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Fate and Uptake of Pharmaceuticals in Soil−Earthworm Systems

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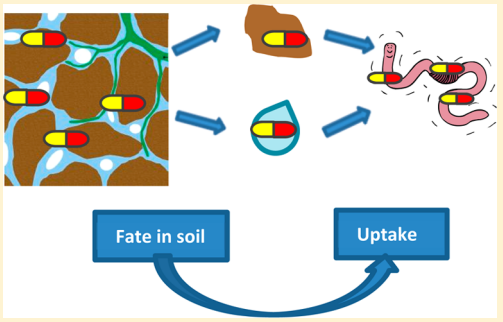
Supporting Information

ABSTRACT: Pharmaceuticals present a potential threat to soil organisms, yet our understanding of their fate and uptake in soil systems is limited. This study therefore investigated the fate and uptake of ^{14}C-labeled carbamazepine, diclofenac, fluoxetine, and orlistat in soil−earthworm systems. Sorption coefficients increased in the order of carbamazepine < diclofenac < fluoxetine < orlistat. Dissipation of ^{14}C varied by compound, and for orlistat, there was evidence of formation of nonextractable residues. Uptake of ^{14}C was seen for all compounds. Depuration studies showed complete elimination of ^{14}C for carbamazepine and fluoxetine treatments and partial elimination for orlistat and diclofenac, with greater than 30% of the ^{14}C remaining in the tissue at the end of the experiment. Pore-water-based bioconcentration factors (BCFs), based on uptake and elimination of ^{14}C, increased in the order carbamazepine < diclofenac < fluoxetine and orlistat. Liquid chromatography−tandem mass spectrometry and liquid chromatography−Fourier transform mass spectrometry indicated that the observed uptake in the fluoxetine and carbamazepine treatments was due to the parent compounds but that diclofenac was degraded in the test system so uptake was due to unidentifiable transformation products. Comparison of our data with outputs of quantitative structure−activity relationships for estimating BCFs in worms showed that these models tend to overestimate pharmaceutical BCFs so new models are needed.



INTRODUCTION

Active pharmaceutical ingredients (APIs) may be released into the soil environment when contaminated sewage sludge, sewage effluent, or animal manure is applied to land.[1,2] Veterinary pharmaceuticals may also be excreted directly to soils by pasture animals. Consequently, a range of APIs has been detected in agricultural soils, with reported concentrations ranging from 0.02 to 15 μg/kg dry soil.[3−6] A number of studies have explored the uptake of APIs into aquatic invertebrates and fish.[7−10] However, much less work has been done to assess the uptake of APIs into terrestrial organisms. The work that has been done has focused on the uptake of human and veterinary APIs into plants[11−14] with only a few studies looking at uptake into terrestrial invertebrates such as earthworms.[15,16]

Earthworms are key organisms in the terrestrial environment, and their presence is central to a healthy and sustainable soil environment; for example, earthworms help to establish and maintain the structure and fertility of the soil.[17,18] The physical motion of earthworm burrowing can bury plant material deep into the soil and is therefore crucial for the recycling of nutrients, whereas the structure of the burrows is important in draining and aerating the soil. Earthworms, being at the base of a food chain, hold an integral position. Uptake and accumulation of contaminants into earthworms not only poses a risk to the earthworm directly but bioaccumulation and contaminant transfer through the food chain to top predators such as birds yield the potential for secondary poisoning.[19] Previous research has demonstrated that earthworms can biomagnify inorganic and organic soil contaminants.[20−24]

The limited data on uptake of APIs into earthworms originates from studies of the bioaccumulation of anthropogenic waste indicators (including the APIs trimethoprim, caffeine, carbamazepine, thiabendazole, and diphenhydramine) from agricultural soil amended with biosolids or swine manure.[15] Trimethoprim was the only API detected in the earthworms, at concentrations of 127 μg/kg dry weight in earthworms from a biosolid-amended field and 61 μg/kg dry weight in earthworms from the manure-amended field.[15] Given the paucity of the data and the potential for pharmaceuticals to end up in the soil, further research is therefore required to fully characterize the potential for pharmaceutical uptake into terrestrial invertebrates.

The aim of this study was to investigate the uptake and depuration kinetics of selected pharmaceuticals in the earthworm *Eisenia fetida*, while also exploring the fate of the chemicals in the soil in order to establish relationships between the properties of

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pharmaceuticals and uptake kinetics. The study also explored the suitability of existing models that have been proposed for estimating the uptake of neutral organic compounds into earthworms based on predicted soil pore water concentrations. The study compounds included the antiepileptic drug carbamazepine, the anti-inflammatory drug diclofenac, the antidepressant fluoxetine, and orlistat, which is a lipase inhibitor, used in the treatment of obesity.

MATERIALS AND METHODS

Pharmaceutical Compounds and Reagents. 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 PerkinElmer (Boston, MA, USA), and orlistat [tridecanyl-2-^{14}C] was provided by GlaxoSmithKline (GSK) (Middlesex, UK). Unlabeled fluoxetine, carbamazepine, and diclofenac were obtained from Sigma–Aldrich (Dorset, UK), and unlabeled orlistat was provided by GSK. Acetonitrile (99.9%), methanol (99.9%), and ethyl acetate (99.9%) were obtained from Fisher Scientific (Loughborough, UK).

Test Soil. The study soil was a clay loam soil obtained from LandLook (Midlands, UK). Prior to use, the soil was air-dried for 48 h and then sieved to 2 mm to ensure homogeneity. The soil had an organic matter content of 3%, a pH of 6.3, and a total organic carbon content of 1.89% (detailed characteristics of the study soil are provided in Table SI 1, Supporting Information).

Test Organism. *E. fetida* were obtained from Blades Biological Ltd. (Kent, UK), cultured in a medium of peat and cow manure (50:50) (Dean's Garden Centre, York, UK), and kept moist with deionized water at room temperature (20 ± 3 °C). The animals were fed twice weekly with homogenized mashed potato powder that was added to the surface of the culture. The *E. fetida* were obtained from a single species culture, and cultures were maintained for at least four generations prior to use in the studies. The lipid content of *E. fetida*, determined using the method of Folch et al.,[25] was 5.11 ± 0.29% (wet weight).

Uptake and Depuration Study—Experimental Design. Preliminary studies were carried out to assess the sorption behavior of the study compounds in the test soil as well as to evaluate any toxic effects of the study compounds on *E. fetida* (see the Supporting Information). The uptake and depuration experiments followed OECD Guideline 317 "Bioaccumulation in Terrestrial Oligochaetes."[26] Tests were performed in glass jars containing 50 ± 1 g of test soil as this was an adequate amount to allow sufficient burrowing depth (approximately 4–5 cm) for the *E. fetida*. All exposures were performed in a growth chamber at 20 ± 2 °C and 60% humidity, using a 16:8 h light/dark cycle. Prior to exposure to test chemicals, the earthworms were acclimated to the experimental conditions in the growth chamber for 48 h using nontreated test soil.

Exposure soils were prepared by adding the labeled pharmaceuticals to the soil using 100–200 μL of a carrier solvent to give concentrations of 39, 49, 80, and 65 μg/kg (wet weight) of carbamazepine, diclofenac, fluoxetine, and orlistat, respectively. For carbamazepine and fluoxetine, ethanol was used as the carrier; methanol was used for diclofenac, and acetonitrile was used for orlistat. After spiking, each test beaker was left for 2 h and then mixed to evenly distribute the pharmaceutical through the soil. Following spiking and mixing, the carrier solvents were allowed to evaporate for a period of 48 h. For each study, blank study soils and test soils spiked with carrier solvent only were prepared as controls. The moisture content of the test soils was maintained to within 10% of 60% of the maximum water-holding capacity (MWHC) by daily weighing of test beakers and the addition of deionized water where required.

For each compound, 45 beakers of spiked soil were prepared, along with solvent and nonsolvent controls. At the start of the exposure, one mature adult *E. fetida* (200–500 mg wet weight), with a visible clitellum, was added to each test beaker, and the burrowing time of each of the earthworms was recorded. Beakers were then covered with garden fleece, attached with an elastic band to prevent earthworms from escaping while allowing for sufficient aeration. Both the uptake and depuration phases each lasted for 21 d. Samples were taken at 0 and 6 h and 1, 3, 7, 10, 14, and 21 d of each phase. The pH of the soils was measured at the beginning and the end of the uptake phase and at the end of the depuration phase. *E. fetida* were fed weekly with mashed potato powder.[27]

At each sampling time point, three replicate beakers of the pharmaceutical-exposed earthworms were taken. At the start of the uptake phase and the end of the uptake and depuration phases, four replicates were sampled from the solvent controls to obtain analytical background values. The earthworms were then removed, rinsed with deionized water, blot dried and weighed, and then, placed on moist filter paper for 24 h to allow the earthworm to void its gut contents.[28] After 24 h, the earthworms were reweighed and then frozen (−20 °C) prior to analysis. A supplementary study indicated that maximum purging of gut contents occurred over 24 h with 77% of the soil gut contents being eliminated (unpublished data). A correction (assuming a soil content of 23% of the body weight) was therefore applied to the experimental earthworm concentration measurements to account for the soil remaining in the earthworm gut and ensure the analysis focused on tissue concentrations only. Samples of soil were also taken for soil analysis and for immediate extraction of soil pore water.

Three replicates of soil spiked with radiolabeled pharmaceutical but containing no earthworms were also prepared and sampled at the end of the uptake phase to check for changes in the concentrations of the study compounds in soil and pore water in the absence of the test organism.

Preparation of Samples for Analysis. Pore water, soil, and earthworm extraction methods are described in full detail in the Supporting Information. Briefly, pore water was extracted by a centrifugation method, and pharmaceuticals were extracted from the test soils and earthworms by liquid extraction. Methanol, ethyl acetate, acetonitrile/water (70:30 v/v), and acetonitrile were used as solvents for the *E. fetida* and soil extractions for carbamazepine, diclofenac, fluoxetine, and orlistat, respectively. Method validation studies showed that average recoveries ranged from 82.8% (diclofenac) to 100.6% (carbamazepine) from the soils and from 86.3% (fluoxetine) to 100.9% (carbamazepine and diclofenac) from the earthworms.

Despite the high extraction recoveries for diclofenac, the concentration at the start of the experiment was significantly lower than expected. A large amount of dissipation of radioactivity from the orlistat test beakers was also observed, which unlike with the other test compounds, could not be explained by uptake into the *E. fetida*. Combustion analysis of the diclofenac and orlistat soils (see the Supporting Information) was therefore performed to determine the radioactivity remaining in the soil (i.e., nonextractable residues), which could account for the observed discrepancies.

Liquid Scintillation Counting. Radioactivity in soil pore water, soil and earthworm extracts was determined using liquid

Table 1. Test Pharmaceutical Physico-Chemical Properties

pharmaceutical	class	CAS no.[a]	molar mass (g/mol)	log K_{ow}[b]	acid/base	pK_a[c]	sorption coefficient (K_d) (L/kg)[d]	specific activity (GBq/mmol)
carbamazepine	antiepileptic	298-46-4	236.30	2.25	base	14	4.83 ± 0.68	0.74
diclofenac	anti-inflammatory	15307-79-6	318.13	4.02	acid	4.12	28.7 ± 3.27	2.30
fluoxetine	antidepressant	54910-89-3	345.80	4.65	base	9.53	608 ± 87.6	2.04
orlistat	weight loss aid	96829-58-2	497.74	8.19	neutral	N/A	1494 ± 103	2.05

[a]CAS no. obtained from the Chemical Abstracts Service. [b]Log K_{ow} values obtained from KOWWIN v. 1.68 database, USEPA EPI suite 4.1 program. [c]pK_a values were predicted using the University of Georgia SPARC database v. 4.2. http://ibmlc2.chem.uga.edu/sparc (accessed May 25, 2012). [d]K_d values were determined experimentally following OECD 106; average values are provided ± standard deviation (unpublished data).

scintillation counting (LSC) using a Beckman LS 6500 LSC counter (Beckman Coulter Inc., Fullerton, CA, USA). Samples were counted three times, each for 5 min. Counts were corrected for background activity by using blank controls. Counting efficiency and color quenching were corrected for using the external standard ratio method. Measured radioactivity of the APIs in the earthworm extracts were corrected to account for soil-associated APIs present in the gut.

Potential Metabolism. To ascertain whether the radioactivity measured in the earthworm samples was that of the parent compound or metabolite/transformation products, additional studies were performed using nonlabeled carbamazepine, diclofenac, and fluoxetine. Studies were performed at 20 times the soil concentration in the radioactive studies. This concentration difference was used to ensure that compounds were detectable in the earthworm tissue. While it is possible that changes in concentration can affect uptake, our previous studies indicate that differences of this magnitude have no significant effect on uptake kinetics of APIs.[10] Extracts were analyzed by liquid chromatography−tandem mass spectrometry (LC-MS/MS) using a Dionex Ultimate 3000 and Applied Biosystems API 3000. Where necessary (e.g., diclofenac), extracts were further analyzed by liquid chromatography−Fourier transform mass spectrometry (LC-FTMS) (solariX 9.4T Bruker) to determine if known transformation products were present in the samples. Due to analytical limitations, studies to ascertain whether metabolism of orlistat had occurred in *E. fetida* could not be performed (see the Supporting Information for further details on LC-MS/MS and LC-FTMS methodology).

Modeling. *Earthworm Kinetic Modeling.* Previous studies have shown that contaminant uptake from soil occurs primarily from pore water.[29−31] Uptake and depuration kinetic modeling was therefore performed based on concentrations in pore water. A first-order one-compartment model was used to estimate the uptake and depuration rates for each test compound from pore water. The toxicokinetic model, as described in eq 1, was fitted to measured internal earthworm concentration data and kinetic bioconcentration factors (BCFs) were then calculated based on principles outlined by Ashauer et al.[32,33] Parameter estimation was carried out in OpenModel (v 1.01; University of Nottingham, 2008; http://www.nottingham.ac.uk/environmental-modelling/OpenModel.htm):

$$\frac{dC_{organism}}{dt} = k_{in}\cdot C_{water}(t) - k_{out}\cdot C_{organism}(t) \quad (1)$$

where t is time (h), k_{in} is the uptake rate constant (L/(kg d)), C_{water} is the concentration in the pore water (Bq/L), k_{out} is the depuration rate constant (d^{-1}), and $C_{organism}$ is the concentration in the organism (Bq/kg).

Modeling Dissipation of Study Compounds in Soil. A simple first-order degradation kinetic model was fitted to the results of the soil analysis during the uptake phase. Model parameters were optimized according to recommendations by FOCUS,[34] using the least-squares method with Microsoft Excel Add-Inn Solver. Half-lives (DT_{50}, the time for a 50% decline in the concentration of pharmaceutical) were then calculated using a true replicates FOCUS[34] spreadsheet.

Comparison of Data to Predictive Models. Models exist to predict environmental exposure scenarios such as those outlined in the Technical Guidance Document (TGD) on Risk Assessment (Part 2).[35] Pore water concentrations obtained in this study were compared to estimated concentrations (PEC_{pw}), calculated using the sorption coefficients (K_d) derived for each pharmaceutical compound (Table 1) and using equations in the TGD[35] (for PEC_{pw} equation, see Figure SI 2, Supporting Information). BCFs obtained in this study were compared to estimated BCFs using quantitative structure−activity relationships (QSARs) outlined in Belfroid et al.[36] and Jager[37] to evaluate predictive models based on pore water only exposure.

Statistical Analysis. Statistical analysis of the data was performed using SigmaPlot (v. 12). For each compound, data on burrowing times and percentage weight gain from the toxicity study were subject to Shapiro−Wilk and Levene−Mediane tests, to test for normality and equal variance, respectively. If they passed, then a one-way analysis of variance (ANOVA) was performed to assess the differences in the values among the treatment groups; where normality failed, ANOVA was performed using a Kruskal−Wallis analysis on ranks. Differences between the measured and predicted pore water concentrations were first tested for normality using a Shapiro−Wilk test. As normality failed for each API, the difference between measured, and predicted values was then evaluated by a Mann−Whitney U Rank test. The relative accuracy of the estimated results was estimated by calculating proportional deviation from the measured to the estimated value.

RESULTS AND DISCUSSION

Sorption of Study Pharmaceuticals to Test Soil. The sorption coefficients (K_d), based on batch experiments, increased in the order of carbamazepine < diclofenac < fluoxetine < orlistat (Table 1). Although the sorption of a pharmaceutical can vary considerably depending upon the soil type,[38] the values for carbamazepine, diclofenac, and fluoxetine fall within the ranges previously reported in the scientific literature.[39−42] The results suggest that orlistat has a particularly strong sorption capacity to the soil; this may be due to its being particularly hydrophobic (the log K_{ow} for orlistat is 8.19).

Uptake and Depuration of Pharmaceuticals in *E. fetida*. *Main Trends in Soil and Pore Water Data from Uptake Phase.*

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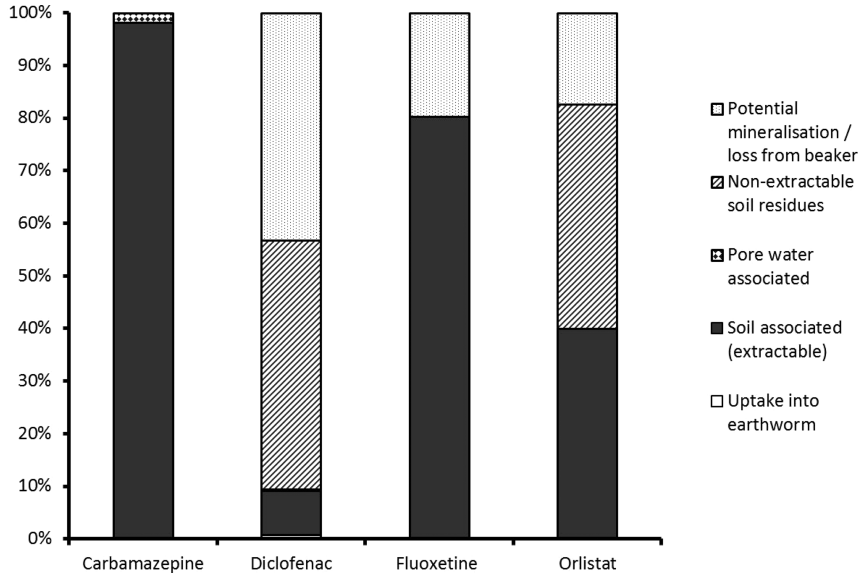

Figure 1. Percentage of radioactivity associated with different compartments in the soil−earthworm system at the end of the uptake phase of the experiment in comparison to applied radioactivity at 0 h.

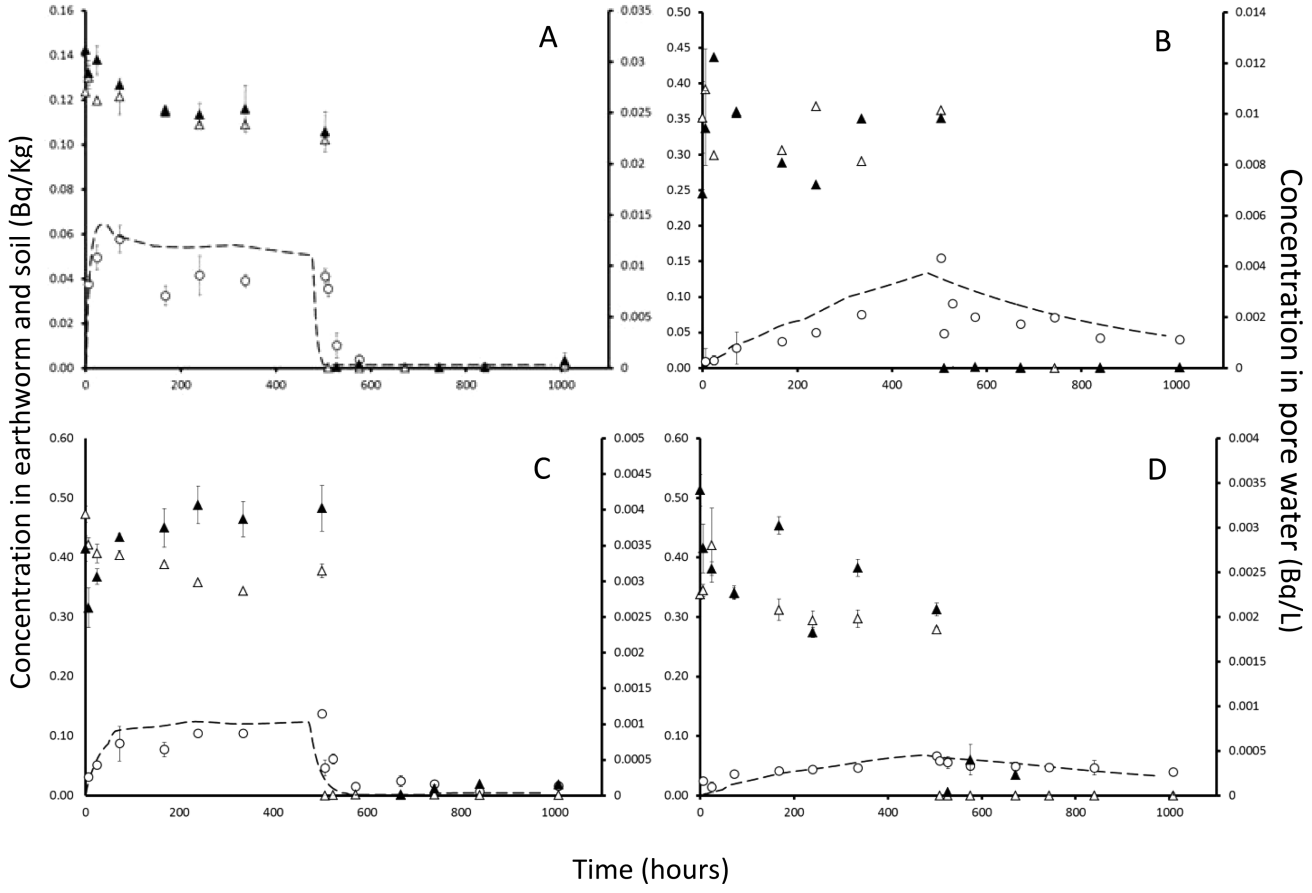

Figure 2. Uptake and depuration curves for *Eisenia fetida* exposed to (A) carbamazepine, (B) diclofenac, (C) fluoxetine, and (D) orlistat. Mean (±SE) measured concentrations in the earthworm are represented by the circles, and the data lines represent the first-order model fit (wet weight). Mean concentrations (±SE) in the soil and soil pore water are represented by the open and closed triangles, respectively.

For all pharmaceuticals, throughout the exposure phase, there was a decrease in radioactivity in the soil followed by an increase in radioactivity in the earthworm (Figures 1 and 2). Almost all the applied radioactivity was recovered from the test soil in the earthworm free beakers (>94% recovery) for carbamazepine, fluoxetine, and orlistat. Thus, if a decline in ^{14}C activity was seen in the earthworm treatments, this was likely to be related to the presence of the organisms.

While solvent extraction data show dissipation of orlistat- and diclofenac-derived radioactivity during the exposure phase, the

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Table 2. Summary of Key Results from Uptake and Depuration Experiments, Including pH Range of Soil throughout Each Exposure and the Time Taken for 50% and 90% Degradation of the Pharmaceuticals in Soil[a]

pharmaceutical	pH	DT_{50} (d)	DT_{90} (d)	k_{in} (uptake rate) (L/(kg d))	k_{out} (depuration rate) (d^{-1})	$BCF_{pore\ water}$ (LCI−UCI)
carbamazepine	6.3 ± 0.2	68	226	0.24	0.14	2.2 (1.3−3.5)
diclofenac	6.2 ± 0.1	N/A	N/A	0.036	0.0021	21.5 (13.9−30.6)
fluoxetine	6.3 ± 0.2	66	220	1.11	0.047	30.8 (25.4−35.8)
orlistat	6.2 ± 0.2	48	159	0.071	0.0016	51.5 (40.0−65.3)

[a]The modelled *E. fetida* uptake and depuration rates including the pore-water-based BCF with lower and upper confidence intervals (LCI−UCI) (all based on wet weight) are also provided. Diclofenac soil concentrations could not be modelled.

soil combustion data for orlistat showed that this was not due to mineralization of the compound but due to the formation of bound residues in the soil matrix. However, for diclofenac, combustion and solvent extraction data could only account for greater than 56% of the applied radioactivity, suggesting perhaps a loss of ^{14}C carbon dioxide released via mineralization of this API (Figure 1). Formation of nonextractable residues in soils has been investigated since the 1980s;[43] however, little research has explored pharmaceutical residues bound in soil.[44,45] It is important to note that a chemical that may be irreversibly sorbed to soil may remain bioavailable for uptake by soil organisms.[46]

Carbamazepine activity decreased slightly in the pore water over the period of the exposure phase, which can probably be explained by the decrease in activity in soil over 21 d (Figure 2). Only in the fluoxetine study was an increase in radioactivity measured in the pore water throughout the uptake phase (Figure 2). Nevertheless, the rapid uptake into the earthworms observed in the fluoxetine and carbamazepine studies would suggest these chemicals were bioavailable. By the end of the uptake phase, only 50% of the initial activity of orlistat was measured in the pore water, possibly due to the strong sorption of orlistat to the soil, as demonstrated by the high K_d for this chemical (Table 1). Even with the high sorption to soil, orlistat was still bioavailable but to a limited extent, given that uptake did occur throughout the 21 days.

Of all the compounds, carbamazepine had the highest activity in the pore water, which might explain the initial rapid uptake into the earthworms, while the slow uptake of orlistat could potentially be explained by the lowest activity in the pore water suggesting that this compound was not bioavailable.

Pharmaceutical Degradation. Dissipation of activity from the test soil for each of the API treatments (with exception of diclofenac) was modeled using single first-order kinetics (Figure SI 1, Supporting Information). For carbamazepine, fluoxetine, and orlistat, the soil dissipation data were well explained by single first-order kinetics with χ^2 values all below the accepted level recommended by FOCUS[33] (Table SI 4, Supporting Information). For diclofenac, there was variation in measured activity in soil between the replicates and no consistent dissipation (Figure 1) that resulted in a poor model fit and thus was not included in the analysis of degradation rates. The half-lives (DT_{50}) in the carbamazepine and fluoxetine studies would suggest these are the most stable chemicals relative to the other chemicals assessed (Table 2). These studies are in agreement with previous work where little to no degradation was observed for these two compounds with measured half-lives greater than 60 d.[2,47,48] Previous research has shown a strong influence of soil type, in particular clay content, on the degradation of diclofenac in soils.[41] The high clay content of the test soil in the present study and the negative regression between clay content and diclofenac degradation may explain the minimal diclofenac degradation observed in this study in comparison to previous research where half-lives of up to 20.44 d have been reported.[39]

Uptake and Depuration of Pharmaceuticals. As no toxic effects, in terms of burrowing behavior, weight change, or mortality, on the earthworms were observed at ×10 and ×100 the proposed test concentrations for all pharmaceuticals (detailed results can be found in the Supporting Information), effects of toxicity on uptake can likely be excluded. The uptake and depuration studies also passed the validity criteria, based on mortality and change in earthworm mass, as set out in the OECD 317 protocol.[26] *E. fetida* accumulated radiolabeled material from all treatments, but the degree and pattern of uptake varied (Figure 2). For carbamazepine, uptake increased over the first 168 h of exposure, after which time it declined, possibly due to the observed dissipation in the soil and soil pore water (Figure 2A). Similarly, in the fluoxetine treatment, internal radioactivity in *E. fetida* increased over the first 168 h of the exposure phase and then appeared to reach a steady state (Figure 2C). For diclofenac and orlistat treatments, uptake continuously increased and did not appear to have reached a steady state by the end of the exposure phase (Figure 2B, D).

As soon as the depuration phase began, *E. fetida* immediately eliminated radiolabeled material in all four pharmaceutical treatments. For carbamazepine and fluoxetine, this was rapid with complete elimination within 72 and 168 h of the start of the depuration phase, respectively (Figure 2). Although elimination from the diclofenac treatment was also rapid at the start, the accumulated material was not completely eliminated from the earthworm, as by the end of the depuration phase, more than 20% of the accumulated radiolabel remained in the tissue. Elimination of radiolabeled material in the orlistat treatment was the slowest from *E. fetida*. By the end of the depuration phase, greater than 60% of accumulated radiolabel remained in the earthworm. Accounting for soil, earthworm, and pore water concentrations, a mass balance calculation was performed to account for the radioactivity in the experiment. More than 80% of the radioactivity was recovered from the test beakers for carbamazepine, fluoxetine, and orlistat treatments, whereas as little as 56% of the radioactivity was accounted for in the diclofenac treatment (Figure 1).

The first-order one-compartment model, based on pore water measurements, was successfully fitted to the uptake and depuration data for all four study compound treatments (Figure 2A−D). Uptake and depuration rates are provided in Table 2. The calculated pore-water-based BCFs increased in the order of carbamazepine < diclofenac < fluoxetine < orlistat. The relatively large BCF of 51.5 for the orlistat treatment may be attributed to the minimal elimination of labeled material from the earthworm in the depuration phase while for carbamazepine the fast elimination of 0.14 d^{-1} can account for the smaller BCF of 2.21. The BCFs increase in a similar order to the increase in the log octanol−water partition coefficient (K_{ow}) for the respective

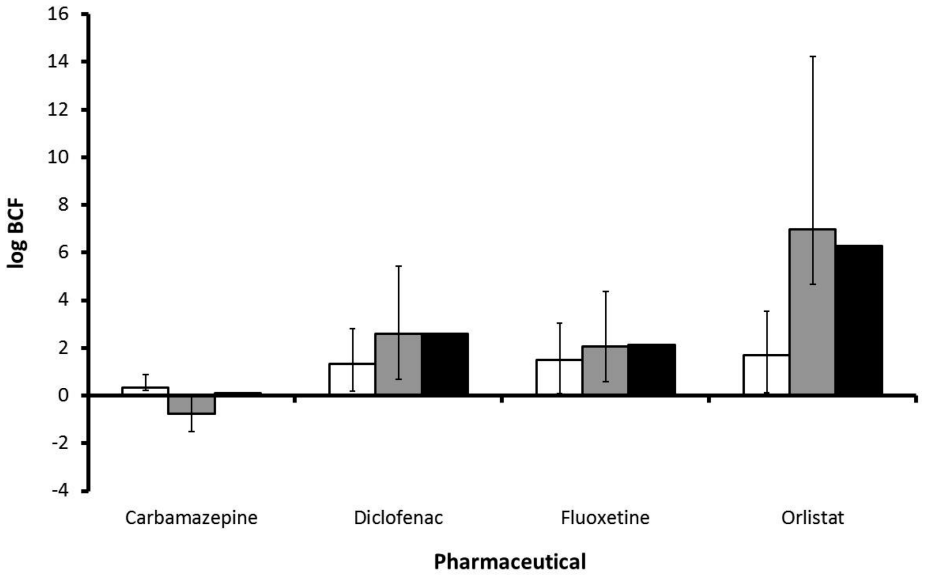

Figure 3. Comparison between earthworm BCFs obtained from the model in this study (white), predictions from the QSAR described in Belfroid et al.[36] (gray), and predictions from the QSAR in the TGD based on Jager[37] (black) for carbamazepine, diclofenac, fluoxetine, and orlistat. Error bars represent the upper and lower confidence intervals of the BCFs.

compounds, supporting previous research that has suggested that the degree of hydrophobicity has a key role to play in the uptake of pharmaceuticals into organisms (e.g., Ashauer et al.[33]).

In comparison to aquatic BCFs for pharmaceuticals published in the scientific literature, earthworms seem to have lower BCF values. For example, BCFs for fluoxetine and diclofenac have been reported at values much larger than calculated for the earthworms in this study.[8,49,50] Specifically, for fluoxetine, the BCFs have been reported up to 185 900 in the fresh water shrimp (*Gammarus pulex*)[10] that is over 6000 times greater than the BCF generated for earthworms. However, this is not surprising as many aquatic organisms have gills, which have a large surface area, and together with water actively being moved through the organism there is an increased potential for the uptake of chemicals in comparison to earthworms. Both of these compounds are ionizable at environmentally relevant pH ranges, so it is possible that differences in uptake between this study and aquatic studies are explained by differences in environmental conditions such as pH. BCFs for carbamazepine in aquatic organisms are similar to the BCF of 2.21 obtained in this study[10,50] with results from Vernouillet et al.,[51] for example, showing that the algae (*Pseudokirchneriella subcapita*) has a BCF of 2.2.

It is important to recognize that these uptake findings are based on tracking a ^{14}C label, irrespective of whether it was a parent or transformation production. Therefore, in a separate, unlabeled study, LC-MS/MS and LC-FTMS analyses were used to determine whether or not the observed uptake was due the parent API or to their metabolites or transformation products. Both carbamazepine and fluoxetine were detected in the earthworm tissue at concentrations slightly greater than expected from the results of the radiolabeled studies, indicating that the parent APIs are the compounds being taken up and eliminated in the radiolabeled studies (Table SI 3, Supporting Information). In previous studies, the major metabolite of fluoxetine, norfluoxetine, has been detected in fish;[7] however, the results presented in this study suggest that fluoxetine was not metabolized in earthworms and thus species-specific metabolism may occur. However, diclofenac was not detected (Table SI 3, Supporting Information), and LC-FTMS analysis, in which extracted ion chromatograms for known diclofenac metabolites and transformation products collated from literature sources (Table SI 5, Supporting Information) were plotted, resulted in no peaks for such chemical species. Although unlabeled experiments were not carried out for orlistat, previous published research has demonstrated the presence of two major orlistat metabolites (primary metabolite (M1) and secondary metabolite (M3)) in human plasma, which together comprised a total of 42% of the total radioactivity in plasma.[52]

Biological attributes such as species size, feeding habits, and reproduction may play a key role in uptake and bioconcentration of pharmaceuticals. Previous work has suggested that an increase in organism size results in a decrease in BCF,[53] and while this is true for the insecticide *p,p′*-DDE as bioaccumulation in the smaller *E. fetida* was up to 6 higher than in *Lumbricus terrestris*,[54] pharmaceutical uptake into different earthworm species has not yet been evaluated to explore this concept further. Mercury accumulation in earthworms has demonstrated that species length and age is important in chemicals assimilating in tissues, with decreased mercury content following increased growth and development.[55] In the aquatic environment, a positive relationship between lipid content and bioconcentration of chemicals has also been suggested.[53,56,57] This would suggest differences in accumulation of pharmaceuticals in earthworms as lipid contents can range between 1% and 20% for different species.[58]

Evaluation of Existing Predictive Models. It has been suggested that uptake (and effects) of chemicals in soils and sediments can be predicted based on estimates of pore water concentrations, determined from K_d values from batch sorption studies and BCF values predicted using QSARs.[35] We therefore used data to determine whether such an approach would work for pharmaceuticals and earthworms.

Pore water concentrations of pharmaceuticals throughout the uptake and depuration phases were estimated using a combination of soil concentrations in the uptake and depuration studies and the results of batch sorption experiments. The estimated pore water concentrations were then compared to the measured pore water values obtained in the studies. For

fluoxetine and orlistat, which had the highest K_d values, pore water concentrations were significantly underestimated (U = 699, $p < 0.05$ and $U = 654$, $p < 0.05$, respectively, Figure SI 2 C, D, Supporting Information). For carbamazepine and diclofenac, which were the APIs less strongly sorbed to the soil, the estimated pore water concentrations were closer to the measured data but still significantly overestimated ($U = 761$, $p < 0.05$ and U = 755, $p < 0.05$, respectively). These results indicate that K_d values obtained from batch studies may not be appropriate for estimating pore water concentrations. Thus, the equations for estimating PEC_{pw} provided in, for example, the TGD on Risk Assessment,[35] for use in exposure modeling scenarios, may not be appropriate for APIs and further refinement of model equations, using parameters in addition to K_d, may necessary.

The QSARs generally overestimated the pore water BCFs, particularly for orlistat where the estimated BCFs were up to 6000 times greater than the kinetic BCF (Figure 3). This may be because of the molecular weight cutoff that is generally seen for compounds with a high log K_{ow} such as orlistat. It is also important to note that neither of the QSARs was developed specifically to predict pharmaceutical uptake. For example, the QSAR determined by Belfroid et al.[36] has a limited log K_{ow} window (4.2−5.7 that was later extrapolated to 2−7) and was developed specifically for neutral compounds. The application of approaches that consider the ionized state of the molecule might improve predictions of earthworm BCFs for pharmaceuticals. A number of models have been proposed, which account for impacts of ionization on uptake.[59−61] For example, the cell model uses Fick's first law of diffusion for the neutral molecules and the Nernst−Planck equation for the ionizable fraction of molecules to predict the movement, by diffusion, of molecules into a living cell.[61] The equilibrium concentration ratio between the inside and outside of the cell calculated in this model can currently be used to predict fish BCFs and with further research may hold some potential to accurately predict earthworm BCFs.

■ ASSOCIATED CONTENT

Supporting Information

Detailed information on sorption of study compounds to soil, *E. fetida* toxicity experiment, preparation of samples for analysis, detailed methods of metabolism study, soil properties, and dissipation modeling. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. Conceived and designed the experiments: L.J.C., A.B.A.B., J.R.; performed the experiments: L.J.C., C.D.G.; designed and developed MS methods: L.J.C., A.D., E.B., J.T.O.; analyzed the data: L.J.C., A.B.A.B., J.R., J.T.O., E.B., A.D.; wrote the paper: L.J.C., A.B.A.B.

Notes

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