Stockholm University

This is an accepted version of a paper published in *Environ. Sci. Technol.*. This paper has been peer-reviewed but does not include the final publisher proof-corrections or journal pagination.

Citation for the published paper:

Kunkel, U., Radke, M. (2008)

"Biodegradation of acidic pharmaceuticals in bed sediments: insight from a laboratory experiment"

Environ. Sci. Technol., 42: 7273-7279

URL: <http://dx.doi.org/10.1021/es801562j>

Access to the published version may require subscription.

Permanent link to this version:

<http://urn.kb.se/resolve?urn=urn:nbn:se:su:diva-18998>

<http://su.diva-portal.org>

Biodegradation of acidic pharmaceuticals in bed sediments: insight from a laboratory experiment

*Uwe Kunkel and Michael Radke**

Department of Hydrology, University of Bayreuth, 95440 Bayreuth, Germany

*Corresponding author: Michael Radke, University of Bayreuth, Department of Hydrology, 95440 Bayreuth, Germany. Phone +49/921/552297; fax +49/921/552366; E-Mail: michael.radke@uni-bayreuth.de

This document is the Accepted Manuscript version of a Published Work that appeared in final form in Environmental Science and Technology, copyright © American Chemical Society after peer review and technical editing by the publisher. To access the final edited and published work see <http://pubs.acs.org/doi/abs/10.1021/es801562j>

ABSTRACT. Pharmaceutical residues are commonly detected micropollutants in the aquatic environment. Biodegradation in sediments is a potentially significant removal process for these compounds in rivers which is constrained by the transfer of water and solutes into the sediment. Aim of this study was to determine the combined effect of flow velocity and sediment dynamics and thus of water-sediment interactions on the attenuation of 6 acidic pharmaceuticals. We carried out experiments with river water and

sediment in a bench-scale annular flume at two different hydraulic boundary conditions (flat sediment surface vs. moving sediment). The effective biodegradation half-lives of 4 compounds (diclofenac, bezafibrate, ibuprofen, naproxen) were in the range of 2.5 to 18.6 days and much shorter when the exchange of surface and pore water was fast. For gemfibrozil, a half-life of 10.5 d was determined in the experiment with moving sediment, whereas no degradation was observed with flat sediment bed. These findings can be attributed to limited transfer of water and solutes into the sediment at low flow velocity and flat sediment bed which rapidly induced anaerobic conditions in the sediment. The only compound that was efficiently removed in deeper, anoxic sediment layers was naproxen. Calculated half-life distances in rivers ranged from 53 to 278 km. Our results indicate that it could be favorable to increase the rate of exchange between surface and pore water during river restoration to enhance the attenuation of organic micropollutants like acidic pharmaceuticals.

KEYWORDS. Surface water-sediment interactions; acidic pharmaceuticals; biodegradation; flume; flow velocity; sediment transport; elimination kinetics

38 **Introduction**

39 The application of pharmaceuticals in human and veterinary medicine is an essential
40 part of modern life. Generally, large amounts of medical drugs are produced, prescribed,
41 and consumed. For example, in 1999 328 of the 2754 in Germany licensed active
42 pharmaceuticals ingredients were sold with an amount >5,000 kg (1). In the human body
43 pharmaceuticals are often only incompletely eliminated, so metabolites, conjugates, or
44 the unmodified substances are excreted (2). This excretion via urine and faeces is the
45 most important source of pharmaceuticals in the aquatic system (3). Due to incomplete
46 removal in sewage treatment plants (STP) (4,5), pharmaceuticals are entering rivers and
47 streams via STP effluents. In rivers, typical concentrations of pharmaceuticals are in the
48 range between 10 and 100 ng L⁻¹ (6,7), but concentrations up to a few µg L⁻¹ have also
49 been reported (6). To maintain the desired concentrations for an adequate period,
50 pharmaceuticals are usually designed to be relatively stable in humans; therefore it can be
51 assumed that many pharmaceuticals are rather persistent under environmental conditions,
52 too (8). Recent ecotoxicity studies mainly stated no acute risk for organisms at
53 environmental concentrations (9,10), but chronic effects could not be excluded especially
54 when organisms were exposed to a cocktail of pharmaceuticals for a longer period of
55 time (9,10).

56 Many studies are available on the elimination of pharmaceuticals in STPs, and there
57 exists also an extensive number of monitoring studies on pharmaceuticals in rivers and
58 lakes. However, only few authors investigated the fate of these compounds in the aquatic
59 environment from a mechanistic point of view. Since a lot of pharmaceuticals are very
60 polar, findings for long-studied pollutants which are relatively unpolar can not readily be
61 transferred to this rather new class of contaminants. Microbial degradation is supposedly

the most important removal pathway for pharmaceuticals in the environment, especially if the compounds of interest are resistant against photodegradation and hydrolysis. Due to the low abundance of microorganisms in river water, the sediment compartment with its high density of microorganisms is a potential major sink of pharmaceuticals. Therefore, the interaction of surface water with the sediment compartment is of crucial importance for the elimination of pharmaceuticals from rivers since it controls the mass transfer of solutes from the river to the sediment (and vice versa).

On the catchment scale, fluxes across the surface water-sediment interface are controlled by regional hydraulic gradients. On smaller scales (pool-riffle-sequences, ripples etc.) interactions between surface water and sediment are driven by pressure irregularities caused by river bed geometry and flow dynamics in the surface water. Two dominant mechanisms determine the flux of water and solutes across the water-sediment interface. The first one known as “pumping” is driven by advective flows caused by pressure head gradients (11,12). Such gradients are evoked by the river bed morphology and depend on shape and size of bed form irregularities. In streambeds with an uneven morphology the exchange rate, expressed as intrusion depth of particles, was reported to be several times higher than in flat beds (13). The second mechanism called “turnover” is caused by moving bed sediments which periodically trap and release water (11,12). Special attention should be paid to the effects of flow velocity on exchange fluxes, as increasing flow velocity transforms an initially flat river bed into a two dimensional rippled bed (14) which causes higher pressure gradients and thus an increased exchange of water and solutes. Besides fluxes that are caused by hydraulic gradients, biota can also cause interface exchange. However, this process is only relevant when advective flow of pore water is negligible (15).

For a small stream, results of a modeling study show that - besides the macroscopic hydraulic gradients - mainly flow velocity and morphology of the river influenced the

environmental fate of the analgesic diclofenac (16). Both factors constrain the interactions of surface water with sediment and thus the residence time of surface water in the subsurface. Wörman et al. (17) showed that the basic relationship between topographic features and this residence time is independent of scale; thus, these factors should also control the fate of pharmaceuticals on a much smaller scale. In the present study, we investigated the effect of hydraulic conditions on the behavior of pharmaceuticals in rivers with special attention to the interactions of surface water with pore water. This pilot study was based on the basic hypothesis that hydraulic interactions between surface and pore water control - to a certain extent - the elimination kinetics of pharmaceuticals from surface water. To test this hypothesis, we optimized a bench-scale laboratory flume to carry out the experiments with reasonable consumption of resources and minimized production of liquid and solid waste. Six acidic pharmaceuticals (the analgesics diclofenac, ibuprofen and naproxen, and the lipid lowering agents bezafibrate, clofibric acid and gemfibrozil; all negatively charged under environmental conditions) were studied. The reported persistence of these compounds in a sediment-water test ranged from rapidly biodegradable (ibuprofen) to highly persistent (clofibric acid) (8). However, information on persistence of pharmaceuticals in the environment is still limited, especially because results obtained for biodegradation in STPs can not readily be transferred to the environment. This becomes obvious for diclofenac: in experiments with activated sludge treatment no biological degradation was observed (4), whereas it was biodegraded in a fixed bed reactor filled with river sediments within several days (18). Based on the elimination kinetics from surface water, half-life times (t_H) and distances (d_H) were determined. Experiments were run at two different flow velocities inducing slow (experiment WS.slow) and fast (WS.fast) exchange of surface water with sediment. Moreover, by the simultaneous analysis of pore water taken from two different depths the distribution of pharmaceuticals in the sediment compartment was determined.

Experimental Methods.

Chemicals. All pharmaceuticals (purity > 97 %) and the internal standard fenoprop were purchased from Sigma-Aldrich (Seelze, Germany). The D-labelled surrogate standards D₄-bezafibrate, D₄-clofibric acid, D₄-diclofenac, D₃-ibuprofen, and D₃-naproxen were purchased from Toronto Research Chemicals (New York, ON, Canada). LC-MS-grade Acetonitrile (ACN) and acetic acid (HAc) were purchased from Sigma-Aldrich, LC-MS-grade water from J.T. Baker (Deventer, The Netherlands).

Sediment and water. Sediment and water were collected prior to each experiment from the river *Roter Main* upstream the city of Bayreuth, Germany. This is a small river with an average discharge of $0.54 \text{ m}^3 \text{ s}^{-1}$ (<http://www.hnd.bayern.de>, accessed 04.02.2008). The sampling site it is nearly unaffected by sewage inputs and thus no contamination with pharmaceuticals has to be taken into account. Regarding discharge and size or type of its catchment, this river is typical for small rivers throughout Central Europe. Sediment was taken from the top 20 cm and wet sieved < 2 mm. Water was used as sampled. Both water and sediment were stored for less than 7 days at 4 °C until use. As can be deduced from analyses of the effect of sample storage on soil microbial activity, storing the sediment at this temperature should not influence its microbial community pattern and activity (19). The sediment was classified as pure sand (92.9 % sand, 3.3 % silt, 3.8 % clay) with a low content of organic carbon (0.08 %) and a mean porosity of 0.43. The initial concentration of total organic carbon (TOC) in the water was approx. 40 mg L^{-1} , the pH of the water was 8.1.

Bench-scale flume. Experiments were conducted in a specifically designed bench-scale annular flume that allowed continuous sediment and water transport. Channel-width was 15 cm, channel-height was 50 cm and the extent of the flume was 1.40 m x 0.70 m. The walls were made of stainless steel (in the bends) and acrylic glass (straight

sections). Preliminary experiments proved the adequacy of these materials for the purpose of this study since no interactions of the pharmaceuticals with these materials occurred. A sketch of the flume set-up is available as Supporting Information. The flow velocity was regulated with a water propeller connected to an electrical motor located at the middle of a straight section. At the straight opposite to the propeller, six pore water sampling ports were installed. The three upper ports were in a depth of approximately 5 cm below the sediment surface, the three lower ones approximately 10 cm. Pore water was sampled with slotted stainless steel needles (diameter 1.8 mm; 64 slots of 0.4 mm width evenly distributed along the length of 10 cm; in-house manufactured). The needles were installed during the initial preparation of the flume and remained in place throughout the whole experiment.

Flume experiments. Two experiments were carried out with sediment and water from the river *Roter Main*. In experiment WS.slow (flow velocity $v = 0.12 \text{ m s}^{-1}$), the sediment surface was flat and no sediment movement occurred, whereas in experiment WS.fast ($v = 0.31 \text{ m s}^{-1}$) sediment transport occurred and thus bedforms were variable. The flow velocities applied are typical for the river *Roter Main* for low and intermediate discharges, respectively. Moreover, one abiotic control experiment (WS.abiotic; $v = 0.12 \text{ m s}^{-1}$) using tap water and sterilized quartz sand, and another experiment with river water but without sediment (W; $v = 0.12 \text{ m s}^{-1}$) were conducted. Details on the experimental conditions are summarized in Table 1. Experiments were carried out at a temperature of approx. 18°C, which is a typical water temperature for the river *Roter Main* in summer. To avoid photodegradation, all experiments were carried out in darkness. Prior to each experiment, the flume was filled with sediment (height approx. 16 cm) and water (26-30 cm), the sediment surface was leveled and the flow velocity was adjusted. The hydraulic properties of the sediment (e.g. porosity, hydraulic conductivity) should be similar to those of (homogeneous) sandy sediments in rivers since the same transport mechanisms,

mainly sedimentation and resuspension, occur both in the flume and in rivers. After equilibration of the system, an aqueous solution (1 L; $c = 6 \text{ mg L}^{-1}$) containing the six pharmaceuticals was added to the surface water to obtain an initial concentration of approximately $30\text{-}50 \text{ } \mu\text{g L}^{-1}$ in the surface water. To minimize sample volume and preconcentration steps, these concentrations were 2-3 orders of magnitude higher than typical environmental concentrations of the pharmaceuticals. For each set of experimental conditions, representative depth profiles of oxygen concentrations with a vertical resolution of 2 mm were measured using a needle-type microsensor (Unisense, Aarhus, Denmark).

Table 1: Details of the experimental conditions. Height of sediment and water represents initial height at start of the equilibration period; v : flow velocity; Re : Reynolds number.

	Experiment			
	W	WS.abiotic	WS.slow	WS.fast
Sediment type	-	quartz sand	Roter Main	Roter Main
Sediment height (cm)	-	16.7	15.9	16.5
Water type	Roter Main	tap water	Roter Main	Roter Main
Water height (cm)	29.1	27.0	26.5	28.4
$v \text{ (m s}^{-1}\text{)}$	0.12	0.12	0.12	0.31
$Re \text{ (-)}$	n.d.	n.d.	7823	19387

n.d. : not determined

Sampling and Analysis. Samples from surface and pore water were collected periodically during the experiments. Surface water was sampled (2-3 replicates) with a steel needle connected to a glass syringe at random locations. Pore water was sampled with a glass syringe that was connected to the sampling ports. The dead volume of the total sampling device (0.5 mL) was discarded before at most 9.5 mL of pore water were sampled. Thereby, water from a cylindrical area with a radius of less than 1 cm around

the tube was sampled and no interferences between the upper and the lower sampling port occurred. Since the sampling volume had to be minimized to avoid excessive advective flows, no replicates of pore water were sampled. 3 mL of sample were transferred to a glass tube and 15 μ L of the surrogate standard containing D-labeled bezafibrate, clofibric acid, diclofenac, ibuprofen and naproxen ($c = 5 \text{ mg L}^{-1}$) were added. Samples were then centrifuged for 5 minutes at 4000 rpm (Sepatech Labofuge AE, Heraeus, Hanau, Germany), filtered with a 0.45 μ m Nylon syringe filter (Rotilabo, Roth, Karlsruhe, Germany), and 1 mL was transferred to a glass vial. Prior to analysis, 5 μ L of a solution containing fenoprop (internal standard; $c = 10 \text{ mg L}^{-1}$) were added to the sample. Pharmaceuticals were determined with a HPLC-MS/MS system (VARIAN Inc, Palo Alto, CA, USA) consisting of two HPLC pumps (Prostar 210), an autosampler (Prostar 410) and a triple quadrupole mass spectrometer (1200 L). Separation was achieved by a binary gradient with H_2O and ACN containing 2.5 mM HAc on a Synergi Polar-RP 80A column (150 x 2 mm, Phenomenex, Aschaffenburg, Germany). Relative standard deviations for replicate surface water samples were generally $< 10 \%$ for all compounds analyzed ($n = 3$). For pore water, the results of experiment WS.fast can be used to assess the analytical reproducibility since we observed homogeneous conditions throughout the sediment; for these "pseudo-replicates", RSD was also $< 10 \%$ ($n = 5$) for all compounds.

Calculations.

For calibration of HPLC-MS analyses, the internal standards method was applied. Concentrations of target compounds were corrected for recovery by the corresponding surrogate standards. For gemfibrozil no isotopically labeled internal standard was available; therefore, recovery was corrected using the mean recovery of the five D-labeled surrogates. Evaporative losses of water during the experiment were also taken into account.

Dissipation times (DT_{50}) - i.e. the time until 50% of the initial concentration has disappeared from the water phase - were calculated by fitting a first-order kinetics to the pharmaceutical concentrations in the surface water. However, at the beginning of the experiments the concentration of pharmaceuticals in the sediment was zero. Thus, even without any degradation the concentration in the surface water was expected to decrease until concentrations in surface and pore water were equal. This equilibrium concentration (c_{eq}) after complete mixing of surface and pore water was calculated by relating the total amount of each pharmaceutical to the total volume of water (sum of pore and surface water) in the system. Because of this initial equilibration, the calculated DT_{50} does not allow differentiating between primary degradation and concentration decreases due to equilibration of the system. To correct for the equilibration and to infer biodegradation rates, we recalculated the results using clofibric acid as tracer. The concentrations of each compound were normalized to its initial concentration in the surface water and then corrected by the proportional loss of clofibric acid for each time step. Again, a first order kinetics was fitted to these corrected concentrations to calculate half-life times t_H . To calculate dissipation distance DD_{50} and half-life distances d_H , DT_{50} and t_H were multiplied by the mean flow velocity. Details of these calculations are available as Supporting Information.

Results and Discussion

Control experiments. In experiment W (w/o sediment), the concentrations of bezafibrate, clofibric acid, diclofenac and gemfibrozil remained at the initial level for 12 days. Therefore, sorption to suspended matter or to the flume, microbial degradation in the water, and hydrolysis were of minor importance. The concentration of ibuprofen remained constant for about 8 days; then ibuprofen was completely eliminated within the next 4 days. The only plausible explanation is the onset of microbial degradation of ibuprofen after a time lag of approx. 8 days. The concentration of naproxen decreased

slightly throughout the whole experiment; after 13 days, approx. 25 % of the initial concentration was removed from the system. In experiment WS.abiotic, the behavior of the studied compounds was similar. At the end of the experiment, the concentrations of bezafibrate, clofibric acid, diclofenac and gemfibrozil were equivalent to c_{eq} . The final concentrations of ibuprofen and naproxen were slightly lower than c_{eq} . Either insufficient sterilization of the system or weak adsorption to the quartz sand could be the reason for this observation. However, the concentration decrease of ibuprofen and naproxen was small compared to the experiments with river water and sediment, so no correction for such losses was applied. Based on the control experiments, the set-up of the flume and the experimental design were appropriate for the scope of this study.

Figure 1: Concentration time trend of bezafibrate, gemfibrozil, and ibuprofen in surface water. Left: experiment WS.slow; right: WS.fast. Concentrations were normalized to the initial concentration of each compound in the surface water. Error bars represent standard error of replicate samples; dashed line represents c_{eq} .

255

256 **Surface water.** The concentration of all pharmaceuticals in the surface water
257 decreased during experiment WS.slow, but final concentrations of the individual
258 pharmaceuticals differed considerably. This is shown for bezafibrate, gemfibrozil, and
259 ibuprofen in Figure 1. The final concentration of clofibric acid was equal to c_{eq} (not
260 shown), so for clofibric acid neither primary degradation nor sorption did occur. The
261 gemfibrozil concentration reached a constant level which was slightly below the c_{eq} after
262 4 days (Figure 1), pointing to minor adsorption to the sediment or limited biodegradation
263 of gemfibrozil. The concentrations of all other compounds were continuously decreasing
264 (Figure 1). In combination with the results of the abiotic control experiment where no
265 significant removal of these compounds was observed, this decrease can be attributed to
266 primary biodegradation. From the concentration trends in the surface water, removal
267 rates in the order ibuprofen > bezafibrate > naproxen $\approx$ diclofenac $\gg$ gemfibrozil were
268 estimated. Similar concentration trends were observed in the experiment with moving
269 bedforms (WS.fast). Generally, all compounds disappeared faster from the surface water
270 than in experiment WS.slow (Figure 1). While clofibric acid again approached c_{eq}
271 (Figure 2), in this experiment the concentration of all other compounds including
272 gemfibrozil was continuously decreasing, which again can be attributed to
273 biodegradation. From the final concentrations in the surface water, removal rates in the
274 order ibuprofen > bezafibrate > diclofenac > naproxen > gemfibrozil were estimated.
275 Obviously, conditions in the sediment were favorable for the degradation of gemfibrozil
276 in experiment WS.fast, whereas no or only very slow degradation of gemfibrozil took
277 place in experiment WS.slow. In both experiments, the general chemical properties
278 (TOC, pH) of the surface water remained on a constant level, thus indicating uniform
279 chemical boundary conditions in the surface water throughout the experiment.

Pore water. The influence of flow velocity on the exchange between surface and pore water is reflected in the time when the pharmaceuticals arrived at the lower sampling ports. In experiment WS.fast, all compounds were detected after 22 hrs, whereas they arrived only after 49 hrs in the lower ports during experiment WS.slow. This is in agreement with general observations previously reported by Packman and Salehin (20). In experiment WS.fast, we observed a rapid equilibration between surface and pore water. Within approx. 1 day, the concentration of clofibric acid in the pore water reached the level of the surface water (Figure 2). Generally, the pore water concentration of all compounds was similar at all sampling ports regardless of horizontal or vertical position, indicating intense exchange of surface and pore water and fast transport of water and pharmaceuticals in the sediment. Figure 2 illustrates the concentration trends for 4 compounds with different biodegradation behavior. While the concentrations of the persistent compound clofibric acid were identical in surface and pore water after the initial equilibration, the concentrations of gemfibrozil, bezafibrate, and ibuprofen were always lower in the pore water. The elimination rate of bezafibrate was obviously similar to the rate of supply from the surface water, as can be deduced from the parallel concentration trends in surface and pore water from day 1 onwards. In contrast, the degradation rate of ibuprofen was much faster than the delivery into the sediment as the pore water concentrations were very low throughout the whole experiment and did not follow the trend in the surface water.

303

304 **Figure 2:** Concentration trend of clofibric acid, bezafibrate, gemfibrozil, and ibuprofen
305 in surface and pore water (mean concentration of all sampling ports) during experiment
306 WS.fast. Error bars represent standard error of replicate samples (surface water: n = 3;
307 pore water: n = 5). Dashed line represents c_{eq} .

308

309 In experiment WS.slow, the pore water concentrations of all compounds were
310 depending on depth of the sampling port. Pharmaceuticals were determined first in the
311 upper sampling ports at all sites. This is illustrated for site 1 in Figure 3. All
312 pharmaceuticals arrived at the upper port after 5 h whereas it took 49 h until they were
313 detected in the lower port. Thus, the travelling times of surface water and
314 pharmaceuticals until they reached the lower sampling ports was much longer than to the
315 upper ports. The pharmaceutical concentrations also peaked markedly later in the lower
316 sampling ports. These observations confirm the limited mixing of surface and pore water
317 due to the flat sediment bed and the low flow velocity in this experiment. As a result of

the longer travelling time to the deeper sediment layers, we had expected considerably lower concentrations in samples from the lower ports if biodegradation rates were constant throughout the sediment. However, this was only true for naproxen where concentrations in the lower port closely followed the time trend in the upper port but at much lower concentration (not shown). For all other compounds, towards the end of the experiment concentrations in the lower ports were constantly higher than in the upper ports (Figure 3). This can only be explained by slower biodegradation of these compounds in the lower sediment layers due to more reducing conditions there. This conclusion is also based on the rapid dissipation of oxygen within the first millimeters of the sediment profile (see Supporting Information) and on the results for other redox species (data not shown). It has been reported that biodegradation of several pharmaceuticals is much faster under aerobic than under anaerobic conditions (18,21), so this backs up our conclusion of slower biodegradation in the deeper sediment. Moreover, our finding is consistent with results from Carballa et al. (5) who reported a high removal rate of naproxen during anaerobic digestion of sewage sludge compared to diclofenac and ibuprofen. However, more systematic information on the behavior of pharmaceuticals under well defined reducing conditions is needed to fully explain our observations.

Figure 3: Concentration trends of gemfibrozil, bezafibrate, and ibuprofen in surface and pore water (sampling site 1; see Supporting Information for location) in experiment WS.slow. Error bars represent standard error for replicate samples of surface water. Dashed line represents c_{eq} .

Kinetic analyses. Dissipation kinetics of pharmaceuticals from surface water were calculated by the application of a first-order kinetic model. For clofibric acid and gemfibrozil in experiment WS.slow and clofibric acid in experiment WS.fast no DT_{50} was calculated due to their persistent behavior. The calculated regressions were significant (gemfibrozil, experiment WS.fast; $p < 0.05$) or highly significant (all other compounds, both experiments; $p < 0.01$), and the coefficient of determination was always > 0.9 . The calculated DT_{50} and DD_{50} are shown in Table 2.

Table 2: Calculated dissipation times (DT₅₀), dissipation distances (DD₅₀), half-life times (t_H), and half-life distances (d_H) for experiments WS.slow and WS.fast.

	Experiment WS.slow				Experiment WS.fast			
	DT ₅₀ (d)	DD ₅₀ (km)	t _H (d)	d _H (km)	DT ₅₀ (d)	DD ₅₀ (km)	t _H (d)	d _H (km)
Bezafibrate	4.3	45	8.4	87	2.5	67	4.3	113
Clofibric Acid	n/a	-	n/a	-	n/a	-	n/a	-
Diclofenac	8.5	88	18.6	193	3.2	84	5.5	144
Gemfibrozil	n/a	n/a	-	-	5.6	147	10.5	278
Ibuprofen	2.5	26	5.1	53	1.2	32	2.5	66
Naproxen	6.9	72	13.9	144	5.4	144	10.3	272

n/a: 1st order kinetics not applicable

The calculated DT₅₀ for experiment WS.fast were always shorter than for experiment WS.slow. Under conditions of experiment WS.fast, the exchange of surface and pore water was higher due to uneven sediment morphology (13), a higher flow velocity (22), and sediment turnover (11,12), thus maintaining aerobic conditions and favoring biodegradation in the sediment. Calculated DT₅₀ for experiment WS.slow ranged from 2.5 d (ibuprofen) to 8.5 d (diclofenac); the range in experiment WS.fast was 1.2 d (ibuprofen) to 5.6 d (gemfibrozil). The calculated DD₅₀ ranged from a few tens of kilometers for ibuprofen up to approximately 150 km for gemfibrozil (Table 2).

At the beginning of the experiment, the water-sediment-system was at disequilibrium since no pharmaceuticals were present in the pore water. Therefore, a major disadvantage of the calculated DT₅₀ is that it does not take into account losses due to equilibration of the system and the calculated dissipation time does not only reflect removal due to biodegradation. To overcome this limitation, we used clofibric acid to correct for the initial equilibration of surface and pore water. The application of clofibric acid was

justified since in all experiments its final concentration matched the calculated equilibrium concentration, so no removal due by sorption or biodegradation occurred. Moreover, the persistent behavior of clofibric acid and its use as tracer has already been reported elsewhere (23).

The kinetic plots with the recalculated concentrations are shown in Figure 4. All calculated linear regressions were significant ($p < 0.05$, $R^2 > 0.8$) except for gemfibrozil in experiment WS.slow. Since the influence of equilibration between surface and pore water was eliminated by the applied procedure, the plots for both experiments should be identical if the degradation kinetics were the same. However, in experiment WS.fast the elimination of all compounds was faster, so the different hydraulic conditions induced changes in the biodegradation kinetics. These changes could be due to either (i) redox conditions favoring biodegradation of pharmaceuticals in the sediment with increasing exchange of water, or (ii) slow advective flow in the pore water in experiment WS.slow and thus a limited transport of pharmaceuticals from surface water to the sediment. The redox conditions in the sediment were obviously influenced by the hydraulic conditions. In experiment WS.slow, oxygen was only present in the uppermost millimeters of the sediment (see Supporting Information). Furthermore, we observed increasing Fe^{2+} concentrations and decreasing concentrations of NO_3^- and SO_4^{2-} over time (data not shown), whereas in experiment WS.fast no evidence for reducing conditions in the sediment was determined. Under anaerobic conditions, the biodegradation of some pharmaceuticals is slower than under aerobic conditions (18,21) so the observed difference in redox conditions could explain the lower degradation rates in experiment WS.slow. On the other hand, there is also some evidence for a transport limitation. In experiment WS.fast the pore water concentrations of all pharmaceuticals were identical in both sediment depths, whereas in experiment WS.slow, we observed differences in the vertical concentration pattern in the pore water. Most likely, both effects contributed to

399 the differences in elimination kinetics. Based on the present knowledge the relative
400 importance of each process can not be estimated, but in any case the hydraulic conditions
401 constrained the biodegradation rates.

402

403

Figure 4: First-order primary biodegradation kinetics of pharmaceuticals from surface water in experiments WS.fast and WS.slow. Plots are based on recalculated concentrations (c^*) relative to clofibric acid and normalized to initial concentrations. Parameters for linear regression (solid lines) are given above. Regression for gemfibrozil in experiment WS.slow was not significant.

From the recalculated elimination kinetics, half-life times (t_H) and distances (d_H) were calculated (Table 2). In contrast to DT_{50} , these values can directly be transferred to the

situation in rivers where pharmaceuticals can be assumed to be at dynamic equilibrium between input, transport, and degradation due to their pseudo-persistent behavior (24). Half-life times ranged from a few days for ibuprofen up to almost 19 d for diclofenac. Respective half-life distances were between about 50 km for ibuprofen and almost 300 km for gemfibrozil. As expected, these half-life times were longer than the calculated dissipation times since the concentration decrease due to the initial equilibration is not incorporated. It should be noted that for bezafibrate, ibuprofen, and naproxen the calculated d_H of experiment WS.fast was greater than that of WS.slow because the smaller t_H was compensated for by the higher flow velocity, whereas for diclofenac the transport distance deduced from WS.fast was shorter than for WS.slow (193 vs. 144 km).

Implications. Up-scaling the findings from laboratory flume experiments to rivers is difficult. The exchange of surface and pore water depends on factors such as sediment texture, river and bed morphology on different scales, and flow velocity (20). To date, quantification of the exchange rate of surface and pore water along river stretches is not easily accessible. Moreover, additional elimination processes are important in rivers (e.g. photodegradation for diclofenac and naproxen (25)), and boundary conditions (e.g. irradiation, discharge, temperature, quality of organic matter, microbial activity) are also variable. And finally, seasonal or daily variations of the elimination kinetics have to be taken into account. For example, Labadie and Budzinski (26) determined a DD_{50} of 1.7 km for a hormone in summer while they detected no significant decrease along a 10 km stretch in winter. Keeping such limitations in mind, the results of this work can be compared with elimination kinetics derived from field studies. Lin et al. (27) determined very short half-life times of 2.7 hours for gemfibrozil, 5.4 hours for ibuprofen and 1.7 – 3.0 hours for naproxen in a Californian river. In contrast, Fono et al. (28) determined DT_{50} of several days in a Texan river (gemfibrozil: 8.6 d; ibuprofen: 4.6 d; naproxen: 4.3 d) that are in the same order of magnitude than half-life times determined in this study, as

are the first-order rate constants for the dissipation/degradation. In both studies, for most compounds biotransformation was reported to be more important than photolysis. Based on the results of our study, the short half-life times reported by Lin et al. (27) seem quite exceptional. However, since a number of factors can contribute to this difference the reason for the large discrepancy has to remain open.

The results of this study indicate that it could be favorable to increase water-sediment interactions during river restoration to enhance the attenuation of acidic pharmaceuticals. However, it should be noted that this could be conflicting with the remediation of other contaminants. For example, Arnon et al. (29) observed the highest denitrification rate in sediments at a relatively low flow velocity and thus low exchange of surface and pore water. At higher flow velocities, the increased mass transfer of NO_3^- into the sediment was counterbalanced by the increased transfer of oxygen which caused a reduced denitrification rate in the sediment due to a shift to aerobic conditions.

Based on the results of this study, experiments in rather small laboratory flumes are suitable to determine the interplay of hydraulic processes with biodegradation of organic contaminants in river water and sediment. Compared to traditional water-sediment test systems for the evaluation of the behavior of chemicals where usually hydraulic conditions are not taken into account, the bench-scale flume provides a more realistic simulation of environmental processes. Especially the combined analysis of surface and pore water allows a more detailed analysis of the behavior of polar trace organic chemicals in rivers. To improve the transfer of results from such studies to the field scale, more information is needed on factors affecting the exchange of water and solutes with river sediments (e.g. river and sediment morphology, heterogeneity), but also on the reaction of physical and chemical processes in bed sediments on dynamically changing hydraulic and chemical conditions in rivers.

Acknowledgements

The help of the staff at the mechanical workshop at the University of Bayreuth with construction and improvements of the flume is acknowledged. We also thank two anonymous reviewers for their helpful comments on an earlier version of the manuscript.

Supporting Information Available. A sketch of the flume, details of the recalculation procedure based on clofibric acid, and vertical profiles of oxygen saturation in the sediment are available as Supporting Information. This information is available free of charge via the Internet at <http://pubs.acs.org>.

- 478 (1) Bund/Länder-Ausschuss Chemikaliensicherheit "Report *Arzneimittel in der*
479 *Umwelt - Auswertung der Untersuchungsergebnisse*," **2003**, [http://blak-](http://blak-uis.server.de/servlet/is/2146/P-2c.pdf)
480 [uis.server.de/servlet/is/2146/P-2c.pdf](http://blak-uis.server.de/servlet/is/2146/P-2c.pdf).
- 481 (2) Ashton, D.; Hilton, M.; Thomas, K. V. Investigating the environmental transport
482 of human pharmaceuticals to streams in the United Kingdom. *Sci. Total Environ.* **2004**,
483 333, 167-184.
- 484 (3) Heberer, T. Occurrence, fate, and removal of pharmaceutical residues in the
485 aquatic environment: a review of recent research data. *Toxicol. Lett.* **2002**, 131, 5-17.
- 486 (4) Joss, A.; Zabczynski, S.; Göbel, A.; Hoffmann, B.; Löffler, D.; McArdell, C. S.;
487 Ternes, T. A.; Thomsen, A.; Siegrist, H. Biological degradation of pharmaceuticals in
488 municipal wastewater treatment: Proposing a classification scheme. *Water Res.* **2006**, 40,
489 1686-1696.
- 490 (5) Carballa, M.; Omil, F.; Ternes, T.; Lema, J. M. Fate of pharmaceutical and
491 personal care products (PPCPs) during anaerobic digestion of sewage sludge. *Wat. Res.*
492 **2007**, 41, 2139-2150.
- 493 (6) Ternes, T. A. Occurrence of drugs in German sewage treatment plants and rivers.
494 *Wat. Res.* **1998**, 32, 3245-3260.
- 495 (7) Tixier, C.; Singer, H. P.; Oellers, S.; Müller, S. R. Occurrence and fate of
496 carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen, and naproxen in surface
497 waters. *Environ. Sci. Technol.* **2003**, 37, 1061-1068.
- 498 (8) Löffler, D.; Römbke, J.; Meller, M.; Ternes, T. A. Environmental fate of
499 pharmaceuticals in water/sediment systems. *Environ. Sci. Technol.* **2005**, 39, 5209-5218.
- 500 (9) Cunningham, V. L.; Buzby, M.; Hutchinson, T.; Mastrocco, F.; Parke, N.; Roden,
501 N. Effects of human pharmaceuticals on aquatic life: Next steps. *Environ. Sci. Technol.*
502 **2006**, 40, 3456-3462.
- 503 (10) Ferrari, B.; Paxeus, N.; Lo Giudice, R.; Pollio, A.; Garric, J. Ecotoxicological
504 impact of pharmaceuticals found in treated wastewaters: study of carbamazepine,
505 clofibric acid, and diclofenac. *Ecotox. Environ. Saf.* **2003**, 55, 359-370.
- 506 (11) Cardenas, M. B.; Wilson, J. L.; Zlotnik, V. A. Impact of heterogeneity, bed forms,
507 and stream curvature on subchannel hyporheic exchange. *Water Resour. Res.* **2004**, 40,
508 W08307.
- 509 (12) Elliott, A. H.; Brooks, N. H. Transfer of nonsorbing solutes to a streambed with
510 bed forms: Theory. *Water Resour. Res.* **1997**, 33, 123-136.
- 511 (13) Huettel, M.; Ziebis, W.; Forster, S. Flow-induced uptake of particulate matter in
512 permeable sediments. *Limnol. Oceanogr.* **1996**, 41, 309-322.
- 513 (14) Robert, A.; Uhlman, W. An experimental study on the ripple-dune transition.
514 *Earth Surf. Proc. Land.* **2001**, 26, 615-629.
- 515 (15) Work, P. A.; Moore, P. R.; Reible, D. D. Bioturbation, advection, and diffusion of
516 a conserved tracer in a laboratory flume. *Water Resour. Res.* **2002**, 38, 1088.
- 517 (16) Freier, K. P.; Brüggemann, R.; Nützmann, G. Pharmazeutisch aktive Substanzen
518 in kleinen Fließgewässern. Modellierung unter Berücksichtigung des Einflusses von
519 Grundwasser. *UWSF – Zeitschrift für Umweltchemie und Ökotoxikologie* **2007**, 19, 189-
520 196.
- 521 (17) Wörman, A.; Packman, A. I.; Marklund, L.; Harvey, J. W.; Stone, S. H. Fractal
522 topography and subsurface water flows from fluvial bedforms to the continental shield.
523 *Geophys. Res. Lett.* **2007**, 34, L07402.
- 524 (18) Gröning, J.; Held, C.; Garten, C.; Claußnitzer, U.; Kaschabek, S. R.; Schlömann,
525 M. Transformation of diclofenac by the indigenous microflora of river sediments and
526 identification of a major intermediate. *Chemosphere* **2007**, 69, 509-516.

- (19) Lee, Y. B.; Lorenz, N.; Dick, L. K.; Dick, R. P. Cold storage and pretreatment incubation effects on soil microbial properties. *Soil Biol. Biochem.* **2007**, *71*, 1299-1305.
- (20) Packman, A.; Salehin, M. Relative roles of stream flow and sedimentary conditions in controlling hyporheic exchange. *Hydrobiologia* **2003**, *494*, 291-297.
- (21) Preuss, G.; Willme, U.; Zullei-Seibert, N. Verhalten ausgewählter Arzneimittel bei der künstlichen Grundwasseranreicherung – Eliminierung und Effekte auf die mikrobielle Besiedlung. *Acta Hydrochim. Hydrobiol.* **2001**, *29*, 269-277.
- (22) House, W. A.; Denison, F. H.; Smith, J. T.; Armitage, P. D. An investigation on the effects of water velocity on organic phosphorus influx to a sediment. *Environ. Pollut.* **1995**, *89*, 263-271.
- (23) Matamoros, V.; Caselles-Osorio, A.; Garcia, J.; Bayona, J. M. Behaviour of pharmaceutical products and biodegradation intermediates in horizontal subsurface flow constructed wetland. A microcosm experiment. *Sci. Total Environ.* **2008**, *394*, 171-176.
- (24) Daughton, C. G. Cradle-to-cradle stewardship of drugs for minimizing their environmental disposition while promoting human health. I. Rationale for and avenues toward a green pharmacy. *Environ. Health Persp.* **2003**, *111*, 757-774.
- (25) Packer, J. L.; Werner, J. J.; Latch, D. E.; McNeill, K.; Arnold, W. A. Photochemical fate of pharmaceuticals in the environment: Naproxen, diclofenac, clofibric acid, and ibuprofen. *Aquat. Sci.* **2003**, *65*, 342-351.
- (26) Labadie, P.; Budzinski, H. Determination of steroidal hormone profiles along the Jalle d'Eysines River (near Bordeaux, France). *Environ. Sci. Technol.* **2005**, *39*, 5113-5120.
- (27) Lin, A. Y. C.; Plumlee, M. H.; Reinhard, M. Natural attenuation of pharmaceuticals and alkylphenol polyethoxylate metabolites during river transport: Photochemical and biological transformation. *Environ. Toxicol. Chem.* **2006**, *25*, 1458-1464.
- (28) Fono, L. J.; Kolodziej, E. P.; Sedlak, D. L. Attenuation of wastewater-derived contaminants in an effluent-dominated river. *Environ. Sci. Technol.* **2006**, *40*, 7257-7262.
- (29) Arnon, S.; Gray, K. A.; Packman, A. I. Biophysicochemical process coupling controls nitrate use by benthic biofilms. *Limnol. Oceanogr.* **2007**, *52*, 1665-1671.

559 **Figures**
560
561

Figure 1

569 **Figure 2**

577 **Figure 3**
578

579
580

582
583

Figure Captions:

Figure 1: Concentration time trend of bezafibrate, gemfibrozil, and ibuprofen in surface water. Left: experiment WS.slow; right: WS.fast. Concentrations were normalized to the initial concentration of each compound in the surface water. Error bars represent standard error of replicate samples; dashed line represents c_{eq} .

Figure 2: Concentration trend of clofibric acid, bezafibrate, gemfibrozil, and ibuprofen in surface and pore water (mean concentration of all sampling ports) during experiment WS.fast. Error bars represent standard error of replicate samples (surface water: $n = 3$; pore water: $n = 5$). Dashed line represents c_{eq} .

Figure 3: Concentration trends of gemfibrozil, bezafibrate, and ibuprofen in surface and pore water (sampling site 1; see Supporting Information for location) in experiment WS.slow. Error bars represent standard error for replicate samples of surface water. Dashed line represents c_{eq} .

Figure 4: First-order primary biodegradation kinetics of pharmaceuticals from surface water in experiments WS.fast and WS.slow. Plots are based on recalculated concentrations (c^*) relative to clofibric acid and normalized to initial concentrations. Parameters for linear regression (solid lines) are given above. Regression for gemfibrozil in experiment WS.slow was not significant.

608 **Tables**

609

610 Table 1: Details of the experimental conditions. Height of sediment and water represents
 611 initial height at start of the equilibration period; v: flow velocity; Re: Reynolds number.

	Experiment			
	W	WS.abiotic	WS.slow	WS.fast
Sediment type	-	quartz sand	Roter Main	Roter Main
Sediment height (cm)	-	16.7	15.9	16.5
Water type	Roter Main	tap water	Roter Main	Roter Main
Water height (cm)	29.1	27.0	26.5	28.4
v (m s ⁻¹)	0.12	0.12	0.12	0.31
Re (-)	n.d.	n.d.	7823	19387

612 n.d. : not determined

613

614

615

616 Table 2: Calculated dissipation times (DT_{50}), dissipation distances (DD_{50}), half-life times
 617 (t_H), and half-life distances (d_H) for experiments WS.slow and WS.fast.

	Experiment WS.slow				Experiment WS.fast			
	DT_{50} (d)	DD_{50} (km)	t_H (d)	d_H (km)	DT_{50} (d)	DD_{50} (km)	t_H (d)	d_H (km)
Bezafibrate	4.3	45	8.4	87	2.5	67	4.3	113
Clofibric Acid	n/a	n/a	-	-	n/a	n/a	-	-
Diclofenac	8.5	88	18.6	193	3.2	84	5.5	144
Gemfibrozil	n/a	n/a	-	-	5.6	147	10.5	278
Ibuprofen	2.5	26	5.1	53	1.2	32	2.5	66
Naproxen	6.9	72	13.9	144	5.4	144	10.3	272

618 n/a: 1st order kinetics not applicable

619

620 BRIEF. The influence of surface water-sediment interactions on the biodegradation of
621 acidic pharmaceuticals is studied in bench-scale flume experiments.

622