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Flume Experiments To Investigate the Environmental Fate of Pharmaceuticals and Their Transformation Products in Streams

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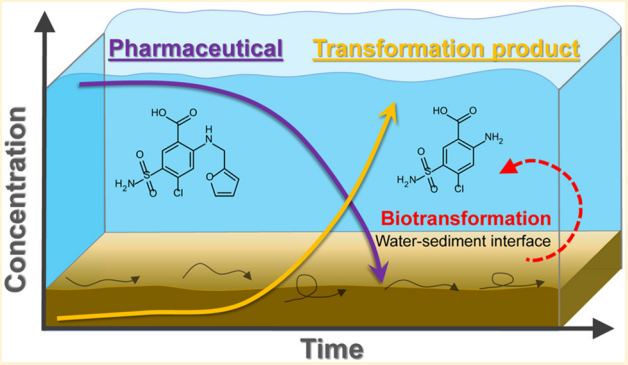
Supporting Information

ABSTRACT: The hyporheic zone—the transition region beneath and alongside the stream bed—is a central compartment for attenuation of organic micropollutants in rivers. It provides abundant sorption sites and excellent conditions for biotransformation. We used a bench-scale flume to study the fate of 19 parent pharmaceuticals (PPs) and the formation of 11 characteristic transformation products (TPs) under boundary conditions similar to those in hyporheic zones. The persistence of PPs ranged from readily degradable with a dissipation half-life (DT_{50}) as short as 1.8 days (acetaminophen, ibuprofen) to not degradable (chlorthalidone, fluconazole). The temporal and spatial patterns of PP and TP concentrations in pore water were heterogeneous, reflecting the complex hydraulic and biogeochemical conditions in hyporheic zones. Four TPs (carbamazepine-10,11-epoxide, metoprolol acid, 1-naphthol, and saluamine) were exclusively formed in the sediment compartment and released to surface water, highlighting their potential to be used as indicators for characterizing hyporheic transformation of micropollutants in streams. The accumulation of certain TPs over the experimental period illustrates that we might face a peak of secondary contamination by TPs far from the point of release of the original contaminants into a stream. Such TPs should be considered as priority candidates for a higher-tier environmental risk assessment.



INTRODUCTION

During the last two decades, particular scientific focus has been put on pharmaceuticals in aquatic systems. Their occurrence at concentrations in the ng L^{-1} to μg L^{-1} range has been reported in receiving waters (e.g., rivers and lakes)[1−3] as well as in groundwater.[4,5] Despite the frequent detection of pharmaceuticals in the environment, we have substantial knowledge gaps with respect to their environmental fate, which in combination with limited long-term monitoring data prevent us from performing scientifically sound environmental risk assessments for many of these chemicals.

Biotransformation, photolysis, and sorption to sediments and biofilms are the most important dissipation processes for pharmaceuticals in surface water.[6−10] Among those processes, microbial transformation in the hyporheic zone, which is the transition zone between surface water in streams and groundwater and which provides excellent conditions for turnover of nutrients and organic matter,[11] is hypothesized to be the most significant removal pathway for many compounds.[12] Experiments on the laboratory scale have proven the essential role of sediment during the elimination of certain pharmaceuticals.[10,13] However, transformation mechanisms and kinetics on the field scale are still poorly understood due to (i) difficulties in extrapolating results from lab-based batch experiments to the field and (ii) a lack of comprehensive information on the nature of transformation products (TPs), which is indispensable for generating a holistic understanding of the environmental fate of pharmaceuticals. While transformation is frequently associated with detoxification of contaminants, products that are less degradable and/or more toxic than their parent compounds can be also formed.[14] The generation of TPs in activated sludge has been investigated in several studies,[e.g.,15−18] while much less work has been carried out on TP formation in sediments.[19−21]

In the present study, we carried out experiments in a bench-scale flume to investigate the relationship between hydraulic conditions and transformation of pharmaceuticals. Our aim was to link the fate of parent pharmaceuticals (PPs) to the behavior of their TPs under defined yet realistic hydraulic conditions. The flume has an annular channel, allowing for continuous transport of sediment and water and thus approximating conditions in streams. Nineteen pharmaceuticals selected from a wide physical−chemical property range were studied along with their TPs to provide comprehensive information on their distribution and fate in the water/sediment system. We periodically collected surface water samples, pore water samples at different depths, and also sediment samples for analysis of

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both PPs and TPs. Experiments were conducted with two different sediment bed morphologies, which were hypothesized to generate different exchange rates between the water and the sediment compartments.

EXPERIMENTAL METHODS

Chemicals and Reagents. Details on all chemicals are provided as Supporting Information.

Water and Sediment. The experiments conducted in this study required sediment neither too fine nor too coarse, and water and sediment with a negligible background level of PPs and TPs. As no suitable stream could be identified within reasonable distance from our laboratory that fulfilled these requirements, we conducted the experiments with water and sediment from Lake Largen (N 59°35′32″, E 18°32′09″) located north of Stockholm, Sweden. The lake has no known sources of pollutants other than atmospheric deposition. Water was used as sampled. Sediment was collected from the top 20 cm and wet sieved <2 mm; the porosity of the sieved sediment was 40%. Water and sediment were stored in darkness at 5 °C for a maximum of 1 week before use.

Experimental Design. The experiments were carried out in a recirculating flume. A sketch of the flume is presented in Figure 1, and a schematic drawing is provided as Figure S1 in the Supporting Information. Details on the flume construction and parametrization are discussed elsewhere.[22] Briefly, the flow velocity of surface water is regulated by a propeller, and collection of pore water is possible through sampling ports installed at two depths in the sediment compartment.

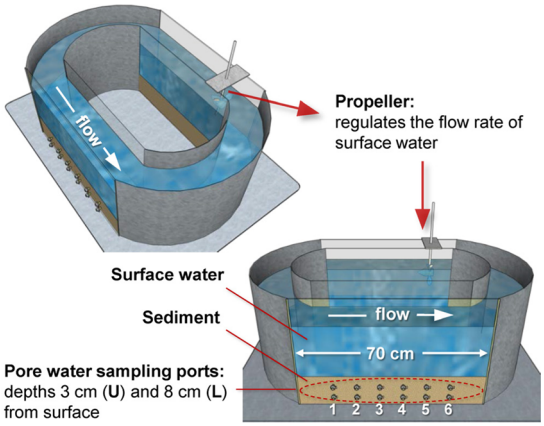

Figure 1. Sketch of the flume. Direction of surface water flow is from left to right. Pore water sampling ports are available at both upper (U) and lower (L) sediment layers.

Two experiments were conducted: experiment FLAT with even sediment surface (Supporting Information Figure S2-A) and experiment RIPPLE with artificial sediment ripples (Supporting Information Figure S2-B). In both experiments the flume was filled with water (V = 100 L) and sediment (V = 60 L). The sediment surface was shaped as desired prior to the experiments, and the surface water was set to a streaming velocity (0.13 m s^{-1}) at which no visible transport of sediment occurred with either morphology. The experiments were carried out in darkness in a climate-controlled room at a temperature of approximately 17 °C, representing the long-term average atmospheric temperature in July in the sampling region.[23] The system was equilibrated for 1 week before background surface and pore water samples were collected. The length of this period was based on experience[22] and is a compromise between equilibrating the system from a physical and biogeochemical point of view and avoiding thermodynamic limitations due to consumption of nutrients and electron acceptors. After equilibration, an aqueous solution containing 19 PPs was spiked into the surface water to yield an initial concentration of approximately 10 μg L^{-1} of each compound.

We did not carry out replicate flume experiments as replication would only be possible with two flumes filled with identical water and sediment that are run in parallel. Given the investment and the required lab space, this could not be realized. Sequential replicate experiments were not possible as (i) storing water and sediment for long time would inherently modify the microbial community as well as the availability of nutrients and trace elements between the two replicates, and (ii) collecting fresh water and sediment from Lake Largen after termination of the first replicate (i.e., at a time difference of 3–4 months) would not provide identical conditions due to the transient nature of biogeochemical conditions (e.g., due to temperature differences and nutrient concentrations).

Salt tracer experiments were carried out to determine the time for the flume system to approach equilibrium through mixing of surface and pore water (see Figure S3 in the Supporting Information). A sodium chloride (NaCl) solution was added into the surface water (final concentration: 0.5 g L^{-1}) after terminating the experiments, and electric conductivity was recorded automatically at a time interval of 15 min for 8 days with a probe (Hach, Düsseldorf, Germany) that was permanently installed in surface water.

In parallel to each flume experiment, water-only control experiments (for both experiments) and a sterile control experiment (for experiment RIPPLE only) were carried out. For water-only controls, 1 L of surface water was taken from the flume 24 h after spiking, transferred to a glass bottle, and used as it was. The sterile control was prepared by autoclaving (121 °C, 20 min) 900 mL of surface water (taken from the flume 24 h after spiking) together with 350 g of fresh sediment in a glass bottle, after which sodium azide (NaN_3, final concentration: 0.1%) was added to the water phase to inhibit microbial growth.

Sampling and Analysis. *Surface and Pore Water.* Both surface water and pore water from the upper and lower sediment layers were sampled at increasing time intervals over 29 days (at hour 2, day 1, 3, 7, 10, 15, 22, and 29 after spiking). Three 5 mL aliquots (replicates) of surface water were sampled at random locations. Pore water was withdrawn with a peristaltic pump (flow rate: 3 mL min^{-1}) connected to multislotted stainless steel needles which were permanently installed through the sampling ports. The first 1 mL (dead volume) of pore water was discarded before 5 mL were collected from each port. This pore water sampling scheme was chosen to avoid interferences between individual sampling ports and to avoid induction of a flow of surface water into the sediment (see also Kunkel and Radke[22]). Additionally, three replicate water samples from the control experiments were collected at the same times as the flume samples were taken. Immediately after sampling, a 900 μL aliquot was transferred to a glass beaker, and 100 μL of an acetonitrile (ACN) solution containing a mixture of 23 isotope-substituted internal standards (final concentration: 5 μg L^{-1}) and acetic acid (HAc, final concentration: 10 mmol L^{-1}) was added before the sample was filtered (0.45 μm PTFE syringe filter).

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Sediment. Sediment was only sampled prior to and after termination of each experiment to avoid major disturbance of the system during the experiments. Due to difficulties in obtaining representative grab samples over the whole depth, sediment microcores were collected from 10 random locations and pooled. Three replicates of wet sediment (4 g) were analyzed; each was spiked with 10 μL of a solution containing 5 ng of each isotope-substituted internal standard. Extraction was performed by adding 5 mL of ACN and vortexing the slurry for 2 min; afterward, the samples were centrifuged for 5 min at 4000 rpm (centrifuge C312, Jouan, Germany), and the supernatant was transferred to a new tube. The extracts were evaporated to dryness under a nitrogen stream (35 °C) and thereafter dissolved in 1 mL of H_2O:ACN (90:10, v:v) containing 10 mmol L^{-1} HAc.

Analysis. All samples were stored at −18 °C before being thawed and analyzed with ultrahigh performance liquid chromatography (Acquity system, Waters, Manchester, U.K.) coupled with a triple quadrupole mass spectrometer (Xevo TQ-S, Waters) using electrospray ionization. Separation was achieved on a HSS T3 column (100 mm × 2.1 mm, particle diameter 1.8 μm; Waters) with a binary gradient. Details on the analytical method are provided as Supporting Information.

The analytes included 19 PPs as well as 11 TPs that were identified in a previous study using batch incubation of water and sediment. Authentic standards were available for all TPs.[19] The internal standards method was used for quantification. Calibration curves were obtained by a weighted ($1/x$) linear least-squares regression of a series of 10 calibration standards with the analytes in the concentration range from 0.01 to 10 μg L^{-1}. This calibration series was measured at the beginning and end of each sequence. Intraday precision of the instrument was tested by analyzing a calibration standard (concentration: 1 μg L^{-1}) before each sequence. Four of the calibration standards and solvent blanks (H_2O:ACN 90:10, v:v, 10 mmol L^{-1} HAc) were measured every 12 samples for quality control purposes. The limit of detection (LOD) and limit of quantification (LOQ) for each analyte were determined on the basis of the lowest calibration standard in which the product ion used for quantification had a signal-to-noise ratio >3:1 and >10:1, respectively (see Table S2 in Supporting Information for details).

Routine Parameters. Total organic carbon (TOC) in surface and pore water was measured at the beginning and end of the experiments using a TOC-VCPH analyzer (Shimadzu, Kyoto, Japan) following the nonpurgeable organic carbon (NPOC) protocol. Conductivity and pH in surface and pore water and dissolved oxygen in surface water were measured during the experiments using hand-held probes. Details on the routine parameter measurements are available in the Supporting Information.

Calculations. In the initial phase of the experiments decreasing concentrations in surface water—where the PPs were spiked—were expected due to mixing with sediment pore water. The equilibrium concentration was calculated as the spiked amount of a compound divided by the total volume of water. In order to calculate dissipation rates (DT_{50}) not influenced by mixing, PP concentrations in surface water at each time step were corrected for dilution using fluconazole as a reference compound. According to its physical−chemical properties and to preliminary experiments fluconazole was estimated nonsorbing and persistent,[24] which is supported by the results of this study (see Results and Discussion below). Compared to typically used conservative tracers such as bromide, using a pharmaceutical for this purpose has the advantages that the tracer had similar diffusion properties as the analytes and that no additional analytical method with its associated uncertainties had to be applied. DT_{50} values were calculated by fitting first-order kinetics to the corrected surface water concentrations (see Supporting Information equations S1−S3),[22] excluding the data from day 0 to avoid artifacts from initial inhomogeneity. Multiple t tests with Bonferroni correction were performed to compare DT_{50} of the PPs between the two experiments. To calculate the mass of analytes sorbed to sediment, we subtracted the mass stemming from the dissolved fraction of the sediment sample (i.e., the pore water) from the total mass determined in sediment (see Supporting Information equations S4−S5). Formation of TPs is reported as relative yield, calculated by dividing the molar concentration of a TP by the initial molar concentration of its corresponding PP. Evaporation losses of water during the experiments were taken into account by correcting the absolute surface and pore water concentrations with the ratio of surface water height at the sampling time to the initial value.

Quality Control and Quality Assurance. None of the analytes was detected in the sediment collected prior to start of the experiments or in surface and pore water collected before spiking. The intraday coefficient of variation of the analytical instrument was <4%. The standard deviations derived from the quality control samples including four calibration standards were <15%. The recoveries of the analytes in sediment (n = 3) ranged from 86 ± 5% (metoprolol acid) to 112 ± 8% (chlorthalidone) with three exceptions; low recoveries were obtained for ibuprofen, 2-hydroxyibuprofen, and carboxyibuprofen. Relative standard deviations for replicate sediment samples (n = 3) were <14%, calculated for all PPs but those removed completely from the system at the end of the experiments (i.e., acetaminophen, bezafibrate, ibuprofen, ketoprofen, and naproxen). Relative standard deviations for replicate surface water samples (n = 3) were <8% for all analytes except diclofenac, for which quantification was more uncertain due to analytical difficulties. Further, glimepiride had an unexpected behavior in surface water (increasing concentration until day 10, followed by a decrease), which we believe was due to problems in dissolving glimepiride in the spiking solution. Both diclofenac and glimepiride were excluded from further interpretation.

RESULTS AND DISCUSSION

Control Experiments and Boundary Conditions. In the water-only controls for both experiments hydrochlorothiazide and four acidic pharmaceuticals (acetaminophen, bezafibrate, ibuprofen, and ketoprofen) dissipated with overall losses ranging from 7% to 28% during the 29-day period, and respective TPs were detected for bezafibrate and hydrochlorothiazide, as illustrated in Supporting Information Figure S4. As for these five PPs, the results from the sterile control were similar to those of the water-only controls, we conclude that their loss in the control experiments can be attributed to abiotic transformation. Moreover, the concentration of hydrochlorothiazide decreased by up to 28%, with its TPs accumulating over the entire period. This observation agrees with the results from previous studies,[13,19] showing that the loss of hydrochlorothiazide in a water/sediment system can to a large extent be attributed to abiotic transformation processes such as cleavage of the thiazide ring in a hydrolytic reaction. In

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the sterile control an approximate 20% loss was observed for each of the three beta-blockers metoprolol, propranolol, and sotalol, while their concentrations in the water-only controls remained constant throughout the experiment. Sorption by electrostatic interactions of the positively charged beta-blockers to negatively charged sites in sediment such as natural organic matter[13,25] is likely the dominant dissipation process for these compounds in the sterile control. All routine parameters were constant in surface and pore water in both flume experiments (Supporting Information Table S3), indicating uniform biogeochemical boundary conditions during the 29-day experimental period.

Dissipation of Parent Pharmaceuticals from Surface Water. Concentration time trends of all investigated PPs in surface water are illustrated in Figure 2. In general, these PPs

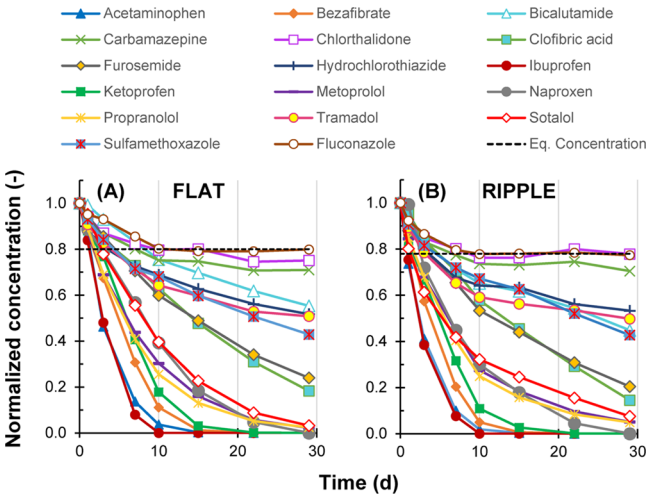

Figure 2. Time trends of parent pharmaceuticals (PPs) in surface water for experiment FLAT (A) and RIPPLE (B). Concentrations were normalized to the measured initial value (t = 2 h) for each compound. Dashed lines represent the calculated equilibrium concentrations in surface water accounting for the complete mixing of surface water and pore water.

spanned the range from being not detectable after 10 days (acetaminophen, bezafibrate, ibuprofen) to being persistent throughout the 29-day experiment (carbamazepine, chlorthalidone, and fluconazole). At the end of both experiments, the concentrations of the majority of the PPs in surface water were significantly lower than the calculated equilibrium levels, although dissipation rates differed considerably among the PPs. The concentration of fluconazole was constant from day 10 onward, and its final concentration agreed well with the calculated equilibrium concentration in both experiments (dashed lines in Figure 2). In combination with its behavior in the sediment compartment (Supporting Information Figure S6) and in the control experiments (Supporting Information Figure S4), this documents that fluconazole was neither transformed nor sorbed to sediment and that it can thus be used as reference compound to correct for dilution. Moreover, the behavior of fluconazole shows that within 10 days the predominant part of pore water was exchanged with surface water in both experiments, which also agrees with the results of the salt tracer experiments (see Supporting Information Figure S3; the somewhat faster equilibration of the salt tracer can be attributed to a 20% evaporation loss of water prior to start of the salt tracer experiment).

The acidic PPs (acetaminophen, bezafibrate, ibuprofen, ketoprofen, and naproxen) were removed completely from surface water during both experiments, with hardly any presence in pore water (Supporting Information Figures S7–S11). In combination with their behavior in the control experiments (Supporting Information Figure S4), we conclude that the removal of these PPs, except naproxen, was due to both biotic and abiotic transformation processes, with a substantial contribution from biotic processes. The loss of naproxen—which did not dissipate in either control experiment—can exclusively be attributed to biotic transformation processes in the sediment compartment. All beta-blockers (metoprolol, propranolol, and sotalol) had similar final concentrations in surface water, with an average loss of 98% in experiment FLAT and 94% in experiment RIPPLE (Figure 2). These beta-blockers were as well detected in the upper layer pore water and at some occasions also in the lower layer pore water (Supporting Information Figures S12–S14). Furthermore, 45–62% of their initial mass was present in sediment at termination of the experiments (Supporting Information Table S4), indicating that sorption to sediment particles played an important role in the dissipation of these PPs from surface water, with biodegradation being responsible for the remaining mass loss. Accordingly, Radke and Maier (2014) demonstrated that the dissipation of metoprolol and propranolol from water is caused by a combination of microbial transformation and sorption.[13]

Generally, the fluconazole-corrected concentrations of all PPs but chlorthalidone decreased continuously from surface water (see Supporting Information Figures S7–S22 for individual substances), following first-order kinetics in both experiments (see Supporting Information Figure S5 for kinetic plots). The DT_{50} values of the PPs are reported in Table 1 and cover a wide range, i.e., from <2 days (for some acidic compounds) to 1–2 months (for bicalutamide, sulfamethoxazole, and tramadol) to far beyond the 29-day experimental time frame. For most PPs, the DT_{50} values determined in the two experiments were not significantly different. However, the three beta-blockers had longer DT_{50} in experiment RIPPLE, which we hypothesize to be caused by differences in establishing sorption equilibrium depending on the small-scale hydraulic conditions in the sediment compartment, even though the gross water/sediment exchange was similar between the two experiments.

The concentrations of fluconazole (Supporting Information Figure S6), carbamazepine (Supporting Information Figure S15), and chlorthalidone (Supporting Information Figure S16) remained stable in surface water after initial equilibration. While the final concentrations of chlorthalidone and fluconazole agreed with the calculated equilibrium values, there was a 5% loss of carbamazepine. Although it is not possible to derive a reliable DT_{50} for carbamazepine due to the need to extrapolate far beyond the time frame of our experiments, the detection of its TP carbamazepine-10,11-epoxide in both experiments confirms that this mass loss can indeed be attributed to transformation processes, as discussed below. The short DT_{50} for the acidic pharmaceuticals ibuprofen (1.8 d), bezafibrate (1.9 d), and naproxen (5.3 d) agree well with previous reports using the same experimental system but different boundary conditions.[22] In contrast, clofibric acid, an acidic compound shown to be stable in various aquatic systems[2,10,26,27] and used as a persistent reference compound for 8-day experiments by Kunkel and Radke,[22] dissipated by more than 80% in our study

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Table 1. Dissipation Half-Lives of Parent Pharmaceuticals (PPs) (DT_{50}, Calculated from Fluconazole-Corrected Concentrations) and Detected Transformation Products (TPs) in Experiments FLAT and RIPPLE

	experiment FLAT		experiment RIPPLE	
PP[a]	DT_{50} (d)[b]	detected TP[c]	DT_{50} (d)[b]	detected TP[c]
acetaminophen	1.8	–	1.8	–
bezafibrate	1.9	4-chlorobenzoic acid	1.9	4-chlorobenzoic acid
bicalutamide	49	–	37	–
carbamazepine	∞	carbamazepine-10,11-epoxide	∞	carbamazepine-10,11-epoxide
chlorthalidone	∞	–	∞	–
clofibric acid	14	–	12	–
fluconazole	∞	–	∞	–
furosemide	16	saluamine	15	saluamine
hydrochlorothiazide	57	4-amino-6-chloro-1,3-benzenedisulfonamide chlorothiazide	57	4-amino-6-chloro-1,3-benzenedisulfonamide chlorothiazide
ibuprofen	1.8	(2-hydroxyibuprofen) (carboxyibuprofen)	1.9	(2-hydroxyibuprofen) (carboxyibuprofen)
ketoprofen	2.2	–	2.0	–
metoprolol*	5.5	metoprolol acid (α-hydroxymetoprolol)	7.2	metoprolol acid (α-hydroxymetoprolol)
naproxen	5.3	–	5.2	–
propranolol*	5.6	1-naphthol	7.1	1-naphthol
sotalol*	6.0	–	9.4	–
sulfamethoxazole	34	–	34	–
tramadol	49	–	49	–

[a]PPs marked with an asterisk indicate DT_{50} values in experiments FLAT and RIPPLE were significantly different at 95% significance level. [b]Infinite sign indicates DT_{50} cannot be estimated from the 29-day study period. [c]Dashed line indicates that no TP for the specific PP was analyzed in this study; TPs in brackets indicate the TP was analyzed but below LOQ in all samples.

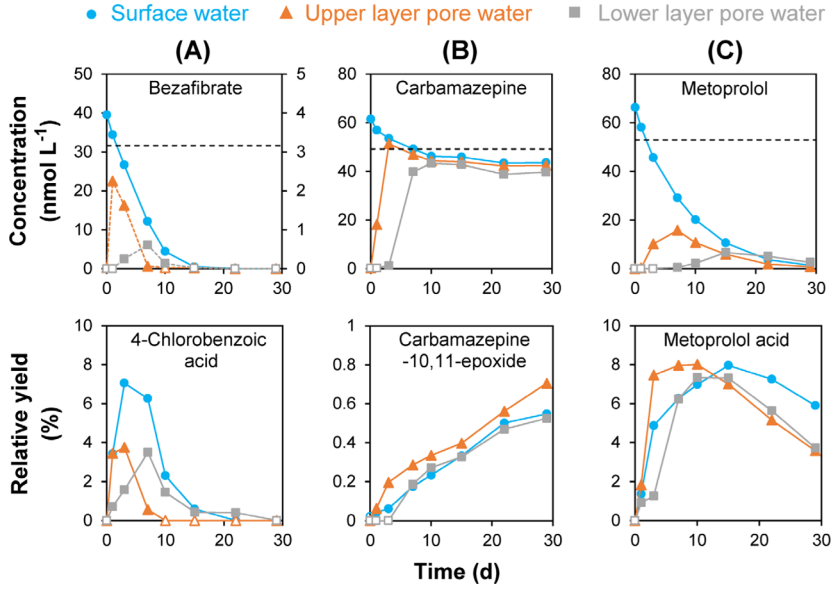

Figure 3. Time trends of three parent pharmaceuticals (PPs) and a corresponding transformation product (TP) for each PP in surface and pore water during experiment FLAT: (A) bezafibrate (top) and 4-chlorobenzoic acid (bottom), (B) carbamazepine (top) and carbamazepine-10,11-epoxide (bottom), and (C) metoprolol (top) and metoprolol acid (bottom). Relative yield of TP refers to the ratio of the molar concentration of a TP to the initial molar concentration of its corresponding PP. Data for pore water refers to sampling ports U6 and L6 (see Figure 1). Hollow symbols indicate concentrations <LOQ and are only shown for visual clarity. Dashed curves indicate that data are shown on the secondary *y*-axis. The dashed horizontal lines represent the calculated equilibrium concentrations.

(see Supporting Information Figure S17). Our observation however is consistent with the findings from a tracer study carried out in a small stream in Sweden, where clofibric acid dissipated with a DT_{50} of 2.5 d.[28] These deviating results regarding clofibric acid illustrate that persistence data obtained from different experiments can vary significantly depending on system-specific conditions such as microbial population and biogeochemical boundary conditions. Given that the concentration of clofibric acid was constant over time in all control experiments, we conclude that its dissipation here can be attributed to microbial transformation in the sediment compartment. All beta-blockers (metoprolol, propranolol, and sotalol) had a similar DT_{50} of 5–6 d. Although sorption contributed to a large extent to their dissipation (Supporting

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Information Table S4), their dissipation still followed first-order kinetics (Supporting Information Figure S5). This was unexpected since sorption as an equilibrium process should be completed once the system is physically equilibrated, which in principle leads to a non-first-order dissipation. Two possible explanations of this inconclusive observation are the following: (i) sorption equilibrium was not reached for beta-blockers in our experiments (e.g., due to their retardation in the upper sediment layer); (ii) there was a rate-limiting process (e.g., the exchange across the water/sediment interface) that controlled the behavior of the beta-blockers. Additional experiments and analysis of depth-resolved sediment samples would be necessary to further explore this phenomenon.

Storck et al. (2012) investigated microbial degradation of various pharmaceuticals in a bioreactor containing surface water and biofilms but no sediment.[29] For the compounds in common to both studies (bezafibrate, carbamazepine, ibuprofen, metoprolol, naproxen, and sotalol), the degradation in their bioreactor was slower by a factor of 3−5 compared to this study. Additionally, the lag time—an initial phase for adaption of the microbes—was generally longer (0−13 days) than our observations (<1 day, see Supporting Information Figure S5). Even though it is difficult to compare the numbers directly due to the two substantially different experimental concepts, the tendency of faster elimination and faster microbial adaption in the current study supports the important role of sediment for the transformation of certain pharmaceuticals.

Transformation Products. Eleven TPs of eight PPs were analyzed in this study, seven of which were detected (Table 1). This confirms that the dissipation of these PPs was at least partly attributed to transformation processes. Three TPs were formed also in the control experiments (Supporting Information Figure S4): 4-chlorobenzoic acid, 4-amino-6-chloro-1,3-benzenedisulfonamide, and chlorothiazide. It is therefore not possible to allocate their formation to either surface water or the sediment compartment, or both. The remaining four TPs (carbamazepine-10,11-epoxide, saluamine, metoprolol acid, and 1-naphthol) were generated exclusively in sediment and were then transported back to the surface water. Figure 3 illustrates the behavior of three pairs of PPs and TPs which are representative for all investigated substances (see Supporting Information Figures S13-A and S13-B; S21-A and S21-B; S22-A, S22−B, and S22-C for the other pairs).

Bezafibrate was one of the acidic compounds that was completely removed during the experiment (Figure 3A). The concentration of its TP 4-chlorobenzoic acid increased rapidly during the first week, after which the parent compound bezafibrate was soon eliminated, and therefore no further production of the TP occurred. Thereafter 4-chlorobenzoic acid was eliminated from the system within a few days.

At the end of the experiment carbamazepine had decreased by 25% from surface water (by 5% when compared to the calculated equilibrium concentration), which was to a great extent due to initial dilution by pore water (Figure 2). Nevertheless, the appearance of the TP carbamazepine-10,11-epoxide and its consistently increasing concentration (Figure 3B) confirm that the decrease of carbamazepine can be partly attributed to transformation in the sediment compartment. Moreover, the similar concentrations of carbamazepine-10,11-epoxide in surface and pore water (both layers) suggest a faster exchange rate between surface water and pore water compared to the rate of transformation of carbamazepine into this TP.

Metoprolol was not detected in the lower layer pore water until day 7 (Figure 3C), as compared to the other two PPs that were both detected there from day 3 onward. The peak concentration of metoprolol at day 15 was also delayed compared to bezafibrate (day 7) and carbamazepine (day 10). The retardation of metoprolol during transport within the sediment compartment can be attributed to sorption to the sediment particles.[25] In contrast to its parent compound, the TP metoprolol acid was detected in the lower layer pore water only 1 day after the start of the experiment, and its final concentration in the surface water was 40% higher than that in pore water at the end of the experiment. This higher mobility of metoprolol acid compared to metoprolol can be attributed to (i) their different polarity, which is mirrored in the log D_{OW} values of 0.14 (metoprolol) and −0.44 (metoprolol acid) at the ambient pH of 8.0 in the surface water, and (ii) the opposite charges of the dominant species: at this pH, metoprolol is present as a cation which is attracted to the abundant negatively charged sorption sites in sediment, while metoprolol acid is present in deprotonated (anionic) state for which only limited sorption sites are available.[30]

In general, the surface water concentrations of the seven TPs detected in the flume system were comparable to or even higher than those in the pore water (see Figure 3 and Supporting Information), and their relative yields ranged from 1% for carbamazepine-10,11-epoxide (Figure 3B) to approximately 30% for saluamine (product of furosemide, Supporting Information Figure S21-B) and one of the hydrochlorothiazide TPs (4-amino-6-chloro-1,3-benzenedisulfonamide, Supporting Information Figure S22-B). Three TPs (4-chlorobenzoic acid, metoprolol acid, and 1-naphthol) were not present any more in the system at termination of the experiments; the pore water peak concentrations of 4-chlorobenzoic acid and 1-naphthol occurred simultaneously with those of their corresponding PP, while metoprolol acid in pore water (Figure 3C) reached its maximum concentration much earlier than metoprolol due to a higher mobility of the TP, as discussed above. Constantly increasing concentrations over time were observed for the remaining four TPs, i.e., carbamazepine-10,11-epoxide, saluamine, and the 2 hydrochlorothiazide TPs, which is in agreement with previous findings from batch experiments.[19] This accumulation might be explained by a faster transformation rate of the PPs than of the TPs, but it also implies that these TPs have the potential to be persistent or to accumulate in the environment under certain conditions. Consequently, the parent contaminants release TPs into the environment as potential secondary pollutants, and depending on transport and transformation conditions, this release can occur far from the primary point of discharge of the parent compounds into the aquatic environment.

Effects of Sediment Bed Morphology on the Behavior of Pharmaceuticals. Small-scale variations in sediment bed topography such as ripples induce pressure gradients along the water/sediment interface, which drive advective water flow into, through, and out of the sediment bed.[31] This small-scale advective transport mechanism is referred to as "pumping".[32] Savant et al. (1987) showed that exchange caused by pumping generally dominates diffusive transport by at least 2 orders of magnitude at the investigated surface water flow velocity of approximately 0.2 m s^{-1} with no transport of sediment and that diffusive transport within the sediment is negligible compared to advection.[33] Consequently, we expected to observe a substantially faster equilibration (i.e., time until persistent and

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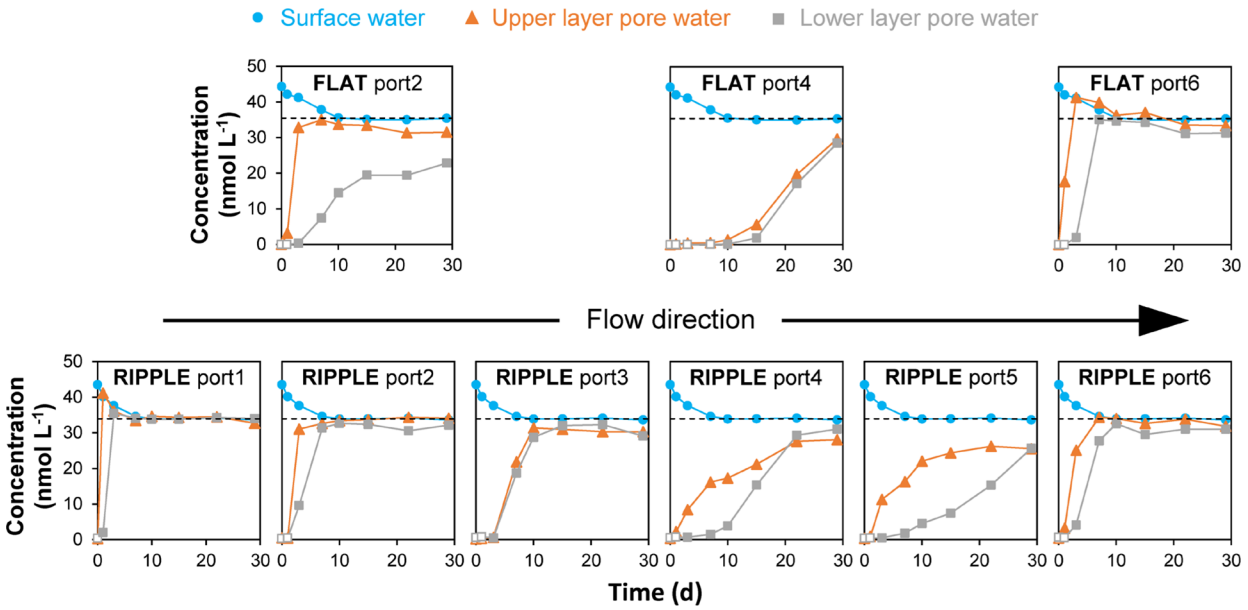

Figure 4. Time trends of the reference compound fluconazole in surface water and pore water at all sampling ports in experiment FLAT (top) and RIPPLE (bottom). Hollow symbols indicate concentrations <LOQ and are only shown for visual clarity. The dashed horizontal lines represent the calculated equilibrium concentrations.

nonsorbing compounds reach constant concentrations in surface water) of the system in experiment RIPPLE than in experiment FLAT, as pumping should be more effective under conditions in the former. However, for fluconazole (Figure 4) as a persistent and nonsorbing reference compound as well as for the independent salt tracer (Supporting Information Figure S3) the time to reach equilibrium concentrations was rather similar between the two experiments. This might be explained by the flow across the water/sediment interface being sensitive to bed topographical features such as amplitude and wavelength of ripples, which consequently might lead to similar water/sediment exchange for different bed configurations.[34−36] As the surface/pore water exchange rate was obviously similar in the two experiments, it is not surprising that the dissipation rates for the majority of the PPs are not significantly different between experiments FLAT and RIPPLE (Table 1).

In experiment FLAT, we expected that the exchange processes were dominated by diffusive transport and consequently that the temporal concentration trends at all pore water sampling ports from one depth were similar. This however was not the case, as is illustrated for fluconazole in Figure 4 (figures for the other compounds are available in the Supporting Information). Based on the temporal trend in the surface water, equilibration was complete after 10 days while the pore water spatial data show that the system was equilibrated to different degrees. At port 2, the upper sediment layer equilibrated with the surface water within 5 days, while the lower layer did not approach equilibrium conditions until the experiment was terminated. At port 4, both depths did not reach this equilibrium stage yet showed an almost identical behavior. Further, at port 6 the concentrations in both sediment layers were identical to those in the surface water from day 5 onward. Obviously, in contrast to our expectations, the conditions in the sediment were heterogeneous: there was sufficient exchange of surface water and pore water throughout the two depths at port 6 and in the upper sediment layer at port 2, while the exchange was slower at port 4 where equilibrium was hardly achieved at the end of the experiment. We therefore conclude that the major part of the sediment compartment was well equilibrated, while some areas were cut off from the exchange with surface water (situation at port 4 and in lower depth at port 2). This can most probably be attributed to nonoptimal hydraulic conditions due to two reasons: (i) the flume was designed to allow experiments with fresh water and sediment and continuous sediment transport, which led to hydraulic conditions that may not be as well-defined as in flumes designed primarily from a pure physical point of view (e.g., the bends create turbulence that alters exchange of surface and pore water);[37,38] (ii) the sediment surface in experiment FLAT was not completely even due to practical reasons (Supporting Information Figure S2), which might have caused some small-scale pumping and consequently a more efficient exchange of water across the water/sediment interface.[32,35]

In experiment RIPPLE, we observed the expected heterogeneity of the pore water concentrations with respect to the position of the sampling ports relative to the ripples. Ideally, surface water enters the sediment compartment at the stoss side of a ripple due to a locally elevated pressure at the water/sediment interface, travels through the sediment, and leaves the sediment at the lee side of the ripple in a local low-pressure zone.[31] Exactly this pattern was observed at ports 1−3. At port 1 (stoss side), equilibration between the two compartments was quick. The longer travel distance within sediment to port 2 below the crest of the ripple is reflected by the longer equilibration time of fluconazole, which is also causative for the comparatively late equilibration of fluconazole at port 3 located on the lee side of the ripple with the longest travel distance in the sediment compartment. For ports 4−6 located below the second ripple, the observed pattern is dissimilar, with long equilibration times in the pore water at ports 4 and 5 and a rather fast equilibration at port 6 (pattern comparable to port 2). This discrepancy from the expected behavior can be attributed to either nonideal pressure conditions at the second ripple, caused by, e.g., its location on the lee side of the first one in a rather short distance, or again the bends of the flume

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creating turbulence that alters exchange of surface and pore water.

Environmental Relevance. Four of the detected TPs (carbamazepine-10,11-epoxide, saluamine, metoprolol acid, and 1-naphthol) were formed exclusively in the sediment compartment. Their detection in surface water is thus indicative of the exchange between surface and pore water. Such TPs have the potential to be used as indicators for characterizing the hyporheic transformation of pharmaceuticals at the reach scale, which would address a methodological shortcoming in determining transformation processes in flowing waters. Moreover, this study shows that we might face a secondary contamination by TPs far from the point of release of PPs, as previously highlighted by Writer et al. (2013).[8] Based on their persistence properties, three pharmaceuticals (carbamazepine, chlorthalidone, and fluconazole) as well as the four TPs with increasing concentrations over the entire experiments (4-amino-6-chloro-1,3-benzenedisulfonamide, carbamazepine-10,11-epoxide, chlorothiazide, and saluamine) should be considered as priority candidates for a higher-tier environmental risk assessment.

Our experiments were designed to simulate conditions in the hyporheic zone as realistically as possible, but simplification is inherent to any laboratory experiment. Direct extrapolation of our results to real systems is complicated by temporal variability and spatial heterogeneity of hydraulic and biogeochemical boundary conditions in hyporheic zones, such as composition and activity of microbial communities, redox conditions, hydraulic conductivities, and large-scale hydraulic gradients, but also by the absence of plants and larger animals in flume experiments. In order to better bridge the gap between lab and field studies, it is also important to put emphasis on the identification of microbial species or enzymes responsible for individual transformation pathways. Consequently, this study provides important insight into several aspects that are important for transformation of pharmaceuticals and formation of TPs in hyporheic zones, but well-designed field experiments are indispensable to obtain the complete picture of hyporheic transformation of polar organic micropollutants.

■ ASSOCIATED CONTENT

Supporting Information

Schematic drawing and photos of the flume, details of chemicals and reagents, analytical method, routine data, results of salt tracer experiments, concentrations of beta-blockers in sediment, results of control experiments, kinetic plots for PPs, and concentration trends of individual compounds. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b00273.

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Notes

The authors declare no competing financial interest.

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