Environmental Pollution xxx (2016) 1–9

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

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

Elena Brezina [a], Carsten Prasse [a, c], Johannes Meyer [b], Harald Mückter [b], Thomas A. Ternes [a, *]

[a] Federal Institute of Hydrology (BfG), Am Mainzer Tor 1, 56068 Koblenz, Germany
[b] Walther-Straub-Institute, LMU, Goethestraße 33, 80336 Munich, Germany
[c] Department of Civil & Environmental Engineering, University of California at Berkeley, Berkeley, CA, USA

ARTICLE INFO

Article history:
Received 18 August 2016
Received in revised form
28 October 2016
Accepted 30 October 2016
Available online xxx

Keywords:
Anticonvulsant
Human metabolites
Aquatic environment
Genotoxicity prediction
Advanced wastewater treatment

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

☆ This paper has been recommended for acceptance by Dr. Chen Da.

* Corresponding author.
E-mail address: ternes@bafg.de (T.A. Ternes).

http://dx.doi.org/10.1016/j.envpol.2016.10.106
0269-7491/

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-

Please cite this article in press as: Brezina, E., et al., Investigation and risk evaluation of the occurrence of carbamazepine, oxcarbazepine, their human metabolites and transformation products in the urban water cycle, Environmental Pollution (2016), http://dx.doi.org/10.1016/j.envpol.2016.10.106