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Effects of soil properties on the uptake of pharmaceuticals into earthworms☆

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Pharmaceuticals can enter the soil environment when animal slurries and sewage sludge are applied to land as a fertiliser or during irrigation with contaminated water. These pharmaceuticals may then be taken up by soil organisms possibly resulting in toxic effects and/or exposure of organisms higher up the food chain. This study investigated the influence of soil properties on the uptake and depuration of pharmaceuticals (carbamazepine, diclofenac, fluoxetine and orlistat) in the earthworm *Eisenia fetida*. The uptake and accumulation of pharmaceuticals into *E. fetida* changed depending on soil type. Orlistat exhibited the highest pore water based bioconcentration factors (BCFs) and displayed the largest differences between soil types with BCFs ranging between 30.5 and 115.9. For carbamazepine, diclofenac and fluoxetine BCFs ranged between 1.1 and 1.6, 7.0 and 69.6 and 14.1 and 20.4 respectively. Additional analysis demonstrated that in certain treatments the presence of these chemicals in the soil matrices changed the soil pH over time, with a statistically significant pH difference to control samples. The internal pH of *E. fetida* also changed as a result of incubation in pharmaceutically spiked soil, in comparison to the control earthworms. These results demonstrate that a combination of soil properties and pharmaceutical physico-chemical properties are important in terms of predicting pharmaceutical uptake in terrestrial systems and that pharmaceuticals can modify soil and internal earthworm chemistry which may hold wider implications for risk assessment.

1. Introduction

Following use, pharmaceuticals are typically excreted to the sewage system and are then transported to wastewater treatment plants. As many pharmaceuticals are resistant to degradation in wastewater treatment processes they will be present in the wastewater treatment effluents and in the sludge by-products (Jelic et al., 2011). The land application of sewage sludge (biosolids) as a fertiliser and use of reclaimed waste water for irrigation purposes therefore provides a route of entry for pharmaceuticals into the terrestrial environment (Dalkmann et al., 2012; Duran-Alvarez et al., 2009; Kinney et al., 2006a, 2006b; Siemens et al., 2008). Concerns have therefore been raised over the potential uptake of pharmaceuticals into terrestrial organisms and the potential effects on soil-dwelling organisms and organisms that feed on these (Arnold et al., 2014). A handful of studies have recently demonstrated that pharmaceuticals can be taken up from soils and accumulate in invertebrates such as earthworms (Berge and Vulliet, 2015; Carter et al., 2014b; Kinney et al., 2008).

Earthworms are key terrestrial invertebrates with respect to the role they have in maintaining a fertile soil environment (Edwards, 2004). Earthworms are also a key food source for many predator species such as birds. Understanding the uptake of chemicals into earthworms is therefore not only a prerequisite to understanding the risks chemicals pose to earthworm populations, but also the potential effects of secondary poisoning on predators. Earthworms are at the base of many food chains and thus if chemicals are taken up into the earthworms they can facilitate the movement of chemicals into the food web via bioaccumulation and biomagnification processes (Shore et al., 2014).

We have previously investigated the uptake and depuration kinetics of four pharmaceuticals in the earthworm, *Eisenia fetida* (Carter et al., 2014b). Pore-water based bioconcentration factors (BCFs) increased in the order of carbamazepine < diclofenac < fluoxetine < orlistat and ranged

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between 2.2 and 51.5. This study highlighted that, unlike neutral organic compounds, uptake of ionisable pharmaceuticals was not driven by the hydrophobicity (log K_{ow}) of the chemical alone. Our previous study exposed the earthworms to pharmaceutical residues in one soil type only. It is well known that pharmaceuticals can behave very differently in different soil types (Drillia et al., 2005; Monteiro and Boxall, 2009). For example, distribution coefficients (K_d) between soil particles and soil pore waters are known to vary by several orders of magnitude for a range of pharmaceuticals in soils with varying properties (Kodesova et al., 2015; ter Laak et al., 2006). As the distribution of pharmaceuticals between the soil and pore water influences the bioavailable fraction of these chemicals and thus uptake by earthworms, it is therefore likely that uptake of pharmaceuticals could also vary significantly across soils.

However, knowledge of the relationships between soil properties and pharmaceutical uptake in terrestrial species is very limited. There is therefore a real need to generate data on the uptake of pharmaceuticals into terrestrial invertebrates from soils with different characteristics in order to identify key drivers affecting uptake. This will help to develop uptake modelling approaches for use in environmental risk assessment. Therefore, in this study we build upon our previously published results demonstrating pharmaceutical uptake by the earthworm *Eisenia fetida* in a single soil type (Carter et al., 2014b) and explore the effects of soil properties on the uptake and depuration of pharmaceuticals in order to help elucidate the relationships between soil properties and uptake. The study focused on one acidic (diclofenac), one basic (fluoxetine) and two neutral (carbamazepine and orlistat) pharmaceuticals, from a variety of therapeutic uses and covering a range of physico-chemical properties (e.g. log K_{ow} 2.25–8.19) (Table 1). With the exception of orlistat, these pharmaceuticals have been previously detected in wastewater irrigated soils in concentrations <7 μg/kg and therefore it is important to understand the potential uptake of these chemicals by soil dwelling organisms. To help explain any potential differences in uptake and depuration, parallel studies were performed to assess the fate and distribution of the study pharmaceuticals in test soils.

2. Materials and methods

2.1. Pharmaceuticals and reagents

All studies were performed using ^{14}C labelled compounds. Radiolabeled fluoxetine [methyl-^{14}C] and carbamazepine [carbonyl-^{14}C] were obtained from American Radiolabeled Chemicals (St. Louis, MO, USA), diclofenac [U − ^{14}C] was obtained from Perkin Elmer (Boston, MA, USA) and orlistat [tridecanyl-2-^{14}C] was provided by GlaxoSmithKline (GSK) (Middlesex, UK). Physico-chemical properties and specific activities for the pharmaceuticals can be found in Table 1. Acetonitrile (99.9%), methanol (99.9%) and ethyl acetate (99.9%) were obtained from Fisher Scientific (Loughborough, UK).

2.2. Test soils

Five standard test soils were obtained from LUFA Speyer (Speyer, Germany). The soils, 2.1, 2.3, 2.4, 5M and 6S, included clay loam, silty sand and loamy sand varieties and were chosen to provide a range of soil characteristics including varying soil pH, organic carbon content, cation exchange capacity and particle size distributions (Table 2). Soils were air dried and sieved to 2 mm prior to testing to ensure homogeneity.

2.3. Test organism

E. fetida were obtained from Blades Biological Ltd. (Kent, UK) and cultured in a medium of peat and cow manure (50:50) (Dean's Garden Centre, York, UK), kept moist with deionised water at room temperature (20 ± 3 °C). The earthworms were fed twice weekly with homogenised mashed potato powder. *E. fetida* were obtained from a single species culture and cultures were maintained for at least four generations prior to use in the uptake studies. The lipid content of *E. fetida*, determined using the method of Folch et al., (Folch et al., 1957), was 5.11 ± 0.29% (wet weight) (Carter et al., 2014b).

2.4. Fate studies

For each pharmaceutical, triplicate beakers of each soil (2.1, 2.3, 2.4, 5M and 6S) (35 ± 1 g) were prepared to sample at eight time points (0 and 6 h, 1, 3, 7, 10, 14 and 21 d) where pore water and soil samples would be analysed for radioactivity and pH. Detailed sample preparation and analysis techniques can be found in Carter et al. (2014b). Briefly, labelled pharmaceuticals were added, individually, to each of the five soils using 125–165 μl of a carrier solvent to create nominal concentrations of 26, 25, 28 and 44 μg/kg of carbamazepine, diclofenac, fluoxetine and orlistat respectively. For carbamazepine and fluoxetine, ethanol was used as the carrier solvent; for diclofenac, methanol was used and orlistat was applied in acetonitrile. After spiking, each test beaker was left for 2 h and then mixed by hand to create an even distribution of the pharmaceutical within the sample. Following spiking and mixing, the carrier solvents were allowed to evaporate from the test beakers for 48 h. Blank and solvent controls were also prepared. The moisture content of all soils was adjusted, and maintained at 40–60% of the maximum water holding capacity (MWHC) by addition of deionised water on a daily basis. All experiments were undertaken at 20 ± 2 °C, using a 16:8 light/dark cycle [600 lx] and 60% humidity.

2.5. Uptake and depuration studies

The uptake and depuration studies followed the 'minimised' approach described in Carter et al. (2014a). The experiments consisted of exposing a single earthworm to each pharmaceutical in each of the five soil types. There were six replicates per treatment. Soils were prepared in glass jars (50 ± 1 g) and spiked with the four

Table 1
Test pharmaceutical physico-chemical properties.

Pharmaceutical	Class	CAS[a]	Molecular weight (g mol^{-1})	Log K_{ow}[b]	Acid/Base	pKa[c]	Specific activity (GBq $mmol^{-1}$)
Carbamazepine	Anti-epileptic	298-46-4	236.30	2.25	Neutral	N/A	0.74
Diclofenac	Anti-inflammatory	15307-79-6	318.13	4.02	Acid	4.12	2.29
Fluoxetine	Anti-depressant	54910-89-3	345.80	4.65	Base	9.53	2.04
Orlistat	Weight loss aid	96829-58-2	497.74	8.19	Neutral	N/A	2.05

[a] CAS obtained from the Chemical Abstracts Service.
[b] Log Kow values obtained from KOWWIN v. 1.68 database, USEPA EPI suite 4.1 programme.
[c] pKa values were predicted using the University of Georgia SPARC database v. 4.2 (http://ibmlc2.chem.uga.edu/sparc) Accessed: 25/05/2012.