Science of the Total Environment 443 (2013) 870–876

Contents lists available at SciVerse ScienceDirect

Science of the Total Environment

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

Ecotoxicity of carbamazepine and its UV photolysis transformation products

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HIGHLIGHTS

- Carbamazepine is highly recalcitrant to standard wastewater treatment practices.
- This study investigated the ecotoxicity of carbamazepine UV-photolysis degradation products.
- Carbamazepine was less toxic to three standard test organisms than the tested degradation products.
- Recalcitrant mixture toxicity of the UV-treated solution was still observed at the end of treatment.
- UV-photolysis of carbamazepine may form toxicologically relevant transformation products.

ARTICLE INFO

Article history:
Received 26 July 2012
Received in revised form 14 November 2012
Accepted 15 November 2012
Available online 14 December 2012

Keywords:
Pharmaceuticals
Environment
Toxicity
Carbamazepine
Acridine
Acridone

ABSTRACT

Carbamazepine, an anti-epileptic pharmaceutical agent commonly found in wastewater, is highly recalcitrant to standard wastewater treatment practices. This study investigated the mixture toxicity of carbamazepine transformation products formed during ultraviolet (UV) photolysis using three standard ecotoxicity assays (representing bacteria, algae and crustaceans). UV-treatment of 6 mg L^{-1} carbamazepine solution was carried out over a 120 min period and samples were removed periodically over the course of the experiment. Quantification results confirmed the degradation of carbamazepine throughout the treatment period, together with concurrent increases in acridine and acridone concentrations. Ecotoxicity was shown to increase in parallel with carbamazepine degradation indicating that the mixture of degradation products formed was more toxic than the parent compound, and all three ecotoxicity endpoints were still inhibited >60% relative to control populations upon dosing with 90 + min UV-treated carbamazepine solution. Single compound toxicity testing also confirmed the higher toxicity of measured degradation products relative to the parent compound. These results show that transformation products considerably more toxic than carbamazepine itself may be produced during UV-treatment of wastewater effluents and/or photo-induced degradation of carbamazepine in natural waters. This study highlights the need to consider mixture toxicity and the formation and persistence of toxicologically relevant transformation products when assessing the environmental risks posed by pharmaceutical compounds.

1. Introduction

There are large knowledge gaps concerning the environmental fate and effects of most active pharmaceutical ingredients (APIs). For regulators, this translates to a high degree of uncertainty about the risks associated with pharmaceutically derived contaminants in the environment (Kümmerer, 2009a,b). The quantities of APIs released to the environment may be relatively low in comparison with other types of pollutants such as pesticides and industrial pollutants, but there is a tendency for their environmental release to be continuous (via wastewater effluents) and the potential for environmental accumulation and/or chronic ecotoxicity has thus been noted (e.g. Besse and Garric, 2008; Fent et al., 2006; Ferrari et al., 2003; Hernando et al., 2006; Escher et al., 2011).

Carbamazepine, an antiepileptic pharmaceutical compound and mood stabilising drug, has attracted particular attention in recent years due to its widespread detection in municipal wastewaters (e.g. Ternes, 1998; Ollers et al., 2001; Falås et al., 2012), surface waters (e.g. Ternes, 1998; Metcalfe et al., 2003; Andreozzi et al., 2003), and drinking waters (e.g. Heberer et al., 2004; Stackelberg et al., 2004, 2007; Togola and Budzinski, 2008). It is also noted to be highly resistant to biodegradation and thus highly recalcitrant under standard biological wastewater

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http://dx.doi.org/10.1016/j.scitotenv.2012.11.059

treatment conditions (Clara et al., 2005; Kosjek et al., 2009; Falås et al., 2012). Under these circumstances, advanced oxidation processes (AOPs) may offer additional treatment value (von Gunten, 2003; Huber et al., 2005; Macova et al., 2010; Kosjek et al., 2011; Hey et al., 2012; Keen et al., 2012), however complete oxidation and mineralisation is not always achievable and stable transformation products formed during AOPs may also pose environmental risks (Agüera et al., 2005). The stability and ecotoxicity of transformation products formed during treatment and environmental transport are of major importance when assessing the suitability of water treatment options and in determining environmental risks associated with APIs in the environment.

This paper presents the results of research investigating the ecotoxicity of carbamazepine and two of its UV transformation products (Kosjek et al., 2009), acridine and 9(10H)-acridone. Kosjek et al. (2009) have also identified several other compounds which may form via UV photolysis of carbamazepine, however authentic standard compounds are not commercially available for these other degradation products. Thus, in addition to single compound testing of carbamazepine, acridine and acridone with three different standard test organisms (bacteria, algae, and crustacean), an experiment was also conducted to investigate the changes in ecotoxicity occurring during UV-treatment of water spiked with 6 mg L^{-1} carbamazepine. This experiment, conducted by using a bench-top circulating flow UV-reactor system, was designed to compare the ecotoxicity of the initial carbamazepine-spiked solution with that of samples collected during the treatment process. These samples contained mixtures of carbamazepine and its UV-transformation products (including, but not limited to, acridine and acridone).

This study provides an example of the 'effect-driven approach' for determining the risks associated with pollutant transformation products. The 'effect driven approach', described by Escher and Fenner (2011) in their paper on advances in environmental risk assessment of transformation products, focuses on ecotoxicity testing of reaction mixtures in which a parent compound is undergoing degradation (e.g. during UV-treatment). This approach facilitates prioritisation of APIs and their transformation products for risk assessment purposes without requiring the prior identification and quantification of individual transformation products and is thus ideal for situations such as this one in which standard compounds are not available for all relevant transformation products. In 'effect driven' assessments, compounds are prioritised for transformation product isolation, purification, and further study if the reaction mixture increases in toxicity in parallel with decreasing parent compound concentration.

This investigation into the relative toxicity of carbamazepine and its phototransformation products is relevant to future discussions regarding the treatment, control and fate of carbamazepine and carbamazepine-derived contaminants in the environment. Knowledge about carbamazepine's UV-induced degradation pathway is also of interest from the perspective of sunlight-induced photochemical oxidation, as this may be a significant process controlling the gradual ongoing breakdown of carbamazepine released to receiving waters and natural aquatic environments. Indeed, for non-biodegradable, non-sorbing APIs such as carbamazepine, sun-induced photodegradation may be one of the most important long-term process affecting persistence and toxicity in the environment (Doll and Frimmel, 2003; Agüera et al., 2005).

2. Methods

2.1. Standards, solvents, and other chemicals

Carbamazepine (99%, CAS 298-46-4) and N-Methyl-N- [tert-butyldimethyl-silyl]trifluoroacetimide (MTBSTFA) were purchased from Acros Organics (New Jersey, USA). Acridine (97%, CAS 260-94-6) and 9(10H)-acridone (99%, CAS 578-95-0) were obtained from Sigma-Aldrich (St. Louis, USA). Stock solutions were freshly made prior to use and the volumetric flasks were covered in aluminium foil to prevent photodegradation.

2.2. Chemical analyses

Solid phase extraction (SPE) was used for sample preparation for all chemical analyses. Concentrated solutions (i.e. stock solutions and selected concentration check samples from the acute toxicity test dilution series) were initially diluted as appropriate (dilution factors ranged from 17 to 600) before loading at neutral pH onto Oasis® HLB reversed-phase sorbent SPE cartridges (Waters, Corp., Milford, MA, USA). These cartridges were also used for preconcentration of the 150 mL subsamples taken throughout the carbamazepine UV-treatment experiment. SPE cartridges were conditioned with 3 mL of ethyl acetate, 3 mL methanol, and 3 mL of tap water. Sample aliquots were loaded on the SPE columns at a flow-rate of 4–5 mL min^{-1}. Each cartridge was then washed with water (3 mL), dried for 30 min under vacuum and eluted with 1 mL acetone, 1 mL of 7/3 ethyl acetate/acetone mixture and 1 mL of ethyl acetate. The combined eluant was evaporated to dryness under a gentle nitrogen stream and reconstituted with 0.5 mL ethyl acetate.

Acridone and carbamazepine were transformed into the tert-butyldimethyl-silyl ether derivatives by adding 30 μL MTBSTFA and maintaining the samples at 60 °C for 12 h. A Varian 3800 gas chromatograph (GC) interfaced with an ion trap Saturn 2000 mass spectrometer (MS) was used for analysis. 10 μL samples were injected (split–splitless) using a PTV injector at 80 °C for 0.30 min before being increased by 200 °C/min to 300 °C and held for 5 min. A Zebron ZB-5 HT INFERNO 30 m × 0.25 mm × 0.25 μm (Phenomenex) column was used for separation. The GC oven temperature programme was originally held at 80 °C for 1 min, then increased by 25 °C/min to 225 °C and held for 1 min; increased by 1 °C/min to 231 °C; increased by 10 °C/min to 280 °C; increased by 45 °C/min to 320 °C and held at 320 °C for a further 3 min. The total runtime was 22.59 min. The mass analyser was operated in electron ionisation (EI) mode, and the following fragment ions were monitored for quantitation: *m/z* 179 for acridine, and *m/z* 252 and *m/z* 193 for the acridone-MTBS and carbamazepine-MTBS derivatives respectively.

For each sample, two parallel subsamples were extracted, derivatised and analysed according to the above protocol. Furthermore, each of these subsamples was injected and analysed twice, with the mean value taken as the relevant concentration. Method performance analyses showed satisfactory linearity ($r^2 > 0.98$) and good repeatability for all three analytes. Blank control samples were also prepared and analysed. The limits of detection (LOD) were 2.3 μg L^{-1} for acridine, 14 ng L^{-1} for acridone and 11 ng L^{-1} for carbamazepine

2.3. Ecotoxicity testing

Three internationally standardised aquatic ecotoxicity tests (Table 1) were used to investigate the ecotoxicity of carbamazepine, acridine, and acridone. The same three assays were also used to monitor changes in ecotoxicity during UV-treatment of the 6 mg L^{-1} carbamazepine solution. All measurements were conducted in triplicate. The selected ecotoxicity tests used organisms from different trophic levels and included the following short-term toxicity tests:

- Inhibition of bioluminescence in the marine bacterium *Vibrio fischeri* (Biotox testing kit; ISO 11348-3, 1998);
- Growth inhibition of the green algae *Pseudokirchneriella subcapitata* (ISO 8692, 2004);
- Immobilisation of the crustacean *Daphnia magna* Straus (Cladocera, Crustacea) (ISO 6341, 1996).

For the Biotox test, 950 μL of sample solution and 50 μL of *V. fischeri* culture were mixed and bioluminescence was measured after 5, 15 and 30 min exposure to the test solution. Algal biomasses were determined by using acetone pigment extractions as described by Mayer et al. (1997). For all three ecotoxicity tests, range finding tests were initially conducted for each compound and test species, prior to