide information about the concentration of TPs formed, meaning that the estimation of toxicity can only be qualitative or semi-quantitative at best. The frequency of occurrence of TPs during the SFMD simulation was used to assess abundance, but this approach requires further validation.

The $TK + TD_{reactive}$ and $TK + TD_{endocrine\ disruptor}$ indices used for the SA analysis and ED are a novel approach to evaluate whether the mixture of formed TPs is likely to be more or less toxic than the parent compound. The most common new SAs were epoxides reactive to both DNA and protein and were present in the TPs of seven of the eight studied parent compounds. Further, the catechol SA was present in acetaminophen, bisphenol A and triclosan. However, the SA analysis approach does not provide any information about how reactivity relates to toxicity and therefore the magnitude of the toxic effect could not be estimated, but it is likely that not every SA leads to a simple 10-fold increase in toxicity as assumed here. In particular, many epoxides are very reactive and it is likely that their TR would be higher than the assumed factor of 10, while others might show lower toxicity. While the QSARs available in MetaDrug are quick and simple to use, the reliability of the results can be influenced by the QSARs themselves, with the number of compounds in the training set varying considerably for different QSARs (372 to 1780). Furthermore, the Tanimoto Prioritization was often very low for the TPs, which means that the structure of the TPs

was very different from the structure of compounds used in the training set. Finally, unlike the other predictive toxicity methods, it was not possible account for the mixture effects using the QSARs.

The disinfection experiments were conducted at environmentally relevant micropollutant concentrations, but the low spiked parent compound concentrations hampered the chemical analysis and bioanalysis. In the current study, a typical disinfectant dose for drinking water treatment was used. Surface waters can contain organic carbon in the range typically from 1 to 10 mg/L and much of this organic carbon will react with the disinfectant. However, the micropollutants were spiked into ultrapure water with a total organic carbon concentration < 0.005 mg/L, thus the micropollutant:disinfectant ratio was not realistic and resulted in extremely high reaction efficiency, contributing to the low observed effects and detected TPs.

Despite these limitations, the current study provided new insights into the assessment of micropollutant TPs, with further work required to improve and validate the current approach.

5. Conclusions

The presence of low concentrations of micropollutants, including PPCPs and EDCs, in drinking water supplies has raised concerns about the formation of TPs after disinfection using common oxidants including chlorine, chlorine dioxide and chloramine. In this study, computational methods were applied to predict TP formation for eight priority chemicals and their likely toxicity compared to the parent compound. This was complemented with an experimental disinfection study with chemical analysis and bioanalysis. The computational methods had advantages and disadvantages, while working at environmentally relevant concentrations meant that the disinfection experiments did not yield as much information as expected. Overall, the applied computational approach indicated that a wide range of TPs can be formed after disinfection of micropollutants, but both predictive and experimental toxicology suggests that disinfection is unlikely to form TPs with increased specific toxicity, though reactive toxicity may increase due to the creation of new reactive functional groups (e.g. SAs or toxicophores). Surface water from a drinking water reservoir was the most responsive in the bioassays after disinfection, indicating that the formation of conventional DBPs is likely to pose a greater risk to health than TPs formed from micropollutants.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.12.106.

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