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Behaviour of pharmaceuticals in spiked lake sediments – Effects and interactions with benthic invertebrates

Ève A.M. Gilroy [a,*], Vimal K. Balakrishnan [a], Keith R. Solomon [b], Ed Sverko [c], Paul K. Sibley [b]

[a] Aquatic Ecosystem Protection Research Division, Water Science and Technology Directorate, Environment Canada, Burlington, Ontario, Canada L7R 4A6
[b] Department of Environmental Biology, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[c] National Laboratory for Environmental Testing, Environment Canada, Burlington, Ontario, Canada L7R 4A6

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ABSTRACT

The behaviour and effects of atorvastatin (ATO), carbamazepine (CBZ), and 17α-ethinylestradiol (EE2) were investigated in spiked lake sediments, at concentrations up to 56.5 mg kg^{-1} dry weight (dw), with the benthic invertebrates *Chironomus dilutus* and *Hyalella azteca*. Desorption constants were calculated in the presence and absence of animals, using linear isotherms, yielding K_d values of 28.2, 189.0 and 125.1 L kg^{-1} (ATO), 73.7, 201.7 and 263.2 L kg^{-1} (CBZ), and 114.9, 114.2 and 519.2 L kg^{-1} (EE2) for *C. dilutus*, *H. azteca*, and without animals, respectively. For ATO and CBZ, K_d values were smaller in the presence of *C. dilutus*, indicating greater desorption to the overlying water from bioturbation, which is consistent with the predominantly benthic occurrence of *C. dilutus* compared to *H. azteca*. In contrast, due to its greater hydrophobicity, bioturbation did not significantly affect desorption of EE2. No significant toxicity was observed, indicating decreased bioavailability of the chemicals sorbed to sediments compared with water-only toxicity assays.

1. Introduction

For several decades, the use of pharmaceuticals has increased globally and recent advances in the development of sensitive analytical techniques have revealed that these chemicals occur in many environmental matrices. Studies have also shown that many pharmaceuticals may be excreted only partially metabolized by target organisms, are poorly degraded during the wastewater treatment process which was not designed to remove large quantities of these organic compounds, and may thus be released in the final effluent of wastewater facilities (Daughton and Ternes, 1999; Kolpin et al., 2002). Consequently, numerous pharmaceuticals are routinely detected at small concentrations in sewage sludge, effluents, and surface waters (Ternes, 1998; Ternes et al., 1999; Kolpin et al., 2002; Wiegel et al., 2004; Lissemore et al., 2006).

Pharmaceuticals are designed to elicit specific effects on target organisms, and thus have the potential to display high biological activity in the environment. Recent efforts to determine the potential impacts of these contaminants have resulted in a significant increase in the number of studies reporting on effects on non-target species (Oetken et al., 2005; Borgmann et al., 2007; Stanley et al., 2007; Dussault et al., 2008; Paterson and Metcalfe, 2008; Péry et al., 2008).

While the majority of pharmaceuticals are not considered to be persistent, high-volume usage patterns along with continuous discharge from sewage treatment facilities constantly replenish these compounds, thus contributing to their apparent environmental persistence (Daughton and Ternes, 1999). Sediments are the final repository for numerous organic contaminants, but few studies to date have investigated the fate of pharmaceuticals in this compartment (Jungclaus et al., 1978; Ternes et al., 2002; Löffler and Ternes, 2003; Kim and Carlson, 2007; Rice and Mitra, 2007). In these studies, concentrations reported in the sediments were often greater than those detected in surface water, which indicates that sediments may act as a sink and long-term source of these compounds in aquatic systems. Moreover, pharmaceuticals present in sediments could affect benthic organisms, which may be exposed via sediments, associated interstitial water, and overlying water.

Sorption or desorption, the processes through which chemicals become associated with, or dissociated from solid phases (Schwarzenbach et al., 2003), are generally visualized as an equilibrium involving two phases, such as, for example, sediment and overlying water, with a net diffusion of zero. However, several studies have revealed that biological activity (bioturbation) by benthic invertebrates can increase desorption of some organic contaminants to the overlying water (Reible et al., 1996; Gunnarsson et al., 1999; Goedkoop and Peterson, 2003), thus increasing their bioavailability, defined as the amount of a chemical that is able to interact with organisms within the compartment of interest (Katayama et al.,

* Corresponding author. Tel.: +1 905 975 0605.
E-mail address: eve.gilroy@bell.net (È.A.M. Gilroy).

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2010). Depending on the behaviour and level of interaction of an organism with the sediment, bioturbation by benthic invertebrates could kinetically enhance desorption of pharmaceuticals from sediments, thereby affecting the overall equilibrium position of the desorption process.

Few studies to date have investigated the effects of pharmaceuticals on benthic invertebrates, and their interactions with pharmaceuticals from contaminated sediments have not been examined. The present study investigated desorption of three pharmaceuticals from spiked sediments, and their effects and interactions with two benthic invertebrates, the midge *Chironomus dilutus* (formerly *Chironomus tentans*) and the freshwater amphipod *Hyalella azteca*. Because *C. dilutus* is an endobenthic deposit feeder inhabiting surficial sediments (≈top 5 cm), which it utilizes as a source of material to build its protective case, and *H. azteca* is an epibenthic invertebrate, which may have limited contact with the sediment, we hypothesized that differences in the intensity of interaction with the sediment would affect the desorption of pharmaceuticals to the overlying water. Hence, desorption studies were completed in the presence of either *C. dilutus* or *H. azteca*, and without the organisms, during which effects on benthic invertebrates were monitored, and after which desorption constants were calculated.

The pharmaceuticals were selected based on usage in Canada, relative environmental persistence, and previous investigations within our laboratory (Dussault et al., 2008). Atorvastatin is the most widely prescribed lipid regulator in Canada, and has been detected in sewage treatment effluents (Miao and Metcalfe, 2003a,b). The antiepileptic drug carbamazepine is a persistent contaminant frequently detected in sewage treatment effluents and surface water, and is poorly removed upon sewage treatment (Ternes, 1998). Half-lives of 82 d (aqueous), 328 d (sediment) and 495 d (soil) have been reported (Lam et al., 2004; Löffler et al., 2005; Walters et al., 2010), thus raising concerns over its potential accumulation and persistence in sediments and soils. The synthetic hormone 17α-ethinylestradiol has been detected in aquatic systems at concentrations that are close to those known to cause endocrine disruption in fish, and has been reported in sediments (Ternes et al., 1999, 2002).

2. Materials and methods

2.1. Chemicals

Atorvastatin (ATO; ⩾99% purity; 134523-00-5) was purchased from Rugao Foreign Trade Corporation (Shanghai, Jiangsu, China), carbamazepine (CBZ; ⩾99%; 298-46-4) was acquired from China Jiangsu Textiles (Nanjiing, Jiangsu China), and 17-α-ethinylestradiol (EE2; ⩾98%; 57-63-6) was obtained from Sigma–Aldrich (Oakville, ON, Canada). Isotopically labelled $^{2}H_5$-atorvastatin (⩾98%) was obtained from Toronto Research Chemicals (North York, ON, Canada), $^{2}H_{10}$-carbamazepine (⩾98%) was purchased from Cambridge Isotope Laboratories (Andover, MA, USA), and $^{2}H_2$-17β-estradiol (atomic purity 98%) was acquired from Sigma–Aldrich. Analytical grade methanol, acetone (distilled in glass) and ammonium carbonate were obtained from Caledon Laboratory Chemicals (Georgetown, ON, Canada).

2.2. Sediment preparation

Reference sediment (Lake Erie site # 112, ph 8.17, Alkalinity 89 mg L^{-1}, 36% clay, 55% silt, 9% sand, 1.9% Total Organic Carbon (TOC), Grapentine, L., personal communication) was spiked with a 0.2% methanol (v ww^{-1}) solution of either ATO, CBZ, or EE2. The sediment was mixed on a rotary shaker at a speed of 6 rpm at 4 °C. In order to achieve a compromise between the need for proper mixing and equilibration, yet minimize compound biodegradation prior to the beginning of the experiment, a mixing duration of 72 h was selected (ASTM, 2004a). Preliminary spiking studies confirmed the stability of the compounds of interest after 2–14 d of mixing (Gilroy, E.A.M., Environment Canada, Burlington, ON, Canada). Aliquots of 25 mL of pharmaceutical-spiked sediment were slowly added to 1-L Imhoff sedimentation cones filled with reconstituted water, using a 60 mL syringe fitted with a pipette tip. The water column of each cone was gently aerated throughout the experiment, using a glass Pasteur pipette, to maintain adequate levels of dissolved oxygen, while care was taken to avoid perturbation of the sediment. The sedimentation cones were equilibrated for 24 h prior to test initiation.

2.3. Experimental design

Each assay was performed in 1-L Imhoff sedimentation cones (Borgmann and Norwood, 1999), which contained 25 mL of sediment spiked with either ATO, CBZ or EE2, and 975 mL of distilled water, reconstituted to achieve an ionic concentration recommended for testing with each organism (*C. dilutus*: 192 mg $NaHCO_3$, 120 mg $CaSO_42H_2O$, 120 mg $MgSO_4$, 8 mg KCl per litre of distilled water (ASTM, 2004b), conductivity ~480 mS cm^{-1}; *H. azteca*: 147 mg $CaCl_2$, 84 mg $NaHCO_3$, 1 mg NaBr, 3.7 mg KCl, and 30.1 mg $MgSO_4$ per litre of distilled water (Borgmann, 1996), conductivity ~360 mS cm^{-1}). For each chemical and organism investigated, testing included eight concentrations (six concentrations + control + solvent control), and seven replicates (five for survival and growth, and two for water and sediment sampling in support of chemical analysis), for a total of 56 cones. Each assay was completed once. An additional series of assays was performed in the absence of organisms, to ascertain desorption coefficients in the absence of bioturbation. Given that sediments are known to be a natural sink for organic contaminants, the range of tested concentrations (0.001–100 mg kg^{-1} dw) included values equivalent to those reported in sediments (Jungclaus et al., 1978; Singer et al., 2002; Antonic and Heath, 2007; Kim and Carlson, 2007; Rice and Mitra, 2007), as well as those equivalent to surface water and toxic concentrations for water-column exposures (Dussault et al., 2008), assuming a worst-case scenario of 1:1 water-to-sediment toxicity ratio.

2.4. Pharmaceutical exposures

Testing with the benthic invertebrates *C. dilutus* and *H. azteca* was performed following standard procedures for 10-d toxicity testing, as outlined by the US Environmental Protection Agency guidelines (US EPA, 2000), with the exception that the tests were performed in Imhoff sedimentation cones, under static conditions (Borgmann and Norwood, 1999). Upon test initiation, eight *C. dilutus* larvae (10–12-d old) or 15 *H. azteca* juveniles (7–14-d old) were added to each sedimentation cone, corresponding to animal densities of approximately 8000 and 15 500 individuals m^{-2}, respectively. These densities are higher than those usually conducted in 250 mL beakers (corresponding to approximately 3200 animals m^{-2}), but below those at which adverse effects on survival and growth are anticipated (Borgmann and Norwood, 1999). Animals were fed daily with 1 mL of a 6 g L^{-1} fish flake slurry (corresponding to 6 mg of food d^{-1}) during the experiments with *C. dilutus*, and 2.5 mg of ground Tetramin fish flakes on alternate days during the experiments with *H. azteca* (Borgmann et al., 1989; US EPA, 2000; Borgmann, 2002). The organic carbon content of Tetramin fish flakes was 45.4% (Golding, L., University of Melbourne, Melbourne, Australia, personal communication), so the amount of organic carbon added to each cone via feeding was estimated to