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Investigation and risk evaluation of the occurrence of carbamazepine, oxcarbazepine, their human metabolites and transformation products in the urban water cycle☆

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ABSTRACT

Trace organic contaminants such as pharmaceuticals, personal care products and industrial chemicals are frequently detected in the urban water cycle, including wastewater, surface water and groundwater, as well as drinking water. These also include human metabolites (HMs), which are formed in the human body and then excreted via urine or feces, as well as transformation products (TPs) formed in engineered treatment systems and the aquatic environment. In the current study, the occurrence of HMs as well as their TPs of the anticonvulsants carbamazepine (CBZ) and oxcarbazepine (OXC) were investigated using LC tandem MS in effluents of wastewater treatment plants (WWTPs), surface water and groundwater. Highest concentrations were observed in raw wastewater for 10,11-dihydro-10,11-dihydroxycarbamazepine (DiOHCBZ), 10,11-dihydro-10-hydroxy-cabamazepine (10OHCBZ) and CBZ with concentrations ranging up to 2.7 ± 0.4, 1.7 ± 0.2 and 1.07 ± 0.06 μg L^{-1}, respectively. Predictions of different toxicity endpoints using a Distributed Structure-Searchable Toxicity (DSSTox) expert system query indicated that several HMs and TPs, in particular 9-carboxy-acridine (9-CA-ADIN) and acridone (ADON), may exhibit an increased genotoxicity compared to the parent compound CBZ. As 9-CA-ADIN was also detected in groundwater, a detailed investigation of the genotoxicity of 9-CA-ADIN is warranted. Investigations of an advanced wastewater treatment plant further revealed that the discharge of the investigated compounds into the aquatic environment could be substantially reduced by ozonation followed by granular activated carbon (GAC) filtration.

1. Introduction

Compounds of emerging concern (CECs) such as pharmaceuticals have been detected in wastewater treatment plants (WWTP), in surface water, in groundwater and, in some cases, even in drinking water (Ternes, 1998; Heberer, 2002; Metcalfe et al., 2003). During their passage through the urban water cycle, CECs are subjected to a variety of elimination processes including sorption to sewage sludge and sediments, biotransformation as well as phototransformation in sunlit waters (Kosjek et al., 2009; Wick et al., 2011). Environmental transformation reactions frequently result in the formation of transformation products (TPs)(Celiz et al., 2009; van Zelm et al., 2010). In addition, metabolism in treated individuals has to be considered to determine the relevance of human metabolites (HMs) that can be additionally present due to their excretion in urine or feces. Several studies have shown that HMs and TPs can exhibit a higher toxicity than the precursor compounds (Boxall et al., 2004), as shown for example by Schlüter-Vorberg et al. (2015) who found that carboxy-acyclovir, a TP of the antiviral drug acyclovir, significantly reduced the reproduction of Daphnia magna (Schlüter-Vorberg et al., 2015).

However, considering the large number of CECs, their HMs and TPs present in wastewater, in-depth toxicity assessment of each individual compound is generally not feasible. To tackle this problem, computational approaches that allow for the prediction of

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toxicities, including quantitative structure activity relationships (QSAR) (Liu et al., 2006; Sinclair and Boxall, 2003) and molecular dynamics simulations, have been proposed as promising alternative for the prioritization of target compounds (Maunz et al., 2013).

In order to minimize the emission of CECs from WWTPs, advanced treatment technologies such as activated carbon filtration or ozonation have been shown to be capable to remove a wide spectrum of CECs (McDowell et al., 2005; Ternes et al., 2003; Hubner et al., 2014; Li et al., 2011). However, the application of oxidants such as ozone or hydroxyl radicals can result in the formation of additional TPs with widely unknown biological activities (McDowell et al., 2005). The high polarity of the oxidative TPs increases their environmental mobility and thus the risk of contaminating groundwater resources. Sorption of CECs to activated carbon does not cause additional TP formation, but highly polar compounds are frequently insufficiently eliminated (Funke et al., 2016).

Carbamazepine (CBZ) and its 10-keto analogue oxcarbazepine (OXC) are antiepileptic drugs which are heavily metabolized in the human body (Bahlmann et al., 2014; Kalis and Huff, 2001). Less than 2% of an administered CBZ dose is excreted as parent drug in human urine, however, due to high number of prescriptions and its persistence in the environment CBZ is one of the most widely detected CECs in the aquatic environment (Patsalos, 2013; Bahlmann et al., 2014). In addition, its HMs such as 10,11-dihydroxy-10,11-dihydroxycarbamazepine (DiOHCBZ), 10-hydroxy-10,11-dihydrocarbamazepine (10OHCBZ), 2-hydroxycarbamazepine (2OHCBZ) and 3-hydroxycarbamazepine (3OHCBZ), which are generated by cytochrome P450 enzymes, are frequently detected in WWTP effluents (Bahlmann et al., 2014; Miao et al., 2005). Conflicting information about the biodegradability of HMs of CBZ has been reported in literature with some studies indicating a high recalcitrance, while others indicate that DiOHCBZ, 10OHCBZ, 2OHCBZ and 3OHCBZ are biodegradable, resulting in the formation of several TPs with unknown biological activity (Bahlmann et al., 2014; Kaiser et al., 2014; Brezina et al., 2015; Jurado et al., 2014).

CBZ is known to pose adverse effects on aquatic organisms including algae, bacteria, fish and invertebrates (Ferrari et al., 2003; Oetken et al., 2005). Furthermore, in comparison to the parent compound, an enhanced toxicity was reported for several of its HMs and their TPs in experiments with *V. fischeri* (Kaiser et al., 2014). In addition, there is strong evidence that the HM CBZ-IQ, which has also been identified as a biotransformation product (Brezina et al., 2015), is responsible for several adverse side effects of CBZ in treated individuals (Ju and Uetrecht, 1999; Pearce et al., 2005). These findings highlight the importance of a comprehensive risk assessment that includes the original drug as well as their HMs and TPs.

In contrast to CBZ, relatively little is known about OXC. In the human body this pro-drug is heavily metabolized by cytosolic arylketone reductases to its active metabolite 10OHCBZ, of which up to 83% is excreted via urine (Flesch et al., 2011; Kalis and Huff, 2001). It has further been shown that OXC is biodegradable and has similar TPs as observed for DiOHCBZ and 10OHCBZ (Kaiser et al., 2014).

The objective of this study was the determination of the occurrence of HMs and TPs of the antiepileptic drug CBZ and OXC (Fig. 1) in the urban water cycle. The toxicological potential of the target compounds was investigated using Distributed Structure-Searchable Toxicity (DSSTox) Database Network predictions focusing on both genotoxicity and reproductive toxicity (Richard and Williams, 2002). Finally, the efficacy of advanced wastewater treatment technologies using ozonation followed by biofiltration and GAC filtration in order to minimize the emission of CBZ, OXC, their HMs and their TPs into the aquatic environment was investigated.

2. Methods

2.1. Environmental sampling

Grab samples were taken from a number of groundwater wells, surface waters and treated wastewater in Germany (Fig. 2). The sampled WWTPs utilize combinations of different treatment steps, including mechanical and biological treatment, nitrification and denitrification, as well as phosphorus elimination. Detailed information on each WWTP can be found in Table 1. In addition, 24 h composite samples were taken from the influent and effluent of one WWTP (see Fig. 2, WWTP I). All samples were filtered (0.45 μm, cellulose acetate filter, Sartorius stedim biotech, Sartorius Biolab Products) and stored at 4 °C until further analysis (which was performed within 2 days). $^{13}C^{15}N$-CBZ, 10OHCBZ-d_3 and OXC-d_4 were used as internal standards (IS; final concentration 200 ng L^{-1}). All samples were analyzed in triplicate. In addition, samples of each matrix were spiked with two different concentrations of target compounds (0.1 or 1 μg L^{-1}) to account for matrix effects.

2.2. Analytical method

Environmental samples were analyzed using a HPLC (Agilent 1260 Series, Agilent Technologies, Waldbronn, Germany, consisting of a G1312B autosampler, a G1312B binary HPLC pump, a G4225A degasser, a G1316A column oven with a G1330B thermostat) coupled to a 6500 QTrap (Applied Biosystems/MDS Sciex, Darmstadt, Germany), equipped with an electrospray ionization interface. For the chromatographic separation a Synergi Hydro-RP column (250 × 3 mm) with a Security Guard column AQ C18 (4 × 2 mm i.d.; both Phenomenex, Aschaffenburg, Germany) was used. For all measurements, an aliquot of 80 μL of each sample was injected into the LC/MS/MS system using purified water with 0.1% formic acid (A) and methanol with 0.1% formic acid (B) both with 10 mM ammonium formate as mobile phases. The gradient program to achieve separation was as follows: 0–4 min, 90% (A); 10 min, 50%; 18 min, 50%; 22 min, 25%; 24 min, 25%; 25 min, 90%. The run time was 35 min, flow rate was 0.45 mL min^{-1} and column temperature was set to 50 °C. Analysis was carried out in positive-ion mode for all substances, using multiple-reaction monitoring (MRM) mode (see Supporting Information Table S1), including CBZ, OXC, their HMs as well as the previously identified TPs (Kaiser et al., 2014; Brezina et al., 2015). For quantification of the target compounds a ten-point calibration curve (0.001–10 μg L^{-1}) was used. Limits of quantification (LOQs) in groundwater were defined as the second lowest calibration point with a signal to noise ratio (S/N) of >10 for the first transition (MRM 1) and >3 for the second transition (MRM 2) (See Supporting Information Table S2). For the other matrices, LOQs were estimated by calculating a S/N ratio of 10 considering the S/N ratios obtained from spiked water samples in comparison to non-spiked samples (0.1 μg L^{-1} and 1 μg L^{-1}).

2.3. Toxicity prediction for CBZ, OCX, their HMs and their TPs

A prediction of the (geno)toxicity of the compounds visualized in Fig. 1 was performed using the Distributed Structure-Searchable Toxicity (DSSTox) Database Network via the *lazar* web interface (Maunz et al., 2013; in-silico gmbh, 2016). To this end, SMILES codes were generated from chemical structures and fed directly into the query form. A database search was initiated and the investigated compounds were analyzed for toxicophores and structural similarities with known genotoxic compounds. Subsequently they were ranked according to the number of positive hits in eight categories (SingleCell, MultiCell, mouse, rat, hamster, ISSCAN (database on experimental chemical carcinogens), DBS mutagenicity and Kazius-

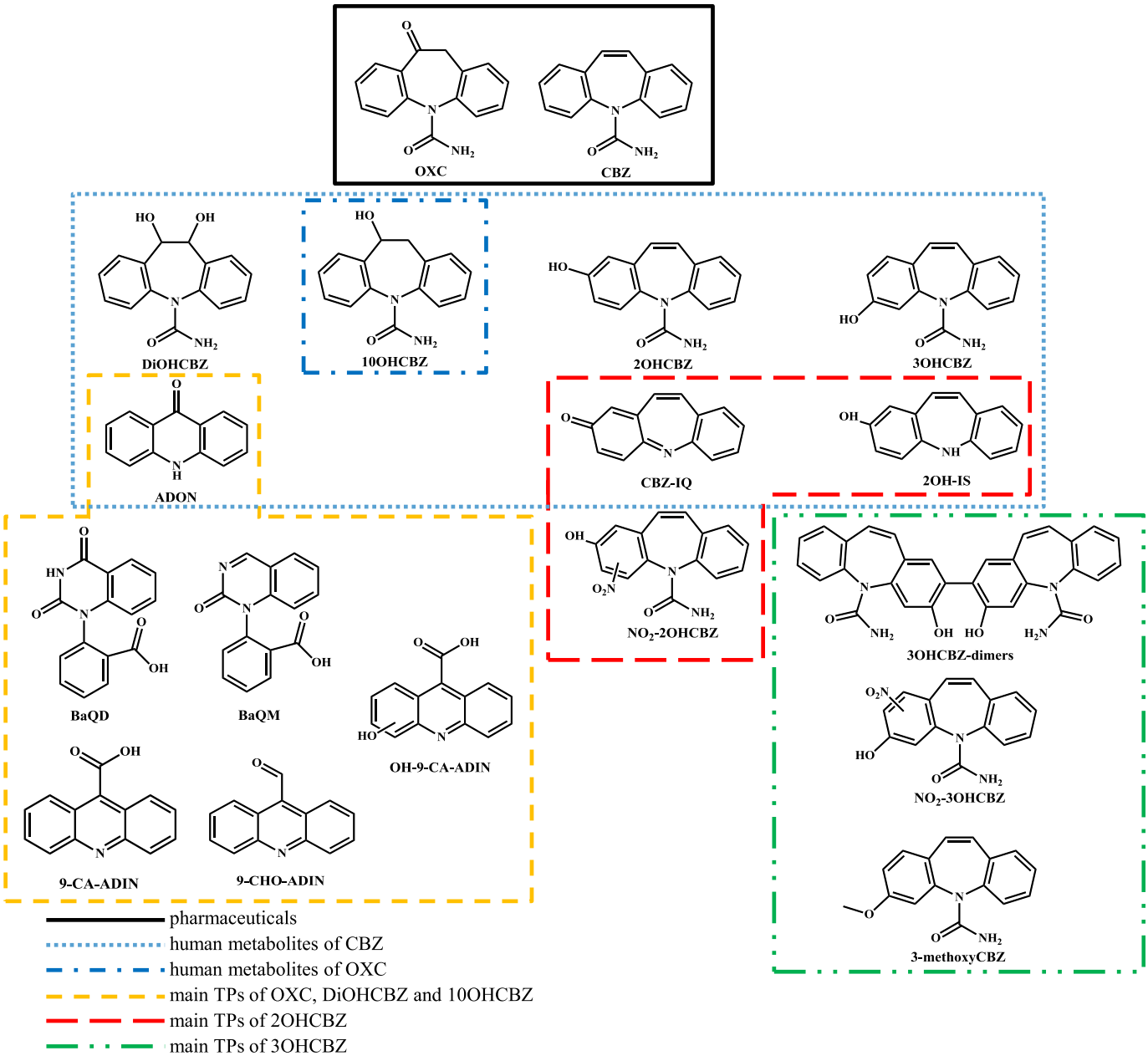

Fig. 1. Transformation products of CBZ and OXC (black box ———): Main human metabolites of CBZ (light blue ·····) and OXC (light green – · – · –) as well as the main (bio-)transformation products of DiOHCBZ, 10OHCBZ and OXC (orange – – – –), 2OHCBZ (red — — —) and 3OHCBZ (dark green — · · —). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Bursi) evaluating the likeliness of (rodent) genotoxicity and mutagenicity, respectively. The output yielded a qualitative prediction of the mutagenicity and carcinogenicity of the queried structures based on structure-activity relationship information.

2.4. Fate during advanced wastewater treatment

In order to investigate the potential of advanced wastewater treatment to minimize the emission of target analytes into the aquatic environment, 24 h composite samples from a pilot WWTP were taken. Briefly, conventional treated wastewater (PP1, 1 m^3 h^{-1}) was subjected to microfiltration (micro sieve, 10 μm, RoDisc®, Huber SE, Berching Germany) (PP2) prior to ozonation (PP3; ozone dose: 0.98 ± 0.24 gO_3 $gDOC^{-1}$ system, HRT of 17 ± 3 min) (see Supporting Information Fig. S1). Afterwards, the wastewater passed either through a non-aerated (PP4) or aerated (PP5) granular activated carbon filter (GAC) or through a non-aerated (PP6) or aerated (PP7) biofilter filled with expanded clay. Detailed information about the pilot plant can be found in Knopp et al. (2016).

3. Results and discussion

3.1. Occurrence of CBZ, OXC, their HMs and TPs in different water matrices

CBZ, OXC, their HMs and TPs were monitored in influents and effluents of WWTPs, rivers, streams and groundwater. The used

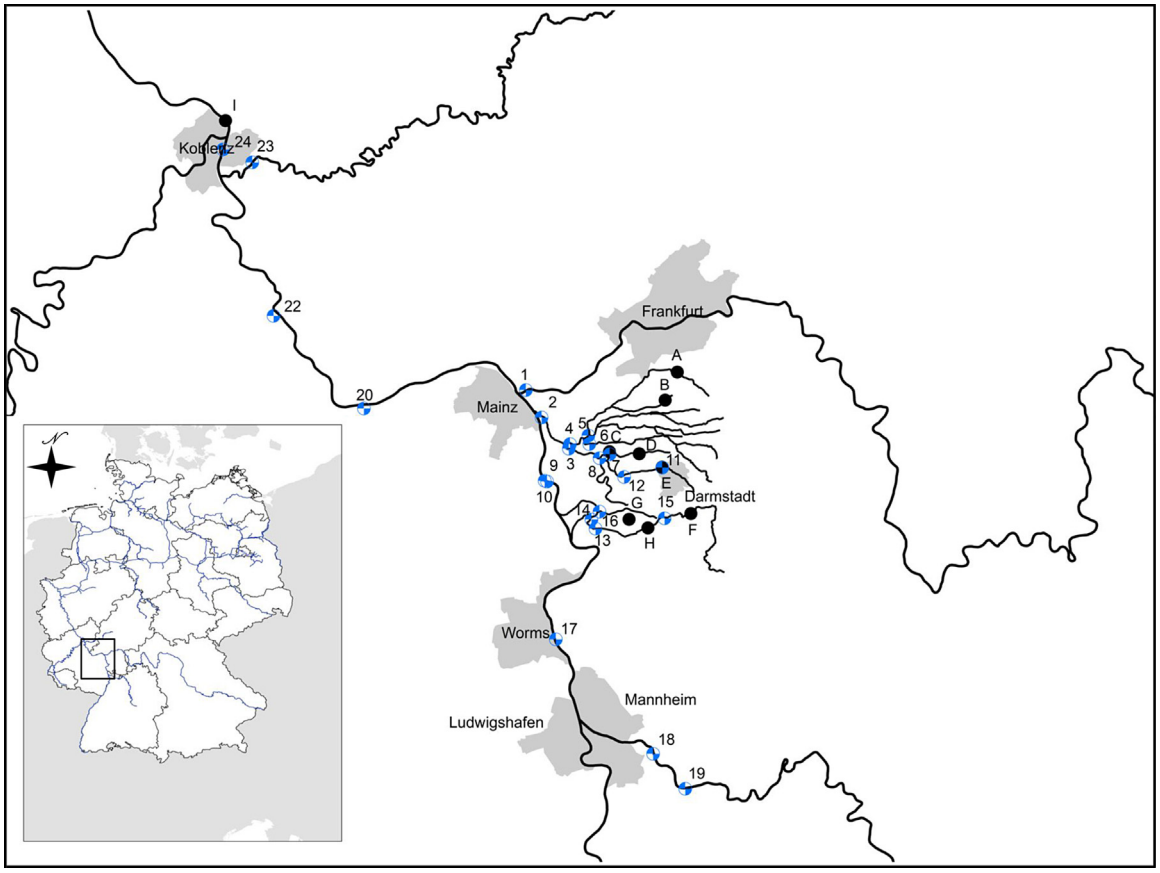

Fig. 2. Map of sampling points of environmental samples. Black dots are WWTP outlets, blue/white dots are indicating surface water sampling points. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Concentrations (μg L^{-1}) of CBZ, OXC, their HMs as well as their TPs in WWTP effluents (for locations see Fig. 2).

	WWT steps	PE	CBZ	OXC	DiOHCBZ	10OHCBZ	epCBZ	1/2OHCBZ	3OHCBZ	BaQD	9-CA-ADIN
A	m/b/n/d/p	85k	0.34 ± 0.002	0.10 ± 0.01	0.52 ± 0.02	0.18 ± 0.01	0.009 ± 0.002	0.049 ± 0.001	0.042 ± 0.003	<LOQ	0.022 ± 0.002
B	m/b/n/d/p	75k	0.28 ± 0.01	0.21 ± 0.01	0.48 ± 0.03	0.09 ± 0.01	0.009 ± 0.001	0.040 ± 0.001	0.035 ± 0.003	0.020 ± 0.005	0.045 ± 0.005
C	b/n/d/p	20k	0.34 ± 0.02	0.121 ± 0.004	0.60 ± 0.02	0.28 ± 0.02	0.013 ± 0.001	0.054 ± 0.002	0.051 ± 0.004	0.018 ± 0.002	0.075 ± 0.008
D	m/b/n/d/p	30k	0.507 ± 0.002	0.15 ± 0.03	0.73 ± 0.06	0.290 ± 0.003	0.013 ± 0.001	0.077 ± 0.004	0.066 ± 0.001	0.010 ± 0.002	0.037 ± 0.005
E	m/b/n/d/p	240k	0.642 ± 0.008	0.29 ± 0.03	1.07 ± 0.05	0.21 ± 0.02	0.030 ± 0.002	0.088 ± 0.005	0.079 ± 0.005	0.040 ± 0.005	0.089 ± 0.006
F	m/b/n/d/p	55k	0.80 ± 0.01	0.35 ± 0.02	1.3 ± 0.1	0.77 ± 0.04	0.038 ± 0.002	0.106 ± 0.001	0.089 ± 0.004	0.056 ± 0.01	0.20 ± 0.02
G	m/b/n/d/p	8k	0.45 ± 0.03	0.08 ± 0.01	0.54 ± 0.04	0.17 ± 0.01	0.009 ± 0.002	0.052 ± 0.002	0.050 ± 0.006	<LOQ	0.032 ± 0.002
H	m/b/n/d/p	45k	0.69 ± 0.03	0.127 ± 0.004	1.06 ± 0.01	0.277 ± 0.004	0.023 ± 0.002	0.098 ± 0.003	0.086 ± 0.002	0.020 ± 0.001	0.075 ± 0.008
I	m/b/n/d	320k	1.46 ± 0.06	0.8 ± 0.1	2.57 ± 0.07	1.20 ± 0.09	0.070 ± 0.005	0.169 ± 0.008	0.177 ± 0.005	0.134 ± 0.005	0.54 ± 0.03

<LOQ: below limit of quantification.
PE = population equivalents (85k = 85 000), m = mechanical cleaning, b = biological cleaning, n = nitrification, d = denitrification, p = phosphorus elimination.

analytical method allowed for a quantification of CBZ, OXC, DiOHCBZ, 10OHCBZ, 10,11-dihydro-10,11-epoxy-carbamazepine (epCBZ), 1/2OHCBZ, 3OHCBZ, BaQD, 9-CA-ADIN, ADON, CBZ-IQ down to 5 ng L^{-1} with recoveries ranging from 73 to 125%. Other previously identified TPs were not detected in any of the investigated WWTP influents or effluents. Thus, these compounds were not included in the final quantification method. For more information about the analytical method see also the Supporting Information. While the occurrence of CBZ, OXC as well as their HMs (DiOHCBZ, 10OHCBZ, 2OHCBZ and 3OHCBZ) has already previously been reported in raw and treated wastewater, this is the first study that also investigates the presence of TPs of HMs of CBZ and OXC, as well as from OXC itself in the urban water cycle.

The concentrations of all detected target compounds are summarized in Tables 1–4. CBZ, OXC as well as their HMs were detected in all matrices, while of the included TPs only BaQD, 9-CA-ADIN and in one case ADON were found. Highest concentrations were observed in raw wastewater for DiOHCBZ, 10OHCBZ and CBZ with concentrations up to 2.7 ± 0.4, 1.7 ± 0.2 and 1.07 ± 0.06 μg L^{-1}, respectively. The occurrence of target compounds in individual investigated matrices is discussed in greater detail in the following:

Raw and treated wastewater

In general, all target compounds listed in Table 1 could be detected in most WWTP effluents, with concentrations ranging from 0.010 ± 0.002 μg L^{-1} (BaQD) up to 2.57 ± 0.07 μg L^{-1} for DiOHCBZ. While CBZ concentrations varied between 0.28 ± 0.01 μg L^{-1} and 1.46 ± 0.06 μg L^{-1} in WWTP effluents, concentrations of OXC were significantly lower, ranging from

Table 2
Concentrations (μg L^{-1}) of CBZ, OXC, their HMs as well as their TPs in 24 h composite sample of a WWTP.

	CBZ	OXC	DiOHCBZ	10OHCBZ	epCBZ	2OHCBZ	3OHCBZ	BaQD	9CA-ADIN	ADON
influent	1.07 ± 0.06	0.25 ± 0.01	2.7 ± 0.4	1.7 ± 0.2	0.058 ± 0.006	0.21 ± 0.02	0.25 ± 0.02	0.12 ± 0.02	0.19 ± 0.02	0.01 ± 0.001
effluent	1.29 ± 0.03	0.198 ± 0.005	2.8 ± 0.2	1.23 ± 0.03	0.082 ± 0.001	0.177 ± 0.001	0.199 ± 0.005	0.205 ± 0.002	0.48 ± 0.03	<LOQ

<LOQ: below limit of quantification.

Table 3
Concentrations (μg L^{-1}) of CBZ, OXC, their HMs as well as their TPs in surface waters (for sample locations see Fig. 2.).

	Estimated proportion of treated wastewater [%][a]	CBZ	OXC	DiOHCBZ	10OHCBZ	epCBZ	1/2OHCBZ	3OHCBZ	BaQD	9-CA-ADIN	ADIN
1	20	0.16 ± 0.02	<LOQ	0.21 ± 0.02	0.075 ± 0.008	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
2	90	1.01 ± 0.01	<LOQ	1.4 ± 0.2	0.132 ± 0.006	0.05 ± 0.01	0.071 ± 0.002	0.042 ± 0.002	0.23 ± 0.01	0.150 ± 0.006	0.065 ± 0.003
3	80	0.95 ± 0.03	0.025 ± 0.004	1.37 ± 0.07	0.12 ± 0.01	0.057 ± 0.004	0.083 ± 0.002	0.072 ± 0.003	0.29 ± 0.03	0.16 ± 0.01	0.060 ± 0.004
4	90	1.15 ± 0.03	<LOQ	1.45 ± 0.09	0.14 ± 0.01	0.065 ± 0.006	0.094 ± 0.006	0.080 ± 0.002	0.22 ± 0.02	0.100 ± 0.001	0.066 ± 0.008
5	80	0.89 ± 0.04	0.127 ± 0.003	1.51 ± 0.09	0.16 ± 0.01	0.053 ± 0.006	0.094 ± 0.003	0.083 ± 0.007	0.19 ± 0.02	0.147 ± 0.05	0.039 ± 0.003
6	90	1.43 ± 0.02	0.44 ± 0.01	1.7 ± 0.3	0.26 ± 0.02	0.074 ± 0.004	0.15 ± 0.01	0.160 ± 0.008	0.41 ± 0.01	0.087 ± 0.05	0.095 ± 0.001
7	95	1.64 ± 0.03	0.57 ± 0.07	2.3 ± 0.3	0.43 ± 0.03	0.079 ± 0.005	0.190 ± 0.005	0.22 ± 0.04	0.33 ± 0.02	0.148 ± 0.07	0.043 ± 0.009
8	90	1.07 ± 0.05	0.05 ± 0.01	1.31 ± 0.05	0.13 ± 0.04	0.060 ± 0.006	0.089 ± 0.006	0.079 ± 0.005	0.19 ± 0.03	0.129 ± 0.009	0.07 ± 0.02
9	5	0.025 ± 0.002	<LOQ	0.034 ± 0.004	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ
10	5	0.021 ± 0.002	<LOQ	0.026 ± 0.008	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ
11	90	1.23 ± 0.05	0.089 ± 0.003	1.6 ± 0.1	0.115 ± 0.009	0.063 ± 0.005	0.120 ± 0.004	0.122 ± 0.007	0.22 ± 0.01	0.111 ± 0.003	0.048 ± 0.004
12	90	1.13 ± 0.04	0.19 ± 0.02	1.5 ± 0.1	0.12 ± 0.01	0.060 ± 0.003	0.144 ± 0.001	0.149 ± 0.001	0.22 ± 0.03	0.103 ± 0.009	0.08 ± 0.01
13	20	0.184 ± 0.003	0.027 ± 0.003	0.30 ± 0.01	0.200 ± 0.005	<LOQ	0.023 ± 0.001	0.019 ± 0.002	0.011 ± 0.001	0.032 ± 0.002	n.d.
14	20	0.170 ± 0.004	0.028 ± 0.005	0.28 ± 0.02	0.14 ± 0.01	<LOQ	0.022 ± 0.002	0.020 ± 0.002	0.011 ± 0.002	0.027 ± 0.002	n.d.
15	15	0.095 ± 0.003	0.008 ± 0.001	0.15 ± 0.01	0.093 ± 0.002	n.d.	0.01 1 ± 0.001	<LOQ	<LOQ	0.013 ± 0.005	n.d.
16	20	0.15 ± 0.01	0.023 ± 0.002	0.22 ± 0.04	0.14 ± 0.01	<LOQ	0.018 ± 0.002	0.016 ± 0.001	<LOQ	0.020 ± 0.008	n.d.
17	5	0.032 ± 0.002	<LOQ	0.041 ± 0.005	<LOQ	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ
18	20	0.12 ± 0.01	<LOQ	0.16 ± 0.02	0.07 ± 0.02	n.d.	<LOQ	<LOQ	<LOQ	n.d.	<LOQ
19	20	0.111 ± 0.001	<LOQ	0.16 ± 0.02	0.058 ± 0.005	n.d.	<LOQ	<LOQ	<LOQ	n.d.	<LOQ
20	5	0.028 ± 0.001	<LOQ	0.04 ± 0.01	0.010 ± 0.002	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ
21	25	0.244 ± 0.005	<LOQ	0.32 ± 0.07	0.074 ± 0.004	0.064 ± 0.001	0.016 ± 0.001	0.013 ± 0.001	n.d.	<LOQ	<LOQ
22	5	0.030 ± 0.003	<LOQ	0.037 ± 0.009	0.010 ± 0.003	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ
23	25	0.208 ± 0.004	<LOQ	0.29 ± 0.01	0.10 ± 0.01	<LOQ	0.014 ± 0.001	<LOQ	<LOQ	<LOQ	<LOQ
24	5	0.032 ± 0.001	<LOQ	0.039 ± 0.009	0.010 ± 0.001	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ

1: Main, 2, 3, 5: Schwarzbach, 4, 8, 11, 12: Landgraben, 6: Mühlbach, 7: Schlimmer Graben, 9, 10, 17, 20, 22, 24:Rhein, 13: Sandbach, 14, 15: Modau, 16: Fanggraben, 18, 19: Neckar, 21: Nahe, 23: Lahn.
<LOQ: below limit of quantification, n.d.: not detected.
[a] Estimation of proportions of treated wastewater were based on detected concentration of CBZ as well as oxypurinol (Funke et al., 2015).

0.08 ± 0.01 μg L^{-1} to 0.8 ± 0.1 μg L^{-1}. This difference can be explained by the lower prescription quantities of OXC in Germany, its extensive human metabolism as well as its biodegradability (Schwabe and Paffrath, 2014; Kalis and Huff, 2001; Kaiser et al., 2014). The major human metabolite of OXC, namely 10OHCBZ could be detected at concentrations up to 1.33 ± 0.06 μg L^{-1} in treated wastewater. Among all previously identified TPs (see Kaiser et al. (2014) and Brezina et al. (2015)), only BaQD, and 9-CA-ADIN were detected in WWTP effluents in concentration above the LOQ (100 ng L^{-1}).

During conventional activated sludge treatment (Table 2) no removal of DiOHCBZ (2.7 ± 0.4 μg L^{-1} in WWTP influent and 2.8 ± 0.2 μg L^{-1} in WWTP effluent) was observed, while CBZ concentrations increased slightly (1.07 ± 0.06 μg L^{-1} and 1.29 ± 0.03 μg L^{-1}). The latter can most likely be attributed to the cleavage of CBZ glucuronides, as observed in previous studies (Bahlmann et al., 2014). Small decreases of OXC, 10OHCBZ, 2OHCBZ and 3OHCBZ concentrations during wastewater treatment of 20%, 27%, 16% and 20%, respectively, indicate an incomplete removal of these compounds during biological treatment. The degradation of OXC and 10OHCBZ most likely results in the formation of the previously identified TPs BaQD and 9-CA-ADIN (Kaiser et al., 2014) as indicated by elevated concentrations of both TPs in WWTP effluents (see Table 2). For 2OHCBZ and 3OHCBZ none of the previously identified TPs that have been identified in experiments with sand taken from a sand filter of a drinking water facility (e.g. CBZ-IQ, 3OH-dimers (TP502 and TP520) or nitrated species) (Brezina et al., 2015) were detected in WWTP effluents. This is, however, not surprising considering the limited removal of both compounds in activated sludge treatment (see Table 2).

In order to obtain additional insights into the occurrence of HMs and TPs, their concentrations in WWTP effluents were plotted against the concentrations of CBZ using linear regression analysis (see Fig. 3). Excellent correlations were observed between CBZ and DiOHCBZ ($r^2 = 0.96$; Fig. 3 A), 1/2OHCBZ ($r^2 = 0.98$; Fig. 3 C), 3OHCBZ ($r^2 = 0.98$; Fig. 3 D) and epCBZ ($r^2 = 0.80$; Fig. 3 B) indicating that these compounds i) are similarly persistent as CBZ in wastewater treatment and ii) that they originate from the metabolism of CBZ in treated patients. In contrast to this, low correlations between CBZ and 10OHCBZ ($r^2 = 0.23$; Fig. 3 F), OXC ($r^2 = -0.11$; Fig. 3 E), BaQD ($r^2 = 0.45$; Fig. 3 G) or 9-CA-ADIN ($r^2 = 0.44$; Fig. 3 H) indicate a different behavior of these substances during biological treatment. This is in agreement with the observed degradation of 10OHCBZ and OXC as well as the formation of BaQD and 9-CA-ADIN (most likely from 10OHCBZ and OXC) in the sampled WWTPs. In addition, OXC is prescribed as a pharmaceutical compound itself and its concentrations are therefore independent of those of CBZ (Kalis and Huff, 2001).

Table 4
Concentrations (μg L^{-1}) of CBZ, OXC, their HMs as well as their TPs in groundwater (for sample locations see Fig. 2).

	CBZ	OXC	DiOHCBZ	10OHCBZ	epCBZ	1/2OHCBZ	3OHCBZ	BaQD	9-CA-ADIN
GW1	0.021 ± 0.001	<LOQ	0.022 ± 0.003	<LOQ	n.d.	<LOQ	<LOQ	n.d.	0.10 ± 0.01
GW2	0.049 ± 0.002	<LOQ	0.078 ± 0.006	n.d.	n.d.	<LOQ	<LOQ	n.d.	n.d.
GW3	0.212 ± 0.001	<LOQ	0.10 ± 0.01	n.d.	n.d.	0.006 ± 0.001	<LOQ	n.d.	n.d.
GW4	0.87 ± 0.01	<LOQ	0.28 ± 0.02	n.d.	0.030 ± 0.001	0.024 ± 0.001	0.011 ± 0.001	0.011 ± 0.004	0.067 ± 0.005
GW5	0.005 ± 0.001	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	<LOQ	n.d.
GW6	0.70 ± 0.02	0.026 ± 0.005	1.1 ± 0.1	0.015 ± 0.002	n.d.	0.084 ± 0.003	0.089 ± 0.004	0.048 ± 0.004	0.40 ± 0.03
GW7	0.26 ± 0.01	0.008 ± 0.001	0.15 ± 0.01	<LOQ	n.d.	0.026 ± 0.002	0.023 ± 0.002	<LOQ	1.2 ± 0.1
GW8	0.70 ± 0.003	0.008 ± 0.001	<LOQ	n.d.	n.d.	0.009 ± 0.001	0.010 ± 0.0005	n.d.	0.077 ± 0.002

<LOQ: below limit of quantification, n.d.: not detected.

Surface water

CBZ, DiOHCBZ and 10OHCBZ were detected in most sampled rivers and streams with concentrations up to 1.64 ± 0.03 μg L^{-1}, 2.3 ± 0.3 μg L^{-1} and 0.43 ± 0.03 μg L^{-1}, respectively (see Table 3). The HM epCBZ was only detected in surface waters with high proportions (>80%) of treated wastewater at concentrations between 0.040 ± 0.003 μg L^{-1} and 0.08 ± 0.01 μg L^{-1}. A similar pattern was observed for all other analytes, thus, clearly emphasizing the relevance of WWTPs as important point sources.

A good correlation of target compounds with CBZ was only observed for DiOHCBZ ($r^2 = 0.98$), indicating that the lower concentrations of this compound compared to WWTP effluents are attributable to dilution and not to (bio)transformation reactions in surface waters. In contrast to this, the low correlations of the other target compounds with CBZ ($r^2 < 0$) indicated that they are partially (bio) degraded in surface waters (see also Supporting Information Table S4).

Groundwater

CBZ was detected in all analyzed groundwater samples (see Table 4) in concentrations ranging from 0.021 ± 0.001 μg L^{-1} to 0.87 ± 0.01 μg L^{-1}. All other investigated compounds were at least detected once, highest concentrations were observed for DiOHCBZ and 9-CA-ADIN up to 1.11 ± 0.01 μg L^{-1} and 1.2 ± 0.1 μg L^{-1}, respectively. Most other target compounds were only detected in low concentrations (<0.089 ± 0.004 μg L^{-1}) if at all (Table 4).

With the exception of one groundwater sample, DiOHCBZ, whose concentrations always exceeded those of CBZ in WWTPs and surface water samples, was detected in groundwater samples only in concentrations that were lower than those of CBZ. This indicates a partial removal of DiOHCBZ, via (bio)transformation or sorption, during soil passage or in groundwater, which is also supported by the low correlation between DiOHCBZ and CBZ ($r^2 = -0.17$).

All other target compounds, including OXC, 10OHCBZ and BaQD, were only detected in a limited number of sampled groundwater, suggesting the (bio)transformation or sorption of these compounds (Kaiser et al., 2014). The reason for the elevated concentration of 9-CA-ADIN (1.2 ± 0.1 μg L^{-1}) observed in one of the groundwater samples remains unclear.

3.2. Toxicity prediction for CBZ, OCX, their HMs and their TPs

In-silico genotoxicity predictions of the target compounds using the DSSTox database resulted in a series of positive hits, although none of them scored in every category. All compounds were positive in the ISSCAN category, and none of them had a positive signal for the Carcinogenic Potency hamster category (see Fig. 4). CBZ itself served as a reference and gathered a score of 4, which means that some genotoxicity is expected either from the compound itself or from some dependent HM. Less genotoxic potential was predicted for oxcarbazepine ("2") and some of the mono- and di-carboxylated and hydroxylated derivatives of CBZ, while eight compounds had more than 4 positive signals. Those with the highest score of "7" were substances containing an iminoquinone moiety or an activated acridine ring system (either by carboxylation, hydroxylation, or as carbaldehyde). This is not surprising as it was previously shown that some acridine derivatives show carcinogenic effects in mammalian and human cells (for review see (Ferguson and Denny, 1991)). In addition, ADON, the phenone derivative, scored "6". These results indicate that some of the investigated compounds may have a potential for genotoxic effects. For example, the formation of the iminoquinone metabolite is most likely linked to the idiosyncratic adverse reactions observed in treated patients with CBZ (Ju and Uetrecht, 1999; Pearce et al., 2005). ADIN and ADON, which are also formed during photodegradation of CBZ in surface waters, have been shown to exhibit an increased ecotoxicity compared to CBZ (Donner et al., 2012).

In summary, the results of the in silico predictions indicate the prioritization of the following compounds for a profound toxicological assessment: CBZ-IQ, 9-CA-ADIN, 9-CHO-ADIN, ADON and the hydroxylated 9-CA-ADIN.

3.3. Toxicological evaluation

Considering both the observed concentrations in surface water and groundwater and the results obtained from in silico genotoxicity predictions, a comprehensive toxicological assessment should be primarily focused on 9-CA-ADIN. Despite the predicted genotoxicity potential of ADON, CBZ-IQ, 9-CHO-ADIN, 3-methoxyCBZ, 3OHCBZ-dimers, 2OH-IS or 1-NO_2-2OHCBZ as well as hydroxylated 2OHCBZ or 3OHCBZ, none of these compounds could be detected in any environmental sample in concentrations > LOQ, thus indicating a low exposure potential. In contrast to this, results of the genotoxicity predictions for DiOHCBZ, 10OHCBZ, OXC, 2OHCBZ and 3OHCBZ suggest a reduced (geno-)toxicity compared to CBZ and thus a lower priority of these compounds even though they were detected in several of the surface water and groundwater samples. However, 9-CA-ADIN, for which a high genotoxicity was predicted, could be detected in WWTP effluents, surface water and groundwater with concentrations of up to 0.16 ± 0.01 μg L^{-1}, 0.54 ± 0.03 μg L^{-1} and 1.2 ± 0.1 μg L^{-1}, respectively. Furthermore, a toxicity towards *Vibrio fischeri* that was higher than with CBZ has been reported previously (Kaiser et al., 2014).

3.4. Fate during advanced wastewater treatment

In order to minimize the discharge of potentially toxic TPs originating from WWTPs into the aquatic environment, a pilot advanced wastewater treatment plant utilizing ozonation followed by granular activated carbon (GAC) or biofiltration was investigated. As it was shown, a complete removal of CBZ can be achieved

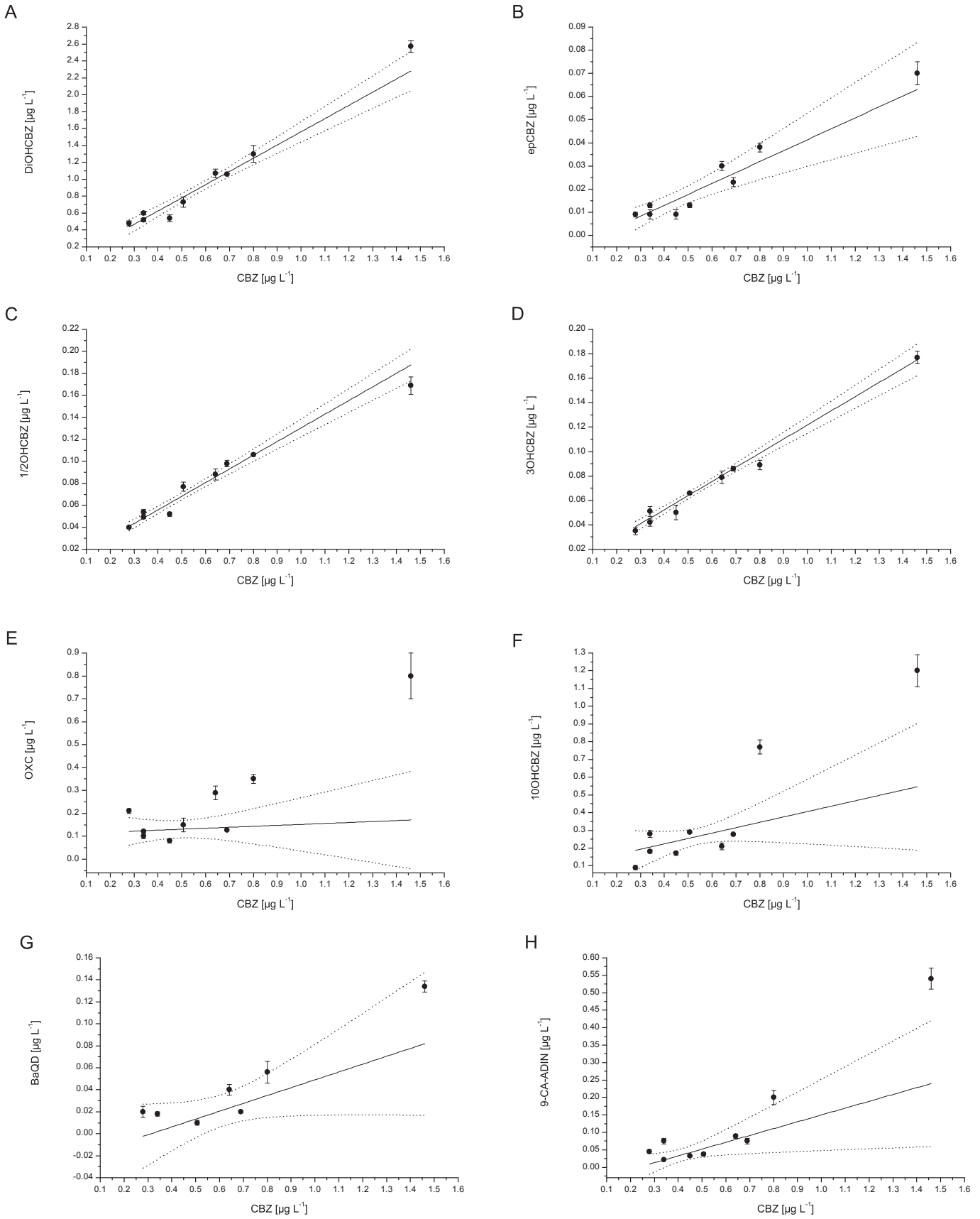

Fig. 3. Correlation between concentration of CBZ an A) DiOHCBZ, B) epCBZ, C)1/2OHCBZ, D) 3OHCBZ, E) OXC, F) 10OHCBZ, G) BaQD and H) 9-CA-ADIN in WWTP effluents. The 95% confidence interval of the measured concentrations is given as error bar. The dashed lines indicate confidence bands of the linear regression analysis.

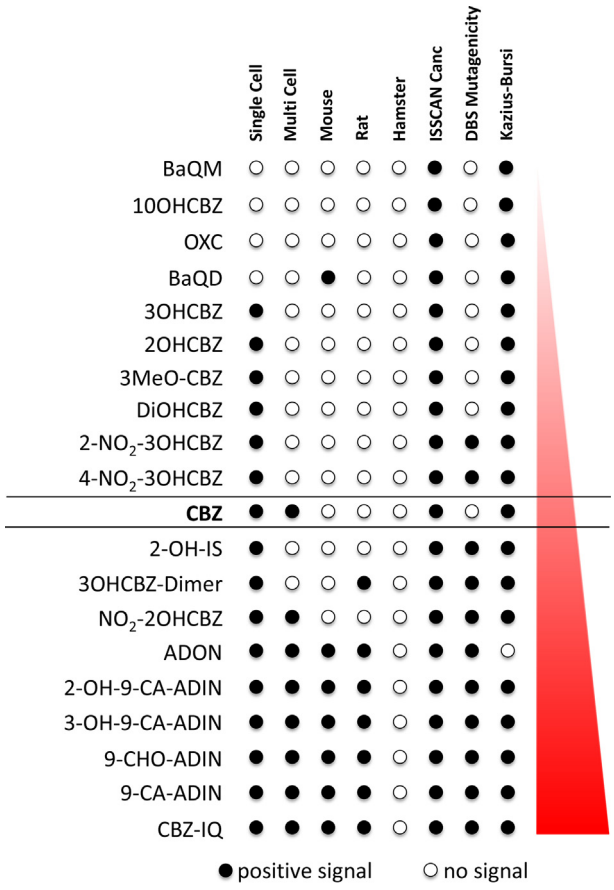

Fig. 4. Lazar prediction of genotoxicity. Investigated compounds were sorted in ascending order with probably innocent structures on top. The number of positive results in the various subsets was counted and interpreted as the likeliness that a given compound may pose a health risk for mammalians including humans and should be prioritized in future screening experiments. CBZ itself served as the reference point.

using ozonation (Fig. 5 and Supporting Information Table S5) which is in good agreement with the high reaction rate constant of CBZ with ozone (McDowell et al., 2005). Similar to CBZ, an extensive elimination of most of the HMs and the TPs was observed during ozonation. epCBZ, 1/2OHCBZ and 3OHCBZ were completely eliminated (concentrations < LOQ), while removal efficiencies of DiOHCBZ, 10OHCBZ, OXC, and 9-CA-ADIN were 93%, 92%, 88%, and 87%, respectively. In contrast to this, concentrations of BaQD did not change significantly. This can be attributed to its formation during the reaction of CBZ and OXC with ozone and/or its persistence to further oxidation (McDowell et al., 2005; Li et al., 2011). The GAC post-treatment (both aerated and non-aerated) led to a nearly complete elimination of residual target compounds present after ozonation (Fig. 5), while the biofilters (aerated and non-aerated) were ineffective with similar concentrations in the influent and effluent. Overall these results indicate that ozonation in combination with GAC treatment is a suitable treatment technology for the removal of CBZ, OXC, as well as their HMs and biological TPs. However, the exact fate of most of the target analytes during ozonation, in particular with regard to the formation of TPs, remains unknown and further investigation regarding their fate is needed.

4. Conclusions

In order to assess the potential adverse health effects of CBZ, OXC, their human metabolites and (bio)TPs formed in wastewater, their genotoxicity potential compared to CBZ was predicted using in silico methods. In addition, a highly sensitive analytical method was developed that allowed for the investigation of the occurrence of the target compounds in wastewater, surface water and groundwater. The DSSTox query identified nine compounds which exhibit a higher toxicological relevance as compared to CBZ. This indicates a potential risk of these compounds to mammalians including humans. CBZ, OXC, DiOHBCZ, 10OHCBZ, epCBZ, 1/2OHCBZ, 3OHCBZ, BaQD as well as 9-CA-ADIN were detected in WWTP effluents, surface waters and groundwater. Highest concentrations were observed in WWTP effluents. However, DiOHCBZ and 9-CA-ADIN were also detected in groundwater up to concentrations of 1.1 ± 0.1 μg L^{-1} and 1.2 ± 0.1 μg L^{-1}, respectively. The synopsis of environmental occurrence and predicted genotoxicity strongly suggest the need for a comprehensive risk assessment for 9-CA-ADIN. Ozonation with subsequent (aerated) GAC filter can

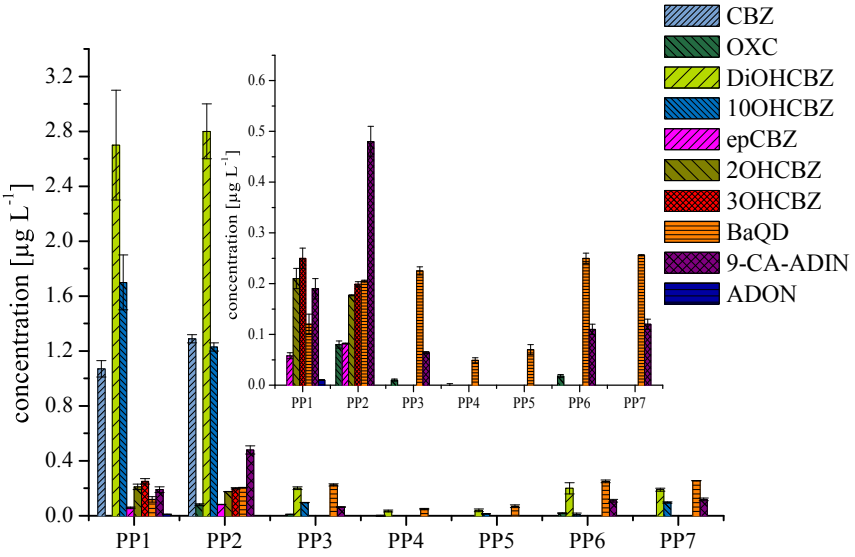

Fig. 5. Concentrations (μg L^{-1}) of CBZ, OXC, their HMs as well as their TPs in pilot WWTP, lower concentrated compounds are visualized in an extra graph (PP1: WWTP influent, PP2 after micro-screen, PP3: after ozonation, PP4: after GAC (non-aerated), PP5: after GAC (aerated), PP6: after biofilter (non-aerated), PP7: after biofilter (areated)).

effectively eliminate the investigated compounds, including those compounds for which a health risk for mammalians is predicted.

Computational methods will be increasingly applied for future drug approvals, taking into account HMs and (bio)TPs which might be harmful and for which a toxicological risk assessment seems to be mandatory. Furthermore, deployment of WWTPs appears to be an effective procedure to minimize the discharge of CECs, including those which are likely to be persistent in the environment.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2016.10.106.

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