Water Research 98 (2016) 19–27

Contents lists available at ScienceDirect

Water Research

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

Effects of carbamazepine and two of its metabolites on the non-biting midge *Chironomus riparius* in a sediment full life cycle toxicity test

Katharina Heye [a, *], Dennis Becker [a], Christian Lütke Eversloh [b], Vedat Durmaz [c], Thomas A. Ternes [b], Matthias Oetken [a], Jörg Oehlmann [a]

[a] Goethe University Frankfurt am Main, Department Aquatic Toxicology, Max-von-Laue-Str. 13, 60438 Frankfurt am Main, Germany
[b] German Federal Institute of Hydrology (BfG), Am Mainzer Tor 1, 56068 Koblenz, Germany
[c] Zuse Institute Berlin (ZIB), Takustraße 7, 14195 Berlin, Germany

ARTICLE INFO

Article history:
Received 23 December 2015
Received in revised form
30 March 2016
Accepted 31 March 2016
Available online 2 April 2016

Keywords:
Carbamazepine-10,11-epoxide
10,11-dihydro-10,11-dihydroxy-carbamazepine
Pharmaceutical
Reproduction
Ecdysone receptor

ABSTRACT

The antiepileptic drug carbamazepine (CBZ) and its main metabolites carbamazepine-10,11-epoxide (EP-CBZ) and 10,11-dihydro-10,11-dihydroxy-carbamazepine (DiOH-CBZ) were chosen as test substances to assess chronic toxicity on the non-biting midge *Chironomus riparius*. All the three substances were tested in a 40-day sediment full life cycle test (according to OECD 233) in which mortality, emergence, fertility, and clutch size were evaluated. In addition, these parameters were considered to calculate the population growth rate which represents an integrated measure to assess population relevant effects. With an LC_{50} of 0.20 mg/kg (time-weighted mean), the metabolite EP-CBZ was significantly more toxic than the parent substance CBZ (LC_{50}: 1.1 mg/kg). Especially mortality, emergence, and fertility showed to be sensitive parameters under the exposure to CBZ and EP-CBZ. By using classical molecular dynamics (MD) simulations, the binding of CBZ to the ecdysone receptor was investigated as one possible mode of action (MoA) but appeared to be unlikely. The second metabolite DiOH-CBZ did not cause any effects within the tested concentration rage (0.17–1.2 mg/kg).

Even though CBZ was less toxic compared to EP-CBZ, CBZ is found in the environment at much higher concentrations and therefore causes a higher potential risk for sediment dwelling organisms compared to its metabolites. Nevertheless, the current study illustrates the importance of including commonly found metabolites into the risk assessment of parent substances.

1. Introduction

Pharmaceuticals play an important role in the treatment of human diseases and are designed to interact with the biological system. Especially due to their high intrinsic biological activity, hardly predictable impacts on the environment can be the result (Martín et al., 2012). It has been estimated that about 3000 different pharmaceuticals are used within the European Union. These pharmaceutical compounds are mainly released through patient excretion into wastewaters or by inappropriate disposal of expired or unneeded drugs whereby they can possibly enter the environment (Gros et al., 2010; Luo et al., 2014).

One of the most frequently detected pharmaceuticals in the environment is the antiepileptic drug carbamazepine (CBZ) (Gros et al., 2010; López-Serna et al., 2012; Ternes, 1998) resulting in a high relevance for ecotoxicological testing. In particular, an insufficient removal during wastewater treatment is considered to be responsible for a widespread occurrence in the aquatic environment (Jelic et al., 2011). Even though its consumption has decreased over the last decade, CBZ is routinely detected in raw wastewater, effluents of wastewater treatment plants (WWTPs), surface waters, and aquatic sediments (Gros et al., 2010; López-Serna et al., 2012; Stein et al., 2008; Vulliet et al., 2014).

Moreover, a variety of metabolites have been detected in the environment (Bahlmann et al., 2014; Jurado et al., 2014). In the liver of patients, most of the parent substance is metabolized, leading to the formation of more than 30 metabolites (Lertratanangkoon and Horning, 1982). While the toxicity of CBZ to aquatic organisms has been studied intensively (Gilroy et al., 2012; Lamichhane et al., 2013; Li et al., 2010), there are currently no studies that investigate the environmental effects of these metabolites, even though in

* Corresponding author.
E-mail address: heye@bio.uni-frankfurt.de (K. Heye).

http://dx.doi.org/10.1016/j.watres.2016.03.071
0043-1354/

several studies higher concentrations of the metabolites compared to CBZ were found (Bahlmann et al., 2014; Fenet et al., 2014; López-Serna et al., 2012). As the exposure to the metabolites could show similar or even stronger effects in organisms than the parent substance, data about the toxicity of commonly found metabolites are strongly required.

Therefore, the current study aimed at conducting the first ecotoxicological assessment of CBZ metabolites in comparison to their parent compound. Two of the main metabolites with a high persistence and high detection frequencies, 10,11-dihydro-10,11-dihydroxy-CBZ (DiOH-CBZ) and carbamazepine-10,-11-epoxide (EP-CBZ) (Jurado et al., 2014; López-Serna et al., 2012; Writer et al., 2013) were chosen for this investigation. To evaluate and compare the toxicity of these three substances, a sediment full life cycle test with *Chironomus riparius* was conducted – a standard test organism, which has already shown to be sensitive to CBZ exposure (Oetken et al., 2005). In addition, the present study is the first to transfer the chronic effects of CBZ and its metabolites to the population level of the species *C. riparius*. The population growth rate (PGR) was calculated as an integrated parameter to summarize and evaluate all the other endpoints (emergence, sex ratio, fertility, and clutch size).

Besides the toxicity of CBZ and its metabolites, we aimed to examine one possible mode of action (MoA) of CBZ. Oetken et al. (2005) observed that CBZ inhibits pupation, in which the ecdysone receptor (EcR) plays a crucial role (Riddiford and Truman, 1993). For this reason, the binding affinity of CBZ to EcR was calculated qualitatively using classical molecular dynamics (MD) simulations. Our knowledge about the active site's location of the EcR is based on the co-crystallized ligand ponasterone A (PONA), an insect hormone and ecdysone analogue with experimentally determined binding affinity. In a further step, this information was used to estimate relative affinities for CBZ to check for a possible MoA of this pharmaceutical in *C. riparius*. Therefore, the present study is the first to investigate MoAs of CBZ in insects.

2. Material and methods

2.1. Test organism and cultivation

The test organism *C. riparius* originated from a culture kept in the Department Aquatic Ecotoxicology at Goethe University Frankfurt am Main, Germany. Organisms were cultured in 2000 mL crystallizing dishes (Kavalier, Simax, Prague, Czech Republic, 19 cm diameter) containing quartz sand (Baumit, Bad Hindelang Germany) and reconstituted water (M4-medium) prepared according to Elendt and Bias (1990). The culture and subsequent tests were kept under controlled conditions of 20 ± 2 °C and a light:dark cycle of 16:8 h.

2.2. Full life cycle test

The test procedure was based on the OECD technical guideline 233 (OECD, 2010) and was performed in 600 mL test beakers (Kavalier, Simax, Prague, Czech Republic, 9 cm diameter). Each beaker contained 120 g artificial sediment consisting of 99% (dw) quartz sand (washed and sterilized by heating to 250 °C), 0.5% (dw) dried stinging nettle (*Urtica dioica*) (particle size < 0.5 mm) (Caelo, Hilden, Germany), and 0.5% (dw) of handpicked black alder leaves (*Alnus glutinosa*, fall foliage from a forest in Oberursel, Germany) (particle size < 0.5 mm). To be able to expose *C. riparius* over the sediment, stock solutions (192 mg/L) of CBZ (CAS number: 298-46-4, Sigma Aldrich, Steinheim, Germany), EP-CBZ (CAS number: 36507-30-9, Sigma Aldrich, Steinheim, Germany), and DiOH-CBZ (CAS number: 58955-93-4, Toronto Research Chemicals, Toronto, Ontario) were prepared with ethyl acetate (CAS number: 141-78-6, AppliChem, Darmstadt, Germany). For each test concentration (CBZ 0.8, 1.6, 3.2, 6.4, 12.8, 25.6 mg/kg; EP-CBZ and DiOH-CBZ 1.6, 6.4, 25.6 mg/kg; nominal concentrations) the stock solution was diluted with an adequate volume of ethyl acetate (total volume of 30 mL) and mixed with the sediment in each test beaker. In addition, a solvent and a negative control were set up in parallel. Each test concentration and control was prepared in eight replicates for CBZ and in four replicates for the two metabolites. After evaporation (48 h) of the solvent, 400 mL of reconstituted water were added into each of the test vessels before the test system was equilibrated for 5 days under gentle aeration.

Subsequently, 20 first instar larvae (<24 h, hatched from 15 egg clutches and then pooled) were added with a Pasteur pipette into each of the test vessels (day 0). During insertion, aeration was stopped and started again after 4 h. Three times a week larvae were fed with finely ground TetraMin® at a rate of 0.25 mg/larvae/day from day 0, 0.5 mg/larvae/day from day 8 and 1 mg/larvae/day from day 13. From day 18 and 20, the amount of food was minimized to 0.5 and 0.25 mg/larvae/day, respectively.

Emergence was recorded daily (time of emergence and sex) and emerged midges were transferred into a gauze covered glass cage (30 × 20 × 20 cm) containing a petri dish (10 × 2 cm, diameter- × height) filled with 35 mL of reconstituted water for oviposition. At this point, two replicates of the sediment exposure were combined into one breeding cage. Each day, produced egg clutches were removed and transferred in 24-well flat bottom plates (Falcon®, Becton Dickinson Labware, Franklin Lakes, New Jersey) (one egg clutch per well, in 2 mL of reconstituted water) for egg clutch analyses. To compensate for evaporation, deionized water was added to ensure a stable volume in the test vessels and petri dishes.

2.3. Chemical analyses

For the chemical analysis of the test substances, water and sediment samples were taken at the beginning (day 0) and the end of the test (day 40). All the water samples were transferred into 50 mL tubes (flat/conical base, polypropylene) (Sarstedt, Nümbrecht, Germany) and were then frozen to −20 °C. The sediment samples were freeze-dried (Alpha 1-4 LSC plus, Martin Christ Gefriertrocknungsanlagen, Osterode am Harz, Germany), transferred into 50 mL tubes, and then stored at −20 °C until the analyses of the samples were conducted.

The sediment samples were extracted by means of an Accelerated Solvent Extraction “ASE” (ASE 200, Dionex, Idstein). Extracts and water samples were then analyzed according to Kaiser et al. (2014) via high-performance liquid chromatography “HPLC” (Agilent 1260 Series, Agilent Technologies, Waldbronn, Germany) coupled with a tandem mass spectrometer (4000 QTrap, AB Sciex, Framingham, Massachusetts) by using an electrospray ionization interface “ESI”. Analysis was conducted in positive ion mode for all the substances, using multiple-reaction monitoring (MRM). For the chromatographic separation, a Synergi Hydro-RP column (150 mm × 3 mm, 4 μm) with a Security Guard column (AQ C18, 4 mm × 2 mm inside diameter (i.d.); both from Phenomenex, Aschaffenburg, Germany) was used. Quantification was performed by means of an external calibration of the respective analytes. Concentrations of the beginning and the end of the test were transferred into time-weighted means (TWM) according to annex 6 of the OECD guideline 211 (OECD, 1998).

As *C. riparius* is a sediment-dwelling deposit-feeder (Rasmussen, 1984; Stief, 2007), it was assumed that larvae were mainly exposed through the sediment. Therefore, sediment and not water concentrations were used to derive effect and lethal concentrations.