

# Evaluation of theoretical and experimental techniques to predict the generation and toxicity of transformation products of trace organic compounds following disinfection of drinking water

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## Introduction

Endocrine active compounds (EACs) and pharmaceuticals and personal care products (PPCPs) are anthropogenic contaminants released into the environment via wastewater. They are frequently detected at ng/L to µg/L concentrations in natural waters. Recent studies have detected a range of EACs and PPCPs in raw drinking water sources, with a few compounds recalcitrant to drinking water treatment and detectable in drinking water itself,<sup>1</sup> albeit typically at concentrations significantly below health concern.<sup>2</sup> These findings raised concern that incomplete mineralization of EACs and PPCPs during disinfection and oxidation treatment steps may lead to the production of reactive (and toxic) transformation products (TPs). As the concentration of the parent compounds in raw drinking water sources is already low, it is currently impossible using conventional chemical analysis methods to screen or detect such potential TPs directly in drinking water.

## Experimental approach

In this project, we applied computational techniques and advanced (bio)analytical methods to assess the formation of TPs of eight priority EACs and PPCPs (namely: estrone, ethinylestradiol, triclosan, bisphenol A, carbamazepine, gemfibrozil, acetaminophen and naproxen) in three different disinfection scenarios (chlorination, chloramination and chlorine dioxide oxidation).

## Predictive chemistry and toxicology

Stepped Forced Molecular Dynamics (SFMD) simulations were used to predict TPs from hypochlorite ion (ClO<sup>-</sup>) reactions for all 8 compounds (Fig. 1). Quantitative structure activity relationship (QSAR) models (MetaDrug,<sup>3</sup> Endocrine Disruptome<sup>4</sup>), toxicokinetic predictions (TK index<sup>5</sup>) and toxicophore analysis were then applied to parent compounds and TPs with more than 1% likelihood of occurrence to predict changes in toxicity (Table 1).

## Laboratory experiments, chemical analysis and *in vitro* bioassays

The eight compounds were spiked at 1 µg/L in 1 L of phosphate buffered ultrapure water and exposed to chlorine (3 mg/L), chlorine dioxide (1 mg/L) or pre-formed chloramine (2-3 mg/L) for one week. The water samples were then extracted by solid-phase extraction with 6 cc / 500 mg Oasis HLB and analyzed by liquid chromatography high resolution mass spectrometry (LC-HRMS) (Table 2) and a battery of *in vitro* assays<sup>6,7</sup> for non-specific toxicity (Microtox, WIL2NS-TOX), reactive toxicity (umuC, micronucleus formation assay), endocrine activity (estrogen, androgen, glucocorticoid and progesterone receptor GeneBLazer assay in agonist and antagonist modes), oxidative stress response (AREC32) and xenobiotic metabolism (Ahr-CAFLUX and CYP1A2 induction) (Table 3).

Fig. 1. Predicted TPs from ClO<sup>-</sup> reactions of selected parent compounds:

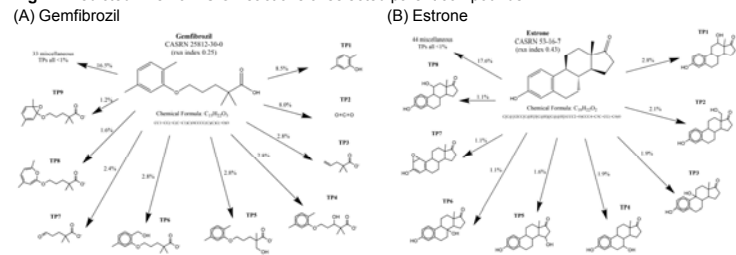


Table 2. Detection of parent compounds and predicted TPs by LC-HRMS from disinfection reaction experiments. Results for bisphenol A are pending.

Compound	No disinfection	Chlorine	Chlorine dioxide	Monochloramine
Estrone	no	no	yes	no
Predicted TPs	no	no	yes	no
Ethinylestradiol	yes	no	no	no
Predicted TPs	no	no	no	no
Triclosan	yes	no	no	no
Predicted TPs	no	no	no	no
Carbamazepine	yes	no	yes	no
Predicted TPs	no	yes	yes	no
Gemfibrozil	yes	no	no	no
Predicted TPs	no	no	no	no
Acetaminophen	yes	no	no	no
Predicted TPs	no	no	no	no
Naproxen	yes	no	no	no
Predicted TPs	no	no	no	no

## Conclusions

- Moderate level of qualitative agreement between predictive and experimental methods, but also some discrepancies.
- In general, disinfection reactions led to a (predicted and actual) decrease in specific toxicity (endocrine effects) and occasionally to an increase in reactive and non-specific toxicity.
- Estrogenicity of steroids was significantly decreased after disinfection, while the lipid regulator gemfibrozil appeared to be more active.

Table 1. Predicted toxicity of parent compounds and their main ClO<sup>-</sup> transformation products. Changes in predicted toxicity are highlighted in green (decreased) or red (increased).

Compound	Respiratory	Mutagenic	Carcinogenic	Genotoxic	Cytotoxic	Toxic to bacteria	TK index	ER binding	AR binding	GR binding
Estrone	No	No	Yes	No	No	No	0	High	High	Moderate
TP1	No	No	Yes	No	No	No	-1	High	High	Moderate
TP2	No	No	Yes	No	No	No	-1	Moderate	Moderate	Moderate
TP3	No	No	Yes	No	No	No	-1	High	High	Moderate
TP4	No	No	Yes	No	No	No	-1	Moderate	Moderate	Moderate
TP5	No	No	Yes	No	No	No	-1	High	High	Moderate
TP6	No	No	Yes	No	No	No	-1	High	High	Moderate
TP7	Yes	No	Yes	No	No	No	0	High	High	Moderate
TP8	No	No	Yes	No	No	No	-1	Moderate	Moderate	Moderate
Ethinylestradiol	No	No	Yes	No	No	No	0	High	High	Moderate
TP1	Yes	No	Yes	No	No	No	-1	Low	Low	Moderate
TP2	No	No	Yes	No	No	No	-1	Moderate	High	Moderate
TP3	No	No	Yes	No	No	No	-1	Moderate	Moderate	Moderate
TP4	No	No	Yes	No	No	No	1	High	High	Moderate
Triclosan	No	No	Yes	Yes	No	Yes	0	Low	Low	Low
TP1	No	No	Yes	Yes	No	Yes	0	Low	Low	Low
TP2	No	No	Yes	Yes	No	Yes	-2	Low	Low	Low
TP3	No	No	Yes	Yes	No	Yes	-2	Low	Moderate	Low
TP4	No	No	Yes	Yes	No	Yes	-1	Low	Moderate	Low
TP5	No	No	Yes	Yes	No	Yes	-1	Low	Moderate	Low
TP6	No	No	Yes	Yes	No	Yes	-1	Low	Moderate	Low
TP7	Yes	No	Yes	Yes	No	Yes	0	Moderate	Moderate	Moderate
TP8	Yes	No	Yes	Yes	No	Yes	-2	Low	Low	Low
Bisphenol A	No	No	Yes	No	No	No	0	Moderate	Moderate	Moderate
TP1	No	No	Yes	No	No	No	0	Moderate	High	Moderate
TP2	No	No	Yes	No	No	No	0	Moderate	High	Moderate
TP3	Yes	No	Yes	No	No	No	-4	Moderate	High	Moderate
TP4	Yes	No	Yes	No	No	No	0	Moderate	High	Moderate
TP5	No	No	Yes	No	No	No	0	Moderate	High	Moderate
TP6	Yes	No	Yes	Yes	No	Yes	-2	Low	Low	Low
TP7	Yes	No	Yes	Yes	No	Yes	-2	Low	Low	Low
TP8	Yes	No	Yes	Yes	No	Yes	-2	Low	Low	Low
Carbamazepine	No	Yes	No	Yes	No	No	0	High	High	Moderate
TP1	No	Yes	No	Yes	No	No	-1	Moderate	Moderate	Moderate
TP2	No	Yes	No	Yes	No	No	-2	Low	Moderate	Moderate
TP3	No	Yes	No	Yes	No	Yes	-1	Low	Moderate	Moderate
TP4	No	Yes	No	Yes	No	Yes	-1	Low	Moderate	Moderate
TP5	No	Yes	No	Yes	No	Yes	-1	Low	Moderate	Moderate
TP6	No	Yes	No	Yes	No	Yes	-1	Low	Moderate	Moderate
TP7	Yes	Yes	No	Yes	No	No	-1	Moderate	High	Moderate
TP8	Yes	Yes	No	Yes	No	No	-2	Moderate	High	Moderate
TP9	No	Yes	No	Yes	No	No	0	Moderate	Moderate	Moderate
TP10	No	Yes	No	Yes	No	No	0	Moderate	Moderate	Moderate
Gemfibrozil	No	No	No	No	No	No	0	Low	Low	Low
TP1	No	No	Yes	Yes	No	Yes	-1	Low	Low	Low
TP2	Yes	No	Yes	Yes	No	Yes	-3	Low	Low	Low
TP3	No	No	Yes	Yes	No	Yes	-1	High	High	Moderate
TP4	No	No	No	No	No	No	-1	Moderate	Low	Low
TP5	No	No	No	No	No	No	-3	Low	Low	Low
TP6	No	No	No	No	No	No	-1	Low	Low	Low
TP7	Yes	No	No	No	No	Yes	-1	Low	Low	Low
TP8	No	No	No	No	No	No	0	Low	Low	Low
TP9	Yes	No	No	No	No	No	-1	Low	Low	Low
Acetaminophen	No	No	Yes	Yes	No	Yes	0	Low	Low	Low
TP1	Yes	No	Yes	Yes	No	Yes	-1	Low	Low	Low
TP2	No	No	Yes	Yes	No	Yes	0	Low	Low	Low
TP3	No	No	Yes	Yes	No	Yes	0	Low	Low	Low
TP4	No	No	Yes	Yes	No	Yes	0	Low	Low	Low
TP5	Yes	No	Yes	Yes	No	Yes	-1	Low	Low	Low
TP6	Yes	No	Yes	Yes	No	Yes	0	Low	Low	Low
TP7	Yes	No	Yes	Yes	No	Yes	-1	Low	Low	Low
TP8	Yes	No	Yes	Yes	No	Yes	-1	Low	Low	Low
TP9	Yes	No	Yes	Yes	No	Yes	-1	Low	Low	Low
TP10	No	No	Yes	Yes	No	Yes	0	Low	Low	Low
TP11	No	No	Yes	Yes	No	Yes	0	Low	Low	Low
TP12	No	Yes	Yes	Yes	No	Yes	-1	Low	Low	Low
TP13	No	No	Yes	Yes	No	Yes	-1	Low	Low	Low
TP14	No	No	Yes	Yes	No	Yes	1	Low	Low	Low
TP15	No	No	Yes	Yes	No	Yes	-2	Low	Low	Low
Naproxen	No	No	No	Yes	No	No	0	Low	Moderate	Moderate
TP1	Yes	No	Yes	Yes	No	Yes	-1	Low	Moderate	Moderate
TP2	No	No	Yes	Yes	No	No	-1	Low	Moderate	Moderate
TP3	No	No	Yes	Yes	No	Yes	-2	Low	Low	Low
TP4	Yes	No	No	Yes	No	No	-3	Low	Moderate	Moderate
TP5	Yes	No	No	Yes	No	No	-1	Low	Moderate	Moderate
TP6	No	No	No	Yes	No	No	-1	Low	Moderate	Moderate
TP7	Yes	No	No	Yes	No	No	-1	Low	Moderate	Moderate
TP8	No	No	No	Yes	No	No	-1	Low	Moderate	Moderate
TP9	No	No	No	Yes	No	No	-1	Low	Moderate	Moderate

Table 3. *In vitro* bioassay responses of the parent compounds and reaction mixtures of transformation products after disinfection reactions. There were no responses with either parent or TP mixtures in the ER(-), AR(+/-) GeneBLazer or CYP1A2 assays.

Disinfection	AREC32	MN	umuC	Cytotox	Microtox	Ahr-CAFLUX	ER(-) GBL	AR(+/-) GBL	GR(+/-) GBL
<b>Estrone</b>	-	-	-	-	-	-	-	-	-
Chlorine	-	-	-	-	-	-	-	-	-
Chlorine dioxide	-	-	-	-	-	-	-	-	-
Monochloramine	-	-	-	-	-	-	-	-	-
<b>Ethinylestradiol</b>	-	-	-	-	-	-	-	-	-
Chlorine	-	-	-	-	-	-	-	-	-
Chlorine dioxide	-	-	-	-	-	-	-	-	-
Monochloramine	-	-	-	-	-	-	-	-	-
<b>Triclosan</b>	-	-	-	-	-	-	-	-	-
Chlorine	-	-	-	-	-	-	-	-	-
Chlorine dioxide	-	-	-	-	-	-	-	-	-
Monochloramine	-	-	-	-	-	-	-	-	-
<b>Bisphenol A</b>	-	-	-	-	-	-	-	-	-
Chlorine	-	-	-	-	-	-	-	-	-
Chlorine dioxide	-	-	-	-	-	-	-	-	-
Monochloramine	-	-	-	-	-	-	-	-	-
<b>Carbamazepine</b>	-	-	-	-	-	-	-	-	-
Chlorine	-	-	-	-	-	-	-	-	-
Chlorine dioxide	-	-	-	-	-	-	-	-	-
Monochloramine	-	-	-	-	-	-	-	-	-
<b>Gemfibrozil</b>	-	-	-	-	-	-	-	-	-
Chlorine	-	-	-	-	-	-	-	-	-
Chlorine dioxide	-	-	-	-	-	-	-	-	-
Monochloramine	-	-	-	-	-	-	-	-	-
<b>Acetaminophen</b>	-	-	-	-	-	-	-	-	-
Chlorine	-	-	-	-	-	-	-	-	-
Chlorine dioxide	-	-	-	-	-	-	-	-	-
Monochloramine	-	-	-	-	-	-	-	-	-
<b>Naproxen</b>	-	-	-	-	-	-	-	-	-
Chlorine	-	-	-	-	-	-	-	-	-
Chlorine dioxide	-	-	-	-	-	-	-	-	-
Monochloramine	-	-	-	-	-	-	-	-	-

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## References

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