MEETING THE CHALLENGES OF QUANTIFYING THE ELIMINATION PROCESSES OF PHARMACEUTICALS IN RIVERS

(BESTIMMUNG DER ELIMINIERUNG VON ARZNEIMITTEL-RÜCKSTÄNDEN IN FLÜSSEN: HERAUSFORDERUNGEN UND LÖSUNGEN)

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Heraklit

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Die Verfügbarkeit von Humanarzneimitteln zur Behandlung von Krankheiten aller Art bildet einen integralen Bestandteil der modernen Gesellschaft. Üblicherweise denken wir allerdings nicht weiter über den Wirkungsmechanismus des Schmerzmittels, der Herpessalbe oder des Hustensafts nach. Genauso kümmern wir uns kaum darum, was mit dem Wirkstoff geschieht, nachdem die gewünschte Wirkung eingesetzt hat. Nach ihrer Einnahme werden Pharmaka im Körper meist nur unvollständig metabolisiert, über Urin und Kot wieder ausgeschieden und ins Abwassersystem eingetragen. Somit werden unsere Kläranlagen nicht nur mit Pathogenen und Nährstoffen, sondern auch mit Tausenden von pharmazeutisch (und oftmals auch biologisch) wirksamen Substanzen konfrontiert. Da Kläranlagen nicht primär dazu konzipiert wurden, organische Mikroverunreinigungen – zu denen Arzneimittelrückstände zählen – zu entfernen, werden die meisten Arzneimittelwirkstoffe während der konventionellen Abwasserbehandlung nur bedingt abgebaut. Zum Teil werden Pharmaka zwar durch Transformationsprozesse während der biologischen Stufe oder durch Sorption an Überschussschlamm entfernt, dennoch wird ein Großteil kontinuierlich über die Kläranlagenabläufe in unsere Bäche und Flüsse emittiert. Konsequenterweise wurden Vertreter verschiedenster Pharmaka-Wirkstoffklassen in Flüssen, Seen, Grundwasser, dem Meer oder sogar im Trinkwasser nachgewiesen. Über die Langzeitwirkung dieses Cocktails an verschiedensten Substanzen auf aquatische Organismen und den allgemeinen ökologischen Zustand der Wasserkörper ist allerdings noch wenig bekannt.

Bisher fokussierten sich die meisten Forschungsprojekte auf das Beschreiben des Vorkommens von Arzneimittelrückständen an verschiedenen Stellen des urbanen Wasserkreislaufs. Diese Studien stellen aber kaum Daten bereit, aus denen zuverlässige quantitative Aussagen über Eliminierungsprozesse von Arzneimittelrückständen in einzelnen Kompartimenten und insbesondere unter den dynamischen Bedingungen in Fließgewässern abgeleitet werden können. Primär sind diese Eliminierungsprozesse: i) photochemische Transformation im Oberflächenwasser, ii) (mikro-)biologische Transformation in den Flusssedimenten und iii) irreversible Sorption an Sedimentpartikel. In den letzten Jahren haben zahlreiche Studien substanzspezifisch quantitative Daten für die einzelnen Reaktionen (Phototransformation, Biotransformation, Sorption) in kontrollierten Laborexperimenten bestimmt. Die Übertragung der Kenngrößen und Erkenntnisse auf die Feldskala gestaltet sich jedoch sehr schwierig, da die unter vereinfachenden Bedingungen im Labor gewonnenen Daten nur bedingt generalisierbar sind. Dennoch wurden bisher nur wenige Studien mit der Zielsetzung, quantitative Daten im Feld zu bestimmen, durchgeführt.

Ziel dieser Arbeit war daher, diese Wissenslücken zu schließen und nachvollziehbare quantitative Daten über das Verhalten von Arzneimittelrückständen in Fließgewässern zu gewinnen. Im Gegensatz zu bisherigen Arbeiten wurden aufeinander abgestimmte systemorientierte Feldkampagnen inklusive *in situ* Abbauexperimenten mit angepassten Laborstudien, die die komplexen hydraulischen Bedingungen in Flüssen und deren Sedimente besser abbilden, kombiniert. Besonderes im Fokus standen dabei i) das Aufstellen von Massenbilanzen und die Bestimmung von Eli-

minierungsraten in einzelnen Flussabschnitten, ii) die Beschreibung der Dynamik der Eliminierung von Pharmaka in Fließgewässern unter verschiedenen hydraulischen Randbedingungen, iii) die quantitative Bewertung der einzelnen Eliminierungsmechansimen in Fließgewässern sowie iv) die Ermittlung des Einflusses von Oberflächenwasser/Porenwasser-Interaktionen auf die Eliminierung von Pharmaka in Fließgewässern. Dazu wurde eine Auswahl an Pharmaka aus verschiedenen Wirkstoffklassen (Antibiotika, Antiepileptika, Betablocker, Lipidsenker, Schmerzmittel) betrachtet, die gewöhnlich in Fließgewässern in höheren Konzentrationen nachzuweisen ist. Die ausgewählten Substanzen decken ein breites Spektrum physikalisch-chemischer Eigenschaften ab und unterliegen in der Umwelt unterschiedlichen Eliminierungsprozessen. Durch quantitative Unterschiede in der Eliminierung der Zielsubstanzen kann somit zwischen verschiedenen Eliminierungsmechanismen für die einzelnen Pharmaka differenziert werden.

Sowohl zeitlich hoch dynamische Größen wie der Abfluss eines Flusses und die Konzentrationen an Pharmaka in Flüssen, als auch unbekannte Eintragspfade erschweren die Bestimmung von Massenbilanzen entlang bestimmter Flussabschnitte. Um diesem Problem zu begegnen wurde ein Tracerversuch mit Arzneimittelwirkstoffen in einem kleinen Bach (Säva Brook) ohne Hintergrundbelastung mit Pharmaka in der Nähe der schwedischen Stadt Uppsala durchgeführt. Hierzu wurden neben zwei Fluoreszenzfarbstoffen (Uranin, Rhodamin WT) sechs Pharmaka (Bezafibrat, Clofibrinsäure, Diclofenac, Ibuprofen, Metoprolol und Naproxen) als Dirac Puls in den Bach gegeben. Die eingesetzten Mengen wurden so gewählt, dass sich nach vollständiger Durchmischung umweltrelevante Konzentrationen im mittleren ng L^{-1}-Bereich einstellten. Entlang eines 16 km langen Flussabschnittes wurden an fünf Messstellen mit automatischen Probennehmern Wasserproben entnommen. Die Proben wurden über Festphasenextraktion (SPE) angereichert und die Konzentrationen der Pharmaka über Hochleistungsflüssigchromatographie gekoppelt mit Tandemmassenspektometrie (HPLC-MS/MS) bestimmt. Die Konzentrationen der Farbtracer wurden mittels Fluoreszenzspektroskopie ermittelt.

Das Schmerzmittel Ibuprofen wurde rasch mit einer Halbwertszeit von etwa zehn Stunden aus dem Oberflächenwasser entfernt und konnte an der letzten Messstelle nicht mehr detektiert werden. Analog nahmen die gemessenen Frachten von Clofibrinsäure mit der Fließstrecke ab und eine Halbwertszeit von ca. 2,5 Tagen wurde berechnet. Für die anderen vier betrachteten Pharmaka (Bezafibrat, Diclofenac, Metoprolol, Naproxen) konnte keine Entfernung nachgewiesen werden. Basierend auf den sehr geringen hydraulischen Leitfähigkeiten der tonigen Sedimente und der annähernd symmetrischen Form der Durchbruchskurven gibt es nur einen sehr geringen Austausch an Oberflächenwasser mit Speicherzonen im Säva Brook. Somit sollten Prozesse in den Flusssedimenten/der hyporheischen Zone nur zu einem geringen Teil zu einer (potentiellen) Eliminierung von Stoffen entlang der Fließstrecke beitragen können. Photoabbau wurde als wichtiger Eliminationsmechanismus für Ibuprofen und Clofibrinsäure ausgeschlossen, da die als wesentlich photolabiler beschriebenen Pharmaka Diclofenac und Naproxen nicht entfernt wurden. Nur für den Betablocker Metoprolol wurde eine signifikante Retardierung entlang der Fließstrecke festgestellt (reversible Sorption). Daher ist eine Entfernung von Ibuprofen und Clofibrinsäure aufgrund dauerhafter Sorption an Sedimenten ebenfalls sehr unwahrscheinlich. Vielmehr wurde

geschlussfolgert, dass die beiden Stoffe durch biologische Transformation in Biofilmen, die an Wasserpflanzen und auf der Sedimentoberfläche in hohem Ausmaß vorkamen, entfernt werden.

Basierend auf den Messdaten des Tracerversuchs wurde ein reaktives Stofftransportmodell entwickelt. Die Kopplung von physikalischen und biogeochemischen Prozessen ermöglichte die Quantifizierung von Prozessen innerhalb der fließenden Welle („main channel“) und Speicherzonen („transient storage zones“). Dadurch konnte der Beitrag von Prozessen innerhalb dieser beiden Zonen an Gesamtrückhalt und Eliminierung der Pharmaka während des Tracerversuchs bestimmt werden. Dies ermöglichte mittels inverser Modellierung erstmals die Ermittlung quantitativer Daten für Sorption und Transformation von Arzneimittelrückständen in Fließgewässern. Die modellierten Halbwertszeiten von Ibuprofen und Clofibrinsäure in den Speicherzonen (1,6 bzw. 22,1 Stunden) waren sehr viel kleiner als in der fließenden Welle (22,7 bzw. 113,2 Stunden). Jedoch sind durch die geringen Interaktionen der fließenden Welle mit den Speicherzonen die Aufenthaltszeiten der Pharmaka in der fließenden Welle während des Transports im Bach wesentlich höher als in den Speicherzonen. Daher trugen beide Zonen in etwa gleich zu der Gesamtentfernung bei.

Viele Pharmaka, die in der Literatur als biologisch abbaubar und/oder photolabil beschrieben sind, wurden während des Tracerversuchs nicht entfernt. Dies war höchstwahrscheinlich auf geringe Interaktionen zwischen dem Oberflächenwasser und den Sedimenten am untersuchten Fluss sowie einer starken Trübung des Wassers und geringe Strahlungsintensitäten während der Studie zurückzuführen. Aus diesem Grund wurde eine weiterführende Feldstudie an einem Fließgewässer, an dem optimale Bedingungen für die Entfernung von Pharmaka vorzufinden sind, durchgeführt. Als optimal wurden eine geringe Wassertiefe und Trübung für photochemische Prozesse und ein intensiver Austausch von Oberflächenwasser und Porenwasser sowie aerobe Verhältnisse in den Sedimenten für biologischen Abbau angesehen. Die Feldarbeiten fanden an einem kleinen Bach, der Gründlach, in der Nähe der Stadt Nürnberg statt. An beiden Enden eines 12,5 Kilometer langen Abschnittes wurden über einen Zeitraum von zwei Wochen Sechs-Stunden-Mischproben entnommen und auf zehn Arzneimittelwirkstoffe (Bezafibrat, Carbamazepin, Clofibrinsäure, Diclofenac, Ibuprofen, Metoprolol, Naproxen, Propranolol, Sotalol und Sulfamethoxazol) analysiert. Darüber hinaus wurde an drei Stellen entlang der Fließstrecke tiefenorientiert das Porenwasser beprobt. An denselben Messstellen wurden *in situ* Phototransformationsstudien durchgeführt indem Glasaquarien in das Flussbett eingebracht, mit Flusswasser befüllt und die Konzentration der Pharmaka über die Zeit bestimmt wurden. Oberflächenwassermischproben, Porenwasserproben sowie die Proben aus den Photoabbauexperimenten wurden mittels SPE aufkonzentriert und die Arzneimittelkonzentrationen durch HPLC-MS/MS bestimmt.

An der ersten Messstelle wurden Konzentrationen im Bereich von 3,5 ng L^{-1} für den Betablocker Propranolol bis hin zu 1400 ng L^{1} für das Schmerzmittel Diclofenac gemessen. Für alle Substanzen konnte eine Abnahme der Konzentrationen entlang der Fließstrecke durch eine Kombination aus Abbauprozessen und Verdünnung durch Zuflüsse von Grundwasser und kleineren Bächen beobachtetet werden. Um diese Verdünnungseffekte zu berücksichtigen, wurden die Eliminierungsraten der untersuchten Pharmaka relativ zu den Konzentrationsänderungen des Antiepilep-

tikums Carbamazepin quantifiziert. Carbamazepin gilt als sehr persistent in Oberflächengewässern und Sedimenten, weshalb sich diese Substanz sehr gut als konservativer Tracer eignet. Darüber hinaus erwies sich Carbamazepin während der Photoabbaustudien als stabil und konnte in allen Tiefen des Porenwassers (bis zu 30 cm) in ähnlichen Konzentrationen wie im Oberflächenwasser nachgewiesen werden. Die Untersuchungen zeigten, dass die Entfernung der meisten Pharmaka während trockener und sonniger Perioden (Zeitraum A) höher als während Zeiträumen mit erhöhtem Abfluss nach Starkniederschlagsereignissen (Zeitraum B) ist. Die errechneten Entfernungsraten lagen zwischen 25 % für das Antibiotikum Sulfamethoxazol (Zeitraum B) und 70 % für den Betablocker Propranolol während der Trockenperiode (Zeitraum A). Photoabbau war nur für das Schmerzmittel Diclofenac und für den Betablocker Sotalol ein relevanter Eliminierungspfad. Für beide Substanzen wurden Halbwertszeiten für photochemische Transformation im Bereich von einigen Stunden in unbeschatteten Bereichen des Flussabschnitts bestimmt. In Dunkelkontrollen wurden weder Diclofenac und Sotalol noch alle anderen Pharmaka entfernt. Neben Photoabbau spielten somit keine weiteren Eliminierungsmechanismen (z.B. Hydrolyse, Bioabbau) im Oberflächenwasser eine entscheidende Rolle. Für Stoffe, wie für den Betablocker Metoprolol und den Lipidsenker Bezafibrat wurde jedoch eine weitgehende Entfernung entlang der Fließstrecke beobachtet. Daher müssen andere Prozesse als Photoabbau hierfür verantwortlich sein. Mit hoher Wahrscheinlichkeit lassen sich die Eliminierungen auf biologischen Abbau in den Flusssedimenten zurückführen. Dies zeigte sich dadurch, dass ihre Konzentrationen im Verhältnis zu Carbamazepin mit der Tiefe im Sediment abnahmen. Darüber hinaus wurde der postulierte Bioabbau für den chiralen Betablocker Metoprolol durch eine Änderung der Verhältnisse der beiden Enantiomere (enantiomer fraction, EF) von der ersten Probenahmestelle (EF = 0,49) zur zweiten Messstelle (EF = 0,43), sowie einer Abnahme des EF bis auf < 0,40 in den tieferen Schichten der Sedimente belegt.

Quantitative Daten für biologische Transformation der Pharmaka in Flusssedimenten wurden zudem in eigens entwickelten Labortestsystemen bestimmt. Standardmäßig wird die biologische Transformation organischer Spurenstoffe in statischen Experimenten bestimmt. In diesen Testsystemen können generelle Aussagen über die biologische Abbaubarkeit von Stoffen sowie die Bildung von Transformationsprodukten getroffen werden. Das Ableiten von auf die Feldskala übertragbarer Transformationsraten ist allerdings problematisch, da die statischen Bedingungen im Gegensatz zu dem hier angewendeten experimentellen Ansatz nicht die komplexen Interaktionen an der Wasser/Sediment-Grenzfläche in Flüssen widerspiegeln. Um diese advektiv dominierten Austauschprozesse in Laborsystemen besser abzubilden und somit Abbauraten unter umweltnahen hydraulischen Bedingungen zu bestimmen, wurden Säulenversuche konzipiert, in denen Wasser aus Vorratsgefäßen mit definierbaren Filtergeschwindigkeiten kontinuierlich durch das Sediment gepumpt werden konnte. Der Ablauf der Säulen wurde in das Vorratsgefäß zurückgeleitet, so dass ein rezirkulierendes System entstand. Mit Hilfe des neu entwickelten Testsystems wurden Kinetiken für den biologischen Abbau ausgewählter Pharmaka (Bezafibrat, Carbamazepin, Clofibrinsäure, Diclofenac, Ibuprofen, Metoprolol, Naproxen und Propranolol) in verschiedenen Sedimenten bestimmt. Ein weiteres Augenmerk lag auf der Überprüfung des Generalisierungspo-

tenzials der Abbaukinetiken aus den Säulenversuchen und der Reproduzierbarkeit der Ergebnisse. Hierzu wurden zahlreiche Wiederholungen der einzelnen Experimente durchgeführt und der Einfluss von Randbedingungen, insbesondere der eingestellten Filtergeschwindigkeiten, systematisch untersucht.

Abgesehen von einer anfänglichen Einstellung eines Sorptionsgleichgewichts für die beiden Betablocker Metoprolol und Propranolol trugen abiotische Prozesse in den Sedimenten nicht zur Eliminierung der Pharmaka bei. Ebenso spielten biotische Prozesse im Oberflächenwasser nur eine untergeordnete Rolle. Die Konzentrationsverläufe der Pharmaka in den einzelnen Replikaten unterschieden sich nur unwesentlich, eine gute Reproduzierbarkeit der Ergebnisse ist daher gewährleistet. Die Eliminierungsrate eines bestimmten Pharmakons unterschied sich in verschiedenen Sedimenten nur unwesentlich. Zudem waren die Eliminierungsraten der untersuchten Pharmaka in den verschiedenen Sedimenten korreliert, was die Erstellung eines generellen Klassifikationsschemas der biologischen Abbaubarkeit der Pharmaka in Flusssedimenten erlaubte. Für alle Substanzen lagen die abgeleiteten Eliminierungsraten deutlich über den aus statischen Batchexperimenten bestimmten Werten. Die Halbwertszeiten für den biologischen Abbau von gut abbaubaren Substanzen wie Ibuprofen oder Metoprolol lagen zwischen 0,5 und 1,8 Tagen bzw. 0,9 und 3,6 Tagen. Darüber hinaus wurden auch Pharmaka, die als relativ persistent in der aquatischen Umwelt beschrieben werden (z.B. Clofibrinsäure), rasch in den Experimenten mit minimalen Halbwertszeiten von 2,9 Tagen transformiert. Die Filtergeschwindigkeit hatte nur einen geringen Einfluss auf die Eliminierungsraten der Pharmaka. Insgesamt konnte gezeigt werden, dass das gewählte Testdesign aus rezirkulierenden Säulenversuchen zwei entscheidende Vorteile gegenüber Standardtestverfahren aufweist. Einerseits liegen die ermittelten Abbauraten wesentlich näher an den wenigen bestimmten *in situ* Abbauraten als die aus Batchversuchen berechneten Werte und stellen somit realistischere Raten dar. Andererseits werden die Abbaukinetiken im Gegensatz zu Batchexperimenten unter definierten hydraulischen Bedingungen bestimmt und können dadurch einfacher auf andere Testsysteme oder die in der Umwelt vorherrschenden Bedingungen übertragen werden. Zudem konnte gezeigt werden, dass das gegenläufige Eliminierungsverhalten von Bezafibrat und Diclofenac in Feldstudien an zwei Flüssen nicht auf ein unterschiedlicheres Potenzial zur biologischen Transformation der vorliegenden Sedimente zurückzuführen. Die Abbauraten lagen in Sedimenten aus den beiden Flüssen jeweils in ähnlichen Bereichen (Halbwertszeiten von Bezafibrat: 1,1 – 9,3 Tage, Diclofenac: 1,5 – 4,1 Tage). Somit sind unterschiedliche Eliminierungsraten im Feld vielmehr durch die jeweiligen hydrologischen Bedingungen, insbesondere der hydraulischen Anbindung des Oberflächenwassers an die hyporheische Zone in Flüssen bestimmt.

Zusammenfassend konnte diese Arbeit einen Teil der nach wie vor vorhandenen Wissenslücke über das Verhalten von Pharmaka in Fließgewässern schließen und wertvolle neue Erkenntnisse über die relevanten Eliminierungsmechanismen liefern. Durch eine geschickte Auswahl an Referenzsubstanzen und die Kopplung von strategischen Messkampagnen mit *in situ* Abbaustudien konnten einzelne und substanzspezifische Eliminierungsprozesse in Fließgewässern quantifiziert werden. Es konnte ebenfalls durch eine Kombination der systematischen Feldstudien mit

innovativen Laborexperimenten gezeigt werden, dass insbesondere (mikro-)biologische Transformationsprozesse in den Flusssedimenten/in der hyporheischen Zone entscheidend zur Entfernung von Arzneimittelrückständen aus Fließgewässern beitragen können. Allerdings wird dieses Reinigungspotenzial der Flusssedimente aufgrund eines mangelnden Austausches von Oberflächenwasser und Porenwasser und den darin gelösten Substanzen oftmals nur bedingt ausgeschöpft. Photochemische Transformationsprozesse im Oberflächenwasser sind nur in klaren und flachen Flüssen sowie bei geringer Beschattung von quantitativer Bedeutung. Daher besitzen vor allem kleine Bäche mit einem turbulenten Fließregime eine gute Selbstreinigungsleistung für Pharmaka.

SUMMARY

The availability of pharmaceuticals for the treatment of all kind of diseases is an essential part of modern society. But normally, the average woman on the street does neither care why the drugs work nor worry about their fate after she has taken the pain killer, the herpes cream or the cough syrup. After medication, pharmaceuticals are only incompletely metabolized and excreted via urine and feces. Hence, wastewater treatment plants (WWTPs) do not only have to deal with pathogens and nutrients but also with high loads of several thousands of pharmaceutically (and biologically) active substances. Since the setup of WWTPs was originally not designed to focus on the elimination of organic micropollutants such as pharmaceuticals, most pharmaceuticals are only incompletely eliminated during conventional wastewater treatment. Partially they can be removed via biological transformation processes or sorption to excess sludge. Nevertheless a big part of the pharmaceutical residues is continuously released into our rivers and streams via the discharge of treated wastewater. Therefore, numerous studies have reported the occurrence of various classes of pharmaceuticals in rivers, lakes, the sea, groundwater, and even in drinking water. While the knowledge on the effects of this cocktail of micropollutants on aquatic organisms and the general ecological status of the water bodies is still scarce, long-term negative effects on organisms cannot be excluded and have already been confirmed for some compounds.

Past research projects concentrated mostly on the detection and monitoring of pharmaceuticals at various locations in the urban water cycle. However, those studies provide only very little data which can be used to derive reliable elimination rates of pharmaceuticals in the aquatic environment. Moreover, there is a lack of systematically derived data of the importance of individual elimination processes to the total elimination of pharmaceutical residues in streaming waters. There, the major potential elimination processes are: i) photochemical transformation in the surface water, ii) (micro-)biological transformation in the river sediments, and iii) irreversible sorption to river sediments. Using laboratory test systems numerous studies have determined substance specific quantitative data for individual processes (phototransformation, biotransformation, sorption). However, the transfer of these rates and constants to the field scale is not straightforward since the simplified laboratory experiments do not properly picture the more complex and dynamic situations at real rivers. Additionally, only a few field studies aiming to derive quantitative data *in situ* for the individual elimination processes at rivers were conducted up to now.

Thereto, the overarching aim of this work was to narrow the knowledge gap on quantitative data for the elimination of pharmaceutical residues in rivers and streams. By a comprehensive approach of combined field studies and adapted experimental laboratory test systems, the importances of individual elimination processes were elucidated. In detail, the main questions and tasks of this thesis were to i) calculate mass balances and elimination rates of pharmaceuticals in selected rivers, ii) determine the dynamic behavior of their mass balances in streaming waters under different hydrologic conditions, iii) quantify the individual elimination processes and evaluate

their relevance in surface waters, and iv) determine the influence of the extent of exchange of surface water and pore water (hyporheic exchange) on the elimination and dynamics of pharmaceuticals in streaming waters. During all the tests, the fate of a set of pharmaceuticals from different therapeutic classes which are frequently detected in the aquatic environment (analgesics, antiepileptics, antibiotics, beta-blockers, lipid regulators) was investigated. The purposeful selection of this set of target substances allowed differentiating between the individual elimination mechanisms based on their different physicochemical properties and reported environmental fate.

Calculating mass balances for specific wastewater-borne substances along river stretches is challenging since their input is governed by various factors and is often strongly variable in time. To account for this problem, a tracer experiment was performed at a small Swedish river (Säva Brook) near the city of Uppsala without substantial background contamination of pharmaceuticals. A defined amount of two fluorescent dyes (uranine and rhodamine WT) were added as Dirac pulse into the stream. Simultaneously, six pharmaceuticals (bezafibrate, clofibric acid, diclofenac, ibuprofen, metoprolol, and naproxen) were injected. The injected amount was chosen to establish concentrations in the stream after completely mixing which are representative for wastewater impacted rivers (mid ng L^{-1} range). Downstream of the injection site, water samples were taken with automated water samplers at five different locations over a total length of 16 kilometers. Subsequently, the water samples were enriched via solid phase extraction (SPE) and pharmaceuticals were determined by high-performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS). The concentrations of the dye tracers were determined via fluorescence spectroscopy.

Ibuprofen was rapidly removed from the surface water and could not be detected anymore at the last monitoring site; a half-life time of approx. ten hours was calculated. The load of clofibric acid also decreased along the river stretch with a calculated half-life time of about 2.5 days. For the four other substances (bezafibrate, diclofenac, metoprolol, and naproxen), no significant removal was observed. Based on the low hydraulic conductivity of the river sediments and on the almost symmetrical shape of the tracer breakthrough curves, we concluded that the exchange of river water with the storage zones was small. Hence, processes in the hyporheic zone/river sediments can supposedly only contribute to a minor extent to the total attenuation along the river stretch. Phototransformation was also excluded as major elimination pathway for ibuprofen and clofibric acid, since the more photolabile substances diclofenac and naproxen were not eliminated. Metoprolol was the only substances that was substantially retarded (by reversible sorption) during river transport. Thus elimination of ibuprofen and clofibric acid due to permanent sorption processes is also highly unlikely. Hence, we concluded that ibuprofen and clofibric acid were potentially transformed and eliminated in biofilms which grew on submerged macrophytes and at the surface water/sediment interface.

In a follow-up study, the data from the tracer test was evaluated using reactive transport modeling. With a coupled physical–biogeochemical modeling framework, we were able to determine the individual contributions of stream channel and transient storage zone processes to the

overall retention and elimination of each of the six pharmaceuticals. Using inverse simulation techniques, for the first time quantitative data for sorption and transformation of pharmaceuticals in both main channel and storage zones were derived. The modeled half-life times of ibuprofen and clofibric acid were much shorter in the storage zones (1.6 hours and 22.1 hours, respectively) than in the main channel (22.7 hours and 113.2 hours). However, due to only small exchange fluxes between the main channel and the storage zones and thus much higher residence times of pharmaceuticals in the main channel compared to the storage zones during river transport, transformation processes in both zones contributed to a similar extent to the total elimination. This example further highlights that the prediction of the elimination of pharmaceuticals in rivers as well as the explanation of an observed attenuation behavior without exact knowledge of the prevailing hydraulics is virtually impossible.

Since conditions at Säva Brook were not optimal for the biotransformation in sediments and photochemical processes (little surface water/pore water interactions, high turbidity of the water and low radiation intensities), an additional field study was carried out at a river which supposedly exhibited best-case conditions for the elimination of pharmaceuticals. We defined these as a shallow stream and with low turbidity for phototransformation, an intense flux of water and solutes across the water/sediment interface and aerobic conditions in the river sediments. The experiments were carried out at a small stream in Northern Bavaria, Germany, near the city of Nuremberg (river Gründlach). Six-hour-composite water samples were taken over a time span of two weeks during summer time at both ends of a 12.5 km long river stretch located downstream of the WWTP Heroldsberg and analysed for a total of ten pharmaceuticals (bezafibrate, carbamazepine, clofibric acid, diclofenac, ibuprofen, metoprolol, naproxen, propranolol, sotalol, and sulfamethoxazole). Moreover, pore water was sampled depth-resolved and *in situ* photolysis experiments were performed at three locations within the river stretch to assess the individual importance of the attenuation mechanisms. Composite surface water samples, pore water samples, and samples from the photolysis experiments were enriched via SPE and pharmaceuticals were determined by HPLC-MS/MS.

The concentrations of the pharmaceuticals in the surface water at the first sampling site ranged from 3.5 ng L^{-1} for the beta-blocker propranolol to 1400 ng L^{-1} for the analgesic diclofenac. The concentrations of all substances decreased from the first to the second sampling site due to a combination of attenuation and dilution processes caused by inflow of unpolluted groundwater and minor creeks. To correct for dilution, attenuation rates were determined in relation to the antiepileptic drug carbamazepine which is known to be persistent in the aquatic environment and whose appropriateness as conservative tracer has been reported before. Moreover, carbamazepine was stable during the phototransformation experiments and present in all depths in the pore water samples in the same concentration range than in the surface water. Relative to carbamazepine, the load of all other pharmaceutical residues decreased along the river stretch. Their elimination was higher during a sunny, dry weather period (period A) in comparison to a second period with an elevated discharge of river Gründlach after a heavy rainfall (period B). Overall, the calculated elimination rates ranged from 25 % for the antibiotic sulfamethoxazole (period B) to 70 %

for the beta-blocker propranolol (period A). Photolysis was only a relevant elimination process for the analgesic diclofenac and for the beta-blocker sotalol. For these two compounds phototransformation half-life times of some hours were determined in the unshaded parts of the river. No transformation of diclofenac, sotalol, and of all other pharmaceuticals was observed in dark controls of the photolysis experiment providing proof that besides photolysis no other process in the surface water (biotransformation, hydrolysis) is relevant for elimination. However, since substances such as the beta-blocker metoprolol or the lipid lowering agent bezafibrate were also eliminated along the river stretch, another process than photolysis must have occurred. This process was most likely biotransformation in the sediments since their concentrations relative to carbamazepine decreased with depth in the sediments. The hypothesis of biological elimination processes in the river sediments was confirmed by a decrease in the enantiomer fractionation (EF) of the chiral beta-blocker metoprolol from 0.49 at the first sampling site to 0.43 at the second sampling site and to < 0.40 in the deeper parts of the sediments.

As a final task of this thesis, an experimental setup was developed aiming to derive more realistic rate constants for the elimination of pharmaceuticals in river sediments than those obtained from standard laboratory test systems. Commonly, the biotransformation of organic micropollutants in river sediments is determined in static batch experiments. While these test systems provide valuable information about the general biodegradability of substances in sediments, the derived rate constants are often not realistic for the transformation in real rivers since the hydraulics in these systems does not mimic the conditions in real rivers. To take this into account, an experimental system where surface water was actively pumped through sediment columns in a recirculating manner was developed. The elimination kinetics of eight commonly detected pharmaceutical residues that were previously studied in the field experiments (bezafibrate, carbamazepine, clofibric acid, diclofenac, ibuprofen, metoprolol, naproxen, and propranolol) were determined in different sediments. To check the robustness of the test systems as well as the generalization potential of the elimination rate constants deduced from the test system, we systematically varied the filter velocities and conducted several replicates of each approach.

Besides an initial equilibrium sorption period for the beta-blockers metoprolol and propranolol, abiotic transformation processes in the river sediments as well as biotic processes in the surface water were negligible for the elimination of pharmaceuticals. Their concentration trends in replicate experiments were similar. Hence, the experimental approach provides reproducible results. Moreover, the elimination rates of each pharmaceutical in the different tested sediments were similar and we were able to rank the substances according to their biotransformability. For all substances, the derived biological elimination rate constants were much faster than literature data from static batch systems. The half-life times of rapidly eliminated substances such as ibuprofen and metoprolol ranged from 0.5 to 1.8 days and 0.9 to 3.6 days, respectively. Even substances with a low biodegradability such as clofibric acid were removed at high rate constants corresponding to half-life times as short as 2.9 days in some of the experiments. The adjusted filter velocity in the column experiments has only a minor influence on the elimination kinetics of pharmaceuticals compared to the sediment characteristics. Moreover, the test design is applica-

ble to derive process based elimination rates in advectively flowed through sediments compared to primarily diffusion controlled rates under less controllable hydraulic conditions in batch experiments. Since the (hydrological) boundary conditions in the test systems can be adjusted and quantitatively described elimination rates can be more easily compared to other test systems and translated to the situation in real rivers. Additionally, the column experiments provide evidence that the contradictive attenuation behavior of bezafibrate and diclofenac at two rivers was not a result of a different biological transformation potential of the respective sediments as both compounds were efficiently eliminated in the column experiments using sediments from both sites (half-life times of bezafibrate: 1.1 – 9.3 days, diclofenac: 1.5 – 4.1 days). Consequently, the observed discrepancy in attenuation at the two river stretches is supposedly a consequence of different hydraulics in the two river systems.

In summary, this thesis provided valuable new insight into the fate of pharmaceuticals residues in streaming waters. By an intelligent selection of reference substances and the coupling of well-designed sampling campaigns with *in situ* transformation experiments quantitative data on individual elimination processes were derived. The combination of the systematic field studies and newly designed innovative laboratory scale experiments elucidated that especially microbial transformation processes in river sediments (hyporheic zone) can act as major player in the attenuation of pharmaceuticals in our rivers. However, these processes are often limited by the lack of exchange of surface water and pore water and hence, the attenuation potentials of the river sediments are not fully exploited. Photochemical transformation processes in the surface water are quantitatively only relevant in shallow and clear streams. Therefore, especially small rivers with a high turbulence provide optimum conditions for the elimination of pharmaceuticals.

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1 GENERAL INTRODUCTION

1.1 Input and Occurrence of Pharmaceutical Residues into the Aquatic Environment and Rivers

The catch phrase technological change is primarily linked to terms like globalization and advances in transportation and telecommunication. However, people often neglect that the development of the modern society is also strongly linked to improvements related to human health due to rapid advances in hygiene and medication. It has been a long way from the application of medicinal herbs in the antique and in the middle age to today's huge pharmaceutical market. However, there are two sides to every coin: we suddenly have to deal with a wide-spread occurrence of the salutary substances in non-target environments (e.g., rivers, soils, drinking water) and are only slowly becoming aware how to deal with this.

Most pharmaceuticals are not completely metabolized during human metabolism. Hence, they are excreted via urine and faeces (Ashton et al. 2004, Ternes 1998) and pharmaceutical residues are commonly detected in raw wastewater (Halling-Sørensen et al. 1998, Heberer 2002b, Heberer et al. 1998, Hirsch et al. 1999, Soulet et al. 2002, Ternes 1998, Ternes and Hirsch 2000). Concentration in hospital effluents can even be orders of magnitudes higher (Gomez et al. 2006, Lindberg et al. 2004). Therefore, pharmaceuticals have become a new class of organic micropollutants our wastewater treatment plants (WWTPs) have to cope with. The two main elimination processes for organic micropollutants such as pharmaceuticals during conventional wastewater treatment are: i) biological degradation during active sludge treatment and ii) sorption to excess sludge that is continuously removed during the treatment (Jelic et al. 2011, Joss et al. 2005, Joss et al. 2006, Maurer et al. 2007, Wick et al. 2011a). Photodegradation during wastewater treatment is negligible due to the high turbidity of the wastewater. Elimination by volatilization is also only of minor importance since pharmaceuticals usually possess only a low volatility (Daughton and Ternes 1999). In contrast to ingredients of personal care products and biocides (Carballa et al. 2008, Wick et al. 2011a), elimination of pharmaceuticals due to sorption to excess sludge is only of importance for some more hydrophobic pharmaceutical substances such as antidepressants and some antibiotics (Jelic et al. 2011). However, most pharmaceuticals are designed to be hydrophilic in order to pass membranes in humans after application (Halling-Sørensen et al., 1998). Therefore, the extent of biological degradation is the crucial factor for the total removal of pharmaceuticals during wastewater treatment. This removal rate is influenced by many factors such as the treatment scheme of the WWTP, season, sludge retention time (SRT) and is also highly substance specific. For most substances, removal during wastewater treatment is only incomplete (Jelic et al. 2011, Joss et al. 2005, Kahle et al. 2008a, Kahle et al. 2008b, Lishman et al. 2006, Petrovic et al. 2009, Quintana et al. 2005, Reif et al. 2011, Tauxe-Wuersch et al. 2005, Wick et al. 2009). Even if pharmaceuticals are partially eliminated, this removal cannot be seen equal to mineralization, i.e., the conversion to CO_2 and H_2O. In contrast, often stable transformation products (TPs) with only minor change in the chemical structure (such as an introduction of a hydroxyl

group or carboxylation of an alcoholic group) are formed (Schulz et al. 2008, Wick et al. 2011b). Therefore, the discharge of treated wastewater constitutes a major source for pharmaceuticals and their TPs into the aquatic environment (Ashton et al. 2004, Heberer 2002a, Lindqvist et al. 2005, Reemtsma et al. 2006).

In recent years, upgrading conventional WWTPs with novel technologies has been discussed to improve their performances. It has been shown that ozonation (Huber et al. 2005, Ternes et al. 2003, Zimmermann et al. 2011), the use the of granular and powdered activated carbon (Nowotny et al. 2007), the combination of ozonation and sand filtration (Hollender et al. 2009), microfiltration (Gabet-Giraud et al. 2010), reverse osmosis (Xu et al. 2005) or combinations of these processes can be suitable to remove pharmaceuticals from (waste)water. While their implication as tertiary treatment step is currently discussed intensively (Joss et al. 2008) and first full-scale plants are upgraded, a lot of water will flow under the bridge until all WWTPs will be re-tooled. Therefore, the discharge of pharmaceuticals into surface waters via treated wastewater will continue. Moreover, it was also shown that an enhanced treatment (resulting in a reduction of the concentration of pharmaceuticals in rivers) such as the chlorination of water can even lead to enhanced ecotoxicological effects by formation of toxic disinfection by-products (Sedlak and von Gunten 2011). Besides WWTPs as point sources for pharmaceuticals there are several other routines that introduce pharmaceuticals into the aquatic environment. Among these, the most important are e.g., the irrigation of treated wastewater on agriculturally used land (Ternes et al. 2007), and the application of manure or sludge to agricultural land (Boxall et al. 2002, Gottschall et al. 2012, Watanabe et al. 2010).

Due to all these releases, pharmaceutical residues are frequently determined in the aquatic environment. The first pilot studies of pharmaceuticals in the aquatic environment were conducted in the 1970^{ies} and for example, the active metabolites of the lipid lowering agent clofibrate (clofibric acid) and of the analgesic drug aspirin (salicylic acid) were reported in WWTP effluents in the U.S. (Hignite and Azarnoff 1977). During, the 1980/90^{ies}, improvements in analytical chemistry and especially the rapid development in both gas chromatography (GC) or liquid chromatography (LC) and mass spectrometry (MS) and their subsequent coupling (GC-MS/LC-MS) were the foundation for an enormous number of studies dealing with the occurrence of the pharmaceuticals residues in the aquatic environment (Bendz et al. 2005, BLAC 2003, Daughton and Ternes 1999, Kolpin et al. 2002, Lindqvist et al. 2005, Peng et al. 2008). The highest concentrations (up to µg L^{-1}) are normally determined in wastewater impacted rivers downstream of WWTPs (Radke et al. 2010, Ternes 1998). Then, due to dilution with unpolluted waters or attenuation processes, the concentrations are usually declining with distance from the WWTP. Nevertheless, pharmaceutical residues have been detected in lakes (Buser et al. 1998a, Buser et al. 1998b, Heberer et al. 1998), the sea (Buser et al. 1998a, Halling-Sørensen et al. 1998, Weigel et al. 2002), groundwater (Müller et al. 2012, Prasse et al. 2011, Reh et al. 2013, Sacher et al. 2001, Ternes and Hirsch 2000) and even in finished drinking water (Heberer 2002b, Jones et al. 2005, Musolff et al. 2007, Prasse et al. 2011). Additionally, TPs of pharmaceuticals that are formed during wastewater treatment have also have detected in surface water (Fatta-Kassinos et al. 2011, Schulze et al. 2010) as well as in

groundwater and drinking water (Kormos et al. 2011, Prasse et al. 2011), showing the persistence of these TPs in the aquatic environment.

As shown above, pharmaceuticals are almost ubiquitously present in the aquatic environment. However, the concentrations are usually in the low ng L^{-1} range and therefore several orders of magnitude lower than therapeutic doses. For example, even drinking three litres of water from the river Rhine with an average concentration of diclofenac of 50 ng L^{-1} (Sacher et al. 2008) over a time span of 70 years would only result in a total uptake of 8.2 mg diclofenac which corresponds to only 8.2 % of the defined daily dosage (DDD, 100 mg). As the concentrations in drinking water are even lower, it can be assumed that the indirect exposure with pharmaceuticals via drinking water poses no immediate health threat for humans (Webb et al. 2003). However, in the urban water circle, there is not one single substance present at these low concentrations but rather a complex mixture of pharmaceuticals and TPs has to be evaluated. This mixture can potentially evoke negative effects on multiple levels ranging from enzymes, individuals, species, to the whole aquatic community or ecosystem functions (Borgmann et al. 2007, Cleuvers 2003, Cunningham et al. 2006, Kortenkamp et al. 2009, Kostich and Lazorchak 2008, Lawrence et al. 2012, Luna-Acosta et al. 2012, Pomati et al. 2008). Additionally, it has to be distinguished between acute effects and impacts from continuous exposure (chronic effects). For the analgesic drug diclofenac damages of the kidneys and gills in rainbow routs have been determined when being exposed for 28 days to environmentally relevant concentrations (Schwaiger et al. 2004) leading to its inclusion on the “watch list” of substances that might be included into the list of priority substances of the Water Framework Directive (WFD) during its next revision process.

Pharmaceuticals residues and their TPs are commonly detected organic micropollutants in the aquatic environment, i.e., in wastewater impacted rivers and can potentially evoke adverse effects to aquatic organisms and ecosystem functions. Measures to reduce the input of pharmaceuticals into rivers will take years to be adequately implemented. Therefore, a profound understanding of the processes that govern the fate of pharmaceuticals in rivers is essential to assess their environmental risk.

1.2 Potential Elimination Processes of Pharmaceuticals in Rivers

After the discharge of pharmaceutical residues into rivers and streams – which is mainly attributed to inflow of treated wastewater (see above) – several processes govern their (potential) elimination from rivers including volatilisation, direct and indirect photolysis in the surface water, hydrolysis, sorption to suspended matter and sediments, biotransformation in the surface water and sediments or, loss of substances to groundwater (Figure 1-1).

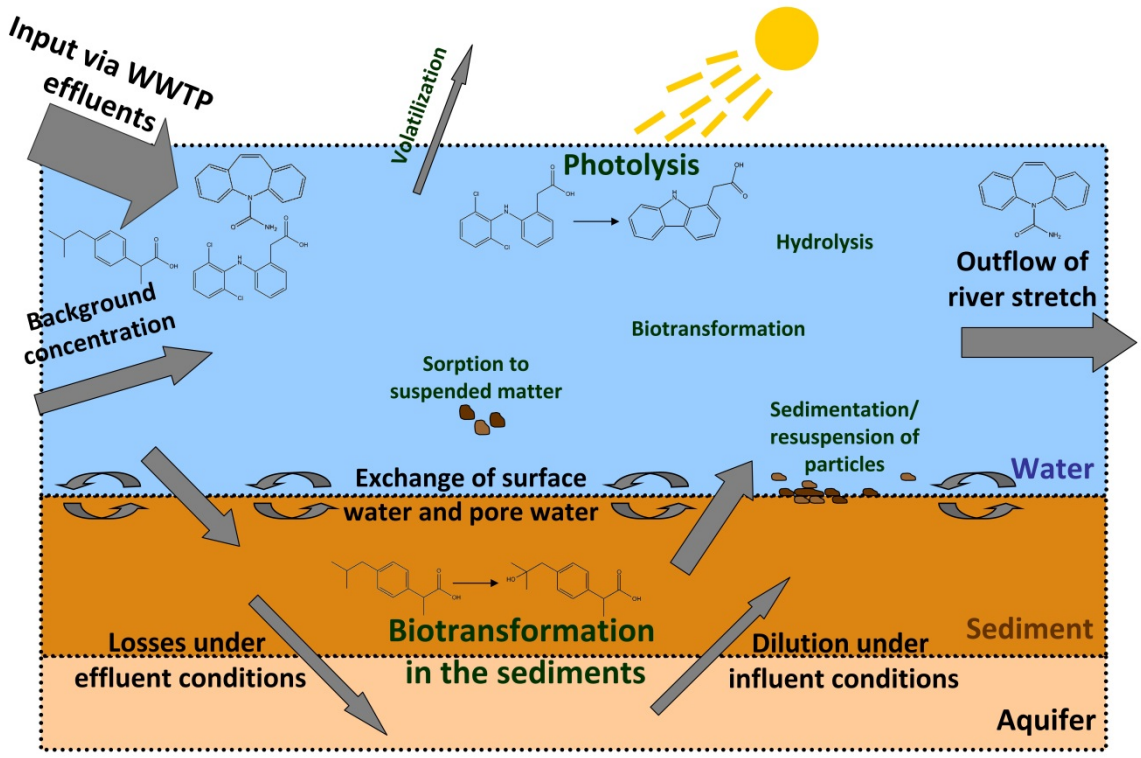


Figure 1-1: Conceptual model of the elimination of pharmaceutical residues in rivers and river sediments.

However, several of these potential elimination processes in rivers are of minor importance for pharmaceuticals residues. In contrast to other organic micropollutants such as ingredients of personal care products (e.g., musk fragrances) the vapour pressure of most pharmaceuticals is rather low and hence, elimination from rivers by volatilisation is negligible (Bendz et al. 2005, Daughton and Ternes 1999, Paxéus 2004). The same is true for hydrolysis and biotransformation in the surface water (Kunkel and Radke 2008, Lam et al. 2004, Perez-Estrada et al. 2005, Poiger et al. 2003, Wang et al. 2012). The fraction of pharmaceutical residues that is sorbed to suspended matter in rivers is a function of the sorption affinity of the substances and the concentration of suspended solids in the river and independent of the geometry of the river (Figure 1-2). The sorption affinity of a substance is commonly described by the distribution coefficient between the dissolved concentration and sorbed concentration (K_d). Often this distribution coefficient is normalized to the organic fraction of the sediment (K_{oc}). Most pharmaceuticals are hydrophilic (log $K_{OC} < 2$) and therefore only a small proportion is transported downstream sorbed to suspended matter (Figure 1-2). Even for the beta-blocker propranolol – the pharmaceutical with the highest sorption affinity that is addressed in this thesis (log K_{OC} 2.5-3.5 (Drillia et al. 2005b, Maurer et al. 2007, Ramil et al. 2010)) less than five percent of the total load is being sorbed at elevated concentrations of the organic fraction of suspended solids (e.g., 20 mg L^{-1}, Figure 1-2). Consequently, neither settling of particulate matter is an efficient elimination pathway nor resuspension of suspended solids from the sediments constitutes a substantial source for typical pharmaceutical residues in rivers. Hence, for the scope of this thesis, analysis of suspended matter can be omitted

and only the dissolved concentrations of pharmaceuticals are taken into account for the calculation of mass balances along river stretches.

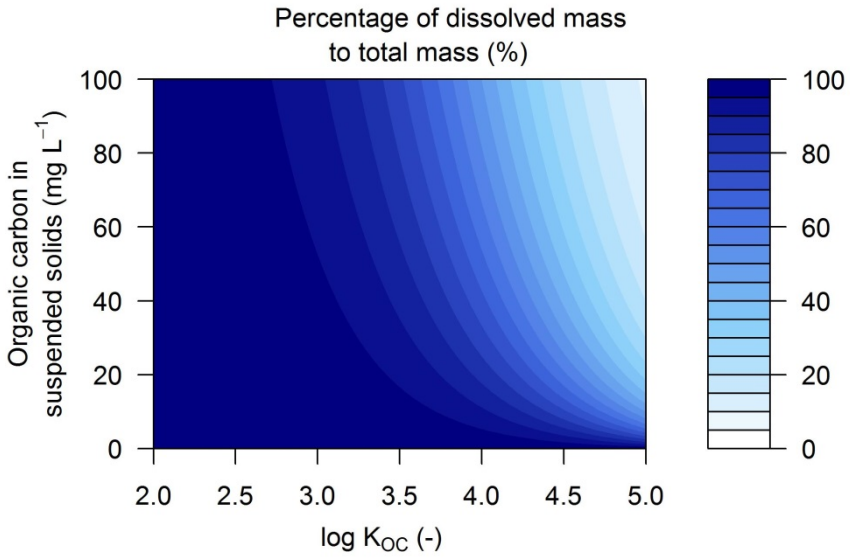


Figure 1-2: A priori estimation of the percentage of dissolved mass compared to the total mass of pharmaceuticals in rivers as function of the K_{OC} value of a substance and the organic carbon in suspended solids.

Most other potential elimination pathways are strongly influenced by the meteorological, hydrological and geomorphologic conditions as well as by the stream characteristics (stream width, water depth, flow velocity, turbidity, and river/groundwater interactions). The rate of direct photolysis of pharmaceuticals due to the absorption of photons is i) governed by the structure and electronic absorption spectrum of the substance, ii) the quantum yield of the photochemical reaction and iii) the solar radiation (a factor of season, latitude, weather, shading of the river, water depth, and turbidity (Zepp and Cline 1977)) to which the substance is exposed (OECD 2008). Additionally, pharmaceuticals can be eliminated by indirect phototransformation reactions i.e., interacting with reactive species in the water produced by solar radiation. These species include photosensitizers and singlet oxygen which are mainly formed by chromophoric dissolved organic matter (CDOM) or $^{\bullet}$OH radicals which are primarily formed by nitrate (Zepp et al. 1987).

Biological transformation of pharmaceuticals in river systems is mainly restricted to the sediments (Kunkel and Radke 2008). Also elimination due to sorption processes is restricted to the sediment particles. Therefore, the prevailing hydraulic conditions in rivers, i.e., the type and extent of interactions between the surface water and the pore water, are an important factor governing the elimination of pharmaceutical residues from rivers. On a larger scale, fluxes across the surface water/sediment interface are driven by the hydraulic gradients between the river and the groundwater. However, besides these general gaining or losing conditions caused by larger scale differences in the hydraulic head, additional small-scale interactions of surface water and pore water exist. Theses interactions are controlled by pressure irregularities at the sediment surface which are a result of both river bed geometry and flow dynamics in the surface water. This transi-

tion zone between the surface water and the groundwater, where water and solutes are pumped into the sediments and eventually released again into the surface water is also called “hyporheic zone”. The fluxes within the hyporheic zone (also called hyporheic flow) are highly dynamic in space and time (Angermann et al. 2012, Lewandowski et al. 2011a). The depth of the hyporheic zone as well as the mean residence time of water and solutes within the hyporheic zone are additionally influenced by the ambient groundwater flow conditions (Trauth et al. 2013). In fluid mechanics, two main mechanisms that induce the transport of water, solutes and particulate matter between surface water and pore water are described. The first mechanism is driven by advective flows that are generated by pressure head gradients (“pumping”). The second mechanism (“turnover”) is caused by transport of sediment during which water is periodically trapped and released from the moving riverbed (Cardenas et al. 2004, Elliott and Brooks 1997). The contribution of both mechanisms to the hyporheic exchange is depending on various factors such as surface water flow velocity characteristics (House et al. 1995, Packman and Salehin 2003, Precht and Huettel 2004), bed form geometry (Kasahara and Hill 2006, Marion et al. 2002, Meysman et al. 2007, Saenger et al. 2005), sediment characteristics (Tonina and Buffington 2007), as well as in-stream obstacles such as stones and wood (Mutz et al. 2007). These interactions also influence the microbial community (Halda-Alija et al. 2001, Olsen and Townsend 2003) as well as the biogeochemical conditions (Huettel et al. 2003) in the river sediments. Moreover, the rate of hyporheic exchange governs the turnover kinetics of nutrients within the sediments and effects on net removal of nitrogen and phosphorus from the surface water have been reported (Gu et al. 2007, House et al. 1995, Lautz and Siegel 2007). The hyporheic zone is regarded as hotspot of microbiological activities (Boulton et al. 1998, Lansdown et al. 2012) and reactions taking place in the hyporheic zone are believed to contribute a significant part to the respiration of the whole aquatic ecosystem (Ingendahl et al. 2009) and to impact the ecological balance of rivers (Brunke and Gonser 1997, Hester and Gooseff 2010). Consequently, due to this high potential of substance turnover and subsequently also of pollutant attenuation, the hyporheic zone was called as the river’s liver (Fischer et al. 2005).

Typical stream reached averaged fluxes across the sediment surface range from a few L m^{-2} d^{-1} to some hundred L m^{-2} d^{-1} (Schmidt et al. 2006). The percentage of stream water (and solutes) flowing into the sediments at a given groundwater recharge rate within a certain time frame strongly depends on the geometry and size of the river as well as on the flow velocity (Figure 1-3). In large rivers (Figure 1-3a), only at very high hyporheic exchange rates and small flow velocities a substantial proportion of surface water is flowing into the sediments within a travel distance of 20 kilometers. Hence, biotransformation of pharmaceuticals in sediments in this type of river is potentially limited by an insufficient transfer into the sediments. In contrast, in small rivers, the proportion of hyporheic flow to total downstream flow is orders of magnitude higher under at the same exchange rates and flow velocities (Figure 1-3b). Therefore, small rivers seem to constitute favorable conditions for attenuation of pharmaceuticals in river.

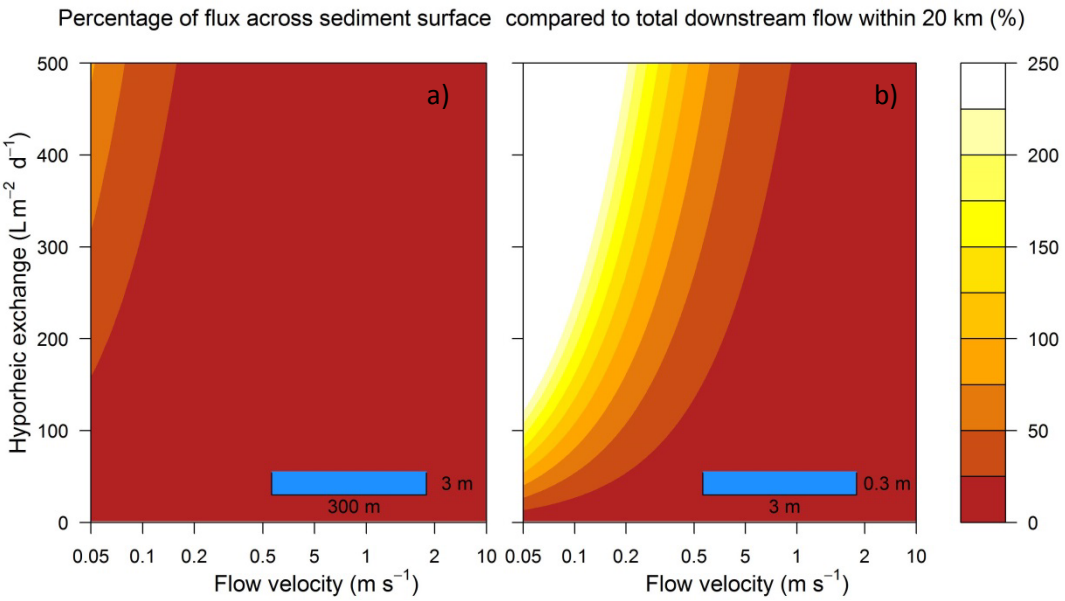


Figure 1-3: A priori estimation of the percentage of surface water that is flowing across the sediment surface within a travel distance of 20 km as function of the flow velocity and hyporheic exchange rate; a) large river: width: 300 m, depth: 3 m b) small river: width: 3 m, depth: 0.3 m

Besides phototransformation in the surface water, biotransformation in the river sediments constitutes the major potential attenuation mechanism for pharmaceuticals in rivers. Therefore, the hydrological connections between surface water and pore water are crucial. These hydraulic interactions also influence the biogeochemical composition of the sediments, the microbial activities, and transformation of substances in the hyporheic zone. Rivers with a high hyporheic exchange should therefore be favorable for the remediation of pharmaceutical residues.

1.3 Determining the Fate of Pharmaceuticals in Rivers – State of the Art

1.3.1 Laboratory Studies – Concepts, Limitations and Gained Insight

The easiest way to assess the fate of pharmaceutical residues in rivers is to conduct laboratory studies under simplified conditions. These studies allow predicting the general behavior of substances in rivers and river sediments as well as looking in detail on influencing boundary conditions. Moreover, experiments are often conducted at elevated concentrations to minimize the analytical effort and uncertainty. Since generated results strongly depend on the experimental setup, well established guidelines for testing of chemicals exist. Usually, experimental setups are prepared analogously to e.g., the OCED guidelines for sorption (guideline No. 106, OECD (2000)), aerobic and anaerobic transformation in aquatic sediment systems (guideline No. 308, OECD (2002)), transport and leaching in soils and sediments (guideline No. 312, OECD (2004)) or direct phototransformation in surface waters (guideline No. 316, OECD (2008)). The basic information

that can be gained with this standardized test systems are if the test substances are susceptible to photolysis, can be biotransformed in soils and sediments or tend to sorb to sediments.

For assessing the transformation of chemicals in sediments (OECD guideline 308) static batch systems with water and sediments are recommended. With this setup the elimination of substances from the water phase, the distribution of the substances between liquid and solid phase, and the formation of TPs can be determined (Berkner and Thierbach 2013, Radke et al. 2009, Ramil et al. 2010). The guidelines explicitly advise the use of radio labelled (^{14}C, ^{3}H) substances. This additionally allows determining mineralization rates of the substances as well as the formation of non-extractable residues (NER) in the sediments (Löffler et al. 2005, Prasse et al. 2009). However, transfer of solutes into the sediment where the transformation occurs is mainly driven by diffusion. Therefore, if a substance is readily biodegradable, the elimination might be kinetically limited by a slow transport of the substances into the sediment. Moreover, the static setup and little fluxes across the sediment surface also can result in a rapid formation of anaerobic zones in the sediments (Ericson 2007). Hence, especially the elimination and transformation kinetics of substances that are only transformed under aerobic conditions might easily be underestimated.

The standard experimental tests for adsorption of pharmaceuticals onto sediments are based on equilibrium sorption. Test flasks are typically filled with a standard solution (10 mM $CaCl_2$) and a defined amount of sediment. After equilibration, the target substances are spiked into the supernatant. The flasks are shaken over a certain time period until sorption equilibrium has been reached (typically max. 48 hours) and residual liquid (and sorbed) concentrations are determined. Tests have to be performed at different concentration levels ranging over at least two orders of magnitude in order to calculate sorption isotherms. While temperature does not affect the distribution of substances between the dissolved and solid phase (ten Hulscher and Cornelissen 1996), factors like salinity (Ong et al. 2012), pH (Vasudevan et al. 2009) or sediment properties (Ramil et al. 2010) can strongly influence the sorption equilibrium. For beta-blockers a positive correlation of sorption and fine material of the sediment (higher specific surface area) and its TOC content was determined (Ramil et al. 2010).

Typically, phototransformation is assessed in laboratory by exposing solutions of defined chemical composition to a known irradiation (i.e., wavelength and intensity). The pharmaceutical concentration does not influence the phototransformation rate (Piram et al. 2008b). Hence, experiments are often performed at elevated concentrations simplifying the analytical procedure and the detection of transformation products (TPs). Adding isopropanol as quencher allows determining the importance of indirect photolysis by reactions with $^{\bullet}OH$ radicals for the total elimination (Jacobs et al. 2011). Additionally, the influence of nitrate and dissolved organic matter (DOM) such as fulvic and humic acids on the transformation rates can be assessed (Andreozzi et al. 2003, Carlos et al. 2012, Jacobs et al. 2011, Lam and Mabury 2005). This way, for example, the photoelimination of the antibiotic sulfamethoxazole in water (WWTP effluent) was attributed to 48 % direct photolysis, 36 % indirect photolysis by reaction with $^{\bullet}OH$ radicals and to 16 % indirect photolysis by reactions with triplet excited effluent organic matter (Ryan et al. 2011). More straightforward experimental approaches are exposing different water solutions (ultra-pure wa-

ter, WTTP effluent, river water, sea water) spiked with target substance to natural sunlight (see e.g., Matamoros et al. (2009) or Packer et al. (2003)). This method usually provides less insight in details of the phototransformation mechanisms but more realistic data for the actual persistence of pharmaceuticals in rivers.

Transport of chemicals such as pharmaceuticals in river sediments or soils is usually tested in column experiments. Beside the transport and the retardation, column experiments also allow determining subsequent desorption and leaching from the sediments as a function of different parameters such as sediment texture, redox conditions (Baumgarten et al. 2011), DOC content (Borgman and Chefetz 2013), pH (Borgman and Chefetz 2013, Strauss et al. 2011) and chemical composition of the river water (Schaffer et al. 2012a). The target substance can either be applied as Dirac pulse or at constant concentration over the specific time span (Scheytt et al. 2004). Column experiments also allow assessing the biotransformation in river sediments. However, this information can only be gained for rather biodegradable substances (e.g., such as ibuprofen (Mersmann et al. 2002)). Otherwise extremely long sediment columns with a high residence time (Tiehm et al. 2011), serial connections of individual columns (Baumgarten et al. 2011) have to be used. Another suitable option to enhance the residence times in the sediment is conducting column experiments in a recirculating manner (Gröning et al. 2007).

A rather novel tool to directly determine biotransformation kinetics within river sediments is applying miniature push-pull tests. To this end, a known amount of target substances is injected into the sediments. After a defined residence time, the pore water is extracted and the residual amount is determined. Based on the recovery of a conservative reference substance elimination rates can be calculated. While this techniques are routinely applied to determine elimination possesses in aquifers (Haggerty et al. 1998, Huntscha et al. 2013, Phanikumar and McGuire 2010), the setup has to be downsized to address small-scale processes within the hyporheic zone or the dimension of laboratory studies. Knecht et al. (2011) successfully developed such a system and subsequently determined the transformation of citrate (half-time time (t_H): 0.5 h) in sediments.

Most complex but also most realistic experimental conditions provide larger scale systems such as microcosms or flumes. These systems bridge the scale gap between manageable batch incubations and field studies. They also allow the contemporaneous evaluation of biotransformation, phototransformation and sorption. For example, Lam et al. (2004) assessed the aquatic persistence of eight pharmaceutical residues in large microcosms (12,000 L) comprised of river water, fish, submerged plants, and phytoplankton. Originally, flumes were primarily used to understand and describe the flow regime in surface water (Thompson et al. 1998), the details of sedimentation and sediment transport (Baas 1999, Robert and Uhlman 2001, Roberts et al. 2003), as well as processes determining the fluxes of water and solutes (Elliott and Brooks 1997, Marion et al. 2002) or particles (Huettel et al. 1996, Ren and Packman 2005) across the sediment surface. In recent years, focus shifted to investigating redox zonation (Huettel et al. 1998), occurrence of biogeochemical reactions (Huettel et al. 2003), nutrient turnover (Barlow et al. 2004) or microbial communities (Frossard et al. 2013) as functions of the prevailing hydraulics. In a pilot study, Kunkel and Radke (2008) described the elimination of acidic pharmaceuticals as function of the

flow velocity in the surface water. The elimination rate of acidic pharmaceuticals from the surface water as well as their biotransformation rate in sediments increased with the flow velocity in the surface water due to a higher transfer rate into the sediments as well as a better oxygen supply to the sediments. In a similar approach, a deeper intrusion of antibiotics into marine sediments in running waters was observed compared to stagnant waters (Xu et al. 2009). In accordance to the acidic pharmaceuticals addressed in Kunkel and Radke (2008), the antibiotics were more persistent in the sediments under stagnant surface water.

Laboratory studies have shown that phototransformation in surface water and biotransformation in sediments are potent processes to eliminate pharmaceuticals from rivers. However, these (kinetic) results cannot directly be transferred to the field scale as their relevance in rivers is governed by complex hydrological and biogeochemical boundary conditions.

1.3.2 Field Studies – Concepts, Challenges and Gained Insight

Compared to the large number of laboratory studies examining the fate of pharmaceuticals, only a small number of mechanistic field studies exist. In contrast, there are many studies balancing the loads of pharmaceutical residues in WWTPs and attributing their elimination during wastewater treatment to different removal processes (Carballa et al. 2004, Göbel et al. 2005, Jelic et al. 2011, Joss et al. 2006, Maurer et al. 2007, Möhle et al. 1999, Strenn et al. 2004). First studies on pharmaceuticals in the aquatic environment focussed predominantly on the detection of pharmaceuticals in different receiving waters (Halling-Sørensen et al. 1998, Heberer et al. 1998, Hirsch et al. 1999, Jones et al. 2001, Ternes 1998). Persistence data for the investigated organic micropollutants from the detection patterns in different compartments were derived for processes such as river bank filtration or aquifer recharge (Heberer 2002b, Heberer et al. 1998, Pang and Close 2001, Petrovic et al. 2009). Even in relatively simple hydraulic systems such as WWTPs where the water fluxes are commonly well described, very well designed sampling campaigns and a sufficiently high number of (flow-proportional composite) samples are required to obtain sound mass balances of pharmaceuticals during wastewater treatment (Ort et al. 2010). In rivers with highly time-variable discharge (Kolpin et al. 2004, Osorio et al. 2012, Pailler et al. 2009), spatio-temporal occurrence patterns of pharmaceuticals (Gomez et al. 2012, Madureira et al. 2010), complex interactions of surface water and groundwater (Reh et al. 2013), inflow of minor creeks (Radke et al. 2010), discharge of combined sewer overflows (CSO) or transient storage of water and solutes in the river sediments the situation even gets worse (Gasperi et al. 2008, Osenbrück et al. 2007, Willems 2008). Moreover, the hydrograph after a rain event is often decoupled from the chemograph (Kurtenbach et al. 2006), an effect also known as “first flush” (Barco et al. 2008, Bertrand-Krajewski et al. 1998, Pailler et al. 2009). Hence, taking samples on the tide of a flooding event at several locations can easily lead to false mass balances for pharmaceuticals. Often even

finding a river suitable for the investigation purpose – i.e., determining the mass balance of pharmaceuticals along a certain river stretch – itself is a Herculean task.

Most field studies that deduce fate data for pharmaceuticals in rivers base their conclusion on a very small number of grab samples. For example, Gross et al. (2004) calculated the removal of pharmaceutical residues at Santa Ana River (California) only from three grab samples taken over a time span of eight months. A follow-up study (Lin et al. 2006) at the same river tracked three water parcels downstream based on previously determined flow times. Both studies report a rapid elimination of naproxen and ibuprofen due to photolysis (naproxen) and biotransformation (ibuprofen). However, concentrations at the first sampling sites strongly varied between the three sampling dates showing the variability and also some kind of randomness of concentrations in grab samples. Therefore, even if the travel time was only slightly different than previously determined, enormous errors in the mass balance calculations are possible (see also Ort et al. (2010)). The work by Fono et al. (2006) at a river in Texas also consists only of four grab samples taken at five sites downstream of a WWTP – without considering the travel time between the sampling sites. However, the concentrations at the sampling sites are less scattering indicating a better reliability of the results. They derived much longer dissipation times of ibuprofen and naproxen compared to the above stated studies (Gross et al. 2004, Lin et al. 2006). Generally, the quality and explanatory power of the results from these three studies is lowered by the sampling procedure (grab samples) and the low number of total samples.

Representative samples can be achieved by using automatic samplers and combining individual samples to composite samples (Radke et al. 2010) and potentially also by passive sampling techniques. In laboratory, the suitability of this technique with Polar Organic Chemical Integrative Samplers (POCIS) has been successfully applied for hydrophilic substances such as pharmaceuticals (Zhang et al. 2008). However, getting quantitative data in rivers is not straightforward (Zhang et al. 2008) since POCIS require intense calibration procedures and results can be distorted by changes in flow velocities (Vermeirssen et al. 2008), water temperature (Söderström et al. 2009, Togola and Budzinski 2007), and water chemistry (Togola and Budzinski 2007). Therefore, passive sampling was excluded as sampling technique in this thesis. Another possibility to generate a more reliable database for the calculation of elimination of pharmaceuticals along river stretches is to enlarge the number of samples per sampling site (Radke et al. 2010) and/or the application of Lagrangian sampling (Barber et al. 2011, Brown et al. 2009, Writer et al. 2012) requiring “real-time data for flow conditions and travel times between sampling locations ”(Volkmar et al. 2011). The most reliable method to ensure Lagrangian sampling is by manually injecting the target substances into the river, thus performing reactive tracer tests. While tracer tests to address the fate of e.g., pesticides in aquifers are a well-established practice (Davis et al. 2000, Pang and Close 1999, 2001), tracer tests in rivers are primarily conducted to determine travel times and to quantify transient storage processes using conservative tracers like tritium, bromide, chloride or fluorescent dye tracers (Johansson et al. 2001, Jonsson and Wörman 2001, Wörman et al. 2002). In a recent tracer test, Lemke et al. (2013) additionally injected resazurin, a substance that can be used to identify surface water/sediment interactions in natural waters since it is exclusively trans-

formed in river sediments to a single TP (Haggerty et al. 2008). By the formation of this TP (resorufin), the extent of transient storage and microbial activities in a river sediment can be determined (Haggerty et al. 2009). Ideally, such as tracer would be injected together with a mix of target pharmaceuticals and the breakthrough curves would be recorded at downstream locations. However, since pharmaceuticals are almost ubiquitously present in rivers, depending on the size of the river an enormous amount of substances would have to be injected in order to increase the background concentrations substantially. The permission for this usually is not given by authorities in charge. Therefore, naturally occurring tracers have to be used for calculating mass balances of pharmaceuticals in rivers. Relating the concentration decrease of a reactive substances (e.g., ibuprofen, diclofenac) to inorganic compounds such as bromide, boron or potassium (Nödler et al. 2011) or persistent and non-sorbing organic substances such as the artificial sweetener acesulfame (Engelhardt et al. 2013, Scheurer et al. 2011) allows the calculation of mass balances along river stretches if precise discharge measurements at both site are not available. Moreover, this relative calculation also accounts for dilution effects due to groundwater inflow or confluences with other rivers of lower pollution levels.

However, even well designed sampling campaigns as stand-alone monitoring studies do not allow distinguishing between individual elimination processes. For attributing the total elimination to individual processes, additional experiments have to be performed. Phototransformation in lakes was successfully assessed by measuring concentration profiles of target pharmaceuticals and a subsequent coupling of these depth-resolved concentration profiles with a hydraulic model (Buser et al. 1998a, Buser et al. 1998b, Poiger et al. 2001, Tixier et al. 2003). It was concluded that diclofenac and naproxen were eliminated from lakes by phototransformation whereas substances like clofibric acid or carbamazepine were resistant against photolysis in the epilimnion of lakes. Phototransformation of pharmaceuticals rapidly decreases with depth in rivers or lakes. Quartz tubes exposed to natural radiation and installed in different depths (e.g., Bartels and von Tümpling (2007) or Radke et al. (2010)) showed that transformation is almost completely restricted to the upper half meter of the water column. Kinetic values determined in laboratory or field studies beneath the water surface have to be extrapolated over the whole column to obtain realistic rates for the respective river or lake (Fono et al. 2006, Schwarzenbach et al. 2003). As a consequence, the deeper the river is the less important phototransformation processes will supposedly be for the total elimination.

Assessing the biotransformation of (polar) pharmaceuticals in rivers is not as straightforward as determining phototransformation kinetics. Simultaneous quantification of surface water and pore water concentration without knowing the prevailing surface water/pore water interactions can only be used to check if the substances are reaching the sediments at the respective depths. Since surface water concentrations can be highly dynamic (Huntscha et al. 2013, Radke et al. 2010) the exact travel times from the surface water to the sampling location in the sediments has to be known to calculate elimination rates. Depth-resolved sampling of pore water also often provides no conclusive results for biotransformation within sediments (Lahti and Oikari 2012, Lewandowski et al. 2011b). If a sufficiently high number of data in the stream and numerous sam-

pling points in sediments as well as a hydrological model for the site are available, the transport and elimination in river sediments (and aquifers) can be determined by reactive subsurface flow modeling (Engelhardt et al. 2013, Huntscha et al. 2013). A promising approach might be to scale down classical push-pull tests (Haggerty et al. 1998, Kim et al. 2004, Tomich et al. 1973) – commonly applied in aquifers – to the required small injection volumes and pumping rates in the river sediments.

Field experiments and monitoring studies have shown that pharmaceuticals can be eliminated in rivers. However, the results are often contradictory and the design of most of these studies does not allow distinguishing between individual elimination processes. Moreover, the boundary conditions that affect the removal efficiencies are often not explicitly addressed. Therefore, for most pharmaceuticals no profound quantitative data on their elimination in rivers exist and hypothesis driven field work has to be performed to obtain reliable data.

1.4 Objectives of this Work

As shown in chapter 1.3, a large discrepancy exists between in-depth insight on the persistence and transformation pathways of pharmaceuticals in rivers and river sediments derived from elaborate laboratory experiments and actual fate data for pharmaceuticals in rivers. Therefore, the overarching aim of this thesis was to close this knowledge gap and to derive quantitative information on the fate of commonly detected pharmaceutical residues in rivers. Special focus was laid on the contribution of biotransformation in river sediments to the total elimination and on determining the factors that govern the elimination kinetics of pharmaceuticals in rivers.

The work on this thesis is based on two main pillars (see Figure 1-4): i) systematic field experiments supplemented by detailed analysis of the prevailing hydrological and meteorological conditions to calculate the mass balances and elimination of single substances along specific river stretches and ii) the design and application of more realistic laboratory experiments to assess sorption and biotransformation in river sediments.

A first task was to determine exact mass balances of pharmaceuticals in rivers in relation to the prevailing hydrological conditions, i.e., the exchange processes between the main channel of the surface water and transient storage zones (Chapter 2). To this end, a reactive tracer experiment was performed by injecting six pharmaceuticals and two dye tracers into a river. The breakthrough curves of the pharmaceuticals and dye tracers at five downstream locations were recorded to determine their elimination along the river stretch. A subsequent modeling study of the tracer test was conducted to calculate substance-specific elimination kinetics and retardation coefficients in surface water and sediments and to assess the importance of processes in transient storage zones such as the hyporheic zone for the total elimination.

A second objective was to determine the elimination of pharmaceutical residues in rivers under best-case conditions (Chapter 3) and to attribute the overall attenuation to individual elimination processes. Thereto, a system-oriented monitoring campaign with high temporal resolution at a small river was combined with *in situ* phototransformation experiments and depth-resolved pore water sampling.

The final aim was to design a laboratory experimental setup that enables determining more realistic biotransformation kinetics for pharmaceutical residues in river sediments (Chapter 4). This test system was subsequently used i) to systematically investigate the biodegradability of pharmaceutical in different river sediments, ii) to determine robustness of the test systems regarding the elimination kinetics of pharmaceuticals in the sediments against changing boundary conditions such as the filter velocities, and iii) to cross-check if the contradicting observed attenuation rates of pharmaceutical residues in field studies can be explained by different elimination kinetics in the sediments of these river systems.

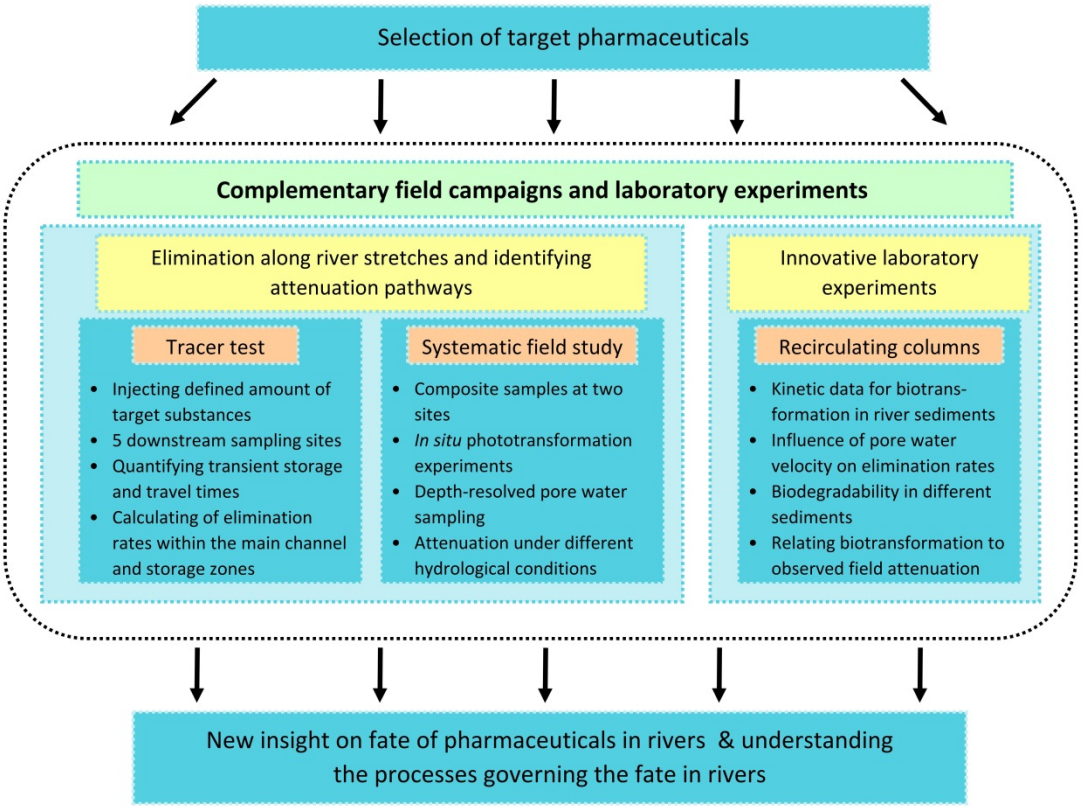


Figure 1-4: Overview on the individual studies of this thesis and how their combination generates new quantitative data for the fate of pharmaceuticals in rivers.

To meet the challenge of the quantification of elimination processes of pharmaceuticals in rivers and river sediments a set of target compounds had to be chosen. The selection was based on occurrence data of the pharmaceutical residues, their physico-chemical properties as well as their expected/reported fate in the environment (see Table 1-1). Differing overall fate and elimination kinetics of the target compounds will then allow attributing their total elimination on actually occurring elimination pathways (biotransformation, phototransformation, and sorption). A

detailed description on the reported occurrence and environmental fate of the pharmaceuticals addressed in this thesis is given chapter 1.5.

Table 1-1: Overview of the pharmaceuticals addressed in this thesis and expected contribution of the individual elimination pathways to the overall fate in rivers (++: very important; +: important; 0: of minor importance; -: not important)

Substance	Biotransformation		Phototrans-formation	Sorption
	aerobic	*anaerobic*		
Bezafibrate[1,2,3]	+	0	-	-
Carbamazepine[2,3]	-	-	-	0
Clofibric acid[1,2,3]	-	-	-	-
Diclofenac[1,2,3]	+	0	++	0
Ibuprofen[1,2,3]	++	+	-	-
Metoprolol[1,2,3]	+	0	0	+
Naproxen[1,2,3]	0	+	+	0
Propranolol[2,3]	0	0	+	++
Sotalol[2]	+	0	+	+
Sulfamethoxazole[2]	-	+	0	-

[1] pharmaceutical investigated in study 1, [2] pharmaceutical investigated in study 2, [3] pharmaceutical investigated in study 3.

1.5 Detailed Information on the Individual Substances

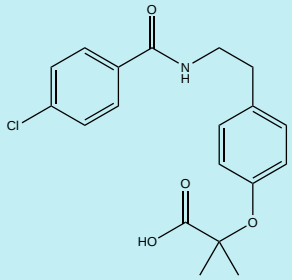
In the following chapters the input, occurrence, and reported environmental fate of the pharmaceuticals that are investigated in detail in this thesis are described. Since data for rivers and river sediments is often scarce, comparable data for WWTPs and soils is discussed as well.

1.5.1 Bezafibrate

Bezafibrate (2-(4-(2-((4-chlorobenzoyl)amino)ethyl))phenoxy)-2-methylpropanoic acid) is a fibrate drug that is used as an lipid lowering agent. Like for all fibrate drugs, its prescribed amounts have slightly decreased over the last few years in Germany from 11.5 tons per year in 2008 to 9.0 tons per year in 2012 (Schwabe and Paffrath 2009, 2010, 2011, 2012, 2013). After medication, 95% of the applied amount is excreted within 48 hours via urine as bezafibrate or its glucuronide (Abshagen et al. 1979). Due to this high excretion proportion, the bezafibrate concentration in raw wastewater are relatively high and concentration of several µg L^{-1} are determined (Lindqvist et al. 2005, Quintana et al. 2005, Stumpf et al. 1999, Ternes et al. 2007). During conventional wastewater treatment, elimination of bezafibrate ranges commonly from 40-100 % (Clara et al. 2005, Sacher et al. 2008, Stumpf et al. 1999, Ternes 1998, Ternes et al. 2002) and concentrations in the effluent of WWTPs of up to several µg L^{-1} are reported (Andreozzi et al. 2003, Ternes 1998). Bezafibrate can be efficiently eliminated from waters by ozonation (Dantas et al. 2007, Ternes et

al. 2002) which however can lead to an increase in toxicity of the treated water (Dantas et al. 2007).

Table 1-2: Structural formula and physicochemical properties of bezafibrate

Bezafibrate		
	CAS number	41859-67-0
	formula	$C_{19}H_{20}ClNO_4$
	M (g mol^{-1})	361.82
	water solubility (mg L^{-1})	9.8-7600[1]
	pK_a (-)	3.2[2]
	log K_{OW} (-)	4.25[3]

[1] Dantas et al. (2007), [2] Beausse (2004), [3] Mersmann (2003)

In surface water, concentrations of bezafibrate in the high ng L^{-1} to the low µg L^{-1} range are commonly reported (Montforts 2001, Radke et al. 2010, Ternes 1998, Ternes et al. 2006, Zuccato et al. 2000). Bezafibrate has also been detected in pore water (Lewandowski et al. 2011b) and drinking water (Stumpf et al. 1996) but concentration are generally low compared to wastewater and surface waters supposedly due to transformation processes.

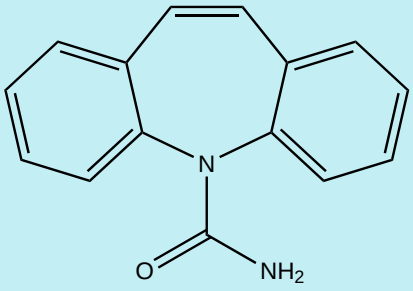
In field and laboratory experiments, it has been shown that phototransformation is no efficient elimination mechanism from surface waters (Cermola et al. 2005, Radke et al. 2010). Moreover, no removal was observed in batch experiments with aerobic and anoxic groundwater, as well as with surface water over 28 days (Ternes et al. 2002). Even the addition of gravel or sand to these systems resulted in no substantial increase of the elimination indicating only a minor sorption to solid matrices. Negligible sorption to river sediments was also reported in previous laboratory studies (Kunkel and Radke 2008) since bezafibrate is deprotonated and highly polar at typical river pH values (pK_a = 3.2, Table 1-2). In microbially active systems, bezafibrate can be degraded and 4-chlorobenzoic acid was identified as the major TP (Quintana et al. 2005). Consequently, Vieno et al.(2005) reported a slightly decreasing load of bezafibrate with distance from a WWTP outfall in a Finnish river. In contrast, in a French river no clear evidence of elimination during river transport was observed (Comoretto and Chiron 2005). In a small German stream, bezafibrate was also not removed at a 13.6 km long river stretch (Radke et al. 2010). However, in flume experiments with sediment and water from this river bezafibrate was transformed with half-life times (t_H) in the range of a few days (Kunkel and Radke 2008). Moreover, (almost) complete removal during river bank filtration (Heberer and Adam 2004, Heberer et al. 2004, Preuß et al. 2002) and during soil passage was reported (Ternes et al. 2007).

The lipid lowering agent bezafibrate is believed to be biodegradable in river sediments under favorable boundary conditions. Sorption and phototransformation constitute no major elimination pathways in rivers. Therefore, bezafibrate will be regarded as an indicator substance to identify biological transformation processes in rivers and river sediments.

1.5.2 CARBAMAZEPINE

Carbamazepine (5H-dibenzo[b,f]azepine-5-carboxamide) is an anticonvulsant drug that is primarily used for the treatment of bipolar disorder and epilepsy. The prescribed amounts of carbamazepine in Germany are about 50 million DDDs or 50 tons per year (Schwabe and Paffrath 2009, 2010, 2011, 2012, 2013). Only about 2-3 % of the intake is excreted unmetabolized (Clara et al. 2004) while most is excreted as carbamazepine 10,11-epoxide (van Rooyen et al. 2002). However, also the parent compound has been frequently detected in high concentrations in the aquatic environment and the concentration in raw wastewater are usually in the low µg L^{-1} range (Heberer 2002b, Ternes 1998). Carbamazepine is behaving (almost) recalcitrant during wastewater treatment (Bendz et al. 2005, Bernhard et al. 2006, Clara et al. 2004, Gao et al. 2012, Heberer 2002b, Paxéus 2004, Ternes 1998, Wick et al. 2009) as it is neither substantially removed by biodegradation processes nor by sorption to excess sludge. Hence, only low sorption coefficients (e.g., K_D = 25.5 L kg^{-1} for sludge (Jones et al. 2002), K_{OC} = 70 L kg^{-1} (Wick et al. 2009)) are reported. However, during a potential tertiary treatment, carbamazepine be transformed and mineralized by ozonation (Andreozzi et al. 2002).

Table 1-3: Structural formula and physicochemical properties of carbamazepine

Carbamazepine		
	CAS number	298-46-4
	formula	$C_{15}H_{12}N_2O$
	M (g mol^{-1})	236.27
	water solubility (mg L^{-1})	2.5-3.0[1]
	pK_a (-)	13.9[2]
	log K_{OW} (-)	2.25[2], 1.51[3]

[1] Stella and Nti-Addae (2007), [2] Jones et al. (2002), [3] (Scheytt et al. 2005a)

Due to the bad removal efficiencies during conventional wastewater treatment, concentrations in WWTP effluents are almost equal to the influent concentrations and up to several µg L^{-1} are reported (Andreozzi et al. 2003, Bendz et al. 2005, Heberer 2002b, Ternes 1998). In wastewater receiving surface waters, concentrations of more than 1 µg L^{-1} have been reported (Bendz et al. 2005, Stolker et al. 2004, Ternes 1998) while the typical concentrations in large rivers like the river Rhine (Sacher et al. 2008) or the river Elbe are in the mid ng L^{-1} range (Wiegel et al.

2004). Carbamazepine was also detected in pore water (Huntscha et al. 2013), groundwater (Clara et al. 2004, Heberer et al. 2004, Rabiet et al. 2006, Reh et al. 2013, Sacher et al. 2001) and drinking water (Hummel et al. 2006).

Carbamazepine is occurring as neutral molecule in the aquatic environment (Table 1-3) and described as extremely persistent. In column experiments, minimal retardation due to sorption to sediments but no elimination was determined (Mersmann et al. 2002, Scheytt et al. 2006). However, results from laboratory batch systems revealed significant sorption to soils and sediments with log K_{OC} values in the range of 1 – 3.5 (Löffler et al. 2005, Ternes et al. 2004). Higher sorption to sediments in rivers after heavy rainfalls due to higher DOC loads in the water caused by combined sewer overflow (CSO) was also reported (Osenbrück et al. 2007). In sediment/water batch systems, a high resistance against biotransformation (DT_{50} > 300 days) was reported (Löffler et al. 2005). In aerobic and anaerobic batch experiments with soil, no transformation occurred (Lin et al. 2011). Carbamazepine was also not degraded by photochemical or microbial processes in outdoor microcosms (Lam et al. 2004). However, there might be some microbes that are potentially able to degrade carbamazepine since a slow elimination during aerobic and anaerobic batch experiments with sediments (t_H > 150 days, Conkle et al. (2012)) was detected and a degradation under anoxic conditions was postulated (Hai et al. 2011). The phototransformation of carbamazepine can be enhanced by the presence of nitrate (Andreozzi et al. 2003), chloride (Chiron et al. 2006), humic substances (Carlos et al. 2012) and under low pH values (Calisto et al. 2011). Hence, it is believed that carbamazepine can be mainly degraded via indirect photolysis (Jasper and Sedlak 2013, Lam and Mabury 2005). De Laurentiis et al. (2012) postulated acridine and 10,11-dihydroxy-10,11-dihydro-carbamazepine (DHDH-CBZ) as major photo-TPs. However, in general the transformation rates are small and range from a few days to more than 100 days depending on latitude and season (Andreozzi et al. 2003, De Laurentiis et al. 2012).

So far, all field data confirmed the persistence of carbamazepine in the aquatic environment. Grab samples taken along different river stretches downstream of WWTPs revealed no decrease in carbamazepine loads (Bendz et al. 2005). Also no or only minor removal of carbamazepine was observed during soil passage (Ternes et al. 2007), river bank filtration (Clara et al. 2004, Heberer et al. 2004, Massmann et al. 2006), subsurface flow constructed wetland (SFCW) systems (Matamoros et al. 2012, Matamoros et al. 2008a, Matamoros et al. 2008b, Matamoros and Salvado 2012), groundwater transport (Clara et al. 2004) and during push-pull tests performed at a Swiss river (Huntscha et al. 2013). Hence, carbamazepine is often used to identify wastewater influence in rivers (Clara et al. 2004, Nakada et al. 2008, Scheurer et al. 2011) and groundwater (Müller et al. 2012) as well as for the detections of leakages in the wastewater system (Wolf et al. 2012). The conservative behavior of carbamazepine is taken advantage of during the second study (Chapter 3).

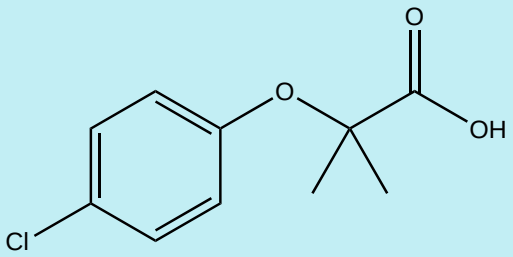
The anticonvulsant drug carbamazepine is very persistent in rivers and river sediments. Neither bio- and phototransformation nor sorption to sediments constitutes substantial elimination pathways. Hence, carbamazepine can be used as reference substance when complex hydraulic conditions prohibit the direct calculation of mass balances of pharmaceuticals along river stretches.

1.5.3 CLOFIBRIC ACID

Clofibric acid (2-(4-Chlorophenoxy)-2-methylpropanoic acid) is the pharmaceutically active metabolite of the three lipid lowering agents clofibrate, etofibrate and etofyllinclofibrate. Like bezafibrate, clofibric acid decreases the plasmatic content of triglycerides and cholesterol. While clofibric acid was one of the first pharmaceuticals identified in domestic wastewaters (Hignite and Azarnoff 1977), its use has been declining (BLAC 2003). Additionally, the use of clofibrate was prohibited in 2002. Reported elimination rates during conventional wastewater treatment range from 0 to 50 % (Heberer 2002b, Tauxe-Wuersch et al. 2005, Ternes 1998). Elimination due to sorption to excess sludge is only of minor importance due to the low sorption affinities (K_D for sludge: 4.8 (Ternes et al. 2004)). A more recent study reported a higher removal in wastewater treated with biolfim carriers than in activated sludge (Falas et al. 2012).

Table 1-4: Structural formula and physicochemical properties of clofibric acid

Clofibric acid



CAS number	882-09-7
formula	$C_{10}H_{11}ClO_3$
M (g mol^{-1})	214.65
water solubility (mg L^{-1})	582.5[1]
pK_a (-)	3.2[1]
log K_{OW} (-)	2.57[2], 2.88[3]

[1] Mersmann (2003), [2] Beausse (2004), [3] Scheytt et al. (2005a)

During the 1990[ies] and first years of the 21[st] century, the reported concentration of clofibric acid in WWTPs effluents ranged from no detection and concentrations of only a few ng L^{-1} to up to a few µg L^{-1} (Andreozzi et al. 2003, Ternes 1998, Tixier et al. 2003). Clofibric acid has been detected in rivers in the mid to high ng L^{-1} range (Heberer 2002b, Winkler et al. 2001), lakes (Buser et al. 1998a), and groundwaters (Heberer 2002b, Heberer et al. 2004). Even in the North Sea, concentrations of up to 28 ng L^{-1} were measured (Buser et al. 1998a, Weigel et al. 2002). However, due to the decreasing application rate during the last years, the occurrence and environmental relevance of clofibric acid has decreased. For example, it was not determined in WWTP effluents and receiving rivers in the U.K. (Hilton and Thomas 2003) and rainwater channels in the U.S. (Boyd et al. 2004). In contrast, clofibric acid was sporadically detected in a Spanish river between October

2003 and Summer 2004 (Kuster et al. 2008) while it was permanently detected in a small German river in Summer 2009 (average concentration: 15 ng L^{-1} in Radke et al. (2010)).

Clofibric acid is completely present in its dissociated form under environmental pH values of > 5 due to its low pK_a of 3.2 (Table 1-4). Therefore, clofibric acid is highly polar and only exhibits negligible sorption to soil and sediments. Hence, no sorption was observed in batch and column experiments with sandy sediments (Löffler et al. 2005, Scheytt et al. 2004) and only minor sorption to soils (Drillia et al. 2005b) was reported. Consequently, also a rapid desorption and subsequent leaching from soil was reported (Oppel et al. 2004) and K_{OC} values are consequently very low (14-30 L kg^{-1}; Löffler et al. (2005), Ternes et al. (2004), and Scheytt et al. (2005a)).

Clofibric acid is believed to be strongly resistant against biotransformation processes under natural conditions. In batch experiments with river biofilms (Winkler et al. 2001), sediment and river water (Löffler et al. 2005), oxic and anoxic groundwater (Ternes et al. 2002), in flume experiments with river sediments (Kunkel and Radke 2008) as well as in saturated column experiments (Scheytt et al. 2004) clofibric acid was not or very slowly degraded (DT_{50} > 50 days). However, in aerobic sequencing batch reactors, it was shown that the main aerobic biological TPs are α-hydroxyisobutyric acid, lactic acid and 4-chlorophenol (Salgado et al. 2012) and thus revealing a potential of biotransformation of clofibric acid. Under natural conditions phototransformation of clofibric acid is very slow (Andreozzi et al. 2003, Packer et al. 2003). Consequently, no elimination in the epilimnion of a lake was observed (Tixier et al. 2003). *In situ* phototransformation experiments at a German river also reported only slow transformation rates (t_H = 2.3 days the near water surface; Radke et al. (2010)). In contrast, Carlos et al. (2012) reported a rapid phototransformation when incubated as single compound in pure water under laboratory conditions. However, in the presence of other pharmaceuticals and/or humic acids the degradation velocity decreased by orders of magnitude. The main photo-TPs are 4-chlorophenol, phenol, hydroquinone as well as α-hydroxyisobutyric acid and lactic acid – the same TPs which can also be formed biologically (Doll and Frimmel 2003, 2004, Salgado et al. 2012).

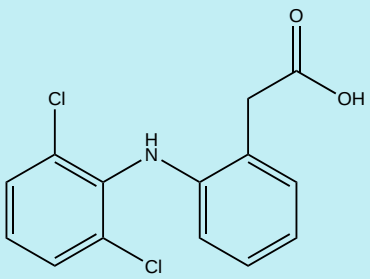
Since the application rate of the pro drugs of clofibric acid has decreased over the last few years – contrary to the occurrence of more systematic mass balancing studies of pharmaceuticals in rivers – reliable data on the fate of clofibric acid is very scarce. No transformation during river bank filtration has been reported (Preuß et al. 2002). Clofibric acid was detected in the pore water of sediments in depths up to 100 cm in 2009 (Lewandowski et al. 2011b). During transport in constructed subsurface flow wetlands (CSFW) only minor removal (in the same order of magnitude than carbamazepine) was observed (Matamoros et al. 2008b). This tracer-like behavior of clofibric acid (Mersmann et al. 2002) was also used to assess the removal of three other pharmaceuticals relative to clofibric acid along a river stretch (Radke et al. 2010).

Clofibric acid, the active metabolite of several lipid lowering agents, is very persistent in rivers and river sediments since it is resistant against bio- and phototransformation. Clofibric acid does also not substantially sorb to the solid matrices. However, its tracer like behavior can only be partly taken advantage of during field studies since the concentrations of clofibric acid are generally very low due to the decreasing application rate of fibrate drugs over the last decade.

1.5.4 DICLOFENAC

Diclofenac (2-(2-(2,6-dichlorophenylamino)phenyl)acetic acid) is a non-steroidal anti-inflammatory drug (NSAID) that is widely used for the treatment of swellings and pain. Besides ibuprofen, it might be the most publicly known pharmaceutical. In Germany, its prescribed amount has been slightly decreasing over the past five years (Schwabe and Paffrath 2009, 2010, 2011, 2012, 2013). Nevertheless, still about 500 mg per inhabitant and year are prescribed which corresponds to about 5 DDDs per inhabitant and year. Moreover, an additional amount of diclofenac is also applied as tablet or ointment without prescription, so that the total consumption in Germany is believed to be near 1000 tons per year (Fent et al. 2006). After consumption, diclofenac is rapidly metabolized in the body (Forth et al. 1992). Five different human metabolites have been described in literature (Wiesenberg-Boettcher et al. 1991). Most metabolites are mono- and dihydroxlated TPs of diclofenac and most important excreted species are the parent compound and 4-OH-diclofenac (Degen et al. 1988). Consequently both the parent compounds as well the TPs have been detected frequently in raw wastewater in the µg L^{-1} range (Buser et al. 1998b, Gonzalez-Barreiro et al. 2003, Heberer 2002b, Perez and Barcelo 2008). During wastewater treatment, elimination rates of diclofenac strongly vary depending on the specific setup of the WWTP and the sludge age (Ternes and Joss 2006). Removal rates between 0 % and 70 % have been reported (Bernhard et al. 2006, Buser et al. 1998b, Heberer 2002b, Lindqvist et al. 2005, Tauxe-Wuersch et al. 2005, Ternes 1998). Sorption to excess sludge accounts for up to 10 % to the total elimination (Ternes and Joss 2006) and is restricted to primary settling (Carballa et al. 2005, Ternes et al. 2004). During tertiary treatment, diclofenac is efficiently eliminated by ozonation (Ternes et al. 2002, Vieno et al. 2007).

Table 1-5: Structural formula and physicochemical properties of diclofenac

Diclofenac		
	CAS number	15307-79-6
	formula	$C_{14}H_{12}Cl_2NO_2$
	M (g mol^{-1})	296.15
	water solubility (mg L^{-1})	0.82[1]
	pK_a (-)	4.51[1], 4.2[2]
	log K_{OW} (-)	0.72[2] ,1.90[3]

[1] Avdeef et al. (2000), [2] Jones et al. (2002), [3] Scheytt et al. (2005a)

Commonly, very high diclofenac concentrations of several µg L^{-1} are detected in treated wastewaters (Andreozzi et al. 2003, Scheytt et al. 2004, Ternes 1998). Hence, concentration in receiving streams are also relatively high spanning concentration ranges from the mid ng L^{-1} range (Letzel et al. 2009, Radke et al. 2010, Sacher et al. 2008, Wiegel et al. 2004) to several µg L^{-1} (Hilton and Thomas 2003, Ternes 1998, Tixier et al. 2003). Diclofenac was also detected in pore water (Lewandowski et al. 2011b), lakes (Buser et al. 1998b, Tixier et al. 2003), and groundwaters (Heberer 2002b, Heberer et al. 2004, Sacher et al. 2001) in substantial concentrations. Additionally to diclofenac, its main human metabolites are frequently detected in WWTP effluents and rivers (Scheurell et al. 2009, Stülten et al. 2008).

Sorption of diclofenac to sediments and soils is strongly related to the TOC of the solid matrix (Borgman and Chefetz 2013, Drillia et al. 2005b). Consequently, in column experiments with sandy sediment and a low TOC only minor retardation was observed (Mersmann et al. 2002). Sorption estimations purely based on log K_{OW} are false and overestimates due to the presence of the dissociated form of diclofenac at environmental pH (Table 1-5) which increases the polarity (Scheytt et al. 2005b). The biotransformation pathways of diclofenac in rivers and river sediments are relatively well investigated. However, the results are often contradicting. Some studies reported a resistance against biotransformation (Mersmann et al. 2002, Onesios and Bouwer 2012, Quintana et al. 2005, Ternes et al. 2002) while other observed a pronounced transformation under anaerobic conditions (Rauch-Williams et al. 2010). However, studies which determined the fate under different redox conditions obtained higher elimination rates under aerobic conditions (Kunkel and Radke 2008, Lahti and Oikari 2011, Mersmann et al. 2002). These results are backed up by the rapid elimination of diclofenac in unsaturated and therefore aerobic soil studies (Al-Rajab et al. 2010, Kreuzig et al. 2003, Ternes et al. 2007). It was shown that the p-benzoquinone imine of 5-OH-diclofenac is one of the major TPs in river sediments (Gröning et al. 2007) under aerobic conditions. Under denitrifying conditions a reversible transformation into NO_2-diclofenac has been proposed (Barbieri et al. 2012a). Diclofenac can be efficiently photolytically transformed predominantly by direct photolysis (Canonica et al. 2008, Packer et al. 2003) while high concentrations of nitrate or humic acids can act as inner filters and reduce phototransformation kinetics (Andreozzi et al. 2003). *In situ* phototransformation experiments with river water and ultra-pure water led to similar elimination kinetics (t_H ~ 1.5 hours near the water surface; Radke et al.

(2010)). Phototransformation of diclofenac has been shown to be a relevant elimination mechanism in lakes (Bartels and von Tümpling 2007, Buser et al. 1998b, Tixier et al. 2003). Several phototransformation pathways have been described such as a dechlorination leading to hydroxycarbazole-1-acetic acid and subsequent oxidative transformation (Poiger et al. 2001) or breaking of the amide bonding (Bartels and von Tümpling 2007). Moreover, for some of the formed photo-TPs an elevated toxicity compared to diclofenac was reported (Encinas et al. 1998, Schulze et al. 2010).

Field observations provide no clear picture about a removal of diclofenac in rivers. Based on grab samples taken downstream of WWTPs, an elimination was concluded (Bendz et al. 2005, Vieno et al. 2005) while a more systematic study revealed no elimination along a 13.6 km stretch (Radke et al. 2010). No elimination of diclofenac was observed in SFCWs (Matamoros and Bayona 2006) while 93 % were eliminated in a water reclamation pond-constructed wetland system (Matamoros et al. 2012). The analysis of pore water also resulted in no clear indication of biotransformation in river sediments (Banzhaf et al. 2013, Lewandowski et al. 2011b). In contrast, in a push-pull test in river sediments, a rapid elimination (t_H ~ 1 hour) was observed (Huntscha et al. 2013). Sorbed concentration in sediments and soils are negligible (Langford et al. 2011, Vazquez-Roig et al. 2010) confirming the hypothesis that sorption is no relevant elimination pathway for diclofenac in rivers.

As shown, diclofenac is one of the most commonly detected pharmaceutical in WWTP effluents and receiving waters and also possesses a relatively high aquatic toxicity (Schwaiger, 2004). Therefore, diclofenac was included on the “watch list” for potential candidates for the update of the list of priority substances according to the Water Framework Directive. The proposed Environmental Quality Standard (EQS) is 100 ng L^{-1}. Based on recent modeling calculations, this EQS will only be exceeded in 1 % of the total European rivers (Johnson et al. 2013).

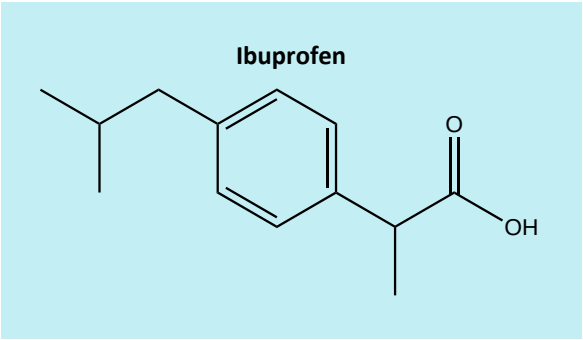
The anti-inflammatory drug diclofenac is detected in high concentrations in rivers. It is highly susceptible to photolysis and can also be biologically transformed, presumably mostly restricted to aerobic conditions. Therefore, diclofenac is used as indicator compound that can be used to identify occurring phototransformation processes and aerobic conditions in river sediments.

1.5.5 IBUPROFEN

Ibuprofen ((RS)-2-(4-(2-methylpropyl)phenyl)propanoic acid is an NSAID that is used for pain relief, fever reduction, and against swelling. It is classified as a core medicine by the World Health Organization. Ibuprofen is prescribed and applied as racemate while only S-ibuprofen is pharmaceutically active (Winkler et al. 2001). The prescription in Germany has significantly risen over the last few years and in 2012 more than 500 tons or 450 DDDs of ibuprofen were prescribed (Schwabe and Paffrath 2009, 2010, 2011, 2012, 2013). Only less than 10 % of ibuprofen are ex-

creted via urine as parent compound (Ternes 1998). The main human metabolites are hydroxy ibuprofen, carboxy ibuprofen, and carboxy hydratropic acid (Winkler et al. 2001, Zwiener et al. 2002). Ibuprofen and the major human metabolites have been frequently detected in raw wastewater in concentrations of several µg L^{-1} (Bendz et al. 2005, Buser et al. 1999, Clara et al. 2005, Roberts and Thomas 2006). During wastewater treatment ibuprofen is efficiently eliminated (> 90 %) by biological transformation processes in the secondary treatment (Buser et al. 1999, Carballa et al. 2004, Tauxe-Wuersch et al. 2005, Ternes 1998, Thomas and Foster 2005). Losses due to sorption to excess sludge are neglectable (Carballa et al. 2004, Clara et al. 2005, Joss et al. 2005). In WWTPs, the pharmaceutically active enantiomer S-ibuprofen is preferentially degraded (Buser et al. 1999) and the same TPs as during human metabolism are formed. Zwiener et al. (2002) observed that hydroxy ibuprofen was formed primarily during aerobic treatment, carboxy hydratropic acid during anaerobic treatment and carboxy ibuprofen in both treatment options while in a complementary study only the formation of hydroxy ibuprofen was determined (Quintana et al. 2005).

Table 1-6: Structural formula and physicochemical properties of ibuprofen



CAS number	525-66-6
formula	$C_{13}H_{18}O_2$
M (g mol^{-1})	206.29
water solubility (mg L^{-1})	49[1]
pK_a (-)	4.42[1]
log K_{OW} (-)	4.13[1], 2.48[2]

[1] Avdeef et al. (2000), [2] Scheytt et al. (2005a)

The concentrations of ibuprofen in WWTP effluents are highly variable depending on the performance of the WWTP. Concentrations between some ng L^{-1} and 28 µg L^{-1} have been measured (Ashton et al. 2004, Bagnall et al. 2012, Bendz et al. 2005, Drewes et al. 2003, Hilton and Thomas 2003, Lindqvist et al. 2005, Ternes 1998). Consequently, the concentrations of ibuprofen in receiving rivers also span large concentration ranges (Ashton et al. 2004, Bataineh et al. 2006, Bendz et al. 2005, Boyd et al. 2004, Hilton and Thomas 2003, Nödler et al. 2011, Roberts and Thomas 2006, Ternes 1998, Tixier et al. 2003, Wiegel et al. 2004). Especially in larger streams with a low proportion of wastewater and fully developed WWTPs as for example at the river Rhine, only very low concentrations in the (sub) ng L^{-1} are reported (Sacher et al. 2008). Nevertheless, ibuprofen has been detected in lakes (Buser et al. 1999), groundwater (Gottschall et al. 2012) and drinking water (Loraine and Pettigrove 2006).

Ibuprofen is present in the dissociated form at natural pH (Table 1-6). Hence, its tendency to sorb to sediments and soils is low (Conkle et al. 2012, Lin and Reinhard 2005, Mersmann et al. 2002) and log K_{OC} values of 2.0-2.2 were determined in river sediments (Scheytt et al. 2005b). Consequently, ibuprofen was also not detected in river sediments at rivers receiving substantial

proportions of treated wastewater (Langford et al. 2011). In batch experiments with river sediments and radio-labelled ibuprofen, about 15 % of total elimination after 100 days was attributed to sorption based on remaining radioactivity in the solid phase (Löffler et al. 2005). However, this high apparent loss by sorption can most likely be attributed to a biogenic fixation of radioactive TPs of or even of mineralized ^{14}C-ibuprofen (Nowak et al. 2013). Like in WWTPs, ibuprofen can be rapidly biologically transformed in river sediments. The reported half-life times in laboratory-scale sediment/water systems are usually in the range of only some hours to a few days depending of the type of sediment and the experimental setup (Kunkel and Radke 2008, Lin et al. 2006, Löffler et al. 2005, Mersmann et al. 2002, Nakada et al. 2008, Onesios and Bouwer 2012, Scheytt et al. 2007, Winkler et al. 2001). Biotransformation of ibuprofen is higher under aerobic conditions than under anaerobic conditions (Conkle et al. 2012, Preuß et al. 2002). In contrast to WWTPs, R-ibuprofen was preferentially degraded in river biofilms (Winkler et al. 2001). Compared to the rapid biotransformation, the phototransformation rates in rivers are small. In lakes no phototransformation (Tixier et al. 2003) or only very slow photolysis of preferentially S-ibuprofen was observed (Buser et al. 1999). Under natural sunlight the half-life times are reportedly in the range of tens of days (Matamoros et al. 2009). Phototransformation of ibuprofen is primarily achieved by indirect mechanisms (Lin and Reinhard 2005, Packer et al. 2003, Szabó et al. 2011). In the presence of fulvic acids the photo-TPs isobutylacetophenone and 1-(4-isobutylphenyl)ethanol were detected (Jacobs et al. 2011).

Despite being readily biodegradable, no substantial elimination of ibuprofen was observed in two rivers downstream of a WWTP (Bataineh et al. 2006, Bendz et al. 2005). Only a slow removal in a river in Texas with a half-life time of 4.6 days was observed and the elimination was mainly attributed to photolysis (Fono et al. 2006). Contrarily, in a Californian river, an 85 % removal within a 12 km stretch due to biotransformation was observed (Lin et al. 2006). Moreover, the analysis of pore water profiles indicated a transformation of ibuprofen in river sediments (Lewandowski et al. 2011b). In subsurface horizontal flow constructed wetlands, a 50 % removal of ibuprofen was reported (Matamoros and Bayona 2006).

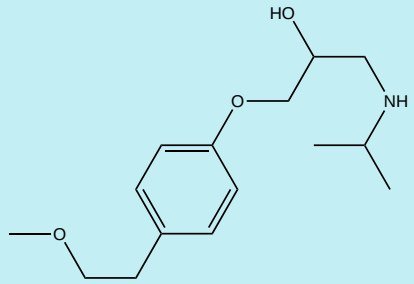
The analgesic drug ibuprofen is reported to be readily biodegradable in river sediments, while photolysis in surface waters is slow and sorption to (sandy) sediments is negligible. Therefore, ibuprofen will be used as an indicator substance to identify biological transformation processes in rivers and river sediments.

1.5.6 METOPROLOL

Metoprolol ((RS)-1-(Isopropylamino)-3-[4-(2-methoxyethyl)phenoxy]propan-2-ol) is a selective beta-blocker that is widely used in cases of malfunction of the cardiovascular system. For example, it is applied for the treatment of hypertension, irregular heartbeat, and also in case of acute myocardial infarction. In Germany, the prescribed amounts of metoprolol have been almost stable over the last five years: about 130 tons are prescribed per year (Schwabe and Paffrath 2009,

2010, 2011, 2012, 2013). Only 5 % of metoprolol are excreted as the active compound (Maurer et al. 2007). Three metabolites account for 85% of total excretion and are formed by oxidative de-amination, O-desalkylation and subsequent oxidation (leading to the formation of atenolol acid), and by aliphatic hydroxylation (Hernando et al. 2007). Usually concentrations of metoprolol in raw wastewater are in the high ng L^{-1} to the low µg L^{-1} range (Ternes et al. 2007, Thomas et al. 2007, Wick et al. 2009). Reported elimination of metoprolol during wastewater treatment ranges from almost no removal (Alder et al. 2010, Bendz et al. 2005, Thomas et al. 2007, Vieno et al. 2006, Wick et al. 2009) and moderate removal (Gabet-Giraud et al. 2010, Maurer et al. 2007) to almost complete removal (Ternes 1998). The elimination is attributed to biological transformation and not to sorption to excess sludge (Maurer et al. 2007, Scheurer et al. 2010). Consequently, only concentrations in the low ng g^{-1} range in sludge were determined (Scheurer et al. 2010).

Table 1-7: Structural formula and physicochemical properties of metoprolol

Metoprolol		
	CAS number	37350-58-6
	formula	$C_{15}H_{25}NO_3$
	M (g mol^{-1})	267.36
	water solubility (mg L^{-1})	49[1]
	pK_b (-)	9.7[2]
	log K_{OW} (-)	1.9[2]

[1] Avdeef et al. (2000), [2] Ternes and Joss (2006)

Metoprolol is commonly detected in WWTP effluents in the range of some hundred ng L^{-1} to several µg L^{-1} (Bendz et al. 2005, Miege et al. 2006, Ternes 1998, Wick et al. 2009). Concentrations in receiving waters are generally lower. Depending on the dilution factor, values in the low/mid ng L^{-1} or few µg L^{-1} range are reported (Alder et al. 2010, Ternes 1998, Vieno et al. 2006, Wiegel et al. 2004). However, metoprolol was not detected in a wastewater receiving lake (Alder et al. 2010) and groundwater (Sacher et al. 2001, Ternes et al. 2007) indicating an elimination of metoprolol in the aquatic environment.

Under environmental pH values (pH 5-8.5) metoprolol is predominately protonated (Table 1-7). Hence, metoprolol can sorb to negatively charged surface such as humic substances or iron and manganese oxides in river sediments. In column experiments with river sediments it was shown that the sorption of metoprolol is competitive to Ca^{2+} ions (Schaffer et al. 2012a). In sediments log K_{OC} values of 2-2.5 were determined indicating substantial sorption (Ramil et al. 2010). However, sorption of metoprolol to solid matrices is weaker than of propranolol (Kibbey et al. 2007). The reported biotransformation rates for metoprolol are inconclusive. While Ramil et al. (2010) calculated half-life times of 5-10 days based on experiments in sediment/water batch systems, metoprolol was not transformed in microcosm experiments with river water (Fono et al. 2006) and in aquifer material under denitrifying conditions (Barbieri et al. 2012b). In contrast, Huntscha et al. (2013) determined half-life times of less than one hour using push-pull tests in

river sediment. Phototransformation of metoprolol in pure water is slow with half-life times of weeks to years (Fono et al. 2006, Liu et al. 2009, Liu and Williams 2007) and also leads to no change in the ratio of the enantiomer fraction (EF) of metoprolol (Fono et al. 2006). Phototransformation rates can be enhanced by the presence of $^{\bullet}OH$ radicals and natural organic matter (NOM) indicating indirect photolysis. Wang et al. (2012) divided the total transformation of metoprolol into 6 % by direct transformation, 25 % by $^{\bullet}OH$ radicals and 70 % by interactions with NOM. No effect of pH (pH range 4-10) on the transformation kinetics was observed. In contrast, Chen et al. (2012) reported an increasing transformation with increasing pH (pH range 6-10). The main described photo-TPs are formed by a hydroxylation of the ether and cleavage of the tertiary amine (Chen et al. 2012, Liu et al. 2009).

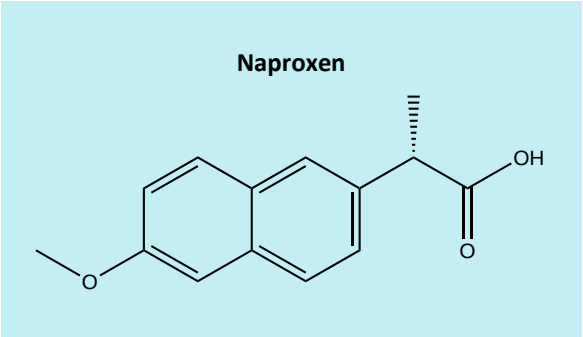
No removal of metoprolol with distance from WWTP in a Swedish river was observed (Bendz et al. 2005). In contrast, based on grab samples of river water, a 60 % loss of metoprolol compared to the sum of discharged loads from five WWTPs was estimated (Vieno et al. 2006). In a Californian river, a half-life time of six days for metoprolol was calculated (Fono et al. 2006). Moreover, the authors observed a decrease in the EF of metoprolol during stream transport indicating that this elimination was caused by biological processes. Concentration of metoprolol in Hessian sediments of up to 30 ng g^{-1} were determined (Ramil et al. 2010).

The beta-blocker metoprolol is reported to be moderately biodegradable in river sediments and also sorbs to a substantial degree to solid matrices. Metoprolol is resistant to photolysis under environmental conditions. Therefore, metoprolol is thought to be used an indicator substance to detect both sorption and biotransformation processes (proved by a change in the EF) in rivers.

1.5.7 NAPROXEN

Naproxen ((+)-(S)-2-(6-methoxynaphthalen-2-yl)propanoic acid) is an NSAID that is commonly applied to reduce moderate stiffness, fever, pain and inflammation. In contrast to the beginning of the 21st century where the consumption of naproxen decreased (BLAC 2003), its prescribed amounts in Germany have increased again over the last five years and accounted for approx. 8.6 tons in 2012 (Schwabe and Paffrath 2009, 2010, 2011, 2012, 2013). Naproxen is metabolized in the human body with a half-life time of 13 hours and excreted almost completely via urine as parent compound, O-desmethyl naproxen and the respective conjugates (Segre 1980, Vree et al. 1993). In WWTPs, influent concentrations of naproxen of up to several µg L^{-1} are reported (Bendz et al. 2005, Carballa et al. 2004, Lindqvist et al. 2005). Elimination of naproxen during wastewater treatment is typically in the range of 50 to 95 % (Bendz et al. 2005, Joss et al. 2005, Lindqvist et al. 2005, Radjenovic et al. 2007, Ternes 1998) and is attributed to biological transformation during secondary treatment (Carballa et al. 2005, Carballa et al. 2004). Good elimination during anaerobic sludge treatment is reported (Carballa et al. 2008). During wastewater treatment also the human metabolite O-desmethyl naproxen is formed (Quintana et al. 2005).

Table 1-8: Structural formula and physicochemical properties of naproxen



CAS number	22204-53-1
formula	$C_{14}H_{14}O_3$
M (g mol^{-1})	230.26
water solubility (mg L^{-1})	14[1]
pK_a (-)	4.18[1]
log K_{OW} (-)	3.24[1], 3.18[2]

[1] Avdeef et al. (2000), [2] Jones et al. (2002)

Concentrations in WWTP effluents usually range from some hundred ng L^{-1} to several µg L^{-1} (Bendz et al. 2005, Carballa et al. 2004, Drewes et al. 2003, Lindqvist et al. 2005, Ternes 1998, Tixier et al. 2003). In receiving rivers, naproxen concentrations are in the range of some ng L^{-1} to the high ng L^{-1} (Boyd et al. 2004, Radke et al. 2010, Ternes 1998, Tixier et al. 2003, Vieno et al. 2005). Naproxen was also detected in storm water channels (Boyd et al. 2004), runoff from a field irrigated with treated wastewater (Pedersen et al. 2005), and lakes (Boyd et al. 2004, Tixier et al. 2003). It was not determined in groundwater (Drewes et al. 2003) but in raw drinking at a concentration of 8 ng L^{-1} (Vieno et al. 2005).

Under natural pH, naproxen is present in its dissociated form and highly polar (Table 1-8); respective log K_{OC} values (determined for sediment, soil and sludge) are only about 2-2.5 (Duran-Alvarez et al. 2012, Jones et al. 2002, Schaffer et al. 2012b) and sorption to river sediments is only of minor importance (Lin et al. 2006). Consequently only minor retardation of naproxen during transport in soils even at high TOC concentration of the soil was determined (Borgman and Chefetz 2013). Biotransformation rates of naproxen span a wide range. Some studies reported no elimination in river water (Peng et al. 2008), river sediments (Lin et al. 2006) or a biofilm reactor (Boyd et al. 2005). Other work determined a slow transformation rate of respective half-life times of some days in biofilm reactors and in aerobic and anaerobic sludge treatment and a subsequent formation of O-desmethyl naproxen (Lahti and Oikari 2011, Quintana et al. 2005) or a biological removal of 80 % within 60 days in batch systems with silica sediment and different nutrients (Maeng et al. 2011). In contrast, naproxen was efficiently eliminated (95 %) in column experiments simulating soil aquifer recharge (Onesios and Bouwer 2012), or in soils by mesophilic aerobic biodegradation also leading to the formation of O-desmethyl naproxen (Topp et al. 2008). In flume experiments with river sediments, naproxen was transformed at equal rates under aerobic and anaerobic conditions (t_H = 10-14 days) in the sediments (Kunkel and Radke 2008). Data on the photosusceptibility of naproxen is more conclusive. Packer et al. (2003) determined a rapid phototransformation of naproxen in both ultra-pure and river water. Naproxen is supposedly rapidly transformed by combined direct and indirect photolysis (Lin and Reinhard 2005) leading to a reduction of the carboxy group to a keto group or to an ethylation of the carboxy group. The formed TPs are more toxic to aquatic organism than naproxen (Isidori et al. 2005). Under natural condi-

tions, half-life times for phototransformation are in the range of 2.5-10 days (Araujo et al. 2011, Radke et al. 2010).

Conclusive to the laboratory studies, field data implies that naproxen can be eliminated within the aquatic environment. Calculations based on a few grab samples taken downstream of WWTPs indicated an elimination of naproxen in rivers (Bendz et al. 2005, Lindqvist et al. 2005, Vieno et al. 2005). More systematic studies were only partially able to solidify this interpretation. Naproxen was rapidly removed (t_H ~ 2 hours) during river transport at a Californian river which was attributed to a combined effect of photolysis and sorption (Gross et al. 2004, Lin et al. 2006). In a German river, naproxen was poorly eliminated along a river stretch under low flow conditions (Radke et al. 2010) while other studies report high removal in rivers due to photolysis (Fono et al. 2006, Nakada et al. 2008). In a lake, naproxen was slowly eliminated (t_H = 14 days) potentially by a combination of direct photolysis and biotransformation. However, the analysis of pore water profiles did not suggest substantial biotransformation in river sediments (Lewandowski et al. 2011b). In contrast, high removal rates during subsurface horizontal flow constructed wetland treatment (Matamoros and Bayona 2006, Zhang et al. 2012) and during aquifer recharge (Drewes et al. 2003) were determined.

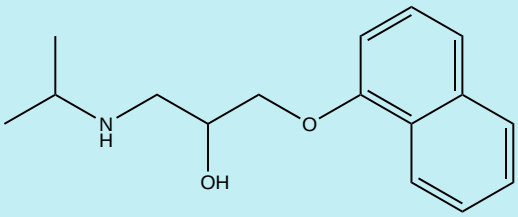
The analgesic drug naproxen is reported to be susceptible to photolysis in rivers. Sorption to (sandy) sediments is negligible and data on the biotransformation potential is inconclusive. In this thesis naproxen is considered as a micropollutant that can be eliminated from rivers by various elimination pathways.

1.5.8 PROPRANOLOL

Propranolol ((RS)-1-(1-methylethylamino)-3-(1-naphthyloxy)propan-2-ol) is a non-selective beta-blocker that is used for the treatment of hypertension. The prescribed amounts in Germany are low compared to other countries and only about 3 tons per year or 0.25 DDDs per year and inhabitant are applied (Schwabe and Paffrath 2009, 2010, 2011, 2012, 2013). About 10 % of the applied amount is excreted as parent compound while about 15 % are excreted as glucuronide (Mehvar and Brocks 2001). Concentrations in raw wastewater are typically relatively low and only some hundred ng L^{-1} are reported (Bendz et al. 2005, Fono and Sedlak 2005, Wick et al. 2009). However, in hospital effluents up to 6 µg L^{-1} were determined (Gomez et al. 2006). During wastewater treatment propranolol can be efficiently eliminated. However, literature elimination rates range from only a few % (Bendz et al. 2005, Gabet-Giraud et al. 2010, Maurer et al. 2007, Wick et al. 2009) to almost complete elimination (Ternes 1998). These inconclusive elimination rates might be explainable by a cleavage of the glucuronide in WWTPs (Liu and Williams 2007). During WWTP treatment the enantiomer fraction (EF) of propranolol is decreasing, indicating that the elimination in WWTPs is caused by biological processes (Bagnall et al. 2012, Fono and Sedlak 2005, Liu and Williams 2007).

Table 1-9: Structural formula and physicochemical properties of propranolol

Propranolol



CAS number	525-66-6
formula	$C_{16}H_{21}NO_2$
M (g mol^{-1})	259.34
water solubility (mg L^{-1})	70[1]
pK_b (-)	9.53[1], 9.49[2]
log K_{OW} (-)	3.48[1]

[1] Avdeef et al. (2000), [2] Beausse (2004)

The concentration of propranolol in WWTP effluents varies strongly between different countries but is generally in the sub µg L^{-1} range (Andreozzi et al. 2003, Ashton et al. 2004, Fono and Sedlak 2005, Hilton and Thomas 2003, Miege et al. 2006, Scheurer et al. 2010, Ternes 1998, Wick et al. 2009, Zhang and Zhou 2007). In receiving rivers, propranolol is typically detected at a few ng L^{-1} (Alder et al. 2010, Ashton et al. 2004, Hilton and Thomas 2003, López-Serna et al. 2012, Roberts and Thomas 2006, Zhang and Zhou 2007). However, individual concentrations of up to 0.6 µg L^{-1} were determined (Ternes 1998). Propranolol was not detected in a groundwater survey in Germany (Sacher et al. 2001).

Sorption constitutes a relevant elimination pathway of propranolol from the water phase in sediment/water test systems. About 80-90 % were removed within 6 hours in (Ramil et al. 2010). Reported log K_{OC} values of 2.3-3.5 in sludge, soil, and sediments were determined (Drillia et al. 2005b, Maurer et al. 2007, Ramil et al. 2010). Sorption to sediments is stronger than for metoprolol (Kibbey et al. 2007) and desorption from solids is only very slow (Drillia et al. 2005b). Compared to sorption, biotransformation is believed to be only of minor importance. Maurer et al. (2007) determined a slow biotransformation in activated sludge (t_H = 10 hours) after a strong initial sorption phase. Consistently, no elimination was observed in river water (Liu et al. 2009) and propranolol was also resistant against biotransformation in batch experiments with aquifer material under denitrifying conditions (Barbieri et al. 2012b). In a different set of sediment/water batch systems, propranolol was rapidly removed from the water phase (t_H = 0.5-2 days), but the sum of dissolved and sorbed propranolol only slowly decreased over time (t_H = 10-30 days) also indicating the superior importance of sorption compared to biotransformation in river sediments (Ramil et al. 2010). Propranolol is susceptible to photolysis. It is eliminated from surface waters by direct phototransformation with rates from less than one day to some weeks depending on latitude and season (Andreozzi et al. 2003, Liu and Williams 2007). Photo-TPs are formed by initial opening and subsequent oxidation of the aromatic structure (Liu and Williams 2007). Higher rates in presence of nitrate and humic acids indicate indirect photolysis, while biotransformation in surface water is negligible (Andreozzi et al. 2003).

Despite the reported sorption affinity and susceptibility to photolysis, no decrease of the propranolol loads downstream of a WWTP over a distance of 7.5 km was observed (Bendz et al.

2005). Sorbed concentration of 10-30 ng g^{-1} (Lahti and Oikari 2012, Ramil et al. 2010) were determined in river sediments while no clear trend of the concentration with depth in the sediments was visible (Lahti and Oikari 2012).

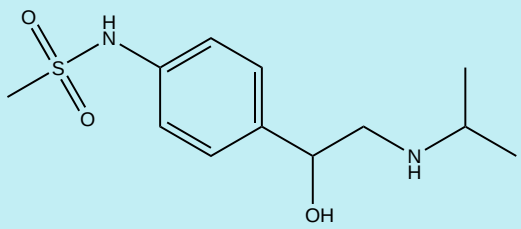
The beta-blocker propranolol is primarily eliminated from rivers by strong sorption to the sediments and phototransformation in the surface waters. Biotransformation is believed to be only of minor importance. Propranolol is used as reference substance that allows determining the maximum contribution of sorption to the total elimination in the studies.

1.5.9 SOTALOL

Sotalol ((RS)-N-{4-[1-hydroxy-2-(propan-2-ylamino)ethyl]phenymethanesulfonamide) is a drug belonging to the class of non-selective beta-blockers. It is applied to treat humans with high blood pressure and arrhythmic heartbeat. In Germany, the application rates of sotalol have slightly decreased over the last years and a total of about 3-5 tons per year were prescribed (Schwabe and Paffrath 2009, 2010, 2011, 2012, 2013). However since sotalol is excreted via urine to about 70 % as parent compound (Maurer et al. 2007) concentration in raw wastewater are still as high as some µg L^{-1} (Gabet-Giraud et al. 2010, MacLeod et al. 2007, Maurer et al. 2007, Piram et al. 2008a, Ternes et al. 2007, Vieno et al. 2006, Wick et al. 2009). Reported elimination rates in WWTPs range from almost no removal (Gabet-Giraud et al. 2010, MacLeod et al. 2007) to almost complete removal (Piram et al. 2008a). But most studies determined a rate of about 30-60 % (Maurer et al. 2007, Ternes et al. 2007, Vieno et al. 2006) and attributed the elimination to a combination of both sorption and biological processes with the latter being the dominant one (Jelic et al. 2012, Scheurer et al. 2010, Wick et al. 2009).

Table 1-10: Structural formula and physicochemical properties of sotalol

Sotalol



CAS number	3930-20-9
formula	$C_{12}H_{20}N_2O_3S$
M (g mol^{-1})	272.36
water solubility (mg L^{-1})	137000[1]
$pK_{a,1}/pK_{a,2}$ (-)	8.2[2]/9.8[2]
log K_{OW} (-)	0.2[2]

[1] Hernando et al. (2007), [2] Ternes and Joss (2006)

In WWTP effluents, sotalol is commonly detected in the mid ng L^{-1} to low µg L^{-1} range (Gabet-Giraud et al. 2010, MacLeod et al. 2007, Maurer et al. 2007, Scheurer et al. 2010, Ternes et al. 2007, Vieno et al. 2006, Wick et al. 2009). In receiving waters, various authors determined concentrations of sotalol of a few to some hundred ng L^{-1} (Nödler et al. 2011, Piram et al. 2008a,

Vieno et al. 2006). Sotalol is also detected sporadically in groundwaters. It was only detected in 3 of 105 groundwater samples in Baden-Württemberg, but the highest the concentration was 590 ng L^{-1} (Sacher et al. 2001). In contrast, sotalol was never detected in groundwater samples after wastewater containing high amounts of sotalol (> 1 µg L^{-1}) was applied to an agricultural soil (Ternes et al. 2007). In Spain, up to 16 ng L^{-1} were detected in groundwater (Huerta-Fontela et al. 2011). Sotalol was also detected in raw and finished drinking water (after chlorination) in the low ng L^{-1} range (Huerta-Fontela et al. 2011, Vieno et al. 2007).

Like metoprolol and propranolol, sotalol is occurring in its protonated form under environmental pH ($pK_{a,1}$ =8.2 for the sulfonamide and $pK_{a,2}$ = 9.8 for amine, Table 1-10). In sediment/water batch systems, 90 % of the initially applied sotalol was removed from the water phase within 6 hours and a log K_{OC} of 2 for this sediments was calculated (Ramil et al. 2010). In contrast, Maurer et al. (2007) reported only minor sorption in batch systems with activated sludge. Sorption to mineral surfaces is highly pH dependent and strongest at slightly acidic and neutral pH values when sotalol is present as cation (Sanchez-Camazano et al. 1987). Quantitative information on biotransformation of sotalol in river sediments is scarce. Ramil et al. (2010) determined total half-life times of 11-30 days depending on the type of sediment. In batch systems with activated sludge, the respective half-life time was 15 hours (Maurer et al. 2007). Phototransformation of sotalol is reportedly low compared to the structurally similar beta-blocker propranolol (Wang et al. 2012). Studies by Piram et al. (2008a) indicate an indirect phototransformation since they observed no elimination in ultra-pure water but transformation in WWTP effluent water (t_H ~ 5 hours, lab-scale with an UV light photo reactor).

Based on twelve grab samples taken on a monthly basis sotalol was partly eliminated in a river downstream of a WWTP in a Swedish watershed since its concentration decreased stronger than the concentration of carbamazepine (Daneshvar et al. 2010). During river bank filtration at the river Rhine 70-90 % of sotalol was removed (Storck et al. 2012). This can be potentially attributed to both sorption and (bio-)transformation since substantial concentrations of sotalol in river sediments have been reported (Ramil et al. 2010).

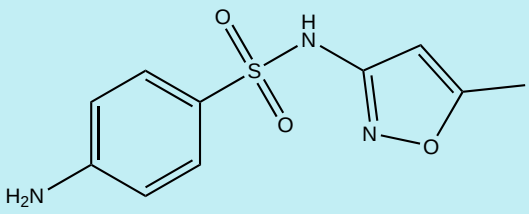
Compared to other substances in focus of this thesis only little data on the fate of sotalol in the aquatic environment is available. Biotransformation as well as sorption and phototransformation can potentially be relevant elimination pathways in rivers. This thesis will generate new insight on the actual fate of sotalol in rivers and river sediments.

1.5.10 Sulfamethoxazole

Sulfamethoxazole (4-amino-N-(5-methylisoxazol-3-yl)-benzenesulfonamide) is an antibiotic pharmaceutical belonging to the group of the sulfonamides. In Germany, it is exclusively prescribed in combination with the other antibiotic trimethoprim (ratio 5:1) under the name co-

trimoxazole. About 50 % of the intaken sulfamethoxazole is usually excreted via urine within 24 hours. Only about 15 % are excreted as the parent compound while the majority is excreted as the human metabolites N4-actetyl-sulfamethoxazole and sulfamethoxazole-N1-glucuronide (Gill et al. 1996, Vanderven et al. 1995). Its consumption in Germany has slightly declined over the past five years from 18.7 million DDD in 2008 to 12.5 million DDD (Schwabe and Paffrath 2009, 2010, 2011, 2012, 2013) corresponding to prescribed amounts of 25-37 tons per year. Nevertheless, sulfamethoxazole has been frequently detected in WWTP influents in concentrations up to the low µg L^{-1} range (Göbel et al. 2004, Hirsch et al. 1999, Renew and Huang 2004). Elimination of sulfamethoxazole during wastewater treatment ranges from 20 to 90 % (Carballa et al. 2004, Gao et al. 2012, Göbel et al. 2005, Ternes et al. 2007) while elimination due to sorption is negligible (Gao et al. 2012, Göbel et al. 2005, Yang et al. 2012).

Table 1-11: Structural formula and physicochemical properties of sulfamethoxazole

Sulfamethoxazole		
	CAS number	723-46-6
	Formula	$C_{10}H_{11}N_3O_3S$
	M (g mol^{-1})	253.28
	water solubility (mg L^{-1})	300-1900[1]
	$pK_{a,1}$ /$pK_{a,2}$ (-)	5.7[2], 1.6[3]
	log K_{OW} (-)	0.89[3]

[1] Lin et al. (1997), [2] Beausse (2004), [3] Boreen et al. (2004), [3] Scheytt et al. (2005a)

Sulfamethoxazole is commonly determined in WWTP effluents and receiving rivers (Heberer et al. 2008, Hirsch et al. 1999, Nödler et al. 2011) as well as in groundwater (Heberer et al. 2008, Hirsch et al. 1999, Sacher et al. 2001). Besides its human use, sulfamethoxazole is also used in veterinary medicine and therefore additionally introduced to soils and subsequently also to the aquatic environment via manure spreading and pasture farming. Therefore, also numerous studies on the transport, sorption, and biotransformation of sulfamethoxazole in soil were conducted.

Sulfamethoxazole is susceptible to photodegradation with 4-amino-N-(5-methyl-2-oxazolyl)-benzenesulfonamide being the major TP (Zhou and Moore 1994). Half-life times derived from laboratory photokinetic studies range from several hours to a few days (Andreozzi et al. 2003, Lam and Mabury 2005, Zhou and Moore 1994). Since the transformation is decreasing with increasing pH values (Zhou and Moore 1994) and the presence of humic acids also decreases the transformation rate (Andreozzi et al. 2003) phototransformation of sulfamethoxazole in rivers with typically relatively high pH values (6-8) and a high content of organic matter (e.g., humic substances) is only of minor importance as elimination pathway. Consequently, Lam et al. (2004) determined only little elimination due to phototransformation in outdoor microcosms (t_H approx. 20 days). Biological transformation of sulfamethoxazole is reported under various redox conditions. Baumgarten et al. (2011) determined higher transformation rates under aerobic compared to anoxic conditions in column experiments mimicking river bank filtration processes. However,

the majority of studies reported a higher removal under more reduced conditions. Mohatt et al. (2011) observed a rapid microbially mediated abiotic transformation of sulfamethoxazole under iron-reducing conditions in soils (t_H ~ few hours) while the elimination under sulfate-reducing and aerobic conditions was much slower (t_H ~ 2 and 5 days, respectively). Under denitrifying conditions, sulfamethoxazole is assumed to be reversibly transformed by a nitration of the primary amine (Barbieri et al. 2012a). Analogously to WWTPs, sorption of sulfamethoxazole to solid matrices constitutes no major sink in rivers (or soil): the reported K_{OC} values for soil range from 0.7 to 530 L kg^{-1} (Drillia et al. 2005a, Strauss et al. 2011, Ternes et al. 2007, Yu et al. 2009). Sorption to sediments was also excluded as major sink (Radke et al. 2009). The sorption affinity of sulfamethoxazole is more pronounced under slightly acidic pH values as shown in column experiments using manure amended soil (Strauss et al. 2011) and sorption tests (Fukahori et al. 2011) since it is then present in its neutral form (Table 1-11).

The experimental results from laboratory work hint to a rather conservative behavior of sulfamethoxazole in rivers. Consistently, only minor dissipation of sulfamethoxazole during downstream transport at the river Seine (Tamtam et al. 2008) and a persistent behavior in a small river in South Sweden (Bendz et al. 2005) was reported. Only 0-20 % are removed during river bank filtration (Storck et al. 2012) and sulfamethoxazole is also frequently detected in run-off from fields irrigated with WWTP effluent (Pedersen et al. 2005) as well as the pore water and groundwater under an agriculturally used site where a mixture of treated wastewater and digested sludge has been applied to soils for more than 50 years (Ternes et al. 2007).

Sorption of the antibiotic drug sulfamethoxazole to sediments and phototransformation in surface water are supposedly no major elimination pathways in rivers under near neutral (and environmentally relevant) pH values. Hence, a potential elimination of sulfamethoxazole will be used to identify (anaerobic) biotic and abiotic transformation processes in rivers and river sediments.

1.6 Outline of this Thesis

A reactive tracer test to evaluate the fate of pharmaceuticals in rivers

Chapter 2 reports a tracer experiment at a small river where pharmaceuticals were manually spiked into a non-polluted stream. Additionally two non-reactive dye tracers were added. This approach allowed determining precise mass balances for substances along river stretches in relation to prevailing hydraulics in the river and the exchange of water and solutes between the stream and storage zones. A subsequent modeling study determined elimination kinetics of pharmaceuticals for different compartments of the stream and evaluated the importance of processes in the storage zone for the total elimination.

Fate of pharmaceuticals in rivers: deriving a benchmark dataset at favorable attenuation conditions

Chapter 3 describes a systematic study at a river with conditions that are believed to be ideal for the elimination of pharmaceuticals. The study combined a systematic monitoring work at two sites along a river stretch with *in situ* phototransformation experiments and the determination of concentrations in river sediments. Moreover, this concept allowed calculating elimination rates of pharmaceuticals along the river stretch and attributing the total elimination to phototransformation in the surface water or biotransformation in the river sediments for individual substances.

Recirculating sediment columns provide generalizable rate constants for the biotransformation of pharmaceuticals in river sediments

Chapter 4 introduces a laboratory-scale experimental setup based on recirculating sediment columns that enables the determination of more realistic elimination kinetics for pharmaceutical residues in river sediments. The experimental concept was applied to several sediments (i.e., from the sites reported in chapter 3 and in Radke et al. (2010)). The effect of pore water velocity on the elimination rates was investigated and the results of the column tests were discussed in light of the elimination rates determined in the field studies.

Final Conclusions

Chapter 5 discusses the main results of studies I-III, highlights the knowledge gaps that were narrowed by the work of this thesis, draws some final conclusions and summarizes the needs for future research.

1.7 Contribution to the Different Studies

Study I

A Reactive Tracer Test to Evaluate the Fate of Pharmaceuticals in Rivers (Environmental Science & Technology 45(15): 6296-6302)

Uwe Kunkel	90 %	Concept, field and laboratory work, calculations, preparation of manuscript
Michael Radke	10 %	Concept, field work, calculations, preparation of manuscript

Study II

Fate of Pharmaceuticals in Rivers: Deriving a Benchmark Dataset at Favorable Attenuation Conditions (Water Research 46(17): 5551-5565)

Uwe Kunkel	90 %	Concept, field and laboratory work, calculations, preparation of manuscript
Michael Radke	10 %	Concept, calculations, preparation of manuscript

Study III

Recirculating Sediment Columns Provide Generalizable Rate Constants for the Biotransformation of Pharmaceuticals in River Sediments (to be submitted to Environmental Science & Technology)

Uwe Kunkel	70 %	Concept, laboratory work, calculations, preparation of manuscript
Stephanie Wilde	20 %	Laboratory work
Michael Radke	10 %	Concept, preparation of manuscript

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2 STUDY I: TRACER TEST TO EVALUATE THE FATE OF PHARMACEUTICALS IN RIVERS

A Reactive Tracer Test to Evaluate the Fate of Pharmaceuticals in Rivers

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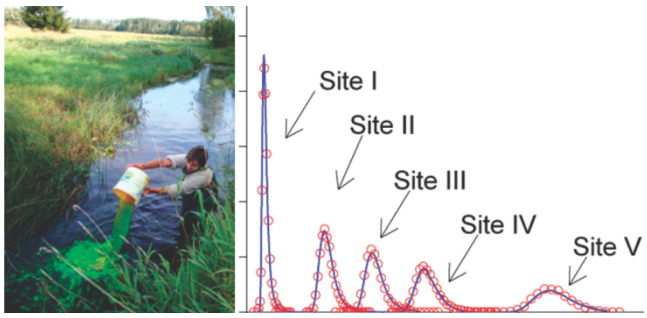

2.1 ABSTRACT

The fate of pharmaceutically active substances in rivers is still only incompletely understood, especially as the knowledge transfer from laboratory experiments to the real world is complicated by factors like turbidity, hydrodynamics, or heterogeneity. Therefore, we performed a tracer test with pharmaceutically active substances to study their fate and the importance of individual attenuation mechanisms *in situ*. The experiment was carried out at a small stream in central Sweden. Two dye tracers and six pharmaceuticals were injected as Dirac pulse and water was sampled at five downstream sites along a 16 km long river reach. Ibuprofen and clofibric acid were the only compounds which were eliminated along the study reach at half-life times of 10 h and 2.5 d, respectively. Based on the shape of the breakthrough curves and the low hydraulic conductivity of the river bed, exchange of river water with the hyporheic zone was minor. Thus, the contribution of processes in the hyporheic zone to the attenuation of pharmaceuticals was low. We hypothesize that ibuprofen and clofibric acid were transformed by in-stream biofilms growing on submerged macrophytes and at the water/sediment interface. Phototransformation and sorption were ruled out as major attenuation processes. No attenuation of bezafibrate, diclofenac, metoprolol, and naproxen was observed.

2.2 INTRODUCTION

Pharmaceutical residues are commonly detected organic micropollutants in the aquatic environment (Ternes 1998, Tixier et al. 2003). Due to incomplete elimination during wastewater treatment (Carballa et al. 2005, Joss et al. 2006), they can reach surface waters where they are present at concentrations in the ng L^{-1} to µg L^{-1} range. From laboratory experiments, we learned that certain pharmaceuticals will be removed or retained by biodegradation in river sediments (e.g., ibuprofen, Kunkel and Radke (2008)), photolysis in the surface water (e.g., diclofenac, see Tixier et al. (2003)), or sorption to sediment (e.g., metoprolol, see Kibbey et al. (2007)). However, the environmental fate of pharmaceutical residues in rivers and the contribution of individual attenuation mechanisms to their elimination are still incompletely understood. This is due to difficulties in transferring laboratory-derived knowledge to the situation in real systems, for example caused by factors such as turbidity of the river water (Robinson et al. 2007), climatic variabilities, river morphology, or the magnitude of hyporheic exchange (Kunkel and Radke 2008). Moreover, in field studies at wastewater impacted rivers additional uncertainties arise from a number of system-inherent properties. For example, spatio-temporal fluctuations of river discharge as well as the temporally variable input of pharmaceuticals (Radke et al. 2010) impair not only the determination of *in situ* attenuation rates, but also the differentiation between individual attenuation processes. Consequently, a wide range of parameters for biotransformation and sorption have been measured for pharmaceuticals in aquatic systems (Table A- 3 in Supporting Information to chapter 2) summarizes some of these observations.

From a process-oriented point of view, on the field scale only an experimental program based on a specifically designed sampling scheme and precisely measured hydraulic parameters can

provide adequate information on micropollutant attenuation (Brown et al. 2009, Ort et al. 2010). Reactive tracer tests are an alternative to such extensive field campaigns since some of the relevant boundary conditions can be assessed more easily. Experiments with a set of organic micropollutants as reactive tracers should enable us to evaluate specific hypotheses and processes under *in situ* conditions at a reasonable effort. However, while such tests are regularly used to study the attenuation of organic contaminants in aquifers (Bahr 1989, Fischer et al. 2006), they are only rarely applied in surface water.

The aim of this study was to determine the influence of individual attenuation processes on the fate of organic micropollutants in rivers. More specifically, we aimed at testing the hypothesis that the interface between surface water and groundwater (i.e., the hyporheic zone) is a major contributor to the attenuation of pharmaceuticals. We performed a tracer test with conservative and reactive tracers at a small river. As reactive tracers, we selected a set of six pharmaceuticals with supposedly different fate in rivers: ibuprofen (easily biodegradable), diclofenac (high photolysis rate, moderately biodegradable), naproxen (moderate photolysis rate, moderately biodegradable), bezafibrate (moderately biodegradable), clofibric acid (persistent), and metoprolol (moderately biodegradable, sorptive).

2.3 Experimental Methods

2.3.1 Chemicals

All pharmaceuticals (purity > 97%) were purchased from Sigma-Aldrich (Seelze, Germany). The surrogate standards bezafibrate-D_4, clofibric acid-D_4, diclofenac-D_4, ibuprofen-D_3, metoprolol-D_7, and naproxen-D_3 were purchased from Toronto Research Chemicals (North York, ON, Canada). LC/MS-grade acetonitrile (ACN) was purchased from Sigma-Aldrich, acetic acid (HAc) and formic acid (FA, both analytical reagent grade), methanol (MeOH, HPLC grade), and ammonium hydroxide (NH_4OH, 25 % in water) were obtained from Merck (Darmstadt, Germany), and LC/MS-grade water from J.T. Baker (Deventer, The Netherlands). Uranine (Fluorescein sodium salt; CAS No. 518-47-8) was purchased from Niepötter Labortechnik (Bürstadt, Germany) and rhodamine WT (CAS No. 37299-86-8; 21% aqueous solution) was purchased from Navarc OY (Turku, Finland).

2.3.2 Study Site

Experiments were carried out at Säva Brook, a small stream in central Sweden where several tracer studies were conducted before (Johansson et al. 2001, Salehin et al. 2003). It drains a watershed of 197 km² and has a total length of about 34 km. Säva Brook originates in forested areas northwest of Uppsala, then flows southward through agricultural lands and finally discharges into Lake Mälaren. Its average inclination is 0.1 %. This study was carried out between 08/31/2009 and 09/02/2009 at a 16.2 km long reach of the stream. Along this reach, most parts of the channel are deeply incised in the landscape. The stream width varied between 3 and 10 m with an average and also predominant width of approx. 5 m. During the experiment, the average water depth was about 1 m but varied from about 20 cm in fast flowing stretches up to 1.5 m in sections with al-

most stagnant water. The hydraulic radius along the study reach varied between 0.2 m and 1.2 m (average/predominant hydraulic radius: 0.7 m). Generally, the flow velocity was low (approx. 10 cm s^{-1}), and at time of the experiment many sections of the stream channel were densely vegetated as described previously (Salehin et al. 2003). The sediment texture was consolidated clayey silt (Table A- 2) overlaid by a thin layer of densely rooted, loosely packed organic material. Only in the surroundings of the few villages and bridges, the banks are reinforced and consequently the flow velocity is higher (up to 60 cm s^{-1}). There, the stream bed consists of rocks with only little in-stream vegetation. During the experiment, the exposure of the channel to sunlight was limited by vegetation on the river banks and in the stream channel as well as by the high and steep banks. The colour of the river water is reddish-brown; an UV/VIS-absorption spectrum of the river water is available as Supporting Information (Figure A- 5). Säva Brook receives no substantial input of wastewater and hence, the background concentration of the investigated pharmaceuticals was insignificant; we verified this by preliminary analyses. Throughout the experiment, the pH of the river water was approximately 5.5. The concentration of dissolved organic carbon (DOC) was about 16 mg L^{-1} (Table A- 2); the water temperature varied between 13 and 16 °C (Figure A- 4). The weather on the first day of the experiment was sunny, became cloudy on the second day, and on the third day there was some slight rainfall.

2.3.3 Tracer Experiment

Approximately 500 mg of each pharmaceutical (bezafibrate, clofibric acid, diclofenac, ibuprofen, metoprolol; 1000 mg of naproxen) and 30 g of uranine were dissolved in river water and injected as Dirac pulse at 08/31/2009 10:10 a.m. into the centre of the stream. Rhodamine WT (32 g) was injected earlier (9:42 a.m.). Pharmaceuticals were dissolved in about 25 mL of ethanol (c ~ 20 mg L^{-1}; 40 mg L^{-1} for naproxen) while the fluorescence tracers were dissolved in about 5 L of river water. The solutions were poured into the stream, and the containers were then quickly rinsed several times with river water. The calculated initial concentrations in the stream were about 170 µg L^{-1} for the pharmaceuticals (naproxen: 340 µg L^{-1}) and 10 mg L^{-1} for the dye tracers. Pictures of the tracer injections are available as Supporting Information to chapter 2. At five sites downstream of the injection site (sites I through V, see Figure 2-1, coordinates are given in Table A- 1), the breakthrough curves of rhodamine WT were measured online (time resolution: 1 min) with a portable fluorometer (sites I, III, V; model 10AU instrument with optical kit 10-041R (excitation/emission wavelengths: 550/580 nm) and continuous flow cuvette, Turner Designs Inc., Sunnyvale, CA, USA) or with a submersible sensor (sites II, IV; Cyclops 7; excitation/emission: 550/590 nm; Turner Designs Inc.). The distance required for complete mixing of the tracers with river water was estimated at 185 m according to an established USGS Guideline (Kilpatrick and Wilson 1989) (for details on the mixing process and its calculation see Supporting Information to chapter 2). As temperature varied by only 3 °C during the experiments, no correction of the fluorescence intensity of rhodamine WT as a function of temperature was necessary; the same applies to the effect of pH on fluorescence.

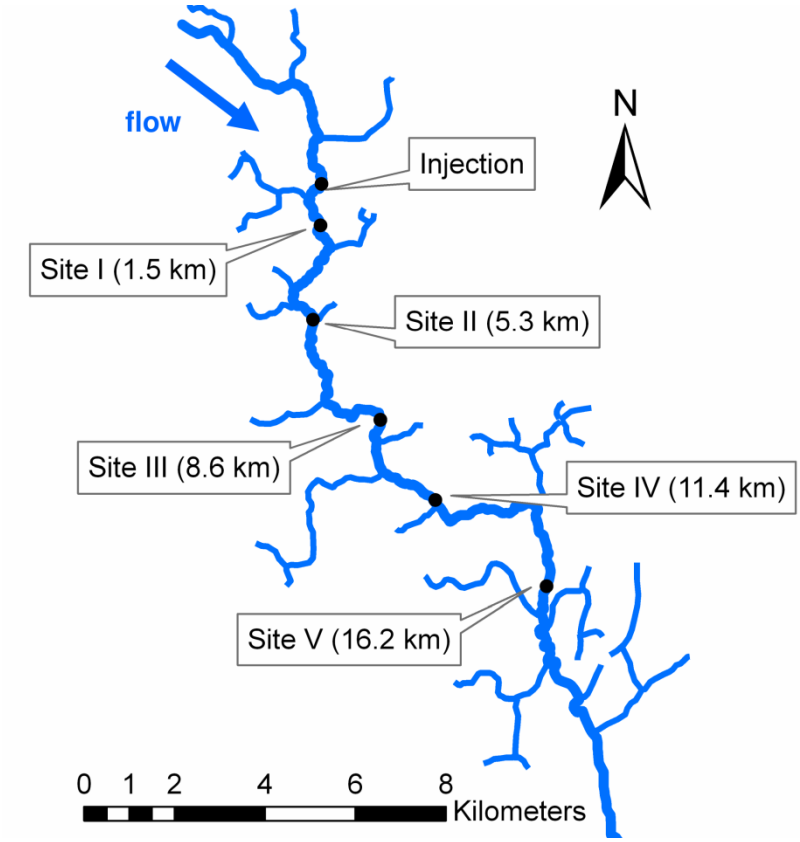

Figure 2-1: Map of Säva Brook with the injection site and the five sampling sites. Numbers in parentheses indicate the distance from the injection point.

Sampling of river water for the subsequent determination of uranine and pharmaceuticals at the five sites was triggered by the increase of the rhodamine WT signal. Approximately 300 mL of water were collected with automatic water samplers (3700 compact, Teledyne-ISCO, Lincoln, NE, USA) at intervals from 10 (site I) to 60 min (site V). Subsequently, a 40 mL subsample was separated for the analysis of uranine and stored at 4 °C, the rest of each sample was stored frozen until further analysis for up to 15 days. Sample stability during deep-freezing was previously confirmed (Radke et al. 2010). Over the whole experiment, discharge was measured at site III with a continuous wave Doppler instrument (model 2150, Teledyne ISCO); at the other sites discharge was measured occasionally with a current meter. Average discharge at site III during the experiment was approximately 350 L s^{-1} with little variations; the time trend at site III is available as Supporting Information (Figure A- 2). Water temperature was continuously measured at several locations along the river stretch with temperature loggers (HOBO UA-001-64, Onset, Bourne, MA, USA). The hydraulic conductivity of the river bed was determined in intervals of approx. 200 m by slug tests as described by Wörman et al. (2002). The exchange of groundwater and surface water was determined at the same spatial resolution with a temperature probe similar to Schmidt et al. (2006).

2.3.4 Analytical Methods

Uranine concentrations were determined in the laboratory with a 10AU Field Fluorometer (Turner Designs Inc.; optical kit 10-086R, excitation/emission wavelengths: 490/580 nm, quartz

tubes) without additional sample pre-treatment. The pharmaceuticals were enriched by solid phase extraction (SPE) as previously described (Lavén et al. 2009). Briefly, water samples were vacuum filtered (GF6, Whatman, Dassel, Germany), 50 ng of each deuterated surrogate standard were added, and the pH was adjusted to 2 by H_2SO_4. Oasis MCX cartridges (60 mg/3 mL; Waters, Eschborn, Germany) were preconditioned with 2 mL MeOH and 2 mL FA (2 % in ultra-pure water), and the samples were extracted on a vacuum extraction manifold. The extracted sample volumes ranged from 220 to 250 mL. Then, cartridges were washed with 2 mL of FA (2 % in ultra-pure water), dried for 1 h by purging with air, and stored frozen. Prior to analysis, cartridges were eluted two times with 1 mL of MeOH (eluate I; fraction containing the acidic compounds) and two times with 1 mL of NH_4OH (2 % in MeOH, eluate II; containing metoprolol). Both eluates were evaporated to dryness under a stream of nitrogen and subsequently redissolved separately in 300 µL ACN:H_2O:HAc (80:20:0.1). Pharmaceutical concentrations were then determined with HPLC/MS/MS (bezafibrate, clofibric acid, ibuprofen, and naproxen) or UPLC/QToF/MS (diclofenac and metoprolol). Details on the choice of the instruments and the LC/MS methods are given as Supporting Information to chapter 2. Concentrations of peaks with S/N < 5 were set to 0 for subsequent data analysis. The operational limits of quantification (LOQ) were 1 ng L^{-1} for clofibric acid, ibuprofen, and metoprolol, 5 ng L^{-1} for bezafibrate and naproxen, and 25 ng L^{-1} for diclofenac.

2.3.5 Quality Assurance

The analytical method for the determination of pharmaceuticals was validated by a standard addition experiment. To this end, river water was spiked with the target compounds to a concentration of 50 ng L^{-1}. This sample was used as river reference and aliquots of this sample were spiked to 100 ng L^{-1}, 150 ng L^{-1}, and 200 ng L^{-1}. Each of these solutions was analyzed in triplicate. To monitor inter-day deviations, a reference sample (100 ng L^{-1}) was prepared in Säva water and extracted; this sample was then measured in every sequence.

2.3.6 Calculations

Dilution along the river stretch was accounted for by normalizing the cumulative concentrations of the pharmaceuticals to the cumulative concentrations of uranine at each site. To this end, the area under the breakthrough curve (BTC) of each compound was calculated using the trapezoidal rule. Based on the uranine BTC area a dilution factor for sites II-V was determined which was used to correct the areas of the pharmaceuticals' peaks. Finally, the BTC areas relative to site I was calculated for each pharmaceutical, which corresponds to the mass recovery relative to site I. Details of these calculations are available as Supporting Information (Chapter 6.1). Attenuation along the river stretch was assessed by testing a log-linear regression between pharmaceutical mass and travel time (i.e., a pseudo first-order kinetics) for significance. The effect of analytical uncertainties on mass recoveries was assessed by a modified Monte Carlo analysis combined with error propagation. Briefly, a randomly generated relative error was superimposed on each measured concentration. BTCs were then integrated as described above. 200 realizations were calculated. Finally, the uncertainty of the relative mass recovery was calculated by error propagation. A

description of this procedure is available as Supporting Information. The slug tests were evaluated for hydraulic conductivity with the method described in Freeze and Cherry (1979). Groundwater/surface water exchanges rates were calculated from the temperature profiles in the sediments as published previously (Schmidt et al. 2006).

2.4 RESULTS AND DISCUSSION

2.4.1 HYDRAULIC CONDITIONS

The BTCs of uranine and rhodamine WT were virtually identical (Figure 2-2). The mean travel time from the injection point to the last sampling site was 44 h, corresponding to a mean flow velocity of 10 cm s^{-1}. Maximum concentrations of both tracers decreased and the width of the BTCs increased with distance due to dispersion in the river channel. The areas under both dye tracer BTCs decreased with distance from the injection site (Table A- 4 as Supporting Information to chapter 2), indicating dilution of river water along the study reach. Current meter measurements confirmed an increase of discharge along the river stretch by about 250 L s^{-1}. This can be attributed to the confluence with 10 small creeks (see Figure 2-1; cumulative discharge: 50 L s^{-1}) and to the exfiltration of groundwater (approximately 200 L s^{-1}; see below).

Transient storage of water and solutes was limited as the BTCs of uranine and rhodamine WT were almost symmetric with only little tailing. The tailing was most probably caused by retention in dead water zones in the stream channel rather than by temporary storage in the hyporheic zone. The minor transient storage can be attributed to the fine sediment texture which limits the exchange of water and solutes between the channel and the river bed. This interpretation is supported by the results of the slug tests as the hydraulic conductivity of the sediment in 7 cm depth was low and typical for clayey and silty sediments (< 10^{-9} m s^{-1}). The exchange rates of groundwater and surface water derived from the temperature profiles (see Figure A- 3) were unexpectedly high for this type of sediments; the calculated fluxes range from 2 to 108 L m^{-2} d^{-1} (average flux: 30 L m^{-2} d^{-1}) and probably overestimate the real value. The flux was directed from the groundwater into the channel at all locations.

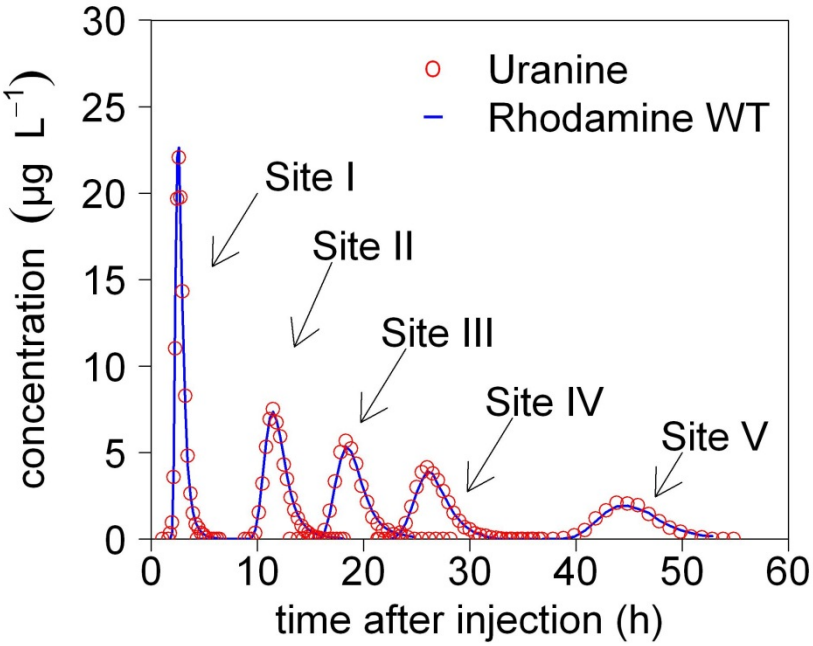

Figure 2-2: Breakthrough curves of uranine and rhodamine WT at the five sampling sites during the tracer test.

From the similar BTCs of both tracers, we conclude that neither photodegradation of uranine nor sorption of rhodamine WT was quantitatively relevant along the river stretch. This is an important prerequisite for the interpretation of our experiment since both tracers are not in all circumstances behaving in a conservative manner. Uranine has been reported to be susceptible to photolysis (Smart and Laidlaw 1977), while sorption is only of minor importance (Koeniger et al. 2010). In contrast, phototransformation is not relevant for rhodamine WT (Tai and Rathbun 1988) while sorption can be significant in porous media (Everts and Kanwar 1994) (see also Table A- 3). However, if sorption of rhodamine WT had occurred its BTC peak should have been delayed compared to uranine at the more downstream sites. Similarly, the uranine concentrations should have decreased substantially in relation to rhodamine WT along the studied river stretch if photolysis had been relevant.

2.4.2 PHARMACEUTICALS

Quality Assurance. The results of the analytical quality assurance indicate a robust and precise method. In the standard addition experiment, the deviation from the expected value was low for bezafibrate (< 10 %) and clofibric acid, ibuprofen, and naproxen (< 5 %; see Supporting Information to chapter 2, Table A- 5 and Figure A- 1). However, the nominal concentration (50 ng L^{-1}) was systematically overestimated for metoprolol (79 ± 6 ng L^{-1}) and diclofenac (59 ± 7 ng L^{-1}). The standard deviation of triplicate analyses was < 13 %.

The inter-day reproducibility of the reference sample was good with a standard deviation < 10% for all substances (Table A- 6). However, the concentration of the reference sample was underestimated by approx. 20 %. The reason for this underestimation is unclear since we did not observe a similar systematic deviation in the standard addition experiment. Nevertheless, such a

systematic absolute deviation would not impair the interpretation of our results since the data analysis is exclusively based on the relative comparison of the sampling sites.

Mass recoveries. The BTCs were well captured for all pharmaceuticals (Figure 2-3). The calculated relative mass recoveries of the reactive tracers are summarized in Table 2-1. The estimated uncertainty was ≤ 20 % for all pharmaceuticals. Only ibuprofen and clofibric acid were attenuated along the study reach: ibuprofen was not detectable any more at site V, while the relative mass recovery of clofibric acid decreased to 65 ± 6 %. For both compounds the slope of the regression lines (ln(mass) vs. time) was significantly < 0 (Figure 2-4a). The changes in the mass recoveries of the other pharmaceuticals were within the range of the methodical (Table A- 6). Due to unknown reasons, the mass recoveries at site III were generally underestimated. This is most pronounced for naproxen, but also applies to the other compounds. Mass recovery of diclofenac at site V is not reported since – due to the high LOQ – only three data points were available there. Due to a contaminated solvent, no data are available for metoprolol at site III.

Table 2-1: Mass recoveries (%) at sampling sites II-V relative to site I during the tracer test (mean ± uncertainty)

	t (h)	x (km)	Bezafibrate	Clofibric Acid	Diclofenac	Ibuprofen	Metoprolol	Naproxen
Site II	8.9	3.8	106 ± 9	102 ± 9	102 ± 18	52 ± 5	96 ± 20	102 ± 12
Site III	15.9	7.1	81 ± 7	84 ± 8	97 ± 19	26 ± 3	n/a	76 ± 9
Site IV	23.9	9.9	95 ± 8	82 ± 8	111 ± 19	21 ± 2	87 ± 18	112 ± 14
Site V	41.4	14.7	85 ± 7	65 ± 6	§	n.d.	95 ± 19	100 ± 12

t: travel time of BTC peak after passage of site I; x: distance to site I; n.d.: not determined; n/a: not available due to analytical difficulties; §: not reported since only 3 valid measurements were available

Bezafibrate, diclofenac, metoprolol, and naproxen. These pharmaceuticals were not attenuated along the studied reach. Based on the compounds which are susceptible to photolysis – diclofenac and naproxen (Packer et al. 2003) – we can conclude that direct photolysis was not a relevant attenuation process for all pharmaceuticals (see Table A- 3). The absence of photolysis can be attributed to the high turbidity of the river water (see also Figure A- 5 for an UV/VIS absorption spectrum), to the limited exposure of the stream channel to sunlight due to morphology and vegetation, and to the cloudy and rainy weather on days 2 and 3 of the experiment.

As bezafibrate, diclofenac, and naproxen were not retarded compared to the dye tracers (Figure 2-3 and Table 2-1), their transport was not affected by sorption. A visual data analysis shows that the breakthrough of metoprolol at sites IV and V might have occurred somewhat later than that of the other compounds (Figure 2-4b), but this was not significant for a number of statistical calculations. The study reach was not long enough to provide solid evidence for the retardation of metoprolol by sorption. As higher sorption coefficients have been reported for metoprolol than for bezafibrate, diclofenac, and naproxen (see Table A- 3), we expected *a priori* to observe

its substantial retardation along the study reach. However, sorption will only be of quantitative relevance at a relatively high solids-to-water ratio as, for example, in sewage sludge or in porous media. This condition was not met at Säva Brook with a low concentration of suspended particles in the river water and a small magnitude of hyporheic exchange.

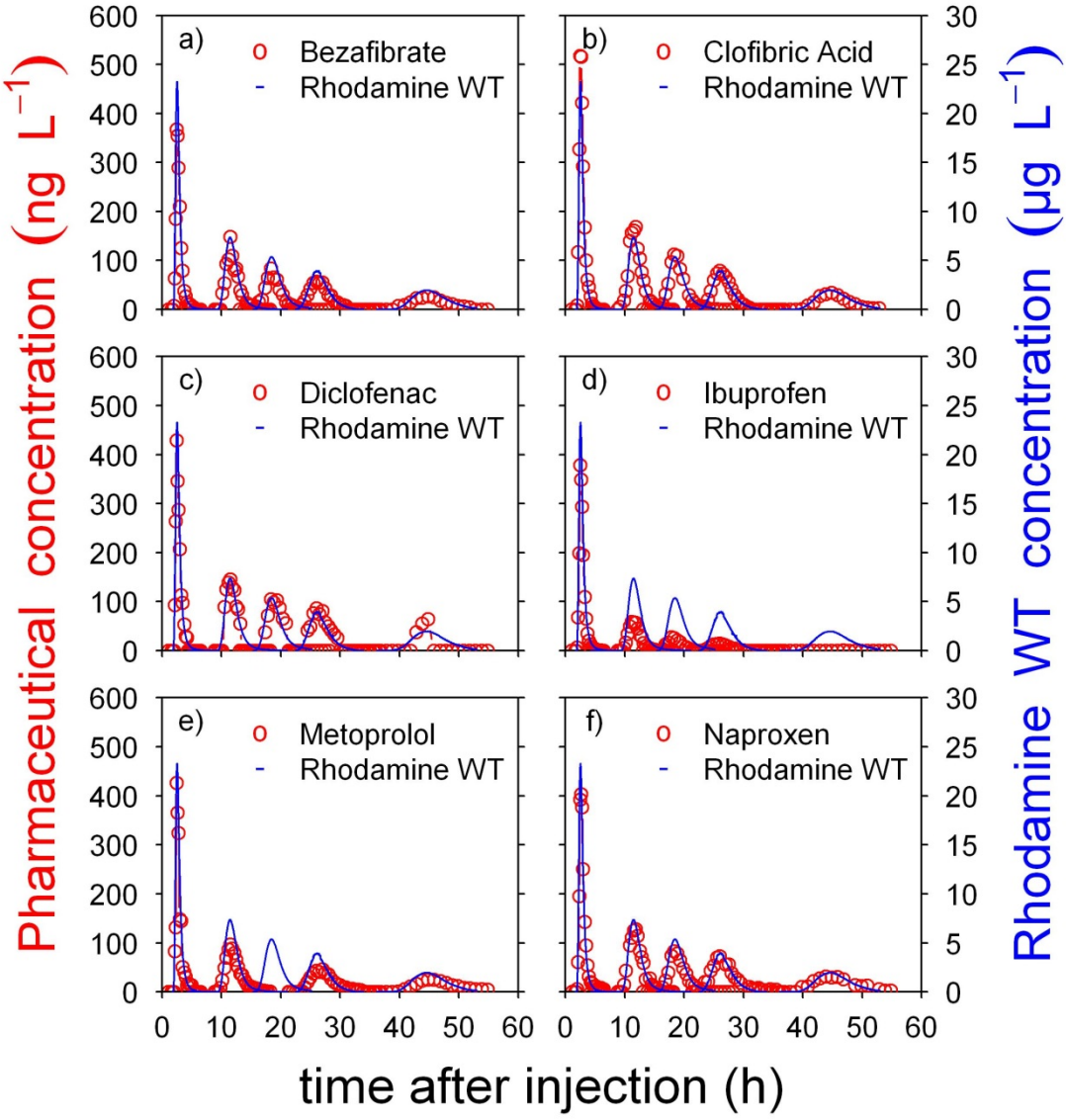

Figure 2-3: Breakthrough curves of pharmaceuticals in comparison to rhodamine WT at the five sampling sites. Left y-axis: concentration of pharmaceutical; right y-axis: concentration of rhodamine WT. Naproxen concentrations have to be multiplied by 2. Metoprolol at site III cannot be reported because of analytical problems.

Clofibric acid and ibuprofen. The recovered mass of ibuprofen and clofibric acid decreased along the river stretch ($p < 0.05$). The attenuation of ibuprofen was rapid and it was not detectable at site V (Figure 2-3d and Table 2-1). Assuming pseudo-first order kinetics, the elimination of ibuprofen proceeded at a half-life time (t_H) of 10 ± 1.3 h (Figure 2-4a) which corresponds to a half-life distance of 4.3 ± 0.4 km. Rapid elimination of ibuprofen in streams was previously reported. Lin et al. (2006) calculated an even shorter half-life time of 2.7 h during river transport, while others reported half-life times of some days (Fono et al. 2006, Kunkel and Radke 2008). For clofibric

acid, we estimated a half-life time of 2.5 ± 0.5 d (Figure 2-4a), equivalent to a half-life distance of 22.3 ± 4.5 km. In contrast to ibuprofen, the attenuation of clofibric acid was unexpected since it was shown to be persistent in the aquatic environment on time scales much longer than in our experiment (Kunkel and Radke 2008, Löffler et al. 2005). For both compounds, sorption can be ruled out as attenuation mechanism since they were not retarded compared to uranine and rhodamine WT and most studies reported only minor sorption of these two compounds (Table A- 3). Moreover, we can rule out direct photolysis as ibuprofen and clofibric acid are not susceptible to this process. Indirect photolysis or enhanced photolysis in the presence of DOM was reported for ibuprofen (t_H: 23 d by Peuravuori and Pihlaja (2009)) and clofibric acid (t_H: 69 d by Radke et al. (2010)). However, based on these studies it is likely that indirect photolysis was of minor importance in our study. Even though we do not have quantitative data on all chemical species involved in such photolytical processes, the rates reported in literature are far too low to explain the elimination of ibuprofen and clofibric acid on the time scale of our experiment. Biotransformation in the flowing water is also unlikely since previous studies reported no or only very slow biodegradation of pharmaceuticals in the water phase on the time scale of our experiment (Buser et al. 1999, Kunkel and Radke 2008, Yamamoto et al. 2009). Therefore, we hypothesize that biofilms growing on submerged vegetation and in the thin, loosely packed uppermost sediment layer were responsible for the attenuation of both compounds. A substantial degradation of ibuprofen in biofilms growing on submerged plants has already been observed by Reinhold et al. (2010) in laboratory experiments. However, they also reported clofibric acid to be persistent. Such a contrasting finding may be explained by differences in the composition and metabolic activity of the biofilms between their laboratory incubation and our field site. To test our hypothesis and to determine the contribution of biofilms on submerged macrophytes to the attenuation of both pharmaceuticals, experiments under the specific conditions of Säva Brook would be necessary.

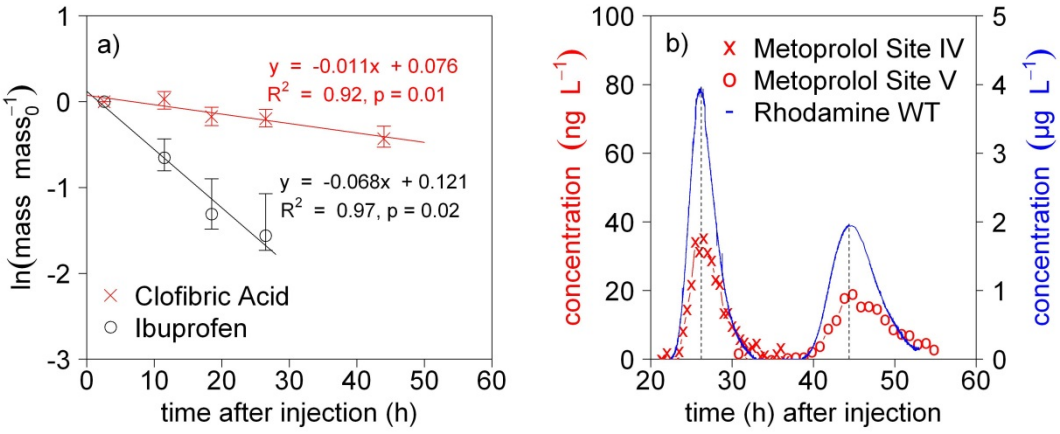

Figure 2-4: a) Pseudo first-order kinetic plots for clofibric acid and ibuprofen relative to site I during the tracer test; error bars indicate the uncertainty determined with the Monte Carlo analyses; b) Metoprolol BTCs at sites IV and V in comparison to rhodamine WT. The dotted vertical lines indicate the maximum of the rhodamine WT BTCs.

So far, the findings on the attenuation of pharmaceuticals in rivers are inconclusive. This is illustrated by the comparison of our results with the few similar studies currently available:

- In a river (discharge 3.3 $m^3 s^{-1}$) located 20 km east of Säva Brook, 75 % of the metoprolol load was attenuated in summer within a distance of only 1320 m (Daneshvar et al. 2010). No information about sediment characteristics and the morphology of the river is available, but given the proximity to our site and the relatively uniform landscape of this area, we assume that river morphology and sediments are similar to Säva Brook (no attenuation of metoprolol).

- Clofibric acid, bezafibrate, and diclofenac were persistent while the load of naproxen decreased along a 13.6 km long stretch of a German river (discharge 2.2 $m^3 s^{-1}$) at a half-life time of 3.6 ± 2.1 d (Radke et al. 2010) (Säva Brook: no attenuation of bezafibrate and diclofenac, but of clofibric acid). Sediments of this river are heterogeneous: most parts are sandy, but there are also reinforced and rocky stretches as well as stretches with clayey sediments and very low flow velocity.

- Along a 300 km stretch of a large river (discharge 21-23 $m^3 s^{-1}$) in Texas, ibuprofen, naproxen, and metoprolol were attenuated at similar half-life times between 4.2 and 5.3 d (Fono et al. 2006) (Säva Brook: ibuprofen half-life time 10 ± 1.3 h, no attenuation of naproxen and metoprolol). A variety of different sediment types and flow characteristics is likely to occur along the studied reach.

- In a Californian stream (discharge 1.4 $m^3 s^{-1}$), ibuprofen and naproxen were eliminated at half-life times < 3 h (Lin et al. 2006) (Säva Brook: no attenuation of naproxen); sediments are coarse gravel, and from the morphology a high exchange of river water with the sediments can be assumed.

While the difference between the Californian stream and Säva Brook can be explained by higher hyporheic exchange (faster elimination of ibuprofen) and higher UV intensity (attenuation of naproxen), our current knowledge does not allow a consistent mechanistic interpretation of all findings. The rivers analyzed in the studies cited above represent a wide variety of environmental characteristics, for example with respect to sunlight exposure, input of nutrients and DOC, pH, and river hydraulics (e.g., with respect to hyporheic exchange and discharge dynamics). Moreover, the microbial communities in some of the rivers might have been adapted to a continuous input of pharmaceuticals, while the microorganisms at Säva Brook were not. This might affect the environmental fate of compounds which are transformed by a specific bacterial strain and not by more general, e.g., co-metabolic processes. Overall, we are currently lacking a holistic mechanistic understanding which allows predicting micropollutant attenuation based on all such site-specific parameters and processes.

At Säva Brook in-stream biotransformation was much more significant than biotransformation and retardation (sorption) in the hyporheic zone. Therefore, we have to reject our initial hypothesis – the hyporheic zone being the major contributor to micropollutant attenuation – for this specific stream. This is due to the consolidated clayey silt sediments underlying the stream channel

and the comparatively low flow velocity which both limit hyporheic exchange. Instead, we attribute the attenuation of ibuprofen and clofibric acid to biofilms attached to these submerged macrophytes and at the water/sediment interface. Future studies should aim at determining the quantitative importance of such in-stream biofilms.

Our study highlights the potential of reactive tracer tests to evaluate the fate of emerging micropollutants in rivers and streams. By comparing compounds with different reactivity and physicochemical properties, we were able to elucidate the importance of sorption, photolysis, and biotransformation for the fate of a set of pharmaceuticals in the studied stream. However, even with well-defined stream hydraulics, a defined input of pharmaceuticals, and state-of-the art analytical methods, uncertainties in the calculated mass recoveries up to 20 % were inevitable. Therefore, the exploratory power of such tests is limited to compounds with a sufficiently high attenuation rate, or to reaches with longer residence times. The application of complementary methods, such as the determination of characteristic transformation products (Quintana et al. 2005), of changes in enantiomer ratios of chiral compounds (Buser et al. 1999), or of compound-specific stable isotope ratios (Penning et al. 2010), could help reducing such uncertainties and provide further insight into the mechanisms of micropollutant attenuation and a means of further differentiating between individual processes. Finally, to complement our study we suggest carrying out similar tracer tests at streams with more intense hyporheic exchange. This would allow us to better understand the mechanisms controlling micropollutant fate in rivers and streams.

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2.6 MODELING OF THE TRACER TEST

In a follow-up study, the data of the tracer test were evaluated from a modeling point of view (Riml, J., et al. **(2013).** *Evaluating the fate of six common pharmaceuticals using a reactive transport model: Insights from a stream tracer test.* Science of the Total Environment 458–460: 344-354). The data was used as input for a newly developed coupled physical–biogeochemical model framework that implemented surface water transport as well as transient storage in slow/immobile zones of the streams and the river sediments. The model enabled to determine river stretch-specific transformation rates and sorption coefficients in both the main channel and the storage zones for each substance. Only a moderate hydraulically based retention of compounds in the hyporheic zone as well as in densely vegetated areas of the river was calculated. All substances except for diclofenac were apparently affected by sorption processes during stream transport and sorption was more pronounced in the storage zones. Consistently to literature data, highest sorption coefficients were determined for the beta-blocker metoprolol. Elimination of ibuprofen and clofibric acid was attributed to (bio-)transformation processes in both main channel and storage zones. The half-life times of were much shorter in the storage zones (1.6 hours for ibuprofen and 22.1 hours for clofibric acid, respectively) than in the main channel (22.7 hours and 113.2, respectively). However, since residence times of pharmaceuticals were about ten times longer in the main channel than in the storage zones, elimination processes of ibuprofen and clofibric acid in both compartments were of equivalent importance for the total elimination during river transport.

The modeling techniques developed for the evaluation of the tracer test enabled us to distinguish between individual elimination processes that govern the fate of pharmaceuticals during stream transport. For the first time, quantitative data for sorption and transformation of pharmaceuticals in both main channel and storages zones could be derived by inverse simulation techniques. By coupling these rates with the mean residence times of substances in the two river compartments during the tracer experiment, we were able to quantify the contribution of in-stream processes as well as reactions in transient storages zones for the overall elimination. Overall, the combined results of the both published manuscripts on the tracer test provide valuable information for evaluating the fate and persistence of organic micropollutants such as pharmaceuticals in rivers.

3 STUDY II: ATTENUATION OF PHARMACEUTICALS IN RIVERS AT FAVORABLE CONDITIONS

Fate of Pharmaceuticals in Rivers: Deriving a Benchmark Dataset at Favorable Attenuation Conditions

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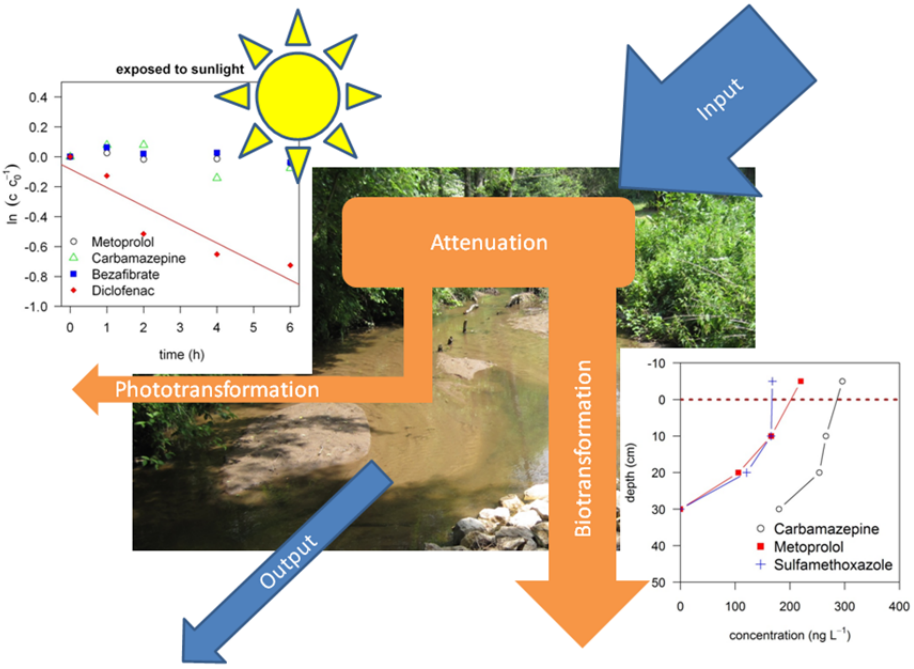
Water Research 46(17): 5551-5565.



3.1 ABSTRACT

Pharmaceutical residues are commonly detected organic micropollutants in the aquatic environment. Their actual fate in rivers is still incompletely understood as their elimination is highly substance specific and studies often report contradictory results. To elucidate the ceiling of attenuation rates of pharmaceuticals in rivers we carried out a study at a river with favorable conditions for the elimination of organic micropollutants.

Experiments were carried out at a small stream in Germany. Composite samples were taken at both ends of a 12.5 km long river stretch located downstream of a wastewater treatment plant (WWTP) and analyzed for ten pharmaceuticals. Moreover, pore water samples were taken and *in situ* photolysis experiments at several sites within the river stretch were performed to assess the importance of these individual elimination mechanisms.

Pharmaceutical concentration in the surface water at the first sampling site ranged from 3.5 ng L^{-1} for propranolol to 1400 ng L^{-1} for diclofenac. In comparison to carbamazepine which was used as persistent tracer, all other pharmaceuticals were attenuated along the river stretch. Their elimination was higher in a sunny, dry weather period (period I) compared to a period with elevated discharge after a heavy rainfall (period II). Overall, the measured elimination rates ranged from 25 % for sulfamethoxazole (period II) to 70 % for propranolol (period I). Photolysis was only a relevant elimination process for diclofenac and potentially also for sotalol; for these compounds phototransformation half-life times of some hours were determined in the unshaded parts of the river. Biotransformation in the sediments was also an important attenuation process since the concentrations of the other pharmaceuticals in the sediments decreased relative to carbamazepine with depth. For the chiral beta-blocker metoprolol this biotransformation was also confirmed by a decrease in the enantiomer fractionation from 0.49 at site A to 0.43 at site B and to < 0.40 in the deeper sediments.

3.2 INTRODUCTION

Human health is one of the important concerns in modern life, and it has been improved substantially during the past decades. This can be attributed to, among other factors, the usage of large amounts of pharmaceutically active substances. Due to incomplete metabolism and subsequent excretion, these substances enter the aquatic environment via wastewater. During wastewater treatment, pharmaceuticals are often only incompletely eliminated and thus are emitted into receiving surface waters. Depending on consumption and metabolism of a substance, season, structure of the wastewater treatment plant (WWTP), and the proportion of WWTP effluent in the river, concentrations in rivers range from a few ng L^{-1} up to tens of µg L^{-1} (Daneshvar et al. 2010, Meyer et al. 2011, Sacher et al. 2008). Today conclusive information on the environmental fate of pharmaceuticals is only available for a small number of compounds. For example, most studies report that the widely used analgesic ibuprofen is readily biodegradable during activated sludge treatment, and its rapid elimination in rivers has also been shown (Daneshvar et al. 2010, Fono et al. 2006, Kunkel and Radke 2011). For other substances, the pic-

ture is less straightforward. Most laboratory studies reported a high to moderate biodegradability of diclofenac (Al-Rajab et al. 2010, Kunkel and Radke 2008) while others found a recalcitrant behavior (Lahti and Oikari 2011); it is generally considered as highly susceptible to photolysis (Buser et al. 1998, Packer et al. 2003). Similarly, findings from field studies range from no or only minor elimination of diclofenac in rivers (Kunkel and Radke 2011, Radke et al. 2010) to moderate attenuation in a lake system on a longer time scale (Tixier et al. 2003). Overall, with our current knowledge and the limited transferability of specific laboratory findings to the environmental scale, it is difficult to predict the behavior of diclofenac in a specific aquatic system based on the physical and/or biogeochemical boundary conditions.

Moreover, it is important to assess the contribution of individual attenuation processes. Factors like turbidity of the water, the hydraulic regime and hyporheic exchange, or the structure of microbial communities in the river strongly influence the importance of potential elimination mechanisms. For example, in deeper rivers only a minor fraction of surface water is exchanged with the hyporheic zone and consequently biotransformation in the sediments will quantitatively be less important for the attenuation of an organic micropollutant from such a river compared to a shallow river. The importance of phototransformation is also depending on the characteristics of the specific system: it is restricted to the uppermost parts of surface waters (Bartels and von Tümpling 2007) and of less importance in turbid waters (Robinson et al. 2007).

In contrast to previous work by Radke et al. (2010) andKunkel and Radke (2011) where the total elimination of pharmaceuticals along river stretches was determined, the aim of the present study was to derive a set of benchmark data for the elimination of pharmaceuticals in a river that has more favorable boundary conditions for the attenuation of organic micropollutants, and to simultaneously study individual attenuation mechanisms *in situ*. Shallower stream depth, lower turbidity, and a higher exposure to sunlight were selected as conditions favoring photolysis; for biotransformation, also a shallower water, aerobic and sandy sediments, and substantial exchange between surface and pore water were selected as criteria. Moreover, a continuous exposure to WWTP effluents was desired. Along a 12.5 km long reach of such a river we monitored the concentration of ten pharmaceutical residues belonging to various therapeutic classes (e.g., analgesics, antibiotics, beta-blockers, lipid-lowering agents). By choosing pharmaceuticals with different environmental fate properties (sorption, biotransformation, phototransformation), we aimed at identifying the relevance of the individual mechanisms for the overall elimination rate of a compound. We combined a mass-balance approach with the analysis of enantiomeric ratios (for metoprolol), *in situ* phototransformation experiments, and analyses of pore water profiles to differentiate between individual processes and to estimate their relative importance.

3.3 Material and Methods

3.3.1 Chemicals

All pharmaceuticals, phenylglyoxylic acid (all purity > 97 %), and acetic acid (HAc, LC-MS grade) were purchased from Sigma-Aldrich (Seelze, Germany). The surrogate standards bezafibrate-D_4,

clofibric acid-D_4, ibuprofen-D_3, diclofenac-D_4, naproxen-D_3, metoprolol-D_7, propranolol-D_7, sulfamethoxazole-D_3, and sotalol-D_6 were purchased from Toronto Research Chemicals (North York, ON, Canada), carbamazepine-$^{13}C^{15}N$ was generously provided by the German Federal Institute of Hydrology (BfG, Koblenz, Germany). LC-MS-grade water and acetonitrile (ACN) were purchased from Th. Geyer (Renningen, Germany), formic acid (FA, analytical grade), methanol (MeOH, LC-MS-grade), and ammonium hydroxide (NH_4OH, 25 % in water) were obtained from Merck (Darmstadt, Germany). For the chiral analysis of metoprolol, ACN (hyper-grade for LC/MS) and H2O (LC-MS grade) were purchased from VWR (Darmstadt, Germany), NH_4-Acetate (purity > 98 %) from Sigma-Aldrich.

3.3.2 Study Site

Experiments were carried out along a 12.5 km long stretch of river Gründlach, a small stream in Northern Bavaria near the city of Nuremberg, Germany. River Gründlach has a total length of about 20 km and discharges into river Rednitz near Erlangen. The main source of organic micropollutants in river Gründlach is the WWTP Heroldsberg which has a capacity of 12,000 population equivalents. Water was concurrently sampled at two sites along the river Gründlach. Site A was located approx. 600 m downstream of the outlet of the WWTP Heroldsberg (49° 31’ 28’’ N, 11° 08’ 12’’ E); site B was located 12.5 km further downstream, close to the town of Boxdorf (49° 31’ 21’’ N, 11° 01’ 26’’ E). Supplementary, we chose three characteristic sites along the river stretch where *in situ* phototransformation experiments were conducted and pore water samples were taken (Exp A, Exp B, Exp C). An overview over the study area is given in Figure 3-1, the coordinates of sampling and experimental sites are given in Table B- 1 (Supplemental Information (SI) to chapter 3). Complete mixing of the WWTP effluent and the river water upstream of site A was verified using an established USGS guideline (Kilpatrick and Wilson 1989). The calculated mixing distance of 182 m was well below the distance of about 600 m between the WWTP outlet and site A. This estimation was additionally verified by measuring the electrical conductivity (EC) at site A. EC was constant along the cross-section, and thus we conclude that the WWTP outflow (high EC) was completely mixed with river water (lower EC).

No additional sources of pharmaceuticals have to be considered between sites A and B as only minor creeks and ditches confluence with river Gründlach in the investigated river stretch. Consequently, discharge increases only marginally between the two sampling sites, as was confirmed by current meter measurements. The first seven kilometers of the investigated river stretch are passing through densely forested areas, in the last 5.5 km river Gründlach is flowing through agriculturally used areas where the river banks are loosely covered by shrubs and low trees (see Figure 3-1). The average inclination between the sampling sites is about 0.4 %, and stream morphology is highly uniform. The river bed is incised into the landscape by about 1 m; during baseflow conditions, average stream width is about 3 m, and average water depth is about 15 cm. The sediments are sandy down to depths of > 30 cm (coarse and medium sand), and mineralic (C < 0.5 %, N < 0.05 %). Water and sediment characteristics during the sampling campaign are listed in Table B- 2.

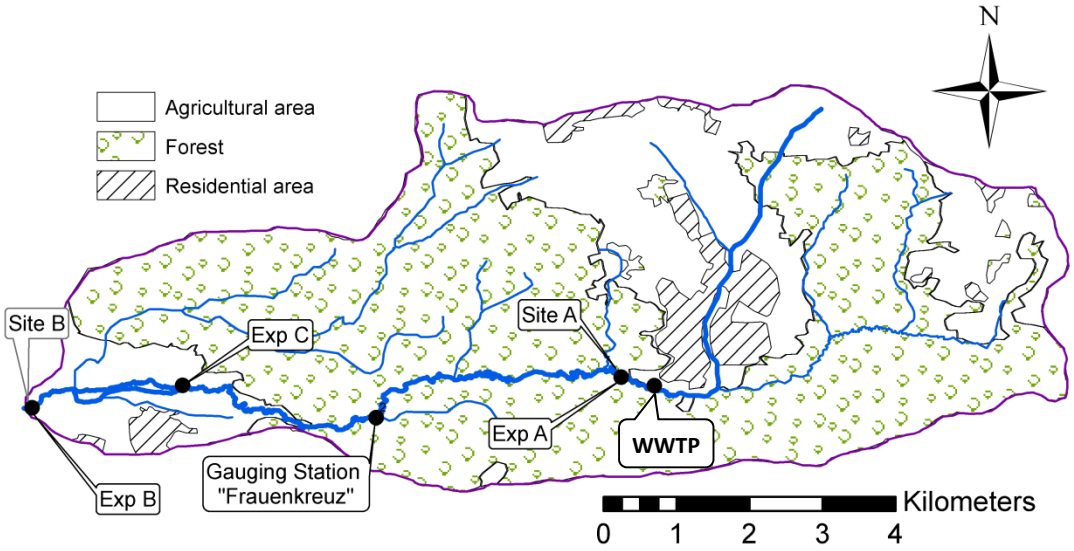


Figure 3-1: Map of the catchment of river Gründlach showing the location of sampling and experimental sites, the wastewater treatment plant (WWTP), and the gauging station “Frauenkreuz”.

3.3.3 Sampling and In situ Measurements

River water was collected at the two sampling sites with automatic water samplers (3700 compact equipped with Teflon suction line and stainless steel inlet filter; sample compartment not exposed to light; Teledyne ISCO, Lincoln, NE) from 2010/07/05 to 2010/07/20. Every hour, 100 mL of water were sampled into a glass bottle that contained NaN_3 (final concentration in sample: 0.1 %) for sample stabilization. The addition of NaN_3 to stabilize surface water samples has previously been reported to be valid for a large number of pharmaceuticals (Vanderford et al. 2011). Samples were then taken to the laboratory after a maximum of 2 days, and 6 consecutive samples were combined to respective 6 h composite samples. Subsamples of 20 mL for the determination of boron (B), potassium (K), and total organic carbon (TOC) were separated, and all samples were stored frozen until further processing. Sampling failed from July, 5th to July, 7th at site A due to a dislocated intake hose of the sampler, and from July, 16th to July, 18th at site B due to power failure. The concentration of dissolved oxygen (DO), EC, and pH were measured online at sampling site A (all instruments from WTW, Weilheim, Germany). Water temperature was logged continuously at both sampling sites with temperature loggers (HOBO UA-001-64, Onset, Bourne, MA).

Discharge at sampling site A was measured with a continuous wave Doppler instrument (model 2150, Teledyne ISCO). Additionally, discharge records were available from a gauging station operated by the local water board (Wasserwirtschaftsamt Nuremberg) 6 kilometers downstream of sampling site A (http://www.hnd.bayern.de, station “Pegel Frauenkreuz”; see Figure 3-1). The daily discharge of the WWTP Heroldsberg was provided by the operator. Hourly data for sunshine minutes, air temperature and precipitation were obtained from the DWD weather sta-

tion Nuremberg (http://werdis.dwd.de, station “Nürnberg”) which is located approximately 2 km south of experimental site Exp C.

Pore water was sampled with self-constructed mini-piezometers made of stainless steel (length: 1 m, outer diameter: 1 cm, inner diameter: 0.2 cm). Near the lower end, the piezometers were slotted over a length of 10 cm (42 slots, size: 0.5 mm). The mini-piezometers were pushed into the river bed to the desired depth, and pore water was sampled into a glass bottle by applying a gentle vacuum with a hand pump. Approx. 300 mL of pore water were sampled at each depth, immediately taken to the laboratory and stored frozen. Po7re water was sampled on 2010/07/20 at the experimental sites (Exp A to C) in depths of 5-15 cm, 15-25 cm and 25-35 cm (only Exp A and Exp C, see Figure 1). Nitrate, sulfate, ferrous iron (using photometric methods) and methane (CH_4) as well as carbon dioxide (CO_2) (using GC with FID and TCD detection) were measured in the pore water samples to characterize the redox conditions in the sediments.

3.3.4 In situ Phototransformation Experiments

Phototransformation experiments were carried out at three locations with different shading intensity (see Figure 3-1): in the agricultural area without any shading (Exp B, near site B), in the agricultural area with partial shading (Exp C), and in the forested area with complete shading (Exp A, near site A). Although experiments were carried out on different days, sunshine duration and radiation intensities were similar for all experiments. A table with the meteorological data during the photolysis experiments recorded by the DWD weather station is given in the SI (Table B- 3). For the experiments, approximately 22.5 L of river water were transferred to a glass container (length: 50 cm, width: 30 cm, height: 30 cm). For Exp A and B, river water was taken directly from corresponding sites A and B, for Exp C river water was collected at site A to obtain higher initial concentrations for the experiments. The water level in the container was equal to the water depth at the experimental site (~ 15 cm at all sites). Additionally, control experiments under dark and sterile conditions were conducted. For the dark control, we filled a second glass container and wrapped it with aluminium foil to shield the water from radiation. For the sterile control experiment, we added NaN_3 (final concentration 0.1 %) as microbial poison to the river water to exclude biological transformation; this third container was also exposed to sunlight. All containers were installed in the river bed (Figure B- 3). Subsamples (approx. 500 mL) from each container were taken immediately after installation (t = 0 h) and after 1, 2, 4, and 6 hours. The samples were stored dark and cool until the experiment was terminated and upon arrival in the laboratory they were frozen until further analysis. Temperature and pH were measured at the time of sampling in each container.

Radiation intensity during the phototransformation experiments was estimated by using phenylglyoxylic acid as chemical actinometer (Defoin et al. 1986). A solution of phenylglyoxylic acid was filled into quartz glass tubes (length: 36 cm; diameter: 2 cm; c = 50 mmol L^{-1} in ACN/H_2O 3:1 v:v) which were immersed directly below the water surface. Samples were taken in short time intervals and measured on site at a wavelength of 380 nm with a portable spectrophotometer (DR 3800, Hach Lange, Düsseldorf, Germany.

3.3.5 Analytical Methods

Samples were filtered (GF6, Whatman, Dassel, Germany) and 250 mL of the filtrate were used for solid phase extraction as previously described (Kunkel and Radke 2011, Lavén et al. 2009). Extractions had to be repeated for carbamazepine and sotalol due to originally inaccurate internal standards; then the residual sample volume was completely extracted (60 – 260 mL). Briefly, 25 ng of each internal standard were added to the sample and pH was adjusted to pH 2 by H_2SO_4. Then, the samples were extracted using Oasis MCX cartridges (60 mg/3 mL; Waters, Milford, MA, USA) which were pre-conditioned with 2 mL of MeOH and 2 mL of 2 % FA (aq.). Then samples were extracted with a flow rate of about 5 mL min^{-1}, dried for 1 h under vacuum, and finally stored frozen until elution. Cartridges were eluted consecutively with 2 x 1 mL of MeOH (eluate I) and 3 x 1 mL of 2 % NH_4OH in MeOH (eluate II). Eluates were dried under a nitrogen stream and redissolved in 500 µL of H_2O:ACN (70:30, v:v, 2.5mM HAc). Eluate I of the phototransformation samples was subjected to an additional clean-up step: the eluate was evaporated, redissolved in 40 mL of ultrapure water and extracted via Oasis MAX (60 mg/3 mL; Waters). MAX cartridges were preconditioned with 2 mL of MeOH and 2 mL of H_2O. After the extraction, cartridges were washed with 2 mL of 0.5 % NH_4OH (aq.), dried under vacuum and stored frozen until elution. Cartridges were eluted separately with 2 x 1 mL of MeOH (eluate III) and 2 x 1 mL of 2 % FA in MeOH (eluate IV), eluates were dried under nitrogen and redissolved in 300 µL of H_2O:ACN (70:30, v:v, 2.5 mM HAc). Metoprolol, propranolol, sotalol, and sulfamethoxazole were collected in eluate II, the other pharmaceuticals in eluate I. If the samples were additionally extracted by the MAX step, carbamazepine was present in eluate III and all other compounds in eluate IV (details see Lavén et al. (2009)). Pharmaceutical concentrations were determined using a HPLC/MS/MS instrument consisting of two HPLC pumps (ProStar 210), an autosampler (ProStar 410) and a triple quadrupole mass spectrometer (1200L, all by Varian Inc., Palo Alto, CA). Separation was achieved by using a binary gradient of H_2O and ACN (both 2.5 mM HAc) with several HPLC columns (150 x 2 mm). The following stationary phases were used (all by Phenomenex Aschaffenburg, Germany): Synergi Fusion-RP 80A (eluates I, III and IV), Luna C18(2)-100A (eluate II, beta-blockers), and Synergi Polar-RP 80A (eluate II, sulfamethoxazole). Calibration by applying isotope-labelled internal standards (c = 50 ng mL^{-1}, isotope dilution) was linear for all substances in the range of 1 – 1000 ng mL^{-1}; the limits of quantification of the overall method (LOQ) ranged from 0.5 ng L^{-1} for propranolol to 45 ng L^{-1} for diclofenac (details are given in Table B- 4) in the water samples. The two metoprolol enantiomers (only a subset of the samples was analyzed) were determined in the SPE extracts (eluate II) by UPLC/MS/MS (Acquity UPLC system; Xevo TQ-S mass spectrometer; all by Waters). Enantiomers were separated on a Reprosil AGP column (5 µm, 100 mm x 2 mm, Dr. Maisch, Ammerbuch, Germany) by an isocratic flow (0.22 mL min^{-1}) of H_2O:ACN (98:2, v:v) containing 10 mM NH_4-Acetate.

Concentrations of boron (LOQ: 0.025 mg L^{-1}) and potassium (LOQ: 0.1 mg L^{-1}) were measured with ICP-OES (Vista-Pro radial, Varian Inc.), TOC was determined with a total organic carbon analyzer (TOC-V CPN, Shimadzu, Duisburg, Germany).

3.3.6 CALCULATIONS

The load of each pharmaceutical at sampling site A was calculated by multiplying the concentration in each sample by the average discharge during the respective sampling time span. Loads are given in mg $6h^{-1}$ to retain the reference to the sampling interval. As no continuous discharge measurements were available for site B, no loads can be calculated for this site. Therefore, we determined the mass balance and attenuation rate of pharmaceuticals along the river stretch by calculating elimination rates relative to a persistent compound. Carbamazepine is commonly used as wastewater marker and considered as persistent in the aquatic environment (Clara et al. 2004, Löffler et al. 2005). Calisto et al. (2011) determined photolysis half-life times for carbamazepine between 5 and 25 days (summer), and Tiehm et al. (2011) reported only little biodegradation in soils. Hence, given the short travel time (< 1 d) of river water between our two sampling sites, the assumption that carbamazepine is persistent is justified. In a previous study (Radke et al. 2010), we used clofibric acid as conservative tracer. Since the concentrations of clofibric acid in river Gründlach were always < LOQ in the current study this compound could not be used as another persistent reference substance.

Elimination of pharmaceutical residues was calculated for two periods with different meteorological and hydraulic conditions. The first period from 2010/07/07 to 2010/07/12 was characterized by low discharge (period I). The second period from 2010/07/12 to 2010/07/14 spans two days of elevated discharge after a heavy rainfall (period II, also see Figure 3-2). These two periods were chosen to determine the elimination rates of pharmaceuticals under best and worst case conditions in summer. In detail, the elimination of a substance within the river stretch was computed by dividing the sum of its concentrations at site B by the sum of its concentrations at site A. This ratio was then normalized by the ratio of the sum concentrations of carbamazepine at the both sites and the percentaged difference from 100 % was taken as elimination of a substance. For calculation purposes, samples with concentration below LOQ at site B were set to the average of the three lowest determined concentrations. This method was chosen as it provides a conservative estimate of the elimination rate (i.e., likely underestimates the elimination rate).

Data from the phototransformation experiments were normalized to the measured initial concentration (t = 0 h) in the respective container. The photolysis rates (k_p) and half-life times (t_H) were then calculated by applying a linear regression of the logarithmic normalized concentrations versus time, i.e., a pseudo first-order kinetics. Photon fluxes were calculated according to a published procedure, assuming a quantum yield for phenylglyoxylic acid of 0.7 (Defoin et al. 1986, Neamţu and Frimmel 2006).

Chiral analyses of metoprolol were evaluated using the enantiomer fraction (EF) after normalizing the peak area (A) of the enantiomer by the peak area of the respective enantiomer of the internal standard metoprolol-D_7 (IS). Enantiomers 1 and 2 were labelled according to the order of elution from the HPLC column (Figure B- 1).

$$EF = \frac{\frac{A_{enantiomer1}}{A_{enantiomer1,IS}}}{\frac{A_{enantiomer1}}{A_{enantiomer1,IS}} + \frac{A_{enantiomer2}}{A_{enantiomer2,IS}}}$$

All statistical analyses and graphical visualizations were done using the open source software R (R Development Core Team 2011). The spearman rank correlation coefficient was used to test correlations between substances. An unpaired, one-sided Wilcoxon rank-sum test was used to test if concentrations of pharmaceuticals and EFs of metoprolol were significantly different at both sampling sites. For all analyses a minimum significance on the 95 % confidence interval was required.

3.4 Results and Discussion

3.4.1 Meteorological and Hydrological Situation

During the sampling campaign, the average air temperature was 22.5 °C, and average daily air temperatures ranged from 17.4 °C to 27.8 °C. Average daily sunshine duration was 11.5 hours. There were one major (July, 12th, 39.4 mm in total, 33 mm within on hour) and two minor rain events on July, 14th/15th and July, 17th (both less than 5 mm). More detailed weather data is shown in Figure 3-2 and Table B- 3. The average water temperature was 19.8 °C with diel fluctuations of about 3 °C; the fluctuations were slightly more pronounced at sampling site B (Table B- 3). Discharge at sampling site A was about 30 – 40 L s^{-1} under dry conditions while the average flow velocity along the river stretch was about 20 cm s^{-1}; at this flow velocity, the corresponding travel time between the two sites was approximately 18 hours. The proportion of wastewater under dry conditions was up to 60% of the total river water. After the rain events, the discharge quickly increased to 500 L s^{-1} on July, 12th and 200 L s^{-1} on July, 17th/18th. For these high discharge conditions, a travel time of approx. 12 hours was derived from the time shift of the occurrence of ibuprofen at the two sampling sites (Figure 3-3, see below). The rainfall on July, 15th and in the morning of July, 17th only resulted in a slight increase of discharge at sampling site A. Discharge at the gauging station “Frauenkreuz” in the middle of the studied river reach was always a little higher and peaks were delayed compared to site A.

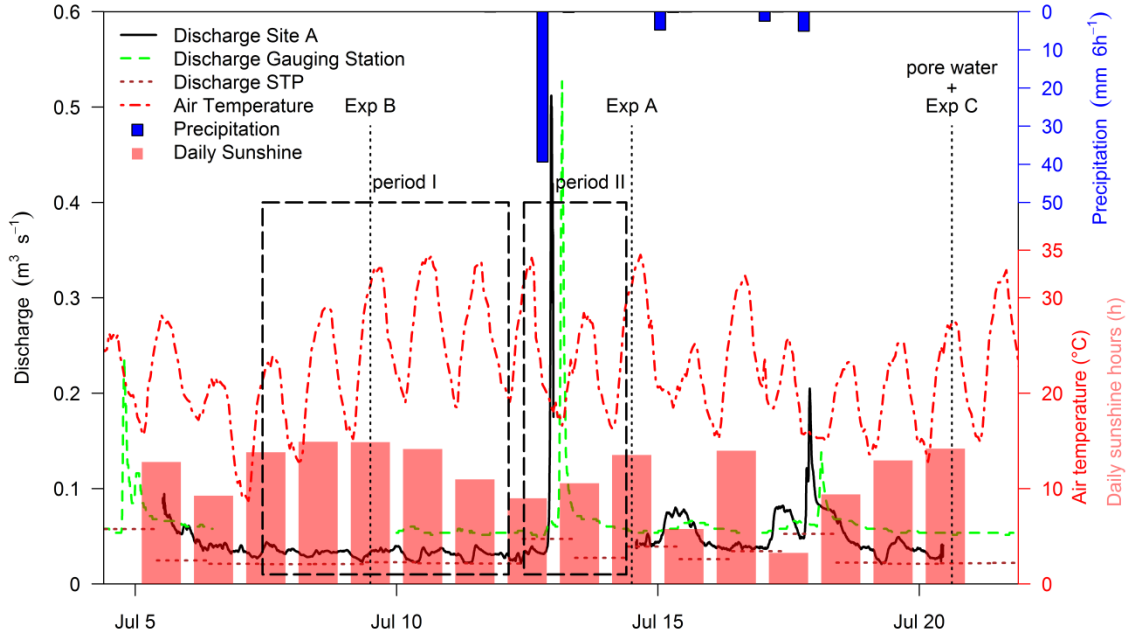


Figure 3-2: Hydrological and meteorological situation during the experiment. Exp A, Exp B, and Exp C mark the date of the phototransformation experiment at the respective site, "pore water" marks the date of the pore water sampling at all three experimental sites.

Under dry conditions, pH at site A was constantly about 7.7, only after the rain events it decreased to 7.5. EC at site A was in the range of 700 µS cm^{-1} und decreased quickly to 250 µS cm^{-1} after the rain events. DO was only measured for two days under dry conditions at site A. It was about 70 % or 6 mg L^{-1} and displayed only little diel fluctuation (< 5 %). The average concentration of total organic carbon (TOC) at sampling site A was 6.6 ± 1.9 mg L^{-1} (n = 51).

3.4.2 Occurrence and Temporal Dynamics of Pharmaceuticals in Surface Water

Of all analyzed compounds, clofibric acid was the only one that was not detected in any sample; it will thus not be discussed further. Average concentrations of the other pharmaceuticals were between the low ng L^{-1} range (propranolol) and the high ng L^{-1} / low µg L^{-1} range (carbamazepine, diclofenac) which is in good agreement to previous studies at rivers that were receiving substantial contributions of wastewater (Bendz et al. 2005, Daneshvar et al. 2010, Radke et al. 2010, Ternes 1998). Table 3-1 summarizes the concentrations at sites A and B and the loads of all pharmaceuticals at sampling site A. Loads could not be calculated for the period following the heavy rainfall due to sedimentation of sand onto the Doppler sensor and resulting malfunction of the instrument. Concordantly with the concentrations, average loads were highest for diclofenac, followed by carbamazepine and ranged from 3.5 ± 3.0 mg $6h^{-1}$ for propranolol to 590 ± 380 mg $6h^{-1}$ for diclofenac. Loads were highest after the heavy rainfall on July, 12th (2.7 g $6h^{-1}$ for diclofenac and carbamazepine) when discharge increased by a factor of 20 while concentrations remained relatively constant. Concentrations of all pharmaceutical residues and of boron and potassium were significantly lower ($p < 0.001$) at site B compared to site A. The ibuprofen data did not allow statistical testing as ibuprofen was only determined in a few samples.

Table 3-1: Concentrations (site A and site B) and loads (only site A) of pharmaceuticals (ng L^{-1} and mg $6h^{-1}$, respectively) and boron and potassium. Data are shown as mean ± stand deviation, the range of values is given in parenthesis; n: number of samples used for calculations. A significantly lower concentration at site B compared to site A ($p < 0.001$) is indicated by an asterisk (*).

	Site A		Site B
	Concentration (ng L^{-1}, n = 51 – 55)	**Loads (mg $6h^{-1}$, n = 46 – 50)**	**Concentration (ng L^{-1}, n = 51 – 52)**
Bezafibrate	120 ± 39 (63 - 280)	120 ± 110 (45 – 830)	22 ± 25 (12^{c} – 180) (*)
Carbamazepine	490 ± 110 (290 – 730)	470 ± 340 (266 – 2700)	310 ± 54 (130 – 420) (*)
Diclofenac	650 ± 220 (250 – 1400)	590 ± 380 (242 – 2700)	170 ± 110 (52 – 680) (*)
Ibuprofen	17 ± 37 (8^{c}– 210)	43 ± 220 (4.0 – 1500)	14 ± 23 (8^{c} – 120)
Metoprolol	310 ± 71 (180 – 440)	290 ± 210 (170 – 1700)	84 ± 28 (42 – 170) (*)
Naproxen	47 ± 18 (20 – 830)	49 ± 77 (16 – 560)	20 ± 11 (16^{c} – 75) (*)
Propranolol	3.5 ± 1.3 (0.7 – 7.8)	3.5 ± 3.0 (0.6 – 21)	1.0 ± 0.8 (0.5^{c} – 4.9) (*)
Sotalol	160 ± 34 (99 – 250)	150 ± 130 (78 – 980)	61 ± 16 (21 – 110) (*)
Sulfamethoxazole	330 ± 120 (110 – 730)	320 ± 150 (141 – 890)	140 ± 53 (52 – 270) (*)
Boron[a]	142 ± 54 (63 – 347)	130 ± 81 (60 – 604)	96 ± 50 (30 – 237) (*)
Potassium[b]	16 ± 5.0 (8.6 – 28)	14 ± 8.8 (7.9 – 68)	10 ± 3.8 (2.2 – 18) (*)

[a] concentration in µg L^{-1}, load in g $6h^{-1}$; [b] concentration in mg L^{-1}, load in kg $6h^{-1}$, [c] LOQ

The concentration dynamics of the investigated pharmaceuticals at both sampling sites are shown in Figure 3-3. The respective time trends for boron and potassium are given in Figure B- 2. Concentrations of pharmaceuticals at site A were generally slightly increasing during the dry period (period I), most likely caused but the slightly decreasing discharge at site A and therefore a higher percentage of WWTP effluent in river Gründlach (Figure 3-2). Thereafter, the concentrations of most pharmaceuticals decreased directly after the rain event on July, 12^{th} due to dilution, but recovered quickly after the discharge returned to previous conditions. The effect of the two other minor rain events was not as pronounced. In contrast, ibuprofen – which was not determined during dry conditions – was present at concentrations up to 200 ng L^{-1} shortly after the three rain events (Figure 3-3). This can either be attributed to input of untreated wastewater through combined sewer overflows (CSOs) or to an incomplete elimination in the WWTP at a decreased short residence time in the treatment plant during the discharge events (Ternes 1998). No data are available on either of these processes.

At site B, the concentrations of pharmaceuticals were generally lower than at site A. Bezafibrate and naproxen were rarely detected during period I, and the concentrations of propranolol were below LOQ in some samples. Ibuprofen was detected only in a few samples corresponding to the rain-driven input observed at site A.

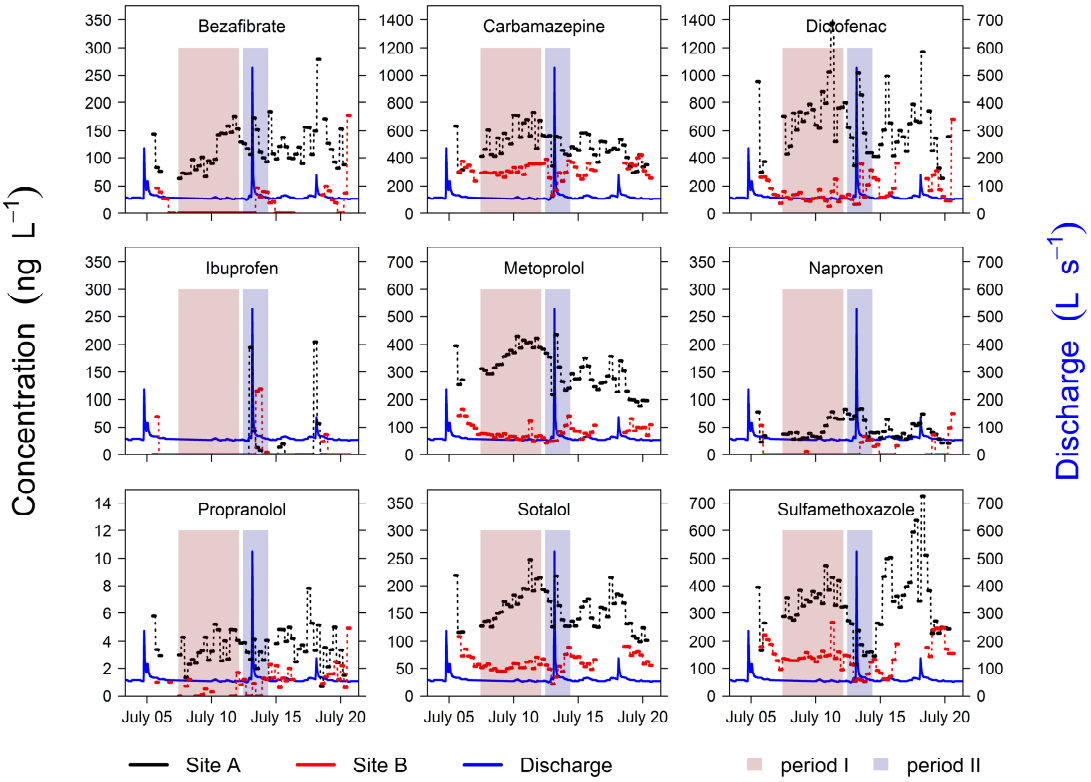


Figure 3-3: Time trends of pharmaceutical concentrations at both samplings sites and discharge at the gauging station “Frauenkreuz” at river Gründlach. For visual clarity concentrations < LOQ were set to 0.

3.4.3 Elimination of Pharmaceuticals Along the River Stretch

The concept we applied to estimate the elimination of pharmaceutical residues along the river stretch by normalizing their concentration to the concentration change of carbamazepine is only completely errorless if the discharge is not changing with time or if the concentrations of carbamazepine and the compound of interest at site A are perfectly positively correlated. A detailed analysis with synthetic examples on the potential errors of our concept for deriving elimination rates is given in Appendix B to chapter 3. Under dry conditions (period I), discharge changed only slightly and consequently correlation of substances (see Table B- 9) is of minor importance for mass balancing; the resulting maximum error of the elimination rate is low (< 2 %; Table B- 9). During the rain events, however, discharge changed substantially and consequently a positive correlation with carbamazepine at site A (see Table B- 5) is crucial for the valid estimation of elimination rates. For period II, this is the case for diclofenac, metoprolol, sulfamethoxazole, and sotalol as well as for potassium ($p < 0.05$), so their elimination rates can be estimated at acceptable uncertainty (maximum error 6 %, see Appendix B-2, chapter 6.2.4). Bezafibrate, naproxen, pro-

pranolol, and boron were not correlated with carbamazepine at site A during period II (Table B- 6) and consequently the elimination rate for these substances are very uncertain (maximum error 28 %). This is indicated by putting their elimination rates in Table 3-2 in parentheses.

The lower concentrations of all pharmaceuticals at site B compared to site A can either be explained by dilution of surface water due to the convergence of small creeks and ditches and to the exfiltration of groundwater into the river, or by the elimination of the pharmaceuticals along the river stretch. The calculation of elimination rates relative to carbamazepine as conservative tracer compensates for dilution, and thus elimination rates > 0 can be attributed to attenuation.

Table 3-2: Relative elimination (%) of pharmaceuticals between sites A and B for periods I and II.

	period I	**period II**
Boron	-19	(-9)
Potassium	5	4
Bezafibrate[b]	63	(57)
Diclofenac	69	41
Ibuprofen[a]	n.a.	n.a.
Metoprolol	68	50
Naproxen[b]	50	(43)
Propranolol[b]	70	(42)
Sotalol	42	36
Sulfamethoxazole	26	25

[a] For ibuprofen no elimination rates could be calculated due to the few data points. [b] Minimum elimination rates; the actual rate can be higher as concentrations < LOQ at site B were set to LOQ for calculation purposes; negative elimination rates indicate increase of mass compared to carbamazepine; values in parenthesis are uncertain due to a lacking correlation with carbamazepine

All pharmaceuticals were eliminated compared to carbamazepine both during period I and period II (Table 3-2) are conservative estimates and likely underestimate the elimination: the concentrations of these compounds in samples from site B were frequently < LOQ, and for calculation purposes their concentration in such samples were set to LOQ. This especially holds true in period I when the concentrations of these three substances in most samples at site B were < LOQ. If we assume a complete elimination (i.e., if concentrations at site B are set to 0), the calculated elimination rates for period I increase to 100 % (from 63 %) for bezafibrate, 99 % for naproxen (from 50 %), and 84 % for propranolol (from 70 %) while elimination rates in period II only slightly increase (see Table B- 8). Therefore, despite these uncertainties in the exact elimination rates of these three substances the elimination during period I still is substantially higher than in period II.

Higher elimination rates during period I are most probably a result of the longer residence time of substances within the river stretch due to a lower flow velocity of the surface water. Travel time between the two sampling sites in period II (high discharge) is assumed to be only approx.

12 hours compared to approx. 18 hours under low flow conditions (period I). Exact numbers for elimination rates per residence time cannot be derived since these travel times are only rough estimates, but based on these times the elimination rates per residence time are in the same range under both flow conditions. A similar finding was obtained in laboratory-scale flume experiments where the elimination rate from surface water under different flow velocities was investigated (Kunkel and Radke 2008).

The mass recovery of potassium at site B under both hydraulic conditions (see Table 3-2) is in good agreement with that of carbamazepine, and thus the elimination rates of pharmaceuticals relative to potassium as conservative reference substance (see Table B- 7) are almost equal to those calculated with carbamazepine. The good correlation of potassium and carbamazepine was reported before (Nödler et al. 2011) and confirms our approach of using carbamazepine as conservative tracer. The apparent mass increase of boron along the river stretch (Table 3-2) during both periods, however, was unexpected. Although boron might follow different concentration/load dynamics in the effluent of a WWTP due to its input via usage in detergents whereas pharmaceuticals and potassium are mostly excreted by humans, its concentration are even better correlated with the pharmaceutical residues than potassium (Table B- 5 and Table B- 6).Since an additional anthropogenic or geogenic input of boron along the river is unlikely, the explanation for the apparent mass increase of boron remains unsettled.

The environmental fate of pharmaceuticals depends on the characteristics of the specific stream as well as on additional boundary conditions. To move forward in our ability to mechanistically explain field observations, we have to further explore the role of individual processes and boundary conditions for the attenuation of pharmaceuticals. Therefore, we complemented the mass-balance approach that provides information on the overall elimination of a compound with the concurrent and *in situ* investigation of individual attenuation processes. The results of these experiments on phototransformation and the analysis of hyporheic processes will be discussed in the following.

3.4.4 PHOTOTRANSFORMATION EXPERIMENTS

The measured photon fluxes at the three experimental sites reflect the characteristics of these sites: photon flux was lowest at Exp A (strong shading, forest; 2.1 x 10^{-9} einstein s^{-1}) and highest at Exp B (open terrain; 9.1 x 10^{-7} einstein s^{-1}). At site Exp C (partial shading), the measured photon flux was intermediate (3.7 x 10^{-7} einstein s^{-1}). For site Exp B, no elimination rates of bezafibrate, diclofenac, and naproxen can be reported as the initial concentration of these compounds were already < LOQ (see Figure 3-3 and Table 3-1). To overcome this limitation, we used water sampled at site Exp A in the subsequent experiment at site Exp C. The elimination rates measured in the sterile controls (with NaN_3) were generally higher than those in the non-sterile experiments (Table 3–3). Obviously, the addition of NaN_3 caused this increase. This might be explained either by pH dependent elimination kinetics (Canonica et al. 2008) since pH was about 0.2 units higher than in the non-sterile approaches or by an influence of NaN_3 on the complex transformation kinetics and pathways (Xu et al. 2009). All conclusions below are consequently based on the ex-

periments without NaN_3 in order to reflect the real situation in the river water. A detailed investigation of this phenomenon was beyond the scope on this study.

None of the pharmaceuticals was eliminated in any of the dark controls (see Figure B- 4), so biotic and other abiotic processes were quantitatively not relevant during the photolysis experiments. Bezafibrate, carbamazepine, metoprolol, naproxen, and sulfamethoxazole were not eliminated in any of the light exposed experiments either. Photolysis did thus not contribute to their attenuation in river Gründlach, and it is also supposed to be only of minor importance in other rivers that experience similar or worse irradiation conditions. Diclofenac, propranolol, and sotalol were the only compounds for which photolysis was observed in at least one experiment; for propranolol this was the case only in the sterile control at site Exp B, while its concentration did not decrease significantly in any of the other experiments. Consequently, we conclude that the contribution of photolysis to the attenuation of propranolol was negligible. For diclofenac, no data are available for the experiment at site Exp B as the diclofenac concentration in the river water used for this experiment was < LOQ (see above). At site Exp C, its photolysis rate was $2.8 \pm 0.7\ d^{-1}$, while it was not eliminated at site Exp A with full tree coverage. The photolysis rate of diclofenac (Exp C) is lower than the near-surface photolysis rate previously reported for a river in close proximity ($11.6 \pm 0.6\ d^{-1}$, Radke et al. (2010)). However, if this near-surface rate is extrapolated for the water depth of 15 cm at river Gründlach (details on calculation see Schwarzenbach et al. (2003) and Fono et al. (2006)), the rate reduces to $2.2 \pm 0.1\ d^{-1}$ which is in good agreement with the rate determined in the present study. Sotalol was affected by photolysis at all three sites, but at Exp C it was not eliminated in the container without NaN_3 (not significant $k_p = 0.70 \pm 0.30\ d^{-1}$). This is contradictory to Exp A where we observed elimination ($k_p = 0.60 \pm 0.15\ d^{-1}$) in spite of the lower photon flux compared to Exp C. It is also in disagreement with the observations made for diclofenac (elimination at Exp C but not at Exp A). Overall, a definitive assessment of the contribution of photolysis to the elimination of sotalol is hindered by these inconclusive results. But since Piram et al. (2008) reported a high susceptibility of sotalol to indirect photolysis in wastewater, we conclude that photolysis very likely contributes to the overall attenuation of sotalol in river Gründlach.

Table 3-3: Photolysis rates (d^{-1}) of pharmaceuticals measured in the *in situ* phototransformation experiments at river Gründlach. For all other pharmaceuticals no rate significantly different from 0 was observed.

	Exp A [a]	Exp B		Exp C	
NaN_3 addition	no	No	yes	no	yes
shading	complete	No	no	partial	partial
Diclofenac	n.s.	n.a.	n.a.	$2.8 \pm 0.7^{*}$	$5.2 \pm 0.4^{**}$
Propranolol	n.s.	n.s.	$1.7 \pm 0.5^{**}$	n.s.	n.s.
Sotalol	$0.60 \pm 0.15^{*}$	$1.8 \pm 0.5^{*}$	$5.5 \pm 0.3^{**}$	n.s.	$1.4 \pm 0.3^{**}$

* $p < 0.05$; ** $p < 0.01$; n.a: not available since initial concentrations were < LOQ; n.s.: slope of regression not significantly different from 0; [a] no sterile control performed at Exp A

Based on the results at the three sites, we conclude that phototransformation was quantitatively not relevant for the elimination in the forested part of the river stretch for any substance due to the efficient shading by the trees. In the agricultural area, photolysis was substantial for diclofenac and potentially also for sotalol. As the observed total elimination rates between site A and site B (Table 3-2) also include night time, the determined phototransformation rates cannot directly be up-scaled for the total travel time between the two sampling sites. However, assuming a travel time of seven hours in the agricultural part during daylight with an average of 13 daily sunshine hours in period I and extrapolating the rate of diclofenac from Exp C ($2.8 \pm 0.7\ d^{-1}$) over the whole agriculturally used stretch, an elimination rate of diclofenac of 28 – 42 % can be estimated for photolysis. This elimination rate is smaller than the calculated elimination of diclofenac along the river stretch during the dry period (69 %; Table 3-2). But overall, we deduce that a substantial part of the total elimination of diclofenac (and potentially also sotalol) in river Gründlach can be explained by phototransformation. For naproxen, which was previously described as photolabile substance (Packer et al. 2003, Radke et al. 2010), we could not derive phototransformation kinetics at the unshaded site (Exp B) since initial concentrations were < LOQ. However, we observed no elimination at Exp C, and previous work reported a three to five times slower phototransformation kinetics for naproxen compared to diclofenac in river water (Radke et al. 2010). Consequently, we conclude that phototransformation contributes only little to the total elimination (50 % in period I) of naproxen in river Gründlach.

For all other compounds, photolysis was not a quantitatively relevant attenuation mechanism along the river stretch. For sulfamethoxazole, this conclusion seems contradictory to the study by Andreozzi et al. (2003) who reported similar phototransformation kinetics for sulfamethoxazole and diclofenac. This contradiction can be explained by the effect of pH on the photolysis rate of sulfamethoxazole. The experiments by Andreozzi et al. (2003) were conducted at pH 5.5, whereas the pH in river Gründlach was about 8. The photolysis of sulfamethoxazole has been shown to be strongly pH dependent and much higher at lower pH around and below the pK_a (5.6 ± 0.5) of the

secondary amine group of sulfamethoxazole (Moore and Zhou 1994), and thus the absence of sulfamethoxazole photolysis in river Gründlach is in agreement with previous studies. However, this example highlights the complexity of phototransformation processes and illustrates challenges when comparing results from different studies.

3.4.5 Biotransformation of Pharmaceuticals in the Sediments

Abiotic (other than photolysis) or biotic elimination processes in the surface water can be excluded as major attenuation mechanisms for all pharmaceuticals since their concentration did not decrease in the dark controls of the phototransformation experiments. This is in agreement with previous studies which also reported no or only minor elimination of pharmaceuticals residues in surface waters under exclusion of sunlight (e.g., Kunkel and Radke (2008)). Therefore, we investigated the presence of pharmaceuticals in the hyporheic zone to estimate the relevance of microbial transformation processes in the sediment. Bezafibrate, ibuprofen, naproxen, and propranolol were not detected in the pore water and will thus not be discussed here. Their concentrations in the surface water were close to the quantification limit, so this observation was to be expected. For diclofenac, no reliable data can be reported due to analytical problems. Hence, the discussion is limited to the depth profiles of metoprolol, sulfamethoxazole, sotalol, and carbamazepine. These compounds were determined in the pore water in all depths (down to 25 – 35 cm) which points to an efficient exchange of surface water with pore water. The pore water concentrations of carbamazepine were equal to the concentrations in the surface water, so dilution by groundwater was little in the sampled sediment profiles, as was biotransformation of carbamazepine. The variations of carbamazepine concentrations with depth are in the same range than concentration fluctuations in the surface water (Figure 3-4a) Therefore, carbamazepine can be considered to be persistent in the pore water as well, and based on the correlation of the other pharmaceuticals with carbamazepine during dry periods (when the pore water sampling was done) we normalized the concentrations of metoprolol, sotalol, and sulfamethoxazole to the respective concentration of carbamazepine in each sample. A decrease in the normalized concentrations thus implies an elimination of a substance in the sediments, which was the case for all three substances (Figure 4b-d). In general, pharmaceutical concentrations (other than carbamazepine) at our three sampling sites decreased more rapidly with sediment depth than at a larger lowland river in Eastern Germany (Lewandowski et al. 2011b). However, a direct comparison is complicated by the different hydraulics and sediments and the somewhat inconclusive results of the cited study.

We attribute the elimination with respect to carbamazepine to microbial transformation, which has been shown for all three substances as potentially relevant process in river sediments (Radke et al. 2009, Ramil et al. 2010). We exclude sorption as a substantial cause for the elimination from the pore water since dynamic sorption equilibrium between the continuously renewed pore water and the sediment particles should have been reached due to the continuous input of pharmaceuticals at relatively constant concentration. For metoprolol, the interpretation of elimination by microbial transformation is backed up by enantiomer ratios (see below).

The analyses of redox parameters (data not shown) indicated that the pore water at Exp B and Exp C was sulfate reducing and methanogenic, respectively, while it was less reducing (denitrifying) at Exp A. This might indicate a faster advection of surface water into the hyporheic zone and thus a more efficient transport of oxygen and pharmaceuticals into the sediment. Such a different hydraulic and biogeochemical situation might also explain differences in the shape of the normalized depth profiles of metoprolol, sotalol, and sulfamethoxazole between Exp A and the two other sites. However, without knowledge on the detailed, small-scale hydraulic conditions; this explanation has to be considered a hypothesis.

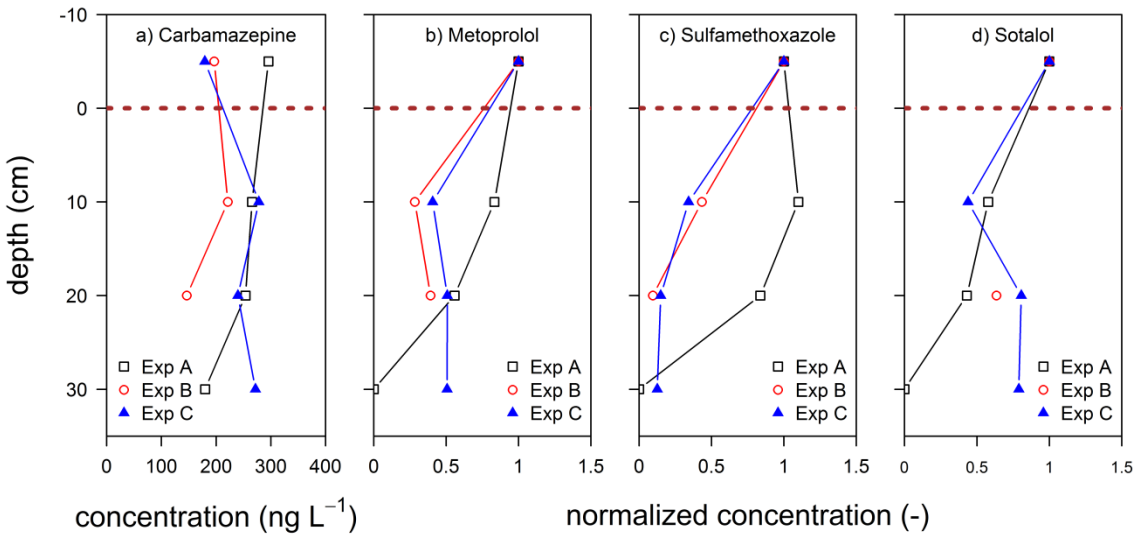


Figure 3-4: Pore water profiles of pharmaceuticals on 2010/07/20 at the three experimental sites at river Gründlach (Exp A, Exp B, Exp C): a) absolute concentrations of carbamazepine, b-d) concentrations of metoprolol, sulfamethoxazole, and sotalol relative to carbamazepine and normalized to this ratio in the surface water.

Unlike in the phototransformation experiments, we are not able to calculate biotransformation rates in sediment from the data of the pore water analyses as no quantitative data on exchange of water and solutes between the river channel and the hyporheic zone are available. Nevertheless, the profiles clearly indicate that attenuation in the hyporheic zone is of relevance at the river Gründlach.

3.4.6 Metoprolol Enantiomer Ratios

The analytical method for determination of metoprolol enantiomers was precise and reproducible. This is documented by constant EFs in the calibrations standards (0.493 ± 0.011, n = 30), in the quality control samples (0.490 ± 0.004, n = 4), and in a subset of samples of the phototransformation experiment from Exp A (0.484 ± 0.005, n = 5) where metoprolol was not transformed. At site A, EF was constant and showed a nearly racemic enantiomer distribution (0.493 ± 0.007, n = 17). This observation is in agreement with previous studies which reported the occurrence of racemic metoprolol in WWTP effluent (Fono et al. 2006). The meteorological and hydrological conditions had no measurable influence on metoprolol EF at site A (Figure 3-5a). Over the whole

period, EFs at site B were significantly lower than at site A (Figure 3-5a, $p < 0.0001$). Abiotic processes usually do not affect enantiomer ratios (Huehnerfuss and Shah 2009) which is reflected in a constant EF in experiments on the sorption of metoprolol to sterilized Gründlach sediment despite a significant sorption (data not shown). Consequently, the decrease of EF supports our interpretation that metoprolol was transformed by microbiological processes along the river stretch. At site B, EFs during period I were significantly lower ($p < 0.001$; 0.429 ± 0.007, $n = 7$) compared to period II (0.463 ± 0.014, $n = 8$). This is in agreement with the higher elimination rate of metoprolol during period I (see above), but also indicates that biotransformation was of relevance even under high discharge conditions. The temporal dynamics of EF in the surface water together with metoprolol concentrations at both sites are available as complementary information in the SI (chapter 6.2). At the time of the highest discharge (in period II), EF at site B was almost equal to EFs at site A, but after discharge decreased again to pre-event values, EFs at site B also returned to pre-rain event values (Figure B- 6).

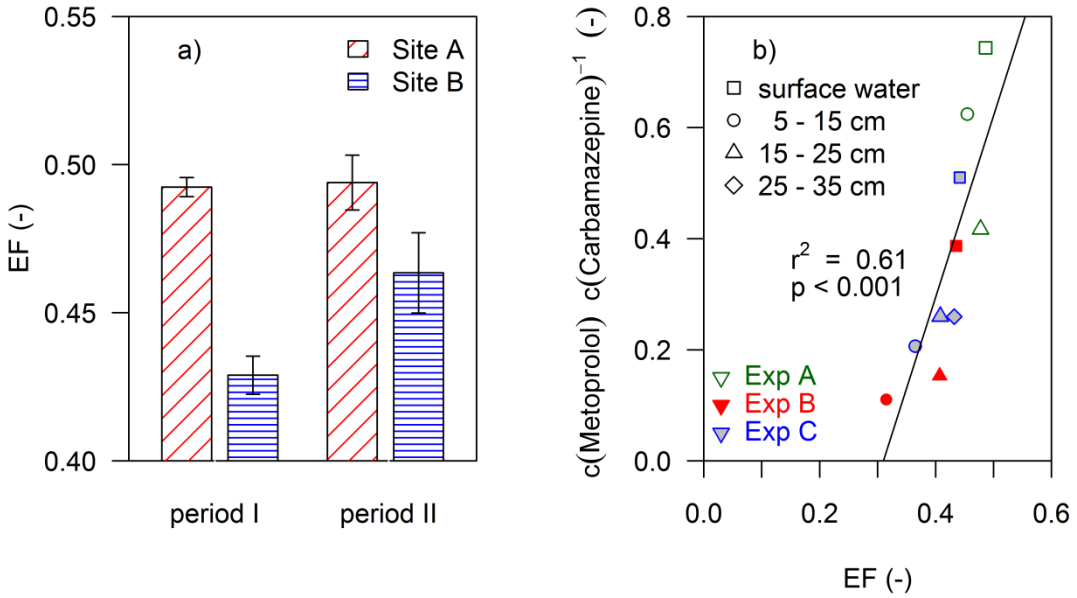


Figure 3-5: a) Metoprolol enantiomer fractions (EF) at sites A and B during periods I and II (only a subset of samples was analyzed for EF); b) correlation of metoprolol EF with normalized metoprolol concentration in the pore water; different symbols represent different depths, different colours different sites.

For the interpretation of EFs in pore water, we normalized the concentration of metoprolol to the concentration of carbamazepine. This compensates for concentration decreases of metoprolol in the hyporheic zone due to dilution which does not change the EF. These normalized metoprolol concentrations were well correlated with EF ($r^2 = 0.61$, $p < 0.01$, Figure 3-5b). Despite this good correlation of EF with the relative concentration of metoprolol, we observed no general decrease of EF with depth at the individual experimental sites (Figure 3-5b). This can be attributed to the complexity of flow paths in the upper layer of the sediment (Cardenas et al. 2004, Lewandowski et al. 2011a) which means that a greater depth in the sediment is not necessarily equivalent to a longer travel (or residence) time of water and solutes. Consequently, the interpre-

tation of a compound's concentration depth profiles alone is complicated. However, in combination with a persistent substance whose concentration in the surface water is correlated with that of a target compound and supplemented by indicators such as the enantiomeric fractionation, processes in the hyporheic zone become accessible to interpretation.

It is difficult to compare the findings of this study to previous studies on the environmental fate of pharmaceuticals in rivers. This is mostly due to the few compounds analyzed in all available studies and to experimental differences. Some studies investigated the fate at much larger rivers than the river Gründlach, so evaluating their findings in light of our results is not straightforward. At a Californian river (discharge 20 times larger than river Gründlach), naproxen was rapidly removed with a half-life time of 1.7 hours by phototransformation processes (Lin et al. 2006). In a Swedish river (discharge approx. 100 higher than a river Gründlach) a rapid removal of metoprolol of up to 75 % within in a flow time of 8 hours was determined (Daneshvar et al. 2010). However, if we apply the assumptions used in this study (i.e., carbamazepine is considered persistent and concentration ratios can be used to estimate removal rate) to the data reported by Daneshvar et al. (2010) elimination of metoprolol in summer months ranges from 63 -88 % along a river stretch of approx. 10 km (from R3 to R7 in their manuscript), which is in better agreement to the results of this study. In contrast, in a large Texan river (discharge about 100 time higher than at river Gründlach) metoprolol and naproxen were eliminated rather slowly with half-times times of 4-5 days (Fono et al. 2006). Probably the best comparable dataset was obtained during a tracer experiment at a Swedish river (Säva) which – based on discharge – is about ten times larger than river Gründlach (Kunkel and Radke 2011). There, ibuprofen was rapidly removed from surface water with a half-life time of 10 hours, and clofibric acid was also eliminated at a half-life of 2.5 days. In contrast, bezafibrate, diclofenac, metoprolol, and naproxen were not attenuated within a travel time of 48 hours. Unfortunately, the elimination of ibuprofen and clofibric acid cannot be assessed for river Gründlach. In contrast to Säva Brook, however, at river Gründlach we observed elimination rates of bezafibrate, diclofenac, metoprolol, and naproxen between 40 % and 70 % within a travel time of 12-18 hours. In a similar mass-balancing approach as in the present work, (hardly) no elimination of bezafibrate, diclofenac, and naproxen was observed at a river (discharge about 20 times larger) located 50 km north of river Gründlach (Radke et al. 2010). The observed differences in the fate of individual compounds in these three rivers (Gründlach, Säva, and Roter Main) are supposedly a result of the different stream characteristics and hydraulics. For example, turbidity in Säva Brook (Figure S5 in Kunkel and Radke (2011)) or Figure A- 5 in this thesis is much higher than in the rivers Gründlach (Figure B- 5) and Roter Main (Figure S1 in Radke et al. (2010)). Additionally, the average water depth of Säva Brook and Roter Main (1 m each) is notably larger than in river Gründlach (15 cm). The combination of these two parameters favors phototransformation in river Gründlach while it is restrained by the larger water depth in river Roter Main and additionally by the high turbidity in Säva Brook. This might partly explain the elimination of diclofenac (which is susceptible to photolysis) in river Gründlach while no elimination was observed in rivers Säva and Roter Main. Another aspect is the exchange of river water and solutes between the stream channel and the hyporheic zone. Although flow velocity under

baseflow conditions are similar in all three rivers (approx. 10 cm s^{-1}), this exchange is (supposedly) much higher in river Gründlach. This can be attributed to the shallow water depth and the resulting smaller ratio of surface water volume to sediment surface area (Packman et al. 2004), to small-scale bedform heterogeneities which were characteristic for the whole stretch a river Gründlach (see Table A- 1) that induce enhanced hyporheic exchange (Elliott and Brooks 1997), and to the comparatively high hydraulic conductivity of the sandy sediments at river Gründlach.

3.5 CONCLUSIONS

In this study we investigated the fate of pharmaceutical residues in a small river. All eight regularly detected pharmaceuticals were attenuated compared to carbamazepine which was used as conservative tracer. Its persistence against photolysis and biodegradation on time scales relevant for this study (maximum travel time of one day) had been shown previously and was also confirmed by this study. The combination of time-resolved composite sampling with *in situ* experiments allows differentiating between potential elimination processes. Hence, this work provides a clear picture of the relevance of individual attenuation processes of organic micropollutants.

Based on the results of this study, we derive the following conclusions.

- The dataset derived for the river Gründlach provides a benchmark for the elimination of the studied pharmaceuticals under favorable yet realistic conditions in Central Europe. Although conditions for photolysis were sub-optimal due to the partial shading of the river stretch, this is typical for the majority of small streams in urban and agriculturally used areas.

- Elimination by photolysis is of minor importance for most substances in rivers. Even under (near) optimal conditions (small stream depth, sparse bank vegetation) photolysis contributes only 50 % to the total elimination for a highly photolabile substance like diclofenac. Therefore, in larger (and deeper) rivers, photolysis of organic micropollutants is supposedly to be even of less importance.

- At favorable hydraulic conditions (intense exchange of surface water and pore water) like at river Gründlach, biotransformation in the bed sediments can be an efficient elimination pathway for pharmaceuticals. For example, the high attenuation of bezafibrate and metoprolol within less than one day can be directly attributed to biotransformation processes in the hyporheic zone since they were persistent to photolysis and transformation in the surface water.

The combination of highly time-resolved sampling and *in situ* experiments that was applied in this study allows elucidating the importance of individual processes and quantifying the overall elimination of organic micropollutants in rivers. However, to provide a better access to *in situ* biotransformation rates of organic micropollutants in river sediments, detailed knowledge on solute transfer rates into and residence times in the sediments are required. Thus, future studies should aim at quantifying this exchange between stream channel and hyporheic zone on both the local and the reach scale.

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4 STUDY III: DETERMINING REALISTIC BIOTRANSFORMATION RATES IN RIVER SEDIMENTS

Recirculating Sediment Columns Provide Generalizable Rate Constants for the Biotransformation of Pharmaceuticals in River Sediments

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4.1 ABSTRACT

Biological transformation processes in river sediments are a major attenuation pathway for pharmaceutical residues in rivers and streams. They have been intensively investigated in recent years. However, most of the previous (laboratory) studies are not appropriate to derive elimination rates that represent conditions in the hyporheic zone. To meet this challenge, we set up a recirculating system where river water was continuously pumped through sediment columns. By this approach, the elimination kinetics of eight commonly detected pharmaceutical residues (bezafibrate, carbamazepine, clofibric acid, diclofenac, ibuprofen, metoprolol, naproxen, and propranolol) were determined in four different sediments. Generally, the derived elimination rate constants (k_{eli}) were much faster than previously determined values with static batch systems. Half-life times for the biotransformation of ibuprofen and metoprolol ranged from 0.5 to 1.8 days and 0.9 to 3.6 days, respectively. Even substances with low reported biodegradabilities such as clofibric acid were removed at rate constants corresponding to half-life times as short as 2.9 days. Additionally, we were able to reveal that a contradictive observed attenuation behavior of bezafibrate and diclofenac at two rivers cannot be explained by a different biological transformation potential of the respective river sediments since bezafibrate and diclofenac were efficiently eliminated in sediments from both field sites (half-life times of bezafibrate: 1.1 – 9.3 days, diclofenac: 1.5 – 4.1 days). Derived k_{eli} values for each pharmaceutical were in the same range in all tested sediments and almost independent from boundary conditions such as the filter velocity in the sediment columns. Moreover, the elimination rates were determined under hydrological conditions that simulate advective exchange process at the surface water/sediment interface. Hence, the derived elimination rates are supposedly more realistic for estimating *in situ* attenuation rates in river sediments than values determined in batch systems and thus can be used as valuable input parameters for reactive transport modeling of pharmaceutical residues in rivers.

4.2 INTRODUCTION

The increasing use of pharmaceuticals has led to a large number of studies on their fate during wastewater treatment (Carballa et al. 2005, Joss et al. 2006, Ternes and Hirsch 2000, Wick et al. 2009). Many pharmaceuticals are only incompletely removed during wastewater treatment, and consequently are discharged into receiving waters where they are commonly detected (Ankley et al. 2007, Boxall et al. 2012, Heberer 2002, Sacher et al. 2008). Besides phototransformation in surface waters (Boreen et al. 2003, Buser et al. 1998, Vione et al. 2011), (bio-)transformation and sorption in the sediment compartment constitute the two major elimination pathways of organic micropollutants such as pharmaceutical residues in rivers. During the last years, several studies addressed their attenuation in rivers of different sizes (Fono et al. 2006, Kunkel and Radke 2012, Lin et al. 2006, Radke et al. 2010, Writer et al. 2012). However, differentiating between the contributions of individual elimination processes to the total attenuation is not straightforward and not always feasible. While concepts for the *in situ* determination of phototransformation rates (e.g., installing test tubes into the river) are available and were successfully tested (Radke et al. 2010), the *in situ* determination of biotransformation in river sediments is still a challenge to be

addressed. Some concepts such as miniature push-pull tests were used for the *in situ* determination of turnover rates of nitrate in river sediments (Knecht et al. 2011), the application to pharmaceuticals has not yet been reported.

Commonly, the fate of organic micropollutants in river sediments is investigated in controlled laboratory test systems. Most test designs consist of static batch systems (simplified versions of experimental test systems described in OECD Guideline 308) containing sediment and a supernatant of water (OECD 2002). While these systems provide valuable information on the general persistence of a compound and also on the formation of transformation products (Löffler et al. 2005, Prasse et al. 2009, Radke et al. 2009, Radke and Maier 2014), they are not appropriate for deriving realistic and universally valid kinetic data. The exchange of substances from the supernatant into the sediment – where the predominant proportion of the transformation or sorption of micropollutants takes place (Kunkel and Radke 2008) – is (almost) exclusively driven by diffusion. In contrast, in reality this exchange is both driven by larger scale groundwater/surface water interactions (infiltrating or exfiltrating conditions, Anibas et al. (2011)) and small-scale fluxes of water and solutes across the surface water/sediment interface (Cardenas et al. 2004, Saenger et al. 2005). Additionally, due to the slow fluxes across the sediment surface in the standard batch systems, oxygen is consumed in the uppermost millimeters of the sediment and rapidly anaerobic zones in the sediment/water batch systems occur. This lack of oxygen is often a limiting factor for the transformation of pharmaceuticals since transformation rates of many organic micropollutants are higher under aerobic conditions (Massmann et al. 2008). In experimental designs with an enhanced exchange between surface and pore water oxygen is still available for microbial respiration processes in greater sediment depths (Kunkel and Radke 2008, Moodley et al. 1998). A second and more complex approach to assess the fate of substances in river sediments is to use column experiments. However, in these experiments the focus often is laid on the transport and sorption of substances and the residence time of substances in the sediment is too short (a few hours) to derive data for calculating reliable transformation rates. Only few studies used very long columns and small pore water velocities (Baumgarten et al. 2011). However, these experiments are more appropriate to simulate processes during river bank filtration and not to mimic the often small scale and rapid exchange processes at the sediment surface of rivers which continuously introduce oxygen and nutrients into the upper parts of the sediments. The third one is to use flume systems representing the most complex system to evaluate the fate of organic micropollutants in rivers. This setup was successfully applied to determine the influence of the flow regime in the surface water on the elimination of six acidic pharmaceuticals (Kunkel and Radke 2008). Yet, the usage of this test design for a battery of different approaches or replicates of the same setup is limited due the large amounts of water, sediments, and laboratory space needed as well as also of the high purchase costs for such a system.

The general aim of the present work was to engineer a test design which merges the advantages of all three commonly used systems (static batch experiments, column experiments, flume experiments) while minimizing the disadvantages of each approach to study transformation kinetics of a suite of pharmaceuticals in river sediments. Special focus was laid on a dynamic and

flow driven exchange of surface and pore water. To this end, we developed a recirculating system where surface water was actively pumped through river sediments following the setup described in Gröning et al. (2007). The four main aspects of this work were i) to differentiate between elimination by abiotic and biotic processes in river sediments, ii) to determine the relationships of elimination kinetics for specific substances in different sediments, iii) to investigate whether the filter velocity has an impact on the elimination of pharmaceuticals, and iv) to relate the derived elimination kinetics to measured elimination along river stretches where the sediments were taken. In total, the fate of eight commonly detected pharmaceuticals (bezafibrate, carbamazepine, clofibric acid, diclofenac, ibuprofen, metoprolol, naproxen, and propranolol) was investigated.

4.3 Material and Methods

4.3.1 Chemicals

All pharmaceutical standards (purity > 97 %) were purchased from Sigma-Aldrich (Seelze, Germany). The isotope-substituted surrogate standards bezafibrate-D_4, clofibric acid-D_4, ibuprofen-D_3, diclofenac-D_4, naproxen-D_3, metoprolol-D_7, and propranolol-D_7 were purchased from Toronto Research Chemicals (North York, ON, Canada); carbamazepine-$^{13}C^{15}N$ was kindly provided by the German Federal Institute of Hydrology (BfG, Koblenz, Germany). Acetonitrile (ACN) was obtained from Th. Geyer (Renningen, Germany), water (H_2O) was supplied by J.T. Baker (Deventer, The Netherlands), and acetic acid (HAc, all LC-grade) was purchased from Sigma-Aldrich.

4.3.2 Sampling Sites and Sediments

Sediments were collected from rivers Roter Main and Gründlach (see Table 4-1). Both rivers are located in Northern Bavaria and represent typical small rivers in Middle Europe. River Roter Main was sampled near the city of Bayreuth where the average annual discharge is about 3 $m^3 s^{-1}$ (http://www.hnd.bayern.de, gauging station “Bayreuth, Roter Main”). Most parts of the sediments of river Roter Main are predominantly sandy except for some slower flowing river stretches upstream of weirs where clayey sediments occur. At some sites the banks and river bed are reinforced and there sediments often are rocky. More information on the river Roter Main and its catchment is given in Radke et al. (2010). Sediments were taken at three different sites of river Roter Main: one sandy sediment was sampled upstream of the city of Bayreuth (no substantial background of wastewater, RM1), and both a sandy sediment (RM2) and a silty sediment (RM3) were collected near the town of Heinersreuth after the WWTP Bayreuth discharges into river Roter Main. Sediment RM1 was sampled on two different dates with a time interval of about eight months. The second river was river Gründlach which is located approx. 10 km north of the city of Nuremberg. River Gründlach is about ten times smaller than river Roter Main with an average discharge of approx. 0.3 $m^3 s^{-1}$ (http://www.hnd.bayern.de, gauging station “Frauenkreuz/Gründlach”) and the sediments are deep sandy in the whole catchment. Sediments were sampled close to “Hundsmühle” downstream of the WWTP Heroldsberg (sediment GR). More details on river Gründlach are given in Kunkel and Radke (2012). All sediments were taken from the uppermost 20 cm and wet sieved (< 2 mm). Surface water was collected as grab sample from

the middle of the river. The sieved sediments were stored at 4°C with some centimeters of the surface water as supernatant until the experiments were conducted; the water was stored at 4°C as well.

Table 4-1: Coordinates of the sampling sites of the four sediments for the column experiments and sediment characteristics.

	RM1	RM2	RM3	GR
River	***Roter Main***	***Roter Main***	***Roter Main***	***Gründlach***
Coordinates	49°54'25.1"N, 11°37'05.1"E	49°58'07.6"N, 11°32'18.0"E	49°57'56.0"N, 11°32'24.7"E	49°31'26.8"N, 11°08'10.8"E
pH of water (-)	7.5 ± 0.2	7.4 ± 0.2	7.4 ± 0.2	7.5 ± 0.2
Coarse sand[1](%)	65.3 ± 5.6	61.0 ± 3.0	0.7	31.2 ± 1.2
Medium sand (%)[1]	33.3 ± 6.2	36.2 ± 2.4	4.1	67.8 ± 1.2
Fine sand (%)[1]	31.4 ± 5.8	2.4 ± 2.3	59.9	0.6 ± 0.1
Silt and clay (%)[1]	0.4 ± 0.3	0.6 ± 0.3	33.7	0.5 ± 0.5
Classification[1]	sand	sand	sandy silt	sand
C (%)	0.08	0.11	0.72	0.2
N (%)	< LOQ[2]	< LOQ	0.06	< LOQ

[1] Classification after AG Boden (1994), [2] LOQ: 0.05

4.3.3 Setup of the Column Experiments

Experiments were conducted in stainless steel columns (length: 30 cm, diameter: 6 cm) previously described by Strauss et al. (2011). The steel columns were incrementally filled with wet sediment from the respective sampling site. To exclude air pockets in the sediment and to obtain a realistic bulk density of the sediment within the steel column each increment was compacted by tapping against the wall of the cylinder. Perforated steel plates were placed at on boths ends of the steel cylinders to retain the sediment within the column. Water was pumped through the columns from bottom to top by a peristaltic pump (IP-8, Ismatec, Wertheim-Mondfeld, Germany) equipped with Pharmed® BPT tubing (Ø 1.85 mm, novodirect, Kehl/Rhein, Germany). The water was sucked out of a storage vessel (1 L amber glass screw cap bottles with a modified cap). The water in the storage vessel was permanently aerated to maintain oxygen saturation. The outlet of the sediment column was piped back into the storage vessel to generate a recirculating system. At the inlet and the outlet of the sediment column, three-way cocks (Fresenius, PSU, Bad Homburg, Germany) were installed to enable sampling of water before and after the sediment passage. All connecting tubes were made of PTFE (Ø 4 mm, VWR, Germany). Coupling of tubes and three-way cocks was done by Luer hose connectors (PP, novodirect). Up to six columns were run in parallel. A scheme and a picture of the whole experimental setup are given in Figure C- 1.

Experiments were run at three different filter velocities (flow rate normalized to the effective cross-section area in the sediment columns): 2.5 m d^{-1}, 5.0 m d^{-1} and 10 m d^{-1} and were kept constant throughout each experiment. An overview of the performed experiments is given in Table 4-2. Experiments (Exp.) 1, 2, 5, and 6 were run in a standard laboratory room. To inhibit both sunlight-induced microbial and algae growth as well as phototransformation of test substances in the semi-transparent PTFE tubes, all PTFE tubes were wrapped in aluminum foil during these experiments. Additionally the windows were coated with an UV light filtering foil. Exp. 3, 4, 7, and 8 were conducted in a dark room. To check for abiotic transformation processes and sorption of the pharmaceuticals to the river sediments, control experiments with sterilized sediment and water were run. To this end, sediments and water were autoclaved (90 min at 125 °C and 1.4 bar) and sodium azide (NaN_3, 0.1 % final concentration) was additionally added to the river water to inhibit microbial re-growth during the course of the experiments. Control experiments without river sediment were conducted to check for biotic transformation processes in surface water. There, the same setup was used, but the steel columns were solely filled with river water. All experiments were run at room temperature (22 ± 2°C).

Table 4-2: Overview of the different performed column experiments

Exp.	Sediment	Control experiments	Filter velocity (m d^{-1})	No. of replicates
1a)[1,2]	RM1	-	5.0	5
1b)[1,2]	RM1	w[3], s[4]	5.0	4
2)	RM2	s[4]	2.5	2
3)	RM2	w[3], s[4]	5.0	2
4)	RM2	s[4]	10	2
5)[2]	RM3	w[3], s[4]	5.0	2
6)	GR	-	5.0	2
7)	GR	s[4]	10	2
8)	GR	s[4]	10	2

[1] experiments were run with sediments collected at site RM1 with a time difference of approx. eight months, [2] carbamazepine was not spiked , [3] w: river water control without sediment, [4] s: sterile control with autoclaved sediment/river and addition of NaN_3 (0.1 %)

4.3.4 Sampling and Determination of Boundary Conditions

After an equilibration phase of seven days to recover microbial activities after storage of water and sediment at 4 °C, the water in the storage bottles was discarded and replaced by fresh surface water. This water was then spiked with a cocktail of pharmaceuticals (c = 2 mg L^{-1} in high purity water) to obtain an initial concentration of 100 µg L^{-1} in Exp. 1 and 5 and 200 µg L^{-1} in Exp. 2-4 and 6-8, respectively. The spiking solution was well mixed with the surface water in the stor-

age bottle, the peristaltic pump was started and after about fifteen minutes – as soon as the water from the storage bottle had completely replaced the non-spiked water in the connecting PTFE tubes – the first sample was taken before the sediment column at position A. The first sampling at position B was done after about approx. one pore volume had been pumped through the column.

Water was sampled at the three-way cocks without applying any additional suction. In total, about 12 mL of water were sampled. Of the total sample volume, 2 mL were directly used for the determination of pH and dissolved oxygen (DO), respectively. 6 mL of the sample were separated and stored frozen for the subsequent analysis of nitrate and sulfate. The remaining sample volume was transferred into 1.5 mL PP centrifuge tubes and stored frozen for the determination of the pharmaceutical concentrations. For the analysis of total organic carbon (TOC) in the water, additional 10 mL were sampled and stored frozen until analysis.

Sulfate (method adapted from Tabatabai (1974)) and nitrate (Spectroquant nitrate kit, Merck, Darmstadt, Germany) were determined photometrically (DR 3800, Hach-Lange, Düsseldorf, Germany). TOC was measured with a total organic carbon analyzer (TOC V-CPN, Shimadzu, Duisburg, Germany). C_{org} and N of the sediments were determined with a TOC/TNb analyzer (multi N/C 2100, Analytik Jena, Jena, Germany).

Experiments were run for up to four weeks. The sampling intervals were short (intervals less than one day) at the beginning and enlarged during the course of the experiments (intervals up to one week). Samples at sampling point B were taken at every sampling date while samples at point A were taken at the beginning of each experiment and then only occasionally. At each sampling day, the filter velocity was measured by clocking the time until 10 mL had flown into the test tube, and the rotation speed of the peristaltic pump was re-adjusted if necessary.

4.3.5 Analytical Methods

In Exp. 1 and 5, 500 µL of the sample were taken, transferred to small reaction tubes mixed with 50 µL of the surrogate solution (c = 0.5 µg mL^{-1}, aq.). Then, 350 µL of this solution were pipetted into HPLC vials and 150 µL of ACN (8.34 mM HAc) were added. Samples were stored frozen until final analysis. In Exp. 2-4 and 6-8 sample processing was slightly different. Here, 5 µL of different surrogate solutions (c = 5 µg mL^{-1}, aq.) were each added to 1 mL of the sample, well mixed, and passed through a 0.45 µm nylon filter (Rotilabo®, Carl Roth, Karlsruhe, Germany). 350 µL of the filtrate was transferred into an HPLC vial and mixed with 150 µL of ACN (8.34 mM HAc). Except for Exp. 2 and 6, pharmaceutical concentrations were determined using an HPLC-MS/MS system consisting of two binary HPLC pumps (Prostar 210), an autosampler (Prostar 410), and a triple quadrupole mass spectrometer (1200L, all by Varian Inc, Darmstadt, Germany). Chromatographic separation was done using an HPLC column (Luna C18(2)-100A, 150 x 2 mm) and a binary gradient of H_2O and ACN (both 2.5 mM HAc). In Exp. 2 and 6 pharmaceutical concentrations were determined with a UHPLC-MS/MS (Acquity UPLC system; Xevo TQ-S mass spectrometer; Waters, Milford, MA). There, the chromatographic separation was achieved by a binary gradient on a HSS T3 column (100 mm × 2.1 mm, Waters). Solvent A consisted of H_2O:ACN, 95:5 and solvent B of ACN:H_2O 95:5 (both containing 10 mM HAc). In Exp. 1, metoprolol-D_7 was not yet available and

metoprolol was quantified using propranolol-D_7 instead. Instrument calibration and sample quantification was performed using the isotope dilution method and calibration was linear in the range from 0.5 µg L^{-1} to 200 µg L^{-1}.

4.3.6 Calculations and Data Analysis

For calculation of elimination rate constants (of microbial transformation, k_{eli}) the highest determined concentration at port B (after the sediment column) was regarded as reference and starting concentration instead of concentrations in the initial samples taken at sampling port A. This procedure was chosen to avoid obtaining altered k_{eli} values by initial mixing and sorption processes. A descriptive example for this procedure is given in the Appendix (chapter 6.3.3, Figure C- 2). Data from replicate experiments (n = 2–5) were aggregated into one combined dataset. Assuming first-order elimination kinetics, all concentrations were normalized to the reference concentration, logarithmized and the elimination rate constant (k_{eli}) was calculated by applying linear regression to the logarithmized data. Moreover, the half-life time for the elimination for each substance was calculated ($t_H = \frac{\ln(2)}{k_{eli}}$). Only elimination rate constants at a significance level of $p < 0.05$ are reported. For all k_{eli} and t_H values, the standard errors as well as the 95 % confidence intervals were calculated.

For some substances, elimination rate constants changed during the course of the experiment. In these cases, a first/initial regression was performed for the first time period and then a second regression was calculated for the time period after the slope of the regression had changed (also see Figure C- 3 in the Appendix). Rate constants were considered statistically different if their 95 % confidence intervals did not overlap. Derived k_{eli} of all pharmaceuticals at the flow velocity of 5.0 m d^{-1} (Exp. 1a, 1b, 3, 5, 7) were ranked using Spearman's rank coefficient for the individual compounds. The same was applied to the respective elimination rate constants for the three filter velocities (2.5, 5.0, and 10 m d^{-1}) with sediments RM2 and GR (Exp. 2-4 and 6-8). Hereby, the highest elimination rate constant was given the lowest rank. All statistical analyses were performed using the open source software package R (R Development Core Team 2013). K_{eli} values were also taken as input variables for a principle component analysis (PCA) and a cluster analysis (using Euclidean distance) to support the ranking of the biodegradability of pharmaceuticals with complementary statistical testing.

4.4 Results and Discussion

4.4.1 Abiotic control experiments and elimination in surface water

Sterile Controls. Generally, the concentration of all compounds did not decrease with time after an initial equilibration phase. This is exemplarily shown for the filter velocity of 10 m d^{-1} (Exp. 4 and 8) in the Supporting Information (Figure C- 4). In a few cases, a significant and continuous elimination was observed. However, the elimination rates were small compared to the rates in the non-sterile approaches and therefore not considered further (for details see Table C- 2 in Appendix 6.3). For the beta-blockers metoprolol and propranolol the first moments of detection as

well as the time point of the maximum concentration at sampling port B were substantially delayed compared to the other pharmaceuticals. Additionally, the final concentration also indicated a substantial removal from the dissolved phase by sorption to the sediment which was previously reported (Radke and Maier 2014, Ramil et al. 2010). However, in accordance to the other compounds, sorption equilibrium for metoprolol and propranolol was reached rapidly (see Figure C- 4 in the Appendix). Therefore, in real river sediments, when the sediments are continuously exposed to rather constant concentrations of pharmaceuticals in the surface water for a prolonged time period, sorption to sediments is no efficient removal pathway for the investigated substances.

Surface Water Controls. In Exp. 1, 3, and 5, control experiments were run without sediments (only surface water from sites where sediments RM1, RM2, and RM3 were taken) to determine the elimination in surface water. Initially, no or only slow elimination of pharmaceuticals was observed and the respective half-life times in surface water were always > 15 days for all pharmaceuticals (Table C-3). However, for some substances, after an initial lag-phase of six to ten days, a rapid elimination was observed. For bezafibrate and ibuprofen, using river water from RM1 and RM3, concentrations then rapidly decreased and were below the LOQ (2 µg L^{-1}) within a few days (< 5 days for ibuprofen, 6-10 days for bezafibrate). However, due to an insufficient quantity of data points during this fast concentration decrease, calculation of k_{eli} was not possible. Nevertheless, the elimination rates during this rapid concentration decrease are presumably as high as in the experimental approaches with sediment (see below). Similar results were also observed during flume experiments with river water (Kunkel and Radke 2008). There, ibuprofen was rapidly eliminated after a lag-phase of eight days, while naproxen was continuously eliminated at a rate constant corresponding to a t_H of about seven days. For carbamazepine no surface water controls were performed. However, since carbamazepine was not eliminated in the approaches with sediment, it is reasonable to assume that carbamazepine would not have been eliminated during the water control experiments.

4.4.2 Elimination Rates of Different Pharmaceuticals in Various Sediments

The elimination kinetics of the individual pharmaceuticals in the different sediments were similar (see Figure C- 5 to Figure C- 12 in the Appendix). Especially for substances that were quickly eliminated in the test systems, the deviation of elimination rates in individual sediment columns was almost indefinite. Thus, the chosen experimental setup of recirculating sediment columns can be regarded appropriate to derive reproducible k_{eli} values.

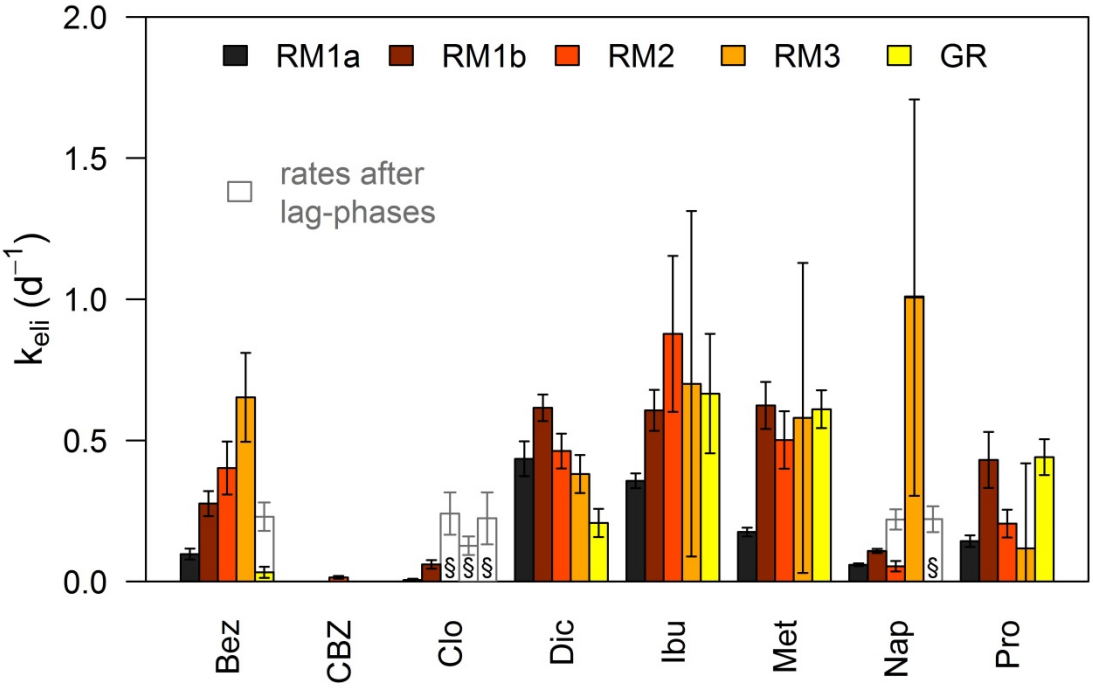

Figure 4-1: Elimination rate constants (k_{eli}, ± 95% confidence intervals, d^{-1}) of pharmaceuticals in different sediments at a filter velocity of 5.0 m d^{-1}. Bez: Bezafibrate, CBZ: Carbamazepine, Clo: Clofibric Acid, Dic: Diclofenac, Ibu: Ibuprofen, Met: Metoprolol, Nap: Naproxen, Pro: Propranolol; CBZ was not spiked in Exp. with RM1a/b and RM3; §: no significant elimination during initial time period; empty bars with grey border: $k_{eli,sec}$ after initial period with slow/no elimination

Except for carbamazepine, all pharmaceuticals were efficiently eliminated during the recirculating columns experiments (Figure 4-1). The concentration decreases were much more pronounced than in the sterile controls and therefore elimination can be attributed to biological processes. The calculated elimination rate constants ranged from relatively slow rates for clofibric acid (0.05-0.25 d^{-1}) up to approx. 1.0 d^{-1} for substances such as diclofenac or ibuprofen (Table C-4). The determined elimination rate constants differed more for the pharmaceutical than with the sediment (Figure 4-1). Therefore, it can be assumed that if a general elimination potential of a substance was determined within a specific river sediment, this elimination behavior can be transferred to other sediments. This hypothesis was confirmed by several statistical analyses. All testing is based on the initial k_{eli}. At first, we calculated the average Spearman's rank coefficient of k_{eli} for the pharmaceuticals in the different sediments (see Figure C- 13a). With the exception of the silty sediment RM3, Spearman's rank coefficients for the elimination rate constants of the pharmaceuticals were correlated (95 % significance level, Figure C- 14). This explicitly means that the elimination kinetics or at least the order of the elimination rate constants of pharmaceuticals in sediments with similar characteristics (i.e., regarding texture and C_{org} content) compared to a reference substance can be potentially predicted. However, it has to be kept in mind, that the sediments were collected only at two river systems and therefore might be not representative for the whole variety of sediments. In contrast, there was no clear correlation between the sediment type and the average rank of elimination rates for all pharmaceuticals in this sediment compared

to other sediments (Figure C- 13b). From a visual analysis, the influence of the sampling the date (RM1a/RM1b) on the elimination kinetics of pharmaceuticals even seems to be more important than the sampling site of the sediments. More specifically, the determined k_{eli} were always higher with the sediment from the second sampling date (RM1b, Figure 4-1). The relationships between k_{eli} of pharmaceuticals and sediment quality were additionally checked by PCA (Figure C- 15a). The first principal component explains about 70%, the second about 18% of the total variance. In the loadings plot of the PCA (black labels in Figure C- 15a) the elimination rate constants of diclofenac, ibuprofen and metoprolol as well as clofibric acid and carbamazepine are grouped. Moreover, the cluster analysis (Figure C- 15b) also strongly differentiated the elimination rate constants of clofibric acid and carbamazepine on the one side and better biodegradable substances (diclofenac, ibuprofen, metoprolol, and propranolol) on the other side. In addition, the PCA also gave evidence that the elimination rate constants in the four sandy sediments (RM1a/b, RM2, GR) are different from the silty sediment RM3 (score plot, red labels in Figure C- 15a). Based on the results from this tool box of different statistical methods, the pharmaceuticals can be divided into three different groups regarding their biotransformation rates in river sediments: i) pharmaceuticals that are rapidly eliminated (diclofenac, ibuprofen, metoprolol), ii) pharmaceuticals that are moderately eliminated (bezafibrate, naproxen, propranolol) and iii) pharmaceuticals with no or only slow elimination (clofibric acid and carbamazepine).

For diclofenac, k_{eli} in all four experiments with sediments from river Roter Main (RM) were not significantly different. However, all rate constants of diclofenac in sediments from river Roter Main were different from the elimination rate determined in river Gründlach sediment (GR). In contrast, the elimination rate constants for ibuprofen and metoprolol in RM1a were significantly smaller than in sediments RM1b, RM2 and GR. If more data points for ibuprofen and metoprolol during experiments with sediment RM3 were available and thus the confidence intervals for the respective k_{eli} had been smaller, most certainly also k_{eli} in RM3 would have been significantly different from those in experiments with sediments RM1a. Generally, the derived elimination rate constants are high and corresponding DT_{50} for the elimination were in the range of about one day for diclofenac, ibuprofen, and metoprolol in various sediments. These DT_{50} are very small compared to DT_{50} values that are commonly determined in static batch experiments (Löffler et al. 2005, Radke and Maier 2014). A more thorough comparison of determined k_{eli} with literature data from different experimental setups is given below.

For some of the substances, a delayed onset of the elimination was observed. For example, clofibric acid was only eliminated in sediments RM1 right from the beginning of the experiment (Table C- 4). During experiments with the other sediments (RM2, RM3, GR) elimination of clofibric acid got going three to seven days after the experiment had been started (also see Figure C- 7 in the Appendix). But then, elimination was fast and half-life times for the degradation of clofibric acid from 2.9 to 5.8 days were determined (white bars with grey border in Figure 4-1). Similar observations of an initial lag-phase with no or only very slow elimination were also made for bezafibrate and naproxen. An overview of these increasing k_{eli} (also called secondary elimination

rate constants from here on, $k_{eli,sec}$) after this initial time span with no or only little elimination is also given in Table C- 5 and discussed in detail in chapter 4.4.4.

4.4.3 Influence of the Filter Velocity on Elimination Kinetics

One aim of this study was to obtain kinetic data for the elimination of pharmaceuticals in river sediments that are less dependent on the experimental setup than data from the commonly performed batch experiments. To this end, the elimination behavior of pharmaceuticals was evaluated at three different filter velocities (2.5, 5.0, and 10 m d^{-1}) in two sandy sediments with similar C_{org} and N content as well as similar texture (RM2, GR, see Table 4-1). In a recirculating and closed experimental system such as the one used in the present study, a change in the filter velocity does not affect the residence time of water and pharmaceuticals in the sediment column compared to tubings and the storage bottle. While the time for the individual passage through the sediment column is longer at a lower filter velocity, the time until is the water is pumped back again from the storage bottle into the sediment is concordantly proportionally increasing. Hence, impacts of the filter velocity on elimination kinetics of pharmaceuticals in our test systems cannot be directly explained by different total residence times in the sediment. However, varying filter velocities can lead to altered boundary conditions (e.g., more reducing conditions at the end of the sediment column due to a longer travel time through the sediment column at the lower filter velocity) and thus influence the elimination kinetics.

The elimination rate constants (k_{eli}, $k_{eli,sec}$) as a function of the filter velocity are shown in Figure 4-2. Since clofibric acid and carbamazepine were only eliminated in some of the experiments, they were omitted from further discussion in this chapter. Their elimination rate constants as well as those for all other pharmaceuticals are given Table C- 6.

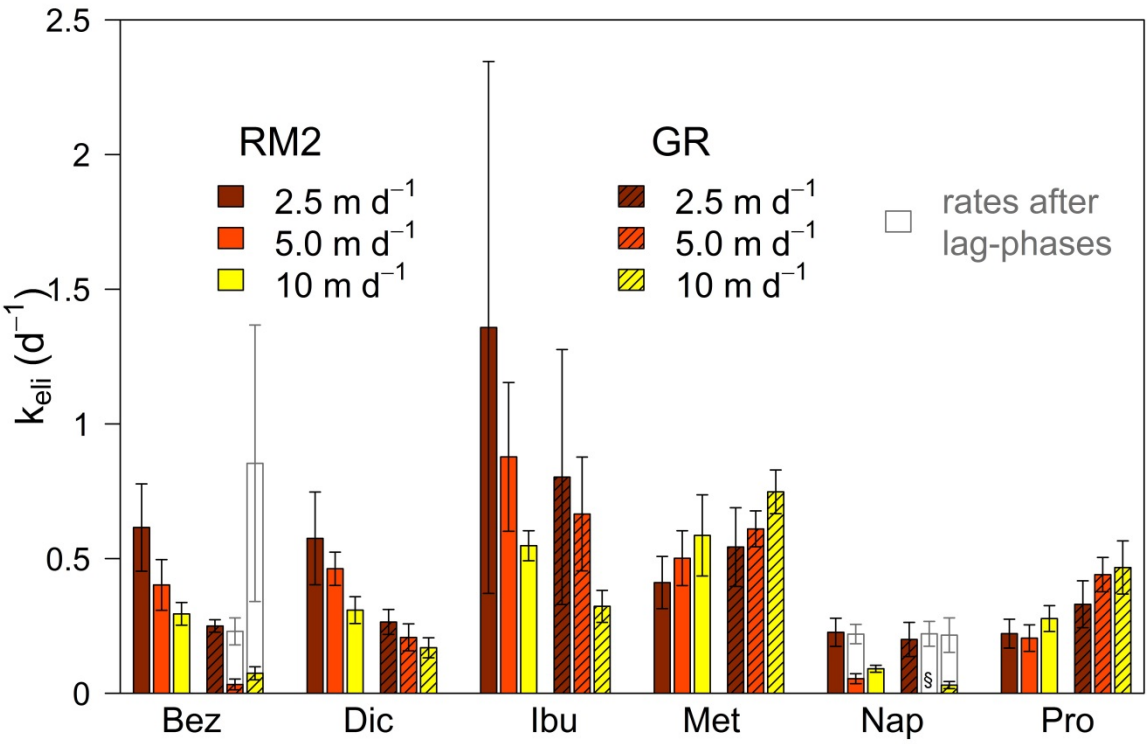

Figure 4-2: Elimination rate constants (k_{eli}, ± 95 % confidence intervals, d^{-1}) of pharmaceuticals at different filter velocities in sediments RM2 and GR.: The elimination rate constants for the filter velocity of 5.0 m d^{-1} are the same as the rates for RM2 and GR in .Bez: Bezafibrate, Dic: Diclofenac, Ibu: Ibuprofen, Met: Metoprolol, Nap: Naproxen, Pro: Propranolol. §: no significant elimination of naproxen during the first three days; empty bars with grey border: $k_{eli,sec}$ after initial period with slow/no elimination

The same relationships between filter velocity and initial elimination rate were observed in experiments with both sediments: pharmaceuticals which were eliminated faster at a higher filter velocity in sediment RM2 were also eliminated with an increased rate constant in experiments with sediment GR. However, variations in the filter velocity changed the elimination rate constants in both sediments only within a factor of 2 and for none of the substances a significant (based on a 95 % significance level) systematic correlation between all filter velocities and elimination rate constants was determined (Table C- 6). But for bezafibrate, diclofenac, and naproxen the elimination rate constants at a filter velocity of 10 m d^{-1} were significantly lower than those in experiments with a filter velocity of 2.5 m d^{-1}. This holds true for both sediments. For example, k_{eli} of diclofenac dropped from 0.575 ± 0.172 d^{-1} to 0.309 ± 0.050 d^{-1} in experiments with sediment RM2 and from 0.265 ± 0.046 d^{-1} to 0.169 ± 0.037 d^{-1} in experiments with sediment GR. Therefore, it can be assumed that the uncertainty of the experiments – due to insufficient data quality and quantity – might prevent from statistically proving a systematic effect of the filter velocity on elimination rate constants. Moreover, while k_{eli} of the less sorptive compounds (bezafibrate, diclofenac, ibuprofen, naproxen) seemed to be inversely related to filter velocity, the elimination rate constants of the more sorptive beta-blockers metoprolol and propranolol slightly increased with filter velocity. Effects of initial sorption processes can be excluded as potential reason for the opposing trend of the beta-blockers since we omitted the first data points – when the sorption equi-

librium had not yet been reached – for the determination of the rate constants (see Figure C- 2 in chapter 6.3.3 in the Appendix). However, the travel time of the beta-blockers for each sediment passage should be longer than for the acidic pharmaceuticals due to permanent sorption and desorption processes. Therefore, microorganisms attached to the sediment matrix might not be able to as efficiently catch and then degrade the hydrophilic pharmaceuticals at higher filter velocities, the reduced effective filter velocity for the beta-blockers might counteract this effect.

The different filter velocities had no influence on the boundary conditions in both sediments. In all experiment the dissolved oxygen concentration at port B was > 7 mg L^{-1} throughout the experiment see Figure C- 18), and no concentration decrease of the electron acceptors nitrate and sulfate was observed during the course of the experiments (see Figure C- 16 and Figure C- 17). Hence, the differing k_{eli} have to be explained by other factors than the prevailing redox conditions. Similar to the experiments with a filter velocity of 5.0 m d^{-1} (previous chapter 4.4.2), an increasing elimination speed over the course of the experiment was observed for some substances at a filter velocity of 10 m d^{-1} (empty bars with grey borders in Figure 4-2). Exemplarily, k_{eli} of bezafibrate in experiments with sediment GR increased after about five days from the initial rate of 0.074 ± 0.024 d^{-1} to 0.854 ± 0.513 d^{-1} (Table C- 6 and Table C- 7). In contrast, at a filter velocity of 2.5 m d^{-1}, elimination in both sediments (RM2 and GR) started right at the beginning and the rate remained constant throughout the experiments. Apparently, there is a delayed onset of biotransformation processes at higher filter velocities. Up to date we cannot give any plausible explanation for this unexpected phenomenon.

However, our experiments generally reveal that the filter velocity has only a minor influence compared to the sediment characteristics on the elimination kinetics of pharmaceuticals. Hence, the test design is applicable to derive process based elimination rates in advectively flowed-through sediments compared to majorly diffusion-limited rates under less steerable hydraulic conditions in batch experiments. Thus elimination rates determined with our introduced test design can be more easily extrapolated to other test systems and the situation in real rivers since it is feasible to precisely quantify and describe the (hydrological) boundary conditions under which the elimination rates were determined.

4.4.4 Assessment of the Lag-Phases and Increasing Elimination Rates over the Course of the Experiments

As shown above, especially for the substances with small initial k_{eli} we often observed an enhanced elimination rate over the time course of the experiments. For some substances (e.g., clofibric acid), we even observed a rapid elimination after an initial phase where no elimination occurred (see Figure C- 7). There are several potential reasons for a change in the k_{eli} during an experiment:

- The concentration and composition of the TOC can affect the transformation rate of pharmaceuticals (Rauch-Williams et al. 2010). While we did not determine the bioavailable TOC fraction, the total TOC over the time span of the experiments in the surface water was stable in all

experiments (8-12 mg L^{-1} with sediment and water from river Roter Main, 4-6 mg L^{-1} with sediment and water from river Gründlach, see Figure C- 16 and Figure C- 17). Hence, we assume that changes in the TOC content are not (exclusively) responsible for the increasing k_{eli}.

- The elimination of pharmaceuticals is often strongly governed by the prevailing redox conditions in the sediments (Conkle et al. 2012). Therefore, the changing k_{eli} might be a result of more/less reducing conditions over time. However, the redox conditions did not change during the course of the experiments as the concentrations of dissolved oxygen, nitrate, sulfate and pH were constant throughout the experiments (see Figure C- 16, Figure C- 17, Figure C- 18 in the Appendix). Hence, changing redox conditions can be excluded as reason for the increasing k_{eli}.

- Often microbial processes need an adaptation phase to substances in order to be able to degrade them (Pieper et al. 2010). Except for RM1, all sediments and surface waters were taken at sites where sediments are continuously exposed to substantial amounts of wastewater. However, there was no systematic deviation in the occurrence of lag-phases in experiments with the different experiments. Hence, adaptation of microbes is unlikely to cause the increasing k_{eli}.

- Due to sampling, the total volume of the water in the recirculating system decreased over time (about 15-20 % of total initial water volume was used for sampling). Therefore, the proportion of water in the storage bottle compared to water in the sediments decreased over time and the relative residence time of pharmaceuticals in the sediments slightly increased. Assuming that the major part of the transformation takes place during the sediment passage (see Kunkel and Radke (2008), also confirmed by the surface water controls of this study) this could lead to an increased k_{eli}, i.e., of those substances that were not removed during the initial stages of the experiment.

- Since the elimination of pharmaceuticals is predominantly caused by microbial processes (confirmed by the sterile controls), an increasing microbial density and/or activity over time might also lead to an increasing k_{eli}. The sediments and water were stored at 4°C before the experiments were conducted. Although, we let the systems equilibrate for one week, this time might have been not sufficient to re-activate the microbes.

- Often, the increase in k_{eli} started when the rapidly biodegradable pharmaceuticals (e.g., ibuprofen) were eliminated from the test systems (see example for bezafibrate during Exp. 7, Figure C- 20). Therefore, it can be hypothesized that the presence of some pharmaceuticals inhibits the elimination/transformation of others, e.g., ibuprofen actively inhibits the bezafibrate transformation or bezafibrate is not transformed by microorganisms until ibuprofen is not present anymore. However, in previous batch experiments with the same substances (Radke and Maier 2014) no change in elimination kinetics during single and mixture incubations was determined for the substances where a $k_{eli,sec}$ was observed during the column experiments.

To address some of the open questions we performed an additional experiment. After Exp 1b was concluded, the surface water was re-spiked with the cocktail of pharmaceuticals (no carbamazepine). The sediment columns were treated in two ways: the experimental setup remained

unchanged (Exp. 1b.a) or the water was disconnected from the sediment (Exp. 1b.b, for details see Table C- 1). Experiments were immediately re-started after the re-spiking. This time experiments were run without replicates. In Exp. 1b.a (no change of experimental setup), all spiked pharmaceuticals were eliminated right from the beginning (no lag-phases, see Table C- 8). For metoprolol the same k_{eli} than in Exp. 1b was calculated (Table C- 8). In contrast, for bezafibrate, clofibric acid, diclofenac, and ibuprofen the elimination kinetics were significantly higher after re-spiking. The elimination of propranolol even was too fast to obtain a sufficient number of data points for calculating a significant k_{eli}. For naproxen, the elimination rate in the re-spiking experiment (0.286 ± 0.060 d^{-1}) was equal to the observed $k_{eli,sec}$ in Exp. 1b (0.220 ± 0.036 d^{-1}). Thus, while the results are not totally conclusive, we cannot exclude that the occurrence of secondary elimination kinetics indeed originated from an adaptation of microorganism to the presence of the pharmaceuticals. In re-spiking experiment 1b.b where the storage bottle had been disconnected from the sediment column, elimination of ibuprofen, metoprolol, naproxen and propranolol started immediately (Table C- 8, Figure C- 19). In the water-only test (Table C- 3), these substances mostly had a lag-phase before the elimination started or they were continuously eliminated at a much smaller k_{eli}. For example, naproxen that was eliminated at a maximum rate constant of 0.039 ± 0.005 d^{-1} during the water controls ($k_{eli,sec}$ in Exp. 1b), while it was now eliminated at a k_{eli} of 0.129 ± 0.020 d^{-1}. Moreover, clofibric acid – that was not eliminated in all three water only controls over a time span of at least 16 days (Table C- 3) – was eliminated at a k_{eli} of 0.110 ± 0.027 d^{-1} starting about four days after the re-spiking. The elimination of bezafibrate started after about six days, and then the elimination rate constant was 0.313 ± 0.091 d^{-1}. This rate is statistically not different from the rate determined in the original experiment (Exp 1b; 0.402 ± 0.094 d^{-1}) and in the re-spiking experiment with the sediment (Exp. 1b.a, 0.509 ± 0.177 d^{-1}). However, in accordance to the water-only controls, diclofenac – which was quickly eliminated in all experiments with sediment contact – was only eliminated slowly. So, while we could observe an enhanced elimination for some pharmaceuticals in surface water after it had been in continuous exchange with the sediments for a prolonged time, this effect seems to be substance specific and not the sole explanation for the occurrence of secondary elimination rate constants.

In conclusion, we cannot give a final explanation for the occurrence of periods with different elimination rates. Most probably, the increased elimination is a combined effect of i) the sampling procedure resulting in a higher relative residence time in the sediments due to decreasing volumes of water in the storage bottle , ii) an onset of degradation processes in the storage bottle presumably by a transport of microorganisms from the sediment to the storage iii) an inhibition of transformation of substances while more favorable substances are present, and iv) an enhanced adaption of microorganisms to the pharmaceuticals leading to degradation of originally unfavorable carbon sources.

4.4.5 Comparison with Static Batch Systems, Flume Experiments, and Modeling Data

In static batch experiments the exchange fluxes across the surface water/sediment interface are driven predominantly by diffusive processes (slightly shaking of the bottles and/or aeration of

the supernatant). Therefore, these studies neglect advective fluxes and probably underestimate the actual transformation rates in river sediments. The elimination rate constants derived in the present study are almost always substantially higher than previously reported. For example, Löffler et al. (2005) reported a DT_{50} value for clofibric acid of 82 ± 12 days (elimination from the water phase) compared to t_H as low as 2.9 ± 0.4 days after an initial lag-phase during Exp. 3 in this study. Also the dissipation of the well biodegradable analgesic drug ibuprofen was substantially faster since half-life times in all experiments ranged from 0.5-1.9 days compared to approx. 6 days in Löffler et al. (2005). In contrast, carbamazepine was not or only very slowly eliminated in both studies (t_H > 100 days, respectively). In sediment/water experiments with a sandy sediment, Ramil et al. (2010) determined DT_{50} values of metoprolol and propranolol from the water phase of 8.7 ± 1 and 1.8 ± 0.3 days which are in the same range than t_H in this work (0.9-3.9 days for metoprolol and 1.5-5.6 days for propranolol). However, in contrast to the present study, they did not exclude the first data points where the sorption equilibrium had not yet been reached. If we calculated DT_{50} from the water phase instead of biological elimination rate constants the respective values for metoprolol and propranolol would drop to 1.1 and 1.6 days during Exp. 3. Hence, it can be assumed that the transformation rates of the beta-blockers are actually higher during the recirculating column experiments. Ternes et al. (2002) reported also no elimination in batch experiments with groundwater and a sandy sediment over a time span of 28 days for carbamazepine, clofibric acid, and diclofenac. Only for bezafibrate, a slight concentration decrease (residual concentration: 76 ± 32 %) was observed. In contrast, during the recirculating column experiments these three pharmaceuticals were completely eliminated within 28 days (except for carbamazepine in all experiments and clofibric acid in Exp. 1a/b).

In static batch experiments with the same sediments than those used in this work much smaller elimination rate constants were derived (Radke and Maier 2014). For example, the DT_{50} values for bezafibrate ranged from 10 to 56 days (in this study: 1.1 days during Exp. 2 and 5 to 21 days in the initial time period with a low transformation rate of Exp. 7) and ibuprofen was eliminated with DT_{50} values of about 5-8 days (0.5-1.9 days in this study). In an experimental setup similar to the approach of this study also very high elimination rate constants of diclofenac were observed (Gröning et al. 2007). The authors calculated dissipation constants for diclofenac of 0.17-1.0 d^{-1} (DT_{50}: 0.7-4.1 days) which agree well with the determined k_{eli} values of 0.2-0.6 d^{1} (DT_{50}: 1.1-4.1 days) of this study. In more complex and realistic flume studies with sediment RM1, half-life times of pharmaceuticals decreased with the flow velocity in the surface water which can be explained by increased exchange fluxes across the sediment surface into the sediments at the higher flow velocity (Kunkel and Radke 2008). However, even at the higher flow velocity the half-life times (bezafibrate: 4.3 days, diclofenac: 5.5 days, ibuprofen: 2.5 days, naproxen: 10.3 days) were still substantially lower than the ones obtained in this study (minimum values for half-life times: bezafibrate: 0.8 ± 0.2 days, diclofenac: 1.1 ± 0.04 days, ibuprofen: 0.5 ± 0.1 days, naproxen: 0.7 ± 0.2 days). During a reactive tracer test at a small river, bezafibrate, diclofenac, metoprolol, and naproxen were not eliminated within the river stretch of 16.2 km (Kunkel and Radke 2011, Riml et al. 2013). In contrast, ibuprofen and clofibric acid were efficiently removed from the sur-

face water. By a modeling approach, elimination rate constants in the storage zones of the river – which can be primarily set equivalent to the sediments – for ibuprofen and clofibric acid of 10.4 d^{-1} and 0.75 d^{-1} were calculated (Riml et al. 2013). These rate constants are substantially higher than the maximum values determined in this study (ibuprofen: 1.4 ± 0.3 d^{-1}, clofibric acid: 0.2 ± 0.03 d^{-1}). Using field-scale push-pull-tests, extremely fast *in situ* elimination kinetics of diclofenac were calculated (Huntscha et al. 2013). The reported half-life times range from 1.0 to 3.5 hours which are more than an order of magnitude faster than those determined in this study (t_H: 17-98 hours). Hence, while the concept of recirculating sediment columns can help to achieve more realistic biotransformation rates in sediment/water systems actual *in situ* attenuation rates in river sediments can still be substantially higher.

4.4.6 Comparison to Observed Field Elimination Data

The final aim of the study was to compare the derived elimination rate constants in the recirculating sediment columns to observed elimination kinetics of pharmaceuticals during field monitoring studies. In field studies performed at the two rivers where the sediments were taken from (river Roter Main, river Gründlach), completely different behavior of the investigated pharmaceuticals was observed. In the field study at river Roter Main (Radke et al. 2010), no elimination of diclofenac and bezafibrate and a minor load reduction of naproxen was observed. In contrast, at river Gründlach diclofenac, naproxen, and bezafibrate were eliminated (up to 70 % of the incoming load, Kunkel and Radke (2012)). The length of the investigated river stretches (13.6 km for river Roter Main vs. 12.5 km for river Gründlach) and the respective travel times of water and solutes (approx. 30 hours at river Roter Main vs. 18 hours at river Gründlach) were in the same range at both rivers. Hence, the enhanced elimination at river Gründlach is not related to a higher residence time of substances in the river stretch. While phototransformation of diclofenac might be a more relevant elimination process at river Gründlach compared to river Roter Main, it can be neglected as major elimination pathway for bezafibrate, naproxen, and the two beta-blockers (Kunkel and Radke 2012). As shown above, both sediments are able to efficiently transform/eliminate pharmaceutical residues (Table C- 4 to Table C- 7, Figure 4-1 and Figure 4-2). Except for carbamazepine, all pharmaceuticals were removed in the test systems. Moreover, the elimination kinetics were by trend even faster in all three sediments from river Roter Main, which is opposite to the findings in the field. Only the elimination kinetics of the beta-blockers metoprolol and propranolol were in the same range for sediments from both rivers. However, the removal of beta-blockers was not addressed in Radke et al. (2010) and thus no comparison between the determined elimination potential in river sediments and observed elimination in field monitoring can be drawn. Overall, it can be stated that the observed contradiction in the elimination behavior in the field studies cannot be caused by different elimination kinetics or elimination potentials of pharmaceuticals in the respective river sediments. In fact, the different elimination rates at the two river stretches are most likely a result of the different hydraulics of the rivers. River Gründlach is a much smaller river with a smaller ratio of surface water volume to sediment cross-section area and therefore, a much more intense exchange of surface water and pore water can

be assumed. Thus the potential for remediation of pharmaceutical residues – which is available in both rivers – is more efficiently used in river Gründlach.

The innovative experimental approach applied in the current study allows addressing the fate of organic micropollutants in river sediments under more realistic (hydraulic) conditions than in usually performed batch experiments under static conditions. Therefore, derived elimination kinetics were generally faster and more realistic than those determined static batch systems. Moreover, our results indicate that the differences in the elimination rates for a specific substance (under aerobic conditions) between various sediments are small. This especially means that substances that are well biodegradable in one sediment are most likely also rapidly transformed in other sediments. However, even with this more dynamic experimental setup it is next to impossible to directly translate the results of the laboratory studies to an actual prediction of attenuation rates due to biological processes in sediments in real river systems. Nevertheless, the more realistic kinetic data on the elimination in river sediments will substantially improve the predictive value of fate models of pharmaceutical residues if the exchange fluxes between surface water and sediments as well as the residence time in the hyporheic zone are known. The insight on the extent of hyporheic exchange as major governing factor for the biological attenuation of organic (micro-)pollutants such as pharmaceutical residues in rivers can and should also be used when setting up new management strategies for rivers and streams. Revitalization of smaller streams with pool-riffle sequences as well as meanders cannot only minimize the risks of flooding catastrophes downstream but also help to reduce the loads of pharmaceuticals residues in streams and thereby improve the surface water quality.

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5 OVERALL SUMMARY AND FINAL CONCLUSIONS

The primary goal of this work was to gain new insight into the fate of organic micropollutants such as pharmaceutical residues in rivers and river sediments by quantitatively determining the individual elimination processes. However, this quantification in rivers is complicated by various spatio-temporally dynamic parameters such as the input of pharmaceuticals into rivers, river discharge, interactions of surface water and groundwater, meteorological and hydrological situation, and sediment properties. Therefore, prior to this work, no systematically derived elimination data for pharmaceutical residues which allowed the differentiation between individual elimination pathways in river systems was available.

This knowledge gap was closed by developing and applying innovative methodologies both in adapted field monitoring campaigns combined with *in situ* experimental studies and during well-designed laboratory work. With the latter it was proven that all tested river sediments possess the potential to degrade of pharmaceuticals rapidly. The derived transformation rates were generally much higher than biotransformation rates reported in studies which used static batch experiments analogously to OECD guideline 308. In these tests the biotransformation potential of river sediments is not fully exploited since it is limited by exclusively diffusive transport into the sediment as well as by the formation of anaerobic zones within the sediment. Hence, these standard experimental approaches are not suitable to determine kinetic transformation rates that can be extrapolated to real river systems.

The concept of injecting pharmaceuticals into a river during the tracer test and downstream sampling of the river water simplified the mass balancing of pharmaceuticals. In contrast to the normal situation in rivers, the amount of each substance entering a river stretch was precisely known. Moreover, the shape of the breakthrough curves of inert tracers revealed that the interactions between the main channel and transient storage zones such as the hyporheic zone were only little. However, pharmaceuticals such as ibuprofen or clofibric acid were eliminated. Phototransformation was negligible due to a high turbidity of the river water and low radiation intensities. Thus their elimination was very likely caused by attenuation processes at river areas with high densities of in-stream biofilms, i.e., at the surface water/sediment interface or submerged macrophytes. A subsequent analysis of the tracer test using a biogeochemical model framework enabled differentiating between elimination processes in the streaming surface water and transient storage zones. There, the importance of the storage zones as major elimination compartment for pharmaceuticals during river transport – even at small interactions of surface water with the sediment – was solidified by the derivation of significantly higher transformation rates in these zones compared to the main channel.

Under favorable conditions, pharmaceuticals can be efficiently eliminated from the surface water within short river stretches and travel times of less than 24 hours. Both phototransformation and biotransformation in the river sediments can contribute substantially to the overall attenuation. Moreover, the use of carbamazepine as reference substance allows the calculation of

actual attenuation rates of eight pharmaceuticals within river stretches independent of dilution processes. A strategic combination of time-resolved composite sampling with *in situ* phototransformation experiments and pore water sampling enabled differentiating between individual elimination processes. Application of cutting-edge analytical methods like chiral analyses helped to definitely attribute the elimination of the beta-blocker metoprolol along the river stretch to biological processes in the river sediments.

In total, this work provided a clear picture of the relevance of individual attenuation processes of organic micropollutants in rivers. It was revealed that a substantial contribution of photolysis to the total elimination is limited to pharmaceuticals that are resistant against biotransformation processes and concurrently are highly susceptible to photodegradation. In contrast, it was clearly highlighted that under favorable conditions – i.e., small rivers with a high hyporheic exchange – biotransformation in the river sediments constitutes the most promising attenuation pathway. The insight on the importance of the exchange fluxes across the surface water/sediment interface for the attenuation of pharmaceutical residues in rivers can and should also be used when setting up new management strategies for rivers and streams to maximally exploit the self-purification potential of rivers. Finally, it was shown that intelligent and thoroughly planned sampling strategies in combination with sophisticated analytical methods can lead to detailed insight into the processes that determine the fate of pharmaceuticals in rivers. Hence, this work provides an unprecedented masterpiece of reliable elimination data of pharmaceuticals in rivers. However, the direct determination of biotransformation rates *in situ* still remains an open challenge for environmental scientists. Potential approaches to face this challenge are coupling of small-scale push pull tests with flume experiments on the laboratory-scale or heat pulse tracer techniques on the field-scale. But until these avant-garde techniques will be fully applicable for routine analysis a lot of water and substances will have flowed under the bridge…

6 APPENDIX

6.1 SUPPORTING INFORMATION TO CHAPTER 2

A Reactive Tracer Test to Evaluate the Fate of Pharmaceuticals in Rivers

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6.1.1 LIST OF TABLES FOR APPENDIX A

6.1.2 List of Figures of Appendix A

6.1.3 Methods

Table A- 1: Coordinates of the injection site and the sampling sites at the tracer test

Site	Latitude	Longitude
Injection Site	*59° 52' 49'' N*	*17° 17' 21'' E*
Site I	*59° 52' 35'' N*	*17° 17' 02'' E*
Site II	*59° 51' 12'' N*	*17° 17' 02'' E*
Site III	*59° 50' 01'' N*	*17° 18' 32'' E*
Site IV	*59° 49' 04'' N*	*17° 19' 40'' E*
Site V	*59° 47' 57'' N*	*17° 22' 20'' E*

Table A- 2: Properties of sediment and water in Säva Brook at the tracer test

Water		Sediment	
pH	5.5	*Sand (%)*	0
T (°C)	12.4-16	*Silt (%)*	69
DOC (mg L^{-1})	16.2 ± 2.2	*Clay (%)*	31
		C (%)	2.4
		N (%)	0.08

LC/MS analyses

Choice of the LC/MS instruments

Samples from preliminary experiments were analyzed with UPLC-QToF/MS, and all samples from the tracer experiment were initially also analyzed with this instrument. Due to a lower sensitivity of the instrument for naproxen than for the other compounds, we decided to inject twice the amount of naproxen compared to the five other pharmaceuticals. Since concentrations at sites IV and V were in the range of the LOQ, we decided to re-analyze the samples with the HPLC-MS/MS system which had a higher sensitivity for all substances. Unfortunately, due to co-elution of other substances, were not able to analyze diclofenac and metoprolol with the HPLC-MS/MS system and therefore report the results obtained by UPLC-QToF/MS.

Bezafibrate, clofibric acid, ibuprofen, and naproxen

Bezafibrate, clofibric acid, ibuprofen, and naproxen were analyzed by an HPLC-MS/MS system consisting of two ProStar 210 solvent delivery pumps, a ProStar 410 autosampler, a column oven, and a 1200L triple quadrupole mass spectrometer (all by Varian Inc., Darmstadt, Germany). HPLC separation was performed on a Synergi Fusion-RP 80A column (150 x 2 mm, Phenomenex, Aschaffenburg, Germany) with a water-acetonitrile gradient elution; column temperature was set to 30 °C, and acetic acid was added to the mobile phase at a concentration of 2.5 mM. The ion source was operated in negative electrospray ionization mode. Calibration curves were generally linear ($r^2 > 0.99$) for all compounds. Standards in the range from 0.5 to 100 µg L^{-1} were used for quantification, and samples were quantified by isotope dilution.

Diclofenac and Metoprolol

An ultra-performance liquid chromatography system coupled to a quadrupole time-of-flight mass spectrometer (UPLC-QToF/MS, Waters, Milford, MA, USA) equipped with an electrospray source was used for the analysis of diclofenac and metoprolol. The column (Acquity HSS T3, 100 mm × 2.1 mm, dp = 1.8 µm; Waters) temperature was kept at 60 °C. 5 µL of sample were injected; the mobile phase consisted of 10 mM acetic acid in water (A) and 10 mM acetic acid in acetonitrile (B). A linear gradient (flow rate 0.3 mL min^{-1}) from 5 % B to 90 % B in 5 min was used, followed by 2 min at 90 % B. Diclofenac was analyzed in negative-ionization mode, metoprolol in positive-ionziation mode. Standards in the range from 5 to 200 µg L^{-1} were used for quantification, and samples were quantified by isotope dilution. Sulfadimethoxine in methanol (100 ng mL^{-1}) was used as lockspray solution in both positive and negative ion mode. The lock-spray frequency was 5 s, with 5 scans to average.

Calculation of mass recovery of pharmaceuticals

To exclude errors due to incomplete dissolution of conservative and reactive tracers, the breakthrough curves at site I was used as reference for calculation of the relative mass recoveries instead of the injected mass. The concentration integral (*Int*) of each substance at a sampling site was calculated using the trapezoidal rule:

$$Int = \frac{1}{2}\sum_{i=2}^{i=N-1}\left(c_{i+1}+c_i\right)\cdot\left(t_{i+1}-t_i\right)+\frac{1}{2}\cdot c_1\cdot\left(t_2-t_1\right)+\frac{1}{2}\cdot c_N\cdot\left(t_N-t_{N-1}\right) \quad \text{Equation A- 1}$$

where N is the number of samples in the respective peaks at each sampling site, t is the time after injection, and c is the concentration.

To correct for dilution along the study reach, a dilution factor ($f_{Dil,site\_x}$) was calculated for sites II through V based on the integrals of the uranine breakthrough curves.

$$f_{Dil,site\_x} = \frac{Int_{Uranine,site\_I}}{Int_{Uranine,site\_x}} \quad \text{Equation A- 2}$$

The mass recovery (*mr*) of each pharmaceutical at sites II-V was then calculated as follows.

$$mr(\%)_{phar,site\_x} = \left(\frac{Int_{\text{Equation A-1,x}}\cdot f_{Dil,site\_x}}{Int_{phar,Site\_I}}\right)\cdot 100\% \quad \text{Equation A- 3}$$

Pictures of the tracer injection

Uranine Rhodamine WT Pharmaceuticals

Estimation of mixing length of tracer solutions with river water

The mixing length of the dye tracers and the pharmaceutical solutions with the river water was calculated as described in a USGS Guideline (Kilpatrick and Wilson 1989). Using strongly conservative assumptions of a mean flow velocity of 30 cm s^{-1}, a channel width of 10 m, an inclination of 0.001, a river depth of 1 m, and a centre channel injection, the mixing distance was 185 m. This mixing length is much shorter than the distance from the injection site to site I (1,500 m) and thus complete mixing up to site I must have occurred. In previous work at another river, we verified this calculation by measurements (Radke et al. 2010).

Additionally, there were some “rapids” between the injection site and site I where the inclination was steeper, flow velocity was higher and flow was turbulent (see picture below). Under these conditions, a rapid and homogeneous mixing can be assumed. [Note: this picture was taken between sites II and III, but the situation there is pretty similar to the one between the injection site and site I.]

Table A- 3: Physicochemical data and reported environmental behavior of pharmaceuticals and fluorescence tracers used at the tracer test

	Bezafi-brate	**Clofibric Acid**	**Diclo-fenac**	**Ibu-profen**	**Meto-prolol**	**Napro-xen**	**Ura-nine**	**Rhoda-mine WT**
pK_a	3.6 (Beausse 2004)	3.2 (Mersmann 2003)	4.16 (Rafols et al. 1997)	4.59 (Avdeef 2007)	9.7 (Jouyban et al. 2003)	4.45 (Avdeef 2007)	1.95, 5.05, and 7.00 (Menzel et al. 2002)	5.1 (Shiau et al. 1993)
			4.14 (Avdeef 2007)			4.2 (Al-Rajab et al. 2010)		
log(K_{OW}) (neutral species)	4.25 (Mompela t et al. 2009)	2.57 (Hansch et al. 1995)	4.51 (Avdeef et al. 1998)	3.97 (Avdeef et al. 1998)	1.88 (Hansch et al. 1995)	3.18 (Jones et al. 2002)	-0.39 (Sabatini and Alaustin 1991)	-1.33 (Sabatini and Alaustin 1991)
		2.84 (Henschel et al. 1997)	4.4 (Cleuvers 2004)	3.5 (Cleuvers 2004)		3.3 (Cleuvers 2004)		
K_d (L kg^{-1})	n. s. (Kunkel and Radke 2008)	5 (Ternes et al. 2004)	0.72 (Jones et al. 2002)	453.79 (Jones et al. 2002)	65 (Wick et al. 2009)	217.2 (Jones et al. 2002)	0.05 - 0.3 (Sabatini and Alaustin 1991)	2.5 - 15.7 (Sabatini and Alaustin 1991)
		0.3 (Löffler et al. 2005)	16 (Ternes et al. 2004)	7.1 (Ternes et al. 2004)	1.75 - 7.3 (Ramil et al. 2010)	13 (Joss et al. 2006)		15 - 114 (Everts and Kanwar 1994)
			n. s. (Lin and Gan 2011)	n. s. (Lin and Gan 2011)		2.9 (Lin et al. 2006)		
				0.7 (Lin et al. 2006)				
Biological half-life time (d)	4 - 8 (Kunkel and Radke 2008)	119 (Löffler et al. 2005)	5.5 - 18.6 (Kunkel and Radke 2008)	2.5 - 5.1 (Kunkel and Radke 2008)		10.3 - 13.9 (Kunkel and Radke 2008)		
	1 (Quintana et al. 2005)		1 - 4 (Al-Rajab et al. 2010)	5 (Quintana et al. 2005)		25 (Quintana et al. 2005)		
			n.s. (Gonzalez et al. 2006)	10 -50 (Lin and Gan 2011)		17 - 69 (Lin and Gan 2011)		
			5 – 30 (Lin and Gan 2011)					
Biological transfor-mation rate (L g^{-1} d^{-1})	2.1 - 4.5 (Joss et al. 2006)	0.1 – 0.8 (Joss et al. 2006)	< 0.1 (Joss et al. 2006)	9 - 35 (Joss et al. 2006)	0.38 (Ramil et al. 2010)	0.4 - 1.9 (Joss et al. 2006)		
				7 – 8 (Smook et al. 2008)				
Phototrans-formation rate k (d^{-1})	n.s. (Radke et al. 2010)	0.01 (Radke et al. 2010)	0.37 (Radke et al. 2010)	1.44 (Packer et al. 2003)	n. s. (Kunkel and Radke 2012)	0.16 (Radke et al. 2010)	0.1 - 0.4 (Smart and Laidlaw 1977)	0.0034 - 0.001 (Smart and Laidlaw 1977)
		0.115 (Packer et al. 2003)	28.37 (Packer et al. 2003)	0.001 - 0.03 (Peuravuori and Pihlaja 2009)	0.096 - 0.475 (Liu et al. 2009)	23.62 (Packer et al. 2003)		

n. s.: no significant observation

6.1.4 RESULTS AND DISCUSSION

Table A- 4: Concentration integrals (ng L^{-1} h) of rhodamine WT and uranine at the sampling sites during the tracer test

	Rhodamine WT	Uranine
Site I	20.5 ± 0.6	20.1 ± 0.5
Site II	19.0 ± 0.5	17.5 ± 0.5
Site III	17.5 ± 0.4	17.4 ± 0.4
Site IV	14.9 ± 0.3	15.5 ± 0.3
Site V	13.4 ± 0.3	13.5 ± 0.4

Quality Assurance

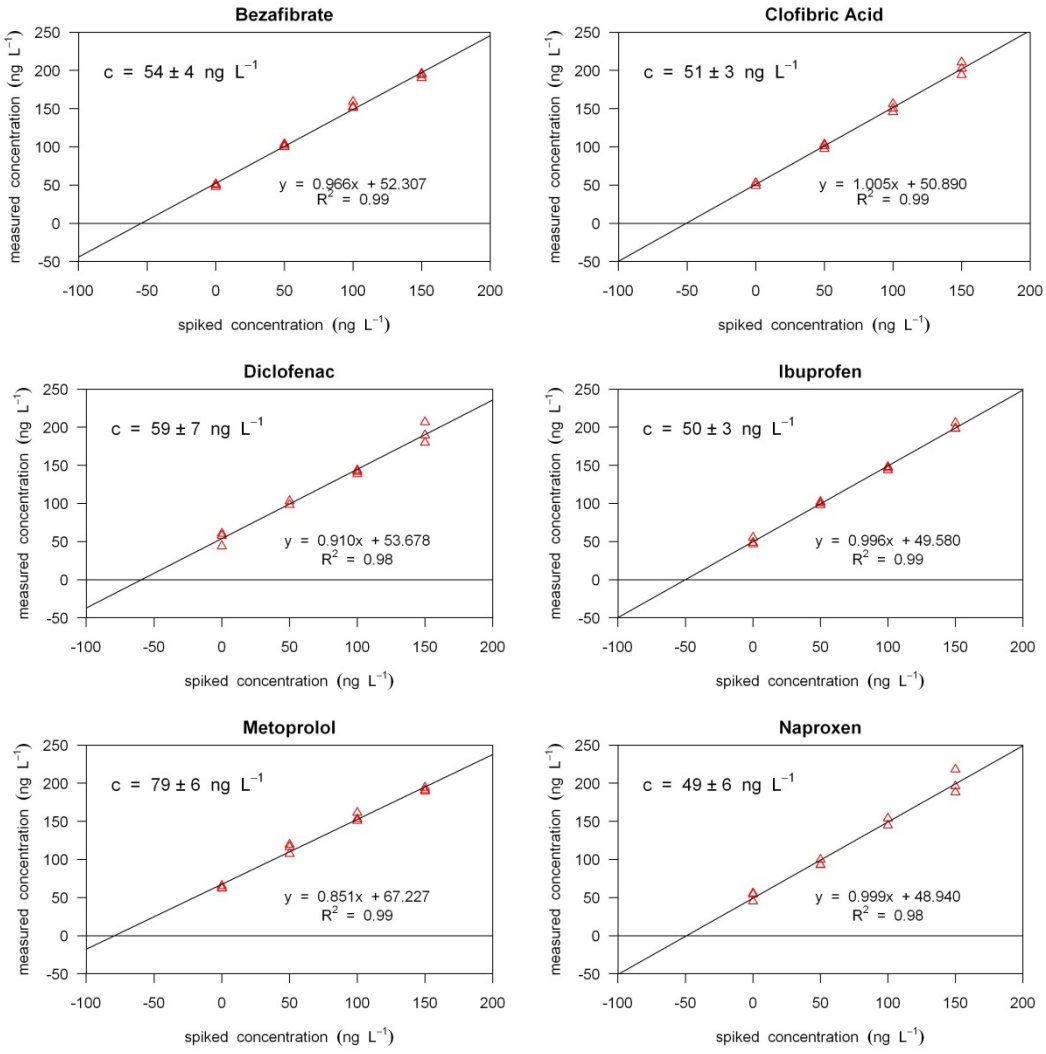

Figure A- 1: Results of the standard addition experiment for the six pharmaceuticals for the tracer test; c: calculated concentration in the reference sample (nominal concentration: 50 ng L^{-1})

Table A- 5: Recovery rates (%, average ± standard deviation) calculated from the standard addition analysis experiment of the substances used at the tracer test

Bezafibrate	Clofibric Acid	Diclofenac	Ibuprofen	Metoprolol	Naproxen
101 ± 3	101 ± 3	100 ± 10	100± 4	110 ± 13	99 ± 7

Table A- 6: Inter-day variation of the concentration (ng L^{-1}) of the reference sample used at the tracer test (average ± standard deviation); in parentheses: number of measurements.

Bezafibrate	Clofibric Acid	Diclofenac	Ibuprofen	Metoprolol	Naproxen
80 ± 7 (10)	86 ± 6 (9)	77 ± 11 (9)	88 ± 6 (10)	66 ± 0 (3)	87 ± 5 (9)

Discharge

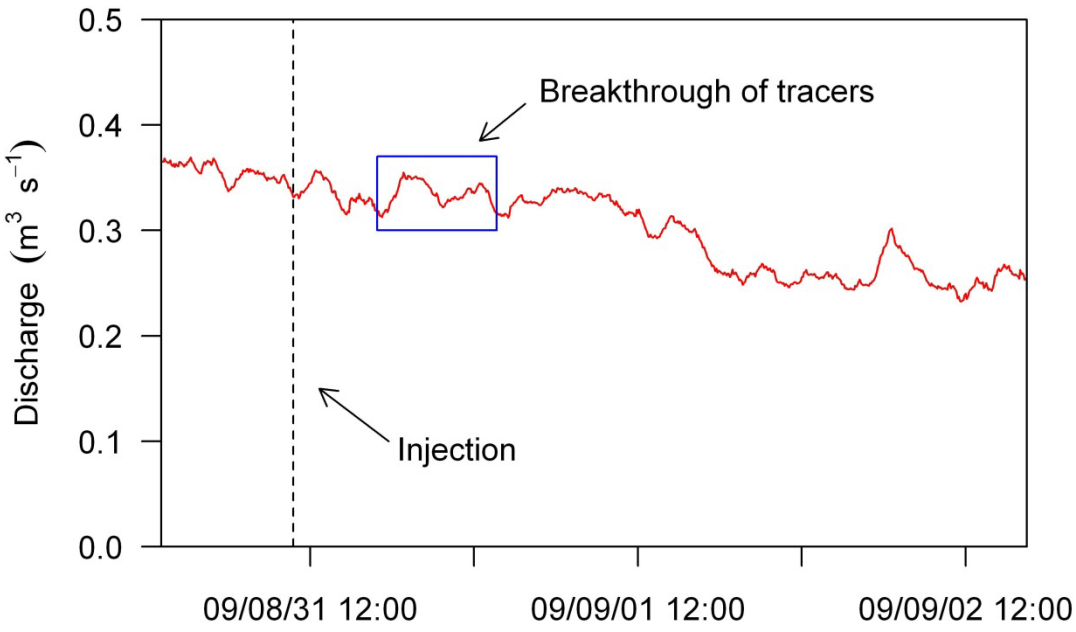

Figure A- 2: Time trend of discharge at site III during the tracer experiment

Temperature profiles in the river bed

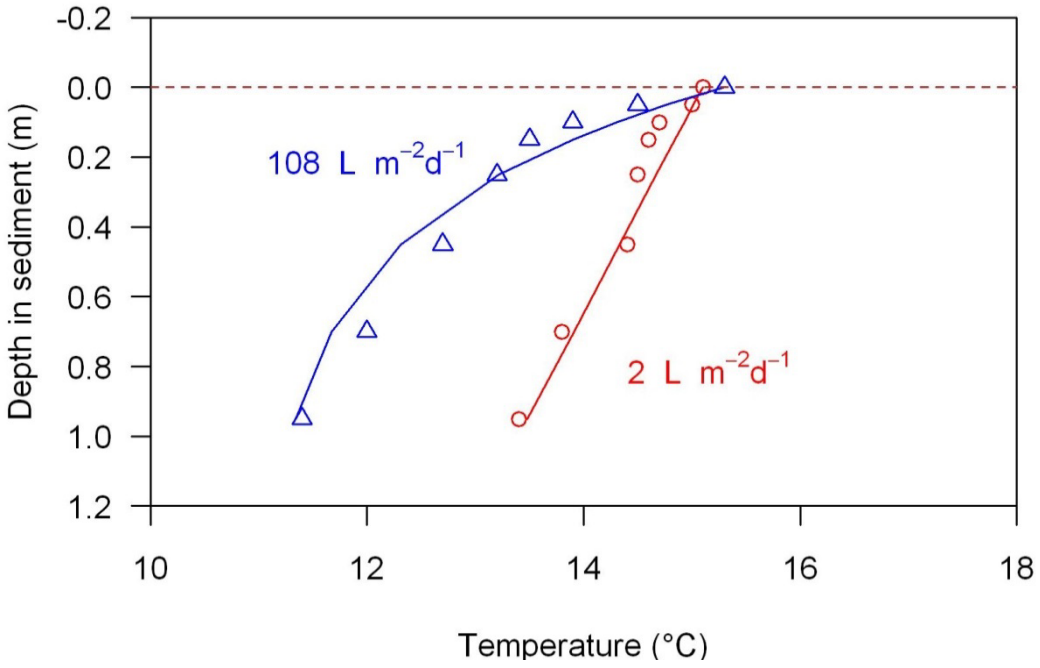

Figure A- 3: Sediment temperature profiles at sites with high and low groundwater discharge into the stream channel at Säva Brook. The solid lines represent the model fit.

River water temperature

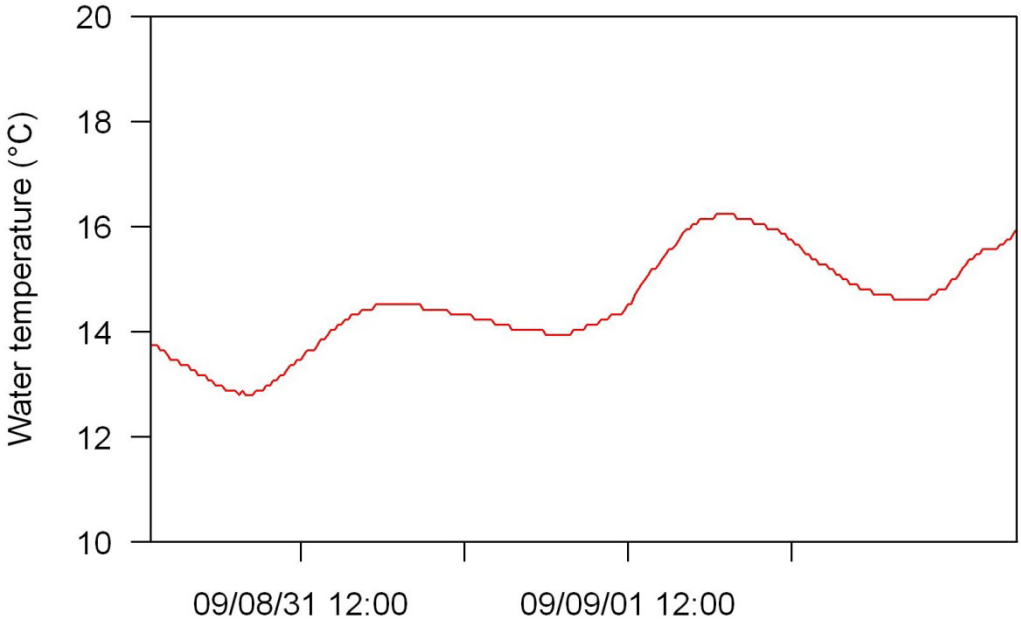

Figure A- 4: Temperature trend of the stream water during the tracer experiment (injection site)

Absorption spectrum

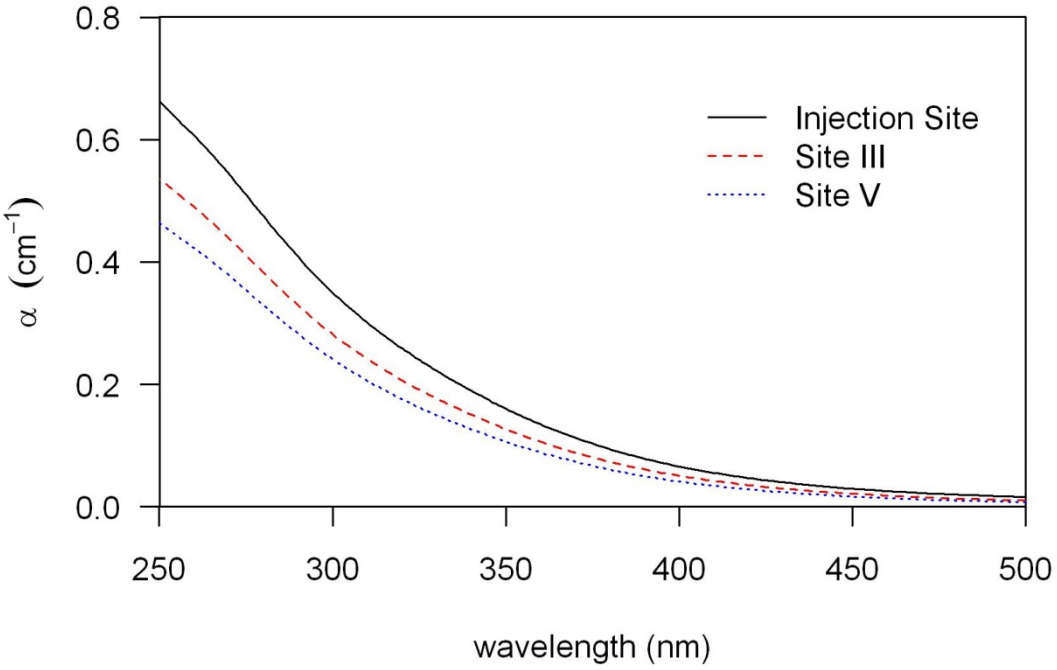

Figure A- 5: Absorption spectrum of the stream water at the tracer test (filtered < 0.45 µm)

Mass balances for pharmaceuticals with conservative behavior

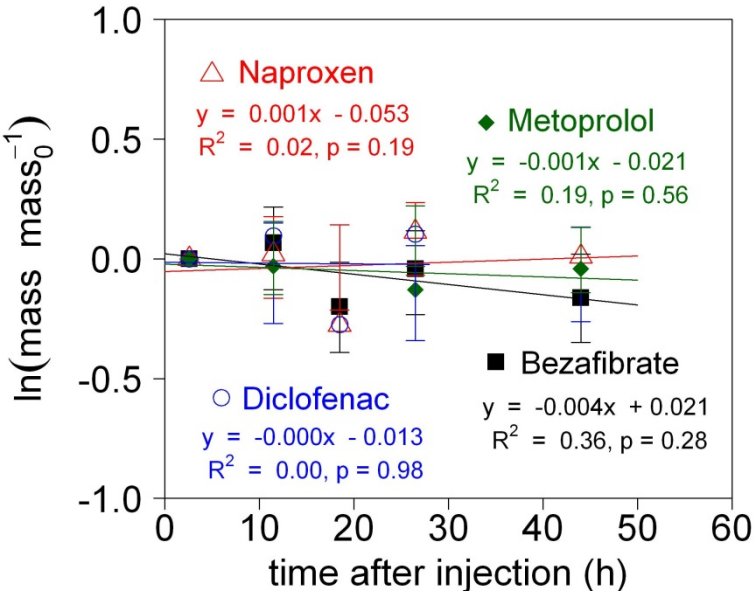

Figure A- 6: Pseudo first-order kinetic plots for bezafibrate, diclofenac, metoprolol, and naproxen, normalized to breakthrough at site I.

6.1.5 REFERENCES TO CHAPTER 6.1

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6.2 SUPPORTING INFORMATION TO CHAPTER 3

Fate of Pharmaceuticals in Rivers: Deriving a Benchmark Dataset at Favorable Attenuation Conditions

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Water Research 46(17): 5551-5565.

6.2.1 LIST OF TABLES OF APPENDIX B

6.2.2 LIST OF FIGURES OF APPENDIX B

6.2.3 APPENDIX B-1: SUPPLEMENTAL INFORMATION ON STUDY SITE, HYDRAULIC AND METEOROLOGICAL DATA, AND ADDITIONAL RESULTS

Table B- 1: Coordinates of sampling and experimental sites at river Gründlach

Name	***Latitude***	***Longitude***
Sampling Site A / Exp A	49° 31' 27'' N	11° 08 '11'' E
Sampling Site B / Exp B	49° 31' 22'' N	11° 01' 27'' E
Gauging station „Frauenkreuz"	49° 31' 15'' N	11° 05' 22'' E
Exp C	49° 31' 31'' N	11° 03' 11'' E
DWD weather station "Nuremberg"	49° 30' 16'' N	11° 03' 26'' E
WWTP Heroldsberg	49° 31' 24'' N	11° 08' 34'' E

Table B- 2: Water and sediment characteristics of river Gründlach

Water [a]				***Sediment*** [b,c]			
	Average	**min**	**max**		**Average**	**min**	**max**
pH	7.69 ± 0.04	7.51	7.79	*Gravel (%)*	2.4 ± 1.6	0.4	5.0
EC (µS cm^{-1})	624 ± 99	246	771	*Coarse sand (%)*	43.4 ± 12.0	24.6	60.9
DO (%)	68.9 ± 3.3	61.3	74.8	*Medium sand (%)*	52.9 ± 34.3	34.3	69.5
DO (mg L^{-1})	6.0 ± 0.3	5.2	6.6	*Fine sand (%)*	0.63 ± 0.41	0.23	1.4
				Silt & clay (%)	0.06 ± 0.08	0	0.2
$T_{site\ A}$ (°C)	19.5 ± 1.35	16.0	22.0	*C (%)*	0.23 ± 0.19	0.06	0.55
$T_{site\ B}$ (°C)	18.9 ± 1.83	14.7	23.9	*N (%)*	< 0.05	n.a.	n.a.

[a] statistics based on measurements at intervals of 15 – 30 minutes during the total sampling period at site A, [b] statistics based on sediment samples (0-30 cm depth) taken at 8 sites along the river stretch [c] texture classification after AG Boden (1994), n.a.: not available as all concentration were < LOQ (0.05 %).

Table B- 3: Overview over hydrological and meteorological conditions during the phototransformation experiments performed at river Gründlach

	Shading	Date	Start time	T_{air} (°C) [a]	T_{water} (°C) [a,b]	SSM [c] (min, %)	pH (-) [a, d]
Exp A	full	2010/07/14	9:30	31.2	19.9	332 (92)	7.7
Exp B	no	2010/07/09	10:40	33.2	23.7	360 (100)	8.0
Exp C	partial	2010/07/20	12:10	27.0	21.2	319 (89)	7.7

[a] average values throughout the phototransformation experiments, [b] water temperature in the containers, [c] sunshine minutes during phototransformation experiments from DWD weather station "Nuremberg", [d] pH in the containers was stable throughout the experiments, addition of NaN_3 increased the pH by approx. 0.2

Table B- 4: Average concentration (ng L^{-1}) of pharmaceuticals in the quality control samples analyzed during the Gründlach study (SD: standard deviation; n: number of samples) and limits of quantification (LOQ)

	Average	SD	n	LOQ[a]
Bezafibrate	110	15	8	12
Carbamazepine	360	22	7	n.d.
Clofibric Acid	0	n.a	8	n.a.
Diclofenac	780	84	8	45
Ibuprofen	0	n.a.	7	8.0.
Metoprolol	290	22	7	n.d.
Naproxen	48	6	8	16
Propranolol	3.2	1	6	0.5
Sotalol	110	13	7	n.d.
Sulfamethoxazole	220	3	8	n.d.

n.a.: not available, n.d.: not determined since concentrations and intensities in all samples were high (Signal-to-noise > 100). [a] LOQ was calculated as average of the three lowest concentrations determined for a specific substance.

Table B- 5: Matrix of Spearman's rank correlation coefficients (r) for concentrations of pharmaceuticals and the elements B and K at sampling site A during the low discharge period (period I).

	Bez	**CBZ**	**Dic**	**Ibu**	**Met**	**Nap**	**Pro**	**SMX**	**Sot**	**B**	**K**
Bez	1.00	n.s.	n.s.	n.s.	0.81***	0.65**	n.s.	0.53*	0.89***	0.59*	n.s.
CBZ		1.00	n.s.	n.a.	0.55*	n.s.	n.s.	n.s.	0.62**	0.66**	n.s.
Dic			1.00	n.a.	0.54*	n.s.	n.s.	0.50*	0.59**	0.59*	0.62**
Ibu				1.00	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Met					1.00	0.52*	0.51*	0.70**	0.87***	0.83***	0.79***
Nap						1.00	n.s.	n.s.	0.67**	0.59*	0.53*
Pro							1.00	n.s.	n.s.	n.s.	n.s.
SMX								1.00	0.61**	n.s.	0.51*
Sot									1.00	0.71***	0.60**
B										1.00	0.88***
K											1.00

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s.: no significant correlation, n.a.: not available since concentrations of ibuprofen were <LOQ in all samples of period I; Bez: bezafibrate, CBZ: carbamazepine, Dic: diclofenac, Ibu: ibuprofen, Met: metoprolol, Nap: naproxen, Pro: propranolol, SMX: sulfamethoxazole, Sot: sotalol, B: boron, K: potassium

Table B- 6: Matrix of Spearman's rank correlation coefficients (r) for concentrations of pharmaceuticals and the elements B and K at sampling site A during the high discharge period (period II).

	Bez	**CBZ**	**Dic**	**Ibu**	**Met**	**Nap**	**Pro**	**SMX**	**Sot**	**B**	**K**
Bez	1.00	n.s.	0.88***	n.s.	0.83*	0.88*	n.s.	n.s.	0.86**	n.s.	n.s.
CBZ		1.00	0.71*	n.s.	0.90**	n.s.	n.s.	0.83**	0.93**	n.s	0.78*
Dic			1.00	n.s.	0.88*	n.s.	n.s.	n.s.	0.862	n.s.	n.s.
Ibu				1.00	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Met					1.00	n.s.	n.s.	0.86**	0.983	n.s.	n.s.
Nap						1.00	n.s.	n.s.	n.s.	n.s.	n.s.
Pro							1.00	n.s.	n.s.	n.s.	n.s.
SMX								1.00	0.83**	n.s.	0.51*
Sot									1.00	n.s.	n.s.
B										1.00	0.99***
K											1.00

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n.s.: no significant correlation; Bez: bezafibrate, CBZ: carbamazepine, Dic: diclofenac, Ibu: ibuprofen, Met: metoprolol, Nap: naproxen, Pro: propranolol, SMX: sulfamethoxazole, Sot: sotalol, B: boron, K: potassium

Table B- 7: Relative elimination (%) of pharmaceuticals between sites A and B for periods I and II based on potassium as conservative tracer at river Gründlach.

	period I	period II
Boron	-22	-13
Bezafibrate[b]	62	(55)
Carbamazepine	-5	-4
Diclofenac	67	(38)
Ibuprofen[a]	n.a.	n.a.
Metoprolol	66	(48)
Naproxen[b]	48	(41)
Propranolol[b]	67	(40)
Sotalol	40	(33)
Sulfamethoxazole	22	22

[a] For ibuprofen no elimination rates could be calculated due to the few data points. [b] Minimum elimination rates; the actual rate can be higher as concentrations < LOQ at site B were set to LOQ for calculation purposes; negative elimination rates indicate increase of mass compared to potassium; values in parenthesis are uncertain due to a lacking correlation with potassium

Table B- 8: Comparison of elimination rates for bezafibrate, naproxen, and propranolol in periods I and II if samples with concentration < LOQ at site B are set to LOQ or 0.

		Bezafibrate	Naproxen	Propranolol
c (Site B) = LOQ	period I	0.63	0.50	0.68
	period II	0.57	0.43	0.42
c (Site B) = 0	period I	1.00	0.99	0.84
	period II	0.65	0.51	0.52

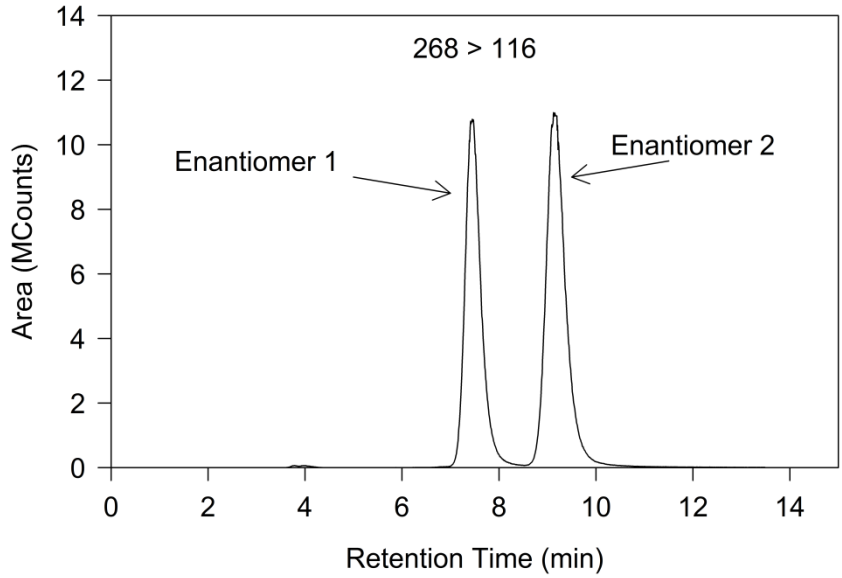

Figure B- 1: Example for chromatographic separation of the two metoprolol enantiomers on the chiral column.

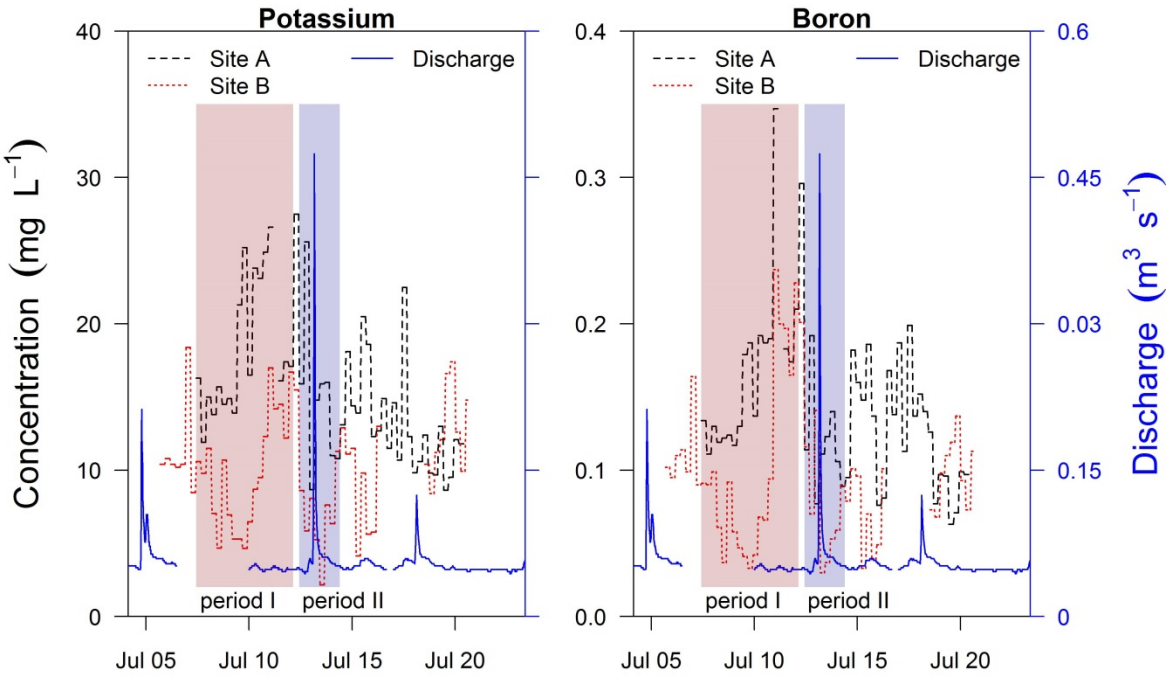

Figure B- 2: Time trends of potassium and boron concentrations at both sampling sites and discharge at the gauging station “Frauenkreuz” at river Gründlach.

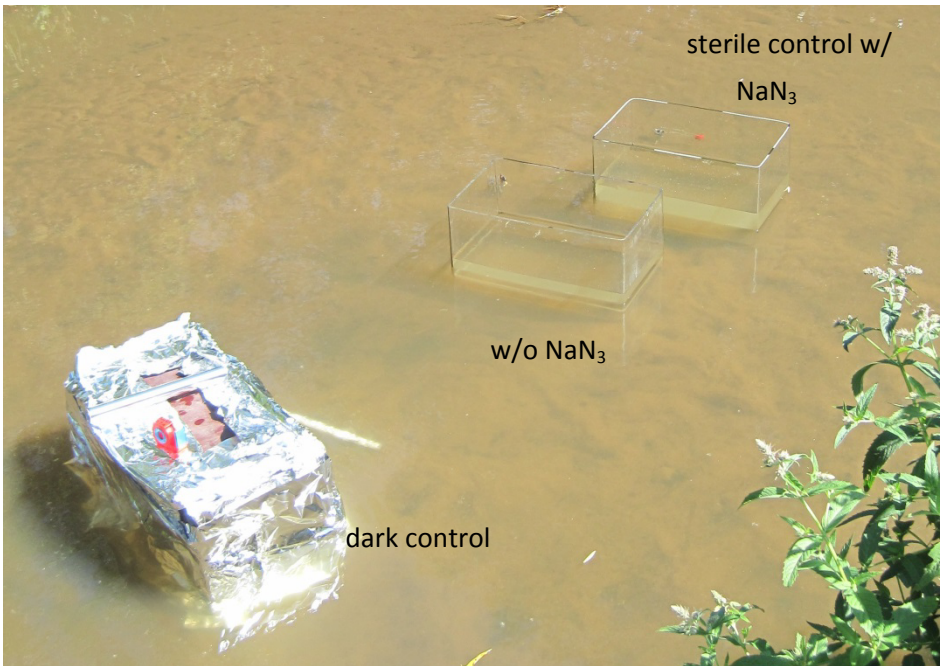

Figure B- 3: Picture of the experimental setup at phototransformation experiment Exp B at river Gründlach.

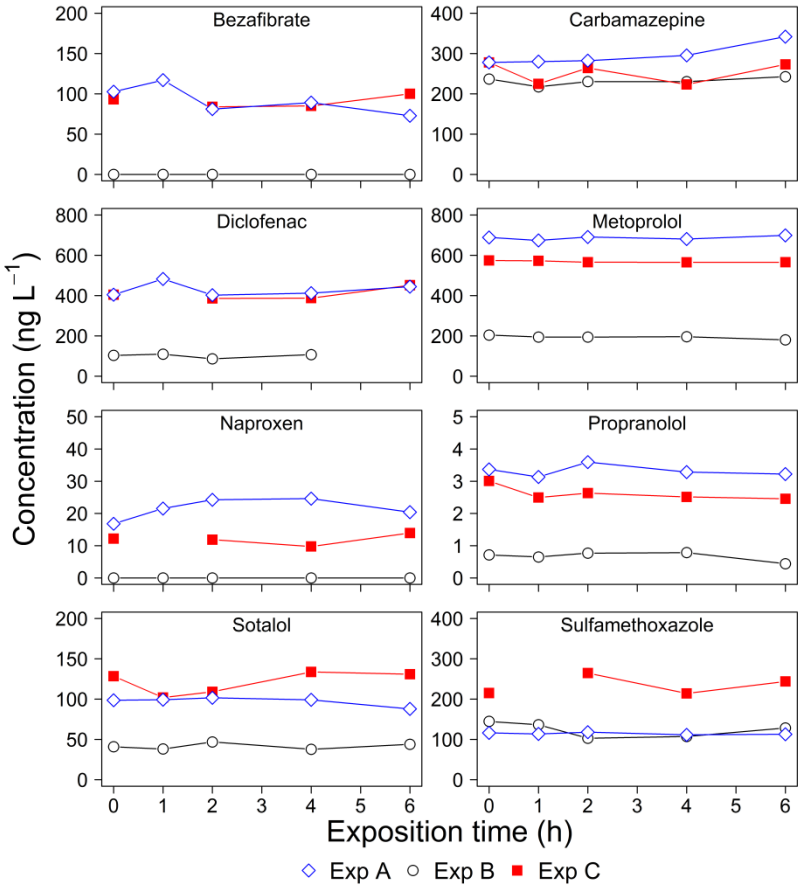

Figure B- 4: Results of the dark controls for phototransformation experiments performed at river Gründlach at sites Exp A, Exp B, and Exp C; initial concentrations of bezafibrate and naproxen were < LOQ at Exp B

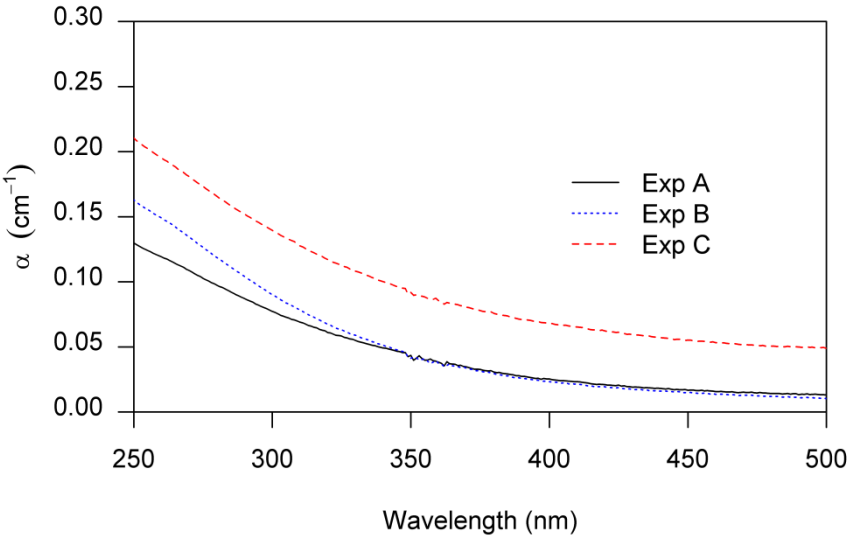

Figure B- 5: Absorption spectra of the river water used for the three phototransformation experiments at river Gründlach.

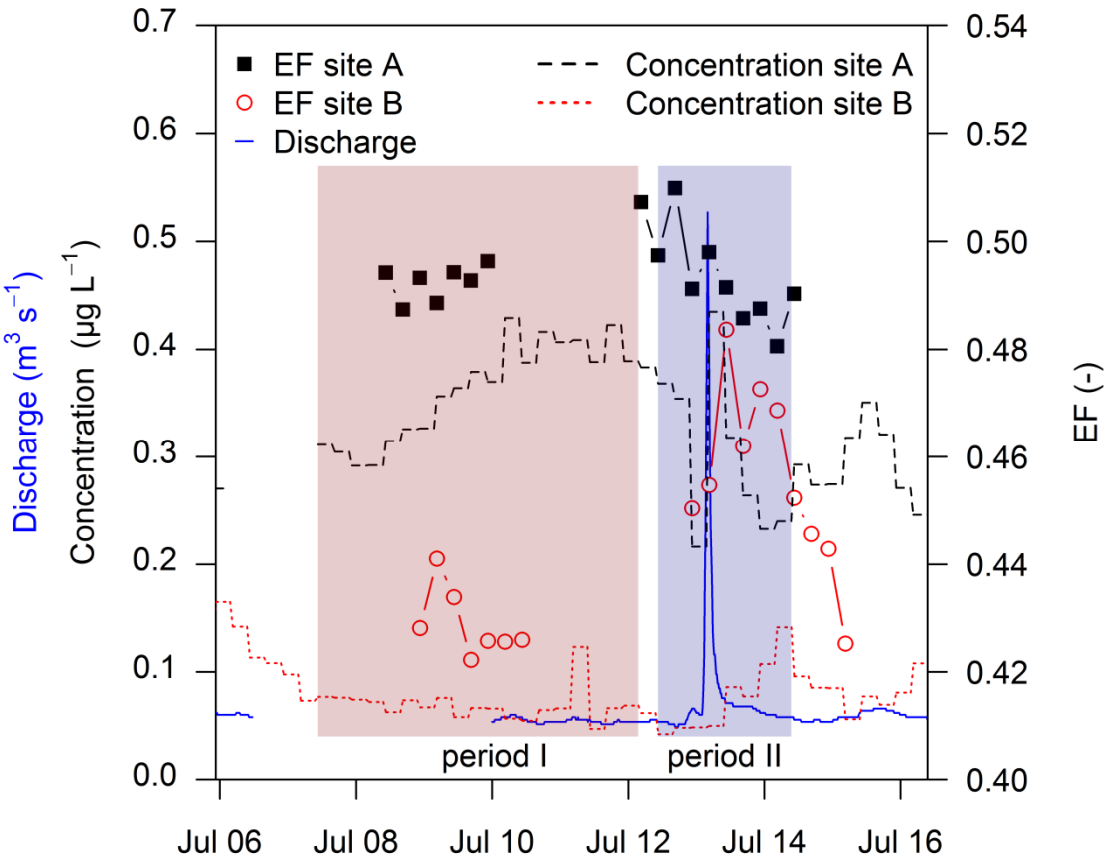

Figure B- 6: Dynamics of the concentration and enantiomer fraction (EF) of metoprolol at both sampling sites at river Gründlach.

6.2.4 APPENDIX B-2: UNCERTAINTY ANALYSIS OF THE PROCEDURE USED FOR CALCULATING ELIMINATION RATES

Methods

The calculation of loads of solutes at two sites along a river stretch and the subsequent calculation of elimination rates requires precise discharge measurements. Since such data were not available at both sites, we calculated the elimination of our target substances indirectly. To this end, we compared the relative change of the cumulative concentrations of a target substance (x) between sites A and B the relative change of the cumulative carbamazepine (CBZ) concentrations. In detail, the elimination of a substance ($Eli_{concen,x}$) in relation to carbamazepine was calculated as follows:

$$Eli_{\,concen,\quad x} = \left(1 - \frac{\frac{\sum c_{x,\,Site\ B}}{\sum c_{x,\,Site\ A}}}{\frac{\sum c_{CBZ,\ \ SiteB}}{\sum c_{CBZ,\ \ SiteA}}} \right) \cdot 100\%$$

If discharge is available at both sites, elimination can be calculated by directly by comparing the loads of a substance at both sites. Hence, elimination based on loads is calculated as follows ($Eli_{load,x}$):

$$Eli_{load,\,x} = \left(1 - \frac{\sum c_{siteB,\,x} \cdot Q_{siteB}}{\sum c_{siteA,\,x} \cdot Q_{siteA}} \right) \cdot 100\%$$

where Q_{siteA} and Q_{siteB} are the discharges at respective sampling sites.

Strictly speaking, the concentration-based approach is only valid if concentrations of a target substance x and carbamazepine as a conservative reference compound are perfectly positively correlated at site A or if discharge is constant over time. Hence, applying this concept is valid (with little errors) in period I (discharge can be considered constant), but can lead to significant errors rates during period II where discharge varied from 40 L s^{-1} to 500 L s^{-1} in case a substance's concentration is not correlated to that of carbamazepine.

To estimate the uncertainties associated with the procedure we used, we can consider four different scenarios: a) constant discharge, correlated substances, b) constant discharge, uncorrelated substances, c) variable discharge, correlated substances, d) variable discharge, uncorrelated substances. To evaluate these four cases, we used a synthetic time series of 15 data points at both sampling sites and compared the calculated elimination rate with the "real" elimination rate based on the actual loads of a substance assuming that discharge was known. We created datasets for the different scenarios and superimposed random variations on each value. We then calculated the elimination rates for 10 realizations of each scenario and compared the mean deviations of the calculated concentration-based elimination rate from the load-based rate.

For each realization in the correlated and uncorrelated scenarios, the same discharge data were used (Figure B- 7).

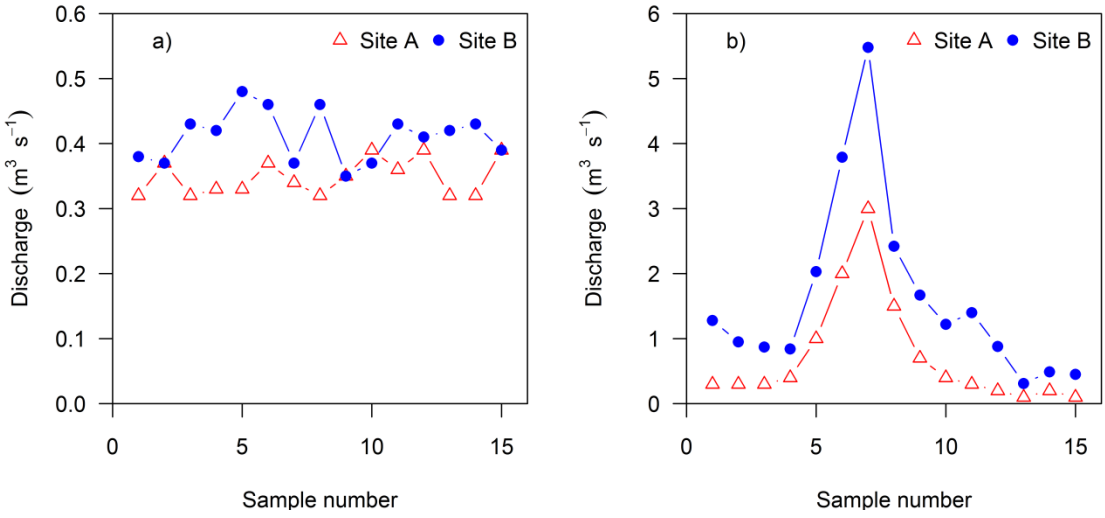

Figure B- 7: Discharge scenarios at sites A and B used for the error analyses; a) constant discharge, b) variable discharge.

An example of one realization of the concentrations used for cases a) and c) (correlated data) and for cases b) and d) (uncorrelated data) is given in Figure B- 8.

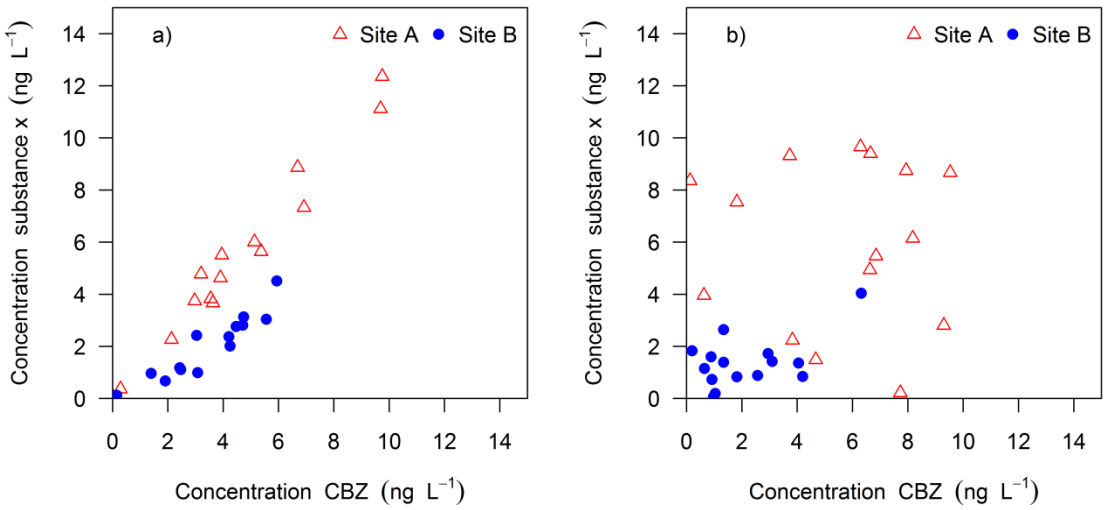

Figure B- 8: Example on correlation of concentration of substances at site A and site B used for the error analyses; a) correlated data, b) uncorrelated data

For all ten realizations, the elimination rate based on concentrations and on loads was calculated. The root mean square error (RMSE) of the elimination based of concentrations (Eli_{concen}) and the elimination based on loads (Eli_{load}) was calculated as a measure of the accuracy of the method. In detail, RMSE was calculated as follows:

$$RMSE = \sqrt{\sum_{i=1}^{10}\left(Eli_{laod,i} - Eli_{concen,j}\right)^2}$$

Results

The mean elimination rates and their standard deviations for the ten realizations of the calculations based on loads and on concentrations are almost equal in all four cases (Table B- 9). Using data based on ten realizations obviously compensates for the uncertainty of using concentrations instead of loads. However, a sampling campaign like the one in this study cannot be repeated under identical conditions and thus it is crucial to examine the deviation between the load- and concentration-based approaches for each individual simulation. This can be done using either the maximum deviation obtained from the 10 realizations or the RMSE that provides information on the overall disagreement between the load-based and the concentration-based concept. If discharge is constant (cases a) and b)) the maximum deviation and hence the maximum error of using concentrations instead of loads is low (<6.1 %) with a low RMSE (4.7 %; Table B- 9). If concentrations are additionally correlated, the maximum deviation was even only 1.3 % (RMSE: 1.2 %). Hence, under constant discharge (like in period I), the use of concentrations instead of loads is valid and the error is negligible regardless if concentrations are correlated or not. Under variable discharge conditions (cases c) and d)), a correlation is more essential for obtaining reliable results when using concentrations. In case concentrations are correlated the maximum deviation was 6.1 % (RMSE: 5.7 %) and the use of concentrations can be considered valid. But in the case of uncorrelated concentrations and variable discharge, the maximum deviation between the concepts obtained from ten realizations was 28 % and the RMSE was largest (17 %). Consequently, under variable discharge conditions (like in period II), the concentration-based concept can be considered reliable if the concentration of the substance and carbamazepine are correlated. If concentrations are not correlated under a period of variable discharge, the concentration-based approach is very uncertain.

Table B- 9: Results of the ten realizations of the errors analyses. Elimination and correlation is given as average ± standard deviation of the ten realizations.

		Elimination based on concentrations (%)	Elimination based on loads (%)	Correlation (-)*	RMSE (%)	Range of deviations (%)[b]
a)	*correlated, constant discharge*	49.3 ± 3.7	49.6 ± 3.5	0.98 ± 0.02	1.2	(0.3 – 1.3)
b)	*uncorrelated, constant discharge*	50.3 ± 4.3	50.2 ± 4.3	0.00 ± 0.26	4.7	(0.1 – 6.1)
c)	*correlated, variable discharge*	51.4 ± 3.4	50.5 ± 4.0	0.97 ± 0.02	5.7	(0.1 – 6.1)
d)	*uncorrelated, variable discharge*	48.0 ± 3.2	46.6 ± 6.3	-0.02 ± 0.14	17	(3.1 – 28)

[a] correlation of concentrations of carbamazepine and substance x at site A, [b] percentaged difference of the elimination based on loads and the elimination based on concentrations

6.2.5 REFERENCES TO CHAPTER 6.2

AG Boden **(1994)** *Bodenkundliche Kartieranleitung [Manual of soil mapping]*. Schweizerbart, Hannover; ISBN 3-510-95804-7.

6.3 SUPPORTING INFORMATION TO CHAPTER 4

Recirculating Sediment Columns Provide Generalizable Rate Constants for the Biotransformation of Pharmaceuticals in River Sediments

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to be submitted to Environmental Science & Technology

6.3.1 LIST OF TABLES OF APPENDIX C

6.3.2 LIST OF FIGURES OF APPENDIX C

6.3.3 Material and Methods

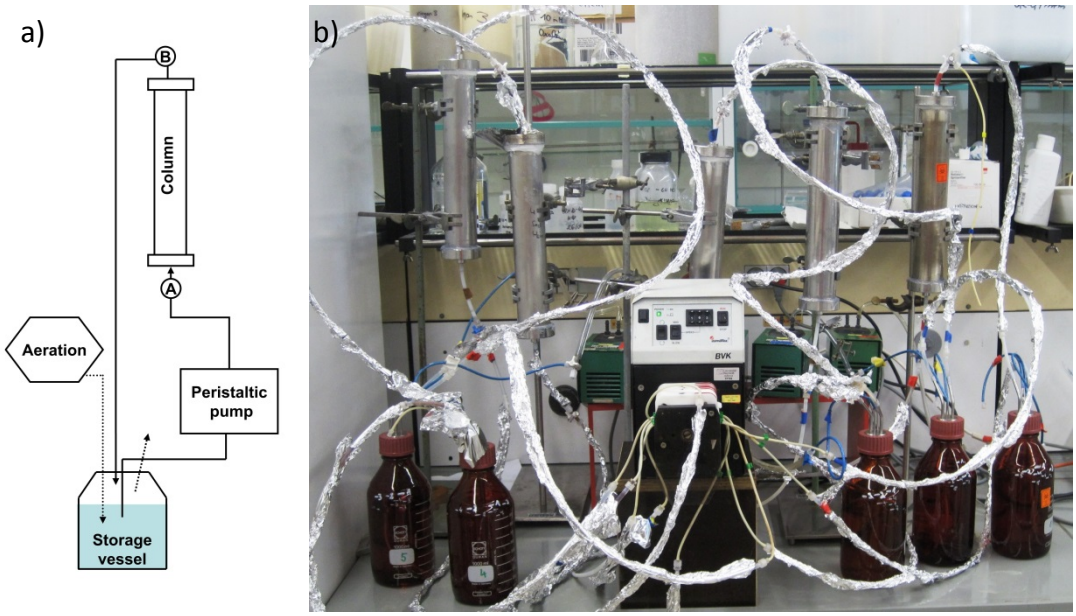

Figure C- 1: a) Scheme of the setup of the column experiments; b) Picture of five sediment columns running in parallel

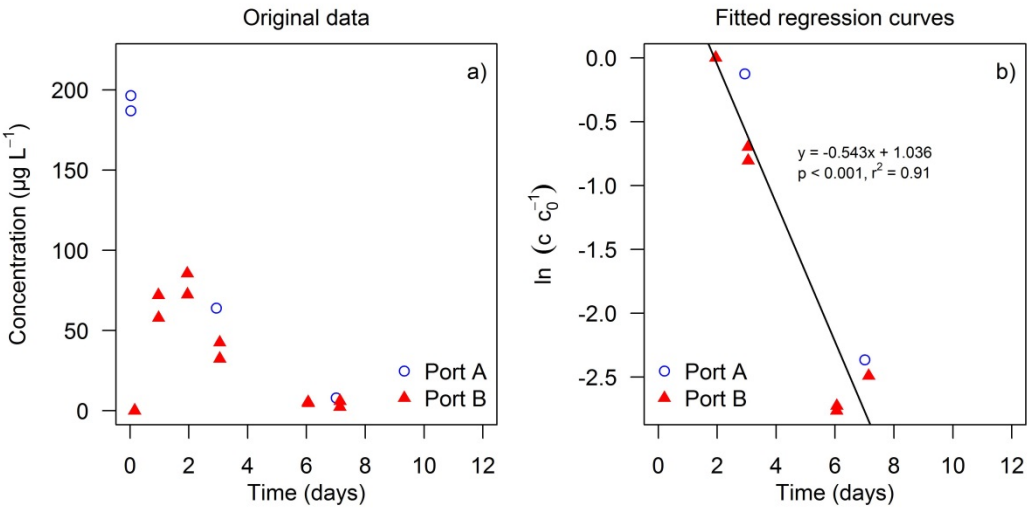

Figure C- 2: Example of the calculation procedure of an elimination rate constant (k_{eli}, data of metoprolol in Exp. 6), a) Original data, b) Calculation of k_{eli} after omission of initial concentrations in port A (incompletely mixing) and port B (no sorption equilibrium)

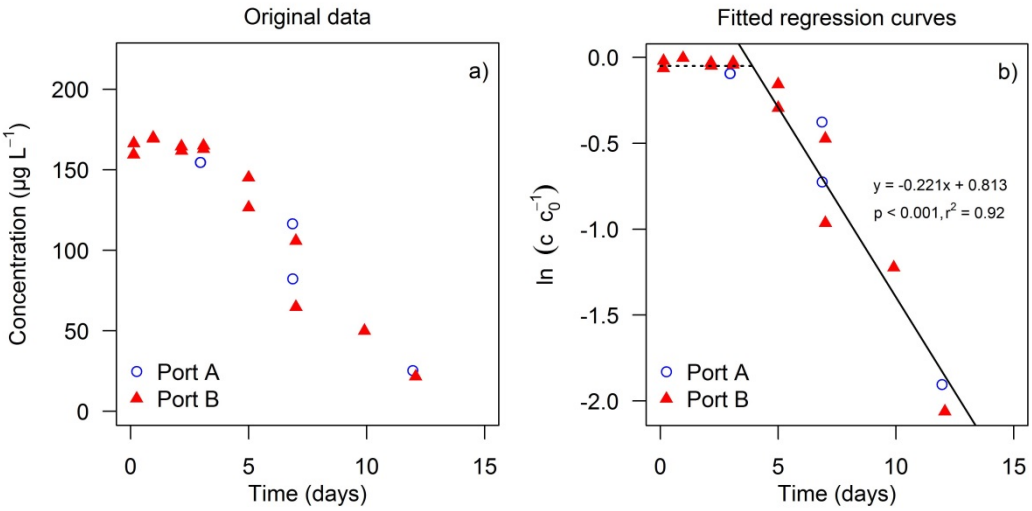

Figure C- 3: Example of the calculation procedure of a secondary elimination rate ($k_{eli,sec}$, data of naproxen in Exp. 7), a) Original data, b) Calculation of $k_{eli,sec}$, solid line: significant removal after a lag-phase, dashed line: no removal with the first x days of the experiment

Table C- 1: Detailed information on the re-spiking experiments

Exp.	Change compared to Exp 1b	Aim	Pharmaceuticals added
1b.a	None	Is there an adaptation phase?	100 µg
1b.b	Disconnection of sediment and water	Is there a transport of microbes into the storage vessel and are pharma-ceuticals not only transformed in the sediment?	100 µg

6.3.4 Results and Discussion

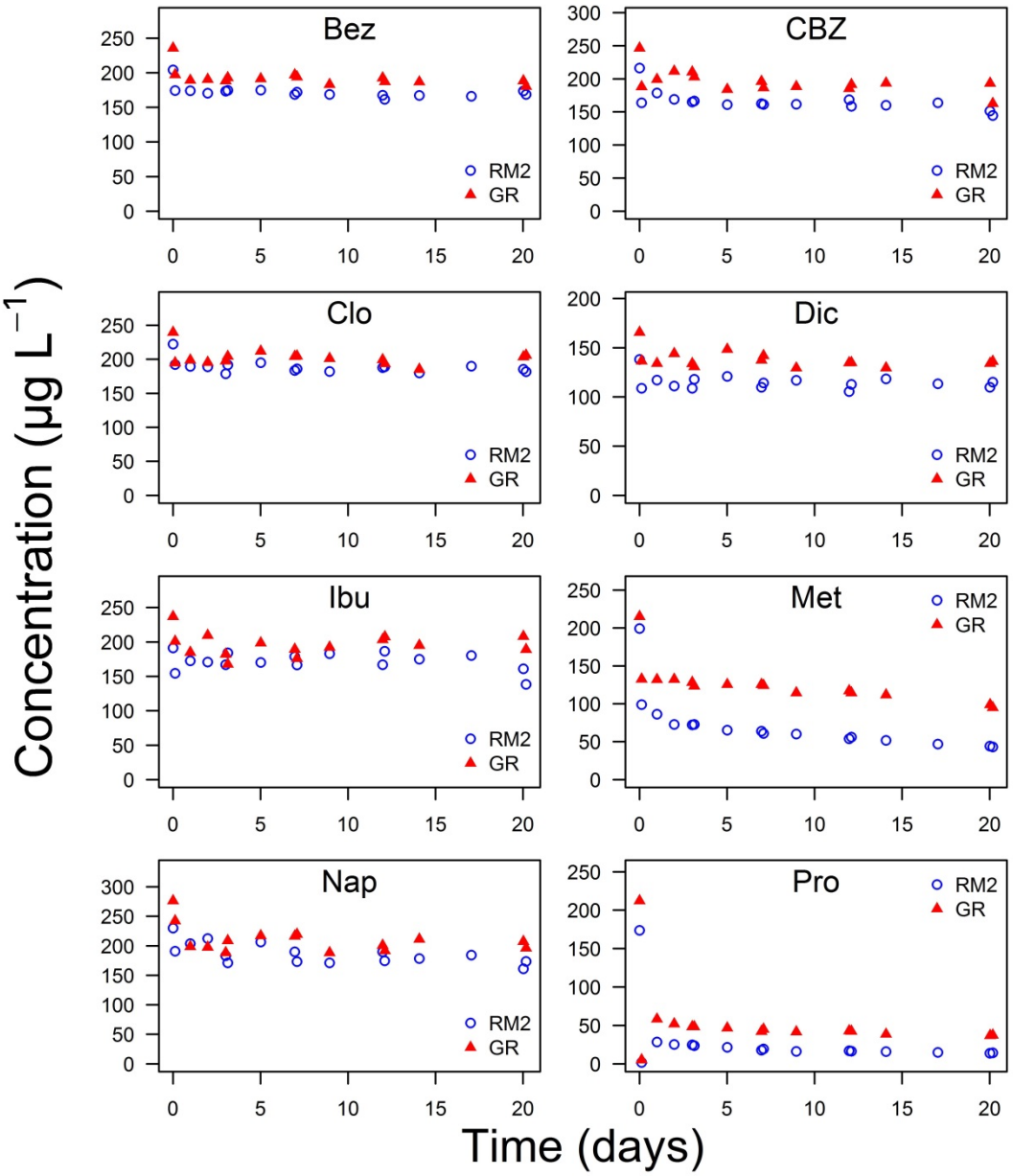

Figure C- 4: Time trends of pharmaceuticals in the sterile controls at a filter velocity of 10 m d^{-1} with sediments RM2 and GR; Bez: Bezafibrate, CBZ: Carbamazepine, Clo: Clofibric Acid, Dic: Diclofenac, Ibu: Ibuprofen, Met: Metoprolol, Nap: Naproxen, Pro: Propranolol

Table C- 2: Elimination rate constants (k_{eli}, ± 95 % confidence intervals, d^{-1}) of pharmaceuticals in the sterile controls after the initial sorption period

	Exp. 1b	Exp. 2	Exp. 3	Exp. 4	Exp. 5	Exp. 6	Exp. 8
Bez	0.021 ± 0.016*	0.010 ± 0.084*	n.s.	0.002 ± 0.002*	0.025 ± 0.007*	0.006 ± 0.005*	0.002 ± 0.002*
CBZ	n.d.	n.s.	n.s.	0.005 ± 0.003**	n.d.	0.009 ± 0.008**	n.s.
Clo	0.007 ± 0.004**	n.s.	n.s.	n.s.	0.015 ± 0.011*	n.s.	n.s.
Dic	0.010 ± 0.004***	n.s.	n.s.	n.s.	0.032 ± 0.020**	n.s.	n.s.
Ibu	n.s.	n.s.	0.012 ± 0.011*	n.s.	0.014 ± 0.007**	n.s.	n.s.
Met	0.015 ± 0.007**	0.040 ± 0.012***	0.023 ± 0.010***	0.034 ± 0.006***	n.s.	0.018 ± 0.009***	0.015 ± 0.003***
Nap	n.s.	n.s.	n.s.	n.s.	0.030 ± 0.019**	n.s.	0.009 ± 0.006**
Pro	0.029 ± 0.009**	0.029 ± 0.016**	0.035 ± 0.028*	0.034 ± 0.004***	n.s.	0.039 ± 0.020**	0.019 ± 0.006***

* < 0.05, ** p < 0.01, *** p< 0.001; n.s.: no significant elimination based on a 95%-confidence level, n.d.: not determined since carbamazepine was not spiked. Bez: Bezafibrate, CBZ: Carbamazepine, Clo: Clofibric Acid, Dic: Diclofenac, Ibu: Ibuprofen, Met: Metoprolol, Nap: Naproxen, Pro: Propranolol

Table C- 3: Elimination rate constants (k_{eli}, ± 95% confidence intervals, d^{-1}) of eight pharmaceuticals in surface water controls

	Exp. 1b				Exp. 3				Exp. 5			
	1st phase		2nd phase		1st phase		2nd phase		1st phase		2nd phase	
	k_{eli} (d^{-1})	duration (d)	$K_{eli,sec}$ (d^{-1})	gone after (d)	k_{eli} (d^{-1})	duration (d)	$K_{eli,sec}$ (d^{-1})	gone after (d)	k_{eli} (d^{-1})	duration (d)	$K_{eli,sec}$ (d^{-1})	gone after (d)
Bez	n.s.	6	c.n.p.	10	n.s.	20	n.s.k.	-	0.023 ± 0.007*	9	c.n.p.	6
CBZ	n.d.	n.d.	n.d.	n.d.	n.s.	20	n.s.k..	-.	n.d.	n.d.	n.d.	n.d.
Clo	0.018 ± 0.007**	20	n.s.k.	-	n.s.	20	n.s.k.	-	n.s.	16	n.s.k.	-
Dic	n.s.	20	n.s.k.	-	n.s.	20	n.s.k.	-	n.s.	16	n.s.k.	-
Ibu	n.s.	6	c.n.p.	< 5	n.s.	20	n.s.k.	-	n.s.	6	c.n.p.	< 5
Met	0.034 ± 0.022*	20	n.s.k.	-	n.s.	20	n.s.k.	-	n.s.	16	n.s.k.	-
Nap	n.s.	6	0.039 ± 0.023*	-	n.s.	20	n.s.k.	-	0.030 ± 0.024*	16	n.s.k.	-
Pro	0.036 ± 0.012**	10	c.n.p.	10	0.014 ± 0.005***	20	n.s.k.	-	0.025 ± 0.012**	16	n.s.k.	-

* < 0.05, ** p < 0.01, *** p< 0.001; n.s.: no significant elimination based on a 95%-confidence level, n.d.: not determined since carbamazepine was not spiked, c.n.p.: calculation not possible since elimination was very quickly and too few measured values were available. n.s.k.: no secondary elimination kinetics. Bez: Bezafibrate, CBZ: Carbamazepine, Clo: Clofibric Acid, Dic: Diclofenac, Ibu: Ibuprofen, Met: Metoprolol, Nap: Naproxen, Pro: Propranolol

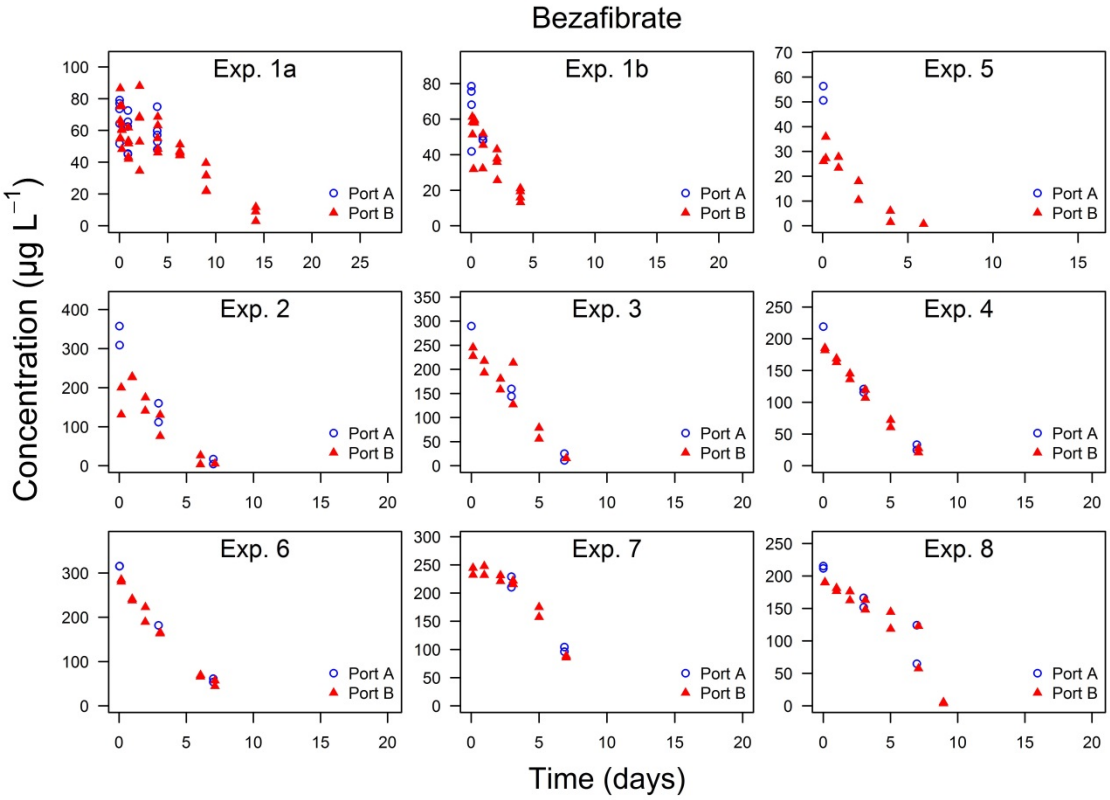

Figure C- 5: Time trends of bezafibrate during all recirculating column experiments

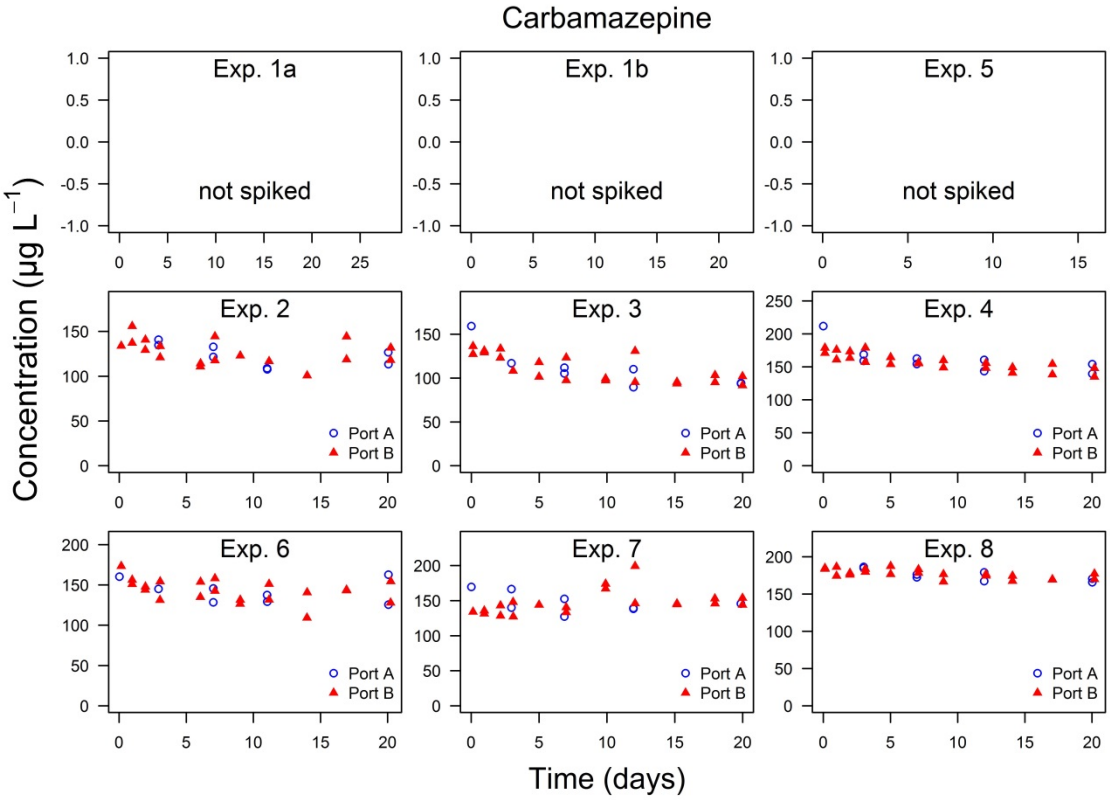

Figure C- 6: Time trends of carbamazepine during all recirculating column experiments

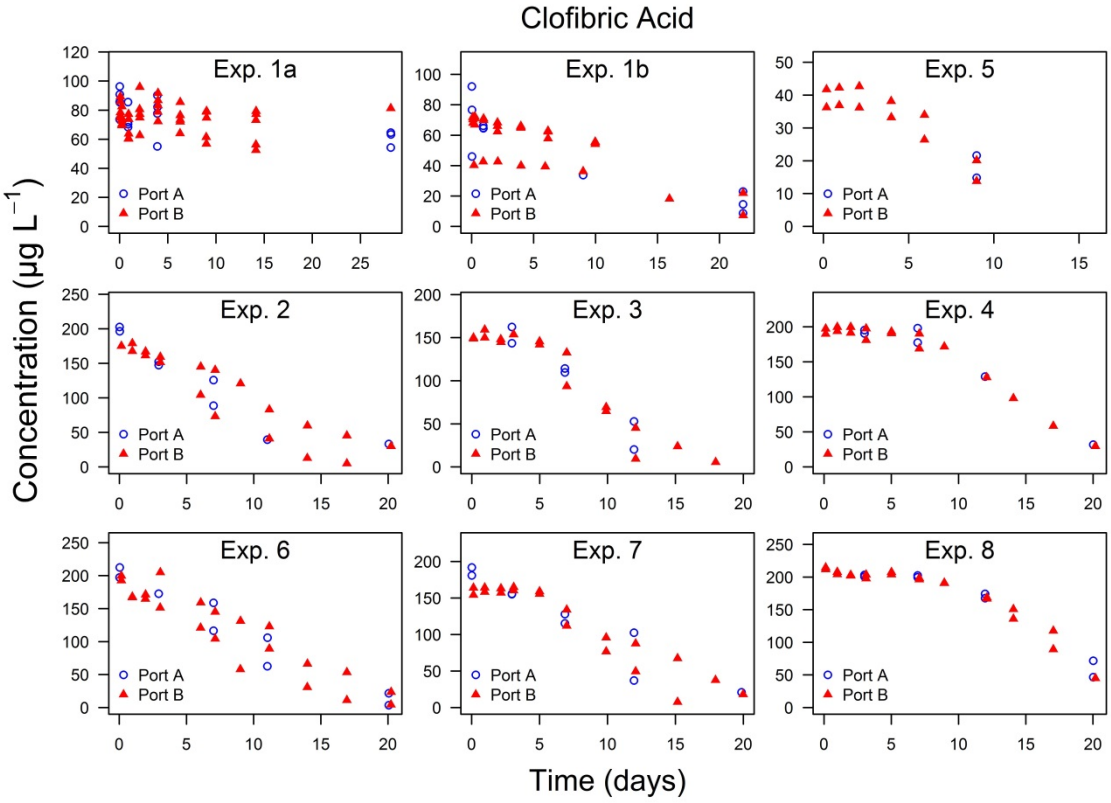

Figure C- 7: Time trends of clofibric acid during all recirculating column experiments

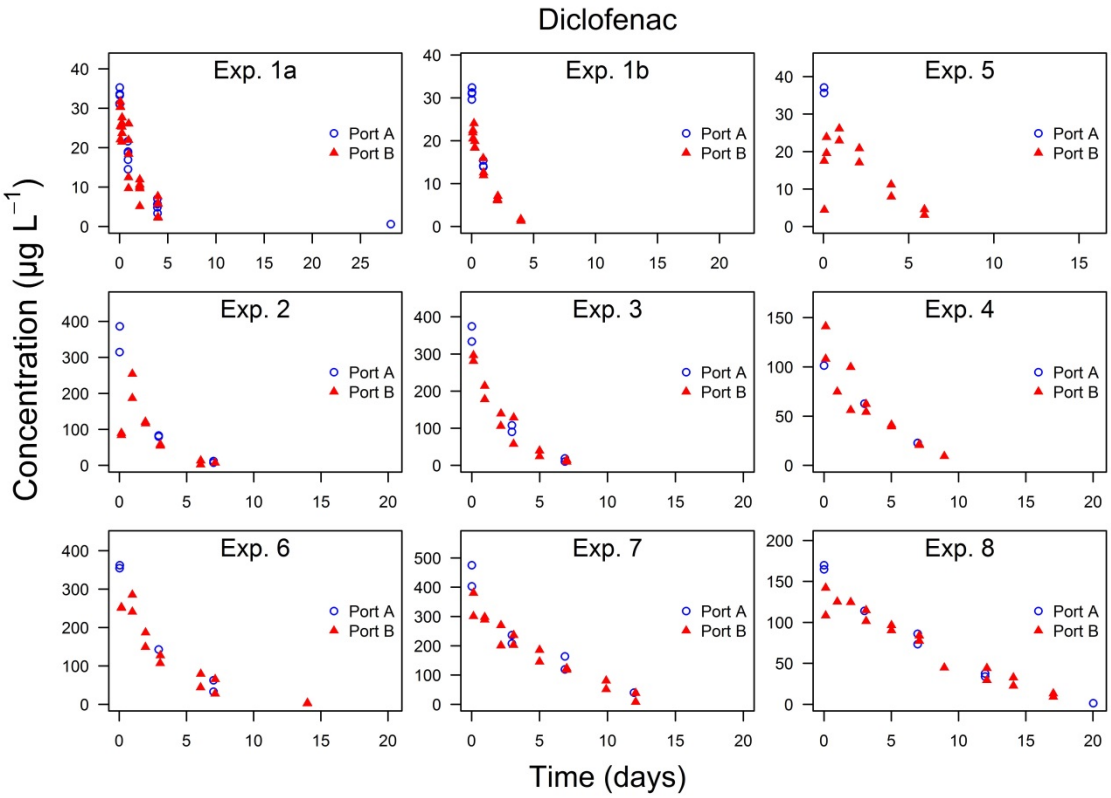

Figure C- 8: Time trends of diclofenac during all recirculating column experiments

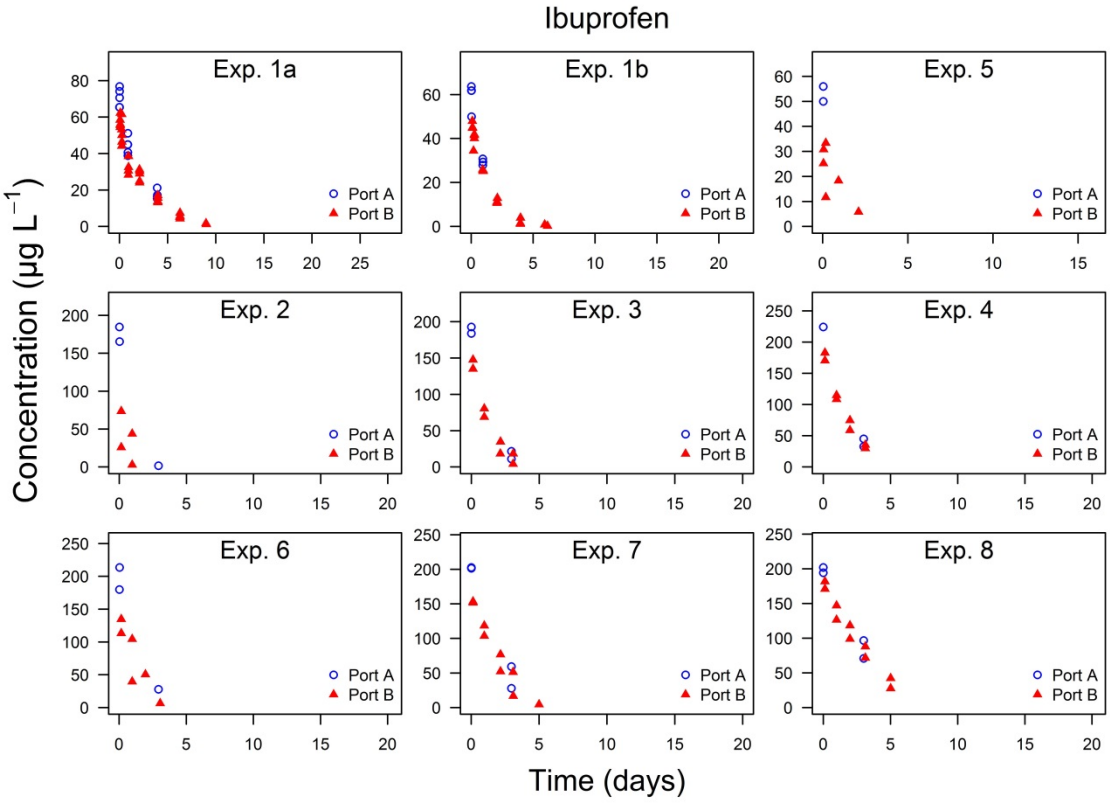

Figure C- 9: Time trends of ibuprofen during all recirculating column experiments

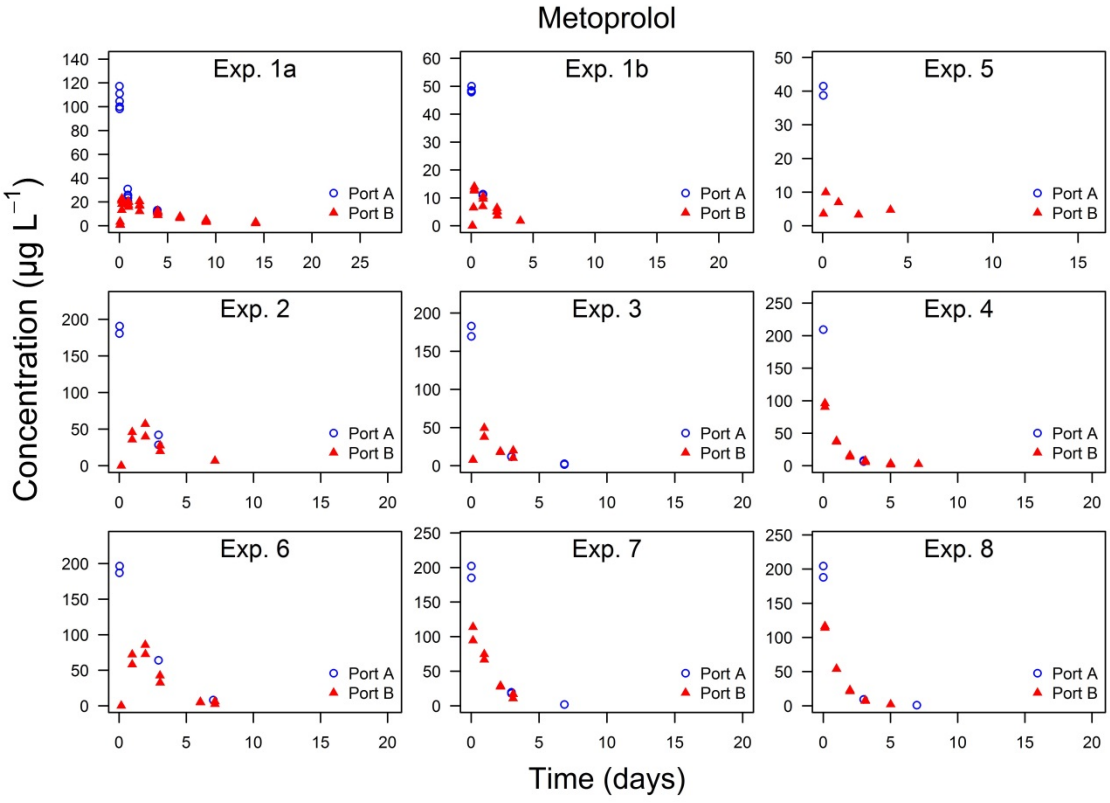

Figure C- 10: Time trends of metoprolol during all recirculating column experiments

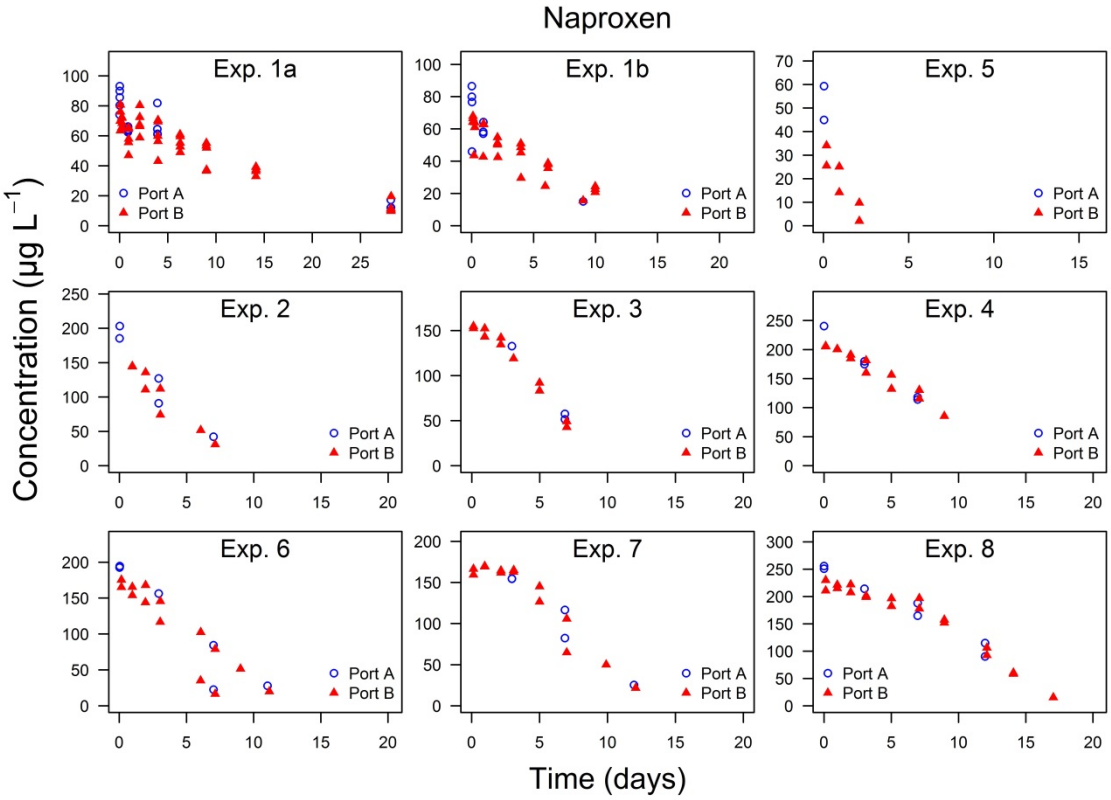

Figure C- 11: Time trends of naproxen during all recirculating column experiments

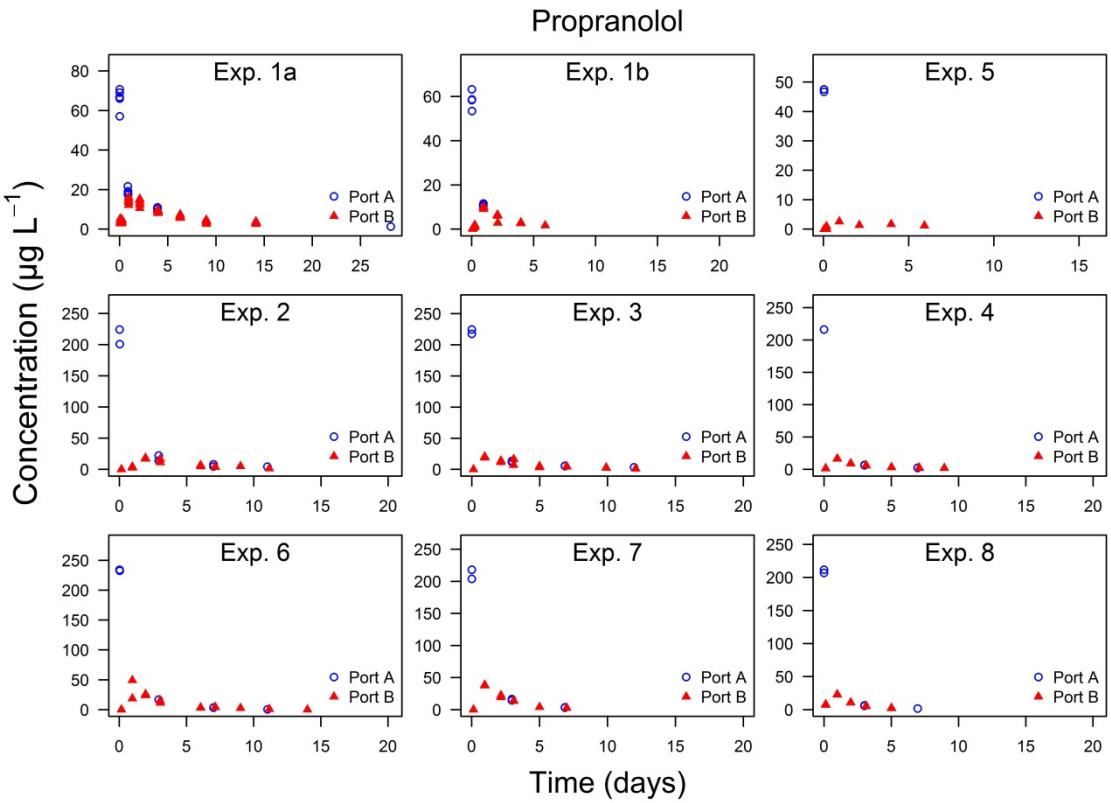

Figure C- 12: Time trends of propranolol during all recirculating column experiments

Table C- 4: Initial elimination rate constants (k_{eli}, ± 95% confidence intervals, d^{-1}) of eight pharmaceuticals in different sediments at a filter velocity of 5.0 m d^{-1}

	RM1a (n=5) (Exp. 1a)	RM1b (n=4) (Exp. 1b)	RM2 (n=2) (Exp. 3)	RM3 (n =3) (Exp. 5)	GR (n=2) (Exp. 7)
Bez	0.097 ± 0.020***	0.276 ± 0.044***	0.402 ± 0.094***	0.653 ± 0157***	0.033 ± 0.020**
CBZ	n.d.	n.d.	0.015 ± 0.005***	n.d.	n.s.
Clo	0.006 ± 0.005*	0.061 ± 0.015***	n.s.	n.s.	n.s.
Dic	0.435 ± 0.062***	0.615 ± 0.047***	0.462 ± 0.017***	0.381 ± 0.067***	0.208 ± 0.050***
Ibu	0.357 ± 0.026***	0.606 ± 0.073***	0.878 ± 0.276***	0.700 ± 0.612*	0.666 ± 0.211***
Met	0.176 ± 0.015***	0.624 ± 0.083***	0.502 ± 0.102***	0.580 ± 0.549*	0.611 ± 0.067***
Nap	0.060 ± 0.005***	0.108 ± 0.008***	0.054 ± 0.018***	1.01 ± 0.702*	n.s.
Pro	0.143 ± 0.020***	0.431 ± 0.099***	0.205 ± 0.049***	(0.118 ± 0.301)	0.441 ± 0.063***

n: number of replicates; * < 0.05, **p < 0.01, ***p< 0.001; n.s.: not significant; (): too few data points for significance in removal, n.d.: not determined since carbamazepine was not spiked in these experiments. Bez: Bezafibrate, CBZ: Carbamazepine, Clo: Clofibric Acid, Dic: Diclofenac, Ibu: Ibuprofen, Met: Metoprolol, Nap: Naproxen, Pro: Propranolol

Table C- 5: Secondary elimination rate constants ($k_{eli,sec}$, ± 95 % confidence intervals, d^{-1}) of the eight tested pharmaceuticals in different sediments at a filter velocity of 5.0 m d^{-1}

	RM1a (n=5) (Exp. 1a)	RM1b (n=4) (Exp. 1b)	RM2 (n=2) (Exp. 3)	RM3 (n =3) (Exp. 5)	GR (n=2) (Exp. 7)
Bez	n.s.k.	n.s.k.	n.s.k.	n.s.k.	0.230 ± 0.050*** (3)
CBZ	n.d.	n.d.	n.s.k.	n.d.	n.e.
Clo	n.s.k.	n.s.k.	0.241 ± 0.075*** (5)	0.127 ± 0.033*** (3)	0.224 ± 0.092*** (5)
Dic	n.s.k.	n.s.k.	n.s.k.	n.s.k.	n.s.k.
Ibu	n.s.k.	n.s.k.	n.s.k.	n.s.k.	n.s.k.
Met	n.s.k.	n.s.k.	n.s.k.	n.s.k.	n.s.k.
Nap	n.s.k.	n.s.k.	0.220 ± 0.036*** (3)	n.s.k.	0.221 ± 0.046*** (3)
Pro	n.s.k.	n.s.k.	n.s.k.	n.s.k.	n.s.k.

n: number of replicates, ***p< 0.001; n.s.k.: no secondary elimination kinetics, n.e.: no elimination, n.d.: not determined since carbamazepine was not spiked; in parenthesis (): estimated starting day. Bez: Bezafibrate, CBZ: Carbamazepine, Clo: Clofibric Acid, Dic: Diclofenac, Ibu: Ibuprofen, Met: Metoprolol, Nap: Naproxen, Pro: Propranolol

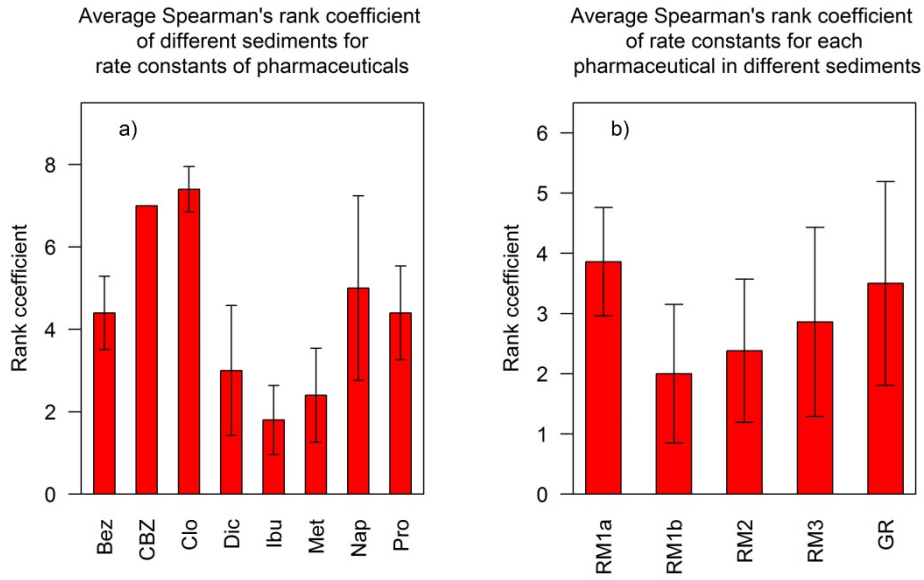

Figure C- 13: Average ranks (± standard deviation) of initial elimination rate constants of (a) different sediments for the elimination of each pharmaceutical and (b) the same pharmaceuticals in different sediments (b). The highest rate was given the lowest rank. Bez: Bezafibrate, CBZ: Carbamazepine, Clo: Clofibric Acid, Dic: Diclofenac, Ibu: Ibuprofen, Met: Metoprolol, Nap: Naproxen, Pro: Propranolol

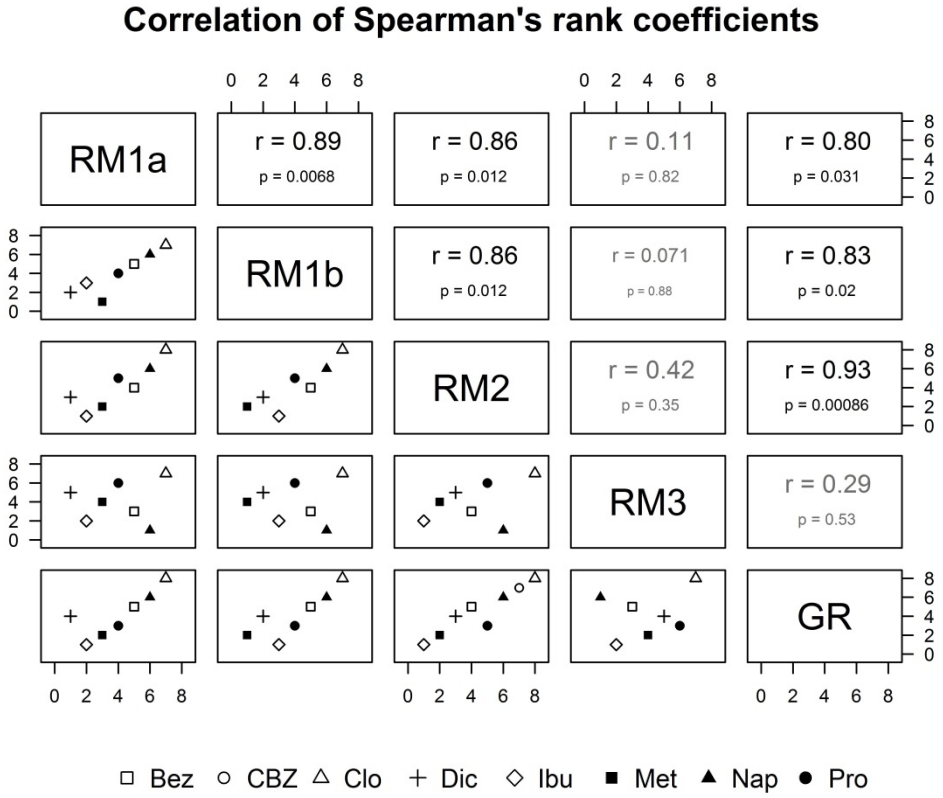

Figure C- 14: Correlation of Spearman's rank coefficients of k_{eli} of pharmaceuticals in different sediments at a filter velocity of 5.0 m d^{-1}. r: correlation coefficient; grey: no correlation based on a 95 % significance level. Bez: Bezafibrate, CBZ: Carbamazepine, Clo: Clofibric Acid, Dic: Diclofenac, Ibu: Ibuprofen, Met: Metoprolol, Nap: Naproxen, Pro: Propranolol

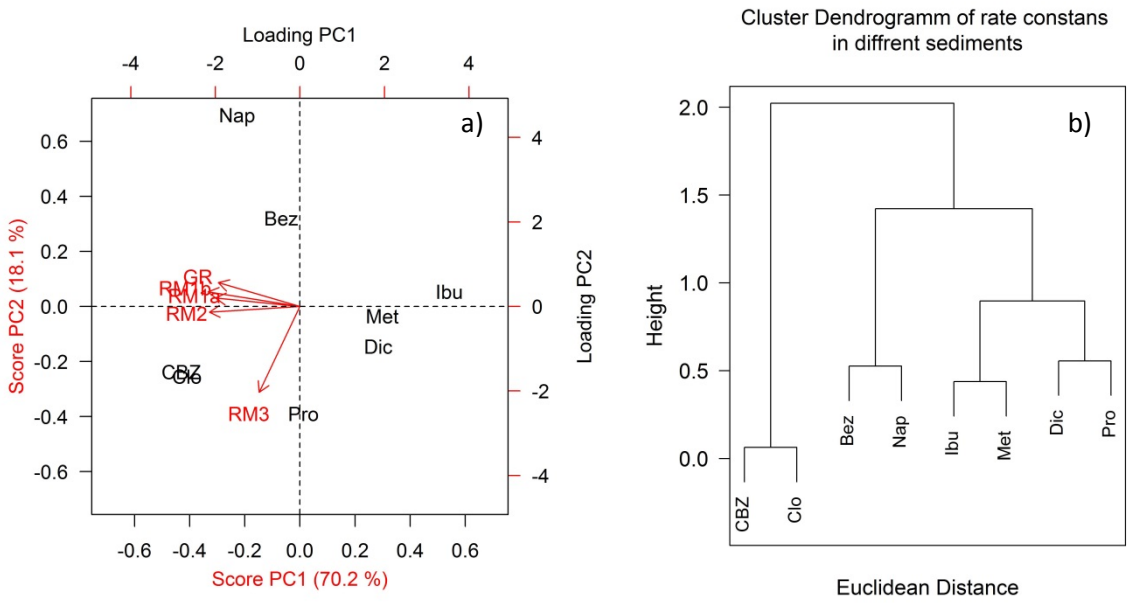

Figure C- 15: Plots for the PCA (a) and Cluster (b) analysis of the initial elimination kinetics of eight pharmaceuticals in the different sediment at a filter velocity of 5.0 m d^{-1}

Table C- 6: Initial elimination rate constants (k_{eli}, ± 95 % confidence intervals, d^{-1}) of eight pharmaceuticals in sediments RM2 and GR at different filter velocities (two replicates)

	RM2			GR		
	2.5 m d^{-1} (Exp. 2)	**5.0 m d^{-1} (Exp. 3)**	**10 m d^{-1} (Exp. 4)**	**2.5 m d^{-1} (Exp. 6)**	**5.0 m d^{-1} (Exp. 7)**	**10 m d^{-1} (Exp. 8)**
Bez	0.615 ± 0.162***	0.402 ± 0.094***	0.295 ± 0.042***	0.250 ± 0.023***	0.033 ± 0.020**	0.074 ± 0.024***
CBZ	n.s.	0.015 ± 0.005***	0.009 ± 0.002***	n.s.	n.s.	0.004 ± 0.001***
Clo	0.122 ± 0.036***	n.s.	n.s.	0.145 ± 0.261***	n.s.	0.008 ± 0.003***
Dic	0.575 ± 0.172***	0.462 ± 0.017***	0.309 ± 0.050***	0.265 ± 0.046***	0.208 ± 0.050***	0.169 ± 0.037***
Ibu	1.358 ± 0.987*	0.878 ± 0.276***	0.548 ± 0.056***	0.803 ± 0.473**	0.666 ± 0.211***	0.323 ± 0.059***
Met	0.411 ± 0.097***	0.502 ± 0.102***	0.586 ± 0.151***	0.543 ± 0.125***	0.611 ± 0.067***	0.748 ± 0.081***
Nap	0.227 ± 0.052***	0.054 ± 0.018***	0.091 ± 0.013***	0.200 ± 0.064***	n.s.	0.031 ± 0.013***
Pro	0.222 ± 0.053***	0.205 ± 0.049***	0.278 ± 0.048***	0.331 ± 0.087***	0.441 ± 0.063***	0.467 ± 0.099***

n: number of replicates; * < 0.05, **p < 0.01, ***p< 0.001; n.s.: not significant; Bez: Bezafibrate, CBZ: Carbamazepine, Clo: Clofibric Acid, Dic: Diclofenac, Ibu: Ibuprofen, Met: Metoprolol, Nap: Naproxen, Pro: Propranolol

Table C- 7: Secondary elimination rate constants ($k_{eli,sec}$, ± 95 % confidence intervals, d^{-1}) of eight pharmaceuticals in sediments RM2 and GR at different filter velocities (two replicates)

	RM2			GR		
	2.5 m d^{-1} (Exp. 2)	**5.0 m d^{-1} (Exp. 3)**	**10 m d^{-1} (Exp. 4)**	**2.5 m d^{-1} (Exp. 6)**	**5.0 m d^{-1} (Exp. 7)**	**10 m d^{-1} (Exp. 8)**
Bez	n.s.k.	n.s.k.	n.s.k.	n.s.k.	0.230 ± 0.050*** (3)	0.854 ± 0.513** (5)
CBZ	n.e.	n.s.k.	n.s.k.	n.e.	n.e.	n.s.k.
Clo	n.s.k.	0.241 ± 0.075*** (5)	0.120 ± 0.022*** (7)	n.s.k.	0.224 ± 0.092*** (5)	0.120 ± 0.030*** (10)
Dic	n.s.k.	n.s.k.	n.s.k.	n.s.k.	n.s.k.	n.s.k.
Ibu	n.s.k.	n.s.k.	n.s.k.	n.s.k.	n.s.k.	n.s.k.
Met	n.s.k.	n.s.k.	n.s.k.	n.s.k.	n.s.k.	n.s.k.
Nap	n.s.k.	0.220 ± 0.036*** (3)	n.s.k.	n.s.k.	0.221 ± 0.046*** (3)	0.216 ± 0.064*** (7)
Pro	n.s.k.	n.s.k.	n.s.k.	n.s.k.	n.s.k.	n.s.k.

$^{***}p < 0.001$; n.s.k.: no secondary elimination kinetics, n.e.: no elimination, in parenthesis (): estimated starting day of increased elimination after initial lag-phase. Bez: Bezafibrate, CBZ: Carbamazepine, Clo: Clofibric Acid, Dic: Diclofenac, Ibu: Ibuprofen, Met: Metoprolol, Nap: Naproxen, Pro: Propranolol

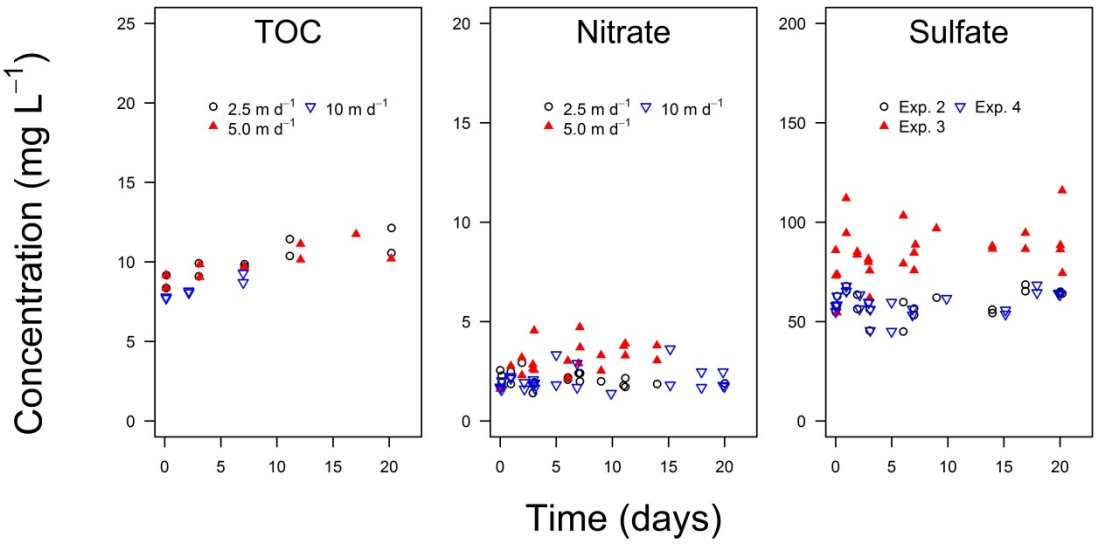

Figure C- 16: Time trends of TOC, nitrate and sulfate in experiments at different filter velocities with sediment RM2; Exp. 2: 2.5 m d^{-1}, Exp. 3: 5.0 m d^{-1} Exp. 4: 10 m d^{-1}.

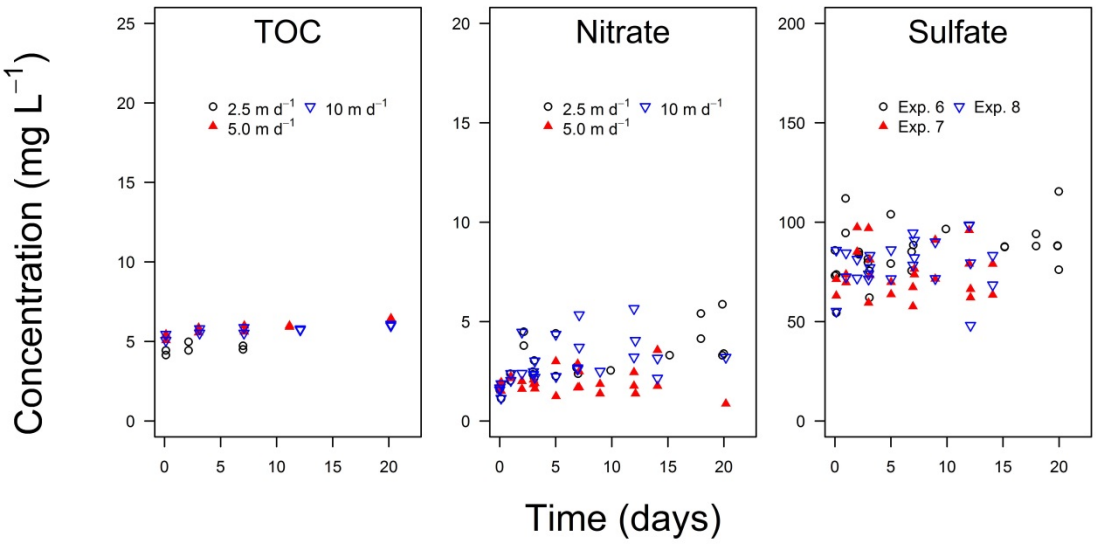

Figure C- 17: Time trends of TOC, nitrate and sulfate in experiments at different filter velocities with sediment GR; Exp. 6: 2.5 m d^{-1}, Exp. 7: 5.0 m d^{-1} Exp. 8: 10 m d^{-1}.

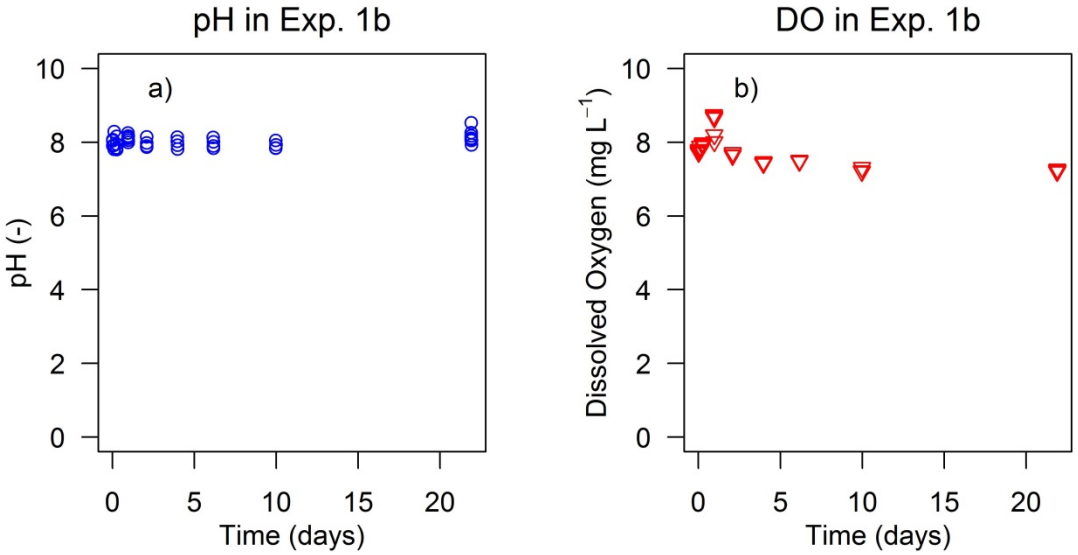

Figure C- 18: Time trends of pH and dissolved oxygen (DO) in experiment 1b. The shown data are aggregate data from all replicate experiments and both sampling ports.

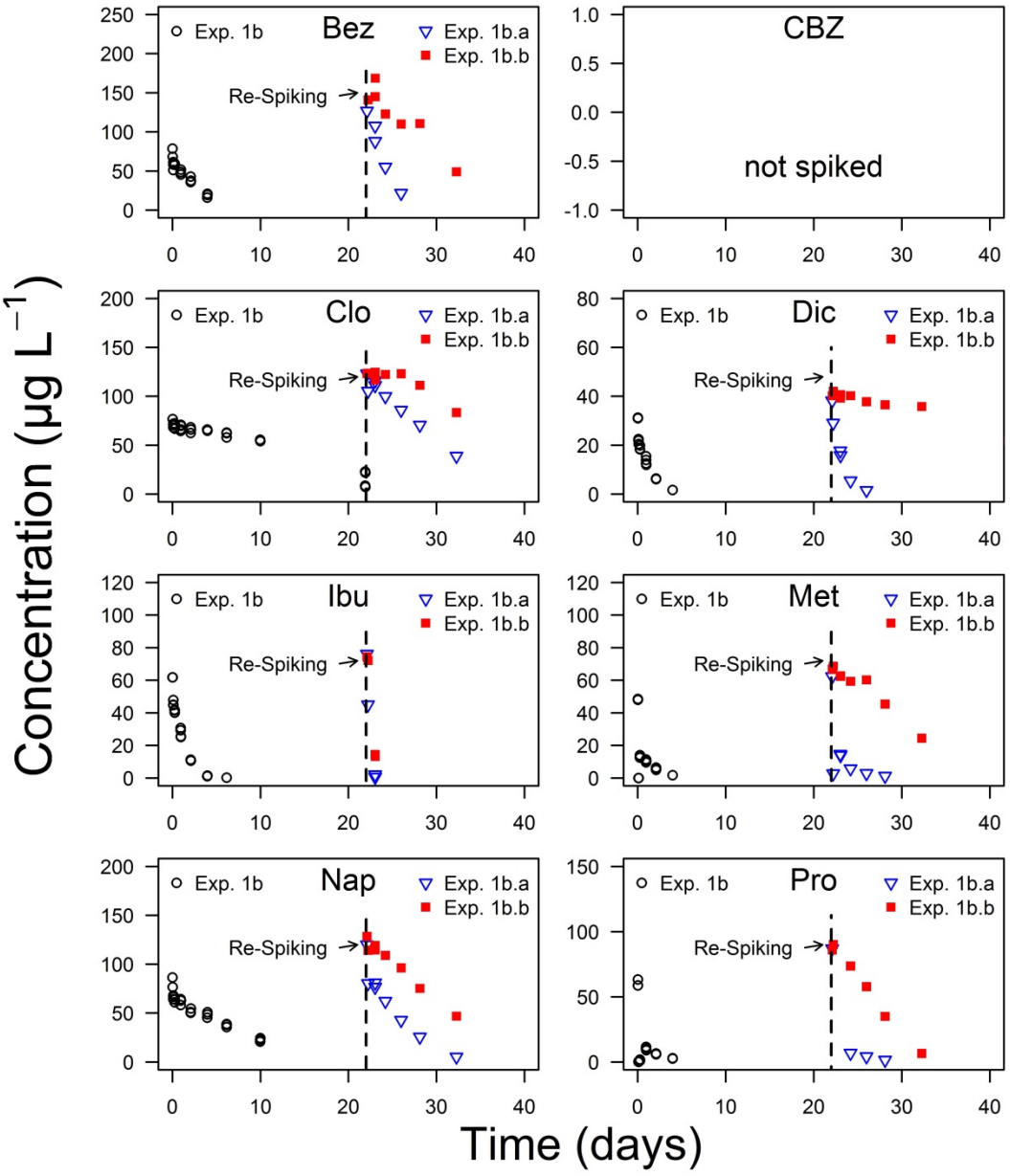

Figure C- 19: Time trends of pharmaceuticals before and after the re-spiking. Exp. 1b.a: no modification compared to Exp. 1b, Exp. 1b.b: storage bottle was disconnected from the sediment columns, Bez: Bezafibrate, CBZ: Carbamazepine, Clo: Clofibric Acid, Dic: Diclofenac, Ibu: Ibuprofen, Met: Metoprolol, Nap: Naproxen, Pro: Propranolol

Table C- 8: Initial and secondary elimination rate constants (k_{eli}, ± 95 % confidence intervals, d^{-1}) of eight pharmaceuticals in the re-spiking experiments

	Exp. 1b.a	**Exp. 1b.b**	
Modification	**No change in setup**	**Storage bottle disconnected from sediment**	
	k_{eli} (d^{-1})	**k_{eli} (d^{-1})**	**$K_{eli,sec}$ (d^{-1})**
Bez	0.509 ± 0.117**	n.s.	0.313 ± 0.091** (6)
CBZ	n.d.	n.d.	n.d.
Clo	0.105 ± 0.025*	n.s.	0.110 ± 0.027*** (4)
Dic	0.806 ± 0.116***	0.014 ± 0.006***	0.049 ± 0.007** (10)
Ibu	4.585 ± 3.164*	1.898 ± 0.450**	n.s.k.
Met	0.459 ± 0.106**	0.093 ± 0.029***	n.s.k.
Nap	0.286 ± 0.060***	0.129 ± 0.020***	n.s.k.
Pro	n.s. [0.403 ± 0.839]	0.265 ± 0.136**	n.s.k.

* < 0.05, ** p < 0.01, *** p< 0.001; n.s.: no significant elimination based on a 95%-confidence level, n.d.: not determined since carbamazepine was not spiked; in parenthesis (): estimated starting day of increased elimination after initial lag-phase, in parenthesis []: elimination but too data quality for significant regression. Bez: Bezafibrate, CBZ: Carbamazepine, Clo: Clofibric Acid, Dic: Diclofenac, Ibu: Ibuprofen, Met: Metoprolol, Nap: Naproxen, Pro: Propranolol

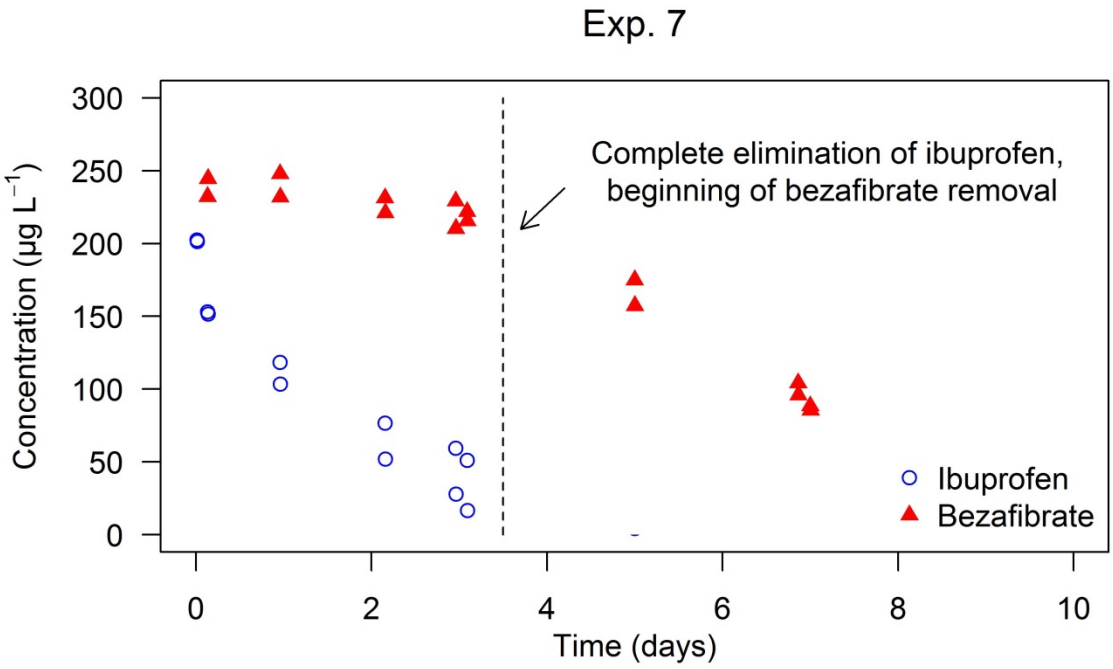

Figure C- 20: Relationship between the time trends of ibuprofen and bezafibrate during experiment 7

7 (EIDESSTATTLICHE) VERSICHERUNGEN UND ERKLÄRUNGEN

(§ 5 Nr. 4 PromO)

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