Temporal and spatial trends of pharmaceuticals in the Rhine

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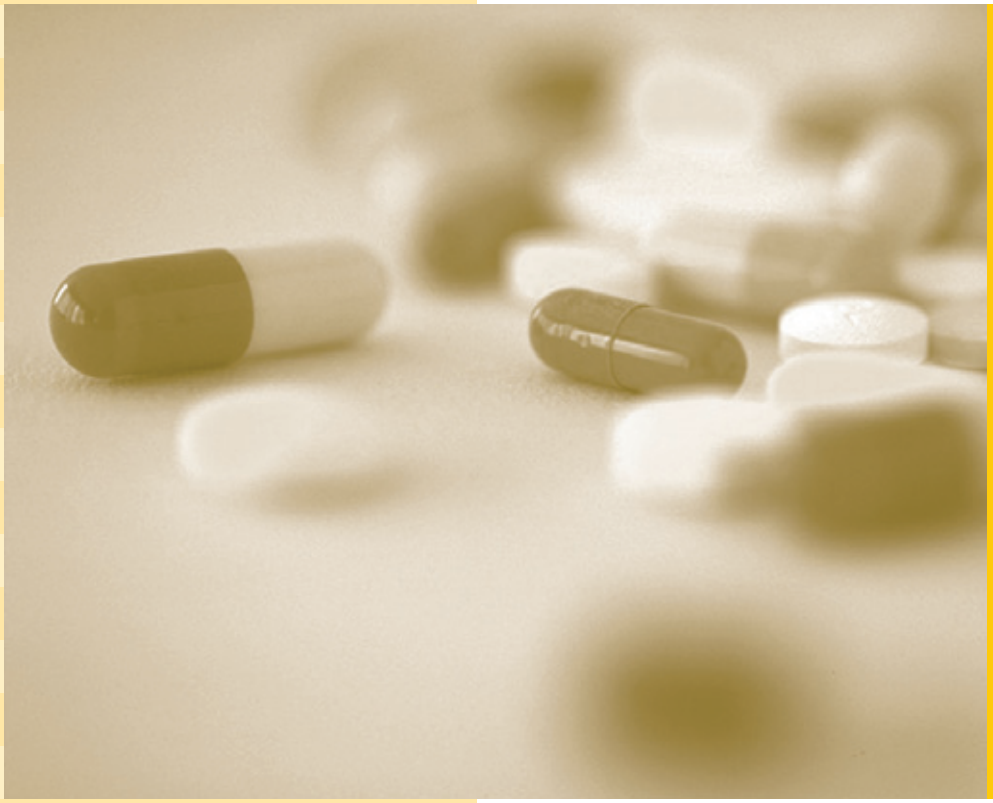

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Consumptie van geneesmiddelen in het Rijnstroomgebied en aangetroffen en voorspelde vrachten in de Rijn bij Lobith

Geneesmiddel of rontgencontrastmiddel	Consumptie (kg/jr)	Vracht (kg/jr)	Uitscheiding door mens in feces en urine (% van totaal)	Verwijdering door afvalwater-zuivering (% van totaal)	Gevonden residue (% van consumptie)	Voorspeld residue (% van consumptie)
Roxithromycin (A)	3665	1073	60%	37%	29.3%	37.7%
Clarithromycin (A)	7784	1055	18%	45%	13.6%	10.0%
Clindamycin (A)	5660	1380	19%	?	24.4%	19.0%
Erythromycin-A (A)	10677	2191	98%	67%	20.5%	32.0%
Sulfamethoxazol (A)	26713	2491	20%	59%	9.3%	8.1%
Trimethoprim (A)	6040	502	45%	16%	8.3%	37.6%
Atenolol (B)	9501	1299	83%	8%	13.7%	76.7%
Metoprolol (B)	32354	2132	11%	10%	6.6%	9.9%
Sotalol (B)	12132	3538	100%	11%	29.2%	94.3%
Pentoxifylline (B)	50930	3906	7%	?	7.7%	7.0%
Bezafibrate (C)	19842	2877	51%	68%	14.5%	16.4%
Carbamazepine (G)	43761	6184	26%	9%	14.1%	23.7%
Ibuprofen (D)	131592	1512	30%	74%	1.1%	7.7%
Diclofenac (D)	41354	4102	16%	32%	9.9%	10.9%
Ioxitalaminic acid (F)	7819	1565	>95%	0%	20.0%	95.0%
Iopromide (F)	36416	14024	>92%	61%	38.5%	35.9%
Iohexol (F)	9764	5938	100%	?	60.8%	100.0%
Iomeprol (F)	24180	12210	100%	9%	50.5%	91.0%
Iopamidol (F)	21181	14922	90%	0%	70.4%	90.0%
Amidotrizoinic acid (F)	25608	12874	>95%	8%	50.3%	87.4%

A = Antibioticum, B = Beta-blocker, C = Cholesterolverlager, D = ontstekingsremmer/pijnstiller,
F = Röntgencontrastmiddel, G = Anti-epilepticum.

Uitgebreide samenvatting

Geneesmiddelen in de Rijn; trends en gevonden concentraties in relatie tot consumptie.

Verschillende drinkwaterbedrijven in Zwitserland, Duitsland en Nederland gebruiken Rijnwater als bron voor drinkwater. De Rijn wordt echter ook gebruikt om (gezuiverd) afvalwater af te voeren naar zee. Het gezuiverde afvalwater bevat resten van geneesmiddelen, waardoor verschillende geneesmiddelen, röntgencontrastmiddelen en endocrien verstorende chemicaliën in de Rijn worden aangetroffen. Het doel van deze studie is om een beter beeld te krijgen van concentraties van geneesmiddelen in de Rijn op basis van een uitgebreide dataset. Hierbij is specifiek gekeken naar variaties van deze concentraties in de ruimte en in de tijd. Daarnaast is ook bestudeerd hoe deze concentraties gerelateerd zijn aan het gebruik van deze middelen door de inwoners van het Rijnstroomgebied. Tevens is onderzocht in hoeverre gebruiksgegevens, metabolisme en afbraak in afvalwaterzuiveringsinstallaties de hoeveelheden in de Rijn kunnen voorspellen.

Om dit te bewerkstelligen is gebruik gemaakt concentraties van geneesmiddelen in de Rijn in Nederland. De meetgegevens zijn verstrekt door RIWA, de Vereniging van Rijnwaterbedrijven. Het Rijnwater is tussen januari 2002 en december 2008 minstens één maal per maand bemonsterd op 9 locaties in Zwitserland (1) Duitsland (4) en Nederland (4). In deze monsters is naar 128 verschillende geneesmiddelen, röntgencontrastmiddelen en endocrien verstorende stoffen gezocht. Van deze stoffen worden enkele tientallen frequent in het Rijnwater aangetroffen in concentraties van 0.01 tot 1 µg/L.

De concentraties van geneesmiddelen en röntgencontrastmiddelen variëren tot wel twee orde grootten. Een deel van deze variatie is toe te schrijven aan de variatie in het debiet van de Rijn. Echter de variatie van de gemiddelde concentraties tussen de 9 locaties is maximaal een factor 7. De vrachten van de ontstekingremmers ibuprofen en diclofenac, de cholesterolverlager bezafibraat en de antibiotica trimetoprim en erythromycine A vertonen duidelijke seizoensgebonden trends, met hogere hoeveelheden in de winter en lagere hoeveelheden in de zomer. Deze seizoensvariatie is waarschijnlijk een gevolg van temperatuurgerelateerde afbraak in de afvalwaterzuivering en milieu, en seizoensgebonden consumptie patronen.

Tientallen tonnen röntgencontrastmiddelen worden jaarlijks door de Rijn afgevoerd. Daarnaast worden ook enkele tonnen van carbamazepine, diclofenac, pentoxifylline, sotalol, etc. door de Rijn afgevoerd. Deze vrachten zijn vergeleken met consumptie van deze stoffen bovenstrooms van Lobith. Gemiddeld blijkt dat 25% van de jaarlijks gebruikte hoeveelheid van de 20 meest gemeten geneesmiddelen en röntgencontrastmiddelen Lobith passeert. Er is echter wel veel variatie tussen de verschillende geneesmiddelen (zie tabel). Het teruggevonden percentage wordt bepaald door de afbraak in de mens door metabolisme, de afbraak in de afvalwaterzuivering en de afbraak in het milieu. Met literatuurgegevens van het metabolisme en de afbraak in de afvalwaterzuivering kan vrij goed voorspeld worden hoeveel van de gebruikte geneesmiddelen jaarlijks in de Rijn terecht komen. De voorspelde hoeveelheden van 15 van de 20 geselecteerde geneesmiddelen verschillen minder dan een factor 2 van de daadwerkelijk aangetroffen hoeveelheden.

Arzneimittelkonsum im Rheineinzugsgebiet und ermittelte sowie vorhergesagte Frachten im Rhein bei Lobith

Arzneimittel oder Röntgenkontrastmittel	Konsum (kg)	Fracht (kg)	Menschl. Ausscheidung - Fäzes und Urin (% der Gesamtmenge)	Entfernung in Kläranlagen (% der Gesamtmenge)	Nachgewiesene Rückstände (% des Konsums)	Vorhergesagte Rückstände (% des Konsums)
Roxithromycin (A)	3665	1073	60%	37%	29,3%	37,7%
Clarithromycin (A)	7784	1055	18%	45%	13,6%	10,0%
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A = Antibiotikum, B = Betablocker, C = Cholesterinsenkende Mittel,
D = Entzündungshemmer/Schmerzmittel, F = Röntgenkontrastmittel, G = Anti-Epileptikum.

Ausführliche Zusammenfassung

Arzneimittel im Rhein; Trends und ermittelte Konzentrationen im Verhältnis zum Konsum.

Verschiedene Wasserversorgungsunternehmen in der Schweiz, Deutschland und den Niederlanden verwenden Rheinwasser als Trinkwasserquelle. Der Rhein wird aber auch genutzt, um (geklärtes) Abwasser ins Meer abzuführen. Das geklärte Abwasser enthält Arzneimittelrückstände, was zum Nachweis verschiedener Arzneimittel, Röntgenkontrastmittel und endokrin wirksamer Chemikalien im Rhein geführt hat. Ziel dieser Studie ist es, auf der Grundlage einer umfangreichen Datenreihe einen besseren Einblick in die Arzneimittelkonzentrationen im Rhein zu erhalten. Besondere Aufmerksamkeit wurde dabei räumlichen und zeitlichen Variationen dieser Konzentrationen geschenkt. Daneben wurde auch untersucht, wie diese Konzentrationen mit dem Gebrauch dieser Mittel seitens der Einwohner des Rheineinzugsgebiets zusammenhängen. Ferner wurde geprüft, inwieweit mithilfe von Benutzungsdaten sowie Informationen über Stoffwechsel und Abbau der Stoffe in Abwasserkläranlagen die im Rhein vorgefunden Mengen vorhergesagt werden können.

Um eine Antwort auf die oben aufgeführten Punkte zu bekommen, wurden die Arzneimittelkonzentrationen im Rhein in den Niederlanden näher betrachtet. Die Messdaten wurden vom Verband der Flusswasserwerke, RIWA, zur Verfügung gestellt. Dem Rhein wurden zwischen Januar 2002 und Dezember 2008 mindestens einmal monatlich an 9 Standorten in der Schweiz, (1) Deutschland (4) und den Niederlanden (4) Wasserproben entnommen. Diese Proben wurden auf 128 verschiedene Arzneimittel, Röntgenkontrastmittel und endokrin wirksame Stoffe untersucht. Einige Dutzend dieser Stoffe wurden häufig im Rheinwasser in Konzentrationen von 0,01 bis 1 µg/l nachgewiesen.

Es wurden Variationen der Arznei- und Röntgenkontrastmittelkonzentrationen in bis zu zwei Größenordnungen festgestellt. Diese Variationen sind zum Teil auf die unterschiedlichen Durchflussmengen des Rheins zurückzuführen. Die Variation der durchschnittlichen Konzentrationen an den 9 Standorten entspricht maximal dem Faktor 7. Die Frachten der Entzündungshemmer Ibuprofen und Diclofenac, des cholesterinsenkenden Mittels Bezafibrat und der Antibiotika Trimetoprim und Erythromycin A lassen deutlich saisonale Trends erkennen, wobei höhere Mengen im Winter und niedrigere Mengen im Sommer ermittelt wurden. Diese saisonale Variation ist wahrscheinlich auf den temperaturbedingten Abbau dieser Stoffe in den Abwasserkläranlagen und der Umwelt sowie auf saisonbedingtes Konsumverhalten zurückzuführen.

Dutzende Tonnen Röntgenkontrastmittel werden jährlich vom Rhein abgeführt. Daneben werden auch einige Tonnen Carbamazepin, Diclofenac, Pentoxifyllin, Sotalol usw. vom Rhein abgeführt. Diese Frachten wurden mit dem Konsum dieser Stoffe stromaufwärts von Lobith verglichen. Es stellte sich heraus, dass durchschnittlich 25% der jährlich verwendeten Durchschnittsmenge der 20 am häufigsten gemessenen Arzneimittel und Röntgenkontrastmittel Lobith passiert. Es gibt allerdings viele Variationen zwischen den verschiedenen Arzneimitteln (siehe Tabelle). Der ermittelte Prozentsatz wird vom Abbau der Stoffe im menschlichen Stoffwechsel, in Abwasserkläranlagen und der Umwelt bestimmt. Mithilfe von Literaturdaten bezüglich des Stoffwechsels und des Abbaus der Stoffe in Abwasserkläranlagen lässt sich die Menge der verwendeten Arzneimittel, die jährlich in den Rhein gelangt, ziemlich genau vorhersagen. Die vorhergesagten Mengen von 15 der 20 ausgewählten Arzneimittel unterscheiden sich weniger als einen Faktor 2 von den tatsächlich nachgewiesenen Mengen.

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Contents

Summary

Over the last decade, various studies have investigated the occurrence of pharmaceuticals in surface waters. Generally studies use a limited number of samples in time and / or space, which do not enable to investigate temporal and spatial trends and annual fluxes of pharmaceuticals in surface waters. This study uses an exceptionally large dataset; 48 to 127 pharmaceuticals, X-ray contrast media and endocrine disrupting chemicals were monitored at 9 sampling locations along the river Rhine in Switzerland (1), Germany (4) and the Netherlands (4) over a period of 7 years, resulting in over 5000 positive detections of pharmaceuticals in the aqueous samples. In this study both spatial variation in concentrations of pharmaceuticals, and temporal variation at the Dutch sampling locations Lobith and Nieuwegein were studied. The obtained information was compared to literature data on the occurrence of pharmaceuticals in the aqueous environment and interpreted in relation to consumption of pharmaceuticals in the Rhine catchment area.

X-ray contrast media (e.g. iomeprol, iopamidol, iopromide) showed the highest concentrations that exceeded 0.1 µg/L, while concentrations of various other pharmaceuticals (e.g. carbamazepine, bezafibrate, diclofenac, ibuprofen, pentoxifylline, sotalol, sulfamethoxazol) varied between 0.2 and 0.01 µg/L. Concentrations below 0.01 µg/L were generally not recorded because of analytical limitations. The monitoring data reveal that concentrations of frequently detected pharmaceuticals slightly increase over the course of the river Rhine. Nevertheless, ratios of the median values of the chemicals never exceed a factor 7 at the different sampling locations. Temporal trends were usually not observed at the Dutch locations. However, concentrations of carbamazepine, bezafibrate and diclofenac significantly decreased with a factor 2 while two X-ray contrast media (iohexol and iomeprol) significantly increased with a factor 2.5 between 2002 and 2008. Additionally, some pharmaceuticals (diclofenac, ibuprofen, bezafibrate, anhydro-erythromicine-A and trimethoprim) showed clear seasonal trends. High loads entered the Netherlands in the winter and up to 10 times lower loads in summer. These trends can be a result of increased degradation in the waste water treatment (and river) as a result of higher temperatures or variations in consumption.

Furthermore, it was observed that 25% (1-70%) of the pharmaceuticals consumed by the inhabitants of the Rhine catchment area was recovered in the Rhine. For 15 out of 20 chemicals the actual recovered fractions deviated less than a factor 2 from predicted fractions based on literature data on consumption, fractions excreted by the users and removal in the wastewater treatment. This analysis illustrates that consumption and information can be used as an initial estimate of average environmental concentrations if no monitoring data are available.

1

Introduction

Various drinking water companies use Rhine water as a source for their drinking water production, or have wells that are in close contact with Rhine water. Approximately 58 million people are living in the catchment area of the river Rhine, of which 5.0 million live in Switzerland, 36.9 million live in Germany, 3.7 million live in France, 11.5 million live in the Netherlands, and 0.8 million live in Luxembourg, Austria, Belgium and Lichtenstein (1). 30 million people in Europe depend on drinking water from the Rhine (IAWR) of which 3.8 million live in the Netherlands. Additionally, another 1 million Dutch citizens consume drinking water from wells that might be in contact with Rhine water (river bank infiltration). The Rhine drains treated sewage effluents of a large fraction of the inhabitants of the Rhine catchment area to the North Sea. These effluents contain numerous contaminants such as human pharmaceuticals. Pharmaceuticals are usually designed to have a specific biological effect at low concentrations, so their presence might pose a threat to both the ecosystem and drinking water production. Consequently, their concentrations in the aqueous environment and aquifers that are used for drinking water sources should be monitored, and the risks should be assessed to guarantee pristine drinking water.

Numerous international and national studies have shown the presence of pharmaceuticals, X-ray contrast media and endocrine disrupting agents in the aqueous environment (2-13). The occurrence of pharmaceuticals in the aqueous environment in rivers depends on various factors. These factors are 1) the amount that is consumed in the catchment area (14), 2) the fraction that leaves the user (human, cattle, pet animal) unchanged or as conjugate (15), 3) sorption and degradation processes in the waste water treatment (to sludge) (16, 17), 4) the volume of water that is drained by a river and 5) sorption and degradation processes in the environment (18). It is, therefore, interesting to investigate how concentrations in the Rhine are related to the consumption of human pharmaceuticals, the hydrological characteristics of the Rhine and the physicochemical properties of the pharmaceuticals.

The extensive dataset obtained from the International Association of Water works in the Rhine Basin (IAWR) was used to study temporal variations and spatial trends of pharmaceuticals in the Rhine. The objective of this study is to reveal spatial, temporal and seasonal trends in concentrations over the course of the river Rhine. Additionally, concentrations in the Rhine are related to the consumption of these pharmaceuticals by the inhabitants of the Rhine catchment area. This gives information on the stability of environmental concentrations, might help to understand processes that affect concentrations in Rhine water, and allows predicting loads in the surface waters from (changing) consumption patterns of pharmaceuticals (14) .

2 Material and Methods

Monitoring data of the period 2002-2008 were obtained from the International Association of Waterworks of the Rhine (IAWR). Samples were taken at 9 locations along the River Rhine, of which 1 was situated in Switzerland (Basel-Birsfelden), 4 in Germany (Karlsruhe, Mainz, Koln, Düsseldorf-Flehe (R), and 4 in the Netherlands (Lobith, Nieuwegein, Nieuwersluis and Andijk). The dataset contains over 5000 individual observations of pharmaceuticals and endocrine disrupting chemicals (Figure 1). The samples of the locations downstream of Lobith were analyzed by Omegam Laboratories, Amsterdam, the Netherlands. All other samples were analyzed by DVGW-Technologiezentrum Wasser, Karlsruhe, Germany.

1 L grab water samples were collected at the nine locations along the Rhine. Samples were taken in pre-rinsed bottles of green glass, kept cool and immediately transported to the laboratory. A volume of one liter of each sample was extracted with solid phase extraction using Waters Oasis HLB cartridges and elution with methanol. An internal standard compound was added to each extract to enable assessment of the recovery of the analysis. Pharmaceuticals were analyzed on a liquid chromatograph coupled to a mass selective detector operated in single ion mode. At least two masses per compound were monitored and their average response was used for quantification. Quantification was performed by comparison with external calibration standards. X ray contrast media were analyzed on a liquid chromatograph coupled to a triple quadrupole mass spectrometric detector operated in multiple reaction monitoring mode (13, 19). Recoveries of the presented compounds in this study fell between 50 and 120 % with the exception of sulfamethoxa-zol (41%) and limits of quantification were 10 ng/L, except of trimethoprim that had a limit of quantification of 5 ng/L.

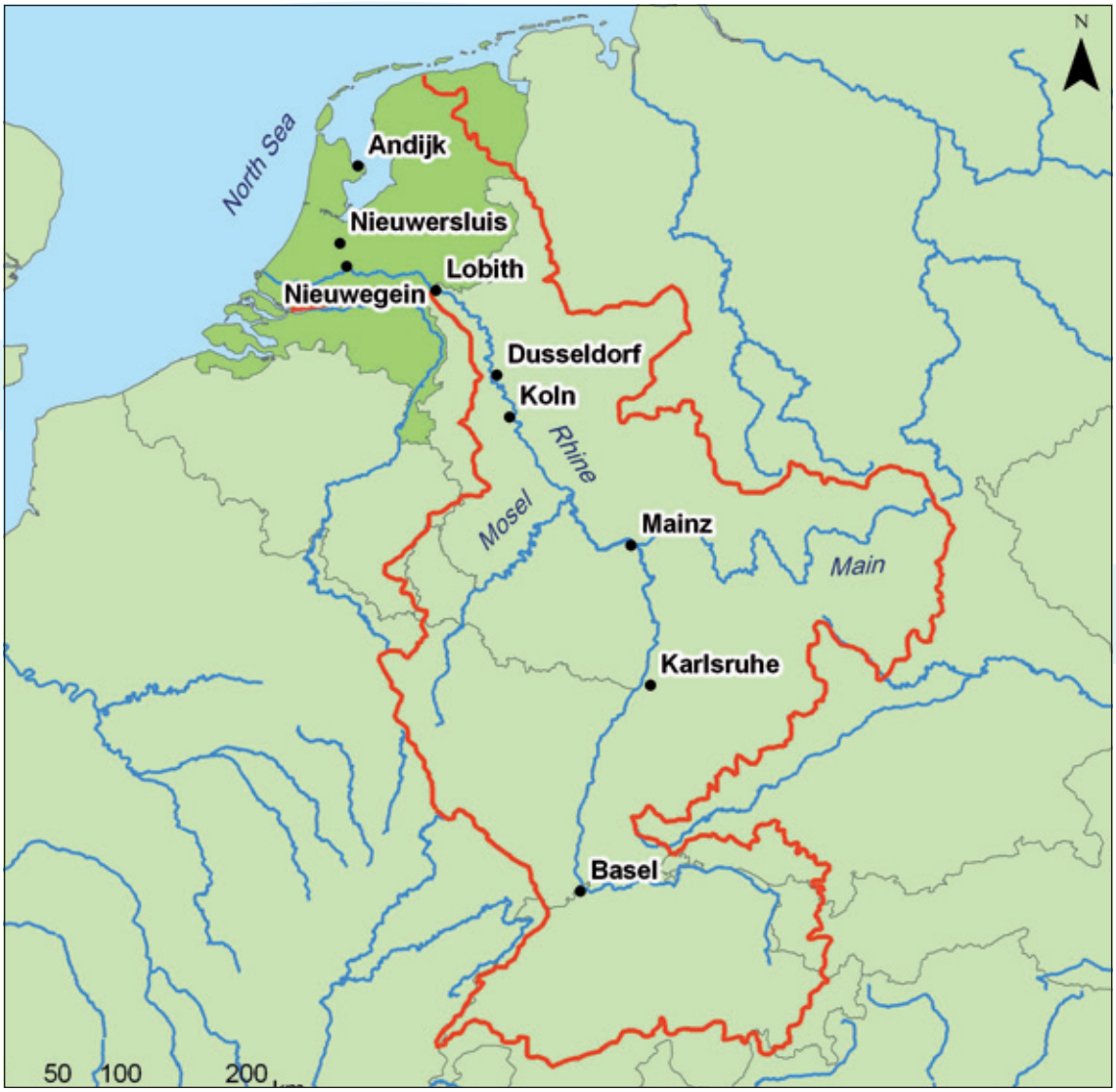

Figure 1: The sampling locations along the Rhine, the red line is the border of the Rhine catchment area. The distances of the sampling locations from the Bodensee are: Basel = 164 km, Karlsruhe = 359 km, Mainz = 501 km, Koln = 686 km, Dusseldorf = 722 km, Lobith = 860 km, Nieuwegein = 950 km.

Table 1: List of analyzed and detected chemicals and their maximum concentrations

Pharmaceutical	Pharm. class	N	Obs. at N	%>LOD	Max. conc. (µg/L)
Amoxicilline	A	74	0	0.0%	
Anhydro-erythromycine-A	A	137	5	67.9%	0.11
Azithromycine	A	251	0	0.0%	
Chloroamfenicol	A	286	1	1.0%	0.051
Chlorotetracycline	A	38	0	0	
Ciprofloxacine	A	38	0	0.0%	
Clarithromycine	A	263	6	18.6%	0.03
Clindamycine	A	135	6	51.1%	0.09
Doxycycline	A	38	0	0.0%	
Enoxacine	A	38	0	0.0%	
Enrofloxacine	A	38	0	5.3%	0.013
Erythromycine	A	193	0	4.7%	0.02
Indometacine	A	471	7	10.8%	0.21
Lincomycine	A	161	0	1.2%	0.01
Metronidazol	A	137	1	0.7%	0.015
Monensin	A	155	0	0.0%	
Norfloxacine	A	38	0	0.0%	
Ofloxacine	A	38	0	0.0%	
Oleandomycine	A	294	0	0.3%	0.08
Oxytetracycline	A	38	0	0.0%	
Ronidazol	A	137	0	0.0%	
Roxithromycine	A	295	3	5.4%	0.018
Spiramycine	A	293	0	0.0%	
Sulfachloropyridazine	A	125	0	0.0%	
Sulfadimethoxine	A	125	0	0.8%	0.02
Sulfamethoxazol	A	303	6	84.5%	0.11
Sulfanilamide	A	12	0	0.0%	
Sulfaquinoxaline	A	125	0	0.0%	
Tetracycline	A	38	0	0.0%	
Trimethoprim	A	295	6	14.2%	0.02
Tylosine	A	137	0	0.0%	
Virginiamycine	A	38	0	0.0%	
Atenolol	B	123	7	45.5%	0.027
Betaxolol	B	123	0	0.0%	
Bisoprolol	B	118	0	0.0%	

N = number of samples in which this chemical was analyzed, %>LOD = percentage of samples in which this chemical was detected above the limit of detection. The chemical classes are A= Antibiotics, B= Beta Blockers, C= Lipid regulators, D= Analgesics / Anti inflammatory pharmaceuticals, E= Endocrine disrupting chemicals, F= X-ray contrast chemicals, G= remaining pharmaceuticals (e.g. anti epileptic pharmaceuticals) and H= Penicillin's.

Pharmaceutical	Pharm. class	N	Obs. at N	%›LOD	Max. conc. (µg/L)
Metoprolol	B	274	6	65.7%	0.2
Pindolol	B	121	1	0.0%	
Propranolol	B	265	0	0.0%	
Sotalol	B	273	7	51.3%	0.2
Bezafibrate	C	457	7	57.5%	0.19
Clofibrate	C	91	0	0.0%	
Clofibrinic acid	C	795	3	3.4%	0.24
Etofibrate	C	38	0	0.0%	
Fenofibrate	C	472	0	0.2%	0.02
Fenofibrinic acid	C	282	4	10.3%	0.055
Gemfibrozil	C	470	4	8.3%	0.042
Pentoxifylline	C	380	4	23.9%	0.58
Simvastatine	C	38	0	0.0%	
Asperine	D	35	0	2.9%	0.03
Diclofenac	D	556	7	61.9%	0.9
Dimethylaminofenazon	D	38	0	0.0%	
Fenacetine	D	262	2	1.1%	0.024
Fenazon	D	187	2	33.7%	0.2
Fenoprofen	D	472	0	0.2%	0.017
Ibuprofen	D	555	7	26.8%	0.1
Ketoprofen	D	470	1	0.2%	0.023
Naproxen	D	196	0	7.1%	0.04
Propyfenazon	D	38	2	7.9%	0.018
17-alfa-ethinylestradiol	E	190	0	0.0%	
17-alpha-Ethinylestradiol-3-methylether	E	40	0	0.0%	
17-beta-Estradiol	E	45	0	0.0%	
4-iso-Nonylphenol	E	40	2	100.0%	0.18
4-nonylphenol	E	51	1	0.0%	
4-octylphenol	E	26	1	88.5%	0.083
4-tert-octylphenol	E	77	1	22.1%	0.21
Activity in rel. to 17-beta-estradiol	E	8	1	100.0%	0.63
Bisphenol A	E	55	2	81.8%	0.18
Butylbenzylphthalate	E	112	1	1.8%	0.06
di(2-ethylhexyl)phthalate (DEHP)	E	150	2	42.7%	1.6
di-(2-methyl-propyl)phthalate	E	39	1	84.6%	1
di(n-octyl)phthalate	E	92	0	1.1%	0.05
Dibutylphthalate (DBPH)	E	92	1	28.3%	0.13
Dibutyltin	E	64	0	14.1%	0.03

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Pharmaceutical	Pharm. class	N	Obs. at N	%>LOD	Max. conc. (µg/L)
Dicyclohexyltin	E	47	0	0.0%	
Diethylphthalate (DEPH)	E	112	1	21.4%	0.9
Difenyltin	E	65	0	0.0%	
Dimethylphthalate	E	92	0	0.0%	
Estriol	E	45	0	0.0%	
Estrone	E	179	0	1.1%	0.002
iso-nonylphenol	E	5	1	80.0%	0.099
N-octacosane	E	52	2	5.8%	21
Nonylphenol	E	37	0	0.0%	
Norethisterone	E	45	0	0.0%	
Octa-methyl-tetra-siloxane	E	8	0	0.0%	
Tetrabutyltin	E	65	0	0.0%	
Tributyltin	E	108	2	4.6%	
Tricyclohexyltin	E	46	0	0.0%	
Trifenyltin	E	65	0	0.0%	
Amidotrizoinic acid	F	294	7	96.9%	1.2
Iodipamide	F	296	2	1.4%	0.53
Iohexol	F	296	7	93.2%	0.4
Iomeprol	F	278	7	97.5%	0.97
Iopamidol	F	296	7	97.0%	0.58
Iopanoïnezuur	F	233	1	1.3%	0.23
Iopanzuur	F	39	0	0.0%	
Iopromide	F	292	7	96.9%	0.67
Iotalaminic acid	F	296	2	5.4%	0.14
Ioxaglinic acid	F	296	4	1.7%	0.073
Ioxitalaminic acid	F	296	7	84.1%	0.23
Aminoantipyrine	G	110	0	0.0%	
Caffeine	G	251	4	78.5%	0.6
Carbamazepine	G	1652	7	74.8%	0.064
Clenbuterol	G	38	0	0.0%	
Cyclofosfamide	G	194	0	0.0%	
Dapsone	G	302	0	0.0%	
Diazepam	G	33	0	0.0%	
Fenoterol	G	148	0	0.0%	
Furazolidon	G	289	0	0.0%	
Ifosfamide	G	35	0	0.0%	
Ioxynil	G	341	1	0.0%	
Lidocaïne	G	126	1	32.5%	0.11

N = number of samples in which this chemical was analyzed, %>LOD = percentage of samples in which this chemical was detected above the limit of detection. The chemical classes are A= Antibiotics, B= Beta Blockers, C= Lipid regulators, D= Analgesics / Anti inflammatory pharmaceuticals, E= Endocrine disrupting chemicals, F= X-ray contrast chemicals, G= remaining pharmaceuticals (e.g. anti epileptic pharmaceuticals) and H= Penicillin's.

Pharmaceutical	Pharm. class	N	Obs. at N	%>LOD	Max. conc. (µg/L)
Meclocycline	G	38	0	0.0%	
Primidon	G	129	1	14.7%	0.03
Progesteron	G	158	0	0.0%	
Salbutamol	G	38	0	0.0%	
Sulfadiazine	G	176	0	0.6%	0.014
Sulfadimidine	G	305	0	1.3%	0.05
Sulfamerazine	G	167	0	1.2%	0.016
Terbutaline	G	38	0	0.0%	
Tiamuline	G	161	0	1.2%	0.03
Tolfenaminzuur	G	160	0	0.6%	0.01
Warfarin	G	39	0	0.0%	
Ampicilline	H	38	0	0.0%	
Cloxacilline	H	234	0	0.0%	
Dicloxacilline	H	234	0	0.0%	
Nafcilline	H	233	0	0.0%	
Oxacilline	H	234	0	1.0%	0.01
Penicilline-G	H	74	0	0.0%	
Penicilline-V	H	74	0	0.0%	

N = number of samples in which this chemical was analyzed, %>LOD = percentage of samples in which this chemical was detected above the limit of detection. The chemical classes are A= Antibiotics, B= Beta Blockers, C= Lipid regulators, D= Analgesics / Anti inflammatory pharmaceuticals, E= Endocrine disrupting chemicals, F= X-ray contrast chemicals, G= remaining pharmaceuticals (e.g. anti epileptic pharmaceuticals) and H= Penicillin's.

All chemicals that could be detected quantitatively were used in further analysis. It should, however, be noted that the detection limits varied between locations and also sometimes within a location, over time. Differences in detection limits might have biased the fractions of positive detections from different sampling sites or at different sampling dates within a location.

Data become less reliable with decreasing fraction of positive identifications. Data of pharmaceuticals are further presented and used in calculations if they were detected in at least 20% of the samples, and for graphical representation, a single representative per chemical class was selected. This representative was selected based on the largest fraction of positive quantifications and number of analyses. Sulfametoxazol, sotalol, bezafibrate, 4-iso-nonylphenol, diclofenac, iopamidol and carbamazepine are chosen as representatives of the pharmaceutical classes; Antibiotics (A), Beta Blockers (B), Lipid regulators (C), Analgesics / Anti inflammatory drugs (D), Endocrine disrupting chemicals (E), X-ray contrast liquids (F) and remaining pharmaceuticals (G), respectively. Trends of Penicillin's (H) are left out of all further analyses since only one of the penicillin's was detected in only two samples.

The concentrations of pharmaceuticals were compared to literature data on environmental concentrations. Furthermore the relation of the (log normalized) concentrations and the distance from the Bodensee was studied by fitting a linear regression trough the data to see if the slope of the regression line deviated significantly ($p<0.05$) from 0. Additionally, temporal trends over the sampling period (2002-2008) were studied in a similar manner, by studying the relation of log normalized concentrations in Nieuwegein and Lobith and the sampling date over the 7 year sampling period. Besides that, seasonal trends of loads entering the Netherlands at Lobith were studied qualitatively. Finally, annual loads of the 20 most frequently observed pharmaceuticals entering the Netherlands at Lobith were compared to the annual consumption of these pharmaceuticals in the Rhine catchment area, and a model that predicts annual loads from consumption and literature data on excretion of pharmaceuticals by the user and removal by the waste water treatment.

Results and Discussion

3.1.1 Pharmaceuticals in the Rhine

Table 2 shows the number of chemicals analysed and determined above the limit of detection (LOD) per sampling location. It can be observed that the number of chemicals analysed varied per location, and that more than half of the chemicals analysed were not detected in the samples.

Table 2: Chemicals analysed and detected per sampling location

	Basel-Birsfelden (164)	Karlsruhe (359)	Mainz (501)	Keulen (686)	Düsseldorf-Flehe (R) (722)	Lobith (860)	Nieuwegein (950)	Nieuwersluis	Andijk	Total
Chemicals analyzed	49	50	50	49	48	99	127	122	107	128
Chemicals detected	22	21	24	21	24	36	41	41	32	67

3.1.2 Comparing concentrations in the Rhine with literature data

Figure 2 and Table 3 show the average and median detectable concentrations of 29 pharmaceuticals, X-ray contrast media and endocrine disrupting chemicals in the river Rhine. The chemicals are only presented if they were detected in ≥5 samples and if they were found in at least ≥20% of the samples.

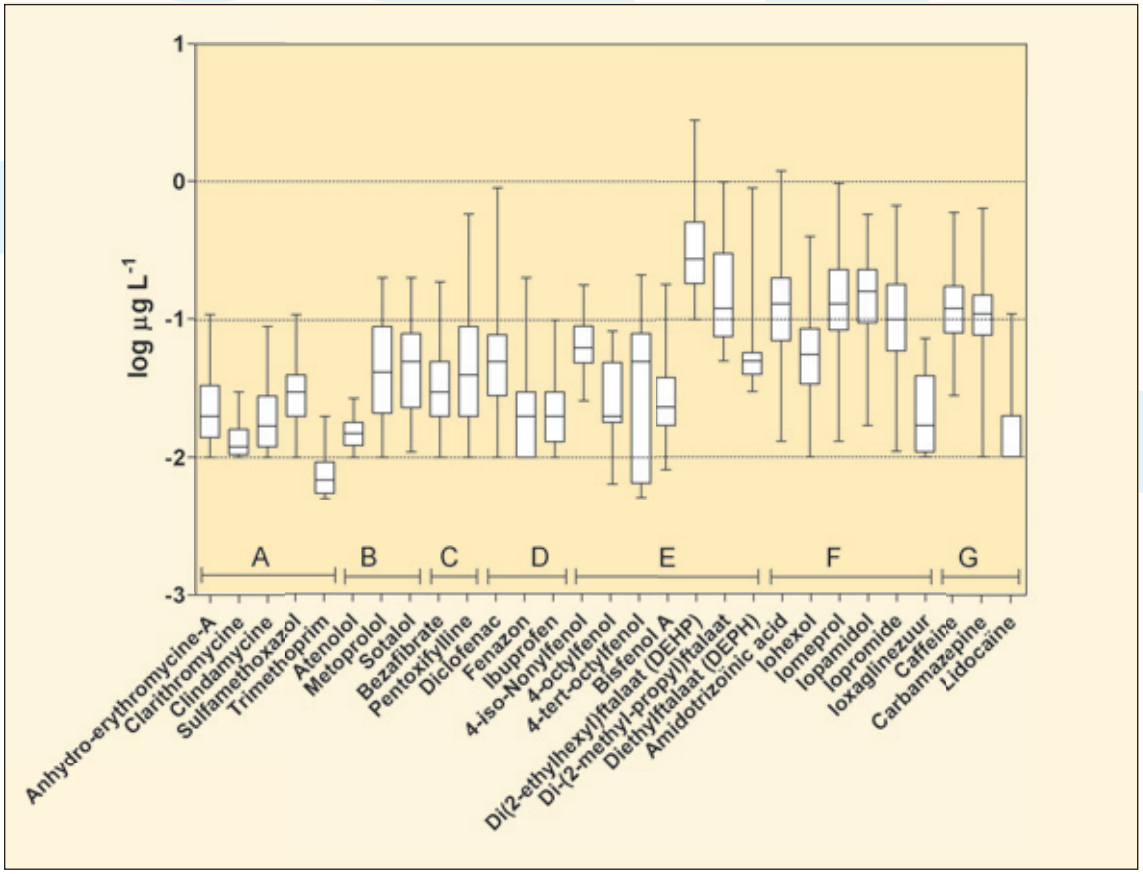


Figure 2: A Box-Whisker plot of the concentrations and variation of concentrations of pharmaceuticals, X-ray contrast media and endocrine disrupting chemicals in the river Rhine.
Legend of groups: Antibiotics (A), Beta Blockers (B), Lipid regulators (C), Analgesics / Anti inflammatory drugs (D), Endocrine disrupting chemicals (E), X-ray contrast liquids (F) and remaining pharmaceuticals (G).

Table 3: Concentrations (µg/L) of the detected chemicals in the river Rhine

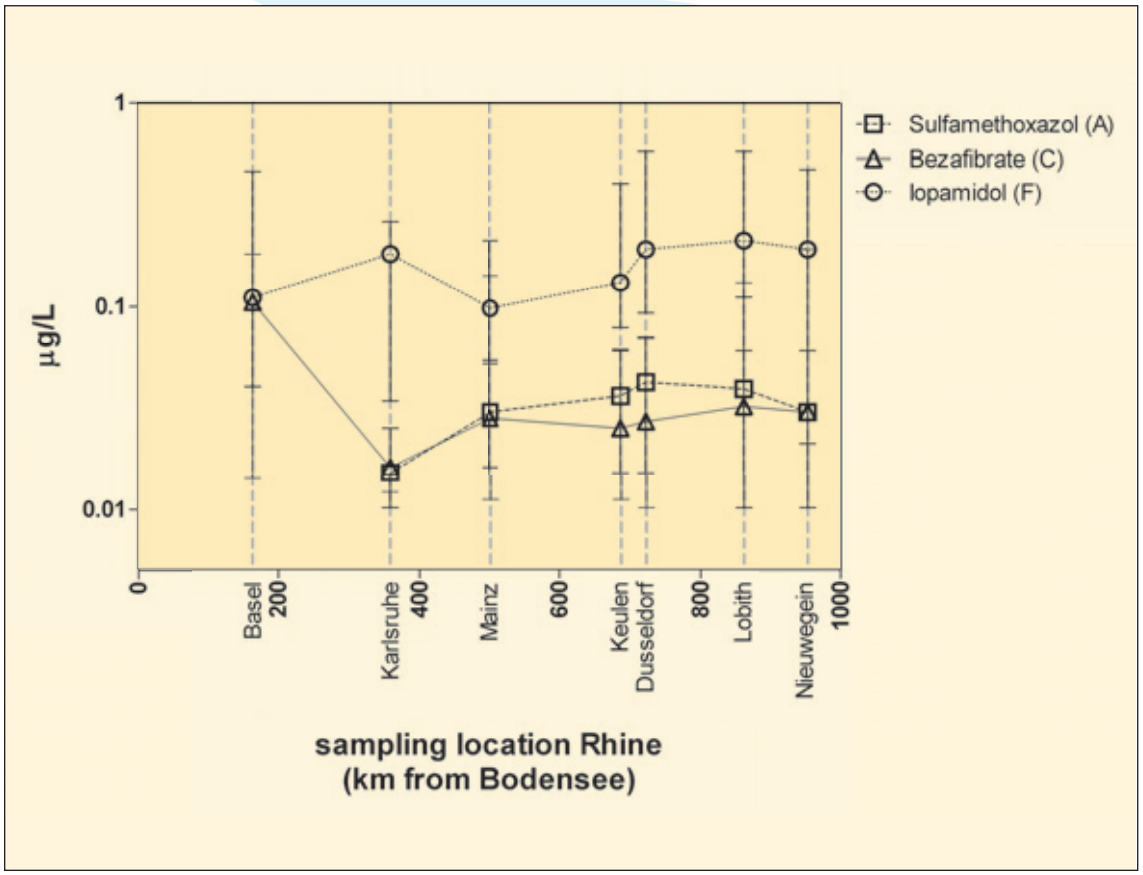
Concentrations in µg/L	Average	median	Max	n> LOD	Lit. average	Lit. max
4-iso-Nonylphenol (E)	0.065	0.063	0.180	40	0.025 [1]	0.08 [1]
4-octylphenol (E)	0.023	0.020	0.083	28		
4-tert-octylphenol (E)	0.028	0.050	0.210	17		
Amidotrizoinic acid (F)	0.118	0.130	1.200	285	0.11 [2]	0.11 [2]
Anhydro-erythromycine-A (A)	0.022	0.020	0.110	93		
Atenolol (B)	0.015	0.015	0.027	56		
Bezafibrate (C)	0.030	0.030	0.190	263	0.0215 [1,2]	0.33 [1,2,3,4]
Bisphenol A (E)	0.027	0.023	0.180	45		
Caffeine (G)	0.120	0.120	0.600	197	0.36 [1,2]	0.72 [2]
Carbamazepine (G)	0.103	0.110	0.640	1235	0.155 [1,2]	0.64 [1,2,3,4]
Clarithromycine (A)	0.013	0.012	0.030	49	0.018 [1]	0.05 [1]
Clindamycine (A)	0.019	0.017	0.090	69	0.02 [1]	0.05 [1]
di(2-ethylhexyl)phthalate (DEHP) (E)	0.319	0.275	2.800	64		
di-(2-methyl-propyl)phthalate (E)	0.160	0.120	1.000	21		
Diclofenac (D)	0.046	0.050	0.900	299	0.06 [1,2]	0.9 [1,2,3,4]
Diethylphthalate (DEPH) (E)	0.058	0.050	0.900	19		
Fenazon (D)	0.020	0.020	0.200	37	0.02 [1]	0.132 [1,2]
Ibuprofen (D)	0.020	0.020	0.100	104	0.08 [2]	0.12 [1,2,4]
Iohexol (F)	0.054	0.055	0.400	235	0.255 [1,2]	0.46 [1,2,3]
Iomeprol (F)	0.134	0.130	0.970	232	0.28 [1,2]	0.47 [1,2]
Iopamidol (F)	0.143	0.160	0.580	245	0.12 [1]	0.4 [1,2]
Iopromide (F)	0.103	0.100	0.670	240	0.1205 [1,2]	0.5 [1,2]
Ioxitalaminic acid (F)	0.020	0.017	0.073	208	0.085 [1,2]	0.14 [1,2]
Lidocaïne (G)	0.013	0.010	0.110	10	0.0313 [2]	0.06 [2]
Metoprolol (B)	0.044	0.042	0.200	141	0.2375 [1,2]	0.43 [1,2,3,4]
Pentoxifylline (C.)	0.040	0.040	0.580	76		0.57 [4]
Sotalol (B)	0.043	0.050	0.200	108	0.25 [2]	0.25 [2]
Sulfamethoxazol (A)	0.028	0.030	0.110	214	0.04 [1]	0.16 [1,3,4]
Trimethoprim (A)	0.007	0.007	0.020	41		

References: 1 = (20), 2 = (21), 3 = (22), 4 = (13). Values are only presented if chemicals were observed in more than 20% of the analyses and in at least 5 samples. Legend pharmaceutical groups: Antibiotics (A), Beta Blockers (B), Lipid regulators (C), Analgesics / Anti inflammatory drugs (D), Endocrine disrupting chemicals (E), X-ray contrast liquids (F) and remaining pharmaceuticals (G).

We have to note that average and median concentrations can be slightly biased because concentrations below detection limits were not included in this analysis. The average and median concentrations will especially be overestimated when chemicals are observed infrequently, because infrequent detection is probably a result of concentrations below the LOD. These estimates should therefore be considered as worst case estimates. Table 3 also shows literature values of average and maximum concentrations observed that in the Rhine in Germany (13) and at various Dutch surface waters (20-22). It can be observed that concentrations from this study are rather similar to concentrations found in literature. Ratios of average concentrations from literature and the current study usually do not exceed a factor 3. Variations can be a result of actual variations in concentrations in the aqueous phase and analytical inaccuracies, especially when these concentrations are near detection limits. However, literature values of concentrations of metoprolol and sotalol are nearly one order of magnitude higher than values observed in this study. This might be explained by the fact that these high concentrations are observed in smaller streams of the Meuse catchment area (22) where the local anthropogenic pressure can be higher or where consumption and waste water treatment might differ.

3.1.3 Spatial trends of pharmaceuticals in the river Rhine

Figure 3 shows the median concentrations of six representatives of the use-categories with maximum and minimum observed concentrations (error bars) at the different sampling locations along the Rhine. The representative of endocrine disrupting chemicals is left out of the figure because these chemicals were only analyzed at the Dutch sampling locations. The Dutch locations 'Andijk' and 'Nieuwersluis' are left out of the analysis as these locations are in the delta, and the Rhine diverts into various rivers and canals and lakes. A separate comparison between the different Dutch locations is made in paragraph 3.1.4.



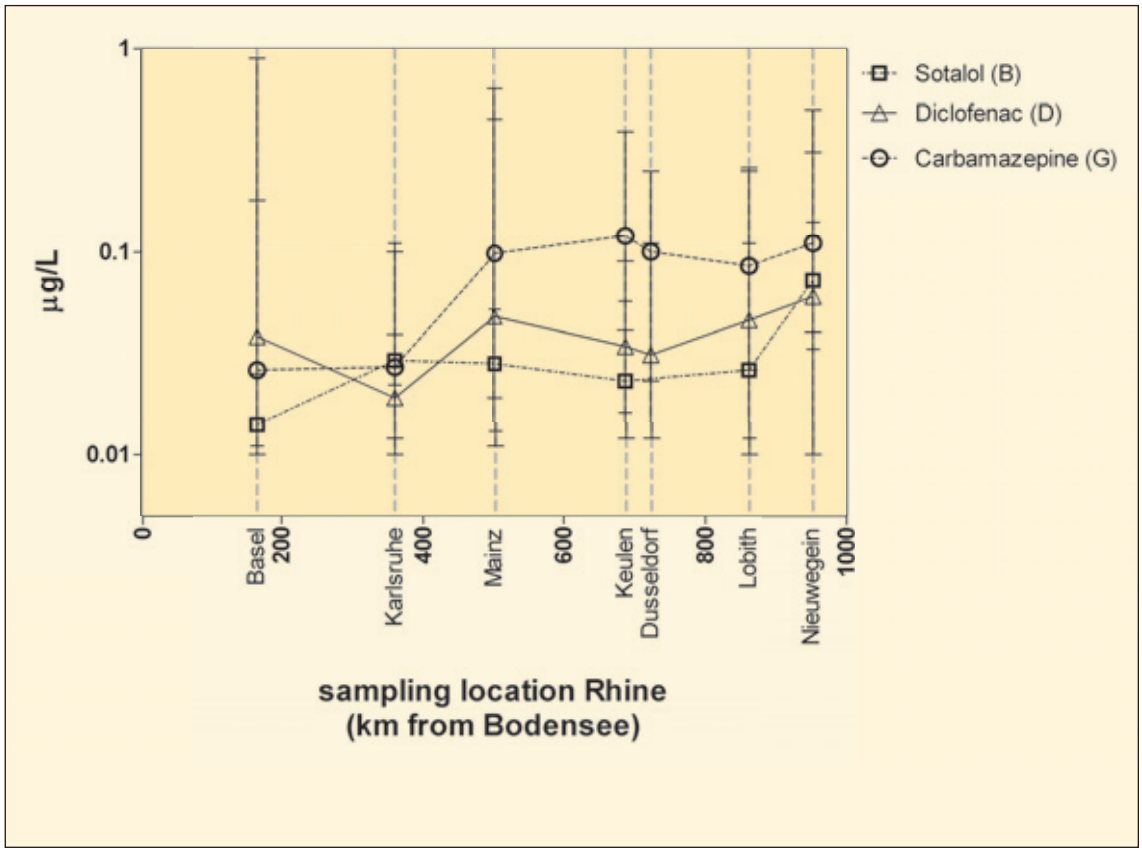


Figure 3: Spatial trends of concentrations of 6 representatives of the pharmaceutical classes.

Figure 3 shows the fluctuations of concentrations over the course of the Rhine. The ratios of the median values of the chemicals never exceed a factor 7 at the different locations. Table 4 shows the results of linear regressions through the logarithm of the concentrations. The concentrations were log transformed. The chosen X-variable is the distance from the Bodensee. The anthropogenic pressure at the different sampling stations can be estimated by dividing the number of inhabitants living upstream in the catchment area by the average flux of water passing at that specific location (Figure 4).
The trend of a pharmaceutical will show a similar increase if consumption is homogeneously spread over all inhabitants in the Rhine catchment area and if there is no degradation or sorption of the pharmaceutical along the course of the Rhine. In this situation, concentrations of pharmaceuticals in the Rhine are expected to increase a factor three to four between Basel and Lobith. The assumptions are obviously not completely true as the consumption of pharmaceuticals varies per country (14) and probably also per region (23), while some sorption and degradation might occur when the chemicals are in the Rhine.

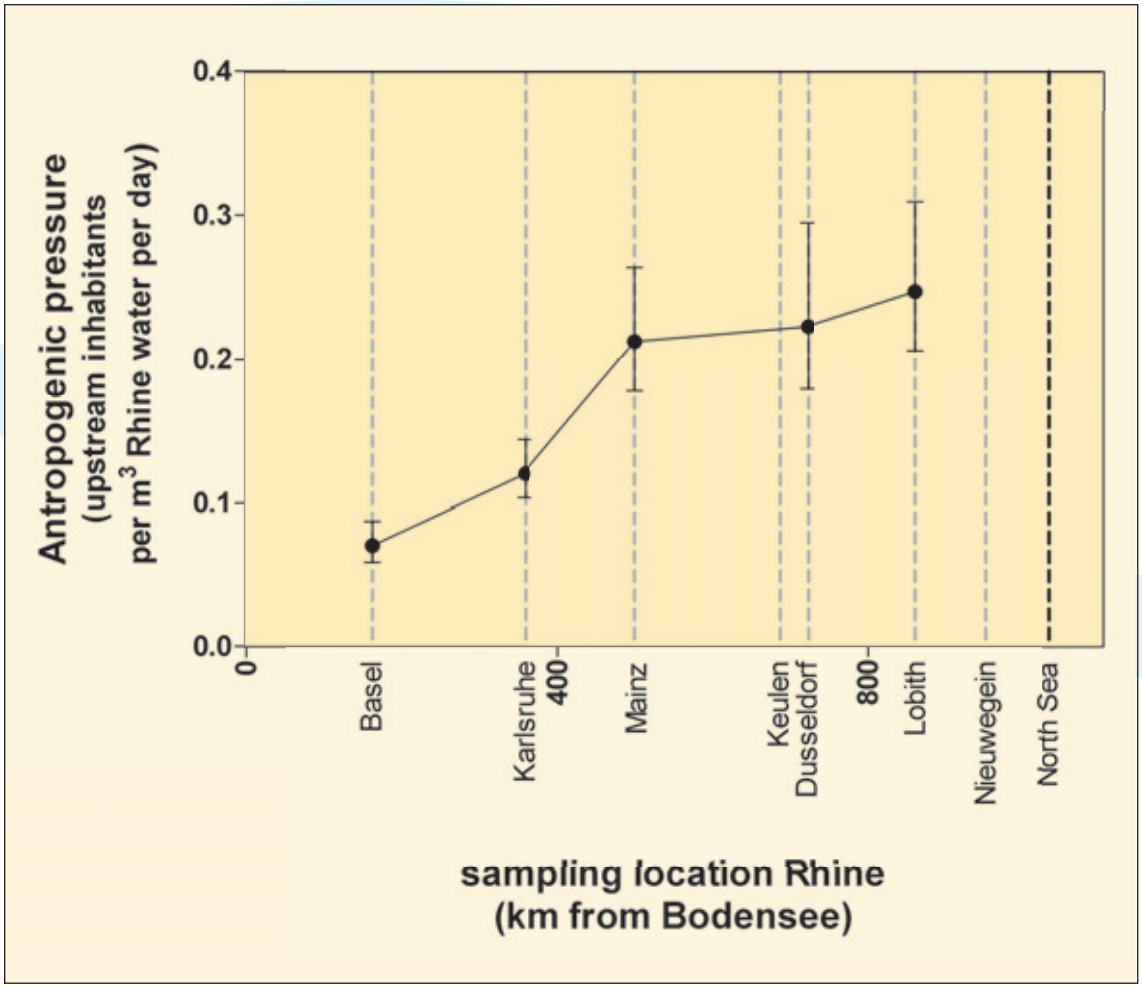


Figure 4: The anthropogenic pressure on the Rhine expressed as inhabitants living upstream per passing m³ of water per day.

Average fluxes are obtained from (13) or the Dutch government (Rijks Waterstaat, www.waterbase.nl). Average fluxes of the Swiss and German locations were from the period 1997 to 2006 while the data of Lobith were from 2002 to 2008. Upstream inhabitants are calculated from maps with regional population densities (1). Error bars represent standard deviations.

Linear regressions of concentrations along the Rhine reveal no significant trend for 9 out of 22 chemicals. Bezafibrate and ioxitalaminic acid even decreased over the course of the Rhine (a factor 1.9 and 1.3 between Basel and Lobith, respectively). The decrease of bezafibrate is likely to be biased, because it is solely based on high concentrations observed in Basel, where bezafibrate was only detected in 6 out of 60 analyses. The decrease of ioxitalaminic acid is only marginal but yet significant. This decrease might have to do with a local preference for this specific X-ray contrast medium in Switzerland (24) compared to Germany (25).

Table 4: Spatial trends of pharmaceuticals in the Rhine.

Pharmaceutical	Trend	P -value	Concentration duplicates per X km Rhine	Factor of increase between Basel and Lobith
Amidotrizoinic acid (F)	Increase	< 0.0001	293	5.2
Anhydro-erythromycine-A (A)	Increase	0.0029	412	3.2
Atenolol (B)	ns	0.2509		
Bezafibrate (C)	Decrease	0.0001	-792	0.54
Carbamazepine (G)	Increase	< 0.0001	646	2.1
Clarithromycine (A)	ns	0.3834		
Clindamycine (A)	ns	0.7329		
Diclofenac (D)	ns	0.554		
Fenazon (D)	ns	0.8249		
Ibuprofen (D)	Increase	0.0309	1813	1.3
Indometacine (A)	ns	0.1279		
Iohexol (F)	Increase	0.0044	1199	1.5
Iomeprol (F)	Increase	0.0006	880	1.7
Iopamidol (F)	Increase	0.0002	1071	1.6
Iopromide (F)	Increase	< 0.0001	438	3.0
Ioxitalaminic acid (F)	Decrease	0.0008	-1672	0.75
Metoprolol (B)	Increase	< 0.0001	259	6.4
Pentoxifylline (C.)	ns	0.0686		
Roxithromycine (A)	ns	0.6888		
Sotalol (B)	Increase	< 0.0001	447	2.9
Sulfamethoxazol (A)	Increase	< 0.0001	796	1.8
Trimethoprim (G)	ns	0.1867		

ns = Not significant; the right column gives an estimate of the distance that is necessary to duplicate the concentration of a specific pharmaceutical. If this value is 500, this means that the concentration of this pharmaceutical will duplicate every 500 km, so concentrations at Karlsruhe (383 km) are expected to be approximately half of concentrations at Lobith (850 km).

However, for 11 out of 22 pharmaceuticals and X-ray contrast media, the level of increase varies from 1.3 to 6.4 between Basel and Lobith (distance 696 km). The average increase of these 11 chemicals is 2.5, this does not significantly deviate from the factor ~3.5 increase expected from anthropogenic pressure alone (Figure 4). However for the other 11 chemicals, no significant trend or even a decrease was observed. This can be explained by sorption and degradation processes in the environment and regional variations in consumption (23, 26).

Summarizing, the water quality, with respect to concentrations of the chemicals, decreases with the course of the Rhine, because half of the pharmaceuticals increase significantly while only a few marginally decrease.

3.1.4 Statistical comparison of the concentrations of pharmaceuticals at the Dutch locations

This chapter discusses the differences in concentrations of pharmaceuticals between the Dutch locations. In the Dutch delta, part of the water flows via the IJssel river (and the Vecht river) into the lake IJsselmeer before it makes it to the North Sea via the IJ canal or 2 openings at the eastern and western part of the dike separating lake IJsselmeer from the Sea (Afsluitdijk), while two other parts flow westward to the North Sea via the Waal and Lek river. Additionally, part of the water from the Lek fils the Amsterdam-Rhine canal (ARK) which is connected to the lake IJsselmeer and the North Sea via the IJ canal. We therefore decided to analyze the data between the Dutch locations separately.

Figure 5 shows a map of the sampling locations in the Rhine delta. The arrows indicate how the Rhine water flows towards the North Sea.

Figure 5: Map of the Dutch sampling locations.

Table 5: Statistical analysis of concentrations of pharmaceutical, X-ray contrast media and endocrine disrupting chemicals at the Dutch sampling locations

	Average concentrations (µg/L)					Tuckey paired analysis					
Pharmaceutical	average Andijk	average Lobith	average Nieuwegein	average Nieuwersluis	ANOVA	Andijk vs Lobith	Andijk vs Nieuwegein	Andijk vs Nieuwersluis	Lobith vs Nieuwegein	Lobith vs Nieuwersluis	Nieuwegein vs Nieuwersluis
4-iso-Nonylphenol		0.030	0.066	0.067	ns						
4-octylphenol			0.024	0.022	ns						
4-tert-octylphenol		0.086	0.008		***				***		
Amidotrizoinic acid	0.079	0.176	0.161	0.155	***	***	***	***	ns	ns	ns
Anhydro-erythromycine-A	0.016	0.025	0.030		ns						
Atenolol		0.014	0.017		ns				ns		
Benz(a)fibrate	0.016	0.032	0.027	0.020	***	***	*	ns	ns	**	ns
Bisphenol A		0.159	0.024	0.027	**				***	**	ns
Butylbenzylphthalate	0.060		0.050		nd						
Caffeine	0.092	0.124	0.131	0.171	***	ns	**	***	ns	ns	*
Carbamazepine	0.064	0.079	0.126	0.093	***	*	***	***	***	ns	***
Clindamycine		0.019	0.013		ns				ns		
Di(2-ethylhexyl)phthalate (DEHP)	0.224	2.698	0.337	0.327	***	***	ns	ns	***	***	ns
Di-(2-methyl-propyl) phthalate	0.133		0.127	0.221	ns						
Dibutylphthalate (DBPH)	0.069		0.059	0.066	ns						
Diclofenac	0.028	0.045	0.057	0.044	**	*	***	ns	ns	ns	ns
Fenazon	0.015	0.023	0.025	0.019	ns						
Ibuprofen	0.010	0.019	0.020	0.028	*	ns	ns	ns	ns	**	ns
Iohexol	0.035	0.074	0.058	0.057	***	***	***	***	ns	ns	ns
Iomeprol	0.081	0.147	0.167	0.221	***	***	***	***	ns	ns	ns
Iopamidol	0.093	0.198	0.181	0.132	***	***	***	*	ns	**	*
Iopromide	0.062	0.174	0.137	0.166	***	***	***	***	ns	ns	ns
Lidocaïne	0.010		0.013	0.013	ns						
Metoprolol	0.033	0.025	0.061	0.118	***	ns	***	***	***	***	***
Pentoxifylline	0.018	0.051	0.037	0.030	**	**	ns	ns	ns	ns	ns
Sotalol	0.031	0.033	0.073	0.090	***	ns	**	***	***	***	ns
Sulfamethoxazol	0.017	0.036	0.030	0.035	***	***	***	***	ns	ns	ns

* = p, 0.05-0.01, ** = p, 0.01-0.001, *** = p ‹0.001, ns = not significant (p ›0.05), nd = not detectable (usually because there was only one positive detection, that did not allow for analysis of variance (ANOVA, Microsoft Excel)) and empty cells are combinations that were not compared because the ANOVA already resulted in no significant difference or because locations could not be compared as the chemical was not found at one or more locations.

Table 5 shows the average concentrations at the four Dutch locations and a statistical analysis (ANOVA) to see whether one or more of the locations significantly differed from each other. Additionally a Tuckey Multiple Comparison test was done (Graphpad Prism, Version 5.01) to see which specific locations significantly differed from the other locations if a significant difference was observed for the ANOVA analysis. The data of pharmaceuticals is only presented if the chemical was observed in more than 20% of the samples taken from these Dutch locations.

It can be observed that concentrations at Andijk are generally a factor 2 lower than at the other Dutch locations. This is observed in the paired Tuckey analysis as well, where the concentrations at Andijk are often significantly different from one or more of the other Dutch locations. Furthermore, concentrations at Lobith are often slightly higher than the concentrations at Nieuwegein and Nieuwersluis, even though differences are marginal and often not significant. There are, however, some exceptions. For example, some endocrine disruptors show up to one order of magnitude higher concentrations at Lobith (4-tert-octylphenol, Bisphenol A, DEHP), while two Beta blockers (metoprolol and sotalol) show about a factor 2 higher average concentrations at Nieuwegein and Nieuwersluis. The higher concentrations of sotalol and metoprolol might indicate that the Dutch consumption is higher than the consumption in Germany (and France and Switzerland). Nevertheless, the general trend is that concentrations decrease downstream of Lobith with the lowest concentrations at Andijk. This is opposite to the increase that was observed from Basel to Lobith (Table 5).

The decrease with the flow of the Rhine in the Dutch delta cannot be fully explained by dilution due to significantly increasing volumes, as the contribution of the catchment area of the Dutch delta is only marginal in comparison to the flux of the Rhine (www.waterbase.nl). Therefore, the reduction that is observed especially at Andijk must be a result of increased sorption and degradation in the Rhine delta. If we consider that deltas contain huge amounts of fine sediment that can act as sorption surface for chemicals, while the residence time of water in a delta is relatively long, losses of pharmaceuticals are expected to be highest in this part of a river basin. The residence time is especially long for the ~18% of the Rhine water (~386 ± 147 m^3/s, www.waterbase.nl) that flows into lake IJsselmeer. This lake has a volume of 5.2 km^3 (27), which means that the average residence time of the water in the IJsselmeer is around 5 months (ignoring the marginal input from the eastern and northern part of the Netherlands and the small river 'Vecht' that diverts from the Rhine west of the IJssel river). This long residence time might enable substantial (bio)degradation and sorption to the fine sediment in this lake, thereby explaining the lower concentrations of pharmaceuticals observed at Andijk.

3.1.5 Temporal trends of pharmaceuticals in the river Rhine

The concentrations of most pharmaceuticals have been monitored every four weeks at Lobith and Nieuwegein. Additionally, some chemicals have been monitored more frequently. Carbamazepine was, for example, analyzed several times a week during the 7 year sampling period at Nieuwegein.
Figure 6 shows the concentrations of the seven representatives of the different chemical classes at Lobith.

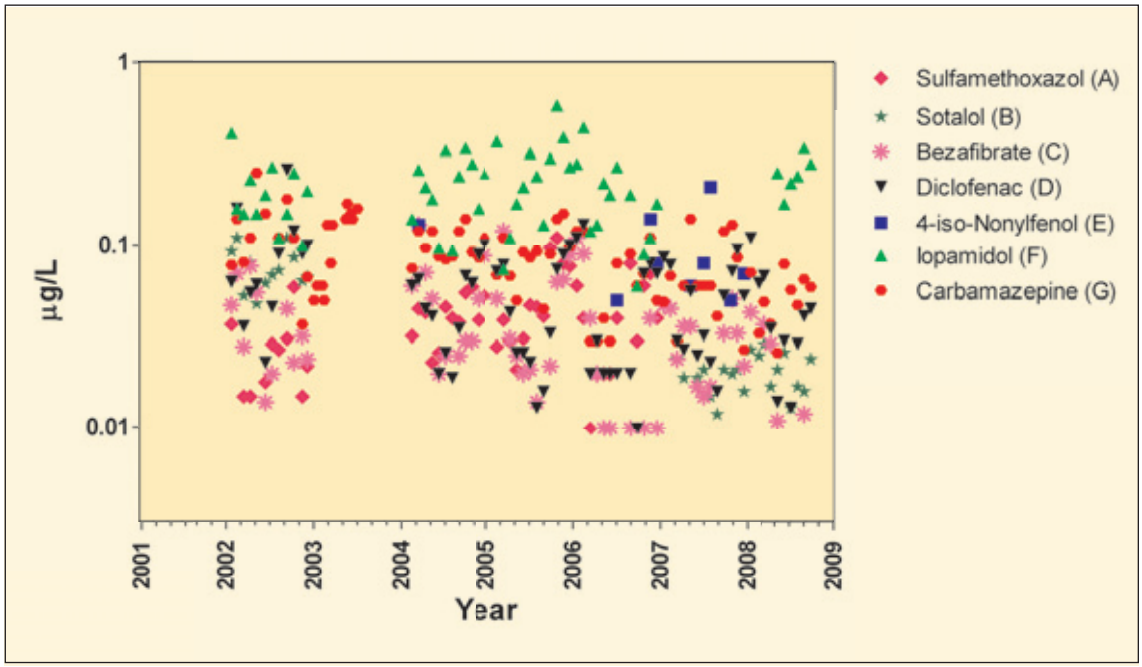


Figure 6: Temporal variations of 7 representatives of the pharmaceutical classes in the river Rhine at Lobith.

The concentrations of most of these chemicals vary around one order of magnitude in time. These variations can be a result of varying fluxes of water in the river, variable consumed amounts by users (14), and varying (bio)degradation in wastewater treatment plants and the environment as a result of environmental conditions (e.g. temperature, light, suspended solids in the water column) (16, 17). Changing consumption patterns between 2002 and 2008 might reveal a trend over these years. Table 6 shows the results of a linear regression that was fitted on the log normalized concentrations at Lobith and Nieuwegein versus time. The relation between the concentration and time is considered a trend if the direction coefficient of the fitted curves significantly deviate form zero. A positive direction coefficient indicates that the concentration significantly increased between 2002 and 2008 and a negative direction coefficient indicates that concentrations decrease with time.

Table 6: Temporal trends of pharmaceuticals in the Rhine at Lobith and Nieuwegein between 2002 and 2008

	Nieuwegein			Lobith		
	Trend	**P value**	**% change /year SE**	**Trend**	**P value**	**% change /year SE**
Amidotrizoinic acid (F)	ns.	0.0954		ns.	0.1179	
Anhydro-erythromycine-A (A)	ns.	0.1561		ns.	0.1635	
Atenolol (B)	ns.	0.3279		ns.	0.2097	
Bezafibrate (B)	Decrease	0.0049	-12 ±4%	Decrease	0.0388	-9 ±5%
Carbamazepine (G)	Decrease	‹ 0.0001	-12 ±1%	Decrease	‹ 0.0001	-12 ±2%
Clarithromycine (A)	ns.	0.5987		ns.	0.9287	
Clindamycine (A)	ns.	0.779		Increase	0.006	21 ±7%
Diclofenac (D)	Decrease	0.0205	-10 ±4%	Decrease	0.0143	-10 ±4%
Fenazon (D)	ns.	0.0989		ns.	0.1009	
Ibuprofen (D)	ns.	0.1574		ns.	0.1832	
Indometacine (A)	na.			ns.	0.6485	
Iomeprol (F)	Increase	0.0018	13 ±4%	Increase	0.0013	14 ±4%
Iopamidol (F)	Increase	0.0034	14 ±4%	Increase	0.0045	14 ±4%
Iopromide (F)	ns.	0.6515		ns.	0.5134	
Ioxitalaminic acid (F)	Decrease	0.0005	-14 ±2%	ns.	0.0563	
Metoprolol (B)	Increase	0.0292	6 ±2%	ns.	0.7149	
Pentoxifylline (C.)	ns.	0.947		Increase	0.0069	50 ±14%
Sotalol (B)	ns.	0.695		Increase	‹0.0001	25 ±2%
Sulfamethoxazol (A)	ns.	0.242		Decrease	0.0414	-10 ±5%
Trimethoprim (A)	na			Decrease	‹ 0.0001	-21 ±7%

ns. = not significant, na. = not analysable. The pharmaceuticals that are printed bold showed a significant trend over time at both locations.

Despite of the large variation of the data, linear regressions of the temporal variations of the log transformed concentrations at Lobith and Nieuwegein revealed that the concentrations of the two X-ray contrast media, iohexol and iomeprol significantly increased with time, while bezafibrate, carbamazepine and diclofenac significantly decreased between 2002 and 2008 at both locations. The magnitude of the increase of these X-ray contrast media is a factor ~2.5 while the decrease of bezafibrate, carbamazepine and diclofenac is almost a factor ~2 over the seven year monitoring period.

The increase of the two X-ray contrast media is likely an effect of increased consumption, since their removal in waste water treatment is marginal (17). Additionally, iomeprol is a relatively new X-ray contrast medium that was introduced less than two decades ago (www.medscape.com/viewarticle/406507). Its consumption in Germany increased a factor 5 between 1996 and 2001 (25), and might have increased further after 2001 when the samples were taken (no data found).

The decrease of the other three chemicals remains unclear, since their consumption does not show a decrease in Germany between 1996 and 2001 (14). Nevertheless, the diclofenac consumption data of Germany do not include the years over which the monitoring took place. Sulfamethoxazol is also used in veterinary practice, while veterinary consumption was not included in this analysis. Possibly, the consumption by live stock has decreased in this period. However, for most pharmaceuticals no significant trend could be observed in time.

3.1.6 Seasonal trends of pharmaceuticals in the river Rhine

Concentrations of chemicals in the Rhine might reveal a yearly trend due to seasonal changes in use (1), variations in sorption and degradation as a result of environmental factors such as light and temperature (2), or variations in the flux of water that is drained by the river Rhine (3). The last factor can be eliminated by multiplying the concentrations in the Rhine by the average load of the sampling date. If this is done, no clear seasonal trend was observed for most pharmaceuticals (data not shown). However, anhydro-erythromicine A, bezafibrate, diclofenac, ibuprofen and trimethoprim showed a clear seasonal trend (Figure 7).

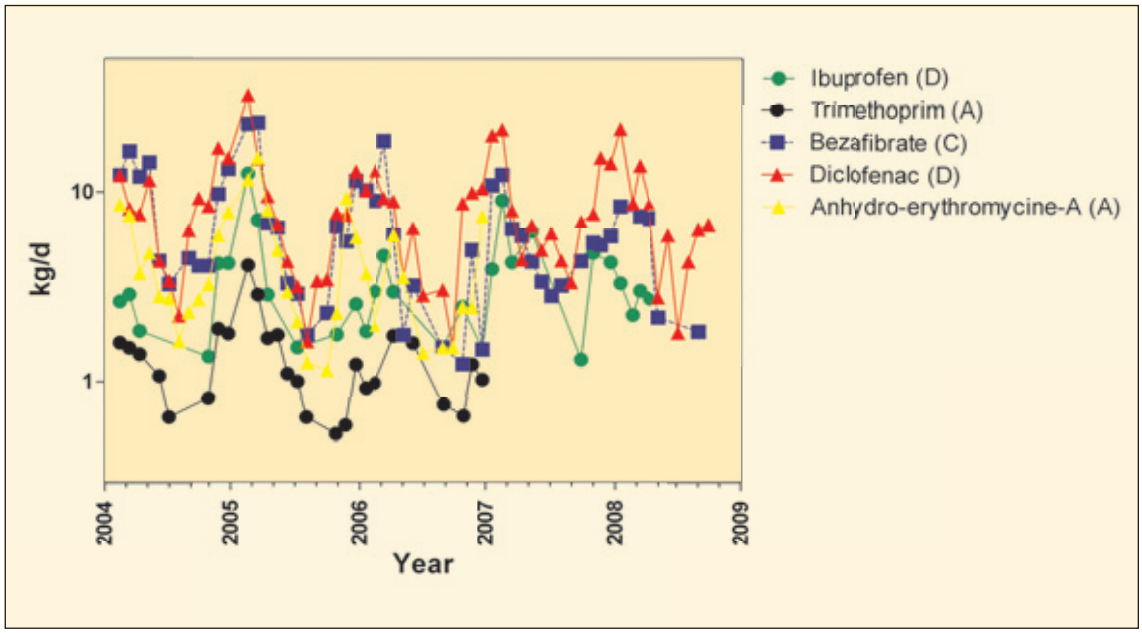


Figure 7: The seasonal trend of daily loads of anhydro-erythromicine, bezafibrate, diclofenac, ibuprofen and trimethoprim in the Rhine at Lobith.

The difference between maximum and minimum daily loads was around one order of magnitude for these pharmaceuticals. Maximum loads were observed in winter and minimum loads in late summer and early autumn. Similar observations were made for bezafibrate, diclofenac and ibuprofen in the Rhine in Germany (13) and for ibuprofen in Canada (28). This trend inversely correlates with environmental temperature fluctuations in Rhine water (29) and probably also in waste water treatment. Variations might therefore be explained by increased degradability at higher temperatures in the waste water treatment plants. However, degradation of some of these chemicals in waste water treatment plants is less than 50% (diclofenac, trimethoprim (30)), while these trends were not observed for other pharmaceuticals that are marginally biodegradable as well (for more details see Table 8, later in the report). This indirectly suggests that not all seasonal variation can be explained by variable degradation in sewage treatment. Additionally, photo-degradation might explain part of the seasonal trend of diclofenac, as this chemical can be rapidly degraded when exposed to light (31). Besides that, part of the seasonal variation in aqueous concentrations can be related to seasonal variations

in consumption. It is not expected that the consumption of bezafibrate varies per season as this is a lipid regulator. However, the ibuprofen and diclofenac might be consumed more frequently in winter (32). Furthermore, various studies show that the antibiotics are more frequently prescribed in winter (33, 34). This might also be the case for anhydro-erythromicine A and trimethoprim.

In conclusion the seasonal variation might be due to both variations in consumption, and variations in removal due to (bio)degradation in the sewage treatment plant and river.

3.1.7 Loads of pharmaceuticals entering the Netherlands via the Rhine

Figure 8 shows the flux of water entering the Netherlands via the Rhine at Lobith. The flux of water that enters the Netherlands at Lobith generally ranges between 700 and 10.000 m^3/s. The average flux is 2167 m^3/s with a 10th and 90th percentile of 1230 and 3532 m^3/s, respectively. The annual load of pharmaceuticals entering the Netherlands at Lobith was determined by taking the average of the product of the measured aqueous concentrations and the volume of water entering the Netherlands on the sampling date, and multiplying this number by 365.25 days (we do not consider leap-years separately).

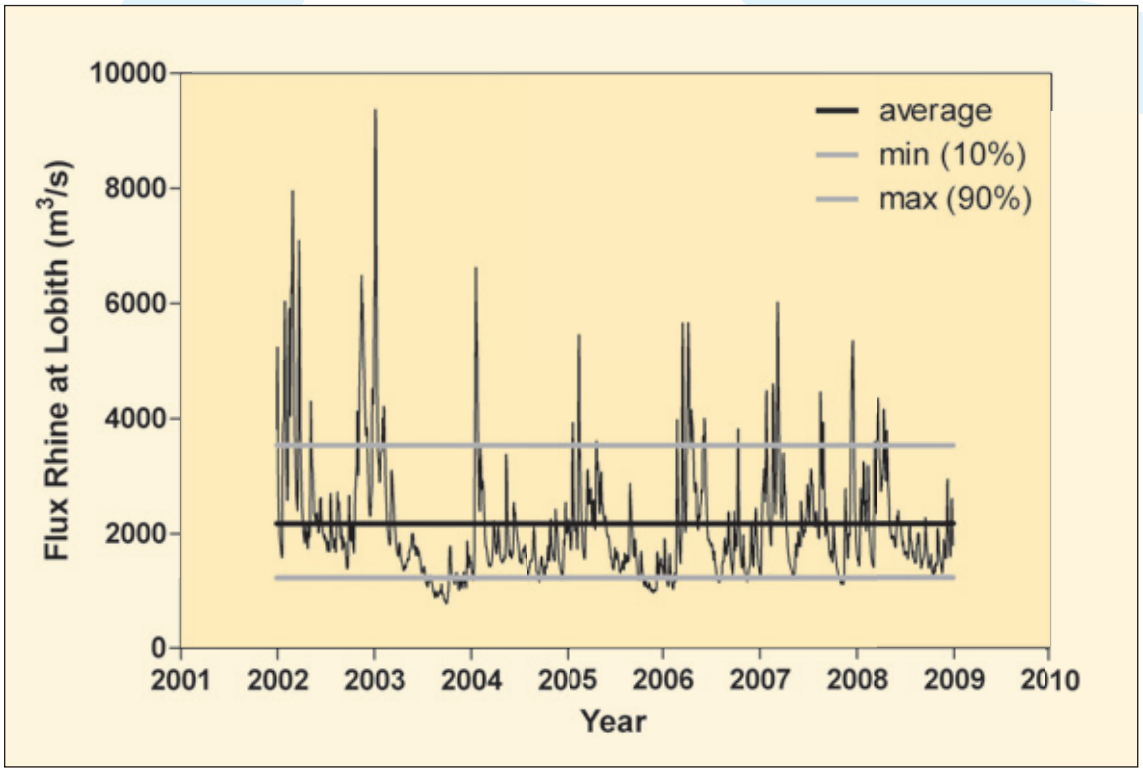


Figure 8: The flux of water entering the Netherlands via the Rhine at Lobith

Table 7 illustrates that the load of pharmaceuticals into the Netherlands via the river Rhine is rather stable over the course of 7 years. It can be observed that the largest loads of pharmaceuticals are from the group of X-ray contrast media (Table 7). Technically these chemicals are not pharmaceuticals in the sense that they are not designed for their specific biological activity; they only help to improve the quality of X-ray photography in hospitals and clinics. These chemicals are all very hydrophilic, and some of them are negatively charged at environmental pH. This results in low sorption to soil, sediments or sludge in sewage treatment plants (17), and also in low retention (and metabolism) in human bodies (17, 35-37). Consequently, their residues in surface water are expected to be high.

The yearly loads are compared to a study of Walraven and Laane (20). The observed loads in the Rhine in our study are expected to be similar or slightly lower than the loads calculated by Walraven and Laane because in the study of Walraven and Laane, loads are calculated for the Rhine, Scheldt and Meuse river together, while our study solely describes loads in the Rhine. Nevertheless, differences are generally expected to be small because the Scheldt and Meuse catchment area have fewer inhabitants than the Rhine catchment area. Table 7 shows that this was generally the case. However, observed loads of iopromide, diclofenac, and pentoxifylline in the Rhine are a factor 2.5 to 3 lower than loads observed in the Rhine, Scheldt and Meuse together, while loads of iomeprol and sotalol were a factor 2 to 3 higher than observed in the Rhine, Scheldt and Meuse together (20). The observed differences can be attributed to the inclusion of the Scheldt and Meuse in the study of Walraven et al, possibly inhabitants of the Scheldt and Meuse catchment areas have different consumption patterns than the inhabitants of the Rhine catchment area for these pharmaceuticals. Furthermore consumption patterns might have changed between 2001 (sampling period of study of Walraven) and 2002-2008 (sampling period of current study). Additionally, differences can be related to inaccuracies as a result of variations of concentrations (see previous paragraphs). Calculated loads are especially vulnerable for variations when they are based on a limited number of observations (Figure 6), or when all samples are taken in one season (Figure 7).

Table 7: Yearly loads of pharmaceuticals and X-ray contrast media in the river Rhine at Lobith.

	2002-2008				2002	2003	2004	2005	2006	2007	2008	Literature[1]
Pharmaceutical	**kg/y**	**SE**	**n>LOD**	**%>LOD**	**kg/y**	**kg/y**	**kg/y**	**kg/y**	**kg/y**	**kg/y**	**kg/y**	**kg/y**
Iopamidol (F)	14922	1194	54	100%	18004		12873	16759	12025		14974	15608
Iopromide (F)	14024	1090	50	96%	18535		17281	14045	8696		10616	42726
Amidotrizoinic acid (F)	12874	760	53	100%	14159		11909	13577	10746		15293	
Iomeprol (F)	12210	1213	54	100%	14196		7230	10633	11864		22049	7442
Caffeine	10202	2579	2	20%		10202						
Carbamazepine (G)	6184	455	84	98%	11481	8016	6360	5474	3896	4479	3392	7467
Iohexol (F)	5938	492	52	96%	5217		4850	6728	5791		8535	6775
Diclofenac (D)	4102		71	100%	8883		3247	3307	2699	3383	2962	10545
Pentoxifylline (C.)	3906	648	21	30%			1764	3415	3886	2268	10560	
Sotalol (B)	3538	615	34	100%	7242					1598	1421	9050
Fenazon (B)	2891	1291	6	50%	2891							
Bezafibrate (C.)	2877	285	60	86%	4085		3295	3121	2134	2149	1999	920
Sulfamethoxazol (A)	2491	127	46	96%	2179		2463	2972	2296			2745
Anhydro-erythromycine-A (A)	2191	295	45	94%	3586		1658	2166	1184			2205
Metoprolol (B)	2132	243	34	100%	2887					1636	1820	2530
Ioxitalaminic acid (F)	1565	154	51	100%	2244		1764	2097	825			2785
Ibuprofen (D)	1512	168	35	49%	2579		1057	1744	946	1776	1044	1344
Clindamycine (A)	1380	145	34	72%	984		1216	1351	1743			
4-iso-Nonylphenol	1368		1	100%					1368			
Atenolol (B)	1299	185	17	50%	1575					924	857	
Roxithromycine (A)	1073	170	9	21%	1360		826	1320	456			1276
Clarithromycine (A)	1055	168	21	44%	1143		904	1668	675			1199
Indometacine (A)	982	195	14	20%			718	796	1368	1397		
Trimethoprim (A)	502	56	26	54%			498	576	414			

Chemicals are only shown when they were identified in more than 20% of the samples and if they were analysed and in at least 5 different occasions between 2002 and 2008.

1) Literature data were obtained from a study of Walraven and Laane (2008) (20).

3.1.8 Relation of loads of chemicals in the Rhine and quantities consumed

Concentrations in the environment are determined by consumption, metabolism by the user, removal of the chemical by wastewater treatment by degradation or sorption to sludge, and degradation or sorption (to soil and sediment) in the environment. The consumption, metabolism by the user and removal in the waste water treatment can be estimated from literature data, and used to predict loads that enter the environment according to:

$$Q_{Rhine(est)} = Q_{Cons} \times f_{Excr} \times f_{WWTP} \qquad 1$$

where $Q_{Rhine(est)}$ is the estimated yearly load in the Rhine (kg), Q_{Cons} is the consumed amount of pharmaceuticals in the Rhine catchment area (kg), and f_{Excr} and f_{WWTP} are the fractions that are excreted by the human body and the fraction that passes the waste water treatment plant, respectively.

Table 8 shows the use of the most frequently observed pharmaceuticals at Lobith, their octanol-water partition coefficients (log K_{OW}), pK_A values, excreted fractions by humans and the removal in the waste water treatment plant. It can be observed that most pharmaceuticals are rather hydrophilic, especially X-ray contrast media have low log K_{OW} values.

Table 8 also shows the removal by waste water treatment plants and excreted fractions by humans. It can be observed that the variation in excretion by humans and the removability by waste water treatment can be large. The waste water treatment removal of the very hydrophilic (low log K_{OW}) X-ray contrast media and Beta blockers is less than 11%, while removal of the other pharmaceuticals is higher. Nevertheless, rather large fractions (≥26%) of all listed pharmaceuticals can pass waste water treatment plants.

Table 8: The selected pharmaceuticals and X-ray contrast media, their general use, the fraction of positive identifications, octanol water partition coefficients pK_A values and the major species of the pharmaceuticals at neutral pH.

Pharmaceutical	% positive id at Lobith	Log K_{OW} [1]	Use	pK_A	Charge major species pH 7	Fraction excreted via in feces and urine	Fraction removed by WWTP
Antibiotics							
Roxithromycin	21%	2.75	H/P	9.1 [3]	+1	0.60 (0.57-0.72) [5]	0.37 (0.09) [10]
Clarithromycin	44%	3.18	H/ P	9.0 [2]	+1	0.18 [8]	0.45 [10]
Clindamycin	72%	2.01	P/V	7.6 [3]	+1 & 0	0.19 (0.14-0.24) [5]	0 [11]
Anhydro-Erythromycin-A	94%	2.48	H/P/V	8.9 [2]	+1	0.98 (0.95-1.00) [6]	0.67 (0.16) [10]
Sulfamethoxazol	96%	0.48	P/V	6.1 [3]	-1	0.20 [6]	0.59 (0.22) [10]
Trimethoprim	54%	0.73	P/V	7.1 [2]	+1 & 0	0.45 (0.40-0.50) [5]	0.16 (0.20) [10]
Beta Blockers							
Atenolol	50%	-0.03	P	9.6 [2]	+1	0.83 (0.69-0.96) [6]	0.08 (0.04) [10]
Metoprolol	100%	1.69	P	9.6 [2]	+1	0.11 (0.05-0.15) [6]	0.10 (0.14) [10]
Sotalol	100%	-0.26	P	9.3 [3]	+1	1.00 (0.85-1.25) [6]	0.11 (0.08-0.12) [9]
Lipid regulators							
Pentoxifylline	30%	0.56	P	-	0	0.07 (0.06-0.09) [7]	0 [11]
Bezafibrate	86%	4.25	P	3.2 [2]	-1	0.51 (0.43-0.53) [6]	0.68 (0.36) [10]
Anti epilepticum							
Carbamazepine	98%	2.25	P	13.9 [2]	0	0.26 (0.21-0.31) [6]	0.09 (0.09) [10]
Analgesics / Anti inflammatory							
Ibuprofen	49%	3.79	P/U	4.9 [2]	-1	0.30 (0.11-0.31) [6]	0.74(0.29) [10]
Diclofenac	100%	4.02	P/U	4.2 [2]	-1	0.16 (0.06-0.26) [6]	0.32 (0.19) [10]
X-ray contrast medium							
Ioxitalamic acid	94%	0.50	H	2.2 [3]	-1	>0.95 [4]	0.00 [10]
Iopromide	96%	-2.05	H	11.1 [3]	0	>0.92 [4]	0.61 (0.50->0.71) [9]
Iohexol	96%	-2.18	H	11.7 [3]	0	1.00 [4]	0 [11]
Iomeprol	100%	-1.35	H	11.7 [3]	0	1.00 [4]	0.09 (0.03-0.86) [10]
Iopamidol	100%	-1.38	H	11.0 [3]	0	>0.90 [4]	0.00 [10]
Amidotrizoinic acid	100%	0.20	H	2.2 [3]	-1	>0.95 [4]	0.08 (0.07-0.22) [5]

Legend of use: H= hospital, P= pharmacy, V= veterinary, U= over-the-counter sale. 1) Log K_{OW} values are estimated with Epi Suite K_{OW} win V1.67a of the U.S. Environmental Protection Agency, 2000. pK_A values are obtained from 2) http://www.srcinc.com/what-we-do/databaseforms.aspx?id=386 or calculated with 3) http://www.chemaxon.com/marvin/sketch/index.jsp if no data were available. In most cases, the pharmaceuticals had more than one pK_A, in that case only the relevant pK_A closest to neutral pH was given. 4) Data obtained from (24), 5) Data obtained from KNMP 2006 (www.knmp.nl), minimum and maximum values are given between brackets. 6) Data obtained from (35), minimum and maximum values are given between brackets. 7) Data obtained from (38), minimum and maximum values are given between brackets. 8) Data obtained from (37). 9) Data obtained from (17), minimum and maximum values are given between brackets. 10) Data obtained from (30), standard deviation is given between brackets. 11) No data found, worst case estimate (0% removal).

The routes of excretion by the human body is complicated. The pharmaceuticals mainly leave the human body via feces and urine. Part of the pharmaceuticals leave the body unchanged, another part leaves the body as conjugate (e.g. glucoronidated, sulphonated, acetylated, methylated, etc.) and yet another part is metabolized to various degradation products. The table lists the fraction that leaves the body unchanged via urine or feces since this fraction will end up at the waste water treatment plant. However, conjugated pharmaceuticals might be transformed back into the parent compound in the waste water treatment or in the environment (39, 40). Only considering the parent compound might underestimate the actual environmental exposure in some cases. Nevertheless, we have decided to use the excretion via urine and feces (if data were available) because insufficient data on conjugation and de-conjugation in the environment are available.

The recoveries and annual loads predicted from consumption, excreted fractions by humans, and removal by wastewater treatment (Equation 1) of 15 out of 20 pharmaceuticals deviated less than a factor 2 from calculated recoveries and annual loads obtained from monitoring data (Figure 9, Table 9). For 5 of these 15 pharmaceuticals, calculated recoveries slightly exceeded predicted recoveries (up to a factor 1.4), while the recoveries of the other 10 pharmaceuticals were slightly overestimated. For the remaining 5 pharmaceuticals, predicted recoveries did not exceed calculated recoveries by a factor 7.

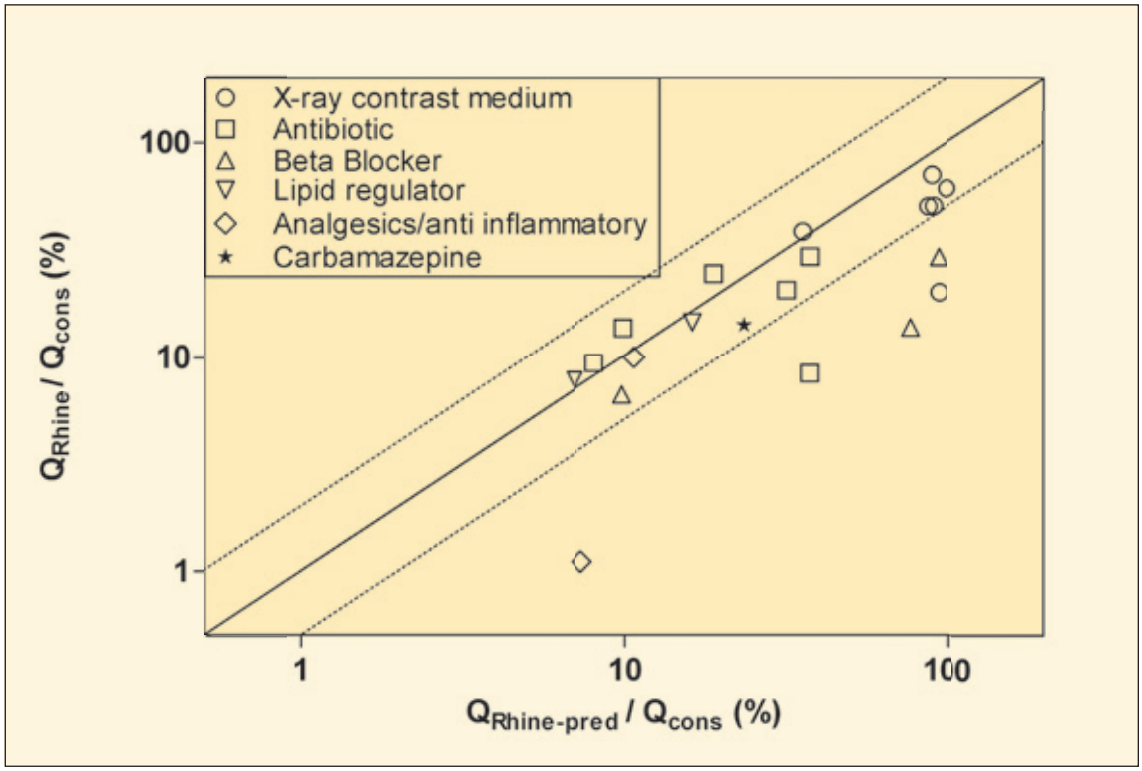


Figure 9: Figure 4: Calculated versus the predicted fractions (%) of the total consumption of the pharmaceuticals recovered at Lobith. The continuous line represents the 1:1 relationship and the dashed lines represent 1:2 and 2:1 ratios between the calculated and predicted recoveries.

Predicted loads can be higher than calculated loads due to environmental loss factors or differences between sales and actual amounts consumed by the users (41, 42). This might, for example explain the higher predicted loads of ibuprofen (factor 7), atenlol (factor 6), trimethoprim (factor 5), ioxitalamic acid (factor 5), and sotalol (factor 3) and than calculated from actual concentrations in the Rhine (Figure 9 and Table 9).

The rather large removal of ibuprofen during sewage treatment (74%) suggests that this pharmaceutical is vulnerable to (bio)degradation and sorption. These processes can continue in the river itself, thereby explaining why predicted loads overestimate calculated loads in the Rhine. However, removal of the other four pharmaceuticals in the waste water treatment is low (>16%). This suggests low environmental degradation as well. Nevertheless, conditions in the waste water treatment environment differ, so other (degradation) processes in the environment might still be relevant in explaining the differences between predicted and calculated loads.

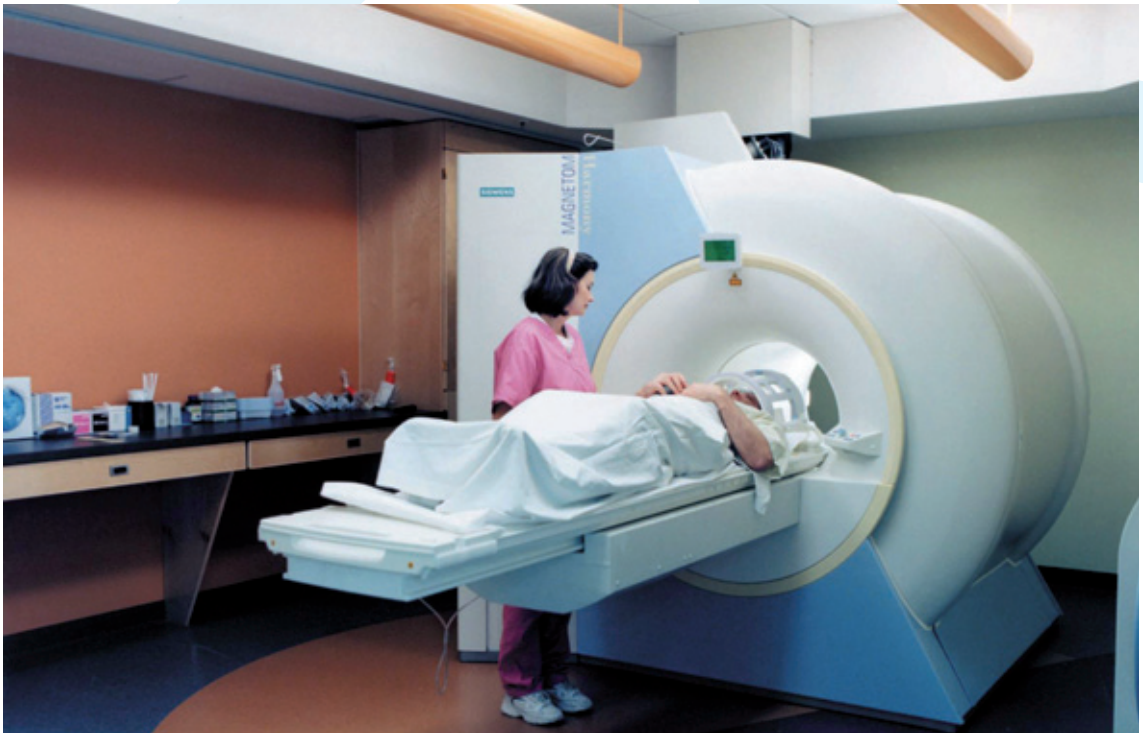


Table 9: Loads and recovered fractions of pharmaceuticals and X-ray contrast media in the Rhine versus consumption in Germany, France and Switzerland

Pharmaceutical	Q_{cons-G} (kg/y)	Q_{cons-S} (kg/y)	Q_{cons-F} (kg/y)	Q_{cons}, Equation 2 (kg/y) [7]	Q_{Rhine} at Lobith, Equation 3 (kg/y)	$Q_{Rhin-pred}$ at Lobith, Equation 4 (kg/y)
Inhabitants per country *10⁶	**82.4**	**7.3**	**58.5**			
Inhabitants Rhine catchment area per country *10⁶ (1)	**36.7**	**5.0**	**3.7**	**45.6** (upstream of Lobith)		
Antibiotics						
Roxithromycin	7359 [1]	149 [2]	4182 [6]	3665	1073	1380
Clarithromycin	12360 [1]	1700 [3]	16889 [6]	7784	1055	777
Clindamycin	11446 [1]	1014 [5]	1236 [6]	5660	1380	1075
Anhydro-erythromycin-A	19958 [1]	1768 [5]	10294 [6]	10677	2191	3420
Sulfamethoxazol	53600 [1]	2300 [3]	17519 [6]	26713	2491	2175
Trimethoprim	12183 [1]	520 [3]	20603 [6]	6040	502	2271
Beta Blockers						
Atenolol	13551 [1]	3071 [3]	10596 [6]	9501	1299	7287
Metoprolol	64699 [1]	3807 [5]	12602 [6]	32354	2132	3199
Sotalol	23945 [1]	877 [3]	24808 [6]	12132	3538	11445
Lipid regulators						
Pentoxifylline	100180 [1]	8875 [5]	19103 [6]	50930	3906	3565
Bezafibrate	39158 [1]	1574 [2]	3508 [6]	19842	2877	3247
Anti epilepticum						
Carbamazepine	83299 [1]	6260 [2, 8]	33364 [6]	43761	6184	10378
Analgesics / Anti inflammatory						
Ibuprofen	250792 [1]	22471 [2, 8]	58353 [6]	131592	1512	10177
Diclofenac	78579 [1]	6819 [2, 8]	22640 [6]	41354	4102	4489
X-ray contrast medium						
Ioxitalamic acid	8895 [1]	6819 [4]	20884 [6]	7819	1565	7428
Iopromide	97817 [1]	8965 [2,4]	12810 [6]	36416	14024	13066
Iohexol	8053 [1]	4614 [4]	46774 [6]	9764	5938	9764
Iomeprol	44727 [1]	1650 [4]	47355 [6]	24180	12210	22004
Iopamidol	38165 [1]	2739 [4]	34540 [6]	21181	14922	19063
Amidotrizoinic acid	50226 [1]	4450 [5]	13179 [6]	25608	12874	22381

1) Data obtained from (25), average sales of pharmacies and hospitals from 1996, 1998 and 2001. 2) Data obtained from (43), average sales in Swiss pharmacies in 2000. 3) Data obtained from (44), average sales in 2000 and 2004 covering all relevant (human) distribution channels in Switzerland. 4) Data obtained from (24),average sales in hospitals in 2004. 5) No data available, consumption assumed to be the same as other countries in the Rhine catchment area. 6) Data of average pharmacy and hospitals sales from 2002-2008 were obtained from (45) of the French medicine agency (AFSSAPS). 7) Calculated upstream of Lobith under the assumption that consumption of pharmaceuticals is homogenously spread over the inhabitants of different regions within a country. 8) Total consumption includes sales at public pharmacies, hospitals, drug stores and self dispensing physicians, which was estimated according to (46), 65% of carbamazepine, 70% of ibuprofen and 57% of diclofenac is sold via public pharmacies in Switzerland.

Predicted loads in the Rhine can also be lower than calculated loads because of using incomplete consumption data in the predictions. For example, the collected data did not include veterinary consumption, while this consumption is relevant for some antibiotics. The route of veterinary pharmaceuticals to surface water does, however, differ from the human route as manure of live stock, that contains these antibiotics, is initially stored in manure tanks and subsequently used as fertilizer on farmland (47). Therefore, the fraction of veterinary pharmaceuticals that enters surface waters is expected to be smaller than the fraction that enters surface waters via sewage treatment plants. Nevertheless, the slightly higher calculated loads of clindamicin and sulfamethoxazol in the Rhine might be explained by additional loads of these antibiotics from veterinary consumption. Additionally, the fraction that leaves the user as conjugate is not included in the current analysis, while part of the conjugates might convert back to the parent compound in waste water treatment or the environment, as is observed for estrogens (48).

The quality of the calculated loads depends on the quality of the monitoring data. The calculated loads might be biased by infrequent observations of the pharmaceuticals. The quality of the predicted loads depends on the quality of the consumption data and the data on excretion by the human body and removal in the waste water treatment plant. The consumption data might be biased because they did not always extend over the same years in which the samples were taken, and were calculated from average national consumption or even estimated from neighboring countries if no data were available. Currently, the pharmaceutical industry is not obliged to publish their production and sales. Better registration of sales of pharmaceuticals via pharmacies, hospitals, drug stores and self dispensing physicians on a regional basis would improve the accuracy of the input data and thereby probably also the prediction of annual loads in surface waters. Additionally, potential variation in the removal of pharmaceuticals by waste water treatment (17, 30) was not included in the estimation. Information on the removal of specific treatment processes applied in different waste water plants along a river, and the incorporation of the effects of the physical and chemical conditions in the treatment on the degradation of the pharmaceutical, can refine these predictions as well.

Environmental degradation and sorption are currently not included the prediction. Models that relate the chemical structure to degradation and sorption in the environment (Boethling and Mackay, 2000) could be applied to predict removal in the environment and could be included for a better prediction of annual loads.
All these uncertainties can bias the comparison between calculated and predicted environmental loads (14, 17, 23). Nevertheless, for 15 out of the 20 pharmaceuticals, the ratio of predicted and calculated concentrations does not exceed a factor two, and all predictions fall within a factor 7. This illustrates that a very simple mass balance (Equation 1) can predict annual loads in the Rhine relatively accurate, despite of all uncertainties and without the incorporation of environmental loss processes. Therefore, this mass balance approach might be useful when no monitoring data is available or when concentrations in surface waters are too low for robust chemical analysis. In addition to that, the mass balance approach might also be used to estimate consumption of substances from monitoring data. This might, for example, be valuable for the estimation of consumed pharmaceuticals for which no reliable consumption data are available, or for the consumption of illicit drugs.

Predicted recoveries can also be used to calculate average annual concentrations in a river. However, one must realize that variations in the flux of water in the Rhine, variations in consumption, and variations in removal efficiency in waste water treatment plants and in the river itself can affect actual concentrations in the environment. Chapter 3.1.2, 3.1.5 and 3.1.6 illustrate the magnitude of this variation for the Rhine.

Figure 10 shows the relationship between the log octanol-water partition coefficient (log K_{OW}) and the recovered fraction at Lobith.

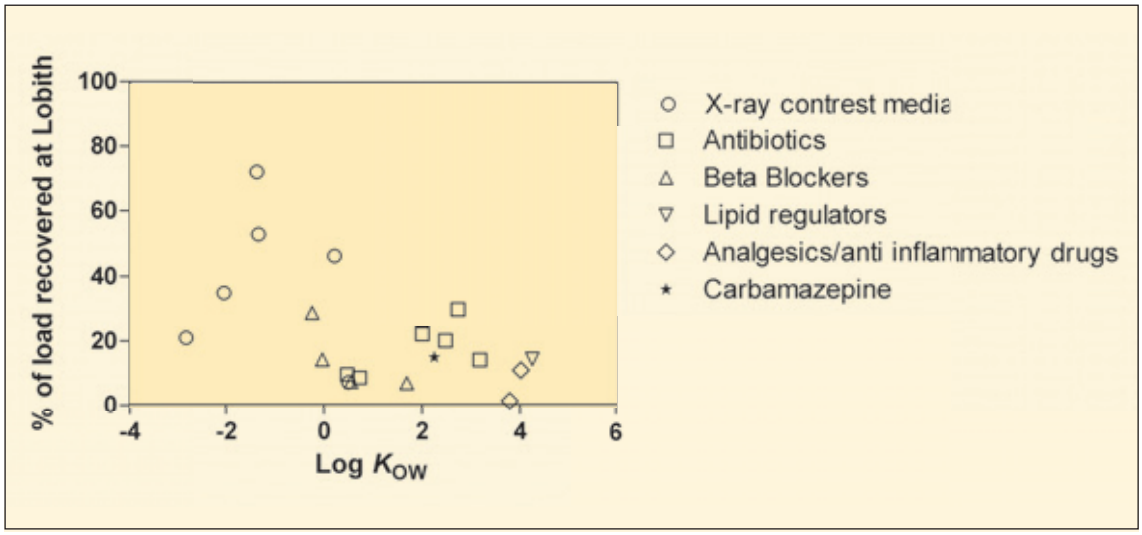


Figure 10: The recovered fractions of the pharmaceuticals plotted against their octanol-water partition coefficients.

The recoveries seem to be higher at lower log K_{OW} values. This trend is mainly governed by the relatively high recoveries of the very polar X-ray contrast media. It should however be noted that the relatively high log K_{OW} values of diclofenac, ibuprofen and bezafibrate hold for their neutral species while these chemicals are mainly negatively charged at neutral (environmental) pH (49). Furthermore, very clear trends are not expected since sorption to sludge (in waste water treatment) and sediment (in river) and the metabolism in the human body is not only determined by the hydrophobicity (i.e. log K_{OW}) of a pharmaceutical (50, 51). Therefore more sophisticated models using various physicochemical properties of the pharmaceuticals need to be developed for a better description (or even prediction) of residues of pharmaceuticals in the environment.

4 Conclusion

Concentrations of pharmaceuticals, X-ray contrast media and Endocrine disrupting chemicals are generally stable or slightly increase with the course of the Rhine following the anthropogenic pressure. However, in the Dutch delta concentrations appear to decrease slightly. Additionally, concentrations of most of these chemicals vary over one order of magnitude, but no significant trends were observed with time between 2002 and 2008. However, some of the monitored chemicals showed clear seasonal trends. These results indicate that several analyses per season remain necessary to retrieve yearly trends and to obtain a good impression of the amplitude of the variation and maximum concentrations in surface waters.

Mass balances of consumption and loads in the Rhine show that substantial fractions of pharmaceuticals consumed can be recovered in the Rhine. The recovered fractions could often be explained by literature data on the removal by waste water treatment and metabolism by the users. For some pharmaceuticals actual recovered concentrations in the Rhine were lower than values expected based on consumption, metabolism and removal in the waste water treatment. This can be explained by sorption and degradation processes in the environment that were not included in the prediction of environmental residues. Nevertheless, this indicates that a simple mass balance model that uses consumption data, excreted fractions, and fractions that pass waste water treatment can be used to make (worst case) estimations of environmental loads and average environmental concentrations. Furthermore, temporal trends in consumption (e.g. as a result of an aging population (14)) can be used to predict current and future concentrations in surface waters.

Knowledge on temporal trends and variations in concentrations, annual loads and models that can predict annual loads are important for drinking water companies, because it provides information on the contamination of drinking water sources, expected variations and trends in these concentrations, and allows us to predict loads and concentrations of future consumption scenario's.

Literature

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1. ICBR International catchment area of the Rhine: Properties, assessment of environmental effects of human activities and economic analysis of water use (part A). “Internationaal stroomgebiedsdistrict Rijn: Kenmerken, beoordeling van de milieueffecten van menselijke activiteiten en economische analyse van het watergebruik (deel A)”; ICBR: 2005, p 83.
2. Ternes, T. A., Pharmaceuticals in surface waters. Vorkommen von Pharmaka in Gewässern 2001, 53, 9-14.
3. Derksen, J. G. M.; Rijs, G. B. J.; Jongbloed, R. H., Diffuse pollution of surface water by pharmaceutical products. In Water Science and Technology, 2004; Vol. 49, pp 213-221.
4. Nikolaou, A.; Meric, S.; Fatta, D., Occurrence patterns of pharmaceuticals in water and wastewater environments. Analytical and Bioanalytical Chemistry 2007, 387, 1225-1234.
5. Snyder, S. A., Occurrence, treatment, and toxicological relevance of EDCs and pharmaceuticals in water. Ozone: Science and Engineering 2008, 30, 65-69.
6. Daughton, C. G.; Ternes, T. A., Pharmaceuticals and personal care products in the environment: Agents of subtle change? Environmental Health Perspectives 1999, 107, 907-938.
7. Kümmerer, K., Drugs in the environment: Emission of drugs, diagnostic aids and disinfectants into wastewater by hospitals in relation to other sources - A review. Chemosphere 2001, 45, 957-969.
8. Snyder, S. A.; Westerhoff, P.; Yoon, Y.; Sedlak, D. L., Pharmaceuticals, personal care products, and endocrine disruptors in water: Implications for the water industry. Environmental Engineering Science 2003, 20, 449-469.
9. Kemper, N., Veterinary antibiotics in the aquatic and terrestrial environment. Ecological Indicators 2008, 8, 1-13.
10. Giger, W.; Alder, A. C.; Golet, E. M.; Kohler, H.-P. E.; McArdell, C. S.; Molnar, E.; Siegrist, H.; Suter, M. J.-F., Occurence and fate of antibiotics as trace contaminants in wastewaters, sewage sludges, and surface waters. Chimica 2003, 57, 485-491.
11. Kasprzyk-Hordern, B.; Dinsdale, R. M.; Guwy, A. J., The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in South Wales, UK. Water Research 2008, 42, 3498-3518.
12. Christian, T.; Schneider, R. J.; FÃ¤rber, H. A.; Skutlarek, D.; Meyer, M. T.; Goldbach, H. E., Determination of antibiotic residues in manure, soil, and surface waters. Acta Hydrochimica et Hydrobiologica 2003, 31, 36-44.

13. Sacher, F.; Ehmann, M.; Gabriel, S.; Graf, C.; Brauch, H. J., Pharmaceutical residues in the river Rhine - Results of a one-decade monitoring programme. Journal of Environmental Monitoring 2008, 10, 664-670.

14. van der Aa, N. G. F. M.; Kommer, G. J.; Versteegh, A., Current and future consumption of pharmaceuticals. "Huidig en toekomstig gebruik geneesmiddelen". H2O 2009, 33-35.

15. Haller, Quantification of veterinary antibiotics (sulfonamides and trimethoprim) in animal manure by liquid chromatography-mass spectrometry. Journal of Chromatography A 2002, 952, 111-120.

16. Jones, O. A. H.; Voulvoulis, N.; Lester, J. N., Human pharmaceuticals in wastewater treatment processes. Critical Reviews in Environmental Science and Technology 2005, 35, 401-427.

17. Schrap, S. M.; Rijs, G. B. J.; Beek, M. A.; Maaskant, J. F. N.; Staeb, J.; Stroomberg, G.; Tiesnitsch, J. Human and veterinary pharmaceuticals in Dutch surface waters and waste waters. "Humane en veterinaire geneesmiddelen in Nederlands oppervlaktewater en afvalwater"; 2003.023; Dutch Institute for Inland Water Management and Waste Water Treatment (RIZA): Lelystad, the Netherlands, Sept 19, 2003, p 86.

18. Löffler, D.; Römbke, J.; Meller, M.; Ternes, T. A., Environmental fate of pharmaceuticals in water/sediment systems. Environmental Science and Technology 2005, 39, 5209-5218.

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

20. Walraven, N.; Laane, R. W. P. M., Assessing the discharge of pharmaceuticals along the Dutch coast of the North Sea. Rev Environmental Contamination Toxicology 2009, 199, 1-18.

21. Derksen, J. G. M.; Roorda, J. H.; Swart, D. Covered Pills "Verg(h)ulde pillen"; 2007-03; STOWA: Utrecht, 2007, p 58.

22. Versteegh, J. F. M.; Stolker, A. A. M.; Niesing, W.; Muller, J. J. A. Pharmaceuticals in drinking water and drinking water sources, results of the monitoring program 2002. "Geneesmiddelen in drinkwater en drinkwaterbronnen. Resultaten van het meetprogramma 2002"; 703719004/2003; Bilthoven, 2003, p 45.

23. van Batenburg-Eddes, T.; van den Berg-Jeths, A.; van der Veen, A. A.; Verheij, R. A.; de Neeling, A. Consumption in the Netherlands, regional variations in consumption of pharmaceuticals. "Slikken in Nederland. Regionale variaties in geneesmiddelengebruik'; 270556005/2002; RIVM: Bilthoven, 2002, p 76.

24. Weissbrodt, D.; Kovalova, L.; Ort, C.; Pazhepurackel, V.; Moser, R.; Hollender, J.; Siegrist, H.; McArdell, C. S., Mass flows of X-ray contrast media and cytostatics in hospital wastewater. Environmental Science & Technology 2009, 43, 4810-4817.

25. Rohweder, U. Pharmaceuticals in the environment: Assesment of results. "Arzneimittel in der Umwelt: Auswertung der Untersuchungsergebnisse"; Bund/Länderausschuss für Chemikaliensicherheit: Hamburg, 2003.

26. van der Aa, N. G. F. M.; Kommer, G. J.; de Groot, G. M.; Versteegh, J. F. M. Geneesmiddelen in bronnen voor drinkwater. Monitoring , toekomstig gebruik en beleidsmaatregelen; 609715002/2008; RIVM: Bilthoven, 2008, p 61.

27. Lemans, J. M. Potentie Meet-en Regeltechniek op grote watersystemen in Nederland: Afvoerverdeling Rijntakken. Technical University Delft, Delft, 2007.

28. Hua, W. Y.; Bennett, E. R.; Maio, X. S.; Metcalfe, C. D.; Letcher, R. J., Seasonality effects on pharmaceuticals and S-triazine herbicides in wastewater effluent and surface water from the Canadian side of the upper Detroit River. Environmental Toxicology and Chemistry 2006, 25, 2356-2365.

29. RIWA, 30 jaar Rijnwater; Deel 1 - Algemene parameters. RIWA Rijnwaterbedrijven: Nieuwegein, 2009.

30. Miege, C.; Choubert, J. M.; Ribeiro, L.; Coquery, M., Fate of pharmaceuticals and personal care products in wastewater treatment plants - conception of a database and first results. Environmental Pollution 2009, 157, 1721-1726.

31. Buser, H. R.; Poiger, T.; Muller, M. D., Occurrence and fate of the pharmaceutical drug diclofenac in surface waters: Rapid photodegradation in a lake. Environmental Science and Technology 1998, 32, 3449-3456.

32. 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. In Water Science and Technology, 2002; Vol. 46, pp 81-88.

33. Neumark, T.; Brudin, L.; Engstrom, S.; Molstad, S., Trends in number of consultations and antibiotic prescriptions for respiratory tract infections between 1999 and 2005 in primary healthcare in Kalmar County, Southern Sweden. Scandinavian Journal of Primary Health Care 2009, 27, 18-24.

34. Davey, P.; Ferech, M.; Ansari, F.; Muller, A.; Goossens, H., Outpatient antibiotic use in the four administrations of the UK: Cross-sectional and longitudinal analysis. Journal of Antimicrobial Chemotherapy 2008, 62, 1441-1447.

35. Lienert, J.; Gudel, K.; Escher, B. I., Screening method for ecotoxicological hazard assessment of 42 pharmaceuticals considering human metabolism and excretory routes. Environmental Science and Technology 2007, 41, 4471-4478.

36. Weissbrodt, D.; Kovalova, L.; Ort, C.; Pazhepurackel, V.; Moser, R.; Hollender, J.; Siegrist, H.; McArdell, C. S., Mass flows of X-ray contrast media and cytostatics in hospital wastewater. Environ. Sci. Technol. ASAP 2009.

37. Besse, J. P.; Kausch-Barreto, C.; Garric, J., Exposure assessment of pharmaceuticals and their metabolites in the aquatic environment: Application to the French situation and preliminary prioritization. Human and Ecological Risk Assessment 2008, 14, 665-695.

38. Smith, R. V.; Waller, E. S.; Doluisio, J. T., Pharmacokinetics of orally administered pentoxifylline in humans. Journal of Pharmaceutical Sciences 1986, 75, 47-52.

39. Clara, M.; Strenn, B.; Kreuzinger, N., Carbamazepine as a possible anthropogenic marker in the aquatic environment: Investigations on the behaviour of Carbamazepine in wastewater treatment and during groundwater infiltration. Water research 2004, 38, 947-954.

40. Leclercq, M.; Mathieu, O.; Gomez, E.; Casellas, C.; Fenet, H.; Hillaire-Buys, D., Presence and fate of carbamazepine, oxcarbazepine, and seven of their metabolites at wastewater treatment plants. Archives of Environmental Contamination and Toxicology 2009, 56, 408-415.

41. Keil, F. Pharmaceuticals for human use: Options of action for reducing the contamination of waterbodies; ISOE GmbH: Frankfurt, germany, 2008, p 52.

42. Keil, F.; Bechmann, G.; Kummerer, K.; Schramm, E., Systemic risk governance for pharmaceutical residues in drinking water. GAIA 2008, 17, 355-361.

43. Alder, A. C.; Bruchet, A.; Carballa, M.; Clara, M.; Joss, A.; Loffer, D.; Mcardell, C. S.; Miksch, K.; Omil, F.; Tuhkanen, T.; Ternes, T. A., Consumption and occurence. In Human Pharmaceuticals, Hormones, and Fragrances: The challenge of micropollutants in urban water management; Thomas, A.; Joss, A., Eds. IWA Publishing: 2006.

44. Ort, C.; Hollender, J.; Schaerer, M.; Siegrist, H., Model based evaluation of reduction strategies for micropollutants from wastewater treatement plants in complex river networks. Environmental Science and Technology 2009, 43, 3214-3220.

45. Cavalié, P. (2009) AFSSAPS Consumption data of human pharmaceuticals in France 2002-2008. personal communication.

46. McArdell, C. S.; Weissbrodt, D.; Kovalova, L.; Ort, C.; Moser, R.; Hollender, J.; Siegrist, H. In Mass flow analysis of pharmaceuticals in hospital wastewater, Xenowac, Xenobiotics in the Water Cycle, Cyprus, 2009.

47. Boxall, A. B. A.; Kolpin, D. W.; Halling-Soerensen, B.; Tolls, J., Are veterinary medicines causing environmental risks? Environmental Science and Technology 2003, 37, 286A-293A.

48. Kummerer, K., Pharmaceuticals in the environment: sources, fate, effects and risks. 3rd ed.; Springer: Berlin, Heidelberg, New York, 2008.

49. Box, K. J.; Comer, J. E. A., Using measured pKa, LogP and solubility to investigate supersaturation and predict BCS class. Current Drug Metabolism 2008, 9, 869-878.

50. ter Laak, T. L.; Gebbink, W. A.; Tolls, J., The effect of pH and ionic strength on the sorption of Oxytetracyclin, Tylosin and Sulfachloropyridazin to soil. Environmental Toxicology and Chemistry 2006, 25, 904-911.

51. Bu, H. Z., A literature review of enzyme kinetic parameters for CYP3A4-mediated metabolic reactions of 113 drugs in human liver microsomes: Structure-kinetics relationship assessment. Current Drug Metabolism 2006, 7, 231-249.

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