Texte

Texte
05/05
ISSN
0722-186X

Final Report Behaviour of selected human and veterinary pharmaceuticals in aquatic compartments and soil

Umwelt Bundes Amt
Für Mensch und Umwelt

ENVIRONMENTAL RESEARCH OF THE
FEDERAL MINISTRY OF THE ENVIRONMENT,
NATURE CONSERVATION AND NUCLEAR SAFETY

Research Report 299 67 401/01

Texte
05/05
ISSN
0722-186X

Final Report

Behaviour of selected human and veterinary pharmaceuticals in aquatic compartments and soil

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On behalf of the Federal Environmental Agency

Berichts – Kennblatt

1. Berichtsnummer	2.	3.

4. Titel des Berichts
(„Verhalten in der Umwelt vorkommender Pharmaka und Metaboliten in Testsystemen für den aquatischen Bereich und Boden anhand ausgewählter Vertreter des Human- und Tierbereiches“)

5. Autor(en) (Name, Vorname(n))	6. Abschlussdatum des Vorhabens
Löffler, D., Meller, M., Römbke J., Ternes, T.	31.07.2003
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12. Fördernde Institution(en) (Name, Adresse) Umweltbundesamt, Bismarckplatz 1, D-14193 Berlin 33	**13. Tabellen und Diagramme** 59 **14. Abbildungen** 11

15. Zusätzliche Angaben

16. Kurzfassung

Eine Auswahl von zehn Pharmaka und Pharmakametaboliten wurde während eines Zeitraums von 100 Tagen in Wasser/Sediment Systemen, angelehnt an die OECD Richtlinie 308, untersucht. Die 14C-markierten Testsubstanzen Diazepam, Ibuprofen, Iopromid und Paracetamol wurden durch Radio-DC bestimmt, wogegen die unmarkierten Substanzen Carbamazepin, 10,11-Dihydro-10,11-dihydroxycarbamazepin, Clofibrinsäure, 2-Hydroxy-ibuprofen, Ivermectin und Oxazepam mittels LC-Tandem MS detektiert wurden.

Da Carbamazepin, Diazepam und Clofibrinsäure im Wasser/Sediment-System eine hohe Persistenz zeigten, liegt eine geringe Abbaubarkeit in natürlichen Oberflächengewässern nahe. Dagegen wurden Oxazepam und 10,11-Dihydro-10,11-dihydroxy-carbamazepin während des Testverlaufs zu mehr als 50 % eliminiert. Für diese beiden Substanzen kann daher von einer prinzipiellen Abbaubarkeit in Oberflächengewässern ausgegangen werden. Das Kontrastmittel Iopromid wurde im Versuchszeitraum vollständig in mehrere stabile Transformationsprodukte umgewandelt. Die Entstehung dieser Transformationsprodukte wurde ebenfalls in ergänzenden Bodensäulenversuchen beobachtet (s. u.); d.h. diese können unter sehr verschiedenen Umweltbedingungen gebildet werden. Die Konzentrationen von Ivermectin und Paracetamol in der Wasserphase der Testsysteme nahmen sehr schnell ab, wobei jedoch Paracetamol in großem Umfang nicht extrahierbare Rückstände bildete. Eine schnelle Elimination aus der Wasserphase aquatischer Systeme ist für beide Substanzen zu erwarten. Das Antiphlogistikum Ibuprofen und sein Humanmetabolit 2-Hydroxy-ibuprofen wurden im Testsystem ebenfalls schnell eliminiert, was eine leichte Abbaubarkeit dieser Substanzen in natürlichen Oberflächengewässern erwarten lässt. Für Diazepam, Oxazepam, Carbamazepin und Ivermectin muss, wegen ihrer Affinität zum Sediment, von einer potenziellen Akkumulierbarkeit in natürlichen Sedimenten ausgegangen werden.

Das Versickerungsverhalten von sechs der zehn ausgewählten Pharmaka wurde in verschiedenen wassergesättigten Böden nach OECD Richtlinie 312 getestet und deren Mobilität, insbesondere hinsichtlich ihres Potenzials zur Grundwasserkontamination, beurteilt. Die Testergebnisse legen nahe, dass das Versickerungspotenzial für Diazepam, Ibuprofen, Ivermectin und Carbamazepin niedrig ist. Das letztgenannte Ergebnis ist überraschend, da Carbamazepin häufig im Grundwasser nachgewiesen wurde. Dieser Widerspruch könnte dadurch erklärt werden, dass die Tests mit Oberboden durchgeführt wurden, während in der Realität der Pharmakaeintrag in das Grundwasser über Infiltration kontaminierter Wässer durch Flusssedimente und Unterböden erfolgt. Clofibrinsäure und Iopromid zeigten sich in den Tests als sehr mobil, sodass von diesen ein deutliches Grundwasserkontaminationsrisiko ausgeht.

Die meisten der hier getesteten Arzneimittel und Arzneimittelmetaboliten wurden, ähnlich wie bei vergleichbaren Tests mit anderen Umweltchemikalien wie Pestiziden, als hoch persistent oder sogar nicht abbaubar eingestuft. Ein Umweltrisiko durch Arzneimittelrückstände in aquatischen Systemen kann daher nicht ausgeschlossen werden. Abschließend bleibt festzuhalten, dass das Umweltverhalten von bereits auf dem Markt vorhandenen Pharmaka generell im Zulassungsverfahren berücksichtigt werden sollte, solange eine Exposition bestimmter Umweltkompartimente nicht auszuschließen ist.

17. Schlagwörter
Pharmaka, Metabolite, Carbamazepin, 10,11-Dihydro-10,11-dihydroxy-carbamazepin, Clofibrinsäure, Ibuprofen, 2-Hydroxy-ibuprofen, Ivermectin, Diazepam, Oxazepam, Iopromid, Paracetamol, Umweltverhalten, Persistenz, Abbau, Sorption, Wasser, Sediment, Bodensäulen, Versickerung, LC Tandem MS, Radio-Dünnschichtchromatographie

18. Preis	19	20.

Document Control Sheet

<table>
<tr><td>1. Report No.</td><td>2.</td><td>3.</td></tr>
<tr><td colspan="3">4. Report Title
Behaviour of selected human and veterinary pharmaceuticals in aquatic compartments and soil</td></tr>
<tr><td colspan="2">5. Author(s) (Family Name(s), First Name(s))
Löffler, D., Meller, M., Römbke J., Ternes, T.</td><td>6. Report Date
31.07.2003</td></tr>
<tr><td colspan="2" rowspan="4">8. Performing Organisation(s) (Name, Address)
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D-65439 Flörsheim</td><td>7. Publication Date
15.09.2004</td></tr>
<tr><td>9. UFOPLAN-Ref. Nr.
UBA-F+E 299 67 401/01</td></tr>
<tr><td>10. No. of Pages
121 + (Annex)</td></tr>
<tr><td>11. No. of References
182</td></tr>
<tr><td colspan="2" rowspan="2">12. Sponsoring Agency (Name, Address)
Umweltbundesamt, Bismarckplatz 1, 14193 Berlin</td><td>13. No. of Tables and Diagrams
59</td></tr>
<tr><td>14. No. of Figures
11</td></tr>
<tr><td colspan="3">15. Supplementary Notes</td></tr>
<tr><td colspan="3">16. Abstract
The fate of ten selected pharmaceuticals and pharmaceutical metabolites were investigated in water/sediment tests according to OECD Guideline 308 over a period of 100 d. The ^{14}C-labelled test substances diazepam, ibuprofen, iopromide and paracetamol were analyzed by radio-TLC, whereas carbamazepine, 10,11-dihydro-10,11-dihydroxy-carbamazepine, clofibric acid, 2-hydroxy-ibuprofen, ivermectin and oxazepam were analyzed via LC-tandem MS.
Carbamazepine, diazepam and clofibric acid were persistent in the water/sediment system, suggesting their low degradability in natural surface waters. Oxazepam and 10,11-dihydro-10,11-dihydroxy-carbamazepine disappeared by more than 50 % which indicates that an appreciable elimination in surface waters might occur. The contrast medium iopromide was totally metabolized in the water/sediment tests under formation of several stable transformation products (TPs) which were also formed in soil column experiments, performed according to OECD guideline No. 312. Obviously, these metabolites can be formed under very different environmental conditions. Ivermectin and paracetamol disappeared rapidly in the water/sediment systems and should also be eliminated to a high degree from aquatic water compartments. A high level of non-extractable residues was formed from paracetamol. Ibuprofen and its' metabolite 2-hydroxy-ibuprofen were rapidly eliminated, suggesting their ready degradability in aquatic systems. The affinity of diazepam, oxazepam, carbamazepine and ivermectin for the sediment compartment indicates a potential risk for accumulation of these compounds in natural sediments.
In addition, the leaching behaviour of six selected pharmaceuticals was tested in different soils. Based on the results of this assessment their mobility in different soils and their potential to contaminate groundwater was evaluated. The test results indicated that the leaching potential could be rated as low for diazepam, ibuprofen, ivermectin and for carbamazepine. The last result is surprising, since carbamazepine is often detected in groundwater. This discrepancy might be explained by the fact that the leaching tests were performed with topsoil, whereas in reality the groundwater contamination occurs mainly over river sediments and subsoils from receiving waters. Clofibric acid and iopromide were very mobile under the experimental conditions and thus, groundwater contamination would be possible if the soil is exposed to these pharmaceuticals.
In comparison to other chemicals, notably pesticides, the distribution pattern of the compounds tested over the persistence classes showed a high percentage of high persistence or even non-biodegradability, leading to the conclusion that an environmental risk cannot be excluded. Summarizing these findings, it is recommended to include the investigation of the fate of pharmaceuticals already available on the market in the drug registration process as long as exposure in a specific environmental compartment is possible.</td></tr>
<tr><td colspan="3">17. Keywords
Pharmaceuticals, metabolites, carbamazepine, 10,11-dihydro-10,11-dihydroxy-carbamazepine, clofibric acid, ibuprofen, 2-hydroxy-ibuprofen, ivermectin, diazepam, oxazepam, Iopromide, paracetamol, fate, degradation, sorption, persistence, water, sediment, soil columns, leaching, test system, LC-tandem MS, Radio-TLC</td></tr>
<tr><td>18. Price</td><td>19.</td><td>20.</td></tr>
</table>

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Part of the

UFO – Framework 1999

Subject

Behaviour of pharmaceuticals and metabolites in environmental systems

("Verhalten von Pharmaka und Metaboliten in Umwelttestsystem")

(299 67 401)

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The results of this project led to the following publications, theses and poster presentations:

Publications in peer reviewed journals:

Löffler D, Ternes TA. Analysis of acidic pharmaceuticals, antibiotics and ivermectin in river sediment using LC-tandem MS. Journal of Chromatography A 2021:133-144 (2003).

Oppel J, Broll G, Löffler D, Meller M, Römbke J, Ternes T. Leaching behaviour of pharmaceuticals in soil-testing-systems: A part of an environmental risk assessment for groundwater protection. The Science of the Total Environment 328:265-273 (2004).

Löffler D, Römbke J, Meller M, Ternes TA. Environmental Fate of Pharmaceuticals in Water/Sediment systems. Environmental Science & Technology (submitted).

Theses prepared in this project:

Löffler D. Fate of Human and Veterinary Pharmaceuticals in Water/Sediment Test Systems by Analysis via LC-tandem MS and Radio-TLC, Dissertation, Johannes Gutenberg Universität in Mainz (2003).

Oppel J. Verhalten von Pharmaka in Bodentestsystemen - Eine Risikoabschätzung für den Boden- und Grundwasserschutz, Diploma Thesis, Westfälische Wilhelms-Universität Münster (2002).

Poster presentations:

Löffler D, Meller M, Römbke J, Ternes T. Verhalten in der Umwelt vorkommender Pharmaka und Metaboliten in Testsystemen für den aquatischen Bereich anhand ausgewählter Vertreter des Human- und Tierbereichs: Projektvorstellung und erste Ergebnisse, SETAC GLB, Berlin (2001).

Löffler D, Meller M, Römbke J, Ternes T. Verhalten ausgewählter Pharmaka in Wasser/Sediment – Testsystemen, Jahrestagung der Wasserchemischen Gesellschaft in der GDCH, Eichstätt (2002).

Löffler D, Meller M, Römbke J, Ternes T. Fate and distribution of selected pharmaceuticals in water/sediment systems, SETAC Europe, Vienna (2002).

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Zusammenfassung

Große Arzneimittelmengen gelangen nach ihrer Anwendung in das Abwasser und erreichen, da sie durch die Abwasseraufbereitung häufig nur partiell eliminiert werden, die aquatische Umwelt. Trotz des intensiven Einsatzes von Pharmaka in der modernen Medizin ist relativ wenig über ihr Vorkommen und Verhalten in natürlichen Gewässer/Sediment-Systemen bekannt.

Entwicklung analytischer Methoden

In der vorliegenden Arbeit wurden analytische Methoden entwickelt, die eine empfindliche Bestimmung von verschiedenen Analgetika, Lipidsenkern, Antibiotika, sowie Carbamazepin, Diazepam, Ivermectin, Iopromid und einigen ihrer Metabolite in Sedimenten von Oberflächengewässern erlauben. Diese Methoden umfassen eine sequenzielle Ultraschall-Lösungsmittelextraktion, Festphasenextraktion und die Detektion der Analyten durch Flüssigkeitschromatographie-Tandem Massen-spektrometrie (LC-Tandem MS). Des Weiteren wurden neue Methoden für die empfindliche Quantifizierung dieser Substanzen in wässrigen Umweltmatrices erstellt. Die Analyten wurden dabei durch Festphasenextraktion angereichert und ebenfalls durch LC-Tandem MS bestimmt. Für die Arzneimittelrückstände im Sediment wurden Bestimmungsgrenzen zwischen 0,4 und 20 ng/g erreicht, während die Bestimmungs-grenzen in wässrigen Matrices zwischen 0,04 und 0,5 µg/l lagen. Zusätzlich wurden analytische Methoden für die Quantifizierung von ^{14}C-markierten Pharmaka in Oberflächenwasser und Sediment durch Radio-Dünnschichtchromatographie (DC) entwickelt.

Verhalten von Arzneimitteln in Wasser/Sediment Systemen

Eine Auswahl von zehn Pharmaka und Pharmakametaboliten wurde während eines Zeitraums von 100 Tagen in Wasser/Sediment Systemen, angelehnt an die OECD Richtlinie 308, untersucht. Die ^{14}C-markierten Testsubstanzen Diazepam, Ibuprofen, Iopromid und Paracetamol wurden durch Radio-DC bestimmt, wogegen die unmarkierten Substanzen Carbamazepin, 10,11-Dihydro-10,11-dihydroxycarbamazepin,

Clofibrinsäure, 2-Hydroxy-ibuprofen, Ivermectin und Oxazepam mittels LC-Tandem MS detektiert wurden.

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Unterböden erfolgt. Clofibrinsäure und Iopromid zeigten sich in den Tests als sehr mobil, so dass von diesen ein deutliches Grundwasserkontaminationsrisiko ausgeht.

Die meisten der hier getesteten Arzneimittel und Arzneimittelmetaboliten wurden, ähnlich wie bei vergleichbaren Tests mit anderen Umweltchemikalien, z.B. Pestiziden, als hoch persistent oder sogar nicht abbaubar eingestuft. Ein Umweltrisiko durch Arzneimittelrückstände in aquatischen Systemen kann daher nicht ausgeschlossen werden. Abschließend bleibt festzuhalten, dass das Umweltverhalten von bereits auf dem Markt vorhandenen Pharmaka generell im Zulassungsverfahren berücksichtigt werden sollte, solange eine Exposition bestimmter Umweltkompartimente nicht auszuschließen ist.

Summary

Pharmaceutical residues are not totally eliminated during municipal sewage treatment and are thus discharged into the aquatic environment via STP effluents. Despite the extensive use of pharmaceuticals in modern medicine, relatively little is known about the occurrence and the fate of pharmaceuticals in sediments and environmental waters.

Development of analytical methods

Analytical methods have been developed in the present work, allowing for the sensitive determination of several analgesics, lipid regulators and antibiotics, as well as for carbamazepine, diazepam, ivermectin, iopromide and some of their metabolites in river sediment. Sediment analysis was accomplished by sequential ultrasonic solvent extraction, solid phase extraction for a clean-up and detection via liquid chromatography–tandem mass spectrometry (LC-tandem MS). Additionally, new methods have been created for the sensitive analysis of these compounds in various environmental waters. The analytes in the aqueous samples were enriched by solid phase extraction and detected via LC-tandem MS. For the determination of pharmaceuticals in sediments LOQs between 0.4 and 20 $ng \cdot g^{-1}$ were attained, whereas those in environmental waters ranged from 0.04 to 0.5 $\mu g \cdot L^{-1}$.

Furthermore, analytical methods were developed for the analysis of ^{14}C-labelled pharmaceuticals in surface water and river sediment samples via radio-thin layer chromatography (radio-TLC).

Fate of pharmaceuticals in water/sediment systems

In the second part of this project, the fate of ten selected pharmaceuticals and pharmaceutical metabolites were investigated in water/sediment tests according to OECD Guideline 308 over a period of 100 d. The ^{14}C-labelled test substances diazepam, ibuprofen, iopromide and paracetamol were analyzed by radio-TLC, whereas carbamazepine, 10,11-dihydro-10,11-dihydroxy-carbamazepine, clofibric acid, 2-hydroxy-ibuprofen, ivermectin and oxazepam were analyzed via LC-tandem MS.

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In addition, the leaching behaviour of six selected pharmaceuticals was tested in different soils. Based on the results of this assessment their mobility in different soils and their potential to contaminate groundwater was evaluated. The test results indicated that the leaching potential could be rated as low for diazepam, ibuprofen, ivermectin and for carbamazepine. The last result is surprising, since carbamazepine is often detected in groundwater. This discrepancy might be explained by the fact that the leaching tests were performed with topsoil, whereas in reality the groundwater contamination occurs mainly over river sediments and subsoils from receiving waters. Clofibric acid and iopromide were very mobile under the experimental conditions and thus, groundwater contamination would be possible if the soil is exposed to these pharmaceuticals.

In comparison to other chemicals, notably pesticides, the distribution pattern of the compounds tested over the persistence classes showed a high percentage of high persistence or even non-biodegradability, leading to the conclusion that an environmental risk cannot be excluded. Summarizing these findings, it is recommended to include the investigation of the fate of pharmaceuticals already available on the market in the drug registration process as long as exposure in a specific environmental compartment is possible.

Table of Contents

Abbreviations and acronyms

APCI	*Atmospheric pressure chemical ionization*
ASE	*Accelerated solvent extraction*
CAN	*Carbamazepine*
CID	*Collision induced dissociation*
CLO	*Clofibric acid*
COH	*10,11-Dihydro-10,11-dihydroxycarbamazepine*
DDD	*Defined daily dose*
DIA	*Diazepam*
DOC	*Dissolved organic carbon*
dpm	*Decays per minute*
DT	*Dissipation time*
ERA	*Environmental risk assessment*
ESI	*Electrospray ionization*
EU	*European Union*
HI	*2-Hydroxyibuprofen*
HPLC	*High performance liquid chromatography*
IAR	*Initial amount of radioactivity*
IBU	*Ibuprofen*
IO	*Iopromide*
ISO	*International Organization for Standardization*
IVR	*Ivermectin*
LC	*Liquid chromatography*
LOQ	*Limit of quantification*
LSC	*Liquid scintillation counting*
m/z	*Mass-to-charge ratio*
MRM	*Multiple reaction monitoring*
MS	*Mass spectrometry*
MS/MS	*Tandem mass spectrometry*
OECD	*Organization for Economic Co-operation and Development*
OXA	*Oxazepam*
PAR	*Paracetamol*
PPCO	*Polypropylene copolymer*
RP	*Reversed phase*
S/N	*Signal-to-noise ratio*
SETAC	*Society of Environmental Toxicology and Chemistry*
SPE	*Solid phase extraction*
STP	*Sewage treatment plant*
TLC	*Thin layer chromatography*
UV	*Ultra violet*
WS	*Water/sediment study*
SD	*Standard deviation*

1 INTRODUCTION

Environmental pollution has become an important issue for the society. Virtually any human activity leads to an environmental contamination with substances of anthropogenic origin (Schwarzenbach et al., 2003). Besides pesticides or heavy metals pharmaceuticals, being extensively used for medicinal purposes, belong to these anthropogenic substances. They were widely ignored as environmental contaminants until the early 1990th. Since then, the environmental risk caused by pharmaceuticals has become an important issue in environmental sciences (e.g. Golet et al., 2002a, Halling-Sorensen et al., 1998, Jones et al., 2002, Römbke et al., 1996, Stuer-Lauridsen et al., 2000, Van Wezel and Jager, 2002, Velagaleti and Robinson, 2001), mainly because an increasing number of pharmaceuticals, i.e. analgesics, antibiotics, antiepileptics, antiphlogistics, beta-blockers, contraceptives, diagnostics, lipid regulators, tranquilizers and corresponding metabolites have been detected in the environment (Golet et al., 2001, Heberer, 2002a, Hirsch et al., 1999, Hirsch et al., 1998, Hirsch et al., 2000, Kolpin et al., 2002, McArdell et al., 2003, Stumpf et al., 1996, Stumpf et al., 1999, Ternes, 1998, Ternes and Hirsch, 2000, Ternes et al., 1998a, Ternes et al., 1999a, Ternes et al., 1999b).

More than 2900 pharmaceutical substances are currently licensed in Germany for human and veterinary medicine. For most compounds, the total amounts sold are not available. Nevertheless, a good estimation of the annual quantities prescribed can be obtained based on the accessible number of prescription items multiplied by the defined daily dose (DDD) of a particular compound. In the case of free available pharmaceuticals, i.e. analgesics, this calculation leads to underestimations, since the amounts dispensed in non-prescription products are not considered.

Detailed data on the prescribed quantities of several selected pharmaceuticals is shown in Table 1.1 (Rote Liste Service GmbH Frankfurt/Main, 2001, Schwabe and Paffrath, 2000). Annual consumption rates of frequently prescribed pharmaceuticals range from a few kilograms, i.e. for hormones, up to more than a hundred tons, i.e. for ibuprofen and iopromide.

Table 1.1: Annual prescription items of selected pharmaceuticals and estimated total amounts used in Germany in 2001

Compound	Therapeutic use	Total prescription items ($\times 10^6$)[a]	Amounts used[c] ($t \cdot year^{-1}$)
Diclofenac	Antiphlogistic	494	86
Ibuprofen	Antiphlogistic	107	344
Erythromycin	Antibiotic	20.6	19
Roxithromycin	Antibiotic	20.8	10
Sulfamethoxazole	Antibiotic	23.6	54
Trimethoprim	Antibiotic	23.6	11
Carbamazepine	Antiepileptic	78.0	88
Metoprolol	Beta blocker	455	93
Sotalol	Beta blocker	120	27
Iopromide	Contrast medium	-	130[b]
Ethinylestradiol	Hormone	355	0.047
Bezafibrate	Lipid regulator	43.2	33
Diazepam	Tranquilizer	44.3	1.1

[a] taken from Schwabe and Paffrath, 2000, Schwabe and Paffrath, 2002, [b] Steger-Hartmann et al., 1999, [c] Bund/Länderausschuss für Chemikaliensicherheit (BLAC), 2003

Corresponding data for England, Denmark and Australia have been published by Jones et al., 2002 and others (Jørgensen and Halling-Sorensen, 2000, Khan and Ongerth, 2002, Stuer-Lauridsen et al., 2000), all displaying the extensive circulation of the pharmaceuticals in developed countries.

1.1 Theoretical background

1.1.1 Pharmacokinetics

A major factor determining the occurrence of pharmaceuticals in the aqueous environment is their pharmacokinetic behaviour which describes the time course of a drug and its' metabolites in the body after any kind of administration (Merck & Co. Inc., 1999). The metabolism of pharmaceuticals occurs in two consecutive phases as shown in Figure 1.1. Phase I reactions involve the formation of new or modified functional groups including oxidation, reduction, hydrolysis, hydration, condensation and isomerization reactions, which usually all result in an increased polarity. In phase II, the metabolites are conjugated with endogenous molecules to obtain an elevated water solubility. The most important conjugation reaction for xenobiotics is glucuronidation. Finally, the water-soluble metabolites are excreted via urine and feces

(Eisenbrand and Metzler, 1994, Forth et al., 1996, Merck & Co. Inc., 1999, Mutschler, 1997).

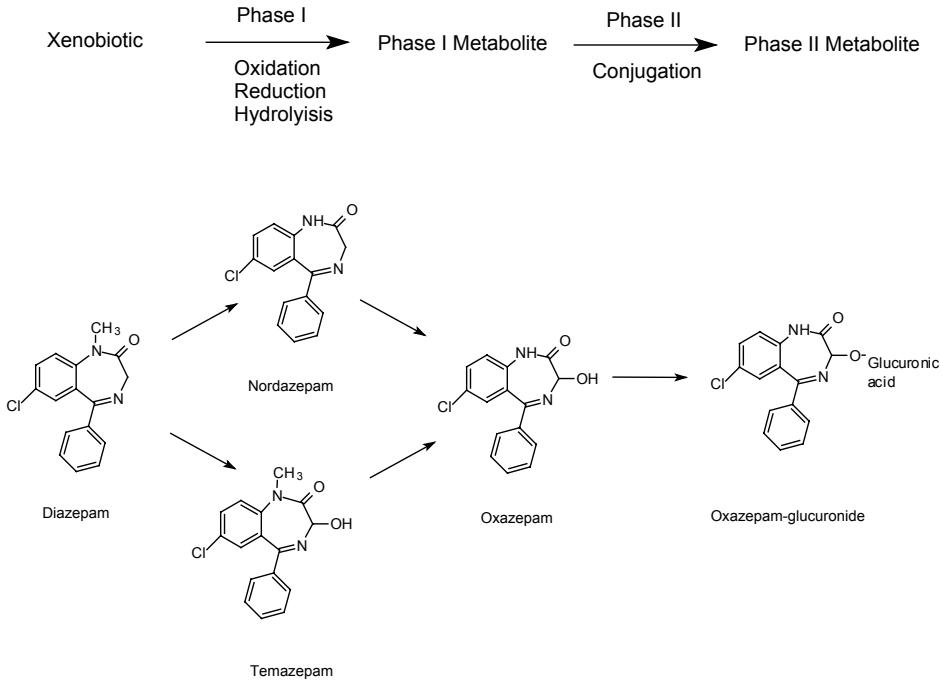


Figure 1.1: General scheme for the metabolization of a xenobiotic and the metabolization of diazepam in humans (Mutschler, 1997)

1.1.2 Exposure pathway

Production and application of human and veterinary pharmaceuticals leads to a potential environmental exposure and potentially to an accumulation in certain environmental compartments. The main exposure routes of human pharmaceuticals are expected to be through their use by patients in private households, in hospitals and by disposal of pharmaceuticals through toilets. After their use, pharmaceuticals are excreted unchanged and/or as metabolites in feces and urine and hence are present in wastewater (Mutschler, 1997).

Similar to other compounds of anthropogenic origin, the fate of the pharmaceuticals residues during sewage treatment can follow one or a combination of three types of behaviour: a) (bio)degradation (mineralization), b) sorption of the residues onto sewage sludge or c) no elimination. The latter results in their presence in treated wastewater (Halling-Sorensen et al., 1998, Hartig et al., 1999, Heberer, 2002b).

Hence, compounds that are not readily degradable enter the environment either with the digested sludge or as dissolved pollutants in the sewage treatment plant (STP) discharges. The latter scenario results in the contamination of the receiving waters and finally, the aquatic environment (Golet, 2002, Golet et al., 2001, Golet et al., 2002b, Heberer, 2002a, Heberer, 2002b, Heberer et al., 2002, Richardson and Bowron, 1985, Stumpf et al., 1999, Ternes, 1998, Ternes et al., 1999a, Zuccato et al., 2000).

Pharmaceutical residues have also been found in groundwater. They were often traced back either to an impact of municipal or industrial wastewater, animal farming, an infiltration of contaminated surface water or landfill seepage over vulnerable water aquifers (Campagnolo et al., 2002, Hirsch et al., 1999, Holm et al., 1995, Lindsey et al., 2001, Sacher et al., 2001, Ternes, 2000). It is further conceivable, that contamination occurs with the discharges of the pharmaceutical industry.

Environmental exposure routes for veterinary pharmaceuticals can mainly be attributed with the distribution and application of dung, urine and manure as fertilizer from medicated animals (Boxall et al., 2001, Campagnolo et al., 2002). Direct carry-over into the water compartment from medical treatment of aquacultures has also been linked (Cannavan et al., 2000, Davies and Rodger, 2000, Hektoen et al., 1995).

In contrast to pesticides, pharmaceuticals are applied during the entire year. Potential steady-state concentrations can thus result in the environmental waters, as they are continuously introduced via the sewage effluents.

1.1.3 Occurrence and fate

Numerous studies have been conducted to investigate various aqueous matrices for the presence of pharmaceutical residues, comprising the target compounds and metabolites. In fact, these residues have been found to be ubiquitous in environmental waters (Daughton and Ternes, 1999, Heberer, 2002a, Jones et al., 2001).

The main contributing factor for the occurrence of pharmaceuticals in the aquatic environment is the elimination efficiency of the sewage treatment process (Alcock et al., 1999, McArdell et al., 2003, Ternes, 1998, Ternes et al., 1999a, Ternes et al., 1999b). Particular weather conditions, i.e. rainstorm events, may result in a reduced elimination efficiency (Ternes, 1998).

Many pharmaceuticals are excreted to a large extent as transformed phase I metabolites and/or after conjugation to hydrophilic groups as phase II metabolites. Conjugates are easily cleaved in the STP, causing a re-formation of the original pharmaceuticals (Belfroid et al., 1999, Ternes et al., 1999a, Ternes et al., 1999b). This might lead to higher concentrations in the STP effluent than in the raw wastewater.

Pharmaceuticals are principally designed to persist in the body after administration. That might be the reason that many pharmaceuticals are relatively resistant towards degradation under environmental conditions and pass through the STP without major elimination, such as the lipid regulator clofibric acid, the antiepileptic carbamazepine or the contrast medium diatrizoate (Putschew et al., 2000, Ternes, 1998, Ternes and Hirsch, 2000).

Residues of various pharmaceuticals are present in the low $\mu g \cdot L^{-1}$ range in STP effluents. Discharge of the STP effluent into the receiving waters leads to a dilution of the pharmaceutical residues which occur up to the high $ng \cdot L^{-1}$ range in contaminated surface water. Once introduced into the surface waters, pharmaceuticals may undergo biodegradation, most likely due to co-metabolic processes. For some pharmaceuticals, i.e. diclofenac, photo induced degradation may occur from natural solar radiation (Andreozzi et al., 2003, Andreozzi et al., 2002, Buser et al., 1998, Lam et al., 2003, Steger-Hartmann et al., 2001). Additionally, depending on the lipophilicity and specific sorption properties of a particular compound, distribution to the sediment and suspended matter occurs (Schwarzenbach et al., 2003). This might result in a change in the transformation behaviour, when sorbed to particular matter, or in a formation of bound residues. However, the extent of pharmaceutical sorption to environmental matter is hardly known.

1.1.4 Fate studies

The determination of the environmental fate of a compound is a complex issue. Transformation and distribution processes are strongly dependant on the specific environmental conditions, which leads to a sophisticated linkage of individual system parameters. In general, there are two major approaches for environmental studies. Field studies allow for the elucidation of a substances behaviour under realistic conditions, whereas laboratory experiments display only certain details of the entire scenario. Since

field studies are quite costly, intensive and frequently the data are difficult to interpret, their realization is mostly limited to certain cases, justifying the effort to be undertaken. In this light, laboratory tests are powerful tools for the elucidation of individual environmental processes, providing high comparability and reproducibility, due to standardized conditions.

In the last several years, numerous test systems have been established allowing for the investigation of a chemical's fate and effects under a variation of relevant environmental conditions in terrestrial, aquatic and other scenarios (Brodsky et al., 1997, Freitag et al., 1985, Freitag et al., 1982, Hill et al., 1994). Respective standardized test procedures are provided by several organizations and institutions, i.e. OECD, 2003b (Table 1.2) and SETAC, 1995. They are an important part of the ERA of environmental relevant chemicals.

Table 1.2: Available OECD Guidelines for the testing of the degradation of chemicals in terrestrial and aquatic laboratory test systems

Test	OECD Guideline No.
Ready biodegradability (several variations)	301
Inherent biodegradability (several variations)	302
Simulation tests – aerobic sewage treatment A: Activated Sludge units B: Biofilms	303
Inherent biodegradability in soil	304
Biodegradability in seawater	306
Aerobic and anaerobic transformation in soil	307
Aerobic and anaerobic transformation in aquatic sediment systems	308
Aerobic mineralization in surface water – simulation biodegradation test	309
Leaching in soil columns	312

1.2 Objectives

Pharmaceuticals are essential for our health system and are applied in high quantities for preventive, curative and diagnostic purposes. The main environmental exposure pathway is the introduction of pharmaceuticals and their metabolites via wastewater into the aquatic environment.

To date, little is known about the fate and the distribution behaviour of pharmaceutical residues in aquatic compartments. Particularly, the role of surface water sediments in the elimination of pharmaceutical contaminants is widely unknown since appropriate analytical methods for sediments are rare. However, the number of pharmaceuticals which are quantifiable in environmental waters is restricted to approximately 100 compounds, including only a few metabolites.

Hence, the major aim of the current study was the sensitive and reliable determination of the environmental fate of selected pharmaceuticals and their metabolites. In detail, the objectives of the present work were:

- the development and the validation of analytical methods for the substance specific determination of pharmaceuticals and corresponding metabolites in river sediment and in the aqueous matrices groundwater, soil leachates, surface water and wastewater, based on LC tandem MS techniques;
- the adaptation of the developed methods for the analysis of ^{14}C-labelled pharmaceuticals in surface water and river sediment using radio-TLC-techniques;
- the elucidation of the fate and distribution behaviour of selected pharmaceuticals in water/sediment test systems according to OECD Guideline 308 (OECD, 2002), using the analytical methods developed;
- the investigation of the leaching behaviour of selected pharmaceuticals in soil column experiments according to OECD Guideline 312 (OECD, 2003a).

2 SELECTION OF ANALYTES

The selection of target analytes was mainly focused on the environmental relevance of the pharmaceuticals and their metabolites which is derived from consumption rates, the environmental occurrence, the environmental persistence and possible (eco)toxic effects.

For the investigation of pharmaceuticals in water/sediment test systems, the number of substances to be tested was restricted to 10 compounds. The leaching behaviour was investigated using six pharmaceuticals.

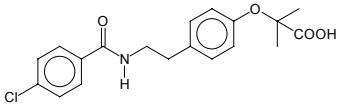
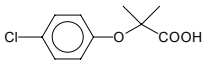
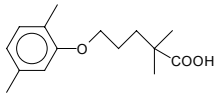
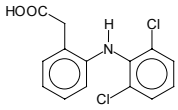
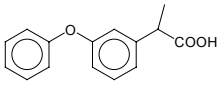
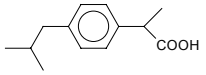
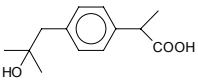
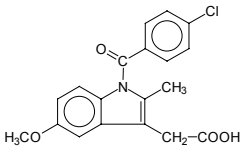
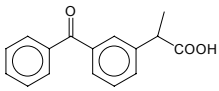
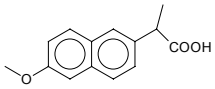
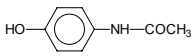
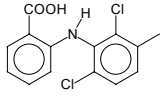
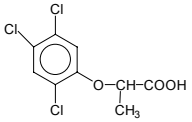
2.1 Lipid regulators and antiphlogistics

Fibric acid-derivatives, such as bezafibrate, gemfibrozil and clofibric acid esters are administered in humans to reduce the blood triglyceride- and cholesterol level. Their structures are shown in Table 2.1. The DDD amounts to 400 – 600 mg for bezafibrate and to 900 mg for gemfibrozil. In 2001, a total of more than 25 tons of bezafibrate has been prescribed in Germany, while gemfibrozil was not registered (Table 1.1). Clofibrate, etofyllinclofibrate and etofibrate are pro-drugs and as such cleaved in the body into the active metabolite clofibric acid. Bezafibrate, gemfibrozil and clofibric acid are excreted largely as glucuronides and/or unchanged with the urine (Forth et al., 1996, Mutschler, 1997).

The non-opioid analgesics display a wide application range, due to their combined analgesic, antipyretic and inflammatory effects (Mutschler, 1997). Their chemical structures are shown in Table 2.1. Their extensive use after prescription (see Table 1.1) is further increased by their dispense over the counter (Jones et al., 2002). In particular, ibuprofen is used in quantities of $> 100\ t \cdot y^{-1}$ and its' human and environmental metabolites 2-hydroxy-ibuprofen and carboxyl-ibuprofen have been identified in environmental waters (Stumpf et al., 1998, Winkler et al., 2001). Paracetamol belongs also to the group of antiphlogistics which are prescribed and used in extremely high quantities. Its fate in the aquatic environment was already studied in 1985 (Richardson and Bowron, 1985). Lipid regulators and especially antiphlogistics have a high

environmental relevance due to their high consumption rates shown in Table 1.1 and the low degradability of several compounds. As a result these pharmaceuticals occur ubiquitous in environmental waters (Heberer, 2002a, Heberer and Stan, 1998, Scheytt et al., 1998, Stumpf et al., 1999, Ternes, 1998, Ternes, 2000).

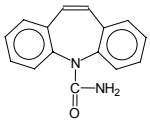
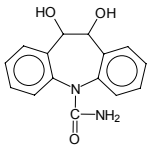
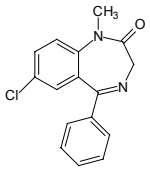
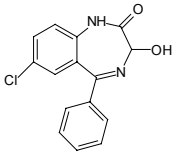
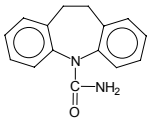
Table 2.1: CAS registry numbers, chemical structure and use/origin of acidic pharmaceuticals

Compound	CAS RN	Structure	Use/origin
Bezafibrate	41859-67-0		Lipid regulator
Clofibric acid	882-09-7		Metabolite of three lipid regulators
Gemfibrozil	25812-30-0		Lipid regulator
Diclofenac	15307-86-5		Antiphlogistics
Fenoprofen	53746-45-5		Antiphlogistics
Ibuprofen	15687-27-1		Antiphlogistics
2-Hydroxy ibuprofen	51146-55-5		Metabolite of ibuprofen
Indomethacin	53-86-1		Antiphlogistics
Ketoprofen	22071-15-4		Antiphlogistics
Naproxen	22204-53-1		Antiphlogistics
Paracetamol	103-90-2		Antiphlogistics
Meclofenamic acid	644-62-2		Surrogate standard
Fenoprop	93-72-1		Surrogate standard

2.2 Carbamazepine and tranquilizers

The antiepileptic carbamazepine is a tricyclic substance and was used at an annual level of 88 t in 2001 (Table 1.1 and Table 2.2). It was thus the most important antiepileptic in terms of quantity. Medicinal application of carbamazepine is indicated in the treatment of epilepsy symptoms, trigeminal neuralgia, diabetic neuralgia, alcohol deterrent and other symptomatologies (Mutschler, 1997, Novartis, 2000). After oral administration, approximately 70 % of the administered carbamazepine is excreted in the urine, composed mainly by the hydroxylated and conjugated carbamazepine metabolite 10,11-dihydro-10,11-dihydroxy-carbamazepine, with only 3 % of unchanged carbamazepine (Mandrioli et al., 2001, Novartis, 2000).

Table 2.2: CAS registry numbers, chemical structure and use/origin of neutral pharmaceuticals

Compound	CAS RN	Structure	Use/origin
Carbamazepine	298-46-4		Antiepileptic
10,11-Dihydro-10,11-dihydroxy-carbamazepine	-		Human metabolite of carbamazepine
Diazepam	439-14-5		Psychiatric drug
Oxazepam	604-75-1		Psychiatric drug and metabolite of diazepam
10,11-Dihydro-carbamazepine	3564-73-6		Surrogate standard

Diazepam and oxazepam belong to the group of 1,4-benzodiazepines and are utilized as tranquilizers (Mutschler, 1997). Their DDD is relatively low at 5 – 40 mg, due to their high potency, resulting in annual consumptions of 400 – 1100 kg. Diazepam is

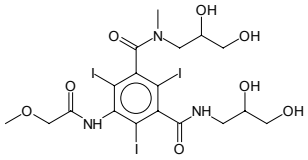
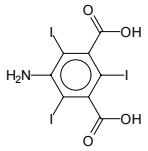
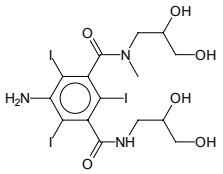
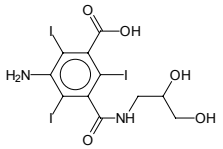
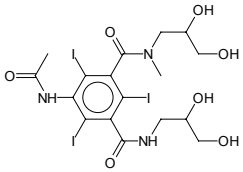
mainly metabolized to oxazepam and oxazepam-glucuronides, which are then renally excreted.

The ubiquitous occurrence of carbamazepine derives from its' relatively high prescription rates and that it is not appreciable removed when passing municipal STPs. In contrast, the consumed amounts of diazepam and oxazepam are relatively low (Table 1.1), but the very high potency of the tranquilizers raises concerns about possible ecotoxicological effects.

2.3 Iopromide and derivatives

Iodinated X-ray contrast media are widely applied as diagnostics for the roentgenoscopy of body tissues, at single doses of up to 200 g (structures given in Table 2.3). As a prerequisite for their use in high quantities in humans, these compounds display minimal toxicity, high water solubility and inertness to biochemical transformations. Iopromide is a non-ionic compound, which is applied in Germany at approximately 130 t per year (Steger-Hartmann et al., 1999). Since iopromide is quite stable, it is poorly eliminated during sewage treatment and thus, is introduced into the receiving waters. Iopromide was detected in surface and groundwater up to the $\mu g \cdot L^{-1}$ level (Putschew et al., 2001, Steger-Hartmann et al., 1999, Ternes, 2000, Ternes and Hirsch, 2000).

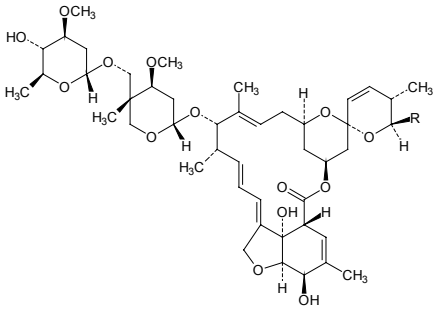
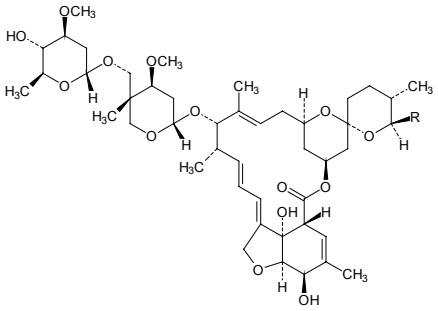
Table 2.3: CAS registry numbers, chemical structure and use/origin of iopromide, iopromide derivatives and paracetamol

Compound	CAS RN	Structure	Use/origin
Iopromide	73334-07-3		X-ray contrast medium
5-Amino-2,4,6-triiod-isophthalic acid (ATI)	35453-19-1		Potential TP of iopromide
Desmethoxyacetyl-iopromide (DAMI)	154361-51-0		Potential TP of iopromide
ATI-(2,3-dihydroxy-propyl)-amide (ATH)	111453-32-8		Potential TP of iopromide
Desmethoxy-iopromide (DMI)	76350-28-2		Surrogate standard

2.4 Ivermectin

Ivermectin is a lipophilic broad-spectrum parasiticide with a macrolide structure used for the treatment of humans, livestock and other animals against endoparasites (structure shown in Table 2.4). In detail, ivermectin is the pharmaceutical of choice for the human therapy of river blindness (onchocerciasis), applied in a single dose of 10 – 15 mg once per year. Additionally, it is used as feed additive in aquaculture to alleviate sea lice infestations in farmed salmon. Hence, ivermectin combines a high pharmacological potency and a direct cross-over into the terrestrial and aquatic environment (Campbell, 1989, Davies et al., 1998, Edwards et al., 2001, Mutschler, 1997, Van Wezel and Jager, 2002).

Table 2.4: CAS registry numbers, chemical structure and use/origin of parasiticides

Compound	CAS RN	Structure	Use/origin
Abamectin	71751-41-2, B1a 65195-55-3		surrogate standard
Ivermectin	B1a 71827-03-7, 70161-11-4, B1b 70209-81-3		parasiticide, pesticide

2.5 Selection of test compounds

The water/sediment studies were conducted with ten compounds from various pharmacological classes (Table 2.5). Compounds were selected considering the annually consumption rates, the environmental occurrence, the biodegradability and the pharmacological properties. The selection included also 4 human metabolites which are excreted in high portions. With the exception of oxazepam, paracetamol, 2-hydroxy-ibuprofen and 10,11-dihydro-10,11-dihydroxy-carbamazepine the same compounds were also tested in the leaching study.

Table 2.5: Selection of compounds for water/sediment studies

Compound	Major key-points for selection	Form applied
Iopromide	extensive application environmental occurrence low biodegradability	^{14}C-labelled
Diazepam	environmental occurrence high potency	^{14}C-labelled
Oxazepam	main human metabolite of diazepam	non-labelled
Paracetamol	extensive application environmental occurrence	^{14}C-labelled
Ibuprofen	extensive application environmental occurrence	^{14}C-labelled
2-Hydroxy-ibuprofen	ibuprofen metabolite	non-labelled
Clofibric acid	environmental occurrence low biodegradability	non-labelled
Carbamazepine	extensive application environmental occurrence low biodegradability	non-labelled
10,11-Dihydro-10,11-dihydroxy-carbamazepine	main human metabolite of carbamazepine	non-labelled
Ivermectin	environmental occurrence high potency	non-labelled

3 MATERIALS AND METHODS

3.1 Methods for the characterization of water, sediment and soil samples

Equipment and materials

- Organic C analyser: TOCOR 2, Maihak AG, Hamburg
- Muffle furnace, Nabler Typ L51/SP, Nabler Industrieofenbau, Lilienthal/Bremen;
- pH-meter: CG 822, Schott-Geräte GmbH, Hofheim;
- Oxygen-meter: Microprocessor OXI 196 (electrode EO 196-1,5), WTW, Weilheim;
- Conductivity measurement: e.g. LF 96-A, WTW, Weilheim;
- Total hardness: Aquamerck® Gesamthärte, Merck Eurolab, Frankfurt/M;
- Nitrate: Spectroquant® Nitrate Test, Merck Eurolab, Frankfurt/M;
- Phosphate: Spectroquant® Phosphate Test, Merck Eurolab, Frankfurt/M;
- Chemicals: $CHCl_3$, $CaCl_2$, HCl conc., Merck Eurolab, Frankfurt/M.

TOC of aqueous samples

The total organic carbon content (TOC) of liquid samples were measured via an automatic TOC analyser (TOCOR 2, Maihak AG, Hamburg). Prior to the measurement 3 drops of HCl conc. were added to each sample (30 mL) to remove carbonate bound carbon.

Nitrate content, phosphate content and hardness of aqueous samples

The total hardness and concentrations of nitrate and phosphate in the overlying water were measured using Aquamerck® and Spectroquant® test kits (Merck Eurolab, Frankfurt/M).

pH and redox potential of sediment and soil

The pH values of sediment was measured directly in the wet substrate according to DIN 38 414, Teil 5. The pH values of soils were determined in a suspension of soil in a solution of 0.01 mol/L $CaCl_2$ according to ISO 10390 (International Organization for Standardization, 1994). The redox potential of sediment was measured directly in the wet substrate according to DIN 38404, Teil 6.

Maximum water holding capacity of soil

The maximum water holding capacity of soil samples were determined according to Annex C of the ISO Guideline 11268-2 (International Organization for Standardization, 1998). Therefore a defined quantity (e.g. 5 g) of the soil substrate was saturated with water for about three hours in a glass tube where the bottom was plugged with filter paper. Afterwards the sample was placed for a period of two hours on a layer of very wet Kleenex for draining in a closed vessel. The sample was weighed, dried to constant mass at 105 °C and re-weighed. The water capacity (WHC) was calculated according to the formula:

$$\text{WHC (in \% of dry mass)} = S - T - D \times 100/D \qquad (3.1)$$

where:

S: water-saturated substrate + mass of tube + mass of filter paper;

T: tare (mass of tube + mass of filter paper);

D: the dry mass of substrate.

Microbial biomass of sediment

The amount of microbial biomass carbon in sediment samples was determined via the fumigation-extraction-method (FE-method) according to ISO 14240-2 (International Organization for Standardization, 1997). For that, sediment samples were divided in two sub samples (equivalent to 25 g dry weight). One sub sample was fumigated prior to the extraction while from one sub sample the organic carbon was extracted immediately by horizontal shaking for 45 min with 100 mL 0.01 M $CaCl_2$. For fumigation the sediment sub sample was filled into a petri dish and incubated for 20 hours at 25° ± 2 °C in a

$CHCl_3$-atmosphere. After removal of all $CHCl_3$ from the sediment sample the organic carbon was extracted by horizontal shaking for 45 min with 100 mL 0.01 M $CaCl_2$. Thereafter the fumigated sample and the untreated one were filtered and the TOC of the extract was measured. The microbial biomass carbon (C_{mic}) was calculated according to the formula:

$$\text{microbial biomass carbon } (C_{mic}) = E_C / k_{EC} \qquad (3.2)$$

where E_C is (organic C extracted from fumigated sediment) - (organic C extracted from non-fumigated sediment) and k_{EC} is 0.45 (Joergensen, 1995, Joergensen, 1996, Joergensen and Mueller, 1996). The results were expressed as µg C_{mic} /g soil (dry weight).

Dry weight and organic carbon content of sediment and soil

Samples of sediment and soil were weighed and dried overnight at 105 ± 2°C. After cooling in a desiccator, the specimens were weighed again. The water content of the samples is expressed in percent of wet weight (ww).

The dried sediment and soil specimens were transferred to pre-heated, pre-weighed porcelain dish, and weighed. HCl (4 mol/L) was mixed with the specimens to remove carbonate bound carbon. After incubating for two to four hours, the specimens were dried overnight at 60 ± 2°C. The specimens were weighed after cooling in a desiccator and ashed in a muffle furnace at 550 ± 10°C. After cooling in a desiccator, the specimens were weighed again. The weight loss (loss on ignition, LOI) in percent of dry weight of the sample was calculated by subtracting the ash weight of the specimen from the dry weight of the specimen. The LOI was divided by the dry weight of the specimen and multiplied by 100 to result in the LOI in percent of dry weight of the specimen. The OC of the specimen was calculated by dividing the LOI in percent of dry weight by the factor 1.72 to correct for volatile sediment and soil components other than CO_2 to result in the OC in percent of dry weight of sediment and soil.

3.2 Water/sediment tests

The OECD Guideline 308 "*Aerobic and anaerobic transformation in aquatic sediment systems*", based on BBA guideline IV 5-1 describes a laboratory test method to assess aerobic and anaerobic transformations of organic chemicals in aquatic sediment systems (Biologische Bundesanstalt für Land- und Forstwirtschaft, 1990, OECD, 2002). In general, this test is required for pesticides and industrial chemicals which are directly applied to water or which are likely to reach the aqueous environment by the routes described in chapter 1.1.2. Since surface layers of sediment can be either aerobic or anaerobic, the test methods include both conditions. The aerobic tests simulate an aerobic water column over an aerobic sediment layer that is underlain with an anaerobic gradient.

Aim of the described tests were :

- Measurement of the distribution of the test substance and its' metabolites between water and sediment compartment.
- Determination of transformation rates and mineralization rates
- Balancing of radioactivity for ^{14}C-labelled test substances
- Quantification and identification (if possible) of transformation products
- Calculation of dissipation times

Deviating from the guidelines described, the tests were conducted with one aerobic sediment only, and due to the limited availability of appropriate ^{14}C-labelled standards also non-labelled analytes were utilized as test compounds.

Sampling and storage of native water and sediment

Sediment and water were taken from the Wickerbach creek in Flörsheim (close to Frankfurt, Southwest Germany) (Figure 3.1) at a sampling site which was located close to the source of the creek. Since the creek is not used as a receiving water for STPs it is widely pristine (Umland-Verband Frankfurt / Region Rhein-Main, 1992).

In order to sample mainly sediment under aerobic conditions, the sampling depth was restricted to 5 cm. The sediment was wet sieved (2 mm mesh) and homogenized with an

electric stirrer. Water and sediment were stored together with a water/sediment ratio of 3:1 at 4°C in the dark for a maximum of 28 d. Water and sediment were characterized according to OECD guideline 308 (Table 3.1).

Figure 3.1: Sampling of sediment and water

Table 3.1: Characteristics of water and sediment used for test systems

Parameter	Sediment	Parameter	Water
pH	7.7	pH	8.5
Redox potential (mV)	269	Redox potential (mV)	382
C_{org} (% dry weight)	1.4	TOC (mg carbon·L^{-1})	4.7
Organic matter (% dry weight)	2.4	Oxygen content (mg·L^{-1})	7.8
Microbial biomass (µg carbon·g^{-1} dry sediment)	41	Nitrate (mg·L^{-1})	6.2
Water content (%)	20	Phosphate (mg·L^{-1})	0.9
Clay (%)	9.9	Hardness (ppm $CaCO_3$)	222
Silt (%)	12.6	Conductivity (µS·cm^{-1})	515
Sand (%)	77.5		
Soil type	Loamy sand		

Equipment and Materials

- Glass flasks amber, 500 mL (Schott, Mainz, Germany)
- Glass attachments for test vessels (Schott, Mainz, Germany)
- PTFE-gasket (Schott, Mainz, Germany)
- Soda lime granules with indicator (Merck, Darmstadt, Germany)

Set-up of the test system

The test system consisted of a 500 mL amber glass flask filled with 200 g sediment and 300 mL creek water (Figure 3.2). A CO_2-trap filled with 30 g granulated soda lime was tidy coupled with the top of the flask. Thus, an exchange of air was possible, while CO_2 was efficiently trapped. Further, the test vessels were wrapped with aluminum foil, to minimize photochemical reactions. Although, non-labelled compounds do not allow for a quantification of the mineralization product $^{12}CO_2$ in the used experimental set-up, CO_2-traps were coupled to the test vessels in all tests to achieve comparability to the tests with ^{14}C-labelled pharmaceuticals.

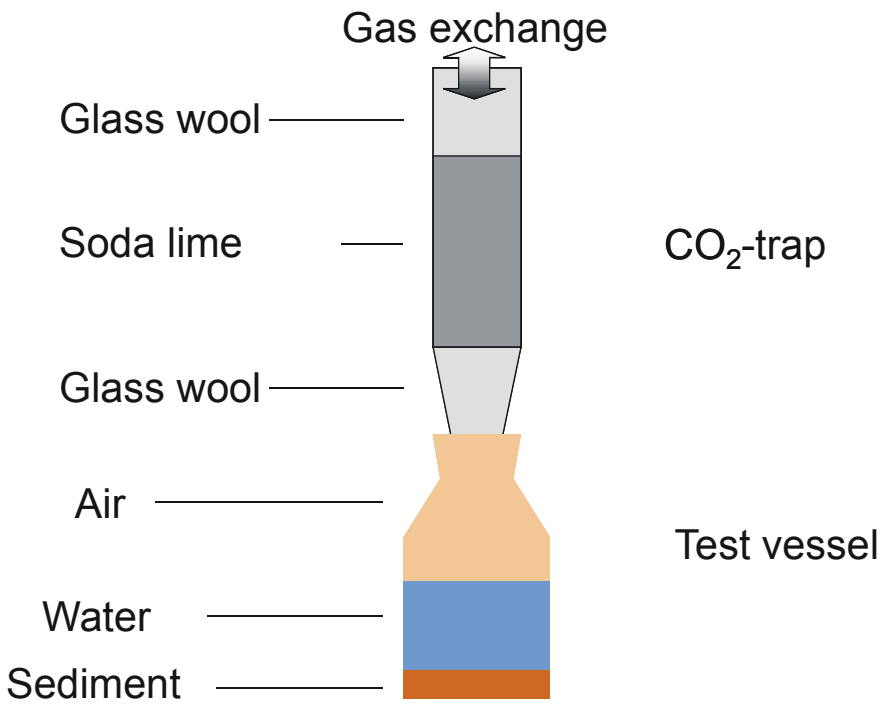


Figure 3.2: Water/sediment test system

Prior to spiking with pharmaceuticals, the water/sediment systems were equilibrated under test conditions for at least 7 d. During the equilibration and the test period the test systems were slowly shaken avoiding a disturbance of the separation of sediment layer and overlying water as well as an excess suspension of sediment fines.

The pharmaceuticals were spiked as aqueous solutions into the water phase of the test systems using water-miscible organic solvents (e.g. ethanol). In any case the concentration of the organic solvent in the overlying water did not exceed 0.5 %. Spiking levels of the individual compounds are listed in Table 3.2. These relatively high concentrations were chosen to enable a detection of 1 percent of the initial analyte concentration. For iopromide and diazepam which were the first compounds tested, a spiking level of 500 $ng \cdot g^{-1}$ µg/kg was chosen. Since the necessary detection limits were attained easily even at lower spiking levels, the spiking level was decreased to 100 $ng \cdot g^{-1}$ for all further test substances. The vessels were slowly shaken for the test period of 100 d in an air-conditioned room at 20 ± 2°C.

For each sampling time two water/sediment vessels were sacrificed in parallel. Hence, 24 test systems were prepared per tested pharmaceuticals, to allow for 10 sampling times, 2 solvent controls and 2 controls (Figure 3.3). Solvent controls were prepared in order to observe the influence of the used organic solvent in the application-solution to the water/sediment system. The error bars in chapter 4.1 show the mean absolute deviation between the two test vessels sampled, their lower and their upper ends refer directly to the individual measurement values made.

Figure 3.3: Test-vessels of the ^{14}C-iopromide test before sampling after 24 h

Table 3.2: Spiking levels of pharmaceuticals

Compound	Concentration ($ng \cdot g^{-1}$ sediment)
^{14}C-Iopromide	500
^{14}C-Diazepam	500
Oxazepam	100
^{14}C-Ibuprofen	100
2-Hydroxy-ibuprofen	100
^{14}C-Paracetamol	100
Carbamazepine	100
10,11-Dihydro-10,11-dihydroxy-carbamazepine	100
Clofibric acid	100
Ivermectin	100

Sampling of the water/sediment test systems

Samples were taken immediately after spiking and after 0.25, 1, 2, 7, 14, 28, 56 and 100 d. For that, the entire water and sediment phase of each test-system were taken. Then, the supernatant water phase was decanted and homogenized using a magnetic stirrer. Sediment was manually homogenized. Water samples containing radio-labelled compounds were filtered through a paper filter, to remove suspended matter, and were divided into 15 mL aliquots. Possible analyte sorption to the filter was always checked. The sediments were divided into portions of 2× 10–20 g and 2× 80–90 g. For non-labelled test compounds, the water phase was divided into aliquots of 75 mL and 225 mL, and the sediments were stored in two portions of 100 g. In the radiotracer tests, the soda lime of the CO_2-traps was transferred into PE-bags, while the soda lime granules were rejected in the tests with non-labelled compounds. All samples were finally frozen at –20°C and stored until analysis. The aliquot size for analysis was selected, to allow for the LOQs listed in Table 3.3.

Table 3.3: LOQs for analysis of system compartments

Analysis	LOQ in water (% C_0)	LOQ in sediment (% C_0)
Quantification of non-labelled analytes	≤ 1	≤ 1
Quantification of radioactivity	≤ 1	≤ 1
Radio-TLC	≤ 2.5	≤ 1

3.3 Soil-column leaching experiments

Equipment and materials

- Cylindrical sectionable glass columns (350 mm long, 50 mm diameter) with glass tubes and glass drip outlets including metal rack;
- Glass sinter discs D1 (diameter 50 mm), Merck Eurolab, Frankfurt, Germany;
- Vibration device used for filling and packing the soil columns;
- Peristaltic pump Ismatec IPS 12 including tubes (Tygon), Wertheim, Germany;
- Chemicals: $CaCl_2$, ethanol, methanol, acetone, (HPLC Grade), Merck, Frankfurt, Germany;
- Standard laboratory equipment: PTFE-tubes, spoons, pipettes, amber glass bottles;

Experimental set-up

All tests were performed according to the OECD guideline No. 312 "*Leaching in soil columns"* (Brodsky et al., 1997, OECD, 2003a).

Air-dried and sieved soils (< 2 mm mesh) were packed in sectional glass columns to a height of approximately 30 cm (Figure 3.4). To obtain uniform packing, the soil was added in small portions under gentle vibration of the column. Subsequently, the soils were saturated with artificial rain (0.01 $mol \cdot L^{-1}$ $CaCl_2$) to their maximal water holding capacities. The test substances were applied on the top of the soil columns as aqueous solution or dissolved in organic solvent at a concentration level of 100 $\mu g \cdot kg^{-1}$ soil (dry weight). When necessary an organic solvent was used for application, the solvent was allowed to evaporate completely from the soil surface prior to the start of the experiment. All tests were performed in the darkness at a temperature of 20 ± 2°C. A total amount of 393 mL artificial rain (0.01 $mol \cdot L^{-1}$ $CaCl_2$) corresponding to a rate of 200 mm was added within 48 h drop wise on each soil column, which simulated an extremely high rainfall. Glass sinter disks on top of the columns ensured an even distribution of the artificial rain.

At the end of the experiments, the amounts of the non-labelled pharmaceuticals, respectively the total radioactivity contained in the leachates were determined. The time dependent leaching behaviour of the test substances was not investigated, since the leachates were collected in one fraction. Additionally, in the studies using ^{14}C-labelled substances, the total radioactivity in different layers of the soil columns was measured. For this purpose, the soil columns were divided in six sections, each about 5 cm high.

Figure 3.4: Cylindrical sectional glass column (350 mm long, 50 mm diameter) with glass tubes and a glass drip outlet including a metal rack

Characterization of applied soils

The soils selected for the leaching studies (Figure 3.5) covered a wide range of the soil spectrum with respect to organic content and pH (Table 3.4). Deviating from OECD guideline No. 312 OECD, 2003a two different soils, *LUFA 2.2* (*LU*) and *Euro Soil 5 (E5*) were used in the studies with non-labelled compounds, whereas in the studies using ^{14}C-iopromide and ^{14}C-diazepam a third soil was tested (*Neuenkirchen;* collected

north of Braunschweig, Niedersachsen, Germany). The third soil was included to improve the comparability with studies performed in parallel to this project. *EuroSoil 5* was sampled at the site near Gatow (Schleswig-Holstein, Germany) where the original *EUROSOIL* (Kuhnt and Muntau, 1992) was taken, the soil *LUFA 2.2* was obtained from the LUFA Speyer (Germany) and the third soil was collected north of Braunschweig (Niedersachsen, Germany). Data on the soil characteristics are given in Table 3.4.

Figure 3.5: Soils used in the leaching studies (LUFA 2.2; EuroSoil 5; Neuenkirchen)

Table 3.4 : Soils applied in leaching experiments

	LUFA 2.2	*EuroSoil 5*	*Neuenkirchen*
pH ($CaCl_2$)	5.8	2.9	7.0
C_{org} [%]	2.3	6.3	1.3
Org. content [%]	4.0	10.8	2.2
C/N-Ratio	13 [(1)]	---	---
N_{total} [mg/kg dw]	---	---	2600 [(3)]
CEC [$cmol_c$/kg]	11 [(1)]	---	---
Water content [%]	10.7	2.2	22.7
WHC [%]	51	25 [(4)]	33
Soil texture	loamy sand [(1)]	loamy sand [(4)]	silty loam[(3)]
Clay [%]	8.2 [(1)]	8.4 [(4)]	17.0 [(3)]
Silt [%]	17.0 [(1)]	17.7 [(4)]	78.4 [(3)]
Sand [%]	74.8 [(1)]	73.9 [(4)]	4.7 [(3)]
Soil type	Gleysol [(2)]	Podsol	Luvisol [(3)]
Sampling depth	0-10 cm [(1)]	0-10 cm	0-10 cm [(3)]
Sampling horizon	A_h [(1)]	A_h	A_p [(3)]
Vegetation	hay meadow [(1)]	pine forest	winter wheat [(3)]
Sampling date	10. July 2001 [(1)]	12. July 2001	10. August 2001

CEC = cation exchange capacity; WHC = water holding capacity; [(1)] data based on standard certificate of analysis, Landwirtschaftliche Untersuchungs-und Forschungsanstalt Speyer; [(2)] pers. comm. Weller, 2001, (Landwirtschaftliche Untersuchungs- und Forschungsanstalt Speyer); [(3)] data provided by Institut für Geoökologie, TU Braunschweig; [(4)] data provided by Fa. Geocomp, Bad Vilbel.

Sampling procedure and sample handling

During the test period the leachates were collected in amber glass bottles (Figure 3.6). After allowing the columns to drain, the concentrations of test substance respectively the total radioactivity were determined by methods described in the chapters 3.5 and 3.6. Additionally, in the studies using ^{14}C-labelled test substances, the total radioactivity was measured in 5 cm soil layers when at the end of the experiments the sectionable glass columns were divided into six layers. After homogenization of the individual soil layers, sub-samples were combusted in a sample oxidizer and the radioactivity were measured by LSC (see chapter 3.6). The amounts of test substance, respectively total radioactivity, were given for each soil layer and leachate in relative concentrations normalized by the applied initial dose.

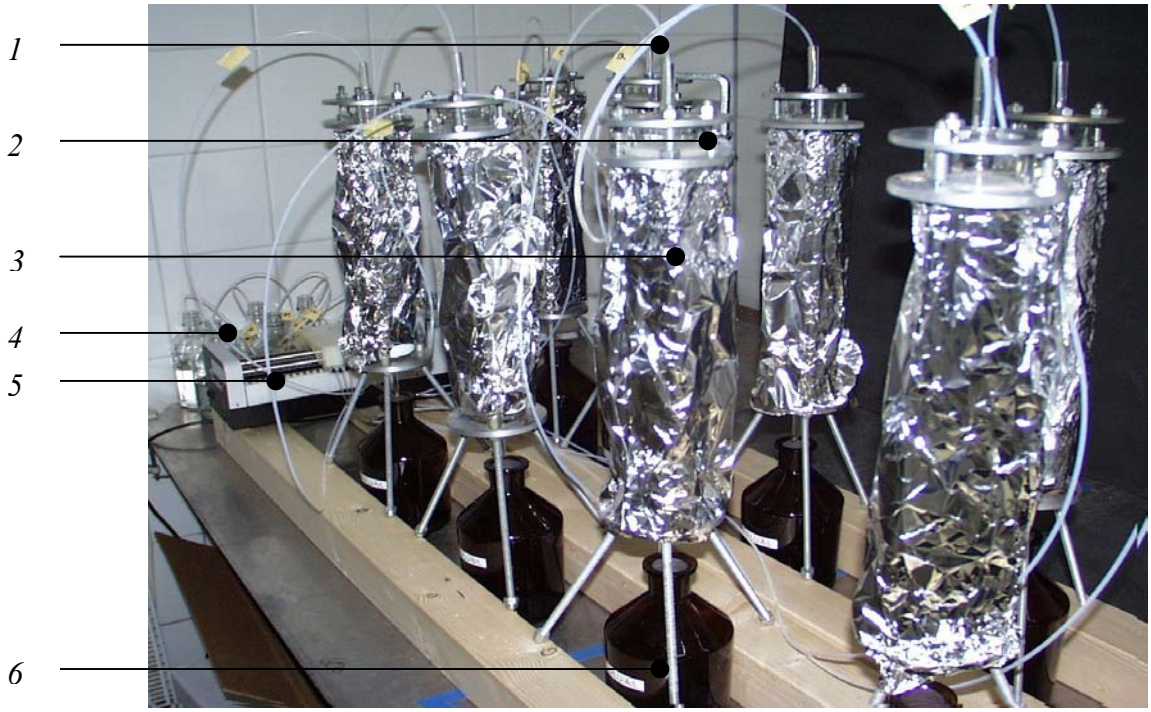


Figure 3.6: Soil columns in a soil leaching study (1: PTFE-tube; 2: glass sinter disk; 3: soil column wrapped with aluminium foil; 4: glass bottle containing artificial rain; 5: peristaltic pump; 6: leachate collecting)

3.4 Materials and instrumentation for chemical analysis

The following reference compounds, chemicals and solvents have been incorporated in this work:

Lipid regulators and metabolites

- Bezafibrate, clofibric acid, gemfibrozil (Sigma, Deisenhofen, Germany)

Antiphlogistics and metabolites

- Diclofenac, fenoprofen, ibuprofen, indomethacin, ketoprofen, naproxen, paracetamol, (Sigma, Deisenhofen, Germany), 2-hydroxy-ibuprofen (µ-Mol, Luckenwalde, Germany)

Parasiticides

- Ivermectin (Sigma, Deisenhofen, Germany)

Antiepileptics and tranquilizers

- Carbamazepine, diazepam, oxazepam, (Sigma, Deisenhofen, Germany), 10,11-dihydro-10,11-dihydroxy-carbamazepine (µ-Mol, Luckenwalde, Germany)

Iopromide derivatives

- Iopromide, desmethoxy-iopromide, ATH, DAMI, ATI (courtesy from Schering, Berlin, Germany)

Internal and surrogate standards

- Abamectin, fenoprop (Riedel-de-Haen, Seelze Germany), 10,11-dihydro-carbamazepine (Alltech, USA), paracetamol-D_4 (Cerilliant, Austin, USA)

^{14}C-labelled reference compounds

- ^{14}C-Diazepam, [2-14C], radiochemical purity 99.8 %, specific activity 7.22 MBq·mg^{-1}, (Amersham Pharmacia Biotech UK Limited, Little Chalfont, UK)
- ^{14}C-Ibuprofen, [carboxyl-14C], radiochemical purity > 99 %, specific activity 8.97 MBq·mg^{-1} (American Radiolabeled Chemicals Inc., St. Louis, MO, US)

- ^{14}C-4-acetaminophen, [ring-UL-14C], radiochemical purity > 99 %, specific activity 1.54 MBq·mg^{-1} (Sigma-Aldrich, Steinheim, Germany)
- ^{14}C-iopromide, [ring-14C], radiochemical purity > 98 %, specific activity 1.86 MBq·mg^{-1} (courtesy from Schering AG, Berlin, Germany)
- ^{14}C-4-nitrophenol, [ring-14C], radiochemical purity 99 %, specific radioactivity 2.66 MBq·mg^{-1} (American Radiolabeled Chemicals Inc., St. Louis, MO, USA)

Chemicals and solvents

- Acetic acid (100 %, p.a.), amyl alcohol, sulphuric acid (suprapur), ammonia (25 %, p.a.), ammonium acetate (p.a.), KH_2PO_4, K_2HPO_4, (Merck, Darmstadt, Germany
- Acetone, acetonitrile, chloroform, ethyl acetate, methanol, 2-methyl-propanol, n-hexane, toluene, all suprasolv (Merck, Darmstadt, Germany)

The following instruments and materials were applied for the analyses and experiments:

LC tandem MS

- Perkin-Elmer Series Autosampler Series 200 connected to a Perkin-Elmer quaternary pump Series 200 and an AS–2000a auto sampler, Perkin Elmer Sciex API 365 tandem MS with ESI and APCI-interface

Columns for HPLC

- EcoCart (125 x 3 mm) LiChrospher® 100 RP-C_{18} endcapped (5 µm) (Merck, Darmstadt)
- EcoCart (125 x 3 mm) LiChrospher® RP-C_{18} (5 µm) (Merck, Darmstadt)
- EcoCart (125 x 3 mm) column cartridge (Merck, Darmstadt)

Radiometry

- Liquid scintillation counter Tricarb 2500 TR (Canberra Packard, Dreieich, Germany)

- LSC-Cocktails: EcoPlus (Roth, Karlsruhe, Germany), Ultima Gold F and Permafluor E+ (Canberra Packard, Dreieich, Germany);
- Sample Oxidizer, TriCarb 307 (Canberra Packard, Dreieich, Germany)
- Carbon dioxide absorbent Carbo-Sorb E (Canberra Packard, Dreieich, Germany)
- Combusto-Cones (Canberra Packard, Dreieich, Germany)
- ^{14}C-Spec-Chec (Canberra Packard, Dreieich, Germany)
- TLC-Radio-Scanner 1052 with Digital Signal Analyzer 1006 (Berthold GmbH, Wildbad, Germany)
- TLC Plates 20 × 20 cm, KG 60, F254 (Merck, Darmstadt, Germany)

Material and equipment for sample preparation

- Lyophilizer Alpha 2-4 (Christ, Osterode am Harz, Germany)
- Rotary evaporator RE 111, water jet pump and vacuum controller (Büchi, Konstanz, Germany)
- Syringe filter Spartan 13/20 0.45 µm (Schleicher & Schuell, Dassel, Germany
- Syringe filter PTFE 0.22 µm (Roth, Karlsruhe, Germany)
- Glass fiber filter (< 1 µm) (Schleicher & Schuell, Dassel, Germany)
- Glass cartridges 3 mL (Mallinckrodt Baker, Griesheim, Germany)
- PTFE-frits (ICT, Bad Homburg, Germany)
- Solid phase materials: Isolute® ENV+, RP-C_{18} end capped (ICT, Bad Homburg, Germany), LiChrolute® RP-C_{18} (40 – 63 µm), LiChrolute® EN (40 – 120 µm) (Merck, Darmstadt, Germany)
- Glass vials 10 mL with stretched tip (0.2 mL) and screw caps, (Glassblowing, Mainz, Germany)
- LC vials 500 µL PP with PTFE caps (A-Z Analysenzubehör, Mainz, Germany)
- Centrifugation flasks 250 mL, PPCO (Nalge Nunc International, Rochester, NY)

3.5 Analysis of pharmaceuticals with mass spectrometry

3.5.1 Acidic pharmaceuticals

Lipid regulators and analgesic pharmaceuticals were presumably the first prescription pharmaceuticals detected in the environment (Garrison et al., 1976, Hignite and Azarnoff, 1977). In recent years numerous methods have been reported for the detection of these pharmaceuticals, often containing carboxyl groups. Predominantly analysis in environmental compartments has been applied via GC/MS (Buser et al., 1998, Heberer et al., 1997, Heberer and Stan, 1996, Sacher et al., 2001, Stumpf et al., 1998, Stumpf et al., 1999, Ternes, 2001b, Weigel et al., 2002) but also by LC/MS (Ahrer et al., 2001, la Farré et al., 2001, Miao et al., 2002) and CE/MS (Ahrer et al., 2001).

Within the present work, the acidic pharmaceuticals were analyzed in leachate samples following a procedure, originally described by Ternes et al., 1998b using SPE enrichment, derivatization with diazomethane and GC/MS detection. Since this method included a derivatization step with the carcinogenic diazomethane, an entirely new method was developed, which allowed for the analysis of water and sediment by LC-tandem MS detection (Löffler and Ternes, 2003).

The new method included an SPE for aqueous samples of various origins, applying a polymer based mixed-bed material, which attained a high extraction efficiency and retained analytes based on reversed phase and cation-exchange mechanisms. This enabled for a limited sample clean-up since cationic compounds extracted remained attached to the SPE-material during elution and were thus removed from the sample extract. Furthermore, an LC-tandem MS method was developed, using *atmospheric pressure chemical ionization* (APCI) for the acidic analytes.

Optionally, a sediment extraction procedure was developed which utilized the newly established SPE step as an clean-up of the sediment extracts. This was in response to the extracts being highly loaded with matrix impurities.

3.5.2 Neutral pharmaceuticals

The term neutral pharmaceuticals relates to those pharmaceuticals, such as carbamazepine and diazepam, which are extractable using reversed phase SPE materials with a neutral pH. These compounds have been found in various environmental waters using gas- and liquid chromatography mass spectrometry (Meisenheimer and Ternes, 2000, Öllers et al., 2001, Sacher et al., 2001).

The analytical method applied for the analysis of various neutral pharmaceuticals is based on methods already reported (Ternes et al., 2001, Ternes et al., 1998a), but were extended to allow for the detection of two additional analytes. These were the main human metabolites of carbamazepine and diazepam, 10,11-dihydro-10,11-dihydroxy-carbamazepine and oxazepam. Again, a solvent extraction procedure was developed for the determination of the analytes in sediment. The SPE, normally applied for the enrichment of the neutral pharmaceuticals in aqueous matrices, served here as a clean-up step for the sediment extracts.

3.5.3 Iopromide derivatives and paracetamol

The iopromide derivatives were analyzed, based on the procedure recently described (Hirsch et al., 2000, Ternes and Hirsch, 2000) for aqueous matrices. The current method was developed for the determination of the analytes in aqueous matrices and sediment. In addition to the iopromide derivatives, the analgesic paracetamol was included into the method. Since the recoveries of the iopromide derivatives ATH and ATI in SPE were generally low, they were excluded as analytes in method development. The SPE was used as an enrichment procedure for aqueous matrices and as clean-up for sediment extracts.

Sediment extraction

All sediment samples were sieved prior to analysis (2 mm mesh), in order to remove the coarse sediment fraction. Sediment samples (50 g) were filled in PPCO flasks and were spiked with 800 ng of DMI, the surrogate standard for the iopromide derivatives, and 800 ng of paracetamol-D_4 the surrogate standard for paracetamol. Sediments were then extracted using 2×45 mL methanol and successively 2×45 mL acetone in an ultrasonic bath. The slurries of the solvent/sediment mixtures were thoroughly hand

shaken and then ultrasonicated for 15 min. After extraction, the slurries were centrifuged for 7 min at 5400 rpm and the supernatant solvent phases were filtered, combined and evaporated at 40°C, 150 – 250 mbar by a rotary evaporator until only water remained. The resulting extracts and methanol rinses (3 × 1 mL) were combined and diluted with 500 mL of uncontaminated groundwater, which is known to be free of anthropogenic organic contamination.

SPE of water samples and aqueous sediment extracts

Water samples or aqueous sediment extracts were, if necessary, filled up to 500 mL with groundwater and adjusted to a pH 2.8 using H_2SO_4 (3.5 mol·L^{-1}). Then, the surrogate standards DMI and paracetamol-D_4 were spiked to the samples. Glass cartridges were manually packed with 250 mg of ENV+ and 100 mg of RP-C_{18}ec-material on the top and were conditioned prior to sample extraction with 6 mL of n-hexane, 2 mL of acetone, 10 mL of methanol and 10 mL of groundwater. Aqueous samples (either water or aqueous sediment extracts) were passed through the SPE cartridges at a flow rate of 10 mL·min^{-1}, since lower flow rates were crucial for good recoveries. Afterwards the cartridges were dried with nitrogen for 1 h. The RP-C_{18}ec material was removed, while keeping the ENV+ material in the cartridges which were then eluted with 4 × 1 mL of methanol. All extracts were evaporated to dryness in a gentle nitrogen stream and the residue was finally dissolved in 50 µL of methanol and 450 µL of phosphate buffer (pH 7, 20 mM KH_2PO_4/K_2HPO_4).

HPLC conditions

Prior to injection all wastewater samples and sediment extracts were filtered (0.45 µm, Spartan 13/20, Schleicher & Schuell, Dassel, Germany). The Perkin Elmer HPLC system consisted of a Series 200 in-line degasser and a quaternary pump connected to an AS–2000a autosampler. The injection volume was always 50 µl. For the chromatography of the X-ray contrast medium and paracetamol a 125 × 3 mm LiChrospher® RP-18ec column (5 µm) (Merck, Germany) was applied and kept at 5°C. A isocratic flow of 0.2 mL·min^{-1} of the mobile phase was used for the LC-analysis containing a 5 mmol·L^{-1} ammonium acetate (pH 5.7) and acetonitrile (90:10, v/v).

MS/MS detection

The iopromide derivatives and paracetamol analyzed using a Perkin-Elmer Sciex 365 triple-stage quadrupole mass spectrometer equipped with turbo electrospray ionization (ESI). Analyses were performed at 400°C in the positive ion mode, with a spray voltage of 4.6 kV. Potential differences between skimmer and orifice varied from 14 to 35 V and ring electrode voltages from 140 to 190 V. Nitrogen was used as the curtain gas and the nebulizer gas at flow rates of 1 and 0.7 $L \cdot min^{-1}$, respectively. The eluent was split 1:10 in the electrospray interface, resulting in a effective spray rate of 20 $\mu L \cdot min^{-1}$ into the mass spectrometer. MS/MS parameters were optimized in the continuous flow mode. Conditions for the measurement of precursor ions were optimized in the single MS scan mode. The protonated molecular ions were used as the precursor ions for subsequent MS/MS experiments. Product ion spectra were recorded by scanning Q_3 over the relevant mass range. After the determination of the product ions, the conditions for the nitrogen-collision-induced dissociation (CID) were optimized. Precursor and product ions of the individual compounds are shown in

Table 3.5.

Table 3.5: Retention times and fragmentation of iopromide derivatives and paracetamol

Compound	Retention time (min)	Precursor and product ions (m/z)	
ATI	3.3	559.5	$[MH]^+$
		432.8[1]	$[M\text{-}HI+H]^+$
ATH	4.9	632.7	$[MH]^+$
		541.8[1]	$[M\text{-}C_3H_9NO_2+H]^+$
DMI	5.6	761.8	$[MH]^+$
		543.1[1]	$[M\text{-}HI\text{-}C_3H_9NO_2+H]^+$
Iopromide	7.4	791.8	$[MH]^+$
		572.8[1]	$[M\text{-}HI\text{-}C_3H_9NO_2+H]^+$
Paracetamol	8.8	152.2	$[MH]^+$
		133.8	$[M\text{-}H_2O+H]^+$
		110.0[1]	$[M\text{-}C_2H_4O+H]^+$
Paracetamol-D_4	8.8	156.0	$[MH]^+$
		113.8[1]	$[M\text{-}C_2H_4O+H]^+$
		95.6	$[M\text{-}H_2O\text{-}C_2H_4O+H]^+$
DAMI	9.5	719.7	$[MH]^+$
		628.9[1]	$[M\text{-}C_3H_9NO_2+H]^+$

1: Product ion used for quantification

Determination of recoveries, LOQs and calibration

Individual recoveries for the SPE procedure were measured by spiking groundwater, soil leachates, surface water and wastewater.

The efficiency of the solvent extraction step was determined for paracetamol using autoclaved sediment, spiked with a ^{14}C-labelled standard, which was extracted after a contact time of approximately 14 h following the described extraction sequence.

Finally, overall recoveries were obtained by spiking autoclaved sediment at two levels and analyzing the samples after a contact time of approximately 14 h as described above. The recoveries were calculated relative to a non-extracted standard. Calibration curves were prepared for each compound from the spiked groundwater samples by plotting the peak area of the respective MRM-transition versus the analyte concentration. In each calibration series, a blank sample and a recovery sample were included. Requiring a S/N-ratio > 10, the LOQs were set as the second lowest calibration point in the linear regression.

Method characterization

Groundwater and soil leachates

Recoveries for the SPE of groundwater were quantitative for the iopromide derivatives and only slightly lower for paracetamol, as shown in Table 3.6. A compensation led to excellent relative recoveries ranging from 89 ± 9 to 104 ± 4 %. In soil leachates the recoveries differed to some extent. Iopromide and DMI were both recovered at the 83 ± 6 % level, while the iopromide metabolite DAMI was over determined with a 123 ± 5 % recovery. A relative recovery of 100 ± 7 % was obtained for iopromide, whereas the compensation increased the recovery to 143 ± 8 %, and thus quantification had to be done without compensation. Nevertheless, all recoveries had a low statistical error of < 10 %, exhibiting a reproducible enrichment procedure.

All LOQs of the analytes in groundwater and soil leachates were 0.04 $\mu g \cdot L^{-1}$. The calibration curves, for the quantification of the iopromide derivatives and paracetamol

in all aqueous matrices and sediment, were prepared from spiked groundwater and attained regression coefficients always > 0.99.

Table 3.6: Recoveries (%) and confidence intervals (P=95 %) of iopromide, an iopromide metabolite and paracetamol for the SPE of spiked groundwater and soil leachates

Compound	Groundwater			Soil leachates		
	LOQ ($\mu g \cdot L^{-1}$)	Absolute Recovery (%)	Relative Recovery (%)	LOQ ($\mu g \cdot L^{-1}$)	Absolute recovery (%)	Relative recovery (%)
DAMI	0.04	97 ± 3	99 ± 5	0.04	123 ± 5	143 ± 8
Iopromide	0.04	87 ± 5	89 ± 9	0.04	83 ± 6	100 ± 7
DMI	0.04	97 ± 7	-	0.04	83 ± 6	-
Paracetamol	-	81 ± 9	104 ± 4	-	-	-
Paracetamol-D_4	0.04	78 ± 8	-	0.04	-	-

Surface water

The SPE method was also well suited for the surface water. Recoveries in surface water ranged from 87 ± 7 to 102 ± 11 % and after compensation between 100 ± 9 % and 115 ± 6 % (Table 3.7). However, the method allowed for the determination of the analytes in groundwater, soil leachates and surface water with a LOQ of 0.04 $\mu g \cdot L^{-1}$.

Table 3.7: Recoveries (%) and confidence intervals (P=95 %) of iopromide, an iopromide metabolite and paracetamol for the SPE of surface water (n=4) spiked with 1 $\mu g \cdot L^{-1}$

Compound	LOQ ($\mu g \cdot L^{-1}$)	Surface water	
		Absolute Recovery (%)	Relative Recovery (%)
DAMI	0.04	87 ± 7	100 ± 9
Iopromide	0.04	92 ± 8	105 ± 10
DMI	-	87 ± 4	-
Paracetamol	0.04	102 ± 11	115 ± 6
Paracetamol-D_4	-	89 ± 5	-

[1] The native analyte concentration of a sample was subtracted from the concentration of spiked samples for the calculation of the recoveries

Sediment

The solvent extraction yielded an almost quantitative recovery of 95 ± 3 % ^{14}C-paracetamol. More than 89 % were attained within the first 2 extraction sequences (Table 3.8).

Table 3.8: Recoveries (%) and confidential intervals (P=95 %, n=4) for paracetamol after extraction of 50 g sediment spiked with ^{14}C-paracetamol at 20 ng·g^{-1}

	Paracetamol recovery (%)
First extraction	73 ± 2
Second extraction	17 ± 2
Third extraction	2 ± 1
Fourth extraction	3 ± 2
Sum	95 ± 3

Overall recoveries of the analytes in sediment were quantitative at a spiking level of 20 ng·g^{-1}, except for the surrogate standard DMI (Table 3.9). The latter was found at a significantly lower level of 54 ± 4 %. Hence, DMI was not appropriate as a surrogate standard for the determination of iopromide and its' metabolite DAMI in sediments. In contrast to that finding, the compensation of the iopromide derivatives and paracetamol using paracetamol-D_4 provided good relative recoveries between 101 ± 19 and 117 ± 6 %.

The recoveries at the lower spiking level of 3 ng·g^{-1} differed from those at the high spiking level. For iopromide and its' metabolite DAMI the recoveries were decreased and ranged between 70 ± 4 and 77 ± 4 %, while the recovery of DMI was constant at 50 ± 4 %. A paracetamol signal was found in non-spiked sediment samples, resulting either from contamination with paracetamol or from interfering matrix components. Therefore, the background signal was subtracted from the paracetamol signal in the spiked samples. A chromatogram of a standard sample is shown in Figure 3.7. Paracetamol was recovered with 52 ± 25 %, while the deuterated surrogate standard provided recoveries of 100 ± 11 %. The strong difference in the recovery rates of paracetamol and its' deuterated standard might have been the result of the different spiking levels applied, since the surrogate standard was spiked in all experiments at the

same concentration of 16 $ng \cdot g^{-1}$ sediment. Hence, neither DMI nor paracetamol-D_4 were appropriate surrogate standards and quantification was always conducted without compensation. The LOQs for iopromide and DAMI was set to 3 $ng \cdot g^{-1}$ while paracetamol's was 20 $ng \cdot g^{-1}$.

Table 3.9: LOQs ($ng \cdot g^{-1}$), recoveries (%) and confidence intervals (P=95 %) of iopromide derivatives and paracetamol for the overall method at two spiking levels (20 $ng \cdot g^{-1}$ and 3 $ng \cdot g^{-1}$), using paracetamol-D_4 as surrogate standard for all analytes (contact time ~ 14 h)

		Spiking level 20 $ng \cdot g^{-1}$ (n=3)		Spiking level 3 $ng \cdot g^{-1}$ (n=4)	
Compound	LOQ ($ng \cdot g^{-1}$)	Absolute recovery (%)	Relative recovery (%)	Absolute recovery (%)	Relative recovery (%)
DAMI	3	92 ± 19	101 ± 19	77 ± 4	79 ± 18
Iopromide	3	100 ± 18	106 ± 17	70 ± 4	71 ± 7
DMI	-	54 ± 11	-	50 ± 4	-
Paracetamol	20	102 ± 21	117 ± 6	52 ± 25	53 ± 29
Paracetamol-D_4	-	88 ± 18	-	100 ± 11	-

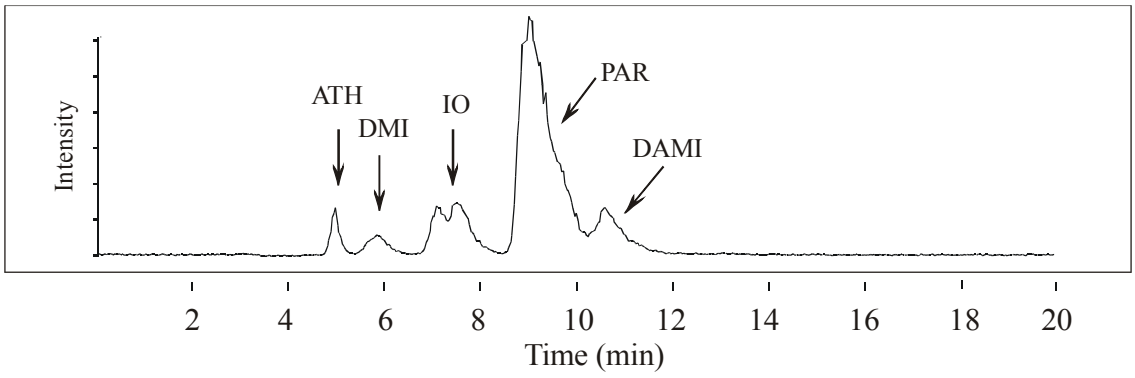


Figure 3.7: Total ion chromatogram of a standard (500 ng) with iopromide derivatives and paracetamol (IO = iopromide, PAR = paracetamol)

3.5.4 Ivermectin

Analytical methods for avermectin derivatives that apply mass spectrometry detection, are available for a wide range of biological matrices, such as vegetables, fruits, tissues and milk (Heller and Schenck, 1993, Tumipseed et al., 1999, Valenzuela et al., 2000, Wu et al., 2001, Yoshii et al., 2000). Unfortunately, most methods for their environmental detection apply fluorescence detection and achieve only a very limited specificity (Cannavan et al., 2000). Recently, Reyzer and Brodbelt published an SPME method for the detection of abamectin in water samples, with LC-tandem MS detection (Reyzer and Brodbelt, 2001). Usually SPME does not allow for a quantitative enrichment of the analyte from the samples and, therefore, the sensitivity was relatively low (LOQ > 1 $\mu g \cdot L^{-1}$).

In the current work, a LC tandem MS method was developed for the determination of the parasiticide ivermectin in environmental waters and sediment (Löffler and Ternes, 2003). As described by Reising, 1998, abamectin was applied as the surrogate standard and allowed for an effective compensation of virtually all analyte losses, since both compounds show very similar properties.

3.6 Radiometric analysis of ^{14}C-labelled pharmaceuticals

Radio tracer techniques are useful tools in chemistry and environmental sciences. The analysis of samples containing radio-labelled compounds is usually divided in two major steps. At first, the total radioactivity in the various system compartments is determined. A balance of radioactivity can then be conducted, comparing the quantity of radioactivity originally applied versus the quantity found after sampling. This provides basic information on the distribution of the radioactivity in the system compartments. The second step is the chemical analysis of the radio-labelled compounds in the different system compartments which allows for the identification and quantification of test compound and its' metabolites.

3.6.1 Analysis of the total radioactivity in environmental samples

Water samples

The total radioactivity in the water samples was determined by liquid scintillation counting (LSC) immediately after sampling. The water aliquots were mixed with an appropriate amount of scintillation cocktail (Ultima Gold F) and measured in the liquid scintillation counter.

Sediment and soil

The total amount of radioactivity included in sediment or soil samples was determined by the combustion of aliquots in a sample oxidizer. Aliquots of 1 g wet sediment were filled in Combusto-cones® (paper-foam tubes) and were mixed with 400 µL of amyl alcohol to enhance the combustion process. The samples were then combusted in a platinum heating coil under an oxygen atmosphere. The released $^{14}CO_2$ was automatically trapped in an absorbent (Carbo-Sorb E) and mixed with scintillation cocktail (Permafluor E+) before it was finally measured by LSC. Recovery rates of the sample oxidizer were determined prior to measurement in 3 replicates and after 8 to 16 samples, by combusting cellulose samples spiked with a defined amount of a ^{14}C-standard (Spec-Check).

All samples were measured in 3 or 4 replicates to minimize the variability. Since a small memory effect occurred, a blank was analyzed after each set of sample replicates. For quantification of the radioactivity in the samples, the recovery rates and the memory effects were considered.

Soda lime CO_2-traps

For the analysis of the trap material, the CO_2 sorbed to the soda lime had to be transferred quantitatively into the liquid phase for LSC-measurement. Soda lime samples of 30 g were mixed with 75 mL of hydrochloric acid and were stirred until complete dissolution of the soda lime pellets occurred. Nitrogen was led gently through the soda lime/hydrochloric acid mixture and the CO_2 in the resulting gas current was

sequentially trapped in an absorbent (Carbo-Sorb E). After addition of the scintillation cocktail, the $^{14}CO_2$ was then quantified by LSC.

3.6.2 Sample preparation for chemical analysis

Prior to analysis, the analytes in aqueous samples had to be concentrated. The enrichment via SPE, used for non-labelled analytes, is more or less specific for compounds of a certain range of polarity. However, the polarity of an analyte and its' TPs usually differ significantly. Thus, an SPE optimized for a certain analyte is presumably less effective for transformation products. A solution for that problem was the use of lyophilization under mild vacuum since a discrimination of polar metabolites was largely avoided.

Lyophilization procedure for surface water containing ibuprofen, diazepam, iopromide and paracetamol

The water samples were lyophilized for 1 – 2 d at –82°C and a vacuum of 0.15 mbar, while keeping the screw cap of the storage vessels semi-closed to prevent losses and cross contamination. Residues were transferred into small glass tubes with 3 × 1 mL of methanol and 1 mL of acetone. Then, the solvent was evaporated under a gentle nitrogen-stream and the samples were dissolved in 1.5 mL methanol. All extracts were filtered using 0.22 µm PTFE syringe filters and refrigerated until measurement.

Evaporation procedure for surface water containing iopromide

Water samples of 15 mL were mixed with 180 mL acetonitrile. The liquid phase was evaporated to dryness by a rotary evaporator at 40°C and 150 mbar. Residues were successively dissolved under ultrasonic conditions in 3 mL of methanol and 3 mL acetone and were transferred into smaller tubes. The solvents were entirely removed in a gentle nitrogen stream and the remaining residues were dissolved in 1.5 mL of methanol. Then, the extracts were filtered using 0.22 µm PTFE syringe filters and refrigerated until measurement.

Extraction of ibuprofen, diazepam and paracetamol in sediment

Analytes in sediment samples were solvent extracted according to the extraction sequences already described in the methods of the non-labelled analytes. After the removal of the extraction solvent, the remaining aqueous phase was diluted 1:12 with acetonitrile and was then azeotropically evaporated to dryness by a rotary evaporator at 40°C and 150 mbar. The residues were successively dissolved under ultrasonic treatment in 3 mL of methanol and 3 mL acetone and were transferred into smaller tubes. The resulting solution was evaporated to dryness in a gentle nitrogen stream and the residues were re-dissolved in 1.5 mL of methanol. Extracts were filtered using 0.22 µm PTFE syringe filters.

Determination of recoveries, LOQ and calibration

The efficiency of the combustion process was checked by combusting sediment samples spiked in triplicate at various levels, and measuring the recovered radioactivity. For further recovery experiments, native surface water (15 mL) was spiked with the radio-labelled analytes. Samples were prepared as described in chapter 3.6.2.

Overall recoveries for the lyophilization procedure were calculated by comparing the quantity of radioactivity found in the final sample extracts with the respective quantity initially spiked to the surface water samples. Efficiencies of the solvent extractions were determined as already described in the methods of the individual non-labelled analytes. Quantification of radioactivity in the liquid samples was attained using a TriCarb 2500 TR liquid scintillation counter (Packard, Dreieich, Germany). The measuring time varied, depending on the radioactivity in the samples ranged between 5 and 20 min. Calibration of the counter was done every day by measuring standard samples and the background radioactivity. The counter TriCarb 2500 TR provided, quench correction via external standardization, an automatic efficiency control and luminescence detection and correction. Luminescence interferences were further minimized by measuring all samples after a decay period of at least 5 h after preparation. The LOQ for quantification with LSC was not compound specific and set to 60 dpm per sample, calculated as the three times the mean ^{14}C-background radioactivity of 20 dpm per sample.

Method characterization

The efficiency of the sediment combustion process was 94 ± 2 % at all spiking levels ranging from 60 – 100,000 dpm·g^{-1} sediment (non-dried) and was considered for quantification. Losses occurred were presumably due to the occlusion of radioactivity into the inorganic sediment matter during combustion and from losses caused by incomplete oxidation caused by the water content in the samples.

The recoveries of the four radio-labelled analytes attained in lyophilization of spiked surface water are shown in Table 3.10 which shows its' excellent suitability for the analytes. However, the recovery of iopromide during lyophilization was strongly dependant on the vacuum applied.

Hence, an alternative concentration procedure was established for iopromide via rotary evaporation. Acetonitrile was added to the aqueous samples, to form an azeotropic mixture with water, which allowed for an evaporation of water at more gentle conditions. This method achieved a recovery of 88 ± 10 % and was comparable with those of the lyophilization procedure. As already mentioned, the solvent extraction procedures attained extraction efficiencies between 94 ± 4 and 95 ± 8 %.

Table 3.10: Recoveries and confidence intervals (P=95 %, n=10) of various ^{14}C-labelled analytes for the lyophilization and the evaporation method for surface water

Compound	Recovery (%) for	
	Lyophilization	Evaporation
^{14}C-Diazepam	95 ± 2	-
^{14}C-Ibuprofen	90 ± 2	-
^{14}C-Iopromide	91 ± 9	88 ± 10
^{14}C-Paracetamol	87 ± 4	-

3.6.3 Radio - thin layer chromatography

For radio-TLC analysis, the ^{14}C-labelled test compound and at least one possible metabolite, either labelled or non-labelled, were used as standards. The TLC conditions were chosen according to the best separation of the respective test compound from its' metabolites or similar derivatives. Silica gel TLC plates, Merck (Darmstadt, Germany) 20 × 20 cm 60 µm, with a 254 nm fluorescence coating and a preconditioning zone were used for all chromatographic separations. The plates were activated for 1 h at 110°C and were divided into six tracks, of which two, were reserved for reference standards. Compositions of the mobile phases for the different analytes are shown in Table 3.11. The gas phase in the developing tank was equilibrated with the mobile phase prior to the development of the plates. Samples and standards were spotted in the pre-concentration zone 1 cm above the bottom of the plate. All plates were developed to a distance of at least 15 cm and were then dried in a fume hood.

The positions of the bands resulting from non-labelled reference compounds were determined using UV light at 254 nm. Then, the TLC-plates were scanned for 10 – 30 min with an autoradiograph (Berthold, Wildbad, Germany) at an amplifier voltage of 900 V, while applying a mixture of argon, methane and methylal (90:10:1.5) as purge gas.

The software Chroma 3D (Berthold, Wildbad, Germany) was used to evaluate the data of the autoradiography. All TLC plates were analyzed by integrating measured values in certain plate areas as rectangular area segments. At first, all signals on a plate were integrated, then all signals in each of the six tracks and finally all signals in each of the four to six track sections. Compounds were identified according to their R_f-values.

Table 3.11: Mobile phase compositions, R_f-values and references

Compounds	R_f-value	Mobile phase for TLC-separation	Method source
Iopromide			
DAMI	0.47	2-Methyl-propanol-1/propanol-2 /ammonia 25 %	Kalsch, 1999
Iopromide	0.37	(50 : 30 : 20, v/v)	
ATH	0.32		
ATI	0.22		
Diazepam			
Diazepam	0.66	Chloroform/acetone	Sarin et al., 1998
Oxazepam	0.31	(85 : 15, v/v)	
Ibuprofen			
Ibuprofen	0.75	Chloroform/methanol/acetic acid	American Radiolabeled Chemicals Inc., 2001
2-Hydroxy ibuprofen	0.58	(85 : 15, v/v)	
Paracetamol			
4-Nitrophenol	0.54	Toluene/methanol/acetic acid	American Radiolabeled Chemicals Inc., 1994
Paracetamol	0.36	(90 : 16 : 8, v/v)	

3.6.4 Calibration and quantification

The aim of the TLC analysis was the separation of the radioactive test compounds from their corresponding metabolites and the quantitative determination of the sample composition.

In contrast to other detection methods, such as mass spectrometry, the response of the autoradiographical detection is not substance specific and depends on quantity of radioactivity present in a certain area of a TLC plate. Hence, the same calibration could be applied for all analytes. The linearity and sensitivity of the detector was determined by measuring a non-developed TLC-plate that was spiked with 6 different quantities of radioactivity in triplicates. A reliable quantification was attained between 250 and

75,000 dpm per spot. For quantification, the background signals per unit area of each track were determined in the upper undeveloped part of a track. Background correction was then achieved by subtracting the respective background signal from the measured values in each segment of a track.

3.6.5 Verification of radiochemical purity

The radio-TLC analysis of the applied radiotracer compounds, exhibited the presence of significant amounts of radio-labelled impurities, despite a higher certified purity in the iopromide, paracetamol and 4-nitrophenol standard (Table 3.12). Therefore, a correction was conducted to avoid incorrect results, from contaminations in the radio-labelled standards. For that, the percentage of contaminations in the radio-labelled standard was determined on each plate. The percentage of possible metabolites in the samples on a plate were then corrected by subtraction of the threefold percentage of observed contaminations with a corresponding R_f-value.

Table 3.12: Certified and measured radiochemical purities of radio-labelled analytes

Compound	Certified purity (%)	Measured purity (%)	Observed contaminations
Iopromide	> 98	91	1
Diazepam	99.8	100	-
Ibuprofen	> 99	100	-
4-Nitrophenol	99	89	2
Paracetamol	> 99	95	1

3.7 Calculation of DT_{50} and DT_{90}-values

The amount of the respective test substance in percent of the initial amount is given in tabular form and graphically. Based on this data, dissipation times (DT_{50} and DT_{90}-values) were calculated separately for the water phase as well as for the whole test system (water and sediment together) using the statistical software Origin 6.0 (Microcal Software Inc., Northampton, MA, USA). According to the EU guidance on the Persistence in Soil (European Commission and Directorate General for Agriculture, 2000) the regressions were accomplished considering at least 5 sampling times. Regression curves were calculated from first order kinetics depending on best fit. When the data did not follow a first order kinetics, a software package obtained from the BBA

(Biologische Bundesanstalt für Land- und Forstwirtschaft) was used. In those very few cases (Ibuprofen) where no fit could be achieved at all the DT-values were estimated. The determination coefficients r^2 obtained were always > 0.95. In addition, the regression coefficients were calculated, indicating also the fit of the curve (again, they were always > 0.95). Finally, the degradation rate per day was determined.

Considering that the duration of the tests was 100 days, numerical dissipation values are given only if the calculated number was not higher than one year. Instead, all values calculated as being between one and two years are given as "> 365 days". All values calculated as being even higher are given as ">> 365 days".

3.8 Quality assurance

Quality assurance plays a major role in the preparation of scientific results. The provision of accurate, reliable and well documented data is central to scientists involved in such areas as i.e. development and manufacture of drugs, testing of the environmental behaviour of chemicals, food control or drinking water analysis.

All data exhibited in this work was attained following several basic criteria to ensure the desired quality of the results. The methods applied and developed were characterized considering specificity, accuracy, precision and the respective limit of quantification, to prove the reliability of these methods and to guarantee the quality of data generated therewith.

An important part of the present work was the development of analytical methods. Additionally, water/sediment tests were conducted and the obtained samples were analyzed using the new analytical methods. The following measurements were taken to assure the quality of the data generated:

- The reproducibility of analytical procedures was verified regularly by standard addition experiments. Further, all sample series included blanks and the recovery rates were checked to compare enriched and non-enriched standard samples.
- All analytical results obtained were verified for their plausibility, i.e. recovery rates of analytes in different matrices.

- Analytical methods included, as far as possible, the use of internal and surrogate standards to compensate analyte losses throughout the sample preparation.
- The stability of the reference compounds was continuously controlled by comparing previously used calibration series with freshly prepared ones.
- The radiochemical purities of the ^{14}C-labelled substances was checked.
- Analytical scales, pH meters, redox meters and oxymeters were calibrated at least monthly, their function verified daily and the results reported in control cards.
- After elucidation of an adequate material for SPE enrichment, the same material charge was used throughout the work
- All LC separations were conducted under thermostatic control of the columns, to preclude temperature related shifts in chromatographic retention times.
- Realization and documentation of all relevant operations in the conducted water/sediment and leaching tests according to the principles of good laboratory practice (GLP).

4 RESULTS AND DISCUSSION

4.1 Water/sediment studies

4.1.1 Principles and limitations of water/sediment tests

Water/sediment studies are usually required for the registration of new active substances in pesticide formulations but they can also be applied to any chemical with a possible relevant occurrence in the aquatic environment. The general aim of those studies is the investigation of fate and persistence of a chemical under controlled laboratory conditions. The generated data is then utilized for conclusions on the environmental behaviour of a chemical and the risks linked therein.

This approach was used to investigate pharmaceuticals which are detected in the aquatic environment of many countries. In fate studies, the test compounds are usually applied as ^{14}C-radiotracers. That allows for a relatively easy and reliable quantification of all relevant processes, such as distribution to the various compartments, metabolization, mineralization and the formation of bound residues. Due to the lack of ^{14}C-labelled standards commercially available, non-labelled pharmaceuticals were also utilized in the test systems. The latter usually allow only for a measurement of their dissipation and not for a process differentiation between transformation, mineralization and formation of bound residues. Therefore, a complete mass balancing is usually not possible using non-labelled test compounds.

Since the fate of a chemical in the water/sediment test system depends not only on its physico-chemical properties but also on the conditions of the test system itself (e.g. the pH in the water phase or the grain size distribution of the sediment used), it is essential to cover a broad range of different waters and sediments. However, due to practical limitations, it was not possible to test more than one sediment. Therefore, a sandy sediment with a low content of organic matter was selected in order to minimize sorption in the experiments. Furthermore, this represents a “worst-case” situation, since such sediments often contain a low microbial biomass, which also means that the

degradation of organic chemicals is usually low (e.g. the persistence of chemicals is higher than in "rich" sediments with a very active micro flora).

The experimental set-up of the water/sediment test system allowed for a widely parallel development of the independent test vessels over a period of 100 d. The test set-up was designed relatively robust, since the test vessels were shaken in batches of 24 flasks for every test compound on an automatic shaker during the entire test period of 100 d. Therefore, very sensitive sets, i.e. continuous gas flow through, were avoided and gas exchange with the environment was attained simply by diffusion through the CO_2-trap.

A weakness of this experimental design became obvious in the tests with ^{14}C-ibuprofen and ^{14}C-paracetamol, where extensive mineralization occurred and the balance of recovered radioactivity was partially incomplete. Especially in the tests with ibuprofen a wide balance gap was observed in the course of the experiment, whereas at the end of the test the balance was rather complete. This was most likely caused by losses of volatile $^{14}CO_2$ during the sampling procedure. After 2 weeks the formation of $^{14}CO_2$ was fairly advanced and $^{14}CO_2$ was present widely dissolved in the water. During the sampling procedure, $^{14}CO_2$ was presumably withdrawn from the balance by volatilization with the gas phase of the test system after opening of the test vessels. An additional volatilization from the water or the sediment compartment cannot be excluded. These assumptions were further supported by the increasing recovery of radioactivity towards the end of the test, considering that losses from the test vessels did not occur in the course of the test. In fact, the progressive demobilization of $^{14}CO_2$ in the trap material reduced the losses by volatilization.

The losses might be at least partly overcome by bubbling a gentle nitrogen stream through the water compartment of the closed test vessels before opening for sampling, so that the demobilization of $^{14}CO_2$ is enhanced. Another deficit of the test set-up can be seen in its´ static conditions, where the consumed nutrients will not be replaced. Hence, it cannot be excluded that a co-metabolic transformation of test compounds stops in the course of the experiment due to a lack of nutrients. In all other respects the test system, originally developed for the investigation of pesticides, is suitable for studying pharmaceuticals.

4.1.2 Behaviour of ^{14}C-paracetamol

In the experiments with ^{14}C-paracetamol, the radioactivity dissipated rapidly out of the water and after 14 d only 13.10 ± 0.03 % of the initial radioactivity was left in the water compartment (Figure 4.1). On the other hand, the percentage of radioactivity in the sediment increased rapidly within 8 days. After that time, 57 ± 3 % of the paracetamol was located in the sediment. The percentage of radioactivity in the sediment remained then constant at approximately 60 % until the end of the experiment. At day 100, 19 ± 3 % of the initially applied radioactivity (IAR) was found in the CO_2 traps. It has to be noted, that the balance of radioactivity recovered was not entirely complete. After day 14, a loss of 20 % of the IAR was observed. This loss remained almost constant until the end of the experiment.

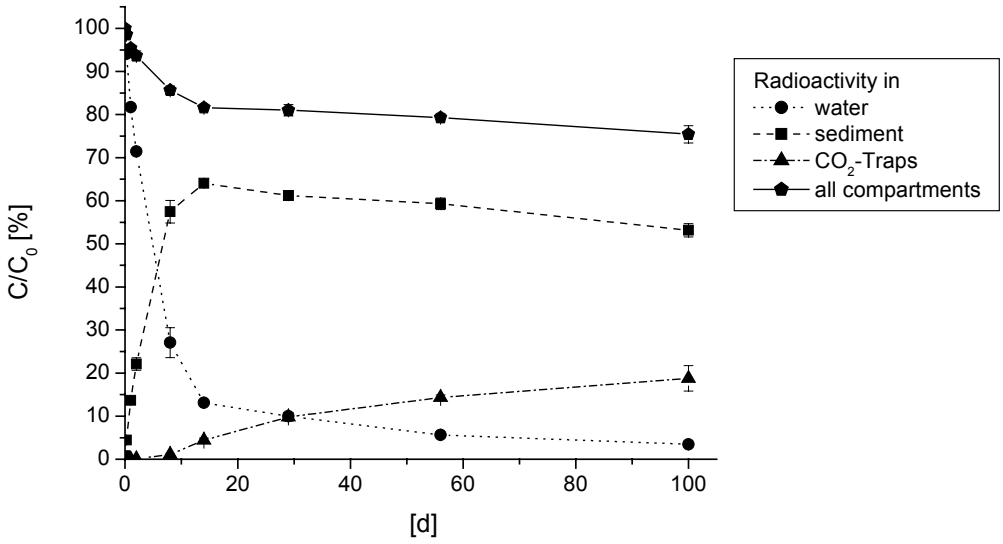


Figure 4.1: Distribution of radioactivity in the water/sediment systems spiked with ^{14}C-paracetamol

The composition of the radioactivity in the water phase was investigated using radio thin layer chromatography after concentration of the water volume by lyophilization (Figure 4.2). Immediately after spiking a transformation product (TP) was found in the water phase. The TP occurred in the course of the first two weeks with a maximum of 4 ± 2 % and was not detected thereafter, suggesting further degradation or sorption.

However, it was observed that at day 7 the recovery of the radioactivity dropped by more than 50 % in the water phase. Later on, only negligible amounts of radioactivity were found in the lyophilized water samples. It can be presumed that during lyophilization the dissipation of volatile degradation products, most likely $^{14}CO_2$, caused the observed losses of radioactivity.

In the water phase paracetamol was found to be quickly eliminated with a DT_{50} of 3.1 d and a DT_{90} of 10.4 d. To some extent it was even mineralized forming CO_2.

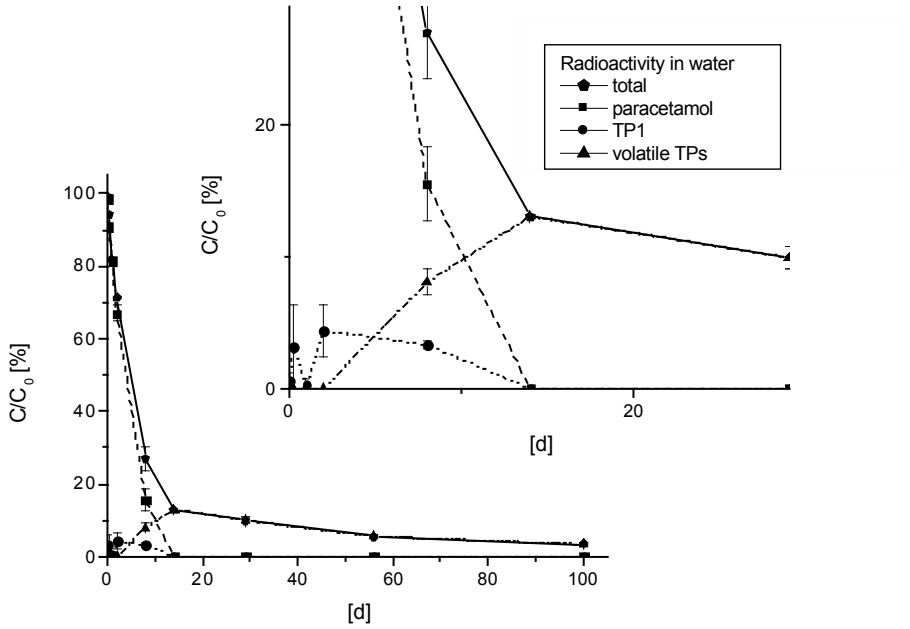


Figure 4.2: Fate of ^{14}C-paracetamol in the water compartment of the water/sediment system detected by radio-thin layer chromatography

Solvent extraction of the sediments with acetone and methanol resulted only traces of radioactivity, while after combustion of the extracted sediment major radioactivity was found. Therefore, most of the initial radioactivity was reserved to compounds sorbed onto sediments and is classified as non-extractable (bound) residues. These bound residues = non-extracted residues are defined as chemical species originating from chemicals that cannot be extracted by methods which do not significantly change the chemical nature of these residues (European Commission and Directorate General for Agriculture, 2000).

This result corresponds to the findings of terrestrial studies in which the sum of the parent compound and its TPs were bound covalently to soil by various binding mechanisms or were entrapped in the soil matrix (Gevao et al., 2000, Northcott and Jones, 2000). Recovery experiments for the solvent extraction of ^{14}C-paracetamol from non-autoclaved sediment showed that only 10 % of the radioactivity applied was extractable after a contact time of 14 h, whereas paracetamol was recovered quantitatively in experiments with autoclaved sediment. That is in good agreement with observations of Kreuzig, 2002, who also reported a rapid bio-transformation of paracetamol into non-extractable residues after an application onto bioactive soils. Paracetamol however, remained entirely extractable in autoclaved soils. With respect to the reported biodegradation of paracetamol, it is important to note that soil bacteria capable for the degradation of paracetamol could be isolated by Ahmed et al., 2001. Ready biodegradability of paracetamol in the environment was first quoted by Richardson and Bowron, 1985. Also Moehle et al., 1999 reported biodegradation of paracetamol in batch experiments with activated sludge and Ternes, 1998 found major elimination of paracetamol during STP passage.

The rapid movement of paracetamol into the sediment cannot be explained by its' lipophilicity, which is relatively low (log K_{OW} = 0.49, pK_a = 9.5) (Stuer-Lauridsen et al., 2000). It is assumed that degradation products of paracetamol were attached covalently to the sediment matrix or were incorporated into the biomass of the sediments.

No DT-values for paracetamol in the water/sediment system could be calculated. However, regarding the high amount of radioactivity bound to the sediment, DT-values of paracetamol or, probably, its TPs of more than 100 d are estimated.

Summary: DT-values for paracetamol

Water phase:

Function and degradation rate:	First order	d = 0.22 per day
DT-values:	DT_{50}: 3.1 d	DT_{90}: 10.4 d
Regression coefficient:	0.9984	Accepted (> 0.95)

Water/sediment system:

Kinetics and degradation rate:	Not possible to determine
DT-values:	Not possible to determine (> 100 d)
Remark:	High amount of bound residues

4.1.3 Behaviour of ^{14}C-ibuprofen

The radioactivity of the ^{14}C-labelled ibuprofen (mixture of R/S-diasteromers) dissipated rapidly from the water compartment as shown in Figure 4.3. After 100 d, less than 5 % of the IAR was detected in this compartment. The radioactivity was found to some extent in the sediment, where a flat maximum of 17 ± 1 % IAR was attained after 14 d. It is remarkable, that the quantity of radioactivity in the CO_2 traps increased very quickly. Total radioactivity found in the test vessels dropped to 73 ± 1 % after 14 d and increased to 90.2 ± 0.5 % after 100 d.

For the correct interpretation of the results it has to be noted that, in contrast to all other radio-labelled pharmaceuticals applied, ibuprofen was ^{14}C-radio-labelled in the carboxyl moiety. Therefore, the radio-label of the [carboxyl-^{14}C]-ibuprofen can be cleaved relatively easy from the original molecule by decarboxylation, while the rest of the molecule remains unchanged. In this respect, it is important to mention that the ibuprofen metabolites known from humans are still containing the carboxyl group (Buser et al., 1999, Stumpf et al., 1998, Winkler et al., 2001, Zwiener et al., 2002).

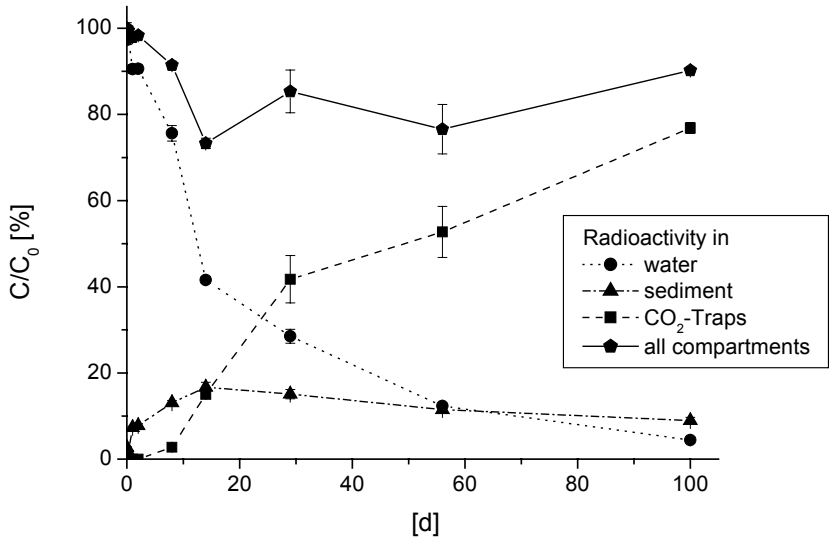


Figure 4.3: Distribution of radioactivity in the water/sediment systems spiked with ^{14}C-ibuprofen

Ibuprofen dissipation in the water phase was minor until day 8 (Figure 4.4). Afterwards, a total loss of radioactivity was observed similar to the observations for paracetamol. It can be assumed that these losses were caused by the presence of volatile radio-labelled degradation products dissolved in the water, such as $^{14}CO_2$. The rapid transformation of ibuprofen is exhibited by a total of 56.61 ± 0.04 % IAR after 14 d and more than 70 % after 100 d present as $^{14}CO_2$ and/or other volatile TPs. After day 8 only volatile TPs of ibuprofen were detected in the water (Figure 4.5). Due to their limited percentage of radioactivity, sediment samples were not further analyzed.

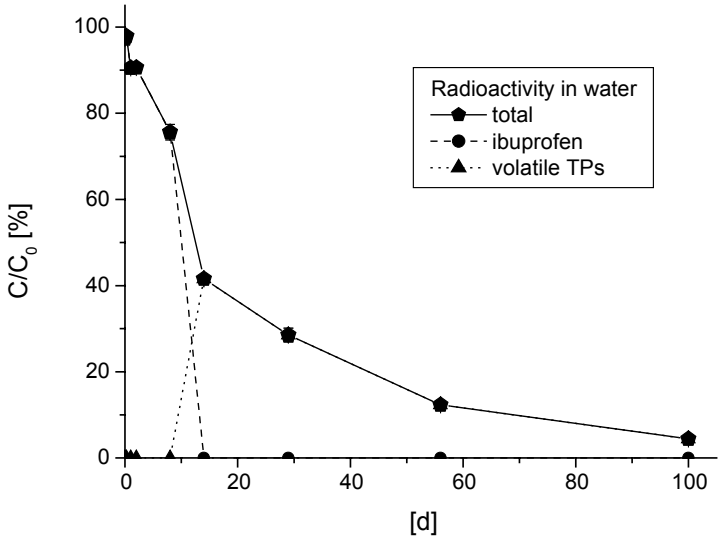


Figure 4.4: Fate of ^{14}C-ibuprofen in the water phase of the water/sediment system

In recovery experiments of the solvent extraction, only 25 % of the radioactivity applied from ^{14}C-ibuprofen was extractable from non-autoclaved sediment after a contact time of 14 h. To the contrary, ibuprofen was recovered quantitatively, when using autoclaved sediment. Hence, it can be presumed that ^{14}C-ibuprofen was susceptible to rapid bio-transformation in the sediment.

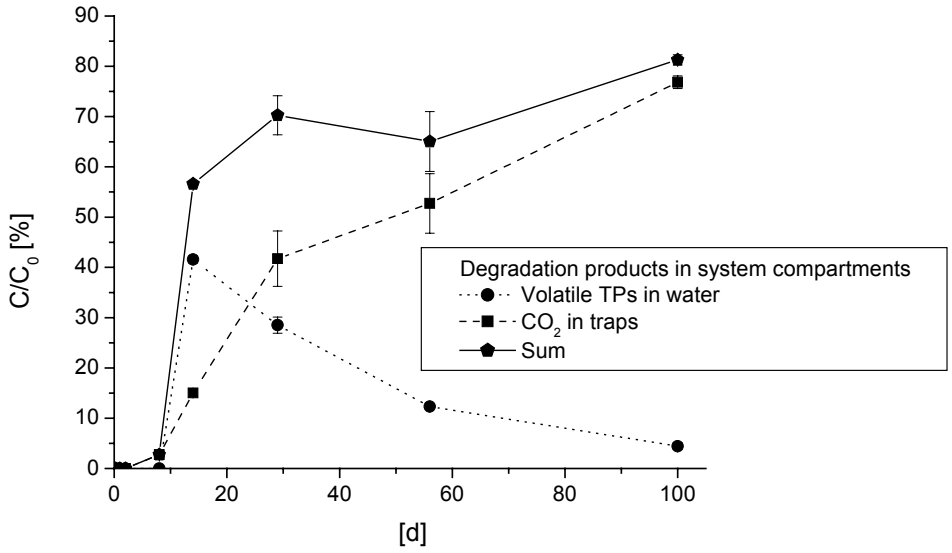


Figure 4.5: Calculated percentage of volatile TPs and measured $^{14}CO_2$ in the test with ibuprofen detected by radio-thin layer chromatography

Ibuprofen showed a low affinity for sorption onto sediment despite a log K_{OW} of 3.5 for the non-dissociated acid. Given its' pK_a-value of 4.9 (Jones et al., 2002), the sorption should be strongly influenced by the pH of the sediment. Under the conditions present in the sediment (pH 7.5), ibuprofen was widely dissociated and displayed therefore a very low lipophilicity (shown in Figure 4.6).

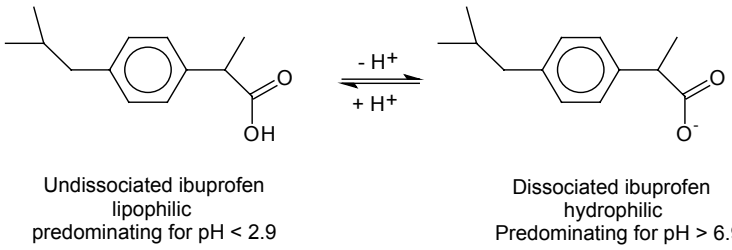


Figure 4.6: Dissociation equilibrium of ibuprofen

Richardson and Bowron, 1985 reported an inherent biodegradability of ibuprofen. Later, Stumpf et al., 1998 isolated the human ibuprofen metabolites 2-hydroxy-ibuprofen and carboxy-ibuprofen and found their occurrence in municipal STP effluents and surface waters. Ibuprofen is degraded to a major extent (> 75 %) during sewage treatment, as recently shown (Stumpf et al., 1999, Ternes, 1998). Furthermore, Winkler

et al., 2001 investigated the biodegradation of ibuprofen in surface water biofilm systems and found DT_{50}-values between 1 and 6 d, depending on the river water applied (Winkler et al., 2001). They and other authors reported the rapid degradation of ibuprofen and the formation of 2 and 3 TPs. These TPs corresponded to approximately 10 % of the initial ibuprofen quantity, varying with the conditions in STP- and surface water experiments (Buser et al., 1999, Zwiener et al., 2002). Hence, for the majority of ibuprofen degraded, the TPs and the transformation pathway is still unknown.

Ibuprofen TPs could not be detected in the aerobic water compartments, presumably due to the rapid degradation process. After a lag period of eight days, complete mineralization towards volatile TPs occurred within the next six days. Therefore, in agreement with other authors (Buser et al., 1999, Winkler et al., 2001, Zwiener et al., 2002), ibuprofen was completely degradable in the water compartment under the present conditions, resulting in a DT_{50}-value of 10 d and a DT_{90} of 13 d. Since the data did not follow a certain kinetic function, these values were estimated using the graphical representation of the raw data.

Summary: DT-values for ibuprofen

Water phase:

Function and degradation rate:	Not possible to determine	
DT-values:	DT_{50}: 10 d	DT_{90}: 13 d
Regression coefficient:	Not possible to determine	
Remark:	DT-values estimated based on raw data	

Water/sediment system:

Kinetics and degradation rate:	Not possible to determine
DT-values:	Not possible to determine (< 20 d)
Remark:	Complete degradation in the water phase

4.1.4 Behaviour of 2-hydroxy-ibuprofen and comparison with ibuprofen

The ibuprofen metabolite 2-hydroxy-ibuprofen dissipated rapidly out of the water compartment under oxic conditions, but was never found in the sediment (Figure 4.7).

After 28 d, its´ concentration was below the limit of quantification. A re-formation of the parent compound can be excluded, since ibuprofen was never detected.

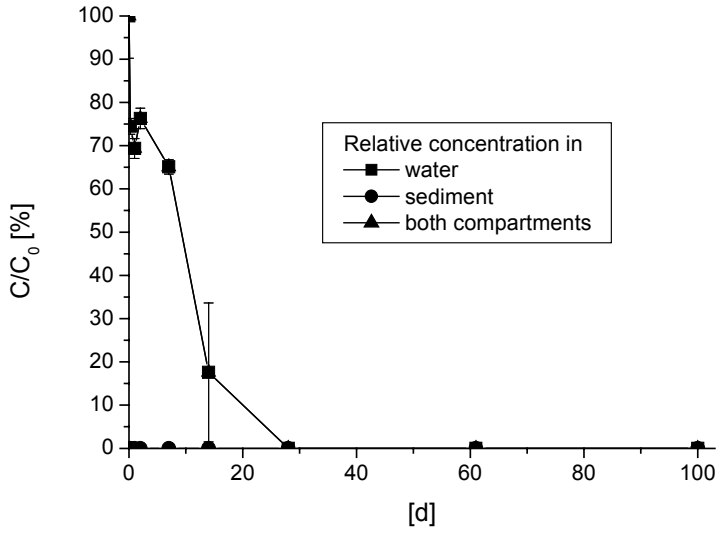


Figure 4.7: Behaviour of 2-hydroxy-ibuprofen in the water/sediment systems

The occurrence of 2-hydroxy-ibuprofen and other ibuprofen metabolites in human urine, wastewater and other environmental waters has already been reported (Buser et al., 1999, Stumpf et al., 1998). In laboratory tests (Winkler et al., 2001, Zwiener et al., 2002), 2-hydroxy-ibuprofen was mainly formed under aerobic conditions.

2-Hydroxy-ibuprofen was not found in the sediment, an it can be assumed that it was rapidly (bio)transformed in the sediment. The DT_{50} of 6.7 d and DT_{90} of 22 d of 2-hydroxy-ibuprofen are low and in agreement with reports on its degradability (Winkler et al., 2001). Again, no different values for the water/sediment system have to be considered. Due to the additional hydroxy moiety 2-hydroxy-ibuprofen possesses a higher polarity than ibuprofen, resulting in a reduced affinity towards the sediment.

A comparison of the degradability of ibuprofen and its' metabolite 2-hydroxy-ibuprofen shows a similar behaviour for both compounds. After a lag phase of about 6 days both compounds are rapidly degraded (Figure 4.8). While ibuprofen was totally degraded until day 14, the metabolite 2-hydroxy-ibuprofen was still present in the water phase on a low level.

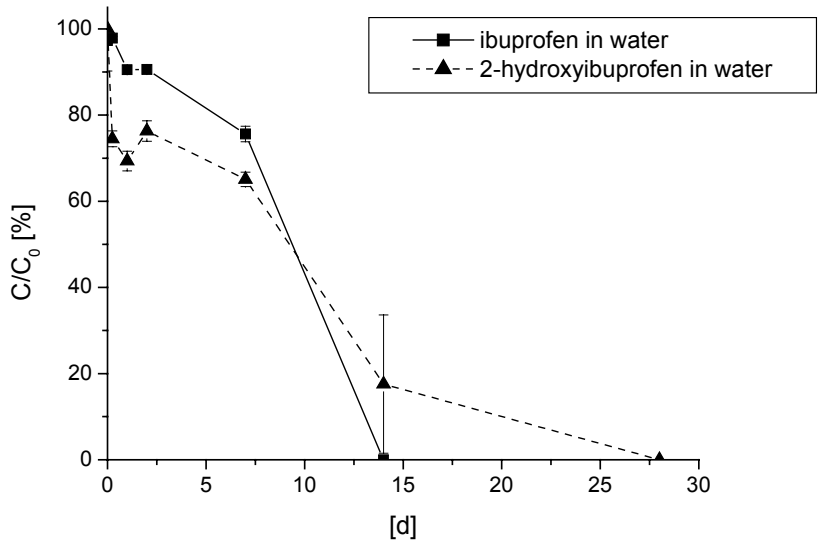


Figure 4.8: Comparison of the behaviour of ibuprofen and 2-hydroxy-ibuprofen

Summary: DT-values for 2-hydroxy-ibuprofen

Water phase:

Function and degradation rate:	First order	d = 0.10 per day
DT-values:	DT_{50}: 6.7 d	DT_{90}: 22 d
Regression coefficient:	0.9682	Accepted (> 0.95)

Water/sediment system:

Kinetics and degradation rate:	Not possible to determine
DT-values:	Not possible to determine (< 30 d)
Remark:	Almost complete degradation in the water phase

4.1.5 Behaviour of clofibric acid

The concentration of clofibric acid in the water decreased steadily until the end of the experiment (Figure 4.9). By that time, 49 ± 2 % of the initial concentration was still present in the water compartment. The sediment compartment never contained more than 12 ± 1 % of the initial quantity. This is equivalent to the ratio of pore water and total water in the system. A total of 55 ± 3 % clofibric acid was found in the entire water/sediment system at the end of the experiment.

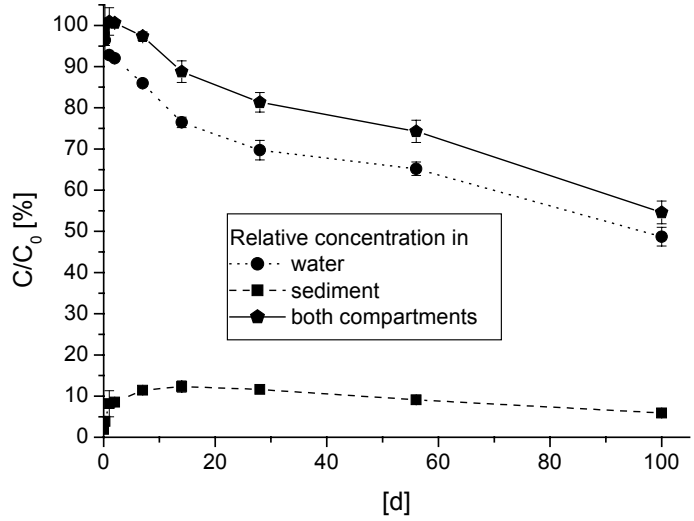


Figure 4.9: Behaviour of clofibric acid in the water/sediment systems

Clofibric acid displayed a low affinity for the sediment. At pH 7.7 in the sediment, clofibric acid was widely dissociated (calculated low pK_a-value of 2.84), and was thus, sorbed onto the sediment only to a negligible extent (Schwarzenbach et al., 2003). This corresponds with the distinct attenuation of clofibric acid in soil leaching experiments with a very acidic soil with pH of 2.9, while only little retardation was observed using a soil with a more common pH of 5.8 (see chapter 4.3.3). These results also are in consistence with other authors (Heberer, 2002a, Lenhard, 2000, Scheytt et al., 1998, Scheytt et al., 2001), who reported an almost tracer-like movement of clofibric acid in soil columns and during river bank filtration.

Clofibric acid was considerably stable in the water/sediment system and disappeared slowly. Based on the time-concentration curves DT_{50} values of 82 d and 119 d and DT_{90} values of 274 d and >365 d were calculated for clofibric acid in the water compartment and the entire water/sediment system, respectively.

That is principally in good agreement with findings of other authors. In batch experiments with fresh field soil Lenhard, 2000 observed no biodegradation of clofibric acid. In wastewater treatment only little or no elimination of clofibric acid was observed (Heberer et al., 2002, Stumpf et al., 1999, Ternes, 1998). Consequently, clofibric acid is introduced to a high portion into surface waters. It is present ubiquitously in the aquatic

environment and was found at concentrations of 1 ng·L^{-1} North Sea (Buser et al., 1998, Weigel et al., 2002).

Summary: DT-values for clofibric acid

Water phase:

Function and degradation rate:	First order	d = 0.0084 per day
DT-values:	DT_{50}: 82 d	DT_{90}: 274 d
Regression coefficient:	0.9912	Accepted (> 0.95)

Water/sediment system:

Kinetics and degradation rate:	First order	d = 0.0058 per day
DT-values:	DT_{50}: 119 d	DT_{90}: > 365 d
Regression coefficient:	0.9992	Accepted (> 0.95)

4.1.6 Behaviour of ^{14}C-diazepam

After the application of ^{14}C-diazepam, the substance was constantly transferred into the sediment (Figure 4.10). Even after 100 d an equilibrium between water and sediment seemed not to be totally attained. The time-concentration curve of the radioactivity in the sediment showed a rapid increase within the first 14 d. By then more than 30 % of the initial radioactivity were localized in the sediment. After that, the percentage of radioactivity in the sediment levelled off and at the end of the experiment, 60 ± 6 % of the initial radioactivity was still found in the sediment. Mineralization was only observed to a minor extent. At the end of the experiment the CO_2-traps contained only 1.49 ± 0.04 % of the initial radioactivity. A balance of radioactivity, at that time, showed that more than 90 % of the initial radioactivity could be recovered in the test systems.

The TLC analysis of water samples exhibited that almost the entire radioactivity in this compartment was still present as diazepam, 95 ± 7 % at day 100. In the course of the experiment, at least two TPs occurred at very low levels (see Figure 4.11). TP 1 appeared only at day 14 and the second TP was initially observed after day 7 for the first time. In the TLC analysis, TP 1 remained entirely at the spot of TLC-application, whereas TP 2 showed a slightly higher mobility but had a diffuse signal without a sharp defined R_f-value. The sum of both TPs never exceeded 5 %. Radio-TLC analysis of the

extracts, suggested that the entire radioactivity in sediment samples was extractable and could be uniformly identified as diazepam.

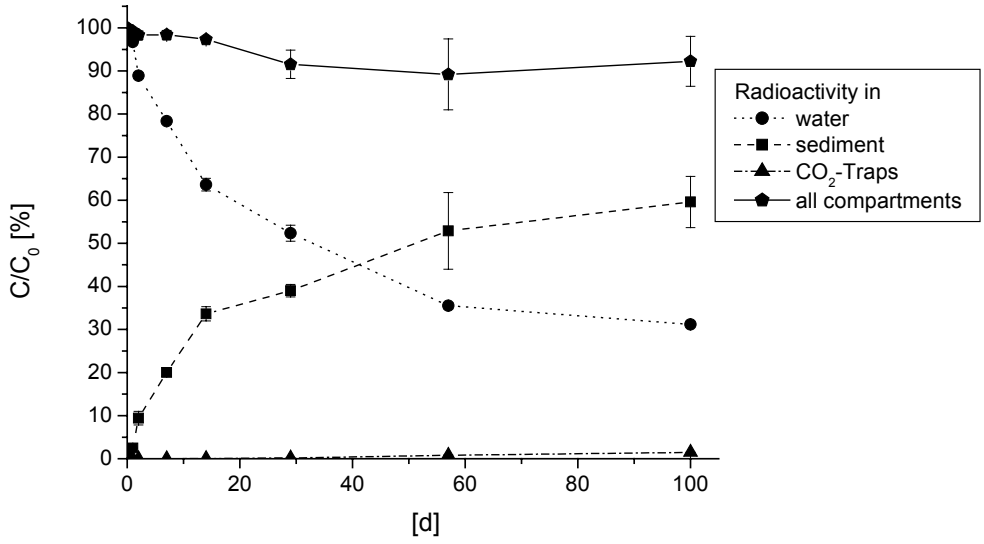


Figure 4.10: Distribution of radioactivity in the water/sediment systems spiked with ^{14}C-diazepam

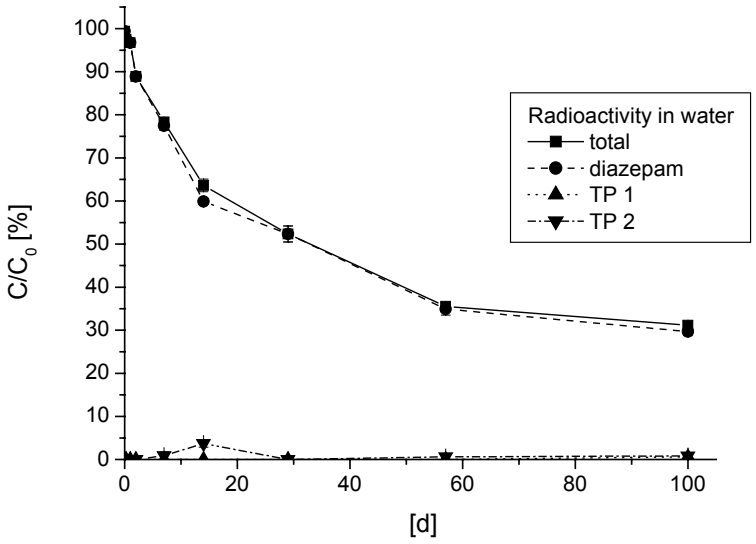


Figure 4.11: Fate of ^{14}C-diazepam in the water phase of the water/sediment system

Under the pH conditions in sediment and water, the weak base diazepam (pK_a = 3.4) (Hilal et al., 1996) should have been present in its' neutral, non-protonated form.

Hence, the rapid sorption of diazepam onto the sediment can be expected to be caused mainly by non ionic interactions with the sediment (log P_{OW} = 2.85) (Stuer-Lauridsen et al., 2000).

A strong sorption of diazepam onto soil particles was also observed in the soil column leaching experiments (see chapter 4.3.4). In these experiments, the entire radioactivity initially applied as ^{14}C-diazepam, remained in the uppermost soil layers.

An elimination of diazepam, respectively the formation of TPs, occurred only to a minor extent in the water/sediment system (Figure 4.12).

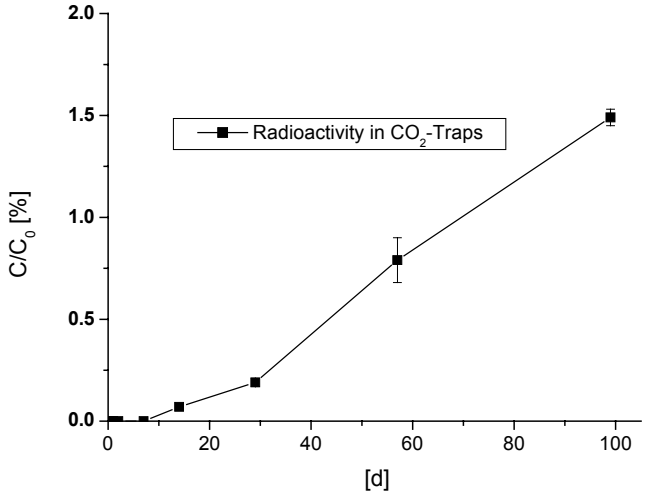


Figure 4.12: Formation of CO_2 by mineralization of ^{14}C-diazepam

More than 87 % of the initial diazepam quantity was recovered at the end of the experiment. In the water compartment, a DT_{50}-value of 34 d and a DT_{90}-value of 113 d were calculated. Corresponding dissipation times in the water/sediment system were calculated to be even higher (DT_{50}-value > 365 d, DT_{90}-value >> 365 d). The tranquilizer diazepam is applied in medicine in relatively low quantities (Table 1.1). Hence, reports on its occurrence in various aqueous compartments, such as STP effluents and rivers (Ternes et al., 2001, Zuccato et al., 2000) indicate a distinct environmental stability.

Summary: DT-values for diazepam

Water phase:

Function and degradation rate:	First order	d = 0.020 per day
DT-values:	DT_{50}: 34 d	DT_{90}: 113 d
Regression coefficient:	0.9841	Accepted (> 0.95)

Water/sediment system:

Kinetics and degradation rate:	First order	d = 0.0016 per day
DT-values:	DT_{50}: > 365 d	DT_{90}: >> 365 d
Regression coefficient:	0.9991	Accepted (> 0.95)

4.1.7 Behaviour of oxazepam and comparison with diazepam

Additionally, oxazepam, the main metabolite of diazepam in humans, was tested with a non-labelled compound in the water/sediment system. The oxazepam concentration in the water compartment decreased relatively fast within the first month and ranged at a level of 14.7 ± 0.4 % of its' initial concentration at the end of the experiments (Figure 4.13). Oxazepam moved distinctly into the sediment compartment, where 27 ± 4 % were localized after two weeks. After that, the oxazepam content of the sediment remained almost constant until the end of the experiment. Finally, 33 ± 1 % of the initial oxazepam quantity was recovered in the test systems.

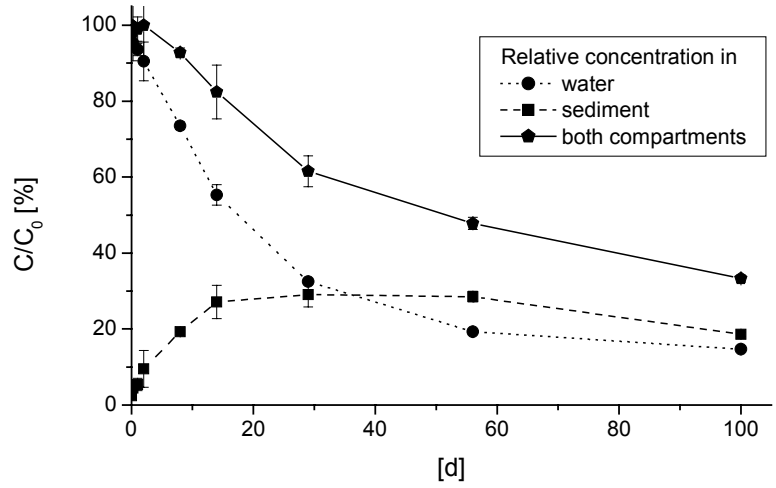


Figure 4.13: Behaviour of oxazepam in the water/sediment system

The partitioning behaviour of oxazepam was presumably influenced mainly by its' lipophilicity, given that oxazepam was present non-protonated in the water/sediment system (log K_{OW} = 2.24, pK_a = 1.7) (Hansch et al., 1990, Hilal et al., 1996). For the water compartment, a DT_{50} of 19 d and a DT_{90} of 63 d was calculated. Dissipation times for the entire water/sediment system, considering oxazepam also sorbed reversible onto the sediment, were significantly higher with a DT_{50} of 54 d and a DT_{90} of 179 d (assuming a second order function, the fit of the regression curve was as good as for a first order one, but in that case both DT_{90} values in the water sediment system would be as twice as high). The data indicates a delayed primary degradation of oxazepam in the water/sediment systems (Beek, 2001). However, the data available for this non-labelled test substance does not allow for a differentiation of transformation, mineralization and the formation of non-extractable or bound residues as cause for the dissipation.

Up till now, few findings of oxazepam have been reported in sewage effluent (250 $ng \cdot L^{-1}$) and river water (70 $ng \cdot L^{-1}$) (Heberer, 2002b, Heberer et al., 2002), indicating a distinct stability in the environment.

Summary: DT-values for oxazepam

Water phase:

Function and degradation rate:	First order	d = 0.036 per day
DT-values:	DT_{50}: 19 d	DT_{90}: 63 d
Regression coefficient:	0.9936	Accepted (> 0.95)
Remark:	Assuming a second order function, the DT_{50} would remain the same but the DT_{90} would be doubled.	

Water/sediment system:

Kinetics and degradation rate:	First order	d = 0.013 per day
DT-values:	DT_{50}: 54 d	DT_{90}: 179 d
Regression coefficient:	0.9981	Accepted (> 0.95)
Remark:	Assuming a second order function, the DT_{50} would remain the same but the DT_{90} would be doubled.	

The elimination rates of the diazepam and oxazepam were very similar until day 8 (see Figure 4.14). Afterwards, the quantity of oxazepam recovered decreased strongly, whereas that of diazepam remained more or less constant.

In humans, diazepam is rapidly demethylated and then metabolized into oxazepam, which is then excreted directly or in the conjugated form (Mutschler, 1997). Under the conditions of the test system, diazepam was not transferred into oxazepam or into any other TP with appreciable quantities. That result underlines the low comparability of human and environmental transformation processes. Nevertheless, oxazepam, as frequently found for phase I metabolites, shows a higher polarity and was more amendable to conversion processes than the parent compound diazepam. Hence, diazepam exhibited a higher persistence than oxazepam in the environmental test system.

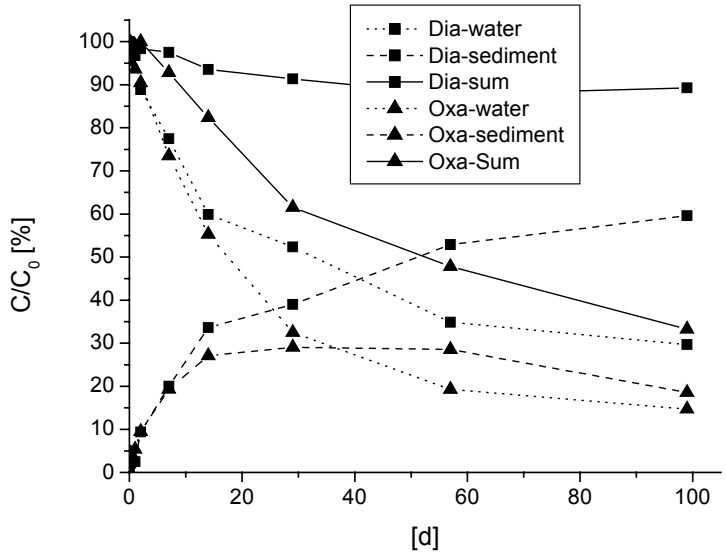


Figure 4.14: Comparison of the behaviour of diazepam and oxazepam in a water/sediment system

4.1.8 Behaviour of carbamazepine

The antiepileptic drug carbamazepine moved from the water phase into the sediment until a steady state level was achieved at about day 58 (Figure 4.15). Carbamazepine displayed a high persistence in the system. An initial slow decrease occurred up until day 57 and a total of 83 ± 6 % of the initial carbamazepine quantity was still present in the system by the end of the experiment.

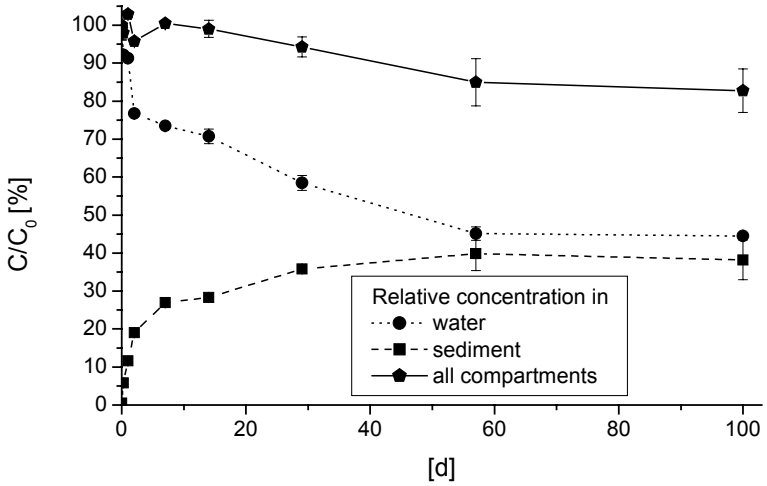


Figure 4.15: Behaviour of carbamazepine in the water/sediment system

Based on its' moderate lipophilicity (log P_{OW}= 2.25) (Jones et al., 2002), and its presence in a non-charged form under environmental conditions sorption could be expected (estimated pK_a-values of 13.9 for the deprotonation (Jones et al., 2002) and < 1 for the protonation of the amino groups). The respective sediment study, the behaviour in soil columns (see chapter 4.3.1) and its´ behaviour in contact with activated carbon (Ternes et al., 2002b) seems to support that assumption. On the other hand, the negligible removal during bank filtration (Sacher et al., 1998), wastewater irrigation (Ternes, 2003), wastewater treatment (Ternes, 1998, Ternes, 2000) or soil passage (Drewes et al., 2002, Lenhard, 2000) were contradictory. The respective sorption mechanisms are still unclear.

Ternes, 2000 found a similar course of the time/concentration curve for carbamazepine in the water phase of water/soil shake flask experiments with field fresh soil. In contrast to the results presented herein, he reported the total elimination of

carbamazepine from the water phase after a lag period of 30 d. Hence, it cannot be excluded that the virtually constant concentration of carbamazepine in the water/sediment systems might have significantly decreased after an adaptation time of yet unknown length. The DT_{50} and the DT_{90} for carbamazepine in the water compartment were calculated as 52 d and 173 d, whereas the respective DT-values for the entire water/sediment system were calculated as 333 d and >> 365 d.

However the high stability of carbamazepine in the water sediment system is consistent with reports of Moehle et al., 1999 who found no primary degradation of carbamazepine in batch experiments with activated sludge. Carbamazepine also was widely resistant towards aerobic or anaerobic biodegradation in batch experiments with surface water and groundwater (Ternes et al., 2002b). Thus, it is ubiquitously present in environmental waters (Ternes, 1998) and was even detected in the marine ecosystem (Weigel et al., 2001).

Summary: DT-values for carbamazepine

Water phase:

Function and degradation rate:	First order	d = 0.013 per day
DT-values:	DT_{50}: 52 d	DT_{90}: 173 d
Regression coefficient:	0.9936	Accepted (> 0.95)

Water/sediment system:

Kinetics and degradation rate:	First order	d = 0.0021 per day
DT-values:	DT_{50}: 333 d	DT_{90}: >>365 d
Regression coefficient:	0.9994	Accepted (> 0.95)

4.1.9 Behaviour of 10,11-dihydro-10,11-dihydroxy-carbamazepine and comparison with carbamazepine

The carbamazepine metabolite 10,11-dihydro-10,11-dihydroxy-carbamazepine dissipated rapidly out of the water compartment in the first 28 d (Figure 4.16). Then, the concentration of the test compound remained constant within the statistical error at 36 ± 1 %. The test compound was found only to a minor extent in the sediment, where at no time more than 6 ± 1 % of the initial quantity could be detected.

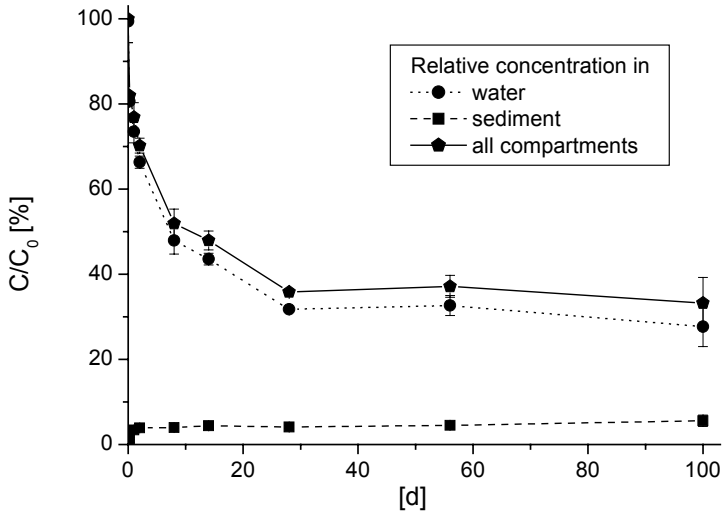


Figure 4.16: Behaviour of 10,11-dihydro-10,11-dihydroxy-carbamazepine in the water/sediment system

Compared to the parent compound carbamazepine, the twofold hydroxylated and thus, more polar metabolite 10,11-dihydro-10,11-dihydroxy-carbamazepine, exhibited a significantly reduced affinity for the sediment. The apparent low level of the test compound in the sediment was checked and confirmed in additional experiments. Calculated DT_{50}-values of 21 and 34 d for the metabolite in the water compartment and the water/sediment system, respectively, were lower than for carbamazepine. DT_{90}-values could not be calculated but they are larger than 365 d. Oxidation and cleavage of the dihydroxy moiety might play a role in the further degradation of 10,11-dihydro-10,11-dihydroxy-carbamazepine. Those reactions have been elucidated in the biodegradation pathway of various aromatic compounds in the environment and involve frequently bacteria and fungi with a broad substrate specificity (Beek, 2001, Díaz et al., 2001, Pointing, 2001, Reardon et al., 2000).

After 28 days, the total quantities of 10,11-dihydro-10,11-dihydroxy-carbamazepine detected remained more or less constant on a level of about 38% of the initial concentration until the end of the test. In natural waters and soils, threshold concentrations for the degradation of contaminants have been reported before (Klimek et al., 2001, Kovar et al., 2002). However, in the case of the described experiments it

cannot be excluded that the static conditions might have lead to a major consumption of essential nutrients and therefore, to a stop of the assumed co-metabolic degradation.

Despite the low DT_{50}-value of 10,11-dihydro-10,11-dihydroxy-carbamazepine, its' stability in the water compartment was relatively high. At a spiking level of 100 $ng \cdot g^{-1}$ of sediment, more than 30 % of the test compound remained non-degraded in the test system at the end of the test. This is supported by Miao and Metcalfe, 2003 who stated a low degradability of 10,11-dihydro-10,11-dihydroxy-carbamazepine in sewage treatment.

Summary: DT-values for 10,11-dihydro-10,11-dihydroxy-carbamazepine

Water phase:

Function and degradation rate:	Root second order	
DT-values:	DT_{50}: 21 d	DT_{90}: > 365 d
Regression coefficient:	0.9111	Best fit available

Water/sediment system:

Function and degradation rate:	Root second order	
DT-values:	DT_{50}: 34 d	DT_{90}: > 365 d
Regression coefficient:	0.8897	Best fit available
Remark:	High portion remained non-degraded in sediment.	

4.1.10 Behaviour of ivermectin

The parasiticide ivermectin moved rapidly from the water compartment into the sediment. After the course of 2 weeks, only 13 ± 6 % of the initial ivermectin concentration was left in the water compartment (Figure 4.17). A maximum concentration of ivermectin in the sediment was attained with 42 ± 2 % at day 7. Afterwards the percentage of ivermectin in the sediment decreased slowly to 16 ± 3 % by the end of the test. The total quantity of ivermectin found in the test system showed a sharp drop after the first 2 weeks and decreased much slower afterwards. The kinetics in both the water phase as well as in the entire water/sediment system followed a root first order function.

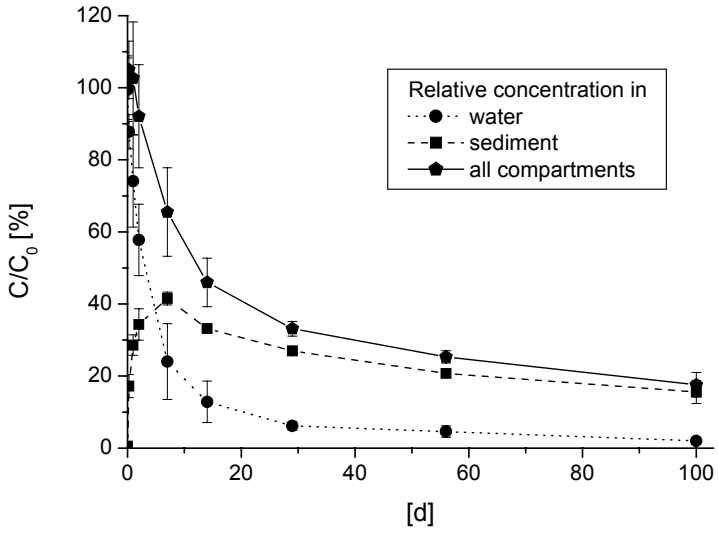


Figure 4.17: Behaviour of ivermectin in the water/sediment systems

The rapid and extensive sorption of ivermectin onto the sediment should have been mainly attributable to its' lipophilicity (log P_{OW} = 3.22; Edwards et al., 2001, K_{OC} = 12,600-15,700; Halley et al., 1989a). Furthermore, additional specific interactions with soil such as a formation of adducts with cations in the sediment are likely (Ali et al., 2000, Cerny et al., 1994, Tolls, 2001).

An appreciable sorption of ivermectin onto sediment was also observed by Cannavan et al., 2000, who recovered a large portion of the ivermectin quantity applied as a feed additive for a marine fish farm in the top layers of the underlying sediment body.

Mainly as a result of the rapid sorption of ivermectin, its' $DT_{50/90}$-values in the water phase were very low, with 2.9 d and 32 d respectively. Ivermectin disappeared within 13 d by 50 % from the water/sediment system and the DT_{90} was estimated to be 144 d.

In contrast, the persistence of ivermectin, reported by Davies and Rodger, 2000 from experiments with marine sediments with $DT_{50} \geq 100$ d, was significantly higher. Strong differences in the DT_{50} of ivermectin under varying test conditions were also reported by Halley et al., 1989a, who found ivermectin half lives in soil of ≥ 93 d under laboratory conditions, whereas half lives under field conditions were about 1-2 weeks. Hence, the specific test conditions, such as temperature, are of high importance for the transformation kinetic.

Since the test was conducted with non-labelled ivermectin, possibly occurring TPs, the formation of non-extractable residues and mineralization products could not be quantified. In literature, an extensive metabolization of ivermectin and the formation of bound residues in soil were reported by Halley et al., 1989a. For abamectin, a closely related avermectin derivative the transformation in soil was studied in more detail by Bull et al., 1984. Using ^{3}H- and ^{14}C-labelled abamectin, he found the formation of at least 13 radioactive degradation products under *aerobic conditions*. Additional mineralization occurred at a level of less than 4 % of the IAR. The major soil degradation products were an 8α-hydroxyl-derivative and the corresponding opened ring aldehyde derivative of abamectin. In contrast to that, during a 3-month test period under *anaerobic conditions*, he found neither an apparent degradation of abamectin nor a formation of non-extractable residues. Hence, the formation of ivermectin TPs under the aerobic conditions in the water/sediment system is likely.

Summary: DT-values for ivermectin

Water phase:

Function and degradation rate:	Root first order	
DT-values:	DT_{50}: 2.9 d	DT_{90}: 10 d
Regression coefficient:	0.9300	Best fit available

Water/sediment system:

Function and degradation rate:	Root first order	
DT-values:	DT_{50}: 13 d	DT_{90}: > 144 d
Regression coefficient:	0.9502	Accepted (> 0.95)

4.2 Determination of K_d-values

The measured concentration levels in water and sediments were used for a calculation of K_{OC}- and K_d-values, determining the distribution pattern between water and sediment phase. For each of the 6 compounds with a complete data set for water and sediment, the maximum values are given in Table 4.13. These maximum values were usually determined after 30 d or later and should therefore be close to distribution equilibrium.

Additionally, K_{OC}-values were predicted using the correlation between the octanol/water distribution coefficient K_{OW} and the sorption coefficient K_{OC}. For that 3 semi-empirical methods were applied following the approaches used by *Stuer-Lauridsen et al., 2000* $K_d = 0.41 \times K_{OW}$, *Karickhoff, 1981* $\log K_{OC} = 0.989 \times \log K_{OW} - 0.346$ and Gerstl, 1990 $\log K_{OC} = 0.679 \log K_{OW} + 0.663$. Since clofibric acid was dissociated in the sediment the D_{OW} was used in the calculation instead of the K_{OW}.

The K_{OC}values of the polar compounds clofibric acid and CBZ-Diol were relatively low < 30 $L \cdot kg^{-1}$ and for carbamazepine, oxazepam and diazepam K_{OC}values obtained were between 83 and 192 $L \cdot kg^{-1}$ (Table 4.13). Ivermectin with the highest K_{OW} exhibited the highest K_{OC} with 1172 $L \cdot kg^{-1}$. All three model approaches attained a high conformity for the 6 compounds, within a factor of 3 which is comparable to typical deviations in K_{OC} reported for a given compound on differing sediments (Delle Site, 2001). Additionally, the experimental K_{OC}-values for clofibric acid and diazepam were very similar to K_{OC}-values measured in sewage sludge (Ternes et al., submitted) and for diazepam in soils (Kreuzig et al., 2003). However, the wide variation in the K_{OC} of carbamazepine are presumably caused by the yet not understood sorption mechanisms. Albeit the K_{OC} determined for ivermectin was relatively high, it has to be noted that Halley et al. reported K_{OC}-values for ivermectin in soils (Halley et al., 1989a) which were even an order of magnitude higher than those from the water sediment study. These deviations in the distribution coefficients might result from variations in the

matrix composition and its impact on sorption processes. Although the main purpose of experiments was not the determination of the distribution coefficients of the pharmaceuticals investigated, it must be noted that the K_{OC}-values obtained were in good agreement with estimated and experimental data previously reported.

Table 4.13: Experimental and predicted K_{OC}- and K_d-values for the sorption onto sediment

L·kg^{-1}	Experimental		Estimated (Lit. [a])		Estimated (Lit. [b])		Estimated (Lit. [c])		Experimental (Lit. [d,e,f])	
	K_{OC}	K_d	K_{OC}	K_d	K_{OC}	K_d	K_{OC}	K_d	K_{OC}	K_d
Clofibric acid	26	0.3	0	0	0	0	0.2	0	14[d]	0.1[d]
Diazepam	192	3.0	290	4.6	297	4.7	396	6.2	62[d] 500[e]	1.0[d] 4-7[e]
Oxazepam	152	2.2	71	1.0	74	1.1	153	2.2	-	
Carbamazepine	83	1.3	73	1.2	76	1.2	155	2.5	3.5[d]	0.1[d]
CBZ-DIOL	29	0.3	-		-		-		-	
Ivermectin	1172	11.7	680	67	690	68	707	69	15700	227

[a] Stuer-Lauridsen et al., 2000; [b] Karickhoff, 1981; [c] Gerstl, 1990; [d] in sewage sludge (Ternes et al., submitted); [e] in soil (Kreuzig et al., 2003); [e] in soil Halley et al., 1989a

4.3 Soil-leaching-Study

4.3.1 Principles and limitations of soil leaching studies

Soil leaching studies are usually required as part of the registration of new active substances in pesticide formulations. As already mentioned (see Chapter 4.1.1), the results of such studies are utilized for environmental risk assessment (ERA) purposes.

This approach was used to investigate pharmaceuticals which might pose a risk for groundwater contamination. This particular test was chosen, since for more realistic scenarios, e.g. covering the possible infiltration of these pharmaceuticals from river water through sediment and subsoil into the groundwater, no adequate methods are available. In fate studies, the test compounds are usually applied as ^{14}C-radiotracers in order to facilitate their quantification. Due to the lack of ^{14}C-labelled standards commercially available, for some test substances non-labelled pharmaceuticals were also utilized in the test systems. In that case it is often more difficult to study the distribution of the test substance in the soil column and to measure the drugs in the leachate, due to analytical reasons.

Since the fate of a chemical in the soil leaching test system depends on its physico-chemical properties as well as on the soil properties used, it is essential to cover a broad range of different soils (the OECD guideline requires at least three soils). However, due to practical limitations, only two different soils, *LUFA 2.2* and *Euro Soil 5* were used in the studies with non-labelled compounds. In the studies using ^{14}C-iopromide and ^{14}C-diazepam a third soil was tested additionally (*Neuenkirchen*). These soils were selected in order to cover a broad range of soil properties:

- *Euro Soil 5* (a soil of a German pine forest) is an acidic sandy soil with a high content of organic matter, representing forest sites of Northern Germany;
- *LUFA 2.2* is a standard sandy loam soil with a medium pH value and a medium content of organic matter, being common at many German crop sites;
- *Neuenkirchen* is also a common agricultural soil but with a low content of organic matter, also being used by the colleagues at the University of Braunschweig (Kreuzig 2002).

Due to the limitations of the experimental set-up the soil leaching studies presented in this report should be regarded as a screening approach. Neither the usage of disturbed soil nor the size of the soil columns nor the duration of the test is directly relevant for field situations. As a result of this simplified laboratory approach the observed variability of the results between the two columns (= replicates) per treatment were usually very low. Therefore, the test system, originally developed for the investigation of pesticides, can be considered as suitable as a first approach to study the leaching potential of pharmaceuticals.

4.3.2 Carbamazepine

In all tests using non-labelled carbamazepine the leachate volumes were in the required range of 92–104 % recovery of the applied artificial rain (OECD, 2003a). Carbamazepine was retained in both soil substrates (Table 4.14). It was not detected in the leachates of *LUFA soil 2.2* and *Euro-Soil 5* and can therefore be classified as non-mobile.

Table 4.14: Leaching of carbamazepine (CAR) in LUFA 2.2 and EuroSoil 5. The recovery in the leachate is given in % of the spiked test substance for each soil column (A and B)

	LUFA 2.2		*EuroSoil 5*	
	CAR_A	CAR_B	CAR_A	CAR_B
Volume leachate [Ml]	368.8	390.6	361.2	366.1
Concentration [ng/leachate]	n.d.	n.d.	n.d.	n.d.
Recovery in leachate [%]	< 1 %	< 1 %	< 1 %	< 1 %

n.d. = not detectable

The mobility of carbamazepine (log K_{OW}=3.5; Jones et al., 2002) did not differ in the two soils investigated. Obviously, the retention of carbamazepine was not decisively affected by the distinctly differing properties of both soils (in particular C_{org} and pH). The latter should be due to the fact that carbamazepine was always present in its neutral form, given pK_a values of 13.9 Jones et al., 2002 for the deprotonation and approximately < 1 for the protonation. However, carbamazepine was frequently detected in groundwater (Heberer, 2002a, Sacher et al., 2001, Ternes, 2001a, Ternes, 2000). This might be explained by the fact that groundwater

contamination occurs mainly over river sediments and subsoil from receiving waters (Mersmann et al., 2002). Drewes et al., 2002 observed no attenuation of carbamazepine in subsoil during bank filtration. Therefore, it might be assumed that the water volume applied was too low for a comparable leaching of carbamazepine. It is also conceivable that the usage of topsoil instead of subsoil resulted in effects as increasing sorption and/or biodegradation, although the latter is less likely. In order to elucidate these assumptions, it would be helpful to repeat the experiments with radio-labelled carbamazepine, which was not available in the study performed, including a chemical analysis of the radioactivity in soils and leachates. Since topsoil and subsoil may differ significantly in C_{org}, bacterial community, and further properties, the results from the current leaching tests performed with top soil have only a limited transferability for subsoil or aquifer scenarios.

4.3.3 Clofibric acid

The leachate volumes from three of four soil columns used in the tests with non-labelled clofibric acid were in the required range of 92–104 % recovery of the applied artificial rain (OECD, 2003a). The leachate volume from one *LUFA 2.2* column (CLO_A) was slightly above this range (106.2%). This deviation from the required quality criteria should have no influence on the overall assessment of the leaching potential of clofibric acid. 60.5 % of the clofibric acid applied to the test substrate was detected in the *LUFA 2.2* leachate (mean of the two columns), while only 3.5 % (mean of the two columns) was detected in the leachate of *EuroSoil 5* (Table 4.15). Based on these results clofibric acid could be assumed as highly mobile in the *LUFA 2.2* and as only slightly mobile in *EuroSoil 5*.

Table 4.15: Leaching of clofibric acid (CLO) in LUFA 2.2 and EuroSoil 5. The recovery in the leachate is given in % of the spiked test substance for each soil column (A and B)

	LUFA 2.2		*EuroSoil 5*	
	CLO_A	CLO_B	CLO_A	CLO_B
Volume leachate [mL]	417.2	387.8	369.0	372.8
Concentration [ng/leachate]	34080	34460	1877	2651
Recovery in leachate [%]	69	52	3	4

The sorption behaviour and thus, the mobility of organic acids (e.g. salicylic acid) in soils is strongly pH dependent (Dubus et al., 2001). Organic acids are widely present in their neutral, undissociated form at pH conditions below their pK_a-value (Schwarzenbach et al., 2003). This results in a distinctly higher lipophilicity and usually in a higher tendency to sorb onto organic soil matter for the undissociated acids compared with their more polar dissociated form.

It can be assumed that the relatively low pH of 2.9 in the *EuroSoil 5* led to an appreciable higher retention of clofibric acid (estimated pKa of 2.84, log P_{OW} = 2.57 Hansch et al., 1990), compared to the *LUFA 2.2* soil, due to enhanced sorption of the undissociated acid onto soil particles. In *LUFA 2.2* soil with a more common pH of 5.8 a significantly higher mobility of clofibric acid was observed. This is in good agreement with findings of Scheytt et al., 2001 and Heberer, 2002a, who reported an almost tracer-like movement of clofibric acid in soil columns and during riverbank filtration.

Although a transformation of clofibric acid in the leaching experiment cannot entirely be excluded, it is very unlikely since clofibric acid remained widely persistent in the water/sediment test and during wastewater treatment, riverbank filtration and under other environmental conditions as reported by various authors (Heberer et al., 2002, Stumpf et al., 1999, Weigel et al., 2002, Winkler et al., 2001). It can be concluded that groundwater contamination due to the infiltration of clofibric acid through topsoil is likely.

4.3.4 Diazepam

Non-labelled

In all experiments using non-labelled diazepam the leachate volumes were in the required range of 92–104 % recovery of the applied artificial rain (OECD, 2003a). The non-labelled diazepam was retained in both soil substrates (Table 4.16). It was not detected in the leachates of *LUFA soil 2.2* and *Euro-Soil 5* and can therefore be classified as non-mobile.

Table 4.16: Leaching of non-labelled diazepam (DIA) in LUFA 2.2 and EuroSoil 5. The recovery in the leachate is given in % of the spiked test substance for each soil column (A and B)

	LUFA 2.2		*EuroSoil 5*	
	DIA_A	DIA_B	DIA_A	DIA_B
Volume leachate [mL]	385.0	382.6	381.7	391.8
Concentration [ng/leachate]	n.d.	n.d.	n.d.	n.d.
Recovery in leachate [%]	<< 1 %	<< 1 %	<< 1 %	<< 1 %

n.d. = not detectable

^{14}C-Diazepam

In the tests using ^{14}C-diazepam the leachate volumes from the *LUFA 2.2* and *EuroSoil 5* columns were in the required range of 92–104 % recovery of the applied artificial rain (OECD, 2003a). The leachate volumes from the *Neuenkirchen* soil columns were slightly above this range (108 %). This deviation from the required quality criteria should have no influence on the comparability of the tests and the overall assessment of the leaching potential of ^{14}C-diazepam. Comparable to the studies using non-labelled diazepam no radioactivity was detected in the leachates of the three tested soils (*EuroSoil 5*, *LUFA 2.2, Neuenkirchen*). In *EuroSoil 5* ^{14}C-diazepam was only measured in the uppermost soil layer (0 – 5 cm), whereas in *Neuenkirchen* soil and *LUFA-Soil 2.2* it was found up to a soil depth of 15 respectively 20 cm (Figure 4.18 - Figure 4.20). Beyond these depths only negligible amounts of radioactivity (< 0.5 %) were detected. Therefore, it can be concluded that ^{14}C-diazepam is totally immobile in *EuroSoil 5* and partly immobile in *LUFA-Soil 2.2* and *Neuenkirchen* soil.

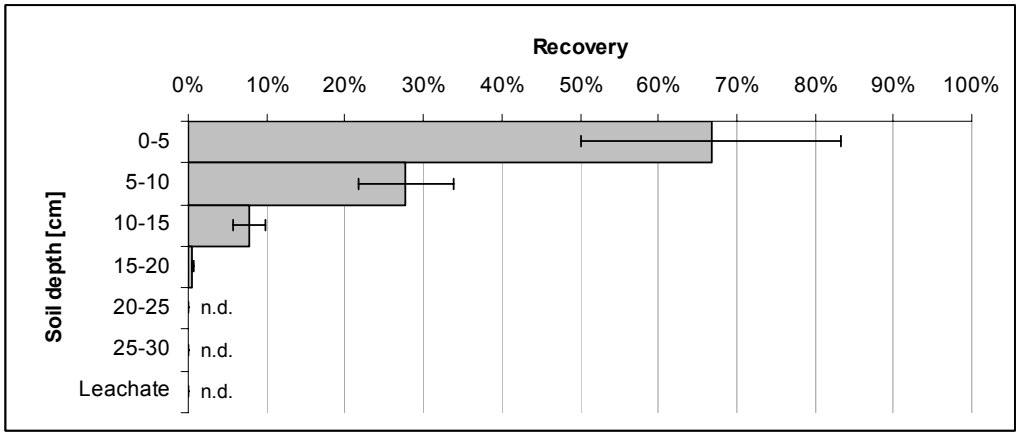


Figure 4.18: Leaching of ^{14}C-diazepam in LUFA 2.2. Recovery rates in % of applied test substance in soil and leachates (means of measured soil aliquots of both columns (n=8); error bars: SD; n.d.: recovery < 0.5 %)

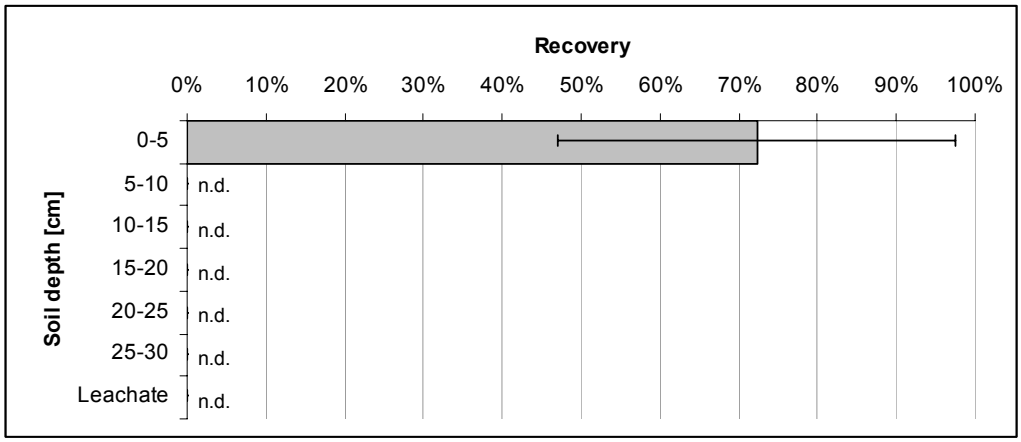


Figure 4.19: Leaching of ^{14}C-diazepam in EuroSoil 5. Recovery rates in % of applied test substance in soil and leachates (means of measured soil aliquots of both columns (n=8); error bars: SD; n.d.: recovery < 0.5 %)

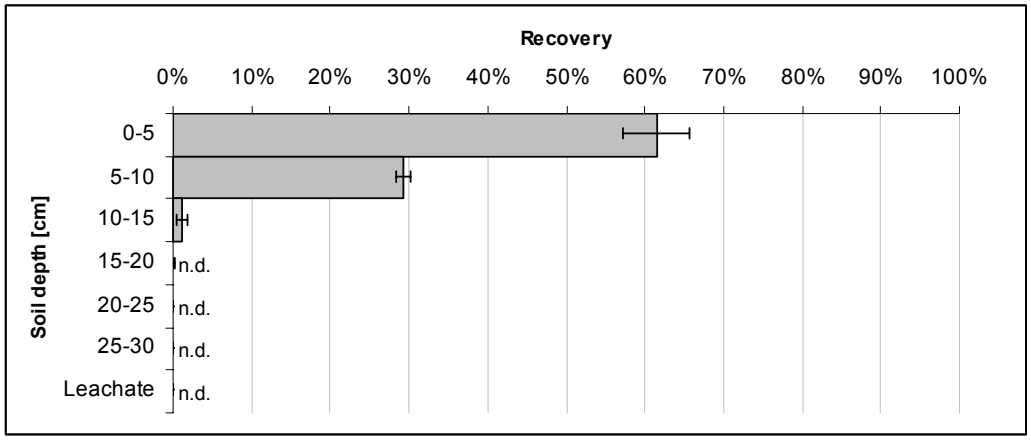


Figure 4.20: Leaching of ^{14}C-diazepam in Neuenkirchen soil. Recovery rates in % of applied test substance in soil and leachates (means of measured soil aliquots of both columns (n=8); error bars: SD; n.d.: recovery < 0.5 %)

The relatively high standard deviations of soil aliquots measured (~ 1 g per aliquot), especially in *EuroSoil 5* (Table 4.17) indicate that the radioactive compounds were very inhomogeneously distributed in the different soil layers. This phenomenon could be explained by the low mobility of ^{14}C-diazepam and may also cause the low total recovery rate in the *Euro-Soil 5* soil columns (mean 77.5 %). According to the OECD draft guideline the recovery should range from 90–110 % for labelled substances. This deviation from the required quality criteria should have no influence on the overall assessment of the leaching potential of diazepam.

Table 4.17: Leaching of ^{14}C-diazepam (^{14}DIA) in LUFA 2.2, EuroSoil 5 and Neuenkirchen soil. Recovery in % of the spiked test substance for each soil column (A and B)

	LUFA 2.2		*EuroSoil 5*		*Neuenkirchen*	
	^{14}DIA_A	^{14}DIA_B	^{14}DIA_A	^{14}DIA_B	^{14}DIA_A	^{14}DIA_B
recovery in soil [%]	97	108	67	88	98	86
recovery in leachate [%]	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
total recovery [%]	97	108	67	88	98	86

n.d.: recovery < 0.5 %

Diazepam is a lipophilic substance with a log K_{OW} of 2.82 and a pKa of 3.3 (Stuer-Lauridsen et al., 2000) and showed a very low mobility in all soils. Even in the relatively acidic *EuroSoil 5* a significantly increased mobility due to a protonated amino moiety was not observed. It can be expected that its' leaching behaviour was mainly determined by the organic carbon content of the soils. An extensive transformation of diazepam in the soil is unlikely, since diazepam was widely stable in the water/sediment test under aerobic conditions and transformation products might have shown certain mobility in the soil due their increased polarity. Therefore, the leaching potential for diazepam is estimated as very low.

4.3.5 Ibuprofen

In all tests using non-labelled ibuprofen the leachate volumes were in the required range of 92–104 % recovery of the applied artificial rain (OECD, 2003a). Ibuprofen was retained in both soil substrates. It was not detected in the leachates of *LUFA soil 2.2* and *EuroSoil 5* and therefore can be classified as non-mobile (Table 4.18).

Table 4.18: Leaching of ibuprofen (IBU) in LUFA 2.2 and EuroSoil 5. The recovery in the leachate is given in % of the spiked test substance for each soil column (A and B)

	LUFA 2.2		*EuroSoil 5*	
	IBU_A	IBU_B	IBU_A	IBU_B
Volume leachate [mL]	371.5	386.1	378.2	378.7
Concentration [ng/leachate]	n.d.	n.d.	n.d.	n.d.
Recovery in leachate [%]	< 1 %	< 1 %	< 1 %	< 1 %

n.d. = not detectable

The weak organic acid ibuprofen contains a carboxylic moiety with a pKa of 4.9 (Merck, 2001) and a distinct lipophilicity in its' undissociated form (log K_{OW} of 3.5; Jones et al., 2002, Stuer-Lauridsen et al., 2000). In both tested soils with a pH of 5.8 (*LUFA-Soil 2.2*) and 2.9 (*EuroSoil 5*), the carboxyl group of ibuprofen should be at least partly protonated leading to a sorption onto soil particles. Furthermore, it is known that ibuprofen can be transformed easily in sediments (see chapter 4.1.3) and under other environmental conditions (Ternes, 1998, Winkler et al., 2001). Since ibuprofen was not found in the soil leachates, it can be expected that the soil passage means an effective barrier for ibuprofen, due to sorption and/or by microbial degradation. However, the leaching potential of ibuprofen in basic soils with a pH clearly above its dissociation constant needs to be further investigated.

4.3.6 Ivermectin

In all tests using non-labelled ivermectin the leachate volumes were in the required range of 92–104 % recovery of the applied artificial rain (OECD, 2003a). Ivermectin was retained in both soil substrates (Table 4.19). It was not detected in the leachates of *LUFA soil 2.2* and *Euro-Soil 5* and can therefore be classified as non-mobile.

Table 4.19: Leaching of ivermectin (IV) in LUFA 2.2 and EuroSoil 5. The recovery in the leachate is given in % of the spiked test substance for each soil column (A and B)

	LUFA 2.2		*EuroSoil 5*	
	IV_A	IV_B	IV_A	IV_B
Volume leachate [mL]	376.2	375.3	379.9	367.5
Concentration [ng/leachate]	n.d.	n.d.	n.d.	n.d.
Recovery in leachate [%]	< 1 %	< 1 %	< 1 %	< 1 %

n.d. = not detectable

Concluding from the experimental data, ivermectin shows no leaching potential in soil columns. Comparable data for ivermectin were reported by Halley et al., 1989b, and for the ivermectin derivative abamectin by Gruber et al., 1990 and by Bull et al., 1984. The immobility of the test compound in the soils tested and its tendency to sorb onto soil particles should at least partly deviate from its high lipophilicity (log K_{OW} = 3.22, K_{OC} = 12,660-15,700 Halley et al., 1989a).

However, Tolls could not assign the soil affinity of avermectins exclusively to their lipophilicity (Tolls, 2001). LC-MS data shows that ivermectin is capable to form adducts with cations, such as ammonium or sodium (Ali et al., 2000). Hence, there is some reason for the presumption of a specific binding of ivermectin to soil, which might deviate from the formation of adducts or complexes with immobile inorganic soil matter.

4.4 A special case: Iopromide and its TPs

4.4.1 Water/sediment study

Iopromide is a very polar, highly water soluble compound (770 $g \cdot L^{-1}$) (Steger-Hartmann et al., 1999) and therefore, the radioactivity initially related to ^{14}C-iopromide remained mainly in the water compartment during the test (Figure 4.21). Even after 100 d more than 65 % of the IAR were still located in the water phase. The quantity of radioactivity in the sediment increased almost linearly to 21 ± 1 % at the end of the experiment. Only negligible amounts of radioactivity were found in the CO_2 traps, and therefore relevant mineralization did not occur. The balance of radioactivity showed a

small deficit, which increased in the course of the experiment but never exceeded 11 ± 5 %.

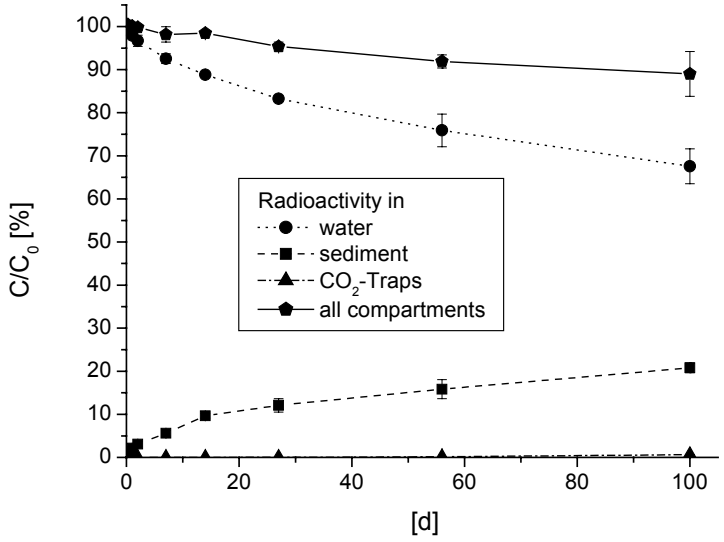


Figure 4.21: Distribution of radioactivity in the water/sediment system spiked with ^{14}C-iopromide

The presence of ^{14}C-iopromide equivalents in the sediment should mainly be attributable to its' occurrence in the sediment pore-water, which contributed about 13 % to the total water amount in the test system. With regard to its' low lipophilicity (log P_{OW} = -2.33) and missing moieties for known specific interactions (Steger-Hartmann et al., 1999), iopromide should have been sorbed onto the sediment particles only to a minor extent.

The radioactivity in the water phase was analyzed by radio-TLC, whereas the sediment was not further investigated. After a lag period of two weeks, ^{14}C-iopromide was transformed, resulting in total transformation after 100 d following a first order kinetic (Figure 4.22).

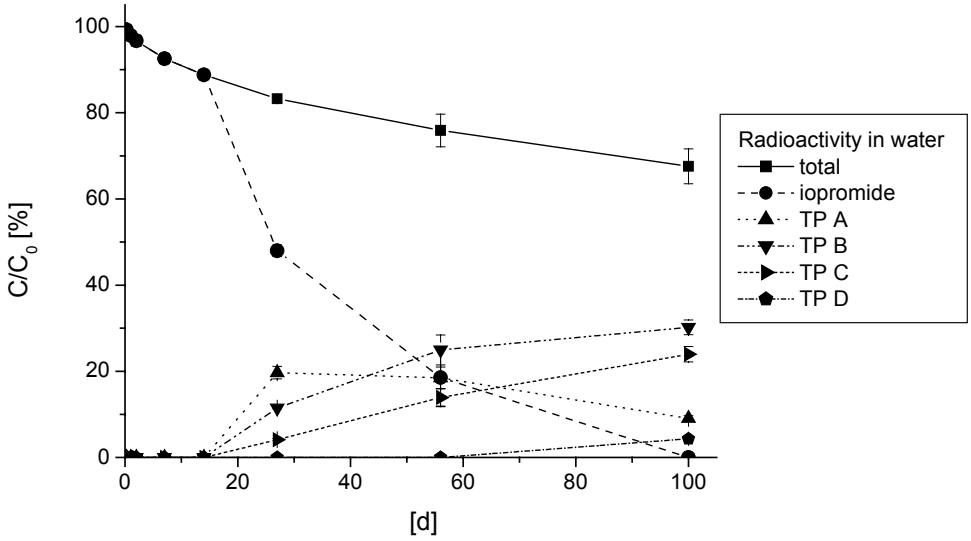


Figure 4.22: Fate of ^{14}C-iopromide in the water phase of the water/sediment system

TP A occurred with a maximum of 20 ± 1 % at day 28 and was object to further degradation. At least three other TPs were formed and occurred in increasing percentages. After 100 d the TPs A, B and C occurred with 9 ± 1, 30 ± 2 and 24 ± 2 %, respectively and TP D appeared with 4.3 ± 0.1 %. The deviations within the individual test vessels were very low, which shows the widely similar development of the independent test vessels.

For TP A and ATI, similar R_f-values were observed, while TP B matched very well with the R_f-value of ATH (shown in Table 4.20, for chemical structures see Table 2.3). A formation of the iopromide TP DAMI, as found by Steger-Hartmann (Steger-Hartmann et al., 1998, Steger-Hartmann et al., 2001) in laboratory-STP's can be excluded. Kalsch, 1992 also observed the formation of at least 3 different iopromide TPs in water/sediment systems and a laboratory STP. He found a similar pattern of R_f-values for the unknown TPs formed, using the same method for TLC- analysis.

Table 4.20: R_f-values for iopromide and TPs of iopromide

Compound	R_f-value
TP A	0.21
ATI	0.23
TP B	0.33
ATH	0.33
IO	0.39
DAMI	0.47
TP C	0.74
TP D	0.89

For iopromide in the water phase, a DT_{50} of 30 d and a DT_{90} of 99 d was calculated. Iopromide is amendable towards microbial degradation (Steger-Hartmann et al., 1998, Steger-Hartmann et al., 1999, Steger-Hartmann et al., 2001). In a somewhat similar study with iopromide, TPs formed were relatively stable, an appreciable mineralization did not occur. Kalsch reported also the degradation of iopromide in water/sediment systems, following a first order kinetic (Kalsch, 1992, Kalsch, 1999). The absence of a lag period in Kalsch's experiments might have been caused by the river water used, which is known to contain iopromide residues (Ternes and Hirsch, 2000). Iopromide can be effectively removed during bank filtration (Putschew et al., 2000) and occurs in groundwater only at the low $ng \cdot L^{-1}$-level (Putschew et al., 2000, Sacher et al., 2001, Ternes and Hirsch, 2000).

Summary: DT-values for iopromide

Water phase:

Function and degradation rate:	First order	d = 0.0233 per day
DT-values:	DT_{50}: 30 d	DT_{90}: 99 d
Regression coefficient:	0.9909	Accepted (> 0.95)

Water/sediment system:

Kinetics and degradation rate:	Not possible to determine
DT-values:	Not possible to determine (< 100 d)
Remark:	Complete transformation in the water phase

4.4.2 Soil-Leaching Study

4.4.2.1 Non-labelled iopromide

In all tests using non-labelled iopromide the leachate volumes were in the required range of 92–104 % recovery of the applied artificial rain (OECD, 2003a). Non-labelled iopromide was retained in *LUFA soil 2.2*. It was not detected in the leachates of *LUFA soil 2.2,* while 37.5 % (mean of the two columns) of the test substance applied was detected in the leachate of *EuroSoil 5* (Table 4.21).

Table 4.21: Leaching of non-labelled iopromide (IO) in LUFA 2.2 and EuroSoil 5. The recovery in the leachate is given in % of the spiked test substance for each soil column (A and B)

	LUFA 2.2		*EuroSoil 5*	
	IO_A	IO_B	IO_A	IO_B
Volume leachate [mL]	389.9	398.4	406.7	401.4
Concentration [ng/leachate]	n.d.	n.d.	20793	30112
Recovery in leachate [%]	0	0	30.6	44.3

n.d. = not detectable

4.4.2.2 Radio-labelled iopromide

In the tests using ^{14}C-iopromide the leachate volumes from the *LUFA 2.2* and *EuroSoil 5* columns were in the required range of 92–104 % recovery of the applied artificial rain (OECD, 2003a). The leachate volumes from the *Neuenkirchen* soil columns were slightly above this range (108 %). This deviation from the required quality criteria should have no influence on the comparability of the tests and the overall assessment of the leaching potential of ^{14}C-iopromide. High percentages of the IAR were found in the leachates of *EuroSoil 5* (59.0 %), *LUFA 2.2* (78.5 %) and *Neuenkirchen* soil (46.5 %) (Table 4.22). The mobility of ^{14}C-iopromide was slightly higher in *LUFA 2.2* soil than in *Euro-Soil 5* and *Neuenkirchen* soil (Figure 4.23 - Figure 4.25). Based on these data it can be presumed that the mobility of ^{14}C-iopromide in the three soils tested is high.

Table 4.22: Leaching of ^{14}C-iopromide (^{14}C-IO) in LUFA 2.2, EuroSoil 5 and Neuenkirchen soil. Recovery in % of the spiked test substance for each soil column (A and B)

	LUFA 2.2		*EuroSoil 5*		*Neuenkirchen*	
	^{14}C-IO_A	^{14}C-IO_B	^{14}C-IO_A	^{14}C-IO_B	^{14}C-IO_A	^{14}C-IO_B
Recovery in soil [%]	21	19	43	50	52	57
Recovery in leachate [%]	78	79	62	56	50	43
Total recovery [%]	99	98	105	106	102	100

n.d.: recovery < 0.5 %

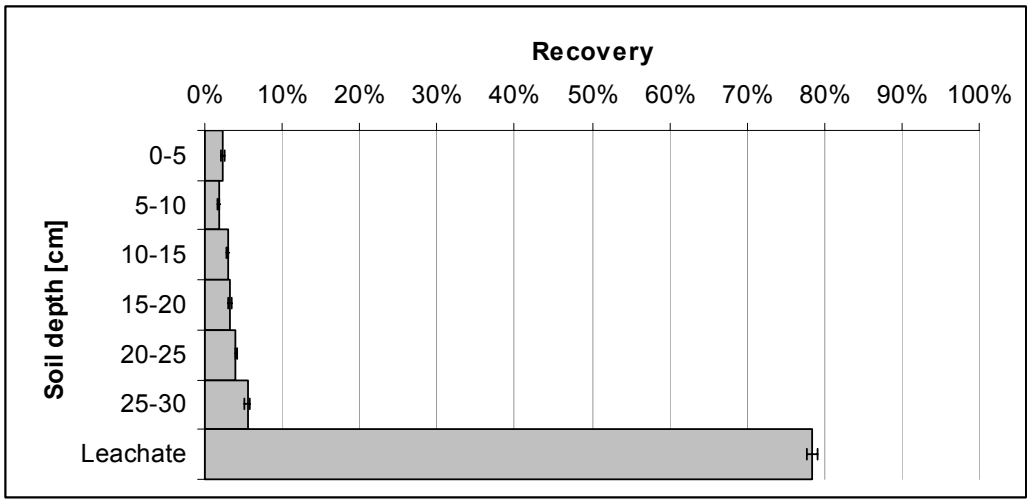


Figure 4.23: Leaching of ^{14}C-iopromide in LUFA 2.2. Recovery rates in % of applied test substance in soil and leachates (means of measured soil aliquots of both columns (n=8); error bars: SD; n.d.: recovery < 0.5 %)

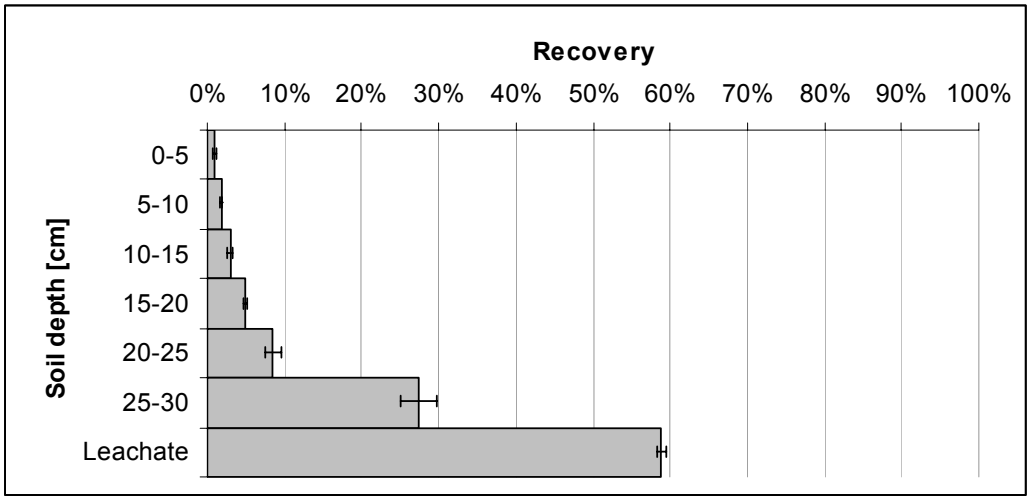


Figure 4.24: Leaching of ^{14}C-iopromide in EuroSoil 5. Recovery rates in % of applied test substance in soil and leachates (means of measured soil aliquots of both columns (n=8); error bars: SD; n.d.: recovery < 0.5 %)

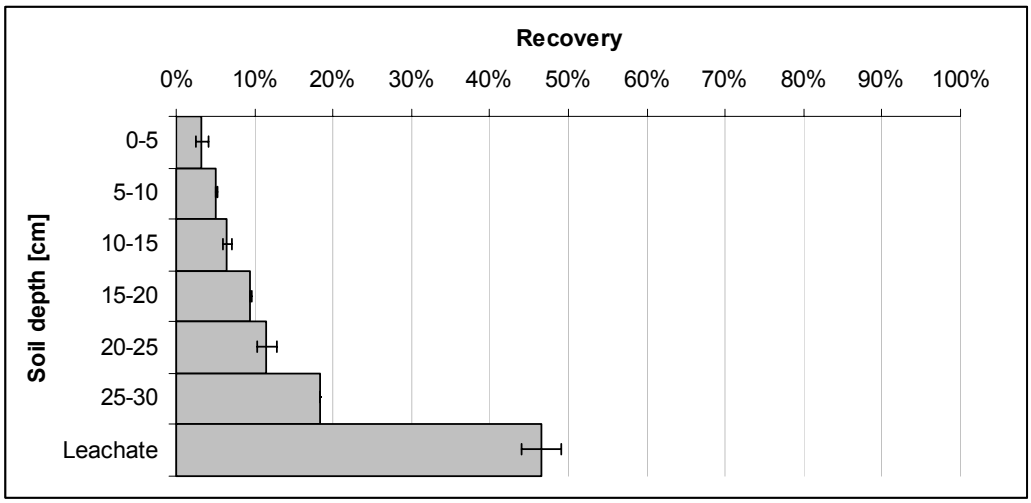


Figure 4.25: Leaching of ^{14}C-iopromide in Neuenkirchen soil. Recovery rates in % of applied test substance in soil and leachates (means of measured soil aliquots of both columns (n=8); error bars: SD; n.d.: recovery < 0.5 %)

Iopromide is a polar compound (log K_{OW} of -2.33) and a high water solubility (Steger-Hartmann et al., 1999). Its tendency for a binding to soil particles can be expected to be very low, since specific interaction with soil particles are unknown for iopromide. Due to its high polarity, iopromide leaches rapidly through the soil, nearly unimpeded by interactions with soil particles.

In experiments with ^{14}C-labelled iopromide, more than 50 % of the radioactivity applied was found in the leachates of both soils. ^{14}C-iopromide was retained slightly more by the *EuroSoil 5* and the *Neuenkirchen soil* than by the *LUFA 2.2*. This could be explained by the coarser soil texture and therefore the faster leaching of the water in the *LUFA 2.2* soil columns.

However, in the study with non-labelled test substance a relative high percentage of the applied amount (38.6 %) was leached when using *EuroSoil 5*, while with *LUFA 2.2* no iopromide could be detected in the leachate with LC tandem MS. To verify this significantly different behaviour of ^{14}C-iopromide and non-labelled iopromide in both soils further experiments were conducted. In a second study using non-labelled iopromide the results of the first experiment were confirmed. The major difference in the performance of the tests using labelled and non-labelled iopromide was the mode of application. ^{14}C-iopromide was kindly provided by the Schering AG and stored in the laboratories of ECT dissolved in ethanol. This ethanol-solution was diluted with water

and the resulting solutions were applied to the soil columns, whereas the non-labelled test substance was applied dissolved in water only. To exclude the possibility that traces of the organic solvent influenced the mobility of the ^{14}C-iopromide in a second experiment the radioactive compound were applied to *LUFA 2.2* soil columns after the ethanol was evaporated in a nitrogen stream and subsequently the ^{14}C-iopromide was dissolved in water. At the end of the test 71.4% (mean of two columns) of the spiked radioactivity was found in the leachates. These results indicate that traces of ethanol in the application solution did not increase significantly the mobility of iopromide.

In order to clarify these virtually contrary results, leachate samples of the experiments with ^{14}C-labelled iopromide were analysed by radio TLC, applying several potential environmental transformation products (TPs) as reference compounds. TLC analysis showed that iopromide was completely transformed in *LUFA 2.2* soil, under formation of at least three TPs at percentages between 14 ± 3% and 63 ± 2% (Table 4.23).

Table 4.23: Composition of radioactivity in leachates of the soils Lufa 2.2 and EuroSoil 5 in % (2 replicates; error = mean absolute deviation)

Compound	R_f-value	*Lufa 2.2*	*EuroSoil 5*
TP A’	0.20	63 ± 2	15 ± 9
ATI	0.22	-	-
TP B`	0.31	23 ± 1	-
ATH	0.32	-	-
IO	0.37	-	85 ± 9
DAMI	0.47	-	-
TP C`	0.70	14 ± 3	-
Total	-	100	100

The R_f-values of the TPs A´ and B´ almost matched with those of the reference compounds ATI and ATH, respectively. In order to determine whether ATI or ATH were formed as TPs, additional LC-tandem MS experiments were conducted using soil leachates from the experiments with non-labelled iopromide. Although parameters for the detection of ATI in MRM experiments were optimized, the sensitivity for ATI remained low. The MRM experiments for ATH were conducted as described by Ternes and Hirsch, 2000.

Leachate samples spiked with 2 µg ATI showed the sharp ATI peak (Figure 4.26) with a retention time of 3.3 min, which was not observed in non-spiked samples. Additionally, a broad TP peak was observed for the MRM-transition of ATI which had a retention time of 4.1 min. The TP peak was also observed in non-spiked samples. Therefore, it is obvious that the TP in the soil leachates was not ATI, but showed a strong cross-selectivity to ATI.

However, it cannot be entirely excluded that ATI was present in the leachate, due to the poor sensitivity in the measurement for this compound. The iopromide derivative ATH which was neither found by Ternes and Hirsch, 2000 nor by Putschew et al., 2001 in various environmental waters, was not observed in the leachates, nor was the iopromide TP DAMI (Steger-Hartmann et al., 2001).

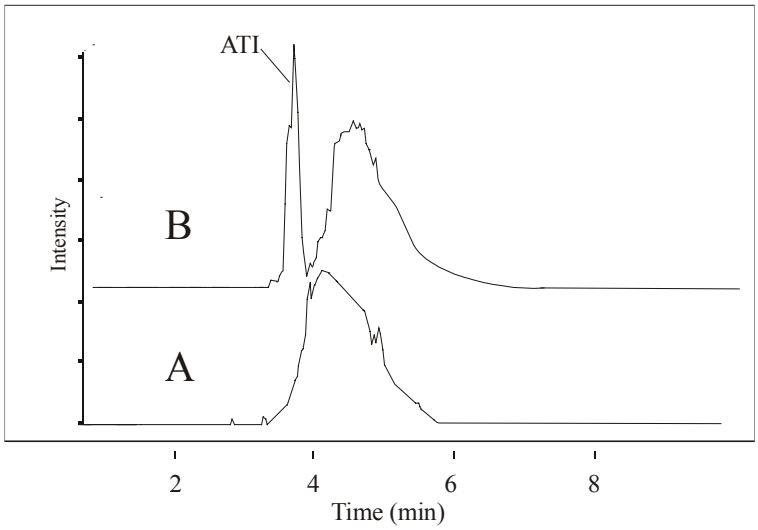


Figure 4.26: Chromatograms showing the MRM-transition of ATI for soil leachates non-spiked (A) and spiked (B) with ATI

During passage of *EuroSoil 5,* ^{14}C-iopromide was transformed only to a minor extent, while 85 ± 9 % of the iopromide remained unchanged (Table 4.23). The TP formed had the same R_f-value as TP A , which was presumably formed in both soils. Additional experiments on the identification of the unknown iopromide TPs were not conducted.

Finally, the results of the experiments with ^{14}C-labelled and non-labelled iopromide were linked to investigate the comparability of the experiments. In both experiments with soil *LUFA 2.2* iopromide was never found in the leachates neither labelled nor non-labelled (Table 4.24), indicating that iopromide was entirely transformed during passage of *LUFA 2.2* soil. In *EuroSoil 5* a partial transformation occurred, resulting in 50 ± 1 % and 38 ± 7 % of the initially applied quantity of ^{14}C-iopromide and non-labelled iopromide, respectively, in the leachates. This shows that both analytical methods led reproducibly to the same results.

Table 4.24: Percentage of initially applied ^{14}C-labelled and non-labelled iopromide recovered unchanged in soil leachates (2 replicates; error = mean absolute deviation)

	LUFA 2.2	*EuroSoil 5*
^{14}C-labelled iopromide	0 %	50 ± 1 %
non-labelled iopromide	0 %	38 ± 7 %

It might be assumed that the varying mobility of the iopromide equivalents in the three soils was mainly a result of the differing extent of transformation.

Hence, the at least partial transformation of iopromide during soil passage gives some evidence, that the removal of iopromide during riverbank filtration is caused by degradation (Putschew et al., 2000). It can be concluded that the introduction of iopromide onto soils means a certain risk for groundwater contamination, since in any case a groundwater contamination with iopromide or its TPs is very likely.

Comparison of iopromide transformation in water/sediment systems and soil columns

The iopromide TPs formed in both test systems displayed almost identical R_f-values (Table 4.25). This might indicate that the TPs A, B, C formed in the water/sediment tests, respectively the TPs A´, B´, C´ of the soil leaching experiments, were identical. Therefore, it can be presumed, that these TPs are frequently formed under a wide range of environmental conditions.

Table 4.25: R_f-values for iopromide and corresponding TPs

Water/sediment test		Leaching test	
Compound	R_f-value	Compound	R_f-value
TP A	0.21	TP A´	0.20
ATI	0.23	ATI	0.22
TP B	0.33	TP B´	0.31
ATH	0.33	ATH	0.32
IO	0.39	IO	0.37
DAMI	0.47	DAMI	0.47
TP C	0.74	TP C´	0.70
TP D	0.89	-	-

The transformation velocity in LUFA 2.2 soil 5 was high in comparison to the transformation of iopromide in the water/sediment system. In the latter experiment TPs appeared not until 28 d and a total transformation was observed after 100 d. It is conceivable that iopromide was transformed in all cases following mainly the same transformation, but with different kinetics. This might give reason for a detailed elucidation of the environmental fate of iopromide in the future.

5 ENVIRONMENTAL RISK ASSESSMENT

5.1 General principle

Pharmaceuticals are potentially harmful environmental contaminants as they are biologically active and often have a low biodegradability (Christensen, 1998). Since 1993, an environmental risk assessment (ERA) for new drugs has been implemented by the European Union, 1993. For the registration of veterinary pharmaceuticals an environmental assessment became obligatory in 1997 (EU, 1997) and VICH guidance paper are available (European Union, 2002, European Union, 2004). A corresponding guideline for human pharmaceuticals is still under discussion (2001, European Union, 2002, Rönnefahrt and Koschorrek, 2002, Straub, 2002). However, an environmental risk assessment for pharmaceuticals follows basically the same rules which have been established for other chemicals (Römbke et al., 2001).

The whole ERA is an iterative, tiered process in that the exposure and effect analysis can be repeated twice with increasing complexity. In general, the ERA consists of the steps shown in Figure 5.1.

For the exposure and effect analysis a number of standardized tests have been published (e.g. by the OECD, 2003b), allowing for a comparable assessment of fate and ecotoxicological effects. In recent years several reports have been published on the effects and the environmental risk of pharmaceuticals in the aquatic and, less often, terrestrial environment (Christensen, 1998, Davies et al., 1998, Henschel et al., 1997, Jones et al., 2002, Pascoe et al., 2003, Schulman et al., 2002, Steger-Hartmann et al., 2001, Stuer-Lauridsen et al., 2000, Van Wezel and Jager, 2002, Wollenberger et al., 2000). However, in comparison to other chemicals (in particular pesticides) still little data is available on the fate and effects of pharmaceuticals in the environment (Golet, 2002, Tixier et al., 2003).

Within the last ten years, several concepts for an environmental risk assessment (ERA) of pharmaceuticals have been proposed (e.g. Römbke et al., 2001, Stuer-Lauridsen et al., 2000). Due to the lack of data in comparison to other chemicals, in particular pesticides, the performance of an ERA is difficult for pharmaceuticals.

Therefore, only few examples of ERAs for this group of chemicals in the compartments sediment, soil or groundwater have been published so far, but the number is increasing recently (e.g. Boxall et al., 2000, Ferrari et al., 2003, Halling-Sorensen et al., 2000, Steger-Hartmann et al., 1999, Webb, 2001).

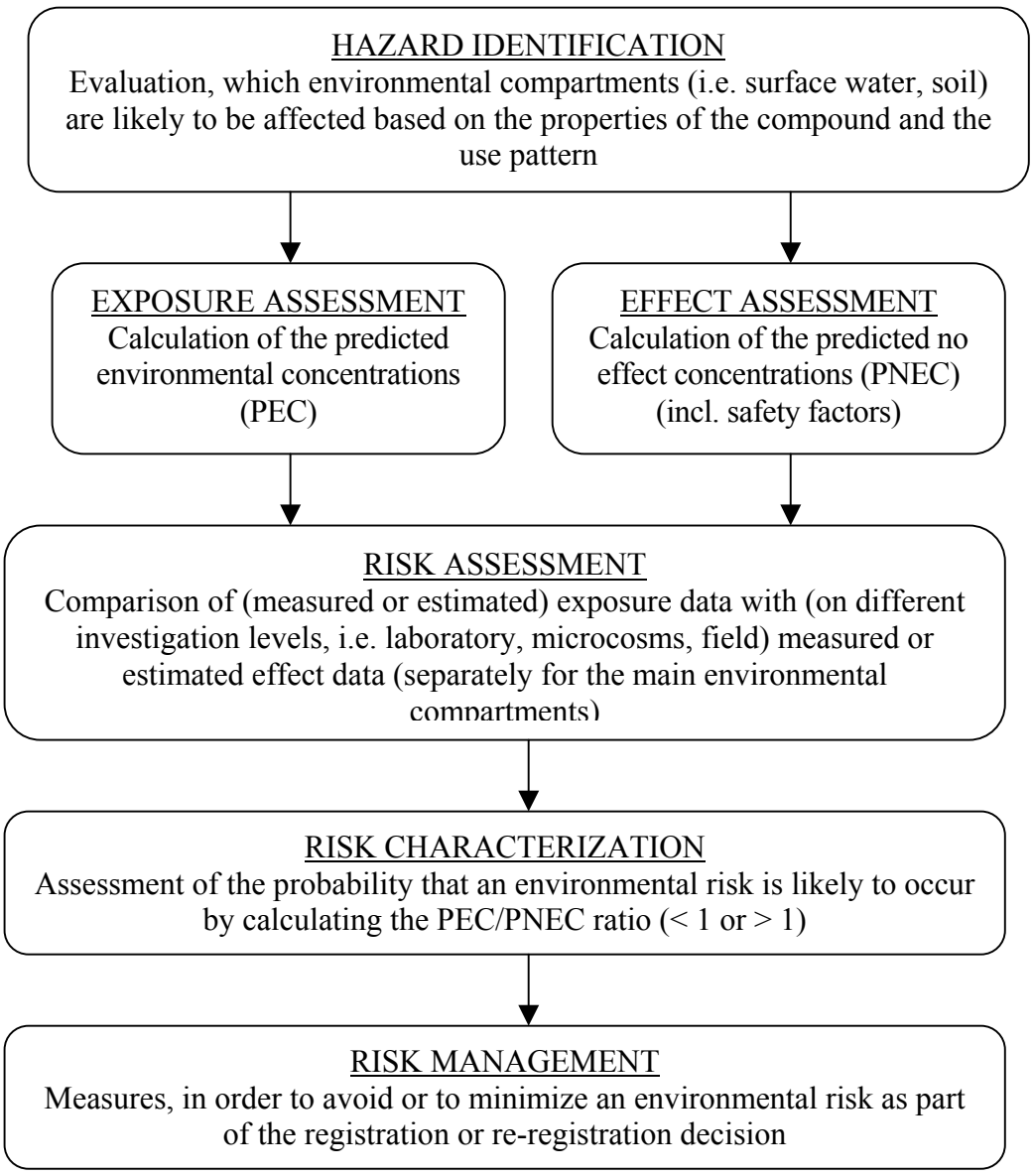


Figure 5.1: General principles of an environmental risk assessment process, adapted from Römbke et al., 2001

In the following, the pharmaceuticals tested in this project will be evaluated concerning their fate in the aquatic environment (surface water and sediment) as well as regarding their potential impact on the groundwater.

5.2 Degradation and metabolization in water/sediment systems

5.2.1 Assessment approach

The results of water/sediment tests, performed as part of the registration of pesticides, are not assessed on their own (i.e. no „Cut-off"-values exists, meaning that no DT-value has been defined by the regulatory authorities which, if exceeded, prevents the registration of a pesticide). Instead, the data is used for the calculation of PECs (Predicted Environmental Concentrations), which are then used for a PEC/PNEC comparison or as a trigger for further effect testing (EU, 1991). Since such a comparison was not possible in this project due to the lack of effect data, another approach based purely on the results of fate tests was chosen.

The characterization of the persistence of a substance from water/sediment tests is done mainly according to Beek, 2001, a procedure which has been proposed for the evaluation of environmentally relevant chemicals in Germany. In Table 5.1 the following classification parameters are shown: i) the time necessary for 50 % dissipation of the test compound (DT_{50}) in the water compartment, ii) the extent of mineralization after 100 d, and iii) the extent of bound residues present after 100 d.

Table 5.1: Persistence classes according to Beek, 2001

First criterion: primary degradation		
DT_{50} in water	Class	Assessment
< 10 d	I	Rapid primary degradation
10 – 30 d	II	Delayed primary degradation
30 – 100 d	III	Slow primary degradation
> 100 d	IV	Negligible primary degradation
Second Criterion: mineralization (after 100 d)		
CO_2	Class	Assessment
> 50 %	I	Extensive mineralization
25 – 50 %	II	Moderate mineralization
10 – 25 %	III	Limited mineralization
< 10 %	IV	Negligible mineralization
Third criterion: bound residues (after 100 d)		
Amount	Class	Assessment
< 10 %	I	Low plateau
10 – 25 %	II	Moderate plateau
25 – 50 %	III	High plateau
> 50 %	IV	Very high plateau

The resulting classes of the three criteria were equally taken for the calculation of the overall persistence category applying the following calculation (average by rounding):

$$\frac{Sum\ of\ the\ classes}{3} = Persistence\ category \qquad (5.3)$$

Depending on the certain degradation kinetic of a substance, the shape of the degradation curve as well as the transformation scheme are considered for obtaining the overall persistence category. Finally the persistence of a chemical is assessed as one of the four persistence categories shown in Table 5.2.

Table 5.2: Persistence categories

Category number	Category name
I	Low persistence
II	Moderate persistence
III	High persistence
IV	Not biodegradable

In most experiments with non-labelled compounds the persistence cannot be entirely assessed according to Beek, 2001, since detailed data on the dissipation processes, such as transformation and mineralization, can usually not be obtained. In such and related cases, the respective substance was preliminarily classified by expert knowledge. It has to be stated again that this approach is not considered to be an alternative to a "full" environmental risk assessment (i.e. a PEC/PNEC comparison), but should be seen as a first evaluation in case effect data are not available.

5.2.2 Assessment of the individual test substances

Before discussing the individual compounds, it has to be noted that the transferability of results attained in lab-scale experiments to large scale systems or even real environmental processes is limited, conditioned by fundamental differences in size, complexity and incubation of the systems (Rönnefahrt et al., 1997). However, the test conditions selected were a worst-case scenario, since the application of organic carbon poor sediment with a relative low TOC is associated with a relatively low microbial activity. Frequently, it is also accompanied with a relatively low extent of analyte sorption onto the sediment. Therefore, real environmental conditions, which include

photo degradation (Andreozzi et al., 2003, Andreozzi et al., 2002, Buser et al., 1998, Lam et al., 2003, Poiger et al., 2001, Tixier et al., 2003) and nutrient replacement, should in general lead to a more efficient elimination of pharmaceutical residues. However, the data obtained allows for a principal estimation of the environmental fate and distribution of the selected pharmaceuticals and TPs which showed a wide range of different behaviours with respect to degradation/dissipation, affinity to the sediment and persistence (Table 5.3).

Table 5.3: Dissipation times, extent of mineralization and sediment sorption and persistence classification of pharmaceuticals tested in water/sediment test systems

	DT_{50} (d)[1]	**DT_{90} (d)[1]**	**Minerali-zation (% C_0)**	**Sorption to sediment (% C_0)**	**Classification[3]**
Paracetamol	-[4] (3.1)	-[4] (10.4)	19	57[2]	*3.5 (Category IV)* *I = Low persistence*
Ibuprofen	-[5] (10)	-[5] (13)	77	9	1.0 (Category I) *I = Low persistence*
2-Hydroxy-ibuprofen	-[4] (6.7)	-[4] (22)	n.d.	< LOQ	1.0 (Category I) *I = Low persistence*
Clofibric acid	119 (82)	> 365 (274)	n.d.	12	3.0 (Category III) *III = High persistence*
Diazepam	> 365 (34)	>> 365 (113)	< 2	60	4.0 (Category IV) *IV = Not biodegradable*
Oxazepam	54 (19)	179 (63)	n.d.	29	3.0 (Category III) *III = High persistence*
Carbamazepine	333 (52)	> >365 (173)	n.d.	40	3.5 (Category IV) *IV = Not biodegradable*
10,11-Dihydro-10,11-hydroxy-carbamazepine	34 (21)	> 365 (> 365)	n.d.	< 10	*2.0 (Category II)* *III = High persistence*
Iopromide	-[5] (30)	-[5] (99)	< 1	< 25	3.0 (Category III) *III = High persistence*
Ivermectin	13 (2.9)	144 (10)	n.d.	16	2.0 (Category II) *II = Moderate persistence*

[1] dissipation times for entire water/sediment system, respective values for the water compartment are shown in brackets, [2] Present as non-extractable residues, n.d. = not determined, [3] numerically according to Beek, 2001 (upper line) and after a plausibility check (i.e. expert knowledge (lower line); final classification is given in *italics,* [4] no value because the compound was never found in the sediment, [5] no value because the compound was not TLC analyzed in the sediment

5.2.2.1 Paracetamol

The results indicate that paracetamol is eliminated from the water phase in the water/sediment system. It can be suggested from the rapid and extensive binding onto the sediment that paracetamol, but more likely its TPs, are incorporated into the biomass and may potentially accumulate in sediments. Since paracetamol or its TPs were not extractable, even not under drastic conditions, it can be expected that the bio availability in the sediment is low.

Consequently, following the classification of Beek, 2001, paracetamol has to be considered as highly persistent in the water/sediment test system (see Table 5.3). However, taking into account what is known about this substance a categorization with "low persistence" is more likely. However, a profound assessment can only be done after elucidation the nature of the non-extractable residues.

5.2.2.2 Ibuprofen

Ibuprofen, which carried a ^{14}C-labelled carboxylic moiety, seemed to be mineralized by more than 75 % in the course of the experiment, displaying a ready degradability in surface water (see Table 5.3). However, de-carboxylation as a major or even solely process cannot be ruled out. In surface waters, a distinct degradation of this substance should be expected. Since ibuprofen is continuously discharged via STP effluents, the micro organisms in the receiving waters should be adapted on its' degradation. Ibuprofen displayed no potential for accumulation in sediments due to its' rapid degradability and its' relatively low lipophilicity under common pH conditions in environmental waters and sediments.

According to Beek`s classification scheme ibuprofen is categorized as "lowly persistent" (Beek, 2001).

5.2.2.3 2-Hydroxy-ibuprofen

With regard to the present data, 2-hydroxy-ibuprofen can be expected to undergo rapid degradation in surface waters and should display neither significant persistence nor accumulation in the sediments and suspended matter (see Table 5.3).

Since a categorization according to (Beek, 2001) was not possible 2-hydroxy-ibuprofen is classified as "lowly persistent".

5.2.2.4 Clofibric acid

The high stability of clofibric acid in surface waters reported in the literature could be confirmed according to the results of the water/sediment test system shown here. The low affinity towards the sediment confirms that clofibric acid shows no potential for accumulation in sediments (see Table 5.3).

Despite the fact that a complete classification according to Beek, 2001 was not possible, clofibric acid is categorized with "highly persistent" due to the high DT-values and the existing monitoring data.

5.2.2.5 Diazepam

Based on the experimental data it is clear that diazepam is hardly degraded in surface waters (excluding light induced degradation) and that it is therefore persistent in the aquatic environment. Due to its' behaviour, an accumulation in sediments, especially in those with high C_{org} levels, can be expected (see Table 5.3).

According to Beek, 2001 diazepam is categorized as "not biodegradable". This is consistent with findings of (Kreuzig, 2003), who found diazepam to be highly stable in soils.

5.2.2.6 Oxazepam

Oxazepam should be eliminated to some extent in surface waters. With respect to its´ moderate lipophilicity and K_d values, the potential for accumulation of oxazepam in natural sediments cannot be ruled out.

The persistence according to Beek, 2001 could not fully be assessed. However, according to the DT-value and its sorption to the sediment a categorization as "highly persistent" is the most appropriate (see Table 5.3).

5.2.2.7 Carbamazepine

The results of the water/sediment test suggest, in agreement with other authors, that carbamazepine is widely resistant to elimination in natural surface waters (excluding light induced degradation). For carbamazepine, a moderate potential for accumulation in sediments, especially in those with a high C_{org} cannot be excluded (see Table 5.3).

Based on the existing data and literature experiences carbamazepine has to be categorized as “not biodegradable” (Beek, 2001).

5.2.2.8 10,11-Dihydro-10,11-dihydroxy-carbamazepine

The compound 10,11-dihydro-10,11-dihydroxy-carbamazepine disappeared quickly in the first days but remained almost stable at a level of 35 % after the first month until the end of the test. This might indicate the presence of threshold concentrations in the degradation of this TP or was caused by a deficit of nutrients which are essential for the respective degrading micro organisms. Significant sorption of 10,11-dihydro-10,11-dihydroxy-carbamazepine onto sediments can be excluded. A degradation of the TP in surface waters might occur, but due to the threshold concentration found a final conclusion is impossible to be taken.

Despite the fact that a classification according to Beek, 2001 is not fully possible, the DT-values, the negligible sorption to the sediment and recent monitoring data (Miao and Metcalfe, 2003) indicate at least a “moderate persistence” (see Table 5.3). In fact, the time course of this substance in the water phase make a categorization as “highly persistent” more likely.

5.2.2.9 Iopromide

Iopromide can be in principal transformed in surface waters, even though the extent of the transformation may be low due to the slow kinetics. An accumulation in sediments can be excluded. For the X-ray contrast medium iopromide, at least four relatively stable TPs were detected of which three occurred at a level of > 10 %. These TPs were also observed in soil column leaching experiments, where iopromide was transformed between 15 and 100 % during soil passage. It is suggested that iopromide is transformed into the observed TPs under various environmental conditions.

According to Beek, 2001, iopromide has to be classified as “highly persistent” in water/sediment systems (see Table 5.3) when considering also the formation of stable TPs.

5.2.2.10 Ivermectin

In surface waters a rapid elimination of ivermectin from the water compartment can be presumed. Due to the elevated sorption properties of ivermectin, an accumulation in natural sediments is likely, in particular for anaerobic sediments with a high C_{org}.

A formal classification according to Beek, 2001 is difficult to be done. However, due to the low DT-values and the transitory sorption in the sediment, it is proposed to categorize this parasiticide as “moderately persistent” (see Table 5.3). This proposal is in accordance with literature data from the compartment soil (Edwards et al., 2001).

5.2.2.11 Final note

The phase I metabolites 10,11-dihydro-10,11-dihydroxy-carbamazepine, 2-hydroxy-ibuprofen and oxazepam showed lower system half lives than the parent compounds, reflecting the comparably elevated accessibility of phase I metabolites towards further degradation reactions. These main human metabolites were never found as TPs in the water sediment systems. Thus, the transformation of the selected pharmaceuticals in humans and in the environmental systems was totally different. Therefore, the transferability of pharmacological data towards environmental behaviour is limited and for a reliable assessment of the environmental fate of pharmaceuticals, appropriate tests are indispensable.

5.3 Mobility and transformation in column leaching systems

Especially the compartment groundwater is a subject of high interest not only from an ecotoxicological point of view but mainly due to toxicological reasons. In most member states of the European Union groundwater is the main source of drinking water. Therefore, the assessment of potential risks of chemicals to human health via groundwater contamination must always be considered. In order to evaluate the risk for this compartment in an ERA, usually adsorption/desorption kinetics and the leaching behaviour are used as the main endpoints.

For the assessment of the leaching potential of pharmaceuticals existing concepts should be applied. Already in the 1980th the Biologische Bundesanstalt für Land- und Forstwirtschaft (Braunschweig, Germany) required for the registration of pesticides the evaluation of their leaching potential. As a first step always a laboratory leaching study

using small disturbed soil columns had to be performed (Biologische Bundesanstalt für Land und Forstwirtschaft, 1986). Depending on the results of this study and taking into account the persistence of the test substance, a data refinement could be required. For substances with a DT_{90} < 100 d, a recovery of more than 10% of the test item and its TPs in the leachate served as a trigger for further tests, e.g. leaching with aged residues, lysimeter tests or even field studies. For substances with a DT_{90} > 100 d a recovery rate of > 5% triggers a data refinement. Later on, in a guideline describing the performance of semi-field lysimeter studies, the BBA specified the trigger values (Biologische Bundesanstalt für Land und Forstwirtschaft, 1990).

A data refinement (i.e. modelling and - depending on the results of the model predictions - further studies) were necessary if one of the following criteria is fulfilled: a) water solubility of the test substance is higher than 30 mg/L; b) adsorption coefficient K_{oc} is below 500; c) K_d-value is below 10 $L \cdot kg^{-1}$ and d) the soil persistence, given as DT_{50} is higher than 21 days.

In the current practice of pesticide registration according to EU guideline 91/414 (EWG, 1991), the investigation of the mobility of these substances in soil starts also with small laboratory columns (EU, 1991). In addition, the same test has to be done with aged residues. Taking into consideration the results of these tests as well as the persistence of the test substance in soil and its adsorption/desorption behaviour, it has to be decided whether a field lysimeter study must be performed. In any case the expected concentration of the test substance in the groundwater has to be calculated. If according to the modeling this concentration is higher than 0.1 μg/L, the substance cannot be registered, unless it has been shown via data refinement, that no unacceptable effects of the substance on the groundwater will occur (EU, 1997). This data refinement is usually focusing on real field conditions (either by performing a field study or by further model calculations) to show that under such conditions the test substance will not exceed the trigger value in the groundwater.

Since it was not possible to model the potential concentration of the six test pharmaceuticals in the groundwater (this would be the most appropriate approach followed by EU as well as national authorities today), the results gained so far were

assessed using the criteria of the BBA guidelines. According to the BBA trigger values a data refinement would be required as follows (Table 5.4):

Table 5.4: Reasons for a data refinement for certain pharmaceuticals

Reason for data refinement	Compounds
Detection of test substance and its TPs in the leachate of disturbed soil columns > 10% (respective 5%)	iopromide, clofibric acid
Water solubility > 30 mg/L	iopromide, clofibric acid, diazepam
K_{oc} < 500	iopromide, clofibric acid, diazepam, carbamazepine
K_d < 10	iopromide, clofibric acid, diazepam, carbamazepine

The DT_{90}-criterion could not be applied, given that no soil DT-values are available for the compounds investigated. Since four of the pharmaceuticals investigated exceed at least one of the trigger values further modelling and testing with iopromide, clofibric acid, diazepam and carbamazepine will be necessary.

The case of carbamazepine shows clearly that the studies with the disturbed soil columns should only be considered as a first screening approach. No carbamazepine was detected in the leachates of the soil columns. However, carbamazepine is known to be present in groundwater very often (Heberer, 2002a, Sacher et al., 2001, Ternes, 2000). Decisions in environmental risk assessment should not be based only on the results of these soil column leaching studies. Therefore - as foreseen in the BBA-scheme - the compound's properties (e.g. water solubility, K_{oc}, K_d) should be regarded in addition to the soil column experiments. In the case of ibuprofen no leaching potential was evaluated using the BBA-approach. However, taken into account that the soil column experiments were performed with non-labelled ibuprofen and also considering the high potential mobility of ibuprofen due to its pH dependent

dissociation, a data refinement is recommended. Especially the pH dependent mobility of ibuprofen should be investigated further. Only the veterinary pharmaceutical ivermectin fulfills the BBA registration criteria for pesticides; i.e. no further investigations are required.

In the cases where data refinements are required, registration restrictions and/or risk reduction measures have to be identified, if the potential to leach cannot definitely be excluded in further studies. However, a direct transfer of the risk mitigation steps developed for pesticides seems questionable due to the medical importance of pharmaceuticals. In other words, the benefits of the use of a certain pharmaceutical have always to be taken into consideration.

The observed mobility of clofibric acid and iopromide (including its TPs) in the soil columns indicates a risk for the occurrence in groundwater. This result could be confirmed by the occurrence of both substances in the groundwater of several German regions (Heberer, 1995, Ternes, 2001a, Ternes, 2000, Ternes and Hirsch, 2000). Substances which enter the aquifer may pose a problem not only for short-term periods. Due to the low microbial activity in the deeper soil layers these contaminants may be persistent in the aquifer over decades (DVWK, 1988) and therefore rather cause a long term risk. In addition, the discrepancy between the lack of mobility in the laboratory columns and literature data (occurrence in groundwater) in the case of carbamazepine requires further investigations. In particular, the hypothesis that the mobility differs between columns filled with top soil in comparison to the use of sub-soils or river sediments.

Despite the fact that these pharmaceuticals were able to enter the groundwater, no concept to assess their ecotoxicological risk in this compartment is available. However, for the assessment of groundwater contaminations (Röder et al., 1999) developed criteria to establish threshold levels, which can be used for a precautionary protection of this precious compartment. Despite an increased contaminant level above the geogenic background, groundwater can be classified as minor polluted, if no relevant ecotoxicological effects will be observed and the quality criteria of the Drinking Water Directive (or analogous values) are fulfilled.

Currently, no data on the effects of pharmaceuticals on organisms in the compartment groundwater are available (Health Council of the Netherlands, 1996). Furthermore, until now no tools are existing to predict the effects of pollutants to groundwater organisms or ecosystematic parameters. Hence, in the year 2002 the German Environmental Agency, Berlin, initiated a project to identify suitable groundwater test-organisms. Based on the results of this project the next step has to be the development of useful test-systems, in order to determine the toxicity of selected contaminants to groundwater organisms. Depending on their ecotoxicological effects threshold levels especially for pharmaceuticals which have a potential to leach into the groundwater (e.g. clofibric acid and iopromide) should be defined in the near future.

With respect to the human-toxicological risk of pharmaceuticals the requirements of the Drinking Water Directive have to be fulfilled (European Union, 1998, Van der Tenck et al., 1999). Herein a threshold level of 0.1 µg/L is defined for pesticides. Such quality criteria do not exist for pharmaceuticals yet. Since various pharmaceuticals like clofibric acid or iopromide could be detected in German drinking water (Heberer, 2002b, Steger-Hartmann et al., 2001, Stumpf et al., 1999, Ternes, 2001a, Ternes et al., 2002a) over the last decade, threshold levels have to be defined in the near future; in particular for those pharmaceuticals which have a potential to leach into the groundwater. Both chemicals are obviously able to pass the elimination procedures involved in drinking water processing. Based on a $PEC_{drinking\ water}$ (Predicted Environmental Concentration), Webb et al., 2003 calculated the uptake via drinking water of clofibric acid and iopromide by a "model human". Assuming a daily consumption of 2 L drinking water the author calculated an uptake of clofibric acid of 14.3 mg (1% of a daily dose) and 112 mg iopromide (< 1% of a daily dose) over a life time of 70 years. Therefore, direct pharmaceutical effects associated with the uptake of both pharmaceuticals by the consumption of drinking water can be excluded, although a general risk for long term effects cannot be ruled out.

6 OUTLOOK

As mentioned already, some results of the current project will require further studies not covered so far. Especially, we propose to consider the following subjects:

6.1 Modeling

The results gained in the water/sediment as well as the leaching tests should be complemented by further tests with differing test conditions, e.g. other sediments, soils, temperatures. This would allow for a more general modelling of the fate of the pharmaceuticals investigated which is necessary for a profound risk assessment of the pharmaceuticals.

6.2 “Ageing” of compounds in soils and sediments

The leaching tests performed here are based on a screening approach. Therefore, the compounds assessed as being mobile should be tested after a period of “ageing” in the same soil according to the OECD draft guideline. For this purpose we propose the mobile compounds clofibric acid and radio-labelled iopromide. In addition, the available test method, currently focusing on top soils, should be modified in order to simulate sub-soils or even river sediments, thus being representative for those environmental compartments relevant for the supplement of groundwater.

7 FINAL CONCLUSIONS

In this study, analytical methods have been developed allowing for the determination of pharmaceutical residues in environmental waters and sediment. The new methods were applied for the investigation of the fate and distribution behaviour of selected pharmaceuticals in aerobic water/sediment systems and in leaching tests.

Several of the selected pharmaceuticals displayed a high persistence in the water/sediment studies conducted and/or a potential for accumulation in sediment compartments. These findings indicate an environmental risk, due to the presence of pharmaceuticals in the aquatic environment. In addition, two of the tested pharmaceuticals proved to be mobile in laboratory soil columns (in a third case, a discrepancy between test results and literature data could be clarified).

For a comprehensive environmental risk assessment of pharmaceuticals, further data on the fate in other compartments, i.e. in soil and anaerobic sediments, and on the (eco)toxicicological effects have to be generated. In general, it should be a future aim of the research to elucidate the environmental transformation of pharmaceuticals to allow for a balancing of their elimination in the various environmental compartments.

It is common sense in the European Union that the environmental risk of new pharmaceuticals and their metabolites should be generally evaluated as an obligatory prerequisite for their registration. The situation for approved pharmaceuticals is different. Many of these have been introduced into the environment for a long time, causing an environmental exposure of widely unknown portion.

In the light of a potential accumulation of pharmaceutical residues in sediments, future monitoring programs are strongly recommended to elucidate the pharmaceutical contamination of aquatic sediments. Based on these results and effect data, priority substances among the approved pharmaceuticals should be identified, to which a supplementary ERA is demanded. In addition, the potential risk of pharmaceuticals to the groundwater has also to be taken into account. Laboratory tests (for new substances) as well as monitoring programs (for existing pharmaceuticals) as well as effect tests are necessary to protect this highly relevant compartment.

8 ACKNOWLEDGEMENT

The authors thank Dr. Klein-Goedicke of the German Environmental Agency for support and excellent cooperation. Furthermore, we would like to acknowledge Schering for providing ^{14}C-labelled iopromide and several analytes.

9 REFERENCES

Ahmed S, Javed MA, Tanvir S, Hameed A. Isolation and characterization of a Pseudomonas strain that degrades 4-acetamidophenol and 4-aminophenol. Biodegradation 2001; 12:303-309.

Ahrer W, Scherwenk E, Buchberger W. Determination of drug residues in water by the combination of liquid chromatography or capillary electrophoresis with electrospray mass spectrometry. Journal of Chromatography A 2001; 910:69-78.

Alcock RE, Sweetman A, Jones KC. Assessment of organic contaminant fate in waste water treatment plants. I: Selected compounds and physiochemical properties. Chemosphere 1999; 38:2247-2262.

Ali SM, Sun T, McLeroy GE, Philuppo ET. Confirmation of Eprinomectin, Moxidectin, Abamectin, Doramectin, and Ivermectin in Beef Liver by Liquid Chromatography/Positive Ion Atmospheric Pressure Chemical lonization Mass Spectrometry. Journal of AOAC International 2000; 83:39-52.

American Radiolabeled Chemicals Inc. Technical Data Sheet p-Nitrophenol [14C-U]. St. Louis, MO, USA, 1994.

American Radiolabeled Chemicals Inc. Technical Data Sheet Ibuprofen, RS [carboxyl-14C]. St. Louis, MO, USA, 2001.

Andreozzi R, Raffaele M, Nicklas P. Pharmaceuticals in STP effuents and their solar photodegradation in aquatic environment. Chemosphere 2003; 50:1319-1330.

Andreozzi R, Raffaele M, Pinto G, Pollio A. Carbamazepine in water: persistence in the environment, ozonation treatment and preliminary assessment on algal toxicity. Water Research 2002; 36:2869-2877.

Beek B. Handbook of environmental chemistry, Vol. 2, Biodegradation and Persistence, Springer, Berlin, Heidelberg, New York, 2001.

Belfroid AC, Van der Horst A, Vethaak AD, Schäfer AJ, Rijs GBJ, Cofino WP. Analysis and occurence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands. The Science of the Total Environment 1999; 225:101-108.

Biologische Bundesanstalt für Land und Forstwirtschaft. Richtlinien für die amtliche Prüfung von Pflanzenschutzmitteln (Nr. IV, 4-1, Teil 1): Bestimmung des Verbleibs von Pflanzenschutzmitteln im Boden (Teil: Abbauverhalten). 1986.

Biologische Bundesanstalt für Land und Forstwirtschaft. Richtlinien für die Prüfung von Pflanzenschutzmitteln (Nr. IV, 4-3): Lysimeteruntersuchungen zur Verlagerung von Pflanzenschutzmitteln in den Untergrund. 1990;

Biologische Bundesanstalt für Land- und Forstwirtschaft. Richtlinie für die Prüfung von Pflanzenschutzmitteln im Zulassungsverfahren, Teil IV 5-1, Abbaubarkeit und Verbleib von Pflanzenschutzmitteln im Wasser/Sediment-System, 1990.

Boxall ABA, Fogg L, Blackswell PA, Kay P, Pemberton E. Review of veterinary medicines in the environment - Technical report. Bristol: Environment Agency, 2001.

Boxall ABA, Oakes D, Ripley P, Watts CD. The application of predictive models in the environmental risk assessment of ECONOR®. Chemosphere 2000; 40:775-781.

Brodsky J, Brodesser J, Bauer C, Römbke J. The environmental fate of six existing chemicals in laboratory tests. Chemosphere 1997; 34:515-538.

Bull DL, Ivie GW, MacConnel JG, Gruber VF, Ku CC, Arison BH, Stevenson JM, VandenHeuvel WJA. Fate of Avermectin B1a in soil and plants. Journal of Agricultural and Food Chemistry 1984; 32:94-102.

Bund/Länderausschuss für Chemikaliensicherheit (BLAC). Arzneimittel in der Umwelt - Auswertung der Untersuchungsergebnisse. Hamburg, 2003.

Buser H-R, Poiger T, Müller MD. Occurrence and Fate of the Pharmaceutical Drug Diclofenac in Surface Waters: Rapid Photodegradation in a Lake. Environ. Sci. Technol. 1998; 32:3449-3456.

Buser H-R, Poiger T, Müller MD. Occurrence and Environmental Behavior of the Chiral Pharmaceutical Drug Ibuprofen in Surface Waters and in Wastewater. Environmental Science & Technology 1999; 33:2529-2535.

Campagnolo ER, Johnson KR, Karpati A, Rubin CS, Kolpin DW, Meyer MT, Esteban JE, Currier RW, Smith K, Thu KM, McGeehin M. Antimicrobial residues in animal waste and water resources proximal to large-scale swine and poultry feeding operations. The Science of the Total Environment 2002; 299:89-95.

Campbell WC. Ivermectin and Abamectin, Springer Verlag, New York, Berlin, Heidelberg, London, Paris, Tokyo, 1989.

Cannavan A, Coyne R, Kennedy G, Smith P. Concentration of 22,23-dihydroavermectin B1a detected in the sediments at an Atlantic salmon farm using orally administered ivermectin to control sea-lice infestation. Aquaculture 2000; 182:229-240.

Cerny RL, MacMillan DK, Gross ML, Mallams AK, Pramanik BN. Fast-Atom Bombardement and Tandem Mass Spectrometry of Macrolide Antibiotics. Journal American Society for Mass Spectrometry 1994; 5:151-158.

Christensen FM. Pharmaceuticals in the environment-a human risk? Regulatory Toxicology and Pharmacology 1998; 28:212-221.

Daughton CG, Ternes TA. Pharmaceuticals and personal care products in the environment: Agents of subtle change? Environmental Health Perspectives 1999; 107 (Suppl. 6):907-938.

Davies IM, Gillibrand PA, McHenery JG, Rae GH. Environmental risk of ivermectin to sediment dwelling organisms. Aquaculture 1998; 163:29-46.

Davies M, Rodger GK. A review of the use of ivermectin as a treatment for sea lice [Lepeophtheirus salmonis (Kroyer) and Caligus elongatus Nordmann] infestation in farmed Atlantic salmon (Salmo salar L.). Aquaculture Research 2000; 31:869-883.

Delle Site A. Factors affecting sorption of organic compounds in natural sorbent/water systems and sorption coefficients for selected pollutants. J. Phys. Chem. Ref. Data. 2001; 30:187-439.

Díaz E, Ferrández A, Prieto MA, García JL. Biodegradation of Aromatic Compounds by Escherichia coli. Microbiology and Molecular Biology Reviews 2001; 65:523-569.

Drewes JE, Heberer T, Reddersen K. Fate of pharmaceuticals during indirect potable reuse. Water Science and Technology 2002; 46:73-80.

Dubus IG, Barriuso E, Calvet R. Sorption of weak organic acids in soils: clofencet, 2,4-D and salicylic acid. Chemosphere 2001; 45:767-774.

DVWK. Filtereigenschaften des Bodens gegenüber Schadstoffen. Teil I: Beurteilung der Fähigkeit von Böden zugeführte Schwermetalle zu immobilisieren. Hamburg:Parey, 1988.

Edwards CA, Atiyeh RM, Römbke J. Environmental Impact of Avermectins. Reviews Environmental Contamination and Toxicology 2001; 171:111-137.

Eisenbrand G, Metzler M. Toxikologie für Chemiker, Georg Thieme Verlag, Stuttgart, New York, 1994.

EU. Council Directive 91/414/EEC concerning the placing of plant protection products on the market. Journal of the European Communities 1991; L230.

EU. Note for Guidance: Environmental Risk Assessment for Veterinary Medicinal Products other than GMO-Containing and Immunological Products. EMEA/CVMP/055/96. European Union, EMEA, London, 1997.

EU. Draft CPMP discussion paper on environmental risk assessment of non-genetically modified modified organism (non-GMO) containing medicinal products for human use. CPMP/SWEP/4447/00, European Union, EMEA. London, 2001.

European Commission, Directorate General for Agriculture. Guidance Document on Persistance in Soil., 2000.

European Union. Council Directive 93/39/EEC of 14 June 1993 amending Directives 65/65/EEC, 75/318/EEC and 75/319/EPIC in respect of medicinal products, Off. J. L214 (24/08/1993), 1993.

European Union. Council Directive 98/83/EC on the quality of water intended for human consumption, 1998.

European Union. Note for guidance on environmental risk assessment of non-genetically modified organism (Non-GMO) containing medicinal products for human use. CPMP/SWP/4447/00. London: EMEA, 2002.

European Union. Environmental impact assessment (EIAs) for veterinary medicinal products (VMPs) - Phase II. VICH Topic GL38. CVMP/VICH/790/03-CONSULTATION. London: EMEA, 2004.

EWG. Richtlinie des Rates vom 15. Juli 1991 über das Inverkehrbringen von Pflanzenschutzmitteln (91/414/EWG). Amtsblatt der Europäischen Gemeinschaften, L 230 1991;

Ferrari B, Paxeus N, Lo Giudice R, Pollio A, Garric J. Ecotoxicological impact of pharmaceuticals found in treated wastewaters: study of carbamazepine, clofibric acid, and diclofenac. Ecotoxicology and Environmental Safety 2003; 55:359-370.

Forth W, Henschler D, Rummel W, Starke K. Allgemeine und spezielle Pharmakologie und Toxikologie, 7th edition, Spektrum Akademischer Verlag GmbH, Heidelberg, Berlin, Oxford, 1996.

Freitag D, Ballhorn L, Geyer H, Korte F. Environmental Hazard Profile of Organic Chemicals. An Experimantal Method for the Assessment of the Behaviour of

Organic Chemicals in the Ecosphere by Means of Simple Laboratory Tests with 14C Labelled Chemicals. Chemosphere 1985; 14:1589-1616.

Freitag D, Geyer H, Kraus A, Viswanathan R, Kotzias D, Attar A, Klein W, Korte F. Ecotoxicological Profile Analysis. VII. Screening Chemicals for Their Environmental Behavior by Comparative Evaluation. Ecotoxicology and environmental safety 1982; 60-81.

Garrison AW, Pope JD, Allen FR. GC/MS Analysis of organic compounds in domestic wastewaters. In: Keith CH edit, Identification and analysis of organic pollutants in water, Ann Arbor Science Publishers, 1976, 517-556.

Gerstl Z. Estimation of organic chemical sorption by soils. Journal of Contaminant Hydrology 1990; 6:307-412.

Gevao B, Semple KT, Jones KC. Bound pesticide residues in soils: a review. Environmental Pollution 2000; 108:3-14.

Golet EM. Environmental Exposure Assessment of Flouroquinolone Antibacterial Agents in Sewage, River Water and Soil, Swiss Federal Institute of Technology, Zurich, Swiss Federal Institute of Technology, Zurich, 2002.

Golet EM, Alder AC, Giger W. Environmental Exposure and Risk Assessment of Fluoroquinolone Antibacterial Agents in Wastewater and River Water of the Glatt Valley Watershed, Switzerland. Environ. Sci. Technol. 2002a; 36:3645-3651.

Golet EM, Alder AC, Hartmann A, Ternes TA, Giger W. Trace determination of fluoroquinolone antibacterial agents in urban wastewater by solid-phase extraction and liquid chromatography with fluorescence detection. Analytical Chemistry 2001; 73:3632-3638.

Golet EM, Strehler A, Alder AC, Giger W. Determination of Fluoroquinolone Antibacterial Agents in Sewage Sludge and Sludge-Treated Soil Using Accelerated Solvent Extraction Followed by Solid-Phase Extraction. Anal. Chem. 2002b; 74:5455-5462.

Gruber VF, Halley BA, Hwang S-C, Ku CC. Mobility of Avermectin B1a in Soil. J. Agric. Food Chem. 1990; 38:886-890.

Halley BA, Jacob TA, Lu AYH. The environmental impact of the use of ivermectin: Environmental effects and fate. Chemosphere 1989a; 18:1543-1563.

Halley BA, Nessel RJ, Lu AYH, Roncalli RA. The environmental Safety of Ivermectin: An Overview. Chemosphere 1989b; 18:1565-1572.

Halling-Sorensen B, Holten Luetzhoft H-C, Andersen HR, Ingerslev F. Environmental risk assessment of antibiotics: comparison of mecillinam, trimethoprim and ciprofloxacin. Journal of Antimicrobial Chemotherapy 2000; 46:53-58.

Halling-Sorensen B, Nors Nielsen S, Lanzky PF, Ingerslev F, Holten Luetzhoft H-C, Jorgensen SE. Occurrence, fate and effects of pharmaceutical substances in the environment-a review. Chemosphere 1998; 36:357-93.

Hansch C, Sammes PG, Taylor JB. Comprehensive Medicinal Chemistry, The Rational Design, Mechanistic Study and Therabeutic Application of Chemical Compounds, Vol. 6, Cumulative Subject Index and Drug Compendium, Pergamon, Oxford, 1990.

Hartig C, Storm T, Jekel M. Detection and identification of sulphonamide drugs in municipal waste water by liquid chromatography coupled with electrospray ionisation tandem mass spectrometry. Journal of Chromatography A 1999; 854:163-173.

Health Council of the Netherlands. Risk of pesticides to groundwater systems. Publ. No. 1996/1 IE. Rijswik, 1996.

Heberer T. Identifizierung und Quantifizierung von Pestizidrückständen und Umweltkontaminanten in Grund- und Oberflächengewässern mittels Kapillargaschromatographie-Massenspektrometrie. Berlin:Wissenschaft und Technik Verlag, 1995.

Heberer T. Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment; a review. Toxicology Letters 2002a; 131:5-17.

Heberer T. Tracking persistent pharmaceutical residues from municipal sewage to drinking water. Journal of Hydrology 2002b; 266:175-189.

Heberer T, Duennbier U, Reilich C, Stan H-J. Detection of Drugs and Drug Metabolites in Ground Water samples of a drinking water treatment plant. Fresenius Environmental Bulletin 1997; 6:438-443.

Heberer T, Reddersen K, Mechlinski A. From municipal sewage to drinking water: fate and removal of pharmaceutical residues in the aquatic environment in urban areas. Water Science and Technology 2002; 46:81-88.

Heberer T, Stan H-J. Determination of Trace Levels of Dichlorprop, Mecoprop, Clofibric Acid, and Naphthylacetic Acid in Soil by Gas Chromatography/Mass Spectrometry with Selected-Ion Monitoring. Journal of AOAC International 1996; 79:1428-1433.

Heberer T, Stan H-J. Arzneimittelrückstände im aquatischen System. Wasser und Boden 1998; 50:20-24.

Hektoen H, Berge JA, Hormazabal V, Yndestad M. Persistence of antibacterial agents in marine sediments. Aquaculture 1995; 133:175-184.

Heller DN, Schenck FJ. Particle beam liquid chromatography/mass spectrometry with negative ion chemical ionization for the confirmation of ivermectin residue in bovine milk and liver. Biological Mass Spectrometry 1993; 22:184-193.

Henschel K-P, Wenzel A, Diedrich M, Fliedner A. Environmental hazard assessment of pharmaceuticals. Regulatory Toxicology and Pharmacology 1997; 25:220-225.

Hignite C, Azarnoff DL. Drugs and drug metabolites as environmental contaminants: chlorophenoxyisobutyrate and salicylic acid in sewage water effluent. Life Sci 1977; 20:337-342.

Hilal SH, El-Shabrawy Y, Carreira LA, Karickhoff SW, Toubar SS, Rizk M. Estimation of the ionization pKa of pharmaceutical substances using the computer program SPARC. Talanta 1996; 43:607-619.

Hill IR, Heimbach F, Leeuwangh P, Matthiessen P. Freshwater Field Tests for Hazard Assessment of Chemicals, CRC Press, Inc., Boca Raton, 1994.

Hirsch R, Ternes TA, Haberer K, Kratz K-L. Occurrence of antibiotics in the aquatic environment. The Science of the Total Environment 1999; 225:109-118.

Hirsch R, Ternes TA, Haberer K, Mehlich A, Ballwanz F, Kratz K-L. Determination of antibiotics in different water compartments via liquid chromatography-

electrospray tandem mass spectrometry. Journal of Chromatography A 1998; 815:213-223.

Hirsch R, Ternes TA, Lindart A, Haberer K, Wilken R-D. A sensitive method for the determination of iodine containing diagnostic agents in aqueous matrices using LC-electrospray-tandem-MS detection. Fresenius J. Anal. Chem. 2000; 366:835-841.

Holm JV, Rügge K, Bjerg PL, Christensen TH. Occurrence and Distribution of Pharmaceutical Organic Compounds in the Groundwater Downgradient of a Landfill (Grindsted, Denmark). Environ. Sci. Technol. 1995; 29:1415-1420.

International Organization for Standardization. ISO 10390 : Soil quality - Determination of pH. Genf, 1994.

International Organization for Standardization. ISO 14240-2: Soil quality - Determination of soil microbial biomass - Part 2: Fumigation-extraction method. Genf, 1997.

International Organization for Standardization. ISO 11268-2 : Soil Quality - Effects of Pollutants on Earthworms (Eisenia fetida). Part 2: Determination of Effects on Re-production. Genf, 1998.

Joergensen RG. The fumigation extraction method to estimate soil microbial biomass: Extraction with 0.01 m CaCl2. Agribiological Research-Zeitschrift Fur Agrarbiologie Agrikulturchemie Okologie 1995; 48:319-324.

Joergensen RG. The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k(EC) value. Soil Biology & Biochemistry 1996; 28:25-31.

Joergensen RG, Mueller T. The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k(EN) value. Soil Biology & Biochemistry 1996; 28:33-37.

Jones OAH, Voulvoulis N, Lester JN. Human pharmaceuticals in the aquatic environment - a review. Environmental Technology 2001; 22:1383-1394.

Jones OAH, Voulvoulis N, Lester JN. Aquatic environmental assessment of the top 25 English prescription pharmaceuticals. Water Research 2002; 36:5013-5022.

Jørgensen SE, Halling-Sorensen B. Drugs in the environment. Chemosphere 2000; 40:691-699.

Kalsch W. Abbau iodhaltiger Röntgenkontrastmittel in der Umwelt, Dissertation, Johannes Gutenberg Universität Mainz, 1992.

Kalsch W. Biodegradation of the iodinated X-ray contrast media diatrizoate and iopromide. The Science of the Total Environment 1999; 225:143-153.

Karickhoff SW. Semi-empirical estimation of sorption of hydrophilic poolutants on natural sediments and soils. Chemosphere 1981; 10:833-846.

Khan SJ, Ongerth JE. Estimation of pharmaceutical residues in primary and secondary sewage sludge based on quantities of use and fugacity modelling. Water Science and Technology 2002; 46:105-113.

Klimek M, Lejczak B, Kafarski P, Forlani G. Metabolism of the phosphonate herbicide glyphosate by a non-nitrate-utilizing strain of Penicillium chrysogenum. Pest Management Science 2001; 57:815-821.

Kolpin DW, Furlong ET, Meyer MT, Thurman EM, Zaugg SD, Barber LB, Buxton HT. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: a national reconnaissance. Environ. Sci. Technol. 2002; 36:1202-1211.

Kovar K, Chaloupka V, Egli T. A Threshold Substrate Concentration Is Required to Initiate the Degradation of 3-Phenylpropionic Acid in Escherichia coli. Acta Biotechnol. 2002; 22:285-298.

Kreuzig R. Verhalten und Verbleib von Pharmakarückständen in Bödenedit. Jahrestagung der GDCH Fachgruppe Umweltchemie und Ökotoxikologie 2002, Braunschweig.

Kreuzig R. 2003;Personal Communication.

Kreuzig R, Kullmer C, Matthies B, Höltge S, Dieckmann H. Fate and behaviour of pharmaceutical residues in soils. Fresenius Environmental Bulletin 2003; 12:550-558.

Kuhnt G, Muntau H. EURO-Soils: Identification, Collection, Treatment, Characterization. Joint Research Centre, European Commission, Ispra. Special Publication No. I.94.60, 1992.

la Farré M, Ferrer I, Ginebreda A, Figueras M, Olivella L, Tirapu L, Vilanova M, Barceló D. Determination of drugs in surface water and wastewater samples by liquid chromatography-mass spectrometry: methods and preliminary results including toxicity studies with Vibrio fischeri. Journal of Chromatography A 2001; 938:187-197.

Lam MW, Tantuco K, Mabury S. PhotoFate: A New Approach in Accounting for the Contribution of Indirect Photolysis of Pesticides and Pharmaceuticals in Surface Waters. Environ. Sci. Technol. 2003; 37:899-907.

Lenhard P. Das Sorptions- und Trasportverhalten ausgewählter Pharmaka während der Bodenpassage. - Experimentelle Studien -, Johannes Gutenberg Universität, Mainz, 2000.

Lindsey ME, Meyer M, Thurman E.M. Analysis of Trace Levels of Sulfonamide and Tetracycline Antimicrobials in Groundwater and Surface Water Using Solid-Phase Extraction and Liquid Chromatography/Mass Spectrometry. Analytical Chemistry 2001; 73:4640-4646.

Löffler D, Ternes TA. Analysis of acidic pharmaceuticals, antibiotics and ivermectin in river sediment using LC-tandem MS. Journal of Chromatography A 2003; 2021:133-144.

Mandrioli R, Albani F, Casamenti G, Sabbioni C, Raggi MA. Simultaneos high-performance liquid chromatography determination of carbamazepine and five of its metabolites in plasma of epileptic patients. Journal of Chromatography B 2001; 762:109-116.

McArdell CS, Molnar E, Suter MJF, Giger W. Occurrence and fate of macrolide antibiotics in wastewater treatment plants and in the Glatt Valley Watershed, Switzerland. Environmental Science & Technology 2003; 37:5479-5486.

Meisenheimer M, Ternes TA. Verfahren zur Bestimmung der Antiepileptika Carbamazepin und Primidon in Wasser mittels GC/MS nach Derivatisierung. Vom Wasser 2000; 94:203-212.

Merck. The Merck Index, Merck Publications, London, 2001.

Merck & Co. Inc. The Merck Manual of Diagnosis and Therapy, Seventeenth Edition, Merck Publishing Group, 1999.

Mersmann P, Scheytt T, Heberer T. Column experiments on the transport behavior of pharmaceutically active compounds in the saturated zone. Acta Hydrochimica Et Hydrobiologica 2002; 30:275-284.

Miao X-S, Koenig BG, Metcalfe CD. Analysis of acidic drugs in the effluents of sewage treatment plants using liquid chromatography-electrospray ionization tandem mass spectrometry. Journal of Chromatography A 2002; 952:139-147.

Miao XS, Metcalfe CD. Determination of carbamazepine and its metabolites in aqueous samples using liquid chromatography-electrospray tandem mass spectrometry. Analytical Chemistry 2003; 75:3731-3738.

Moehle E, Kempter C, Kern A, Metzger JW. Examination of the degradation of drugs in municipal sewage plants using liquid chromatography-electrospray mass spectrometry [German]. Acta Hydrochimica et Hydrobiologica. 1999; 27:430-436.

Mutschler E. Arzneimittelwirkungen, Wissenschaftliche Verlagsgesellschaft, Stuttgart, 1997.

Northcott GL, Jones KC. Experimental approaches and analytical techniques for determining organic compound bound residues in soil and sediment. Environmental Pollution 2000; 108:19-43.

Novartis. Tegretol - Carbamazepine USP - Prescribing information, 2000.

OECD. Guideline for Testing of Chemicals No. 308 Aerobic and Anaerobic Transformation in Aquatic Sediment Systems. Paris, 2002.

OECD. Guideline for Testing of Chemicals. Leaching in Soil Columns. Paris, 2003a.

OECD. OECD Guidelines for Testing of Chemicals:OECD, 2003b.

Öllers S, Singer HP, Fässler P, Müller SR. Simultaneous quantification of neutral and acidic pharmaceuticals and pesticides at the low-ng/l level in surface and waste water. Journal of Chromatography A 2001; 225-234.

Pascoe D, Karntanut W, Müller CTM. Do pharmaceuticals affect freshwater invertebrates? A study with the cnidarian Hydra vulgaris. Chemosphere 2003; 51:521-528.

Poiger T, Buser H-R, Müller MD. Photodegradation of the Pharmaceutical Drug Diclofenac in a Lake: Pathway, Field Measurements, and Mathemathical Modeling. Environmental Toxicology and Chemistry 2001; 20:256-263.

Pointing SB. Feasibility of bioremediation by white-rot fungi. Applied Microbiology and Biotechnology. 2001; 57:20-33.

Putschew A, Schittko S, Jekel M. Quantification of triiodinated benzene derivates and X-ray contrast media in water samples by liquid chromatography-electrospray tandem mass spectrometry. Journal of Chromatography A 2001; 930:127-134.

Putschew A, Wischnack S, Jekel M. Occurrence of triiodinated X-ray contrast agents in the aquatic environment. The Science of the Total Environment 2000; 255:129-134.

Reardon KF, Mosteller DC, Bull Rogers JD. Biodegradation Kinetics of Benzene, Toluene, and Phenol as Single and Mixed Substrates for Pseudomonas putida F1. Biotechnology and Bioengineering 2000; 69:386-400.

Reising KP. Ivermectin Quantitation with an Abamectin Internal Standard. Journal AOAC International 1998; 81:477-483.

Reyzer ML, Brodbelt JS. Analysis of fire ant pesticides in water by solid-phase microextraction and gas chromatography/mass spectrometry or high-performance liquid chromatography/mass spectrometry. Analytica Chimica Acta 2001; 436:11-20.

Richardson ML, Bowron JM. The fate of pharmaceutical chemicals in the aquatic environment. Pharm. Pharmacol. 1985; 37:1-12.

Röder R, Van der Tenck KT, Markard C, Kühl C, Slama H. Ableitungskriterien für Geringfügigkeitsschwellen zur Beurteilung von Grundwasserverunreinigungen. UWSF Z Umweltchem Ökotox 1999; 11:212-218.

Römbke J, Knacker T, Stahlschmidt-Allner P. Studie über Umweltprobleme im Zusammenhang mit Arzneimitteln. Flörsheim, Main, 1996.

Römbke J, Knacker T, Teichmann H. Environmental Risk Assessment of Pharmaceuticals: A Proposal with Special Emphasis on European Aspects. In: Daughton CG, Jones-Lepp T edit, Pharmaceuticals and Personal Care Products in the Environment: Scientific and Regulatory Issues, American Chemical Society, Washington, D.C., 2001, 304-319.

Rönnefahrt I, Koschorrek J. Humanarzneimittel in der Umwelt - Neue Ansätze der Risikobewertung in der EUedit. Jahrestagung der GDCH Fachgruppe Umweltchemie und Ökotoxikologie 2002, Braunschweig.

Rönnefahrt I, Traub-Eberhard U, Kördel W, Stein B. Comparison of the fate of isoproturon in small- and large-scale water/sediment systems. Chemosphere 1997; 35:181-189.

Rote Liste Service GmbH Frankfurt/Main. Rote Liste 2001, Editio Cantor Verlag, Aulendorf, 2001.

Sacher F, Lange FT, Brauch H-J, Blankenhorn I. Pharmaceuticals in groundwaters. Analytical methods and results of a monitoring program in Baden-Württemberg, Germany. Journal of Chromatography A 2001; 938:199-210.

Sacher F, Lochow E, Bethmann D, Brauch H-J. Vorkommen von Arzneimittelrückständen in Oberflächenwässern. Vom Wasser 1998; 90:233-243.

Sarin RK, Gyan PS, Varshney KM, Rasool SN. Determination of diazepam in cold drinks by high-performance thin-layer chromatography. Journal of Chromatography A 1998; 332-335.

Scheytt T, Grams S, Fell H. Vorkommen und Verhalten eines Arzneimittels (Clofibrinsäure) im Grundwasser. Grundwasser, Vol. 2/98 1998; 67-77.

Scheytt T, Mersmann P, Heberer T. Natural Attenation of Pharmaceuticalsedit. 2nd International Conference on Pharmaceuticals and Endocrine disrupting Chemicals in Water 2001, Minneapolis, Minnesota;253-259.

Schulman LJ, Sargent EV, Naumann BD, Faria EC, Dolan DG, Wargo JP. A human health risk assessment of pharmaceuticals in the aquatic environment. Human and Ecological Assessment 2002; 8:657-680.

Schwabe U, Paffrath D. Arzneiverordnungsreport 1999, Springer-Verlag, Berlin, Heidelberg, New York, 2000.

Schwabe U, Paffrath D. Arzneiverordnungsreport 2001, Springer-Verlag, Berlin, Heidelberg, New York, 2002.

Schwarzenbach RP, Gschwend PM, Imboden DM. Environmental Organic Chemistry, John Wiley & Sons, New York, Chichester, Brisbane, Toronto, Singapore, 2003.

SETAC. Procedures for assessing the environmental fate and ecotoxicology of pesticides. Brussels:SETAC-Europe, 1995.

Steger-Hartmann T, Länge R, Schweinfurth H. Umweltverhalten und ökotoxikologische Bewertung von iodhaltigen Röntgenkontrastmitteln. Vom Wasser 1998; 91:185-194.

Steger-Hartmann T, Länge R, Schweinfurth H. Environmental risk assessment for the widely used iodinated X-Ray contrast agent Iopromide (Ultravist). Ecotoxicology and Environmental Safety 1999; 42:274-281.

Steger-Hartmann T, Länge R, Schweinfurth H, Tschampel M, Rehmann I. Investigations into the environmental fate and effects of iopromide (ultravist), a widely used iodinated X-ray contrast medium. Water Research 2001; 36:266-274.

Straub JO. Environmental risk assessment for new human pharmaceuticals in the European Union according to the draft guideline/discussion paper of January 2001. Toxicology Letters 2002; 135:231-237.

Stuer-Lauridsen F, Birkved M, Hansen LP, Holten Lützhoft H-C, Halling-Sorensen B. Environmental risk assessment of human pharmaceuticals in Denmark after normal therapeutic use. Chemosphere 2000; 40:783-793.

Stumpf M, Ternes TA, Haberer K, Baumann W. Isolierung von Ibuprofen-Metaboliten und deren Bedeutung als Kontaminanten der aquatischen Umwelt. Vom Wasser 1998; 91:291-303.

Stumpf M, Ternes TA, Haberer K, Seel P., Baumann W. Nachweis von Arzneimittelrückständen in Kläranlagen und Fließgewässern. Vom Wasser 1996; 86:291-303.

Stumpf M, Ternes TA, Wilken R-D, Rodrigues SV, Baumann W. Polar drug residues in sewage and natural waters in the state of Rio de Janeiro, Brazil. The Science of the Total Environment 1999; 225:135-141.

Ternes T. Pharmaceuticals and metabolites as contaminats of the aquatic environment. In: Daughton CG, Jones-Lepp TL edit, Pharmaceuticals and personal care products in the environment, American Chemical Society, Wachington, DC, 2001a,

Ternes TA. Occurrence of Drugs in German Sewage Treatment Plants and Rivers. Water Research 1998; 32:3245-3260.

Ternes TA. Rückstände von Arzneimitteln, Diagnostika und Antiseptika in Abwasser, Flüssen und Grundwasser, Eine neue Herausforderung für die Wasserwirtschaft, Habilitation Thesis, Johannes Gutenberg University, Mainz, 2000.

Ternes TA. Analytical methods for the determination of pharmaceuticals in aqueous environmental samples. Trends in analytical chemistry 2001b; 20:419-434.

Ternes TA. German Case Study: Fate of PPCPs after irrigation in Braunschweigedit. Symposium of the EU-Project Poseidon 2003, Braunschweig, Germany.

Ternes TA, Bonerz M, Schmidt T. Determination of neutral pharmaceuticals in wastewater and rivers by liquid chromatography-electrospray tandem mass spectrometry. Journal of Chromatography A 2001; 938:175-185.

Ternes TA, Herrmann N, Bonerz M, Knacker T, Hansruedi S, Adriano J. A rapid method to measure the solid-water distribution coefficient (Kd) for pharmaceuticals and musk fragrances in sewage sludge. submitted;

Ternes TA, Hirsch R. Occurence and behaviour of X-ray contrast media in sewage facilities and the aquatic environment. Environ. Sci. Technol. 2000; 34:2741-2748.

Ternes TA, Hirsch R, Mueller J, Haberer K. Methods for the determination of neutral drugs as well as betablockers and ß-sympathomimetics in aqueous matrices using GC/MS and LC/MS/MS. Fresenius Journal of Analytical Chemistry 1998a; 362:329-340.

Ternes TA, Kreckel P, Mueller J. Behavior and occurence of estrogens in municipal sewage treatment plants - II. Aerobic batch experiments with activated sludge. The Science of the Total Environment 1999a; 225:91-99.

Ternes TA, Meisenheimer M, McDowell D, Sacher F, Brauch HJ, Gulde BH, Preuss G, Wilme U, Seibert NZ. Removal of pharmaceuticals during drinking water treatment. Environmental Science & Technology 2002a; 36:3855-3863.

Ternes TA, Meisenheimer M, McDowell D, Sacher F, Brauch H-J, Haist-Gulde B, Preuss G, Wilme U, Zulei-Seibert N. Removal of Pharmaceuticals during Drinking Water Treatment. Environ. Sci. Technol. 2002b; 36:3855-63.

Ternes TA, Stumpf M, Mueller J, Haberer K, Wilken R-D, Servos M. Behavior and occurence of estrogens in municipal sewage treatment plants - I. Investigations in Germany, Canada and Brazil. The Science of the Total Environment 1999b; 225:81-90.

Ternes TA, Stumpf M, Schuppert B, Haberer K. Determination of acidic drugs and antiseptics in sewage and river water. Vom Wasser 1998b; 90:295-309.

Tixier C, Singer HP, Oellers S, Müller SR. Occurrence and Fate of Carbamazepine, Clofibric Acid, Diclofenac, Ibuprofen, Ketoprofen, and Naproxen in Surface Waters. Environ. Sci. Technol. 2003; 37:1061-1068.

Tolls J. Sorption of veterinary pharmaceuticals in soils: a review. Environmental Science & Technology 2001; 35:3397-3406.

Tumipseed SB, Roybal JE, Rupp HS, Gonzales SA, Pfenning AP, Hurlbut JA. Confirmation of Avermectin Residues in Food Matrices with Negative-ion Atmospheric Pressure Chemical lonization Liquid Chromatography/Mass Spectrometry. Rapid Communications in Mass Spectrometry 1999; 13:493-499.

Umland-Verband Frankfurt / Region Rhein-Main. Bericht zur Gewässergüte, 1992;15.

Valenzuela AI, Redondo MJ, Pico Y, Font G. Determination of abamectin in citrus fruits by liquid chromatography-electrospray ionization mass spectrometry. Journal of Chromatography A 2000; 871:57-65.

Van der Tenck KT, Markard C, Kühl C, Slama H, Röder R. Ableitung von Geringfügigkeitsschwellen zur Beurteilung von lokal begrenzten Grundwasserverunreinigungen. In: Rosenkranz D, Bachmann G, Einsele G, Harress H-M edit, "Bodenschutz", ergänzbares Handbuch der Maß-nahmen und Empfehlungen für Schutz, Pflege und Sanierung von Böden, Landschaft und Grundwasser, Kennzahl 3605, BoS 29. Lfg. VII/99, Erich Schmidt Verlag, 1999,

Van Wezel AP, Jager T. Comparison of two screening level risk assessment approaches for six disinfectants and pharmaceuticals. Chemosphere 2002; 47:1113-1128.

Velagaleti RR, Robinson J. Regulatory oversight for the environmental assessment of human and animal health drugs - Environmental assessment regulations for drugs. In: Daughton CG, Jones-Lepp TL edit, PPCPs as Environmental Pollutants, American Chemical Society, Washington, D.C., 2001, 320-332.

Webb S, Ternes T, Gibert M, Olejniczak K. Indirect human exposure to pharmaceuticals via drinking water. Toxicology Letters 2003; 142:157-167.

Webb SF. A data based perspective on the environmental risk assessment of human pharamceuticals II - Aquatic risk characterization. In: Kümmerer K edit, Pharmaceuticals in the environment, Springer-Verlag, Berlin, 2001, 203-219.

Weigel S, Bester K, Hühnerfuss H. New method for rapid solid-phase extraction of large- volume water samples and its application to non-target screening of North Sea water for organic contaminants by gas chromatography-mass spectrometry. Journal of Chromatography A 2001; 912:151-161.

Weigel S, Kuhlmann J, Hühnerfuss H. Drugs and personal care products as ubiquitous pollutants: occurence and distribution of clofibric acid, caffeine and DEET in the North Sea. The Science of the Total Environment 2002; 295:131-141.

Winkler M, Lawrence JR, Neu TR. Selective degradation of ibuprofen and clofibric acid in two model river biofilm systems. Water Research 2001; 35:3197-3205.

Wollenberger L, Halling-Sorensen B, Kusk KO. Acute and chronic toxicity of veterinary antibiotics to Daphnia magna. Chemosphere 2000; 40:723-730.

Wu Z, Li J, Zhu L, Luo H, Xu X. Multi-residue analysis of avermectins in swine liver by immunoaffinity extraction and liquid chromatography-mass spectrometry. Journal of Chromatography B 2001; 755:361-366.

Yoshii K, Kaihara A, Tsumura Y, Ishimitsu S, Tonogai Y. Liquid chromatographic determination of emamectin, milbemectin,ivermectin and abamectin in crops and confirmation by liquid chromatography-mass spectrometry. Journal of Chromatography A 2000; 896:75-85.

Zuccato E, Calamari D, Natangelo M, Fanelli R. Presence of therapeutic drugs in the environment. The Lancet 2000; 355:1789-1790.

Zwiener C, Seeger S, Glauner T, Frimmel FH. Metabolites from the biodegradation of pharmaceutical residues of ibuprofen in biofilm reactors and batch experiments. Anal. Bioanal. Chem. 2002; 372:569-575.

10 ANNEX

Properties of water and sediment at the sampling site in the Wickerbach and at the set-up of the test

Study	Sediment+water sampling Wickerbach						Test set-up						
				water		sediment				water			sediment
	Date	T (°C)	pH	O2 (%)	O2 (mg/L)	pH	Date	pH	Redox (mV)	O2 (%)	O2 (mg/L)	pH	Redox (mV)
WS/IO	26/01/2001	4.6	7.8	100		7.3	02/02/2001			73	7.3	7.3	
WS/DIA	03/07/2001	15.9	6.8	92.9	8.9	7.3	17/07/2001	7.5	459	99.7	10.7	7.7	155
WS/IBU + OXA	06/09/2001	12.9	6.3	106	11.1	7.3	17/09/2001	7.5	354	100.1	10.9	7.5	111
WS/PAR + CAN	17/10/2001	12.8	6.5	91	9.7	7.8	23/10/2001	7.7	501	99.3	10.2	7.6	223
WS/COH	07/12/2001	6.2	7.2	94.1	8.9	7.5	14/12/2001	7.6	473	102	11.1	7.5	198
WS/CLO + HI	27/12/2001	6.0	8.2	99		7.6	10/01/2002	8.1	576	105	11.8	7.4	528
WS/IV	26/02/2002	6.0	8.1	101		7.5	18/03/2002	7.7	431	99.8	10.5	7.5	389

Course of the temperature during the experiments

Study	Test vessel-no:		°C													Mean	SD	Min	Max
		Day:	-20	-17	-16	-14	-6	0	1	2	7	14	27	58	100				
WS/IO		21	22.0	21.8	20.5	20.2	21.8	19.7	20.1	20.1	19.3	19.7	20.5	19.9	21.4	20.5	0.9	19.3	22.0
		22	22.0	21.9	20.4	20.2	21.8	19.7	20.1	20.1	19.3	19.7	20.5	19.9	21.4	20.5	0.9	19.3	22.0
		23	21.9	21.9	20.4	20.2	21.8	19.7	20.2	20.0	19.3	19.7	20.5	20.0	21.4	20.5	0.9	19.3	21.9
		24	21.2	21.8	20.4	20.2	21.8	19.7	20.1	19.9	19.3	19.7	20.5	20.0	21.4	20.5	0.8	19.3	21.8
		25	22.1	21.9	20.3	20.3	21.9	19.7	20.1	19.9	19.3	19.7	20.5	19.8	21.4	20.5	1.0	19.3	22.1
		26	21.8	21.9	20.4	20.2	21.9	19.6	20.3	20.0	19.3	19.7	20.5	20.0	21.4	20.5	0.9	19.3	21.9
	Mean/day		21.8	21.9	20.4	20.2	21.8	19.7	20.2	20.0	19.3	19.7	20.5	19.9	21.4				

		Day:	-12	-8	-5	-1	0	1	2	7	14	27	58	100				
WS/DIA		21	21.9	22.4	22.1	22.9	22.9	22.2	22.9	22.3	22.4	22.2	20.6	20.5	22.1	0.8	20.5	22.9
		22	21.8	22.5	22.1	22.9	22.8	22.2	22.5	22.2	22.9	22.0	20.6	20.5	22.1	0.8	20.5	22.9
		23	21.8	22.6	22.0	22.9	22.9	22.2	22.6	22.2	22.4	22.0	20.5	20.5	22.1	0.8	20.5	22.9
		24	21.7	22.5	22.1	22.9	22.9	22.3	22.5	22.2	22.8	22.0	20.3	20.8	22.1	0.8	20.3	22.9
	Mean/day		21.8	22.5	22.1	22.9	22.9	22.2	22.6	22.2	22.6	22.1	20.5	20.6				

		Day:	-4	-2	1	2	8	14	28	56	100				
WS/IBU + OXA		21	21.1	21.2	20.5	20.0	20.5	20.7	20.3	20.8	19.4	20.5	0.6	19.4	21.2
		22	21.1	21.4	20.7	20.1	20.3	20.7	20.2	20.8	19.5	20.5	0.6	19.5	21.4
		23	21.1	21.3	20.8	20.0	20.5	20.7	20.2	20.6	19.4	20.5	0.6	19.4	21.3
		24	21.1	21.5	20.8	20.1	20.5	20.7	20.4	20.5	19.5	20.6	0.6	19.5	21.5
	Mean/day		21.1	21.4	20.7	20.1	20.5	20.7	20.3	20.7	19.5				

		Day:	-4	-2	0	1	2	7	15	29	57	100				
WS/PAR + CAN		21	19.9	20.6	20.7	20.4	20.1	19.3	20.5	20.0	20.5	19.9	20.2	0.4	19.3	20.7
		22	20.0	20.5	20.7	20.4	20.2	19.3	20.4	20.2	20.4	19.9	20.2	0.4	19.3	20.7
		23	20.1	20.5	20.6	20.4	20.2	19.4	20.5	20.0	20.5	19.8	20.2	0.4	19.4	20.6
		24	20.2	20.3	20.7	20.4	20.1	19.3	20.5	20.1	20.5	20.1	20.2	0.4	19.3	20.7
	Mean/day		20.1	20.5	20.7	20.4	20.2	19.3	20.5	20.1	20.5	19.9				

		Day:	-7	-2	0	1	2	8	14	28	56	100				
WS/COH		21	19.6	19.8	19.9	19.8	20.0	20.2	19.9	20.1	20.0	19.9	19.9	0.2	19.6	20.2
		22	19.6	19.8	19.9	19.9	20.0	20.0	19.9	20.0	20.0	20.0	19.9	0.1	19.6	20.0
		23	19.7	19.7	19.9	20.0	20.0	20.1	19.7	20.1	20.0	19.8	19.9	0.2	19.7	20.1
		24	19.8	19.7	19.9	19.9	20.0	20.0	19.8	20.0	20.0	19.9	19.9	0.1	19.7	20.0
	Mean/day		19.7	19.8	19.9	19.9	20.0	20.1	19.8	20.1	20.0	19.9				

		Day:	-6	-2	0	1	2	7	14	28	56	100				
WS/CLO + HI		21	19.7	19.9	20.1	19.8	19.9	20.0	19.9	20.0	20.4	21.6	20.1	0.5	19.7	21.6
		22	19.8	19.9	20.0	19.7	19.9	20.0	19.9	20.2	20.4	21.5	20.1	0.5	19.7	21.5
		23	19.8	19.9	19.9	19.9	20.0	20.0	19.9	20.0	20.6	21.7	20.2	0.6	19.8	21.7
		24	19.8	19.9	20.1	19.8	19.9	20.1	19.9	20.2	20.5	21.6	20.2	0.5	19.8	21.6
	Mean/day		19.8	19.9	20.0	19.8	19.9	20.0	19.9	20.1	20.5	21.6				

		Day:	-1	0	1	2	7	14	29	56	100				
WS/IV		21	20.0	19.8	19.8	20.1	20.1	20.9	20.2	20.0	20.3	20.1	0.3	19.8	20.9
		22	19.9	19.8	19.9	19.9	20.0	20.9	20.2	20.1	20.1	20.1	0.3	19.8	20.9
		23	19.9	19.7	19.9	19.9	19.9	20.9	20.1	20.0	20.1	20.0	0.3	19.7	20.9
		24	19.9	19.8	19.8	20.1	19.9	20.8	20.1	20.0	20.1	20.1	0.3	19.8	20.8
	Mean/day		19.9	19.8	19.9	20.0	20.0	20.9	20.2	20.0	20.2				

Course of the pH in the water during the experiments

Study	Test vessel-no:		pH													Mean	SD	Min	Max	n
		Day:	-20	-17	-16	-14	-6	0	1	2	7	14	27	58	100					
WS/IO		21	8.3	8.2	8.3	8.3	8.3	8.6	8.6	8.3	8.3	8.5	8.8	8.5	8.6	8.4	0.2	8.2	8.8	13
		22	8.2	8.2	8.3	8.3	8.3	8.6	8.6	8.3	8.3	8.4	8.9	8.6	8.6	8.4	0.2	8.2	8.9	13
		23	8.2	8.2	8.3	8.3	8.3	8.5	8.6	8.5	8.6	8.6	8.9	8.7	8.6	8.5	0.2	8.2	8.9	13
		24	8.2	8.3	8.3	8.3	8.3	8.6	8.6	8.6	8.5	8.5	8.9	8.7	8.5	8.5	0.2	8.2	8.9	13
		25	8.2	8.3	8.3	8.3	8.3	8.6	8.6	8.5	8.5	8.5	9	8.8	8.5	8.5	0.2	8.2	9	13
		26	8.2	8.2	8.3	8.3	8.6	8.5	8.6	8.5	8.5	8.6	9	8.8	8.6	8.5	0.2	8.2	9	13
	Mean/day		8.2	8.2	8.3	8.3	8.4	8.6	8.6	8.5	8.5	8.5	8.9	8.7	8.6					

		Day:	-12	-8	-5	-1	0	1	2	7	14	27	58	100					
WS/DIA		21	8.3	8.3	8.3	8.5	8.7	8.6	8.6	8.5	8.4	8.8	8.8	8.8	8.6	0.2	8.3	8.8	12
		22	8.3	8.3	8.3	8.6	8.6	8.5	8.3	8.4	8.5	8.8	8.8	8.8	8.5	0.2	8.3	8.8	12
		23	8.2	8.3	8.3	8.4	8.5	8.4	8.6	8.4	8.4	8.4	8.6	8.6	8.4	0.1	8.2	8.6	12
		24	8.3	8.3	8.3	8.5	8.7	8.6	8.8	8.3	8.3	8.4	8.5	8.6	8.5	0.2	8.3	8.8	12
	Mean/day		8.3	8.3	8.3	8.5	8.6	8.5	8.6	8.4	8.4	8.6	8.7	8.7					

		Day:	-4	-2	1	2	8	14	28	56	100					
WS/IBU + OXA		21	8.4	8.7	8.6	8.5	8.7	8.7	8.7	8.7	8.8	8.6	0.1	8.4	8.8	9
		22	8.4	8.7	8.6	8.5	8.7	8.7	8.7	8.7	8.7	8.6	0.1	8.4	8.7	9
		23	8.4	8.7	8.6	8.8	8.7	8.7	8.7	8.7	8.7	8.7	0.1	8.4	8.8	9
		24	8.4	8.6	8.6	8.8	8.7	8.7	8.7	8.7	8.6	8.6	0.1	8.4	8.8	9
	Mean/day		8.4	8.7	8.6	8.7	8.7	8.7	8.7	8.7	8.7					

		Day:	-4	-2	0	1	2	7	15	29	57	100					
WS/PAR + CAN		21	8.5	8.5	8.5	8.5	8.6	8.6	8.6	8.7	8.7	8.8	8.6	0.1	8.5	8.8	10
		22	8.5	8.5	8.6	8.6	8.6	8.6	8.7	8.7	8.7	8.7	8.6	0.1	8.5	8.7	10
		23	8.5	8.5	8.5	8.5	8.6	8.6	8.6	8.6	8.7	8.7	8.6	0.1	8.5	8.7	10
		24	8.5	8.5	8.5	8.6	8.6	8.6	8.6	8.7	8.7	8.8	8.6	0.1	8.5	8.8	10
	Mean/day		8.5	8.5	8.5	8.6	8.6	8.6	8.6	8.7	8.7	8.8					

		Day:	-7	-2	0	1	2	8	14	28	56	100					
WS/COH		21	8.3	8.3	8.2	8.4	8.5	8.7	8.9	8.9	8.9	8.9	8.6	0.3	8.2	8.9	10
		22	8.4	8.4	8.5	8.5	8.5	8.6	8.9	8.9	8.9	8.9	8.7	0.2	8.4	8.9	10
		23	8.2	8.5	8.4	8.4	8.5	8.7	8.8	8.8	8.9	8.9	8.6	0.2	8.2	8.9	10
		24	8.4	8.3	8.4	8.4	8.4	8.6	8.9	8.9	8.9	8.9	8.6	0.3	8.3	8.9	10
	Mean/day		8.3	8.4	8.4	8.4	8.5	8.7	8.9	8.9	8.9	8.9					

		Day:	-6	-2	0	1	2	7	14	28	56	100					
WS/CLO + HI		21	8.1	8.4	8.4	8.4	8.5	8.6	8.7	8.7	8.7	8.6	8.5	0.2	8.1	8.7	10
		22	8.1	8.3	8.4	8.4	8.4	8.6	8.6	8.7	8.8	8.9	8.5	0.2	8.1	8.9	10
		23	8.2	8.4	8.4	8.4	8.5	8.5	8.7	8.7	8.8	8.9	8.6	0.2	8.2	8.9	10
		24	8.1	8.5	8.5	8.5	8.6	8.6	8.7	8.7	8.8	8.9	8.6	0.2	8.1	8.9	10
	Mean/day		8.1	8.4	8.4	8.4	8.5	8.6	8.7	8.7	8.8	8.8					

		Day:	-1	0	1	2	7	14	29	56	100					
WS/IV		21	7.8	8.0	8.1	8.2	8.8	8.8	8.8	8.7	8.7	8.4	0.4	7.8	8.8	9
		22	7.7	8.1	8.2	8.2	8.7	8.8	8.8	8.9	8.8	8.5	0.4	7.7	8.9	9
		23	7.8	8.0	8.1	8.1	8.8	8.8	8.8	8.7	8.6	8.4	0.4	7.8	8.8	9
		24	7.7	7.9	8.1	8.2	8.8	8.8	8.8	8.7	8.7	8.4	0.4	7.7	8.8	9
	Mean/day		7.8	8.0	8.1	8.2	8.8	8.8	8.8	8.8	8.7					

Course of the redox potential in the water during the experiments

Study	Test vessel-no:		Redox potential in the water measured by a platinum electrode versus a silver/silverchloride reference electrode , c(KCl) 3 mol/L [mV]													Mean	SD	Min	Max	n
		day:	-20	-17	-16	-14	-6	0	1	2	7	14	27	58	100					
WS/IO		21									417	392	375	416	399	400	18	375	417	5
		22										386	382	408	417	398	17	382	417	4
		23										383	388	406	413	398	14	383	413	4
		24										386	393	404	416	400	13	386	416	4
		25										383	396	401	410	398	11	383	410	4
		26										405	396	398	415	404	9	396	415	4
	Mean/day										417	389	388	406	412					
		day:		-12		-8	-5	-1	0	1	2	7	14	27	58	100					
WS/DIA		21		353	417	405	399	397	365	354	320	358	361	381	386	375	28	320	417	12
		22			428	398	400	400	385	372	341	356	373	378	392	384	24	341	428	11
		23			412	395	406	414	392	376	354	368	407	386	402	392	19	354	414	11
		24			423	394	405	408	383	370	349	372	420	397	375	391	23	349	423	11
	Mean/day			353	420	398	403	405	381	368	341	364	390	386	389					
		day:					-4	-2	1	2	8	14	28	56	100					
WS/IBU + OXA		21					404	406	394	367	351	374	369	390	415	386	21	351	415	9
		22					392	406	395	354	349	365	373	367	412	379	23	349	412	9
		23					388	408	397	346	344	332	376	372	411	375	29	332	411	9
		24					379	406	400	345	342	358	349	398	411	376	28	342	411	9
	Mean/day						391	407	397	353	347	357	367	382	412					
		day:				-4	-2	0	1	2	7	15	29	57	100					
WS/PAR + CAN		21				408	393	382	361	390	400	372	352	316	395	377	28	316	408	10
		22				413	376	326	349	395	400	338	376	350	393	372	29	326	413	10
		23				414	388	332	407	349	312	346	375	380	390	369	33	312	414	10
		24				413	364	315	354	353	349	360	411	350	384	365	30	315	413	10
	Mean/day					412	380	339	368	372	365	354	379	349	391					
		day:				-7	-2	0	1	2	8	14	28	56	100					
WS/COH		21				377	314	424	369	396	381	427	386	311	413	380	41	311	427	10
		22				364	363	420	373	324	409	430	371	333	412	380	36	324	430	10
		23				410	314	419	328	389	378	432	334	393	408	381	41	314	432	10
		24				322	356	417	396	316	382	434	387	334	406	375	41	316	434	10
	Mean/day					368	337	420	367	356	388	431	370	343	410					
		day:				-6	-2	0	1	2	7	14	28	56	100					
WS/CLO + HI		21				318	319	395	382	343	381	442	392	325	352	365	40	318	442	10
		22				392	401	393	375	396	364	443	387	364	410	393	23	364	443	10
		23				391	347	354	403	410	323	445	405	392	312	378	42	312	445	10
		24				332	329	372	342	357	363	445	322	393	365	362	36	322	445	10
	Mean/day					358	349	379	376	377	358	444	377	369	360					
		day:					-1	0	1	2	7	14	29	56	100					
WS/IV		21					425	395	393	405	417	390	356	353	340	386	30	340	425	9
		22					405	423	410	405	424	369	350	392	344	391	30	344	424	9
		23					404	409	328	402	434	314	378	315	315	367	48	314	434	9
		24					409	423	401	401	374	368	306	356	366	378	35	306	423	9
	Mean/day						411	413	383	403	412	360	348	354	341					

Course of the oxygen concentration in the water during the experiments

Study	Test vessel-no:		Oxygen concentration [mg/L]													Mean	SD	Min	Max	n
		Day:	-20	-17	-16	-14	-6	0	1	2	7	14	27	58	100					
WS/IO		21	7.5	8.5	8.0	8.2	7.5	7.4	6.5	5.0	5.6	5.3	5.4	6.9	7.1	6.8	1.2	5.0	8.5	13
		22	7.4	8.4	8.1	8.1	7.7	7.6	6.3	5.0	6.6	5.7	5.9	6.9	7.2	7.0	1.0	5.0	8.4	13
		23	7.4	8.4	7.8	8.0	7.7	7.4	7.4	8.0	7.3	7.5	7.6	7.2	7.4	7.6	0.3	7.2	8.4	13
		24	7.9	8.4	8.0	8.1	7.8	8.0	6.7	7.8	7.4	7.3	7.6	7.3	7.4	7.7	0.4	6.7	8.4	13
		25	7.4	8.6	7.9	8.3	7.8	7.1	6.8	7.6	7.1	7.0	7.2	7.4	7.4	7.5	0.5	6.8	8.6	13
		26	7.0	8.4	7.9	8.0	7.3	7.1	7.6	7.6	7.3	7.2	7.1	7.4	7.3	7.5	0.4	7.0	8.4	13
	Mean/day		7.4	8.5	8.0	8.1	7.6	7.4	6.9	6.8	6.9	6.7	6.8	7.2	7.3					
		Day:		-12	-8	-5	-1	0	1	2	7	14	27	58	100					
WS/DIA		21		7.5	7.5	7.4	6.9	6.6	6.4	4.9	5.6	6.7	8.3	7.5	7.3	6.9	0.9	4.9	8.3	12
		22		7.5	7.6	7.4	6.9	6.6	6.1	4.2	6.1	6.2	8.0	7.4	7.5	6.8	1.0	4.2	8.0	12
		23		7.3	7.1	7.5	6.7	7.0	6.9	6.6	6.7	6.9	7.3	7.8	7.6	7.1	0.4	6.6	7.8	12
		24		7.5	7.5	7.4	6.6	7.2	7.1	6.8	4.4	6.1	7.6	8.1	8.3	7.1	1.0	4.4	8.3	12
	Mean/day			7.4	7.4	7.4	6.8	6.9	6.6	5.6	5.7	6.5	7.8	7.7	7.7					
		Day:					-4	-2	1	2	8	14	28	56	100					
WS/IBU + OXA		21					8.0	7.8	6.7	3.2	6.8	6.9	7.4	6.5	8.7	6.9	1.6	3.2	8.7	9
		22					7.8	7.8	6.7	3.6	6.5	6.7	7.6	6.3	8.6	6.8	1.4	3.6	8.6	9
		23					7.5	7.9	7.7	8.0	7.5	7.4	7.4	7.4	8.6	7.7	0.4	7.4	8.6	9
		24					8.0	7.8	7.7	8.0	7.7	7.9	8.0	6.7	8.6	7.8	0.5	6.7	8.6	9
	Mean/day						7.8	7.8	7.2	5.7	7.1	7.2	7.6	6.7	8.6					
		Day:					-4	-2	0	1	2	7	15	29	57	100				
WS/PAR + CAN		21					8.1	8.1	8.3	8.5	8.7	7.8	7.5	6.9	8.9	9.2	8.2	0.7	6.9	9.2	10
		22					8.2	7.9	8.8	8.9	8.4	7.4	6.7	6.3	8.6	9.5	8.1	1.0	6.3	9.5	10
		23					8.2	8.1	8.9	8.6	7.7	7.6	6.8	7.5	8.4	9.1	8.1	0.7	6.8	9.1	10
		24					8.1	8.7	8.7	8.9	7.6	7.5	8.2	7.5	8.0	9.3	8.3	0.6	7.5	9.3	10
	Mean/day						8.2	8.2	8.7	8.7	8.1	7.6	7.3	7.1	8.5	9.3				
		Day:					-7	-2	0	1	2	8	14	28	56	100				
WS/COH		21					8.0	7.8	8.1	7.6	7.6	8.2	9.0	7.8	8.0	7.6	8.0	0.4	7.6	9.0	10
		22					7.4	7.6	8.5	7.7	8.0	8.5	9.3	8.5	8.8	8.5	8.3	0.6	7.4	9.3	10
		23					8.0	8.2	8.4	7.4	7.8	8.8	9.2	8.1	7.3	8.2	8.1	0.6	7.3	9.2	10
		24					7.8	8.7	8.4	7.5	8.6	8.7	9.2	8.6	7.8	8.2	8.4	0.5	7.5	9.2	10
	Mean/day						7.8	8.1	8.4	7.6	8.0	8.6	9.2	8.3	8.0	8.1				
		Day:					-6	-2	0	1	2	7	14	28	56	100				
WS/CLO + HI		21					7.7	7.7	7.7	7.7	9.0	7.2	8.8	8.4	8.4	8.6	8.1	0.6	7.2	9.0	10
		22					8.6	7.5	8.8	7.5	7.8	7.0	8.6	8.0	8.7	8.5	8.1	0.6	7.0	8.8	10
		23					7.5	8.5	8.8	8.5	8.5	8.0	8.5	7.9	8.5	8.3	8.3	0.4	7.5	8.8	10
		24					7.4	7.7	8.2	7.7	8.2	7.5	8.6	8.8	8.6	8.2	8.1	0.5	7.4	8.8	10
	Mean/day						7.8	7.9	8.4	7.9	8.4	7.4	8.6	8.3	8.6	8.4				
		Day:						-1	0	1	2	7	14	29	56	100				
WS/IV		21						7.9	8.0	8.0	9.1	9.3	7.9	9.6	8.2	7.8	8.4	0.7	7.8	9.6	9
		22						7.9	8.0	9.0	8.4	9.5	8.0	9.5	8.0	8.8	8.6	0.7	7.9	9.5	9
		23						8.4	9.0	7.9	8.5	9.4	7.9	9.3	8.3	8.1	8.5	0.6	7.9	9.4	9
		24						8.7	8.2	7.6	8.6	9.4	7.9	9.4	8.7	8.0	8.5	0.6	7.6	9.4	9
	Mean/day							8.2	8.3	8.1	8.7	9.4	7.9	9.5	8.3	8.2				

Course of the pH in the sediment during the experiments

Study-name:	Test vessel-no:		pH												Mean	SD	Min	Max	n
		Day:	-20	-17	-16	-14	-6	0	1	2	7	14	27	58	100					
WS/IO		21							7.8	7.8	7.7	7.6	7.7	7.6	7.6	7.7	0.1	7.6	7.8	7
		22							7.8	7.7	7.7	7.6	7.7	7.7	7.5	7.7	0.1	7.5	7.8	7
		23							7.7	7.6	7.4	7.5	7.6	7.6	7.5	7.6	0.1	7.4	7.7	7
		24							7.7	7.6	7.5	7.5	7.6	7.5	7.5	7.6	0.1	7.5	7.7	7
		25											7.6	7.5	7.5	7.5	0.1	7.5	7.6	3
		26											7.6	7.7	7.5	7.6	0.1	7.5	7.7	3
	Mean/day								7.8	7.7	7.6	7.6	7.6	7.6	7.5					
		Day:	-12		-8	-5	-1	0	1	2	7	14	27	58	100					
WS/DIA		21	7.5		7.7	7.7	7.7	7.3	7.6	7.1	7.1	7.4	7.2	7.4	7.4	7.4	0.2	7.1	7.7	12
		22			7.7	7.7	7.6	7.7	7.3	7.3	7.3	7.2	7.3	7.6	7.4	7.5	0.2	7.2	7.7	11
		23	7.3		7.6	7.7	7.6	7.8	7.5	7.9	8.0	7.6	7.7	7.9	7.8	7.7	0.2	7.3	8.0	12
		24	7.4		7.6	7.7	7.7	7.7	7.4	7.9	7.8	7.5	7.7	7.9	7.8	7.7	0.2	7.4	7.9	12
	Mean/day		7.4		7.7	7.7	7.7	7.6	7.5	7.6	7.6	7.4	7.5	7.7	7.6					
		Day:					-4	-2	1	2	8	14	28	56	100					
WS/IBU + OXA		21					7.7	7.9	8.0	8.0	7.9	7.9	7.9	7.9	8.0	7.9	0.1	7.7	8.0	9
		22					7.6	7.8	7.8	7.9	7.9	7.9	7.9	7.9	7.9	7.8	0.1	7.6	7.9	9
		23					7.6	8.0	7.8	7.9	7.9	7.9	7.9	7.9	7.9	7.9	0.1	7.6	8.0	9
		24					7.6	7.9	7.8	7.9	7.9	7.9	7.9	7.9	7.6	7.8	0.1	7.6	7.9	9
	Mean/day						7.6	7.9	7.9	7.9	7.9	7.9	7.9	7.9	7.9					
		Day:					-4	-2	0	1	2	7	15	29	57	100				
WS/PAR + CAN		21					7.7	7.7	7.7	7.7	7.7	7.6	7.7	7.8	7.7	7.7	7.7	0.0	7.6	7.8	10
		22					7.7	7.7	7.7	7.6	7.7	7.7	7.7	7.7	7.7	7.7	7.7	0.0	7.6	7.7	10
		23					7.6	7.6	7.6	7.7	7.6	7.7	7.7	7.8	7.8	7.9	7.7	0.1	7.6	7.9	10
		24					7.6	7.6	7.6	7.6	7.7	7.7	7.7	7.8	7.9	7.9	7.7	0.1	7.6	7.9	10
	Mean/day						7.7	7.7	7.7	7.7	7.7	7.7	7.7	7.8	7.8	7.8					
		Day:					-7	-2	0	1	2	8	14	28	56	100				
WS/COH		21					7.5	7.6	7.6	7.6	7.7	7.8	7.9	7.9	8.0	7.9	7.8	0.2	7.5	8.0	10
		22					7.6	7.7	7.7	7.7	7.7	7.8	7.9	7.8	7.8	7.7	7.7	0.1	7.6	7.9	10
		23					7.5	7.7	7.8	7.8	7.8	7.9	8.2	8.0	8.1	8.0	7.9	0.2	7.5	8.2	10
		24					7.5	7.6	7.7	7.7	7.7	7.8	8.0	8.0	8.1	8.1	7.8	0.2	7.5	8.1	10
	Mean/day						7.5	7.7	7.7	7.7	7.7	7.8	8.0	7.9	8.0	7.9					
		Day:					-6	-2	0	1	2	7	14	28	56	100				
WS/CLO + HI		21					7.5	7.7	7.7	7.7	7.6	7.8	7.9	7.8	7.8	7.6	7.7	0.1	7.5	7.9	10
		22					7.4	7.6	7.6	7.6	7.7	7.7	7.9	7.9	7.8	7.7	7.7	0.2	7.4	7.9	10
		23					7.5	7.5	7.5	7.6	7.5	7.8	7.9	7.9	7.9	8.0	7.7	0.2	7.5	8.0	10
		24					7.6	7.6	7.5	7.6	7.6	7.7	7.8	7.8	7.9	7.8	7.7	0.1	7.5	7.9	10
	Mean/day						7.5	7.6	7.6	7.6	7.6	7.8	7.9	7.9	7.9	7.8					
		Day:					-1	0	1	2	7	14	29	56	100					
WS/IV		21					7.7	7.8	7.8	7.8	8.1	7.8	7.6	7.7	7.5	7.8	0.2	7.5	8.1	9
		22					7.6	7.7	7.8	7.7	7.8	7.8	8.0	7.7	7.7	7.8	0.1	7.6	8.0	9
		23					7.8	7.8	7.8	7.9	7.9	7.9	7.8	7.8	7.6	7.8	0.1	7.6	7.9	9
		24					7.6	7.6	7.5	7.6	8.0	7.9	7.6	7.7	7.8	7.7	0.2	7.5	8.0	9
	Mean/day						7.7	7.7	7.7	7.8	8.0	7.9	7.8	7.7	7.7					

Course of the redox potential in the sediment during the experiments

Study- name	Test vessel-no:		Redox potential in the water measured by a platinum electrode versus a silver/silverchloride reference electrode , c(KCl) 3 mol/L [mV]													Mean	SD	Min	Max
		day:	-20	-17	-16	-14	-6	0	1	2	7	14	27	58	100				
WS/IO		21								417	392	375	416	399		400	18	375	417
		22									386	382	408	417		398	17	382	417
		23									383	388	406	413		398	14	383	413
		24									386	393	404	416		400	13	386	416
		25									383	396	401	410		398	11	383	410
		26									405	396	398	415		404	9	396	415
	Mean/day									417	389	388	406	412					
		day:	-12		-8	-5	-1	0	1	2	7	14	27	58	100				
WS/DIA		21	353		417	405	399	397	365	354	320	358	361	381	386	375	28	320	417
		22			428	398	400	400	385	372	341	356	373	378	392	384	24	341	428
		23			412	395	406	414	392	376	354	368	407	386	402	392	19	354	414
		24			423	394	405	408	383	370	349	372	420	397	375	391	23	349	423
	Mean/day		353		420	398	403	405	381	368	341	364	390	386	389				
		day:					-4	-2	1	2	8	14	28	56	100				
WS/IBU + OXA		21					404	406	394	367	351	374	369	390	415	386	21	351	415
		22					392	406	395	354	349	365	373	367	412	379	23	349	412
		23					388	408	397	346	344	332	376	372	411	375	29	332	411
		24					379	406	400	345	342	358	349	398	411	376	28	342	411
	Mean/day						391	407	397	353	347	357	367	382	412				
		day:				-4	-2	0	1	2	7	15	29	57	100				
WS/PAR + CAN		21				408	393	382	361	390	400	372	352	316	395	377	28	316	408
		22				413	376	326	349	395	400	338	376	350	393	372	29	326	413
		23				414	388	332	407	349	312	346	375	380	390	369	33	312	414
		24				413	364	315	354	353	349	360	411	350	384	365	30	315	413
	Mean/day					412	380	339	368	372	365	354	379	349	391				
		day:				-7	-2	0	1	2	8	14	28	56	100				
WS/COH		21				377	314	424	369	396	381	427	386	311	413	380	41	311	427
		22				364	363	420	373	324	409	430	371	333	412	380	36	324	430
		23				410	314	419	328	389	378	432	334	393	408	381	41	314	432
		24				322	356	417	396	316	382	434	387	334	406	375	41	316	434
	Mean/day					368	337	420	367	356	388	431	370	343	410				
		day:				-6	-2	0	1	2	7	14	28	56	100				
WS/CLO + HI		21				318	319	395	382	343	381	442	392	325	352	365	40	318	442
		22				392	401	393	375	396	364	443	387	364	410	393	23	364	443
		23				391	347	354	403	410	323	445	405	392	312	378	42	312	445
		24				332	329	372	342	357	363	445	322	393	365	362	36	322	445
	Mean/day					358	349	379	376	377	358	444	377	369	360				
		day:					-1	0	1	2	7	14	29	56	100				
WS/IV		21					425	395	393	405	417	390	356	353	340	386	30	340	425
		22					405	423	410	405	424	369	350	392	344	391	30	344	424
		23					404	409	328	402	434	314	378	315	315	367	48	314	434
		24					409	423	401	401	374	368	306	356	366	378	35	306	423
	Mean/day						411	413	383	403	412	360	348	354	341				

Water content, microbial, biomass, C_{org} of the sediment and TOC of the water at the beginning and the end of the experiments

Study	Sampling day	Water content % weight of dry weight	Microbial biomass Cmic [µg C/g sediment (TG)]	Corg org. C in % of dry weight	TOC [mg C/L]
WS/IO	0	19.7	27.4	1.7	2.7
	100	22.8	22.6	2.1	3.7
WS/DIA	0	16.6	32.4	1.5	2.3
	100	17.9	33.7	1.7	8.1
WS/IBU + OXA	0	21.3	53.9	1.5	2.2
	100	22.5	21.8	1.5	2.3
WS/PAR + CAN	0	20.6	84.3	1.4	8.2
	100	19.4	51.9	1.7	8.0
WS/COH	0	18.1	52.0	1.0	3.0
	100	18.2	29.1	1.2	3.9
WS/CLO +HI	0	15.8	47.5	1.0	2.3
	100	18.8	36.7	1.0	5.0
WS/IV	0	19.7	43.5	1.0	3.0
	100	19.4	32.5	1.0	4.2