XENOBIOTIC ORGANIC MICROPOLLUTANTS IN URBAN WASTEWATER

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LEVELS, DISTRIBUTION PATTERNS AND THE IMPACT OF ADVANCED TREATMENT TECHNOLOGIES ON THEIR PRESENCE IN WASTEWATER FROM DIFFERENT SOURCES

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To my parents

The best way out is always through.

– Robert Frost (1874-1963), A Servant to Servants, 1914 –

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Gießen, August 2014

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LIST OF ABBREVIATIONS

AChE	acetylcholinesterase
ACN	acetonitrile
ADHD	attention-deficit hyperactivity disorder
AOX	adsorbable organic halogen
APCI	atmospheric pressure chemical ionization
ATV	Abwassertechnische Vereinigung e.V.
BAS	biofilm airlift suspension
BCF	bioconcentration factor
BfArM	Bundesinstitut für Arzneimittel und Medizinprodukte (Federal Institute for Drugs and Medical Devices)
BFB	biofilm fluidized bed
BfG	Bundesanstalt für Gewässerkunde (German Federal Institute of Hydrology)
BFR	biofilm reactor
BIP	bezafibrate infarction prevention
BLAC	Bund/Länderausschuss für Chemikaliensicherheit
BZF	bezafibrate
CAD	collisionally activated dissociation
CAS	conventional activated sludge system, also: conventional activated sludge treatment
CBE	Center for Biofilm Engineering
CBZ	carbamazepine
c_c	solute concentration in the concentrate
CDN	codeine
CE	collision energy
c_{eff}	effluent concentration
c_f	solute concentration in the feed
c_{inf}	influent concentration
CLA	clarithromycin
c_{max}	maximum concentration
COD	chemical oxygen demand
CORESET	Development of HELCOM Core Set indicators (HELCOM project)
COX	cyclooxygenase

c_p	solute concentration in the permeate
CT	X-ray computed tomography
CXP	collision cell exit potential
DCF	diclofenac
DDD	defined daily dose
DHC	dihydrocodeine
DH-CBZ	10,11-dihydrocarbamazepine
DHFR	dihydrofolate reductase
DHH	10,11-Dihydro-10,11-dihydroxycarbamazepine
DMI	desmethoxy-iopromide
DNA	deoxyribonucleic acid
DP	declustering potential
DTZ	diatrizoic acid
DVKW	Deutschen Verbandes für Wasserwirtschaft und Kulturbau e.V.
DXP	doxepin
DZP	diazepam
EAWAG	Wasserforschungs-Institut des ETH-Bereichs (Swiss Federal Institute of Aquatic Science and Technology)
EBPR	enhanced biological phosphorus removal
ECDC	European Centre for Disease Prevention and Control
EFRA	European Flame Retardants Association
EGSB	expanded granular sludge blanket
EMEA	European Medicines Agency
EM-ERY	(E)-9-[O-(2-methyloxime)]-erythromycin
EP	entrance potential
EPA	United States Environmental Protection Agency
EPS	extracellular polymeric substances
ERA	environmental risk assessment
ERQ	environment risk quotient
ESAC	European Surveillance of Antimicrobial Consumption
ESI	electrospray ionization
F/M	food-to-microorganism ratio
FEDESA	European Federation of Animal Health
FS	flat sheet
GABA	gammaaminobutyric acid
GC-MS	gas chromatography-mass spectrometry

GHS	Globally Harmonized System of Classification and Labelling of Chemicals
GPC	gel permeation chromatography
h	hours
H_2SO_4	sulfuric acid; also: sulphuric acid
H302	GHS hazard statement 302: Harmful if swallowed
H315	GHS hazard statement 315: Causes skin irritation
H317	GHS hazard statement 317: May cause an allergic skin reaction
H319	GHS hazard statement 319: Causes serious eye irritation
H351	GHS hazard statement 351: Suspected of causing cancer
H360F	GHS hazard statement 360F: May damage fertility
H410	GHS hazard statement 410: Very toxic to aquatic life with long lasting effects
H411	GHS hazard statement 411: Toxic to aquatic life with long lasting effects
H412	GHS hazard statement 412: Harmful to aquatic life with long lasting effects
HELCOM	Helsinki Commission; also: Baltic Marine Environment Protection Commission
HF	hollow fibre
HLC	Henry's Law Constant
HPV	high production volume
HRT	hydraulic retention time
IBP	ibuprofen
IC	internal circulation
ICM	iodinated x-ray contrast medium
IFA	Institut für Arbeitsschutz der Deutschen Gesetzlichen Unfallversicherung
IMI	iopromide
IMI-TP	transformation product of iopromide
IMS	International Marketing Services
IOP	iomeprol
IPM	iopamidol
IS	internal standard
J	permeate flux
K	membrane permeability

K_a	acid dissociation constant, also: acidity constant, acid-ionization constant
KemI	National Chemical Inspectorate of Sweden
K_{OW}	octanol-water partition coefficient
LC	liquid chromatography
LC_{50}	median lethal concentration: aqueous concentration at which 50% of test organisms dies
LD_{50}	median lethal dose, e.g. by injection or oral administration, causing 50% of test organisms to die
LIF	Swedish Association of the Pharmaceutical Industry
LOEC	lowest observable effect concentration; lowest observed effect concentration
LOQ	limit of quantification
MBR	membrane bioreactor
MDR	multiple drug-resistant
MEC_{max}	maximum concentration measured
MeOH	methanol
MF	microfiltration
MLSS	mixed-liquor suspended solids
MMT	methadone maintenance treatment
MPN	morphine
MRM	multiple reaction monitoring
MRT	magnetic resonance tomography
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MTD	methadone
MW	molecular weight
MWCO	molecular weight cut-off
N_2	nitrogen (gas)
NaCl	sodium chloride
N-Ac-SMX	N4-acetylsulfamethoxazole
NF	nanofiltration
NICE	National Institute for Clinical Excellence
NLM	United States National Library of Medicine
NMR	nuclear magnetic resonance
NO_2	nitrite

NO_3	nitrate
NOEC	no observed adverse effect concentration
NPX	naproxen
NSAID	non-steroidal anti-inflammatory drug
NZP	nordiazepam
OCN	oxycodone
OECD	Organisation for Economic Co-operation and Development
OEHHA	Office of Environmental Health Hazard Assessment
OP	organophosphate ester; organophosphorus compound
OSPAR	Convention for the Protection of the Marine Environment of the North-East Atlantic
OTC	over-the-counter (drug)
OZP	oxazepam
p.e.	population equivalent
PAC	powdered activated carbon
PAN	polyacrylonitrile
PAO	polyphosphate accumulating organism
PBT	persistent, bioaccumulative and toxic substance
PE	polyethylene
PEC	predicted environmental concentration
PES	polyethylsulphone
pH	activity of (solvated) hydrogen ions
pK_a	logarithmic constant, equal to $-\log_{10} K_a$
PLE	pressurised liquid extraction
PMD	primidone
PNEC	predicted no effect concentration
$POCl_3$	phosphorus oxychloride
POP	persistent organic pollutant
PP	polypropylene
PUF	polyurethane foam
PVC	polyvinyl chloride
PVDF	polyvinylidene difluoride
QA/QC	quality assurance and quality control
Q_c	flow rate of the concentrate
Q_f	flow rate of the feed
Q_p	flow rate of the permeate

Qq-LIT-MS	hybrid triple quadrupole linear ion trap mass spectrometer
QSAR	quantitative structure activity relationship
R	resistance; membrane rejection of solutes
r^2	correlation coefficient
RBC	rotating biological contactor
R_c	cake resistance
REACH	Regulation concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (Regulation (EC) No 1907/2006)
R_f	fouling resistance
R_m	membrane resistance
RNA	ribonucleic acid
RO	reverse osmosis
ROX	roxithromycin
RSD	relative standard deviation
RT	retention time
S/N	signal/noise ratio
SCBP	suspended carrier biofilm process
SCHER	Scientific Committee on Health and Environmental Risks
SD	standard deviation
SDI	sulfadimidine
SMA	sulfamerazine
SMI	sulfadimethoxine
SMX	sulfamethoxazole
SPE	solid phase extraction
SRT	solids retention time
SSX	sulfisoxazole
t	exposure duration
t	tonne (= 1000 kg)
T	transition
TAM	tiamulin
TBEP	tris(2-butoxyethyl) phosphate
TCEP	tris(2-chloroethyl) phosphate
TCPP	tris(chloropropyl) phosphate
TDCPP	tris(1,3-dichloro-2-propyl) phosphate
TEHP	tris(2-ethylhexyl) phosphate
TiBP	tri-iso-butyl phosphate

TMP (also: ΔP)	transmembrane pressure
TMP	trimethoprim
TnBP	tributyl phosphate
TP	transformation product
TPP	triphenyl phosphate
TZP	temazepam
UCS	Union of Concerned Scientists
UF	ultrafiltration
UK	United Kingdom of Great Britain and Northern Ireland
UNESCO	United Nations Educational, Scientific and Cultural Organization
USA	United States of America
USB	upflow sludge blanket
UV	ultraviolet light
WHO	World Health Organization
W_s	water solubility
WWTP	wastewater treatment plant
Y	yield; also: recovery or water recovery
η	permeate viscosity

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ABSTRACT

This study investigated the occurrence of 52 xenobiotic micropollutants – pharmaceuticals, metabolites, transformation products, and organophosphorus compounds (OPs) – in hospital and municipal wastewater. It aimed to characterise the xenobiotic fingerprint in raw wastewater from different sources and the impact of different advanced treatment technologies on concentration levels and distribution patterns of xenobiotic micropollutants. Thus, temporal concentration profiles of the influent and effluent of a nanofiltration membrane biofilm reactor (NF-MBR) situated at the influent of a municipal wastewater treatment plant (population equivalent: 300,000) as well as of a particle-supported biofilm reactor (PS-BFR) installed at the main effluent of a municipal hospital (186 beds, about 200 medical staff) were studied. Furthermore, the occurrence of xenobiotics in biosolids was addressed. For this, a multi-residue extraction method was developed and subsequently used to determine the micropollutants in sewage sludge from the NF-MBR and in sludge and carrier material from the PS-BFR. Ultimately, an aquatic environmental assessment for the investigated micropollutants was carried out based on the results of the presented study.

It shows that a wide range of xenobiotics is present in both municipal and hospital wastewater. In summary, it can be said that there are basic differences between wastewater streams originating from the hospital and from mixed municipal sources regarding concentrations for iodinated contrast media, while other pharmaceuticals are more evenly distributed. For OPs, similar overall concentrations in the two wastewater types derived from a very different set of single substances. Regarding the contribution of hospitals to the overall xenobiotic load of municipal wastewater, it was found that even a small hospital can contribute greatly to the overall annual load: 21.5% of the annual load of diatrizoic acid reaching the municipal wastewater treatment plant is estimated to originate from the investigated hospital.

While the NF-MBR revealed a much greater potential for micropollutant removal than the PS-BFR, even this system showed unsatisfactory results (< 75% removal) for 45% of the investigated substances. The NF-MBR was especially efficient with regard to biodegradation, while the removal of non-biodegradable substances (e.g. carbamazepine) was insufficient, which suggests that the filtration capacity of the loose NF membrane yielded retention results that were no better than those previously described for wider membrane types.

The seasoned PS-BFR (start-up time prior to the study: 199 days) successfully adapted to the dynamic matrix which hospital wastewater represents, and demonstrated basic potential for biodegradation by stable removal rates of over 75% for primidone, ibuprofen and morphine, but failed in terms of increased overall xenobiotic degradation. However, high concentrations (ranging up to over 2,000 ng/g d.w.) of clarithromycin found in the biosolids (sludge and biofilm

of the carrier material) might indicate that the system has a high sorption potential for ionic compounds.

The aquatic environmental assessment showed that wastewater, both raw and treated, represents a risk when it reaches receiving waters. High risk levels are being caused by substances from not just a single therapeutic group but several (psycho-active compounds, antibiotics, non-steroidal anti-inflammatory drugs). However, in all wastewater types investigated, the highest risk was found to be the psycho-active compound oxazepam.

Taking all of the above mentioned into account, it is impossible to say with certainty that pharmaceuticals and other xenobiotics in (waste)water are not a threat to the aquatic environment. To compensate for the lack of knowledge, precautionary principles must be applied, and these xenobiotics should be kept from reaching the aquatic environment.

1. THE CHALLENGE OF XENOBIOTIC MICROPOLLUTANTS IN URBAN WATER CYCLES – INTRODUCTION AND SCOPE OF THE STUDY

All the water that will ever be is, right now.
– National Geographic, October 1993 –

We forget that the water cycle and the life cycle are one.
– Jacques-Yves Cousteau (1910-1997) –

WATER, THE SOURCE OF LIFE, IS LIMITED. The earth's water resources are not endless but finite. Water is merely recycled and redistributed within a global water cycle which is influenced by climatic conditions and nowadays, to an alarming degree, by human activity (UNESCO, 2009). Access to safe water is a major subject with regard to disease control and it is recognized as one, maybe *the* one fundamental base for human well-being, economic growth and political stability (Prüss-Üstün et al., 2008). Yet, water is becoming a costly treasure.

WATER CRISIS. Globally, the pressure on fresh water resources is rising, already reaching the level of acute crisis in many regions (UNESCO, 2009). Reasons include the increasing demands of growing populations, rapid urbanisation and rising water consumption for agricultural and industrial production. This is compounded by shrinking water resources brought about by changes in climatic conditions leading to temperature rise and declining rainfall. This causes prolonged drought periods, during which surface water reservoirs are no longer able to match water demand (Hagare, 2012; EPA, 1998). Excessive groundwater abstraction results in water table drawdown, which opens the door to additional environmental problems such as land subsidence and saltwater intrusion (Takizawa, 2008; Rodriguez et al., 2009). Certain geographical situations, such as the dependence of large urban settlements on a single river (for example, London is provided with drinking water mainly by the river Thames), small landmass or missing natural aquifers can exacerbate the water scarcity further (Cho, 2011). In Germany, the Berlin area is expected to experience decline in the replenishment of natural groundwater which currently provides 30% of Berlin's drinking water**.** At the same time the flow rate of regional surface waters, which as a result are becoming more important for the drinking water supply, are expected to drop by 40% (Dünnbier, 2012).

WATER REUSE STRATEGIES. With surface and groundwater sources increasingly failing to provide a long-term, continuous supply, water reuse in an artificially shortened water cycle is rapidly becoming more essential for practical water resources management (Wintgens et al., 2005; Le-Minh et al., 2010). While water recycling for non-potable purposes (e.g. irrigation of agricultural fields, parks and golf courses, process water in industrial contexts or water for toilet flushing in households) is largely established today (EPA, 1998), so-called indirect potable water reuse is becoming increasingly important (Rodriguez et al., 2009). In this process purified wastewater is intentionally used to supplement drinking water supplies by e.g. groundwater recharge or infusion into lakes and water reservoirs. A technique which is to date less common, but considered crucial to meet the challenges of water management, is direct potable reuse, where highly purified wastewater is directly used as raw water for drinking water production without a temporal and spatial buffer (Crook, 2010).

NO SUCH THING AS PURE, NATURAL WATER. Natural water always contains substances in suspended or particular form. Some of them are favourable, such as minerals that give spring water a certain distinct taste (EPA, 1999). However, natural waters are often a habitat for microbial organisms, many of them harmful to human health. The WHO estimates roughly 10% of the global disease burden would be preventable by improving water supply, sanitation, hygiene and the management of water resources (Prüss-Üstün et al., 2008). Safe drinking water is always the result of a production process, its complexity depending on the quality of the feed water and the available financial resources.

XENOBIOTIC ORGANIC MICROPOLLUTANTS. Microbial impact on water is traditionally the major concern regarding drinking water quality, while nutrients are of most concern in wastewater because of their potential to induce eutrophication in receiving waters. However, in the last decades so-called xenobiotic substances, meaning chemicals not naturally occurring in the environment, increasingly came into focus. Firstly, pesticides were recognised as possible threats to water quality (e.g. Greve, 1972; Dawson and Riley, 1977; El-Dib and Aly, 1977; McNeil et al., 1977). Due to better analytical instrumentation allowing the detection of polar substances in the ng/L range, a group of "emerging" organic micropollutants was noticed in the 1990s, among them pharmaceuticals (Ternes, 2007; Busetti et al., 2009; Fatta-Kassinos et al., 2011a).

PHARMACEUTICALS. Soon thereafter pharmaceuticals were recognized as environmentally relevant chemicals (Ternes, 1998), since they are by design biologically effective in human and animal organisms at low doses and to some degree persistent, which makes their presence in the environment a matter of concern (Comeau et al., 2008). In recent years, there have been

frequent reports of pharmaceuticals being identified in environmental samples of wastewater, surface water and drinking water (e.g. Heberer, 2002a; Heberer, 2002b; Kolpin et al., 2002; Kim et al., 2007; Huerta-Fontela et al., 2011; Kleywegt et al., 2011; Wang et al., 2011). Today, the concentrations of some pharmaceuticals in surface water equal the concentrations of common pesticides (Gentili, 2007). Moreover, it is not only the parent drugs which can be detected in the environment but their metabolites, too. Many of the metabolites are biologically active and their ecotoxicological properties unknown (Kolpin et al., 2002; Miao et al., 2005; Bataineh et al., 2006; Leclercq et al., 2009; Fatta-Kassinos et al., 2011b). Furthermore, environmental transformation products of pharmaceuticals are as yet often completely unidentified and have only very recently become subject to research studies. Thus, to date, their environmental pathways are mostly unclear. This basically makes them an entirely unknown hazard to aquatic ecosystems and human health (e.g. Kosjek et al., 2007; Kormos et al., 2009; Kosjek et al., 2009).

INDUSTRIAL CHEMICALS. Another group of xenobiotic substances causing increasing concern are industrial chemicals like flame retardants and plasticisers that are used as high production volume (HPV) substances in a wide range of everyday products and applications (EPA, 2012a). Since even moderately persistent substances among them are constantly found in high environmental concentrations they are regarded as "pseudo-persistent" compounds (Daughton, 2002a).

UNKNOWN LONG-TERM EFFECTS. Little is known about the long-term effects of the environmental presence of xenobiotic micropollutants (Gentili, 2007; Hernando et al., 2007; Busetti et al., 2009), but plain evidence of possible risks was given in 2004, when a study of Oaks et al. (Oaks et al., 2004) proved that the mass extinction of vultures in Pakistan was caused by the massive intake of the painkiller diclofenac which was used to treat the cows that provided the vultures' nutrition base. Furthermore, some xenobiotic micropollutants are considered endocrine disrupting compounds. For these, possible connections between their occurrence in the environment and severe health effects like deteriorating reproductive health in humans and development of cancer are discussed (Gunten et al., 2006; Mückter, 2006). In increasingly tightening urban water cycles, xenobiotics can accumulate rapidly. Thus, in accordance with precautionary principles, the entry of pharmaceuticals and other micropollutants into the aquatic environment has to be prevented (Ternes, 2007; Rechenberg and Dieter, 2009).

REDUCING MICROPOLLUTANTS. One way to reduce micropollutants in water is to prevent their production and use. Efforts have been made to reduce the input of micropollutants into the environment, e.g. regulations for the phase-out of known harmful HVP-substances such as

brominated flame retardants. In an as yet unique attempt to reduce the environmental pressure caused by pharmaceuticals, Sweden developed an environmental classification system for pharmaceuticals (LIF, 2007; LIF, 2012). Still, in many cases, especially regarding pharmaceuticals, end-of-pipe solutions in the form of the treatment of wastewater are the only currently viable way to reduce the occurrence of xenobiotics in the environment.

TREATMENT METHODS. However, conventional wastewater treatment plants (WWTPs) were identified as major point sources of xenobiotic substances and gateways for their entry into the aquatic environment, since they are not designed to remove (polar) persistent micropollutants (Joss et al., 2006; Ternes, 2007). For this purpose, advanced treatment techniques in form of additional steps beyond conventional treatment or as alternative treatment designs are necessary. Various concepts like advanced oxidation, filtration systems and enhanced biological treatment have been investigated recently, but the need for further research is apparent (Huber et al., 2005; Lin et al., 2010; Tambosi et al., 2010; Behera et al., 2011). In addition, decentralised treatment of local hot spots such as wastewater from hospitals, which are considered a possible primary source of certain pharmaceutical contamination, is currently being discussed (e.g. Souza et al., 2009; Chang et al., 2010; Beier et al., 2011; Escher et al., 2011; Verlicchi et al., 2012a; Perrodin et al., 2013).

THE STUDY PRESENTED. This study investigated the occurrence of 52 xenobiotic micropollutants – pharmaceuticals, metabolites, transformation products and HPV organophosphorus compounds – in raw hospital and municipal wastewater. It aimed to characterise a) the xenobiotic fingerprint in wastewater from different sources and b) the impact of different advanced treatment technologies on concentration levels and distribution patterns of xenobiotic micropollutants. For this, temporal concentration profiles of raw municipal wastewater were studied, including both weekdays and the weekend, and the removal of the studied micropollutants during wastewater treatment by a nanofiltration membrane biofilm reactor (NF-MBR) situated at the influent of a municipal WWTP (population equivalent: 300,000) was investigated (Chapter 3). These results were compared to the elimination performance of pure NF-treatment without biological treatment. In the same way as the municipal wastewater stream, untreated wastewater from a municipal hospital was studied and the influence of a particle-supported biofilm reactor (PS-BFR) on the distribution patterns of xenobiotic micropollutants was investigated (Chapter 4). In the third part of the presented work, the occurrence of xenobiotics in biosolids was addressed (Chapter 5). For this, a multi-residue extraction method was developed and subsequently used for the determination of the micropollutants in sewage sludge from the NF-MBR and in sludge and carrier material from the

PS-BFR. The results of the individual studies presented in Chapters 3 to 5 were subsequently contrasted and related to each other with regard to xenobiotic distribution patterns, xenobiotic loads of different wastewater streams and the capacity for xenobiotic removal found for the studied wastewater treatment methods (Chapter 6). Finally, an aquatic environmental assessment for the investigated micropollutants was carried out based on the results of the presented study.

2. FUNDAMENTALS

Access to a secure, safe and sufficient source of fresh water is a fundamental requirement for the survival, well-being and socio-economic development of all humanity. Yet, we continue to act as if fresh water were a perpetually abundant resource. It is not.
– Kofi Annan (1938-), Awake! magazine, June 22, 2001 –

2.1 CHARACTERISTICS OF SELECTED XENOBIOTIC ORGANIC MICROPOLLUTANTS FROM DIFFERENT CLASSES

2.1.1 PHARMACEUTICALS – METABOLISM, CONSUMPTION AND OCCURRENCE IN THE AQUATIC ENVIRONMENT

DESIGN OF PHARMACEUTICALS. Pharmaceuticals are designed to have a pharmacological, i.e. biological, effect at low concentrations (Halling-Sørensen et al., 1998; Hörsing et al., 2011). At the same time, they are meant to withstand biological processes to reach their target organ in human or animal organisms. Thus, they are to some degree persistent by design. These characteristics are the main sources of concern regarding pharmaceutical occurrence in the environment (Kümmerer, 2001).

METABOLISM AND BIOTRANSFORMATION. After intake, pharmaceuticals are completely or partly metabolised before excretion. Metabolism takes place mainly in the liver and also, to a lesser extent, in the gastrointestinal tract, kidneys, lungs or skin. It can be described as a two-phase-process: the first phase (phase I reaction) typically consists of oxidation, reduction or hydrolysis of the compound, before, in the phase II reaction, the metabolite is conjugated in a way that enhances polarity and therefore excretion (Figure 2-1). While phase I metabolites are often bioactive, phase II usually leads to the deactivation of the metabolites (detoxication) (Mutschler et al., 2008). However, these principles do not apply in all cases: some compounds only undergo the phase I reaction and are excreted directly afterwards, others show a phase II reaction without a prior phase I (Ternes, 1998; Alder et al., 2006).
As well as metabolism in the target organism, pharmaceuticals can also be biotransformed in the environment after excretion (Alexy and Kümmerer, 2006; Heberer and Ternes, 2006). Microorganisms use organic compounds directly as an energy source for life sustainment and

growth. Furthermore, organic compounds are co-metabolised without being used as a carbon source by the metabolising microorganism (Nyholm et al., 1996).

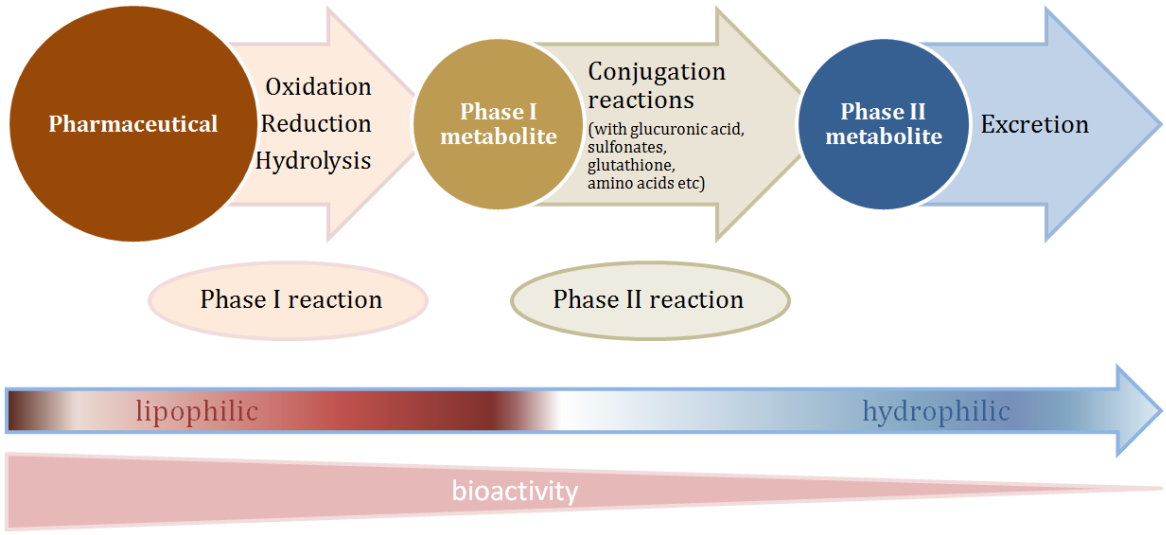


Figure 2-1: Principle human metabolism of pharmaceuticals (adapted from Mutschler et al., 2008).

UNIDENTIFIED PROPERTIES. Despite intensive research during the last decades, the kinetics and mechanisms of drug metabolism are still in many cases not known precisely. This is partly because of the large number of substances used in modern human and veterinary medicine. According to Kümmerer, 2004, at the end of the twentieth century about 50,000 drugs were registered in Germany for human use, of which 2,700 accounted for 90% of the overall consumption, the latter containing approximately 900 different pharmaceutically active compounds. At the same time in the UK, about 3,000 active compounds were registered (Ayscough et al., 2000).

In addition, the identification of metabolites is difficult, time-consuming and costly; it requires high-end analysis equipment and very often the in-house synthesis of metabolites which are not commercially available (Kosjek et al., 2007). Furthermore, the metabolic processes of one pharmaceutical compound can result in a large number of metabolites; e.g. for carbamazepine 33 metabolites were found in human and rat urine (Lertratanangkoon and Horning, 1982).

The extent to which a drug is metabolised during passage through the target organism varies between substances, as does the percentage excreted in either urine or faeces (Table 2-1). However, Alder et al., 2006 estimates that overall approximately 70% of pharmaceutical compounds are excreted via urine and 30% are faeces-bound. In sewage and sewage treatment (or, in the case of veterinary drugs, in manure), metabolite conjugated with e.g. glucuronic acid

or sulphate can be cleaved by enzymes and/or bacteria resulting in a re-introduction of the active parent compound in the wastewater during treatment and subsequently elevated amounts in the receiving water bodies (Heberer and Ternes, 2006). Consequently, several studies reported negative removal rates for various pharmaceuticals in wastewater treatment plants (e.g. Lishman et al., 2006; Barron et al., 2008; Chang et al., 2008c).

In addition to the metabolism that drugs undergo in the target organism, an "environmental" biotransformation of drug residues and metabolites by microorganisms occurs in sewers, WWTPs, receiving water bodies and soils which are treated with sewage sludge or irrigated with treated water. For example, Kormos et al., 2009 described five, seven and eleven transformation products in soils for the contrast media iomeprol, iopamidol and iohexol respectively.

Table 2-1: Excretion forms of selected pharmaceuticals.

Substance	**Excreted unchanged in human urine/faeces [%]**	**Excreted as glucuronide*** [%]**
Clarithromycin	20–30 / 4–11[1]	
Roxithromycin	8 / 55[1]	
Sulfamethoxazole	10 / -[1]	
N4-acetylsulfamethoxazole (metabolite)	(50 / -)*[1]	
Sulfadimidine	10 / -[1]	
Trimethoprim	50 / -[1]	
Carbamazepine	0.5; 1-2**[2]	approx. 30[2]
Bezafibrate	50**[2]	22
Diclofenac	15**[2]	< 1[2]
Ibuprofen	1-8**[2]	15[2]
Iopamidol	100**[3]	
Iomeprol	100**[3]	
Iopromide	100**[3]	
diatrizoic acid	100**[3]	

[1] Göbel et al., 2005b; [2] Alder et al., 2006; [3] Pérez and Barceló, 2007 *percentage of the administered sulfamethoxazole dose; **manner of excretion not further specified; *** phase II metabolite without previous phase I reaction

ENVIRONMENTAL FATE. Until recent years, the release of pharmaceuticals into the environment attracted little attention (Halling-Sørensen et al., 1998). Only when improved analytical methods with considerably lower detection limits became available did these micropollutants start to be (re-)discovered in environmental samples as "emerging compounds", a somewhat misleading term because many of the drugs had been in use for decades (Möller et al., 2011).

Possible pathways of pharmaceuticals into the environment are shown in Figure 2-2. As early as during production, potential input pathways are opened by disposal of solid waste or process waters. After intake, excreted human drugs enter the sewage system and reach wastewater treatment plants, where they are removed (i.e. biotransformed) to very different degrees before

being released into the aquatic environment. The disposal of unused pharmaceuticals via the toilet is another entrance to this pathway. In Germany, a study showed that 23% of prescribed liquid drugs were discarded; the amount of pharmaceuticals flushed into the sewers equals approximately 364 t per year (Lubick, 2010). Subsequently, wastewater treatment plants have been identified as the most important gateway for drugs into the aquatic system (Barceló, 2004; Kümmerer, 2004; Alder et al., 2006). In the assessment of the environmental fate of pharmaceuticals, sorption to sewage sludge, soils and sediments has to be considered. While drugs disposed of with solid waste are destroyed by oxidation during waste incineration, waste disposal in landfills can lead to the occurrence of pharmaceuticals in the aquifer through landfill leakage or by passage with drainage water (Alder et al., 2006). Sewer leakages can open various pathways for pharmaceuticals into groundwater. Veterinary pharmaceuticals are introduced to the environment via manure applied to agricultural land as fertiliser, from where they might be transferred to surface water via run off or infiltrate into groundwater. The same exposure pathway is possible for drugs associated with sewage sludge after application to agricultural soil (Kümmerer, 2004).

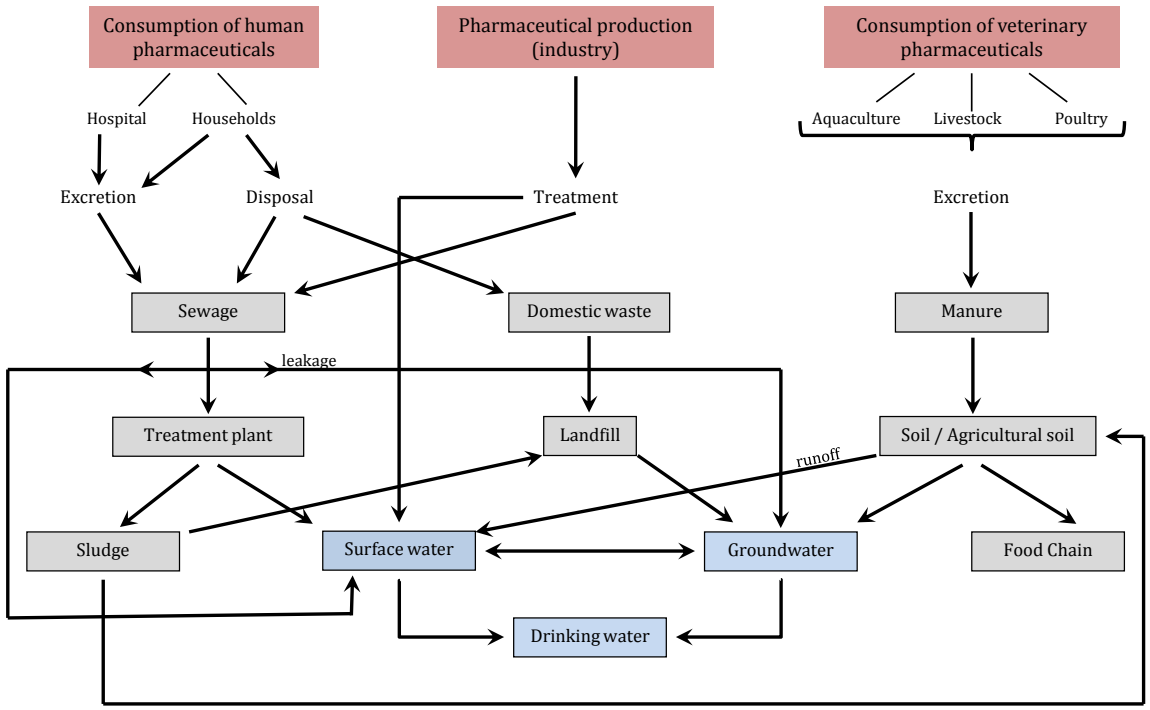


Figure 2-2: Principal exposure routes of human and veterinary pharmaceuticals into the environment (adapted from Barceló, 2004; Alder et al., 2006; Heberer and Ternes, 2006).

2.1.1.1 ANTIBIOTICS

SIGNIFICANCE AND APPLICATION. A therapeutic group of special interest is antibiotics. They are possibly the most important pharmaceutical substances (Alexy and Kümmerer, 2006) for they revolutionised modern medicine by enabling the treatment of a wide range of fatal diseases efficiently for the first time in human history. Furthermore, they enabled the development of sophisticated surgical applications like joint replacements and heart surgery by controlling accompanying infections. Antibiotics are used not only in human medicine, where they are the third most prescribed pharmaceutical class (Schwabe and Paffrath, 2007; see also Table 2-2), but in livestock, poultry production and aqua cultures in quantities equalling those of human medicine applications. In many cases it is difficult to obtain reliable and accurate consumption data. The Union of Concerned Scientists (Union of Concerned Scientists, 2001) estimates that of the approx. 16,200 t of antibiotics produced in the USA in 2000, 70% were used in healthy livestock. In 1996, approximately 50% of the antibiotics used in the European Union (about 10.200 t) were applied in veterinary medicine and as animal growth promoters (FEDESA, 1997). To limit extensive non-medical use, in the EU antibiotics were banned as growth promoters in 2006 (WHO, 2011a). In veterinary use, antibiotics represent 70% of all consumed pharmaceuticals (Barceló, 2004).

PROPERTIES. Antibiotics embody a very diverse range of chemical classes with very different physico-chemical and biochemical properties (Le-Minh et al., 2010). According to the statistics of German Statutory Health Insurance, in 2009 the leading antibiotic classes in use in Germany were beta-lactam antibacterials at 44% (data based on reimbursable pharmaceuticals bought in a pharmacy and available on prescription as well as reimbursable over-the-counter drugs in the case of defined exceptions), followed by tetracyclines (20.7%), macrolides (16.8%), quinolones (9.9%) and sulfonamides and trimethoprim (4.9%) (ESAC, 2009).
Despite being the most abundant group, beta-lactam antibacterials are not detected in the environment, since they are eliminated by biodegradation during conventional wastewater treatment (Le-Minh et al., 2010). Therefore they are not further discussed in this study, which will focus on macrolides, sulfonamides, trimethoprim and tiamulin. The term “antibiotics” hereafter refers to these named antibiotic classes.

MACROLIDES. Macrolides (physico-chemical properties shown in Table 2-3) are mainly applied in the treatment of upper and lower respiratory tract infections (Göbel et al., 2005b) to combat Gram-positive bacteria, the Gram-negative legionella bacterium, mycoplasma pneumonia, campylobacter and chlamydia species (Schwabe and Paffrath, 2007) by inhibiting ribosomal protein synthesis (Bryskier et al., 1993). The name of the compound group derives from the

macrocyclic lactone structure of the parent drug erythromycin, which was discovered in a strain of *Streptomyces erythreus* in 1952 (Mückter, 2006). In the late decades of the twentieth century, several derivates such as clarithromycin and roxithromycin became available and are used today in considerable amounts (Table 2-1). They are applied only in human medicine, whereas erythromycin is used in both human and veterinary applications.

After intake, macrolides are not largely metabolised (Table 2-1), but are mostly excreted in faeces in form of the original drug (Göbel et al., 2005b).

Table 2-2: Estimated oral consumption of selected prescribed drugs in Germany for 2005.

Compound	**DDD oral[1]**	**DDD in Mio. prescribed for oral treatment in Germany, 2005[2]**	**Estimated prescribed oral consumption in Germany in 2005[3]**
	[g]		**[t/year]**
		Antibiotics	
Clarithromycin	0.50	21.5	10.8
Roxithromycin	0.30	19.6	5.88
sulfamethoxazole	2.00	22.4	44.8
		Psychoactive drugs	
Trimethoprim	0.40	23.6	9.44
carbamazepine	1.00	63.5	63.5
Primidone	1.25	5.0	6.25
Codeine	0.10	25.4	2.54
dihydrocodeine	0.15	7.6	1.14
Methadone	0.03	1.2	0.03
Morphine	0.10	15.7	1.57
Oxycodone	0.08	14.4	1.08
Diazepam	0.01	38.3	0.383
Nordiazepam	0.02	n.r.	n.r.
Oxazepam	0.05	19.6	0.98
Temazepam	0.02	9.3	0.186
Doxepin	0.10	55.9	5.59
		Lipid-regulators	
Bezafibrate	0.60	24.7	14.8
		Non-steroidal anti-inflammatory drugs	
Diclofenac	0.10	472	47.2
Ibuprofen	1.20	221	265
Naproxen	0.50	9.1	4.55
		Iodinated x-ray contrast media	
Iopamidol	n.r.	n.r.	43.0 [5] (2001)
Iomeprol	n.r.	n.r.	83.4 [5] (2001)
Iopromide	n.r.	n.r.	130[4]/64.1 [5] (2001)
diatrizoic acid	n.r.	n.r.	60.7 [5] (2001)

[1] (WHO, 2012); [2] Schwabe and Paffrath, 2007; [3] calculated by multiplying defined daily doses for oral application (WHO, 2012) with the prescribed number of defined daily doses in 2005 (Schwabe and Paffrath, 2007); [4] Alder et al., 2006; [5] sales in Germany in 2001 according to BLAC, 2003; n.r. = not reported in the searched databases

SULFONAMIDES. These are a group of synthetic antibiotics which have been used in medical treatment since the 1930s. They are mainly used for the treatment of urinary tract infections, pneumonia and some other infections (Schwabe and Paffrath, 2007). Although thousands of sulfonamides were developed and tested, less than 200 reached the market and today only a few are widely used in human or veterinary medicine. Sulfonamides act against Gram-positive and -negative bacteria through growth blockage by competitive inhibition of bacterial folate biosynthesis (Mückter, 2006).

In human medicine, sulfamethoxazole is the most extensively used sulfonamide – in Germany the only one at all (Schwabe and Paffrath, 2007) – while in veterinary medicine a range of sulfonamides, e.g. sulfamethoxine, sulfisoxazole, sulfamerazin and sulfadimidine are used.

After treatment, sulfonamides are excreted via urine, partly metabolised, partly as the original drug (Table 2-1). Major metabolites are the biologically inactive N4-acetylated forms of the drug, e.g. N4-acetylsulfamethoxazole, which accounts for about 50% of the administered dose of the parent compound sulfamethoxazole (Table 2-1) (Göbel et al., 2005b). The retransformation of N4-acetylsulfamethoxazole back to the active parent drug during wastewater treatment was observed by Göbel et al., 2005b; Berger et al., 1986 found the same for N4-acetylsulfadimidine which was biotransformed to the parent compound sulfadimidine during the storage of manure. This intertransformation between the original drugs and their respective metabolites may account for reported negative removal rates in conventional wastewater treatment (Göbel et al., 2007) (see above).

As Table 2-4 depicts, sulfonamides have two functional groups of which the amine attached to the aromatic group is deprotonated at pH levels under 1.8 – 2.3 (depending on the compound, see $pK_{a,1}$ in Table 2-4) while the amide attached to the sulphur is deprotonated at pHs above 4.8 – 7.5 ($pK_{a,2}$). Consequently, in general sulfonamides are positively charged under acidic conditions and negatively charged under alkaline conditions (Haller et al., 2002).

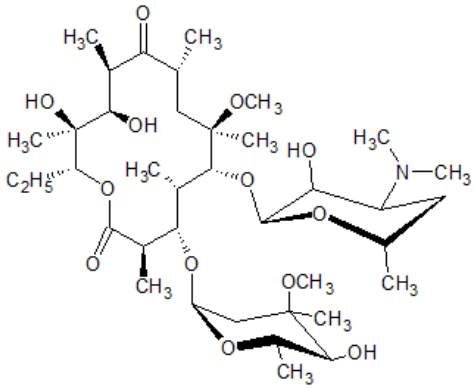
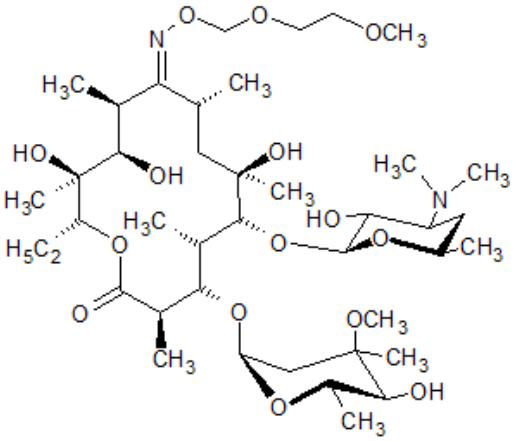
TRIMETHOPRIM. To enhance their therapeutic potential and lessen the risk of resistance development, sulfonamides are often used in combination with trimethoprim. Trimethoprim is a polyalkoxybenzyldiaminopyrimidine derived from pyrimethamine, an anti-malaria drug which hinders the bacterial folate biosynthesis by inhibiting the bacterial enzyme dihydrofolate reductase (DHFR) (Mückter, 2006). Additionally, it has the same half-life in the treated body as the sulfonamides and is likewise excreted renally (Schwabe and Paffrath, 2007). Since it is much more expensive as a mono-drug, trimethoprim is rarely administered in isolation, although it is recommended in medical guidelines as treatment of choice for certain indications, as it is equally potent and better tolerated than the combination (Schwabe and Paffrath, 2007).

The metabolic rate of trimethoprim is similar to that of sulfonamides: approximately half of the administered drug is excreted in metabolised form (Table 2-1).

Like sulfonamides, trimethoprim is charged differently at different pHs; the two pK_a values of trimethoprim ($pK_{a,1}$ 3.3, $pK_{a,2}$ 6.8) are assigned to the basic N3 and N1 sites respectively in the pyrimidine ring (Table 2-5) (Quiang and Adams, 2004).

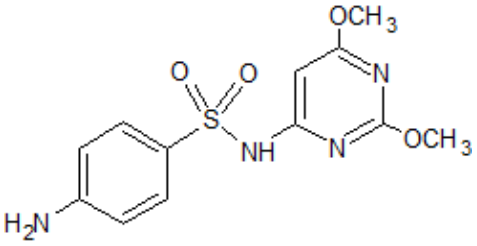
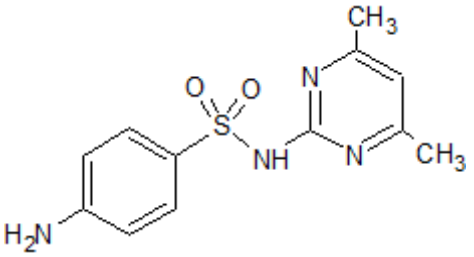
TIAMULIN. Tiamulin (Table 2-5) is a diterpene antimicrobial with a pleuromutilin chemical structure, which mainly targets Gram-positive bacteria and mycloplasma. It is used in veterinary medicine for both treatment and prophylaxis of dysentery, pneumonia and mycoplasmal infections in pigs and poultry (EMEA, 2008).

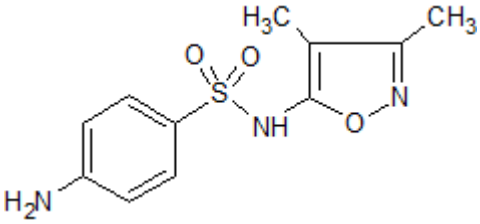
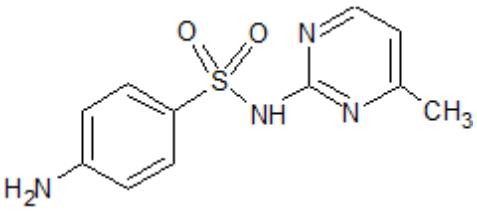
Table 2-3: Physico-chemical properties of macrolides. W_s: water solubility (if not otherwise indicated at 25 °C); MW = molecular weight [g/mol]; HLC = Henry's Law Constant [atm^{-m3}/mole]; W_s = water solubility [mg/L] at 25 °C if not otherwise indicated; ---, not listed in the literature or databases consulted.

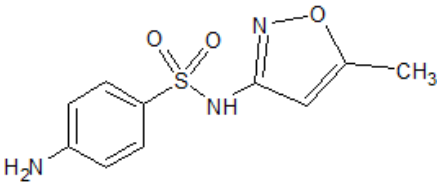
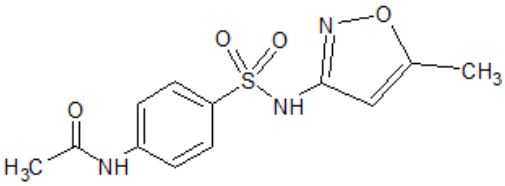
Substance	**Clarithromycin**	**Roxithromycin**
Abbreviation	CLA	ROX
Chemical structure		
CAS-No.	81103-11-9	80214-83-1
MW	747.95	837.05
Log K_{OW}	2.42[a]; 3.16[b]	2.750[b]
pKa	8.99[a,b]	9.2[c]
HLC	$1.73x10^{-29}$[d]	---
W_s	0.342[b]	0.0189[d]

[a] Sangster, 2012; [b] NLM, 2005; [c] Bryskier et al., 1993; [d] SRC Research Corporation; [e] Maria and Reginald, 1993; [f] Gros et al., 2006a; [g] Quiang and Adams, 2004

Table 2-4: Physico-chemical properties of sulfonamides. W_s: water solubility (if not otherwise indicated at 25 °C); MW = molecular weight [g/mol]; HLC = Henry's Law Constant [atm^{-m3}/mole]; W_s = water solubility [mg/L] at 25 °C if not otherwise indicated; ---, not listed in the literature or databases consulted.

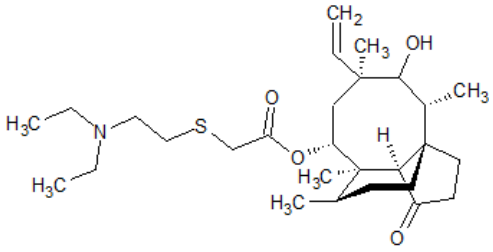
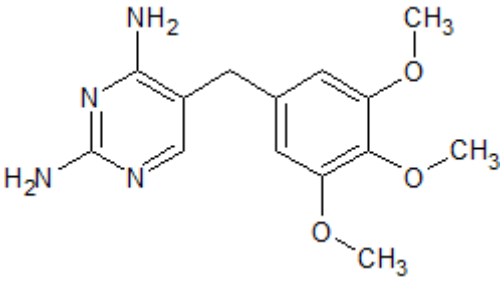
Substance	**Sulfadimethoxine**	**Sulfadimidine; sulfamethazine; sulfadimethylpyrimidine**
Abbreviation	SMI	SDI
Chemical structure		
CAS-No.	122-11-2	57-68-1
MW	310.33	278.33
Log K_{OW}	1.63[a]	0.28[a]; 0.89[b]
$pKa_{,1}$	1.87[e]	2.28[e]
$pKa_{,2}$	5.86[e]; 5.98[a]	7.42[e]; 7.59[a,b]
HLC	$1.30x10^{-14}$[d]	$3.05x10^{-13}$[d]
Ws	343[b]	1500[b]

Substance	**Sulfisoxazole; sulfafurazole**	**Sulfamerazine**; sulfamerathyldiazine
Abbreviation	SSX	SMA
Chemical structure		
CAS-No.	127-69-5	127-79-7
MW	267.30	264.31
Log K_{OW}	1.01[a]	0.14[a,b]
$pKa_{,1}$	---	2.17[e]
$pKa_{,2}$	4.8[a]	6.77[e]; 6.83[a]
HLC	$1.06x10^{-12}$[d]	$1.75x10^{-10}$[d]
Ws	300[b]	202[b]

Substance	**sulfamethoxazole**	**N4-acetylsulfamethoxazole**
Abbreviation	SMX	N-Ac-SMX
Chemical structure		
CAS-No.	723-46-6	21312-10-7
MW	253.28	295.32
Log K_{OW}	0.89[f]; 0.89[a]	1.2[d]
$pKa_{,1}$	1.83[e]	5.0[d]
$pKa_{,2}$	5.57[e]; 6.0[f]; 5.81[a]	
HLC	$6.42x10^{-13}$[d]	$3.1x10^{-15}$[d]
Ws	610 (37 °C)[b]	1220[d]

[a] Sangster, 2012; [b] NLM, 2005; [c] Bryskier et al., 1993; [d] SRC Research Corporation; [e] Maria and Reginald, 1993; [f] Gros et al., 2006a; [g] Quiang and Adams, 2004

Table 2-5: Physico-chemical properties of trimethoprim and tiamulin. W_s: water solubility (if not otherwise indicated at 25 °C); MW = molecular weight [g/mol]; HLC = Henry's Law Constant [atm-m^3/mole]; W_s = water solubility [mg/L] at 25 °C if not otherwise indicated; ---, not listed in the literature or databases consulted.

Substance	**Tiamulin**	**Trimethoprim**
Abbreviation	TAM	TMP
Chemical structure		
CAS-No.	55297-95-5	738-70-5
MW	493.74	290.32
Log K_{OW}	4.750[b]	0.91[f,b]
$pK_{a,1}$	---	3.23[g]
$pK_{a,2}$	---	6.76[g]; 7.12[f,b]
HLC	$4.21x10^{-16}$[d]	$2.39x10^{-14}$[b]
Ws	0.696[d]	400[b]

[a] Sangster, 2012; [b] NLM, 2005; [c] Bryskier et al., 1993; [d] SRC Research Corporation; [e] Maria and Reginald, 1993; [f] Gros et al., 2006a; [g] Quiang and Adams, 2004

ENVIRONMENTAL OCCURRENCE – STATE OF RESEARCH. Antibiotics have been found in domestic and hospital wastewater (Ashton et al., 2004; Carballa et al., 2004; Göbel et al., 2004; Batt and Aga, 2005; Yang et al., 2005; Gros et al., 2006a; Göbel et al., 2007; Kim et al., 2007; McClure and Wong, 2007; Chang et al., 2008b; Gros et al., 2008; Radjenović et al., 2009a) and in agricultural wastewater (Malintan and Mohd, 2006). Reported amounts vary according to local patterns of pharmaceuticals use. In general, reported concentrations in wastewater range from the middle and upper ng/L to the low µg/L range, similarly showing different patterns in different countries. In effluents from pharmaceutical production sites and hospitals, some antibiotics were found in concentrations potentially high enough to cause adverse effects on wastewater bacteria (Al-Ahmad et al., 1999; Kümmerer et al., 2000; Alexy and Kümmerer, 2006). They are greatly diluted by mixing with municipal wastewater before reaching the WWTP. Since antibiotics can diffuse into biofilms such as those present in the pipes of a sewer system, unknown amounts of the pharmaceuticals are held back there, temporarily or permanently, resulting in a sink with the possibility of later re-release of the substances into the water (Alexy and Kümmerer, 2006). Additionally, increased contact times of bacteria and antibiotics in biofilms in sewer systems, especially the effluents of production sites or hospitals, are discussed as source of spread of MDR (multiple drug-resistant) bacteria (see below).

The studies undertaken reported highly diverse removal rates of antibiotics in conventional wastewater treatment, ranging from none to complete biotransformation for the same substance, but in general antibiotics seem to be only partly removed during wastewater treatment (Le-Minh et al., 2010). It is indicated that operating conditions such as solids retention

time (SRT) and hydraulic retention time (HRT) influence the biodegradation processes of micropollutants (Clara et al., 2005; Oppenheimer et al., 2007; Yu et al., 2009); thus variations in elimination could be explained by different operational modes. In contrast to sulfonamides, observed negative removal rates of macrolides are primarily attributed to the release of the unmetabolised parent drug from faeces during wastewater treatment rather than intertransformation of metabolites (Göbel et al., 2007).

Some studies investigated the occurrence of antibiotics in sewage sludge (Göbel et al., 2005a; Göbel et al., 2005b; Yang et al., 2005; Lindberg et al., 2006; Lillenberg et al., 2009; Radjenović et al., 2009b; Li et al., 2013). As shown above, the sorption of antibiotics to sewage sludge is highly dependent on pH, stereochemistry and the chemical nature of the micropollutant and the sorbent as well. Subsequently, results differ between studies, but, generally speaking, the sorption processes of the antibiotic groups presented in this study have been found to be of only minor consequence for the removal of antibiotics during wastewater treatment (Göbel et al., 2005b; Le-Minh et al., 2010).

Due to their incomplete removal, antibiotics are frequently detected in surface water (cf. e.g. Kolpin et al., 2002; Ashton et al., 2004; Yang et al., 2004; Kim et al., 2007; Kim and Carlson, 2007; Chang et al., 2008b; Díaz-Cruz et al., 2008; Gros et al., 2008; Tamtam et al., 2008; Kleywegt et al., 2011), in river sediment (Kim and Carlson, 2007) and groundwater (Hirsch et al., 1999; Kolpin et al., 2002; Díaz-Cruz et al., 2008). Kleywegt et al., 2011 detected antibiotics in finished drinking water in the low ng/L-range. Díaz-Cruz et al., 2008 reported finding sulfadimethoxine in bottled mineral water.

In aquatic environments, sulfonamides have been described as photosensitive and their photodegradation has been observed in natural waters (Boreen et al., 2004).

ADVERSE EFFECTS. One particular concern regarding antibiotics is a possible adverse effect on non-target organisms in the environment (Alexy and Kümmerer, 2006). Only a few studies have investigated this issue so far. In an ecotoxicity study based on bioassays performed on various organisms to determine acute and chronic toxicity as well as the genotoxic potential of sulfamethoxazole and clarithromycin (among others) proved that sulfamethoxazole was mutagenic, while clarithromycin was found to be the most harmful for the aquatic environment (Isidori et al., 2005b). Toxicity details for all investigated antibiotics are shown in Table 2-6.

BACTERIAL RESISTANCE. Besides the ecotoxicological effect on non-target organisms (LD_{50} values see Table 2-6), the wide-spread presence of antibiotics in the environment may increase the development of bacterial resistance to specific compounds (Alexy and Kümmerer, 2006). In recent years, antibiotic resistance in bacteria has become a serious health problem. It is

estimated that in the European Union, Norway and Iceland around 25,000 people died in 2007 of infections connected with antibiotic resistance (ECDC, 2009; WHO, 2011a; WHO, 2011b). According to the European Centre for Disease Prevention and Control (ECDC), two thirds of these deaths resulted from infections due to Gram-negative bacteria. In addition, infections caused by antibiotic-resistant bacteria led to approximately 2.5 million extra hospital days (ECDC, 2009), making antibiotic resistance both a medical and economic burden.

Resistance to antimicrobial drugs is part of the natural adaptation process due to mutation not only in bacteria but in fungi, viruses and parasites as well, causing similar problems for the control of antimicrobial resistance in all these microorganisms (WHO, 2001). It was only four years after the mass-production of penicillin began that the first resistant microbes were found (the first bacterium adapting successfully was *Staphylococcus aureus*) (WHO, 2011a). Although resistance occurs naturally as a result of the application of antibiotics, it is particularly increased by inappropriate use, which includes overprescribing e.g. for treatment of minor or non-bacterial infections, misuse because of false diagnosis or lack of information about alternatives, and also underuse due to lack of access or financial ability, or inadequate compliance with the prescription to complete treatment correctly (WHO, 2011a). Development of resistance may also occur by uptake of genetic material encoding resistance (e.g. in hospital effluents) (Alexy and Kümmerer, 2006) or by information exchange via DNA between bacteria, which is especially easy to accomplish in biofilms or microbial flocs (e.g. in sewers and WWTPs) where large numbers of microorganisms are in close proximity to one another (Karatan and Watnick, 2009; Nadell et al., 2009; CBE, 2013c). Antibiotic resistance is also important in terms of food safety (WHO, 2011b), since resistant bacteria and resistant genes carried by food-producing animals can spread to humans through the food chain as foodborne diseases caused by antibiotic-resistant bacteria like salmonella (WHO, 2011a), through direct contact with the animals, or via environmental pathways such as contaminated water (WHO, 2011b).

Bacterial resistance shows highly differentiated national patterns, often due to varying treatment regulations. For example, while in most EU countries antibiotics are only used as prescribed drugs, they are sold as OTC drugs in some eastern European countries, leading to greatly increased use and mirrored by high resistance rates of up to 50%, while for the EU, Norway and Iceland, antibiotic resistance of up to 25% is reported (WHO, 2011a). Additionally, national actions of surveillance and control are at very different levels. In Denmark, a significant decrease in resistance in some bacteria was observed after the prohibition of using antibiotics as a growth promoter (WHO, 2011a).

To date, the issue of resistance spread in wastewater is controversial (cf. Kümmerer and Henninger, 2003; Michael et al., 2013). Laboratory-scale studies have found that the presence of antibiotics in wastewater – even in microbiologically effective concentrations – had no influence

on the bacterial population in WWTP, which suggests that resistant bacteria are already present in wastewater reaching the WWTP (Al-Ahmad et al., 2009). Municipal wastewater treatment has been reported to be accompanied by significant increases in antimicrobial resistance (Figueira et al., 2011a), while other studies have found significant reduction of antibiotic resistance genes and antibiotic-resistant bacteria – of over 2 log-units – to have been achieved by conventional wastewater treatment. Disinfection steps such as chlorination and UV treatment fail to significantly reduce antibiotic resistant bacteria (Munir et al., 2011) or even seem to increase the incidence (Gao et al., 2012c). Studies suggest that resistance introduced to a stream via WWTP effluents rapidly decreases downstream (Akiyama and Savin, 2010). Nonetheless, antibiotic-resistant bacteria were recently found in drinking water samples (Figueira et al., 2011b). Taking the complexity of microorganism communities in sewage into account, further studies need to be conducted on this issue (Alexy and Kümmerer, 2006).

Table 2-6: Chemical toxicity data of antibiotics for aquatic life (EPA, 2012b). LC_{50}: median lethal concentration; t: exposure duration; LOEC: lowest observable effect concentration; species given in index numbers: 1: *Thamnocephalus platyurus* (Fairy Shrimp); 2: *Oryzias latipes* (Japanese Medaka); 3: *Anguilla Anguilla* (Common Eel); 4: *Morone saxatilis* (Striped Bass); 5**:** *Brachionus calyciflorus* (Rotifer); 6: *Hydra attenuata* (Hydra); 7**:** *Pseudokirchneriella subcapitata* (Green Algae); 8: *Lemna gibba* (Inflated Duckweed); 9: *Daphnia magna* (Water Flea); 10: *Xenopus laevis* (African Clawed Frog).

Substance (CAS-No.)	**LC_{50}**			**LOEC**		
	lowest LC_{50} (µg/L) for the most sensitive indicator species (indicator species)	t	no. of aquatic studies identified	lowest LOEC (µg/L) for the most sensitive indicator species (indicator species)	t	no. of aquatic studies identified
Clarithromycin (81103-11-9)	33,640 (1)	24 h	2	40 (7)	72 h	1
Roxithromycin (80214-83-1)	---	---	0	40 (7)	72 h	1
Sulfadimethoxine (122-11-2)	> 100,000 (2)	24 h	4	300 (8)	196 h	6
Sulfadimidine (57-68-1)	> 75,000 (3)	24 h	4	1,000 (8); 3,125 (9)	21 days; 21 days	4
Sulfisoxazole (127-69-5)	---	---	0	---	---	0
Sulfamerazine (127-79-7)	> 100,000 (4)	1–96 h	5	---	---	0
Sulfamethoxazole (723-46-6)	26,270 (5)	24 h	5	800 (7); > 100,000 (10); 10,000 (6)	72 h; 96 h; 96 h	11
N4-acetyl-sulfamethoxazole (21312-10-7)	---	---	0	---	---	0
Tiamulin (55297-95-5)	---	---	0	---	----	0
Trimethoprim (738-70-5)	> 100,000 (2, 6)	48 h; 96 h	3	20,000 (9)	21 days	6

2.1.1.2 IODINATED X-RAY CONTRAST MEDIA

PROPERTIES AND USE. Iodinated x-ray contrast media (ICMs) are usually derivates of 2,4,6-triiodinated benzoic acid. They are commonly divided into *ionic* (e.g. diatrizoic acid) and *non-ionic* (e.g. iopamidol, iomeprol, iopromide) compounds. The latter are used, for example, in angiography and urography and represent 90% of the applied ICMs (Putschew and Jekel, 2006). Ionic ICMs contain carboxylic moieties in their side-chains, while non-ionic ICM are amide derivates with hydroxyl groups.

Since iodine atoms absorb X-rays, ICMs are used to enhance the contrast between different blood vessels and/or organs which would otherwise be not visible under x-ray examination (Pérez and Barceló, 2007). Treatment doses can reach up to 200 g ICM per application, equalling approximately 100 g iodine (Pérez and Barceló, 2007). In terms of mass, ICMs are the most frequently applied pharmaceuticals in hospitals (Putschew and Jekel, 2006). The global consumption of ICMs is approximately 3,500 t per year (Sprehe et al., 2000). The consumption of selected ICMs in Germany is shown in Table 2-2, where iopromide is seen to be the most heavily used (130 t per year).

EXCRETION AND ENVIRONMENTAL FATE. ICM are designed for metabolic stability: they are inert and do not interact with the treated organism. Therefore, they are excreted completely and unaltered via the patient's urine within 24 h after application (Speck and Hübner-Steiner, 1999). The highest concentration in urine is found within one hour after treatment, reaching up to 70 g/L (Sprehe et al., 2000). Since in consequence of their pharmaceutical purpose ICM are highly persistent and polar (log K_{OW} diatrizoic acid: -1.05; iopamidol: -2.42; iopromide: -2.05; for more physico-chemical data see Table 2-7), they remain in the water phase and reach the sewage system unchanged. ICM are poorly eliminated by conventional wastewater treatment systems (Hirsch et al., 2000; Carballa et al., 2004; Pérez and Barceló, 2007; Ternes et al., 2007). Thus, they are discharged into the receiving waters in quantities up to µg/L levels, where they undergo practically no sorption by sediment or sludge and can be transported, dissolved, over long distances (Löffler et al., 2005; Haiß and Kümmerer, 2006; Seitz et al., 2006). Seitz et al., 2006 found a WWTP to be a point source for ICM concentrations in the river Danube, releasing ICM in fluctuating amounts into the river, with detected concentrations being higher on weekdays than on weekends due to the working hours of hospitals and x-ray units. It can be assumed that ICM occur in all surface waters which are influenced by WWTP effluents (Putschew and Jekel, 2006). Additionally, groundwater can be affected, especially in areas where bank filtration is used for the production of drinking water. Ternes and Hirsch, 2000 found that ICM in surface water influenced ground water in the greater Frankfurt area (Southwest

Germany) and detected ICM in groundwater of agricultural fields receiving wastewater irrigation near Braunschweig (Germany) (Ternes et al., 2007).

BIOTRANSFORMATION AND DEGRADATION IN THE ENVIRONMENT. While no profound biodegradation of ICM is observed in WWTPs, several laboratory studies point to biotransformation processes. Joss et al., 2006 observed the biodegradation of several ICM in batch experiments with activated sludge, revealing no biodegradation for diatrizoic acid, but in the case of iopromide 85% was transformed into 2 metabolites within 54 h in activated sludge. The metabolites were not identified, but partial deiodination is suspected because of rising amounts of inorganic iodine in the sludge batches. Haiß and Kümmerer, 2006 studied the biodegradation of diatrizoic acid during simulated biological sewage treatment (modified OECD 303 A test), observing biodegradation occurring only under special conditions and with long lag times. These and other laboratory studies simulating WWTP treatment found biodegration to be dependent on a) test period length, b) test substance concentration and c) sludge composition (Steger-Hartmann et al., 1999; Steger-Hartmann et al., 2002; Haiß and Kümmerer, 2006; Joss et al., 2006; Pérez and Barceló, 2007). In laboratory tests with river water, the degradation of iopromide was found to be concentration-dependent with the shortest half-life measured being about 3.1 days. Degradation of iopromide showed no lag time, while diatrizoic acid showed exponential decline (with a half-life of approximately 4.5 days) only after an acclimation time of 21 days, forming two water-soluble metabolites (Kalsch, 1999).

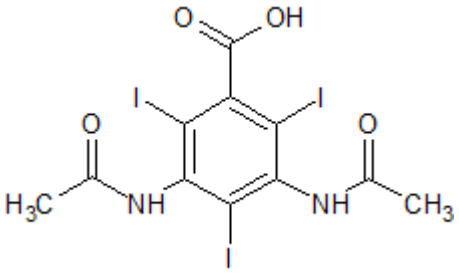
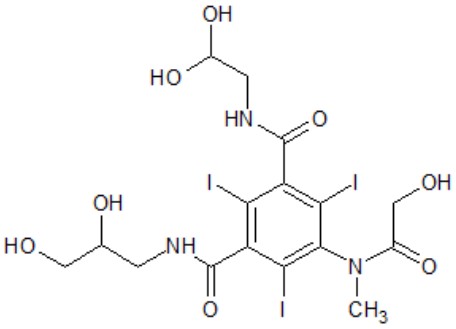
Another study reported moderate persistence of iopromide in laboratory tests with river water sediment systems and its transformation into at least four metabolites after a lag period of two weeks (Löffler et al., 2005).

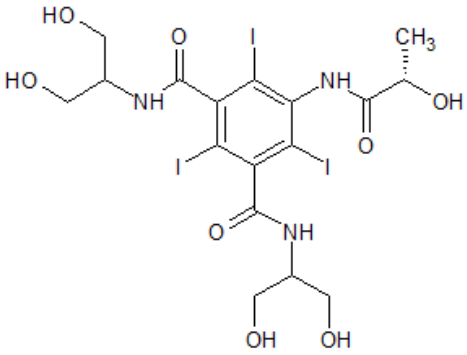
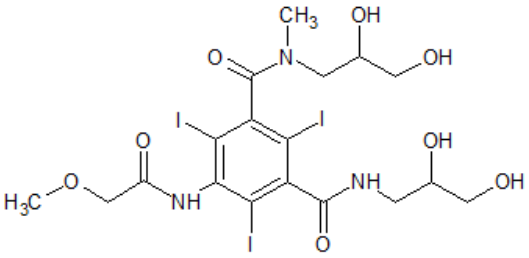
Besides metabolism by microorganisms, photodegradation is another possible breakdown mechanism for ICM in the environment. For example, Doll and Frimmel, 2003 established photodegradation for iomeprol. In general, sorption to particles can also result in the elimination of micropollutants from water, but due to the polar nature of ICM an accumulation in sediments and sludges can be excluded as a major elimination process (Steger-Hartmann et al., 1999; Löffler et al., 2005; Putschew and Jekel, 2006).

TRANSFORMATION PRODUCTS. Until now, few studies have identified transformation products of ICM (Table 2-8). Schulz et al., 2008 and Kormos et al., 2009 used a hybrid triple quadrupole linear ion trap mass spectrometer (Qq-LIT-MS) in combination with nuclear magnetic resonance (NMR) analysis to identify the chemical structures of biotransformation products which the ICM iohexol, iomeprol and iopamidol form in water and soil. A number of these transformation products were detected in surface-, ground- and drinking water, with the highest concentrations

shown for biodegradation products of iomeprol (up to 1,450 ± 110 ng/L in wastewater-influenced groundwater and 289 ± 41 ng/L in drinking water) (Kormos et al., 2009). Steger-Hartmann et al., 2002 reported the transformation of iopromide into one major metabolite, postulating it being 5-amino-N,N'-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-methylisophthalamide. In most cases, identified metabolites are the result of laboratory studies, and their occurrence under real environmental conditions and in environmental concentrations still has to be investigated (Pérez and Barceló, 2007).

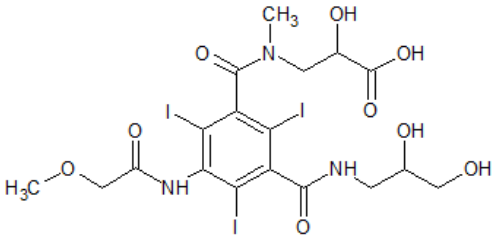
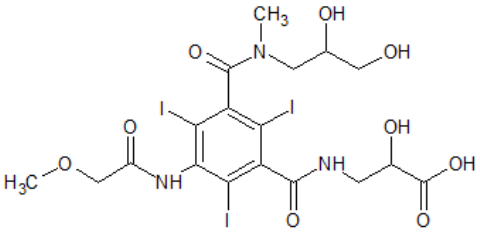
Table 2-7: Physico-chemical properties of iodinated x-ray contrast media. W_s: water solubility (if not otherwise indicated at 25 °C); MW = molecular weight [g/mol]; HLC = Henry's Law Constant [atm-m^3/mole]; W_s = water solubility [mg/L] at 25 °C if not otherwise indicated; ---, not listed in the literature or databases consulted.

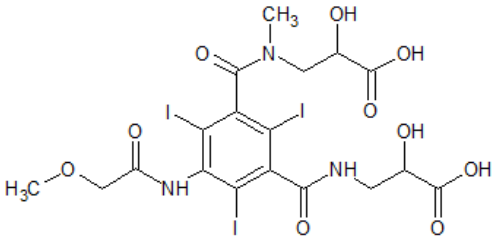
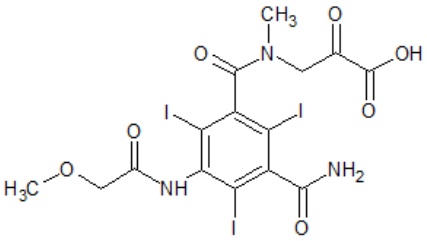
Substance	**Diatrizoic acid (Diatrizoate; Amidotrizoic acid)**	**Iomeprol**
Abbreviation	DTZ	IOP
Chemical structure		
CAS-No.	117-96-4	78649-41-9
MW	613.91	777.09
Log K_{OW}	-1.05[a]	---
pKa		
HLC	2.81x10^{-18}[b]	----
Ws	8.89[b]	----

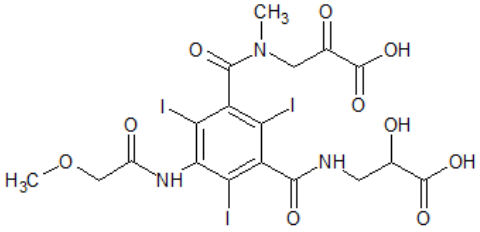
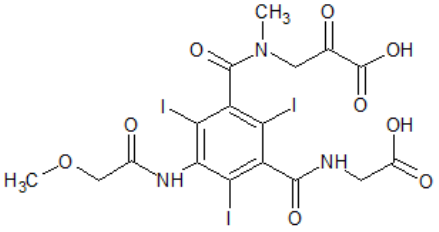
Substance	**Iopamidol**	**Iopromide**
Abbreviation	IPM	IMI
Chemical structure		
CAS-No.	60166-93-0	73334-07-3
MW	777.08	791.11
Log K_{OW}	-2.42[a,c]	-2.05[a,c]
pKa	---	---
HLC	1.14x10^{-25}[b]	1.00x10^{-28}[b]
Ws	1.40x10^{5}[c]	23.8[c]

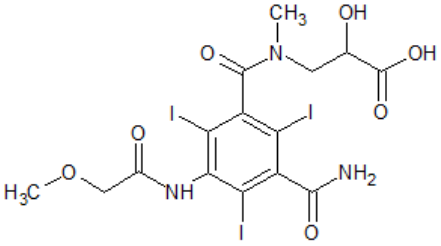
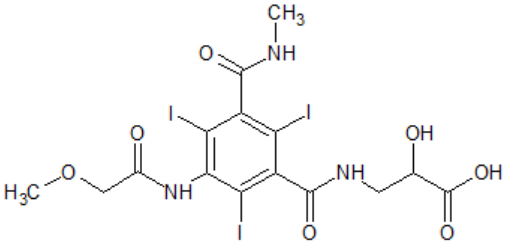
[a] Sangster, 2012; [b] SRC Research Corporation; [c] NLM, 2005

Table 2-8: Chemical structures of transformation products of iopromide (Schulz et al., 2008).

Substance	**Iopromide TP 805 A**	**Iopromide TP 805 B**
Abbreviation	IMI-TP 805 A	IMI-TP 805 B
Chemical structure		

Substance	**Iopromide TP 819**	**Iopromide TP 729 A**
Abbreviation	IMI-TP 819	IMI-TP 729 A
Chemical structure		

Substance	**Iopromide TP 817 A**	**Iopromide TP 787 A**
Abbreviation	IMI-TP 817 A	IMI-TP 787 A
Chemical structure		

Substance	**Iopromide TP 731 A**	**Iopromide TP 731 B**
Abbreviation	IMI-TP 731 A	IMI-TP 731 B
Chemical structure		

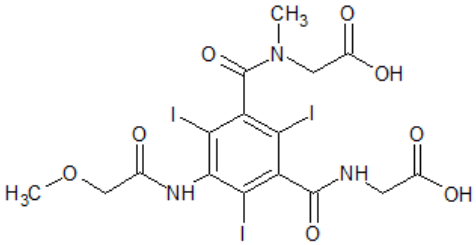
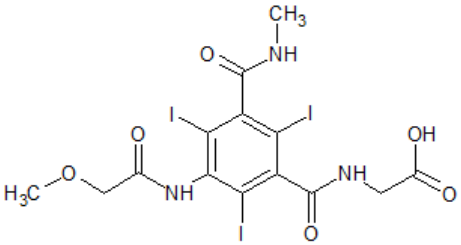
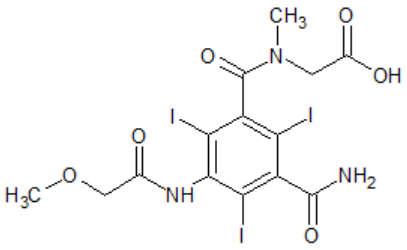
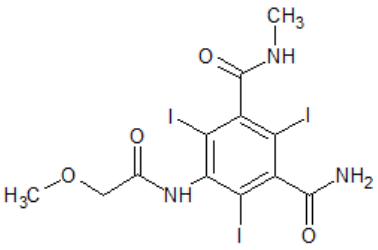
Substance	**Iopromide TP 759**	**Iopromide TP 701 B**
Abbreviation	IMI-TP 759	IMI-TP 701 B
Chemical structure		

Table 2-8: continued.

Substance	**Iopromide TP 701 A**	**Iopromide TP 643**
Abbreviation	IMI-TP 701 A	IMI-TP 643
Chemical structure		

TOXICITY. Besides the still fundamental lack of knowledge about the environmental fate of ICM and their potential adverse health impact, ICM came into focus when studies showed that they contribute profoundly to AOX (adsorbable organic halogen) concentrations in hospital effluents and the municipal sewage system (Putschew and Jekel, 2006). In hospital effluents, up to 90% of the AOX concentration originate from ICM (Pérez and Barceló, 2007).

As micropollutants, ICM are of concern mainly because of their high polarity, resulting in long range transport and high mobility combined with extreme persistence and high consumption quantities, which makes them the "worst case" contaminants in terms of their environmental exposure (Ternes and Hirsch, 2000). Toxicity data of ICM are scarce. Short-term toxicity tests with bacteria (*Vibrio fischeri, Pseudomonas putida*), algae (*Scenedesmus subspicatus*), crustaceans (*Daphnia magna*), and fish (*Danio rerio, Leuciscus idus*) have shown no toxic effects at maximum concentrations of ICM of 10 g/L (Steger-Hartmann et al., 1999; see also Table 2-9). In a chronic toxicity test (> 20 days) with *Daphnia magna*, no effect was observed at concentrations of 1 g/L (Steger-Hartmann et al., 1999). Haiß and Kümmerer, 2006 tested the effects of both diatrizoic acid and its transformation products on microorganisms in sewage sludge, finding no adverse effects. So seemingly, ICM are no immediate threat when released into the environment. Nonetheless, their high environmental concentrations, persistent nature and the lack of information about long-term sublethal effects indicate the need for further investigations (Ternes and Hirsch, 2000).

Table 2-9: Chemical toxicity data of ICM for aquatic life (EPA, 2012b). LC_{50}: median lethal concentration; t: exposure duration; LOEC: lowest observable effect concentration; species given in index numbers: 1: *Leuciscus idus* (Ide, Silver Or Golden Orfe); 2: *Danio rerio* (Zebra Danio).

Substance (CAS-No.)	**LC_{50}**			**LOEC**		
	lowest LC_{50} (µg/L) for the most sensitive indicator species (indicator species)	t	no. of aquatic studies identified	lowest LOEC (µg/L) for the most sensitive indicator species (indicator species)	t	no. of aquatic studies identified
Diatrizoic acid (117-96-4)	---	---	0	----	----	0
Iomeprol (78649-41-9)	---	---	0	---	---	0
Iopamidol (60166-93-09)	---	---	0	---	---	0
Iopromide (73334-07-3)	> 10,000,000 (1;2)	96 h; 48 h	2	---	---	0

2.1.1.3 Lipid regulators

PROPERTIES AND USE. Lipid regulators (also called hypolipidemic agents, antihyperlipidemic agents or lipid-lowering drugs) are designed to counteract high levels of lipidproteins in blood (hyperlipidaemia) which, when untreated, increase the risk of cardiovascular diseases (Schwabe and Paffrath, 2007). Different groups of lipid regulators (e.g. fibrates, statins, niacin) act in different ways on the blood-lipid system. In Germany in 2005, lipid regulators were the second best-selling group of prescription pharmaceuticals (Schwabe and Paffrath, 2007). Worldwide, cholesterol and triglyceride reducers are counted amongst the ten largest therapy classes and show an increase in sales of over 10% year-on-year (IMS, 2005).

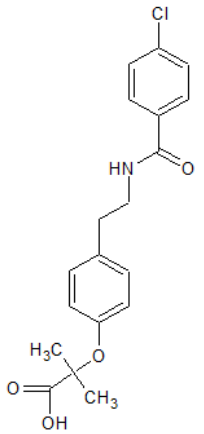
BEZAFIBRATE. Bezafibrate is a fibrate drug used to lower triglycerides and, to a lesser extent, cholesterol (Table 2-10). It was first marketed in 1971 by Boehringer Mannheim. As with other clofibrin acid derivates (clofibrate, fenofibrate), its use in Germany is decreasing due to new studies casting scientific doubt on their effectiveness and the introduction in recent years of new classes of lipid regulating drugs (Schwabe and Paffrath, 2007). In a long-term study with a duration of 6.2 years and with more than 3,000 participants, bezafibrate failed to prevent effects on cardinal end points such as heart attack and sudden cardiac death (BIP, 2000).

EXCRETION AND ENVIRONMENTAL OCCURRENCE. Bezafibrate is readily degraded in WWTPs with reported removal rates of 80–95% (Ternes, 1998; Clara et al., 2005; Radjenović et al., 2009b). The elimination depends on the temperature and solid retention times of the operating WWTP (Clara et al., 2005). Temperature dependence could also explain the more effective removal of bezafibrate observed in summer samples compared to samples taken in winter (Castiglioni et al.,

2005; Sacher et al., 2008). Subsequently, winter accumulation was observed in a Swedish river due to reduced natural transformation processes (Daneshvar et al., 2010). The same study found the mean mass flow of bezafibrate being highest in spring. During investigations of two WWTPs in Spain, Pedrouzo et al., 2011 detected no bezafibrate in any influent samples taken over several months but frequently found the drug in effluent samples in concentrations of up to 0.5 µg/L. This can be explained by the cleavage of metabolites during wastewater treatment (Lindqvist et al., 2005). Other studies reported finding bezafibrate in both influent and effluent of WWTPs. Quintana and Reemtsma, 2004 reported bezafibrate with concentrations of 2,775 ng/L and 565 ng/L in raw and treated municipal wastewater respectively, while in lake Tegel in Berlin (which receives treated wastewater and is also used for the production of drinking water through artificial groundwater recharge) 847 ng/L of the drug was detected. In a study including 24 drug residues, Ternes, 1998 found the highest concentrations of bezafibrate in wastewater-receiving river water.

TOXICITY. In an ecotoxicological risk assessment considering human metabolism, Lienert et al., 2007 found bezafibrate to pose a potential ecotoxicological risk. While studies described LC_{50} values (96 h) of 70,710 µg/L for the indicator species *Hydra attenuata* (Hydra)**,** LOEC was found to be 3.61 µg/L (24 h, indicator species used**:** *Mytilus galloprovincialis* (Mediterranean Mussel)) (EPA, 2012b).

Table 2-10: Physico-chemical properties of the lipid regulator bezafibrate. W_S: water solubility (if not otherwise indicated at 25 °C); MW = molecular weight [g/mol]; HLC = Henry's Law Constant [atm-m^3/mole]; W_s = water solubility [mg/L] at 25 °C if not otherwise indicated; ---, not listed in the literature or databases consulted.

Substance	**Bezafibrate**
Abbreviation	BZF
Chemical structure	
CAS-No.	41859-67-0
MW	361.82
log K_{OW}	4.25[a]; 4.250[b]
pKa	---
HLC	---
Ws	0.355[c]

[a] Gros et al., 2006a; [b] NLM, 2005; [c] SRC Research Corporation

2.1.1.4 NON-STEROIDAL ANTI-INFLAMMATORY DRUGS (NSAIDS)

USE AND PROPERTIES. Analgesics and antirheumatics are the leading pharmaceutical group in terms of their dispensed prescriptions in Germany (Schwabe and Paffrath, 2007) and their use in many other countries (Alder et al., 2006). Among them, non-steroidal anti-inflammatory drugs (NSAIDs) are a widely used group, resulting in consumption rates of over 250 t per year for ibuprofen alone (data for Germany, see Table 2-2; other sources give sale rates of more than 344 t for Germany in 2001 (BLAC, 2003).

The term "non-steroidal" distinguishes them from steroids (especially glucocorticoids) which have similar anti-inflammatory properties (Gentili, 2007). One distinctive characteristic of NSAIDs, which singles them out among analgesics, is their non-narcotic nature which is based on their pharmacologic mechanism: they act as non-selective or selective inhibitors of the isoenzymes cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) which control the formation of prostaglandins and thromboxane. As locally acting messenger molecules, prostaglandins mediate inflammation by acting on the thermoregulatory centre of hypothalamus to produce fever (Schwabe and Paffrath, 2007).

Chemically, all NSAIDs are acidic compounds (see pK_a in Table 2-11) since the acidic group is essential for therapeutic effect.

NON-SELECTIVE COX INHIBITORS. Diclofenac, ibuprofen and naproxen are non-selective COX inhibitors. They are mainly used in human medicine to treat pain (e.g. in post-operative treatment and treatment of cancer pain), fever, inflammatory disorders like rheumatoid arthritis and dysmenorrhea (BfArM, 2007a; BfArM, 2007b; U.S. National Library of Medicine, 2010; U.S. National Library of Medicine, 2012). The propionates ("profens") ibuprofen and naproxen were patented in 1961 (U.S. National Library of Medicine, 2010) and 1968 (Thieme Chemistry, 2009), respectively, and the phenylacetic acid diclofenac was patented in 1966 (Thieme Chemistry, 2009). Ibuprofen in particular is widely used today (see above and Table 2-2) and is part of the WHO's List of Essential Medicines, which lists core drugs for a basic healthcare system (WHO, 2011c). Only naproxen is also used to considerable extent in veterinary medicine (Löscher et al., 1994).

METABOLISM. After administration, NSAIDs are excreted as a mixture of parent drug and metabolites. Diclofenac is completely resorbed in the stomach before rapidly being hydroxylated and conjugated, resulting in a half-life of approximately 2 h. Over 99% of it is excreted in the form of inactive metabolites, with about 30% being excreted with faeces and 70% being excreted renally (BfArM, 2007a).

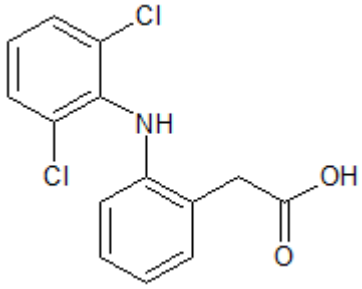
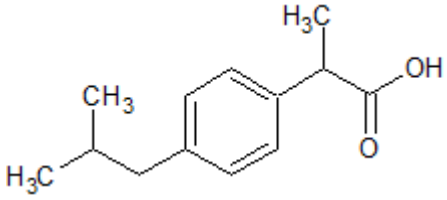
Ibuprofen is renally metabolised with a half-life of about 2 h (IIF, 2002). Weigel et al., 2004 reported 15% of the substance being excreted in original form of the parent drug while nearly 70% is excreted in metabolic form (26% as hydroxyibuprofen, 43% as carboxyibuprofen).
After application, naproxen has a half-life of 16–36 h. Vree et al., 1993 reported it to be metabolised to naproxen acyl glucuronide (51%) and its isomerised conjugate isoglucuronide (6.5%), to O-desmethylnaproxen acyl glucuronide (14%) and its isoglucuronide (5.5%). The same authors found practically no excretion of the unchanged parent compound or of O-desmethylnaproxen (< 1%).

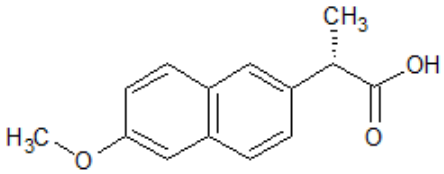
ENVIRONMENTAL FATE. Corresponding to their high consumption rates, their hydrophilicity and stability, NSAIDs are among the most frequently detected pharmaceuticals in wastewater and the environment (Gentili, 2007). Despite relatively high removal rates in conventional wastewater treatment with e.g. 50%, 83%, 85% (Radjenović et al., 2007) and 22%, 99%, 72% (Radjenović et al., 2009b) of diclofenac, ibuprofen and naproxen respectively, they are found globally in both the influent and effluent of WWTPs (Öllers et al., 2001; Carballa et al., 2004; Clara et al., 2004a; Botitsi et al., 2007; Santos et al., 2007; Smook et al., 2008). Reported concentrations vary between countries depending on application patterns and consumption rates, but reach up to the µg/l range, with higher influent amounts for ibuprofen than for diclofenac and naproxen. For example, Pedrouzo et al., 2007 reported the detection of all three NSAIDs in two Spanish WWTPs over several months, with ibuprofen frequently detected in influents (max. 5.99 µg/L) and effluents (0.69 µg/L), while naproxen was less regularly found (concentrations of up to 8.62 µg/L (influent) and 0.42 µg/L (effluent)). Diclofenac was repeatedly detected in influent and effluent in concentrations of up to 0.5 µg/L.
Possibly due to more efficient removal during wastewater treatment in the warmer seasons, Sacher et al., 2008 described seasonal variations of concentrations of diclofenac and ibuprofen in the river Rhine with considerably lower concentrations in summer, while Daneshvar et al., 2010 observed elevated amounts of NSAIDs (among other pharmaceuticals) in WWTP effluents and receiving waters in winter. Busetti et al., 2009 detected diclofenac and ibuprofen in secondary wastewater in Australia. Gómez et al., 2006 reported amounts of 1.5–151 µg/L for ibuprofen and 0.06–1.9 µg/L for diclofenac in hospital effluent: Jose Gomez et al., 2007 found 0.30–5.48 µg/L of naproxen in hospital wastewater.
Kosma et al., 2014 found NSAIDs in sludge samples from three WWTPs in Spain with concentrations of up to 74.9 ng/g, 117 ng/g and 5.9 ng/g of diclofenac, ibuprofen and naproxen respectively. Yu et al., 2009 investigated the removal of NSAIDs in sewage sludge, finding it depended on operational parameters of the wastewater treatment system as well as on the initial concentration of the substances. Lapen et al., 2008 investigated the behaviour of naproxen and ibuprofen in tile drainage and found them not readily washed off.

In two different studies, Radjenović found the elimination of diclofenac, ibuprofen and naproxen from the aqueous phase in conventional wastewater treatment to be 50.1%, 82.5% 81.5% (Radjenović et al., 2007), and 21.8%, 99.1%, 71.8% (Radjenović et al., 2009b) respectively.
Ashton et al., 2004 investigated the fate of diclofenac and ibuprofen through wastewater treatment and in the receiving surface waters for 11 WWTPs in the UK, finding wide differences in efficiency between various WWTPs. Gros et al., 2006a detected all three NSAIDs in influents and effluents in Croatian urban WWTPs and in the Ebro river.
Moldovan et al., 2009 investigated the influence of technological upgrades in WWTPs on the amount of ibuprofen (among others) in a river in Romania. Accordingly, NSAIDs are frequently detected in rivers and streams (Öllers et al., 2001; Antonić and Heath, 2007). In a mass balance approach based on a long-term study of the river Rhine, Sacher et al., 2008 calculated that up to 10% of the prescribed volume of diclofenac end up in the river.
Bataineh et al., 2006 studied the degradation of NSAIDs during sand bank filtration. Togola and Budzinski, 2008 detected all three NSAIDs not only in samples of WWTP effluent and surface water but in drinking water in France as well.
Comeau et al., 2008 detected NSAIDs in wastewater-receiving freshwaters in Canada but not in the connected marine waters or watersheds.

ECOTOXICITY. The environmental fate of NSAIDs has not yet been investigated in depth, but Isidori et al., 2005a reported photodegradation of naproxen. With phototransformation of the drug already reported in laboratory studies (Schmitt-Jansen et al., 2007), Kreuzinger et al., 2004 suggested the same as a possible elimination form of diclofenac in pretreated wastewater. In the case of naproxen, a higher ecotoxicological effect for photoproducts was found compared to the parent drug regarding both acute and chronic toxicity, while no genotoxic or mutagenic effects were found in bioassays performed on algae, rotifers and microcrustaceans (Isidori et al., 2005a). In biotests with the cladoceran *Daphnia magna*, the chlorophyte *Desmodesmus subspicatus* and the macrophyte *Lemna minor*, the aquatic ecotoxicity of diclofenac and ibuprofen was reported to be moderate (Cleuvers, 2003; see also Table 2-12). Nonetheless, investigating the phytotoxicity of diclofenac exposed to natural sunlight, Schmitt-Jansen et al., 2007 revealed fast degradation of the drug with a half-life of 3.3–6.4 h during the first three days of exposure. This was accompanied by increasing toxicity to the unicellular chlorophyte *Scenedesmus vacuolatus* after 3.5 h of sunlight exposure and sixfold enhanced toxicity after 53 h caused by several phototransformation products.

Table 2-11: Physico-chemical properties of non-steroidal anti-inflammatory drugs. W_s: water solubility (if not otherwise indicated at 25 °C); MW = molecular weight [g/mol]; HLC = Henry's Law Constant [atm-m^3/mole]; W_s = water solubility [mg/L] at 25 °C if not otherwise indicated; ---, not listed in the literature or databases consulted.

Substance	**Diclofenac**	**Ibuprofen**
Abbreviation	DCF	IBP
Chemical structure		
CAS-No.	15307-86-5	15687-27-1
MW	296.15 g/mol	206.29 g/mol
log K_{OW}	4.51[a]; 4.40[b]; 4.51[c]	3.97[a,c]; 3.50[b]
pKa	4.14[a]; 4.15[b]	4.91[a,c]; 4.91[b]
HLC	4.73x10^{-12}[c]	1.50x10^{-07}[c]
Ws	2.37[c]	21[c]

Substance	**Naproxen**
Abbreviation	NPX
Chemical structure	
CAS-No.	22204-53-1
MW	230.259
log K_{OW}	3.18[a]; 3.18[b]; 3.18[c]
pKa	4.15[a]; 4.15[b]
HLC	3.39x10^{-10}[c]
Ws	15.9[c]

[a] Gros et al., 2006a; [b] Sangster, 2012; [c] NLM, 2005

Table 2-12: Chemical toxicity data of NSAIDs for aquatic life (EPA, 2012b). LC_{50}: median lethal concentration; t: exposure duration; LOEC: lowest observable effect concentration; species given in index numbers: 1: *Oryzias latipes* (Japanese Medaka); 2: *Planorbis carinatus* (Keeled Ramshorn Snail); 3: *Hydra attenuata* (Hydra); 4: *Daphnia magna* (Water Flea).

Substance (CAS-No.)	**LC_{50}**			**LOEC**		
	lowest LC_{50} [µg/L] for the most sensitive indicator species (indicator species)	t	no. of aquatic studies identified	lowest LOEC [µg/L] for the most sensitive indicator species (idicator species)	t	no. of aquatic studies identified
Diclofenac (15307-86-5)	8,000 (1)*	96 h	1	0.36 (4)	~ 6 days	45
Ibuprofen (15687-27-1)	32 (4)	24-96h	9	1 (1)	42 days	13
Naproxen (22204-53-1)	22,360 (3)	96 h	2	1,000 (3)	96 h	5

* LC_{10}; no LC_{50} values given in searched database

2.1.1.5 PSYCHOACTIVE DRUGS

In Germany, psychoactive drugs were the fourth most prescribed pharmaceutical group in 2005 (Schwabe and Paffrath, 2007); they are often used in quantities of several tonnes per year (Table 2-2). Oxycodone (under its brand name Oxygesic) was among the 30 top-selling pharmaceuticals in Germany in 2005 (Schwabe and Paffrath, 2007).

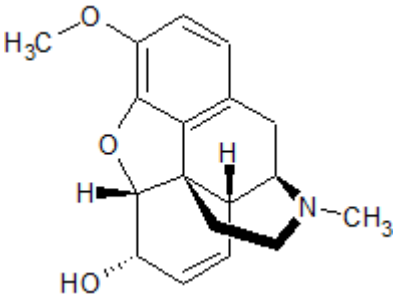
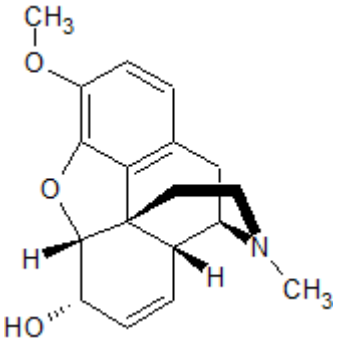
OPIOIDS. Today, strong opioids are mainly used in palliative care, especially to treat cancer pain (Schwabe and Paffrath, 2007). To relieve pain, opioids act directly on the central nervous system (and on the peripheral nervous system and the gastrointestinal tract) by binding to opioid receptors. Some opioids also act as powerful cough suppressants (Schwabe and Paffrath, 2007). Natural plant alkaloids like morphine and codeine count among opioids as well as the semi-synthetic opioid analgesics dihydrocodeine and oxycodone (Table 2-13).

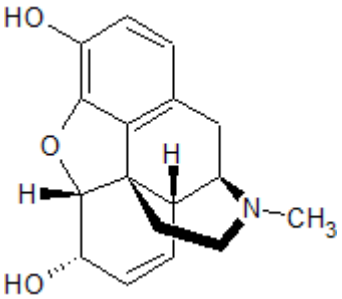
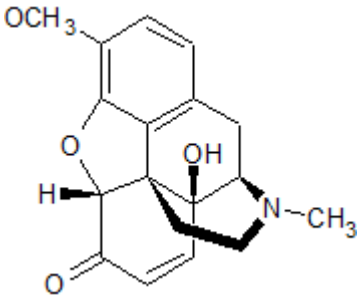
MORPHINE. In clinical medicine, morphine is the benchmark of analgesics applied to relieve agonising pain, and it is the gold standard for cancer pain treatment in the World Health Organization's three-level programme (WHO, 1996). Morphine metabolism mainly occurs in the liver. Approximately 87% of the administered dose is excreted via urine within 72 h after treatment. It is metabolised by phase-II-metabolism into two main products: about 60% is biotransformed into morphine-3-glucuronide, and 6–10% into morphine-6-glucuronide, the latter being half as potent as the parent drug in humans while the former has no analgesic effect (Kilpatrick and Smith, 2005; van Dorp et al., 2006).

CODEINE. Codeine is the prototype of weak to midrange opioids (e.g. dihydrocodeine: see below). After treatment, 80% of it is metabolised to codeine-6-glucuronide by conjugation with glucuronic acid. Codeine-6-glucuronide is mostly responsible for the analgesic effect of codeine. Additionally, 15% of the parent drug is biotransformed to norcodeine while 5% is metabolised to morphine, which thereafter undergoes morphine metabolism (see above) (Vree et al., 2000).

SEMI-SYNTHETIC OPIOIDS. Dihydrocodeine and oxycodone are semi-synthetic opioids with half-lives of 4-6 h. Like the natural opioids, they are metabolised renally and excreted via urine. For oxycodone, the percentage of the unchanged drug excreted is given as 19% (Lalovic et al., 2006). Dihydrocodeine is counted among the less potent opioids and can, among other applications, be used as an alternative to codeine (Schwabe and Paffrath, 2007). Oxycodone (half-life 4–6 h) is used similar to morphine, i.e. used in treatment of severe and agonising pain, but it is more than twice as expensive as morphine generics (Schwabe and Paffrath, 2007).

Table 2-13: Physico-chemical properties of opioids (for details refer to text). W_s: water solubility (if not otherwise indicated at 25 °C); MW = molecular weight [g/mol]; HLC = Henry's Law Constant [atm-m^3/mole]; W_s = water solubility [mg/L] at 25 °C if not otherwise indicated; ---, not listed in the literature or databases consulted.

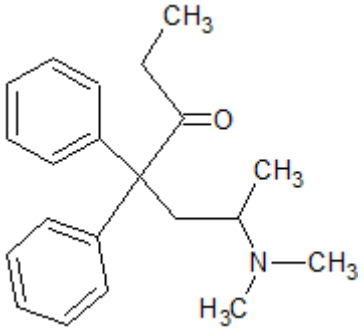
Substance	**Codeine**	**Dihydrocodeine**, also DHC, Drocode, Paracodeine, Parzone
Abbreviation	CDN	DHC
Chemical structure		
CAS-No.	76-57-3	125-28-0
MW	299.36	301.38
Log K_{OW}	1.14[a]	1.490[c]
pKa	8.1[a]	---
HLC	$7.58x10^{-14}$[b]	$8.61x10^{-14}$[b]
Ws	9,000 (20 °C)[b]	6,710[b]

Substance	**Morphine**	**Oxycodone**
Abbreviation	MPN	OCN
Chemical structure		
CAS-No.	57-27-2	76-42-6
MW	285.34	315.364
Log K_{OW}	0.89[c]	0.660[b]
pKa	8.21[c]	---
HLC	$1.33x10^{-16}$[b]	$2.33x10^{-16}$[b]
Ws	149[c]	4,160[b]

[a] Sangster, 2012; [b] SRC Research Corporation; [c] NLM, 2005

SYNTHETIC OPIOIDS: METHADONE. This is a synthetic opioid mostly known for its use in opiate replacement therapy (Methadone Maintenance Treatment, MMT). Furthermore, it is used in the treatment of severe chronic pain. It is lipophilic and very slowly metabolised (half-life 15–60 h, although some studies give half-lives up to 190 h), resulting in extended effect times compared to morphine-based drugs (Eap et al., 1999; Eap et al., 2002; Manfredonia, 2005; see also Table 2-14).

Table 2-14: Physico-chemical properties of methadone. W_s: water solubility (if not otherwise indicated at 25 °C); MW = molecular weight [g/mol]; HLC = Henry's Law Constant [atm^{-m3}/mole]; W_s = water solubility [mg/L] at 25 °C if not otherwise indicated; ---, not listed in the literature or databases consulted.

Substance	**Methadone**
Abbreviation	MTD
Chemical structure	
CAS-No.	76-99-3
MW	309.45
Log K_{OW}	3.93[a]
pKa	8.94[a]
HLC	$4.97x10^{-10}$[a]
Ws	48.5[a]

[a] NLM, 2005

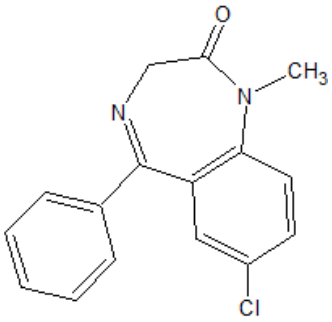
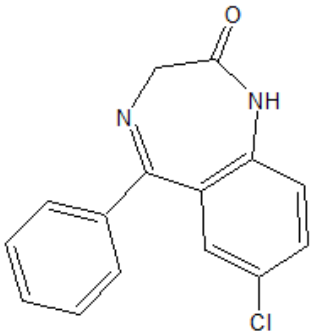
BENZODIAZEPINES. Benzodiazepines consist of a core chemical structure of a benzene ring fused with a diazepine ring (Table 2-15). They increase the effect of the neurotransmitter gammaaminobutyric acid (GABA) and are used as tranquilisers for their sedative, sleep-inducing (hypnotic), anxiolytic (anti-anxiety), amnesic, anticonvulsant and muscle relaxant actions to treat e.g. insomnia, anxiety, agitation, muscle spasms and the effects of alcohol withdrawal. Additionally, they are used in premedication for medical or dental procedures (Olkkola and Ahonen, 2008).

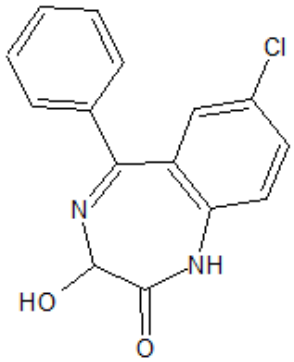
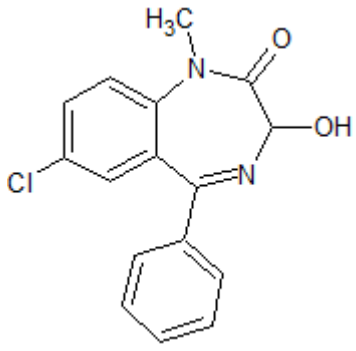
The first benzodiazepine, chlordiazepoxide, was marketed in 1960. Three years later, diazepam (brand name valium) followed. It became rapidly the standard choice of benzodiazepines and is to date still one of the leading drugs used in the treatment of anxiety. The WHO considers it a core medicine for minimum medical needs of a basic health system (WHO, 2005a). It has a long half-life (24–48 h). Therefore, and because of the even longer half-life of its active metabolite nordiazepam (50–80 h, Schwabe and Paffrath, 2007), an accumulation in the body is achieved. This results in long-lasting action which reduces the possibility of panic attacks should the drug intake be interrupted. On the other hand, the downsides of such long-acting benzodiazepines are increasing sedation and inhibition of psychomotor abilities, and the development of addiction. A second metabolite of diazepam is oxazepam, which results from the hydroxylation of desmethyldiazepam and is rapidly renally excreted in form of glucuronides. Most of the long-acting benzodiazepines undergo the described metabolism, being biotransformed to nordiazepam and oxazepam (Schwabe and Paffrath, 2007).

Besides being an active metabolite of diazepam, oxazepam is used directly as a drug itself, entering the market as the third benzodiazepine in 1963. Since its biotransformation takes place without a further active metabolite being produced (direct glucuronidation in a phase-II-reaction, see Chapter 2.1), and because of its relatively short half-life (7–15 h) it shows practically no cumulative effect and is more easily controllable.

With a half-life of 8–20 h, temazepam is another intermediate-acting benzodiazepine which is often used for the treatment of insomnia.

Table 2-15: Physico-chemical properties of benzodiazepines. W_s: water solubility (if not otherwise indicated at 25 °C); MW = molecular weight [g/mol]; HLC = Henry's Law Constant [atm^{-m3}/mole]; W_s = water solubility [mg/L] at 25 °C if not otherwise indicated; ---, not listed in the literature or databases consulted.

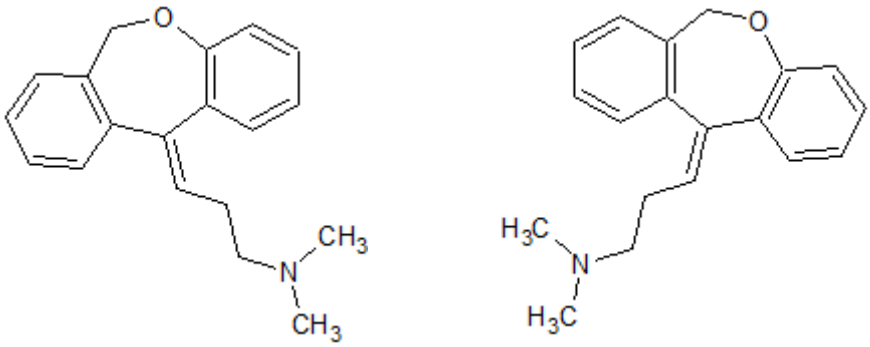
Substance	**Diazepam**	**Nordiazepam also known as nordazepam, desoxydemoxepam and desmethyldiazepam**
Abbreviation	DZP	NZP
Chemical structure		
CAS-No.	439-14-5	1088-11-5
MW	284.7	270.71
Log K_{OW}	2.82[a]	2.93[a]
pKa	3.4[a]	---
HLC	$3.64x10^{-9}$[a]	$1.78x10^{-10}$[a]
Ws	50[a]	57[a]

Substance	**Oxazepam**	**Temazepam**
Abbreviation	OZP	TZP
Chemical structure		
CAS-No.	604-75-1	846-50-4
MW	286.71	300.70
Log K_{OW}	2.24[a]	2.19[a]
pKa	---	---
HLC	$5.53x10^{-10}$[a]	$1.13x10^{-8}$[a]
Ws	179[a]	164[a]

[a] NLM, 2005

TRICYCLIC ANTIDEPRESSANTS: DOXEPIN. Doxepin is a tricyclic antidepressant (Table 2-16) which is used to treat depression, anxiety disorders and insomnia, and is also used in dermatological cream-based preparations. It inhibits the re-uptake of serotonin and norepinephrine (Schwabe and Paffrath, 2007). Doxepin has a half-life of 17 h, its main metabolite desmethyldoxepin one of 51 h, and it is excreted via urine (Thieme Chemistry, 2009).

Table 2-16: Physico-chemical properties of doxepin. W_s: water solubility (if not otherwise indicated at 25 °C); MW = molecular weight [g/mol]; HLC = Henry's Law Constant [atm^{-m3}/mole]; W_s = water solubility [mg/L] at 25 °C if not otherwise indicated; ---, not listed in the literature or databases consulted

Substance	**Doxepin (cis-, trans-)**
Abbreviation	DXP
Chemical structure	
CAS-No.	1668-19-5
MW	279.38
Log K_{OW}	4.29[a]
pKa	---
HLC	$2.77x10^{-9}$[b]
Ws	31.6[a]

[a] NLM, 2005; [b] SRC Research Corporation

ANTICONVULSANT DIBENZAZEPINE: CARBAMAZEPINE. Carbamazepine is an anticonvulsant dibenzazepine which is extensively used in the treatment of epilepsy, bipolar disorder and trigeminal neuralgia (Table 2-17). Its main application is as an antiepileptic drug. Although being one of the oldest antiepileptics used (it was first marketed in 1962), it is the first choice drug for focal seizures in several countries (SIGN, 2003; NICE, 2004; Arzneimittelkommision der deutschen Ärzteschaft, 2006). Other applications are attention-deficit hyperactivity disorder (ADHD), complex regional pain syndrome, neuromyotonia, borderline personality disorder, post-traumatic stress disorder, schizophrenia and others. Carbamazepine is structurally similar to tricyclic antidepressants and, like these, has mood-stabilising properties. Further, it is used for the treatment of alcohol withdrawal (Miao and Metcalfe, 2003).

Daily doses of Carbamazepine range between 400 – 1200 mg/d (BfArM, 2003). The drug undergoes a complex hepatic metabolism via oxidation, hydration and conjunction with glucuronide resulting in a range of at least seven metabolites formed in the human organism (BfArM, 2003). Of these, some are pharmaceutically active, but the most abundant one, 10,11-

Dihydro-10,11-dihydroxycarbamazepine is inactive (Miao and Metcalfe, 2003; see also Table 2-17). The half-life of Carbamazepine is 18 – 65 h, with an average of 36 h. During long-term therapy, which is the main form of treatment due to the fact that it is used lifelong in its primary indications, the half-life is shortened by about 50% (10–20 h). According to BfArM, 2003, after oral treatment approximately 72% of the drug is excreted via urine (of which 2–3% is unmetabolised) and 28% via faeces (partly unmetabolised). Alder et al., 2006 estimates the overall excretion of the unmetabolised parent drug to be less than 3%. Bernus et al., 1995 reported the percentage of unmetabolised carbamazepine in the urine of pregnant women as 1%, while 10,11-Dihydro-10,11-dihydroxycarbamazepine accounts for 35%.

ANTICONVULSANT BARBITURATE: PRIMIDONE. Primidone is an anticonvulsant of the barbiturate class (Table 2-17), which laid the foundations of epileptic therapy almost 100 years ago. It is used to treat a wide range of seizures, but is seldom administered in the treatment of psychiatric problems. Besides its application in human medicine, primidone is also used in veterinary medicine e.g. to prevent aggressive behaviour and cannibalism in young female pigs or to treat nervous disorders in various animals (e.g. dogs) (Thieme Chemistry, 2009; 5m Publishing). According to Schwabe and Paffrath, 2007 it is only recommended as the third choice treatment since it shows sedative effects that interfere with cognitive abilities even at plasma levels at which no other intolerances are displayed. Its still increasing use is explained by its broad spectrum of efficacy, easy application and low costs (Schwabe and Paffrath, 2007).
Primidone acts mostly through its active metabolite, phenobarbital. The parent drug is metabolised renally; its half-life is 5–18 h, while Phenobarbital has a half-life of 75–120 h (Ochoa and Riche, 2012).

ENVIRONMENTAL FATE. With the exception of Carbamazepine, the fate during wastewater treatment and the environmental behaviour of most psycho-active drugs has not yet been thoroughly investigated despite their extensive use.
Carbamazepine has been documented as being ubiquitously present in influents and effluents of WWTPs in Europe, America, and Asia at concentrations of up to the lower µg/l range, finding only marginal removal rates for the parent substance during conventional wastewater treatment (Öllers et al., 2001; Clara et al., 2004b; Kreuzinger et al., 2004; Zuehlke et al., 2004; Gros et al., 2006a; Vieno et al., 2006; Santos et al., 2007; Vieno et al., 2007; Zhang and Zhou, 2007; Radjenović et al., 2009b; Ying et al., 2009; Pedrouzo et al., 2011; Ryu et al., 2011). Miao et al., 2005 additionally investigated the occurrence of carbamazepine metabolites in WWTP water, revealing that 10,11-Dihydro-10,11-dihydroxycarbamazepine exhibit up to three times higher concentrations than carbamazepine itself. Similar ratios of drug and metabolites were found by

Leclercq et al., 2009 when investigating the fate of carbamazepine and several of its metabolites in three different types of WWTP (CAS, trickling filter, and stabilisation ponds). Moreover, no removal of either parent drug or metabolites was found for CAS treatment, while the other treatment systems displayed varying removal rates, indicating that increased biodegradation or sorption as well as enhanced cleavage and re-transformation processes take place in these systems.

Several studies failed to detect diazepam in influents and effluents of WWTPs in Germany and Spain (Ternes et al., 2001; Carballa et al., 2004; Hummel et al., 2006; Wick et al., 2009) while other benzodiazepines as well as several opioids and barbiturates were frequently detected in influents and effluents of German WWTPs (except oxycodone which was present only in influents) in concentrations of up to the low µg/L range (Hummel et al., 2006; Wick et al., 2009).

These studies reveal that while more than 80% of the natural opium alkaloids morphine and codeine are removed during wastewater treatment, several psycho-active drugs such as primidone and oxazepam are not significantly removed.

Gómez et al., 2006 detected carbamazepine and codeine in hospital wastewater in Spain in concentrations of 0.04 and 0.9 µg/L respectively.

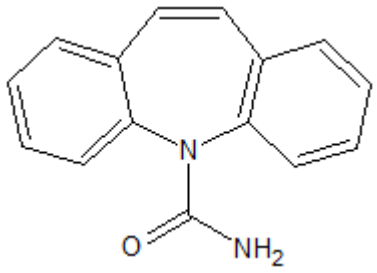
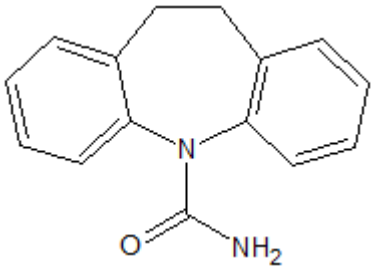
Wick et al., 2009 found sorption into activated sludge to be a negligible removal pathway, with removal rates of less than 3% for several psycho-active and lipid-regulating drugs, thus postulating that the removal of the beta blockers and psycho-active drugs examined could be described solely as biological transformation. Still, because of its log K_{OW} of 2.47 (Table 2-17), carbamazepine shows a moderate sorption potential and subsequently was found in several studies in sewage sludge (Miao et al., 2005; Barron et al., 2008) and in tile drainage (Lapen et al., 2008).

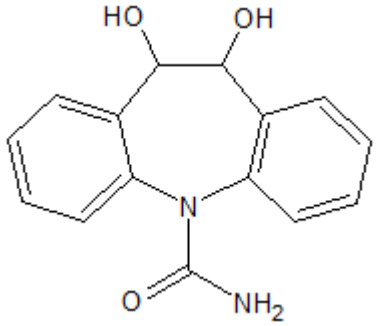
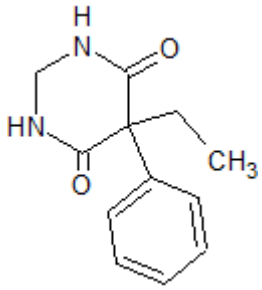
Carbamazepine was frequently detected in surface water in several countries (Tixier et al., 2003; Zuehlke et al., 2004; Gros et al., 2006a; Vieno et al., 2006; Benotti and Brownawell, 2007; Zhang and Zhou, 2007; Conley et al., 2008; ter Laak et al., 2010). In a widespread study in Germany, carbamazepine was found to be among the most frequently detected pharmaceuticals in surface waters (BLAC, 2003) and was also detected in groundwater, with concentrations of up to 0.9 µg/L. Loos et al., 2007 found CBZ in lake water and tap water in Italy.

Hummel et al., 2006 reported the presence of 15 psycho-active drugs in surface waters in Germany in the low ng/L range. In the same study, carbamazepine, 10,11-dihydro-10,11-dihydroxycarbamazepine and primidone were detected in tap water at concentrations of up to 20 ng/L, 13 ng/L and 16 ng/L respectively. Valcárcel et al., 2011 found several psycho-active drugs in surface and tap water in the Madrid region in Spain.

In a laboratory study using river sediment, Löffler et al., 2005 reported elevated levels of sorption of diazepam, oxazepam and carbamazepine into the sediment.

Table 2-17: Physico-chemical properties of anticonvulsant drugs and metabolites (for details refer to text). W_S: water solubility (if not otherwise indicated at 25 °C); MW = molecular weight [g/mol]; HLC = Henry's Law Constant [atm^{-m3}/mole]; W_s = water solubility [mg/L] at 25 °C if not otherwise indicated; ---, not listed in the literature or databases consulted.

Substance	**Carbamazepine**	**10,11-Dihydrocarbamazepine**
Abbreviation	CBZ	DH-CBZ
Chemical structure		
CAS-No.	298-46-4	3564-73-6
MW	236.27	238.29
Log K_{OW}	2.47[a]; 2.30[b]	2.46[c]
pKa	7[a]	---
HLC	$1.08x10^{-10}$[c]	$5.57x10^{-10}$[c]
Ws	17.7[c]	16.8[c]

Substance	**10,11-Dihydro-10,11-dihydroxycarbamazepine**	**Primidone**
Abbreviation	DHH	PMD
Chemical structure		
CAS-No.	35079-97-1	125-33-7
MW	270.288	218.25
Log K_{OW}	---	0.91[b,d]
pKa	---	---
HLC	---	$1.94x10^{-10}$[c]
Ws	---	500 (22 °C)[c]

[a] Gros et al., 2006a; [b] Sangster, 2012; [c] SRC Research Corporation; [d] NLM, 2005

DEGRADATION. Data regarding the degradation of psycho-active drugs in the environment are scarce. Doll and Frimmel, 2003 reported the degradation of carbamazepine through simulated and natural sunlight into several unidentified degradation products.

TOXICITY. In general, the environmental impact and possible adverse effects on non-target organisms of psycho-active drugs is unknown (see Table 2-18), which makes appropriate environmental risk assessments unfeasible. Based on its log K_{OW} value (Table 2-17), BLAC, 2003 postulates a moderate potential of accumulation in aquatic organisms for carbamazepine. The drug was shown to have a chronic toxic effect on crustaceae (*Cerodaphnia dubia*: NOEC (7 d): 0,025 mg/L) and to a lesser extent on fish (*Danio rerio*: NOEC (10 d): 25 mg/L) (Ferrari et al.,

2003). Oetken et al., 2005 found a concentration-dependent decrease in abundance of the non-biting midge *Chironomus riparius* when exposed to carbamazepine-spiked sediment (lowest observed effect concentration (LOEC) 140 mg/kg). Gagné et al., 2006 established adverse effects of carbamazepine and morphine on the immune system of fresh water mussels (*Elliptio complanata*). Additionally, there are indications that carbamazepine may be toxic to reproduction in mammals (BLAC, 2003). Furthermore, its accumulative interaction with other pharmaceuticals is to be considered (BLAC, 2003).

Table 2-18: Chemical toxicity data of psycho-active drugs for aquatic life (EPA, 2012b). LC_{50}: median lethal concentration; t: exposure duration; LOEC: lowest observable effect concentration; species given in index numbers: 1: *Chironomus tentans* (Midge); 2: *Hydra attenuata* (Hydra); 3: *Artemia parthenogenetica* (Brine Shrimp); 4: *Mytilus galloprovincialis* (Mediterranean Mussel).

Substance (CAS-No.)	**LC_{50}**			**LOEC**		
	lowest LC_{50} (µg/L) for the most sensitive indicator species (indicator species)	t	no. of aquatic studies identified	lowest LOEC (µg/L) for the most sensitive indicator species (indicator species)	t	no. of aquatic studies identified
Carbamazepine (298-46-4)	9,900 (1); 29,400 (2)	240 h; 96 h	5	0.1 (4)	168 h	40
10,11-Dihydro-carbamazepine (3564-73-6)	---	---	0	---	---	0
10,11-Dihydro-10,11-dihydroxy-carbamazepine (35079-97-1)	---	----	0	---	---	0
Primidone (125-33-7)	---	----	0	---	---	0
Doxepin (1668-19-5)	---	----	0	---	---	0
Codeine (76-57-3)	---	----	0	---	---	0
Dihydrocodeine (125-28-0)	---	----	0	---	---	0
Methadone (76-99-3)	---	----	0	---	---	0
Morphine (57-27-2)	---	----	0	---	---	0
Oxycodone (76-42-6)	---	----	0	---	---	0
Diazepam (439-14-5)	12,200 (3)	48 h	22	---	---	0
Nordazepam (1088-11-5)	---	----	0	---	---	0
Oxazepam (604-75-1)	---	----	0	---	---	0
Temazepam (846-50-4)	---	----	0	---	---	0

2.1.2 ORGANOPHOSPHORUS COMPOUNDS

ORGANOPHOSPHATE ESTERS. Among organophosphorus compounds, which are widely used as flame retardants, organophosphate esters (OPs) are the most utilized group (WHO, 1997). OPs do not naturally occur in the environment but only reach it as a result of human activity (WHO, 1991a; WHO, 1991b; WHO, 1997; WHO, 1998a; WHO, 2000). The global consumption of flame-retarding substances is directly connected to national and international regulations concerning fire precautions (WHO, 1997).

Structurally OPs are derivates of phosphoric acid. They are produced industrially by a reaction of phosphorus oxychloride ($POCl_3$) with a range of reactants, resulting in a variety of (tri)alkyl- and (tri)aryl-phosphates with diverse physico-chemical characteristics (Fisk et al., 2003; Marklund, 2005) as shown in Table 2-19. Organophosphate esters can also be halogenated combining the chemical properties of halogen and phosphorus components. Furthermore, the halogen reduces the mobility of the OP in the polymer matrix, leading to a longer lifetime of the OP in the end-product and therefore reducing blooming (see below) (Fisk et al., 2003). Halogenated flame retardants are practically exclusively brominated or chlorinated, since fluorinated compounds are ineffective and expensive while iodinated compounds are effective but unstable (Fisk et al., 2003). In this study five non-chlorinated OPs (TBEP, TnBP, TiBP, TEHP, TPP) and three chlorinated OPs (TCEP, TCPP, TDCPP) are investigated.

APPLICATIONS. The variations in characteristics allow the use of OPs in different applications. Besides their use as flame retardants they are commonly utilised as plasticisers to decrease rigidity in polymers, and as solvents. As they are used in e.g. rigid and flexible polyurethane foams (PUF), polyvinyl chloride (PVC), thermoset resins, thermoplastic materials, textile finishes, polyesters and cellulosics (Marklund, 2005), they are incorporated into a wide range of everyday objects as computer housing, furnishings, upholstery and textiles. Additionally, they are used in paint, lubricants, hydraulic fluids and motor and transmission oils (WHO, 1997; Commonwealth of Australia, 2001; Umweltbundesamt, 2012).

Although OPs have been produced since the 1930s and used increasingly throughout the twentieth century with the spread of plastics, their production increased drastically when substitutes for brominated flame retardants needed to be found after the latter had been identified as persistent organic pollutants (POPs), being persistent, bioaccumulative and toxic substances (PBTs). Consequently, the restriction of use or the complete phase-out of some brominated flame retardants was initiated by several national and international regulations, e.g. the Stockholm Convention on Persistent Organic Pollutants. The United States Environmental Protection Agency (EPA) named several OPs such as TPP and TDCPP as possible substitutes for brominated flame retardants in polyurethane foam (EPA, 2005). Hence, the consumption of OPs

is rising globally. In 2007, roughly 209,000 t were consumed worldwide; the overall consumption of OPs in Europe was 83,000 t (compared to 45,000 t of brominated flame retardants) (Clariant International Ltd). As high production volume (HPV) compounds, all OPs investigated in this study (except TiBP) were subject to registration within the EU REACH procedure before the first deadline of 1st December 2010 (registration of substances with production or import amounts of more than 1,000 t per year in the EU). According to Umweltbundesamt, 2012, the production of TCEP and TCPP exceeds 5,000 t per year in the EU, whilst SCHER, 2007 reported a total production of about 10,000 t per year in the EU for TDCPP.

PROPERTIES OF SELECTED OPS. As mentioned above, OPs are used in a wide range of applications. In general, triaryl phosphates such as TPP are more effective flame retardants because of their greater thermal stability, while trialkyl phosphates are better plasticisers, improving the low-temperature flexibility of plastics and synthetic rubber (Green, 1996).

TBEP is commonly used as a plasticiser in PVC, synthetic rubber and plastics e.g. in hoses, seals and soles of footwear, as a levelling compound in floor polishes, waxes and paper coating, as a viscosity modifier in plastisols and as an antifoam agent (WHO, 2000; Marklund, 2005).

Besides its use as flame retardant, TnBP is utilised as an extreme pressure additive in hydraulic fluids, lubricants and transmission and motor oils. Aircraft hydraulic fluids can consist of up to 79% TnBP (Marklund, 2005).

TnBP and TiBP are both used as solvents and antifoaming agents in concrete, textile auxiliaries and paper coating compounds. Additionally, TiBP is used in the production of synthetic resins and natural rubber. In cellulose-based plastics and resins it is used as a flame-retarding plasticiser; in pigment pastes it serves as a pasting agent. Being a very strong polar solvent, it is also utilised as a wetting agent in the production of textiles (LANXESS AG).

TEHP is used as a flame retardant in cellulose acetate and as a flame-retarding plasticiser in PVC and natural rubber for low temperature applications. Furthermore, it serves as a solvent for certain chemical reactions, e.g. in the production of hydrogen peroxide, and is utilised in pigment pastes and as an additive in mineral oils (WHO, 2000; LANXESS AG, 2009).

TPP is often used as a flame retardant in automobiles (e.g. vinyl upholstery) and electrical compounds (e.g. computer housings). It is a non-flammable plasticiser in cellulose-acetate based films as well as in lacquers and varnishes. TPP is used to impregnate roofing paper, and similar to TBP it is used in lubricants and hydraulic fluids (up to 5%) (WHO, 1991a; Marklund, 2005; LANXESS AG, 2008).

Chlorinated OPs are widely used as flame retardants in flexible and rigid polyurethane foam (PUF), rubber, polyester resins and textile coatings (WHO, 1998a). While rigid PUF is mostly utilised for thermal insulation, flexible PUF is primarily used to add comfort in upholstered

furniture, mattresses and car interiors (EFRA). The level of additive in the product is usually 5-15% (EFRA).

TCEP is used in PVC, PUF, plastics, carpet backing, fabric back-coating formulations, cellulose ester compounds and coatings (WHO, 1998a; Fisk et al., 2003). Furthermore, it is largely used in the production of liquid unsaturated polyester resins (WHO, 1998a). TCPP is mainly used in rigid polyurethane foams such as in furniture and furniture upholstery, building insulation and in refrigerator casings. Small amounts of TCPP are used for textile back-coating and various coatings (WHO, 1998a; LANXESS AG, 2011). Recently, it has also been a substitute for TCEP (Umweltbundesamt, 2012). According to WHO, 1998a, TDCPP is used in both rigid and flexible PUF, in plastics and resins as well as in acrylic latexes. Mattresses in prisons and hospitals are commonly flame retarded by TDCPP (KemI, 1996).

TCEP, TCPP and TDCPP were also found in shock- and sound-absorbing materials, wood preservation coatings and foam fillers (Marklund, 2005).

PRODUCT LIFE CYCLE AND ENVIRONMENTAL OCCURRENCE. OPs are mostly used as additives, i.e. they are not chemically bound to the polymer backbone but remain as discrete molecules within the polymer matrix. This results in possible diffusion of the OPs out of these materials over the whole span of the products' life cycle (also referred to as blooming) (Commonwealth of Australia, 2001; Marklund, 2005). The degree to which blooming occurs depends on the chemical properties of the particular OP and the polymer it is incorporated in (e.g. K_{OW}, vapour pressure, surrounding temperature, size and shape of the polymer and OP molecules, stability of the polymer backbone against solvents)(Commonwealth of Australia, 2001). Though detailed studies are lacking, it is suspected that OPs which are moved by diffusion processes to the surface layers of a product can be rubbed or washed off or become airborne either through volatilisation or particle-bound, thus sustaining the diffusion gradient and leading to a constant drain of OPs from the end product. Subsequently, OPs are found in indoor dust (Carlsson et al., 1997; Otake et al., 2001; Hartmann et al., 2004; Staaf and Östman, 2005; Stapleton et al., 2008, Ali et al., 2012) as well as in environmental compartments such as surface water (e.g. Andresen et al., 2004), groundwater (Fries and Puttmann, 2003) and wastewater (Bester, 2005). In a study of 95 organic wastewater contaminants in rivers in the U.S., TCEP was found to be one of the pollutants with the highest concentrations (Kolpin et al., 2002). Several studies showed long-range transport of OPs to remote areas such as polar regions (e.g. Laniewski et al., 1998; Möller et al., 2012) and volcanic lakes (Bacaloni et al., 2008). After disposal, leaching from landfills is another way OPs can get into the environment (Yasuhara et al., 1999). When burning, e.g. during waste incineration, halogenated OPs form highly toxic dioxins and furans, which are only eliminated in state-of-the-art incinerators with multiple combustion zones (DiGangi et al., 2010).

TOXICITY. Although studies regarding the toxicity of phosphorus-containing flame retardants were published as early as in the 1970s (e.g. Liepins and Pearce, 1976), reliable data about the toxicity of OPs and their environmental fate are in most cases still lacking, scarce or incongruent, despite the efforts of REACH and other regulations. However, according to the Globally Harmonized System of Classification and Labelling of Chemicals (GHS (United Nations, 2011)), TiBP, TCEP and TPP are classified as being harmful (H412), toxic (H411) and very toxic (H410) to aquatic life, with long lasting effects, respectively. Three substances (TnBP, TCEP, TDCPP) are suspected of causing cancer (H351). TCEP may, in addition, impair fertility (H360F). TnBP, TCEP and TCPP are harmful if swallowed (H302). TnBP and TEHP cause skin irritation (H315); the latter also provokes serious eye irritation (H319), while TiBP may cause an allergic skin reaction (H317).

In a study surveying commercial substances not currently part of contaminant measurement programs, TDCPP was described as being likely to be persistent and bioaccumulative (Howard and Muir, 2011). A recent study from Canada reports the presence of TCEP in predatory freshwater fish from Canadian lakes (McGoldrick et al., 2014). In general, the physico-chemical properties of OPs which determine their fate in the (aquatic) environment are very heterogenic, e.g. reported log K_{OW} values range between 0.5 and 9.49 (Table 2-19), indicating very diverse potentials for sorption into particles and/or possible bioaccumulation.

Of the substances in this study, TPP and TDCPP are reported to be the most toxic ones to aquatic organisms, with 96 h LC_{50} concentrations of 0.36 and 1.1 mg/L respectively for rainbow trout (WHO, 1991a; WHO, 1998a), while the toxicity of TBP is given as 4.2 – 12 mg/L for rainbow trout. For TCPP, the 96 h LC_{50} concentration in fathead minnow is reported with 51 mg/L; for TCEP in goldfish it is 90 mg/L (WHO, 1998a). Further details of acute toxicity of OPs are given in Table 2-20. Studies showed that OPs also have an adverse effect on algae and plants. TPP was found to completely inhibit algae growth at concentrations above 1 mg/L (WHO, 1991a). TBP increases plants' drying rates and damages their leaf surfaces, thus inhibiting respiration (WHO, 1991b). Furthermore, organophosphate flame retardants seem to alter sex hormone balances in zebrafish (*Danio rerio*) through several mechanisms, including alterations of steroidogenesis and oestrogen metabolism (Liu et al., 2012).

For TDCPP (log K_{OW} 3.65), the EU's Simple Treat Model predicts that 25% of the substance present in water bodies would be associated with sludge, 5% may be released into the air while 70% would remain dissolved (Commonwealth of Australia, 2001). Regarding the HVP character of the OPs, it can be assumed that not only the water compartment is affected by high amounts of TDCPP but air, sludge and soils as well. Similar is to be expected for other OPs (WHO, 1991b; WHO, 1998a; WHO, 2000; Marklund, 2005). In particular, chlorinated flame retardants are the

subject of recent scientific discussion and concern (cf. DiGangi et al., 2010). OPs utilised as flame retardants and plasticisers are of similar chemical structure from organophosphorus insecticides, the latter having an impact on the nervous system by inhibiting the action of acetylcholinesterase (AChE) and other enzymes in nerve cells of not only target organisms but non-target organisms, including humans, as well (e.g. Hayden et al., 2010, Terry, JR, 2012). Thus, many organophosphates are powerful nerve agents, and organophosphate poisoning is a common means of suicide in both developing and developed countries (e.g. Yurumez et al., 2007; Eddleston et al., 2008; Hrabetz et al., 2013). Sublethal exposure to organophosphates triggers transcriptional changes in genes associated with Parkinson's disease (Slotkin and Seidler, 2011); it is suspected of producing lasting neurotoxicity affecting cognitive functions, increasing the risk of dementia and Alzheimer's disease (Santibáñez et al., 2007; Hayden et al., 2010), and causing or encouraging impairments generally recognised as psychologically based disorders, such as Gulf War Syndrome (Terry, JR, 2012) or depression (Beseler et al., 2008). Furthermore, low-level exposure to organophosphates shows the potential to modify the male hormone profile, pointing to their endocrine-disrupting potential (Aguilar-Garduño et al., 2013).

Table 2-19: Physico-chemical properties of organophosphorus compounds. W_s: water solubility (if not otherwise indicated at 25 °C); MW = molecular weight [g/mol]; HLC = Henry's Law Constant [atm-m^3/mole]; W_s = water solubility [mg/L] at 25 °C if not otherwise indicated; ---, not listed in the literature or databases consulted.

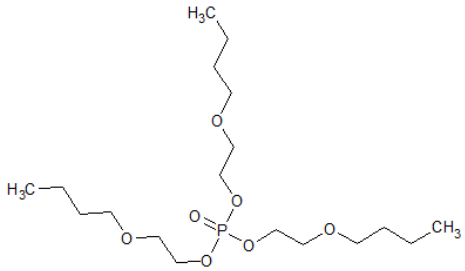
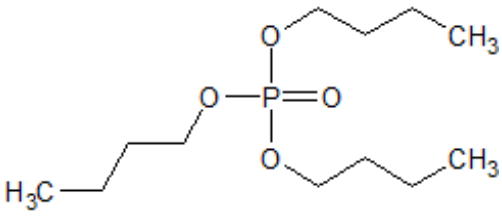
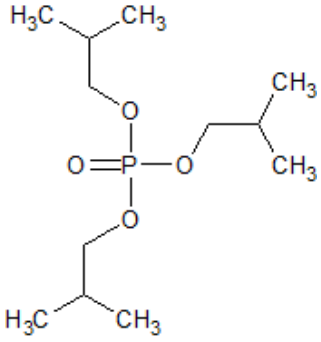
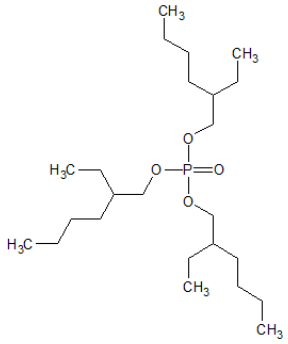
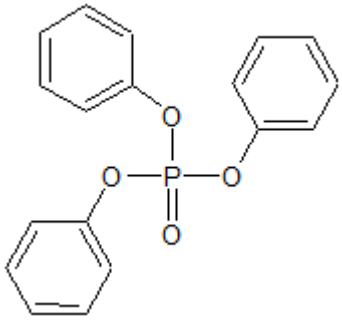
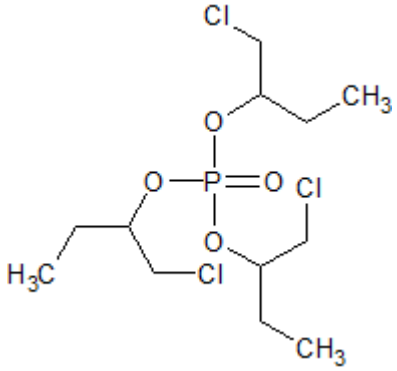
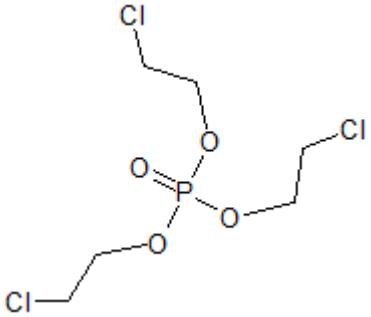
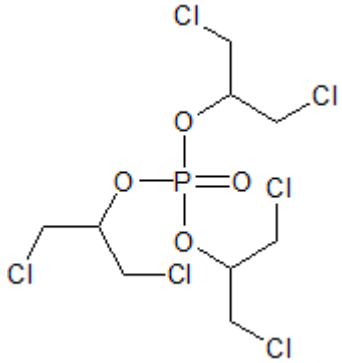
Substance	**tris(2-butoxyethyl) phosphate**	**tributyl phosphate**
Abbreviation	TBEP	TnBP
Chemical structure		
CAS-No.	78-51-3	126-73-8
MW	398.54	266.3141
log K_{OW}	3.75[a]	4[a]; 4.00[b]
pKa	---	---
Ws	1,100[c]	280[a]
HLC	1.20x10^{-11}[a]	1.41x10^{-6}[a]

Table 2-19: continued.

Substance	**tri-iso-butyl phosphate**	**tris(2-ethylhexyl) phosphate**
Abbreviation	TiBP	TEHP
Chemical structure		
CAS-No.	126-71-6	78-42-2
MW	266.3141	434.64
log K_{OW}	3.600[a]	9.490[a]; 4.22[d]
pKa	---	---
Ws	16.2[a]	0.6[c]
HLC	$3.19x10^{-6}$[c]	$7.86x10^{-8}$[a]

Substance	**triphenyl phosphate**	**tris(chloropropyl) phosphate**
Abbreviation	TPP	TCPP
Chemical structure		
CAS-No.	115-86-6	13674-84-5
MW	326.28	327.57
log K_{OW}	4.59[a]; 4.59[b]	2.59[a]
pKa	---	---
Ws	1.9[c]	1,600[e]
HLC	$3.31x10^{-6}$[a]	$5.96x10^{-8}$[a]

Substance	**tris(2-chloroethyl) phosphate**	**tris(1,3-dichloro-2-propyl) phosphate**
Abbreviation	TCEP	TDCPP
Chemical structure		
CAS-No.	115-96-8	13674-87-8
MW	285.49	430.91
log K_{OW}	0.5[b]; 1.44[a]	3.65[a]
pKa	---	---
Ws	7,000[a]; 8,000[e]	7[a]
HLC	$3.29x10^{-6}$[a]	$2.61x10^{-9}$ (25 °C)[a]

[a] NLM, 2005; [b] Sangster, 2012; [c] SRC Research Corporation; [d] WHO, 2000; [e] WHO, 1998a

Table 2-20: Acute toxicity of organophosphorus compounds. BCF: Bioconcentration factor (BFC = concentration on organism/concentration in surrounding environment); a rainbow trout; b zebra fish; c goldfish; d fathead minnow; ---: not described in used the literature.

OP	NOEC [mg/L]	96h LC_{50} [mg/L]	LD_{50} rat (orally) [mg/kg]	BCF	Reference
TBEP	10[a]	24[a]	3,000	---	1
TnBP	---	4.2–12[a]	1,390	11–49[a]	2
TiBP	---	---	---	---	---
TEHP	---	> 100[b]	37,000	250[b]	1
TPP	---	0.36[a]	3,800	324–1,368[a]	3
TCEP	---	90[c]	1,150	---	4
TCPP	9.8[d]	51	1,017	---	4
TDCPP	0.56[a]	1.1	2,380	47–107[a]	4

[a] WHO, 2000; [b] WHO, 1991b; [c] WHO, 1991a; [d] WHO, 1998a.

2.2 Recent and advanced technologies for wastewater treatment

2.2.1 Conventional activated sludge systems (CAS)

These days, wastewater treatment usually consists of a multi-step process (Figure 2-3).

Treatment steps of CAS. During preliminary treatment, wastewater is firstly passed through screens to remove large solids that otherwise could block the downstream mechanics or cause undue mechanical wear (EPA, 2004). Furthermore, grit removal usually precedes primary treatment. Grit consists of sand, gravel, cinder or other solid material with higher specific gravity than organic biodegradable solids (EPA, 2004). In primary (physical) treatment, the wastewater is held in a primary clarifier where larger suspended (organic) material settles at the tank bottom while oily and greasy substances float to the surface (Water UK, 2006). These are removed mechanically and the water passed on to the secondary (biological) treatment where in a controlled, optimised environment (aerated bioreactors) water-borne microorganisms are mixed with the wastewater ("mixed liquor" or "activated sludge") (Radjenović et al., 2008). The main components of the biomass are saphrotrophic bacteria and protozoa. Using the biodegradable material in the water, these microorganisms metabolise dissolved and suspended matter and bind suspended material in the biological flocs they form by assemblage of single cells and micro-colonies (Nicolella et al., 2000a).

This process of aerobic microbial biodegradation, as in every biological wastewater treatment, mirrors the natural process performed by the microbial community in natural water bodies, but in an optimised manner (Radjenović et al., 2008). To keep the growing biomass under control, excess mixed liquor ("secondary sludge") is drawn from the system and treated further by e.g. thickening, dewatering, anaerobic stabilisation, chemical conditioning or thermal reduction, before being disposed of (Radjenović et al., 2008). A portion of the excess sludge ("return activated sludge") is recirculated to the beginning of the secondary treatment to be mixed with fresh wastewater.

In a third stage of treatment (tertiary treatment) the effluent quality is improved further e.g. for discharge into sensitive water bodies or bathing waters (Water UK, 2006). Nowadays the removal of nutrients is widespread. Both households (through urine, faeces, washing agents, etc.) and agriculture (through fertilisers) contribute nutrients to wastewater. In the past, large quantities of nitrogen and phosphate in incompletely treated wastewater discharged into the environment caused excessive eutrophication problems in all receiving aquatic systems (Radjenović et al., 2008). Today, their removal is usually incorporated into the biological treatment in WWTP as a tertiary treatment. Nitrogen is removed by biological nitrification and denitrification. Nitrification is the oxic process of converting ammonia to nitrite (NO_2) and

further nitrate (NO_3). By changing aerobic conditions to anaerobic, NO_3 is then transformed to nitrogen gas (N_2) (denitrification) which is released into the atmosphere and therefore removed from the water (EPA, 2008a). The rate-limiting factor in this process is the slow growth rate of nitrifying bacteria, which demands a certain sludge age, i.e. a long solid retention time (SRT) with the biomass being withdrawn from the system more slowly than the bacterial growth rate (Barnes and Bliss, 1983).

Phosphorous in wastewater is usually found as phosphate. It is usually eliminated either biologically or by chemical precipitation (Radjenović et al., 2008). During enhanced biological phosphorus removal (EBPR) the growth of polyphosphate accumulating organisms (PAOs) is selectively encouraged by alternating between aerobic and anaerobic conditions, which limits the growth of strictly aerobic organisms. The subsequent removal of the phosphate-enriched biomass reduces the phosphorus load of the wastewater. For chemical precipitation, lime or salts of iron (e.g. ferric chloride) or aluminium are used (EPA, 2000; Radjenović et al., 2008).

Before the treated wastewater is released into the environment, it is passed through secondary clarifiers and/or natural ponds (EPA, 2002) to remove remaining bacterial floc (Crown Copyright, 2012). Where especially high effluent quality is demanded, additional 'polishing' processes like UV oxidation or filtration are also included in this treatment step (see following chapters) (Water UK, 2006; Crown Copyright, 2012).

The sludge emerging from the wastewater treatment is dewatered and digested to reduce organic matter and pathogenic microorganisms. Depending on the composition and volume of the sludge, digestion is carried out either aerobically or anaerobically, or a combination of both, with anaerobic treatment being the most common (Radjenović et al., 2008).

LIMITATIONS. A number of drawbacks are connected with CAS. The immense demand for space ("footprint") was identified by Fatone, 2010 as the main factor driving costs in conventional wastewater treatment. Still, there are other limitations to CAS. Firstly, it is restricted with regard to achievable effluent water quality (Radjenović et al., 2008) which can cause problems with demands for higher effluent quality for e.g. indirect potable water reuse. Today, reclaimed water is used in many countries to augment drinking water supplies (Wintgens et al., 2002). The removal efficiency of CAS is entirely based on the separation of treated water and sludge by sedimentation, so that the smallest possible amount of microbacteria is co-discharged into the receiving waters. Poorly settling sludge ("bulking sludge") leads to reduced discharge quality. Furthermore, it can temporarily disable the whole treatment plant. Consequently, good sedimentation behaviour in the sludge is the most important aspect of CAS plant maintenance. This is achieved by operating the CAS in a way that favours the formation of well settling flocs, which is in turn directly connected to the characteristics of the microbial flocs. Since CAS are

operated at low SRTs (usually in the range of several days), substantial microbial communities have to be not only floc-forming but fast-growing, too. In many cases specialised microorganisms are needed for the biodegradation of special compounds like some "emerging compounds". Since many of them are slow-growers, they cannot develop at low SRTs and the removal of certain trace pollutants is subsequently unfeasible in CAS. However, even without the concern of emerging compounds, the production of large volumes of sludge during CAS is one of the main drawbacks of this technique. With rising regimentations of the agricultural use of excess sludge, the handling, treatment and deposition of the material is becoming more and more expensive, reaching up to 60% of the total costs of wastewater treatment (Spellman, 1996; Radjenović et al., 2008). It follows, that to run efficiently, CAS requires constant attention and close monitoring of many parameters (Radjenović et al., 2008) with the multitude of single processes included making it a delicate system, prone to instability.

Since necessary upgrades are often made to CAS treatment plants for the sake of higher effluent quality, conventional wastewater treatment is now becoming more expensive (Radjenović et al., 2008).

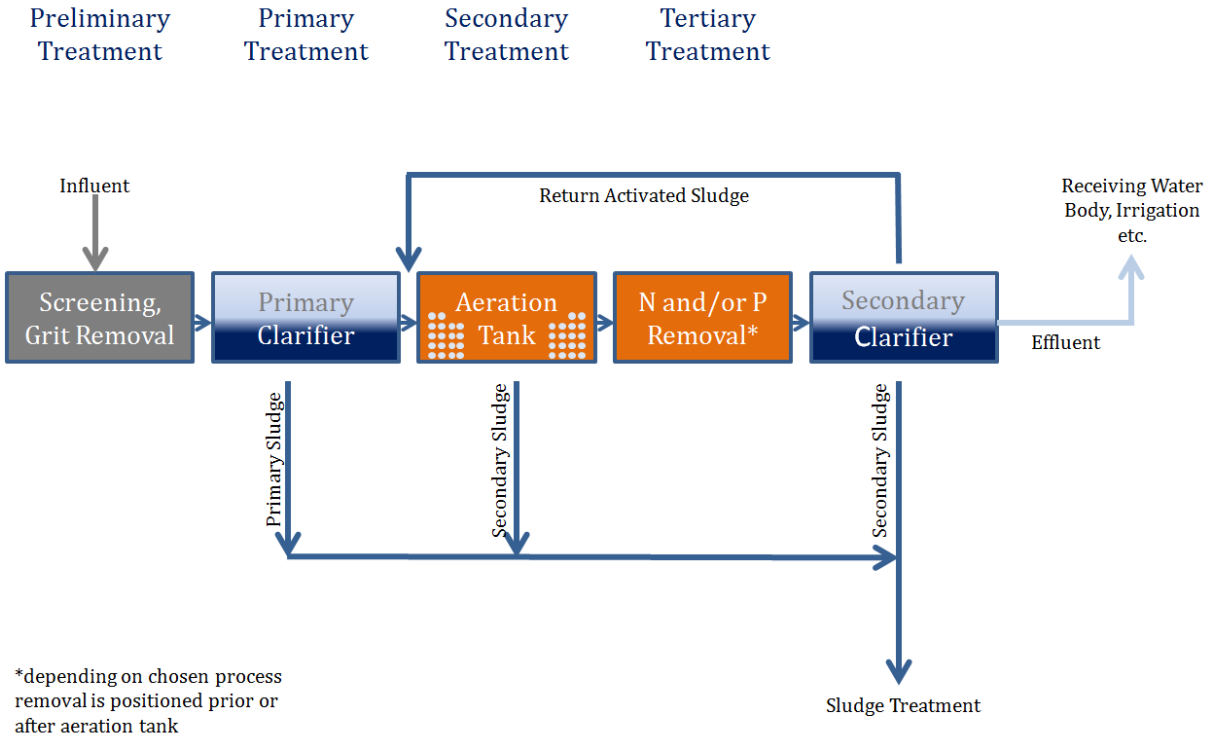


Figure 2-3: Generalised diagram of conventional activated sludge treatment (CAS). For more details refer to text.

2.2.2 BIOFILM REACTORS (BFR)

In biofilm reactors (BFRs), the microorganisms involved in the biological water treatment are immobilised on the surface of an inert support material, which could be a fixed structure such as a trickling filter or freely movable, submerged particles that function as carriers (Nicolella et al., 2000a).

INTRODUCTION TO BIOFILMS. By immobilizing the microorganisms, a disconnection between HRT and SRT is achieved since the separation of water and activated sludge no longer depends on the sludge's settlement properties. Additionally, high biomass concentrations are easily maintained (van Loosdrecht and Heijnen, 1993). A drastic increase of biofilm surface area is achieved by growing biofilms on small particles, and at the same time microorganisms are much less prone to being washed out of the system (Nicolella et al., 2000b). For these reasons, a considerably higher sludge age is achievable, which ultimately allows for a different composition of the microbial community, with e.g. slow growing microorganisms that are disadvantaged in suspended-growth systems of activated sludge treatment (Hall, 1987; van Loosdrecht and Heijnen, 1993; Nicolella et al., 2000b). Fully developed biofilms (see below) often maintain a complex biocenosis, where aerobic microorganisms inhabit the biofilm layers close to the biofilm-water interface, while anaerobic organisms colonise the oxygen-limited and oxygen–free regions further away from the interface. Additionally, slow growers are generally overgrown by fast growers and thereby protected from mechanical influences (Nicolella et al., 2000a).

DEFINITION OF BIOFILMS. While biofilms had already been detected in the 17th century (CBE, 2013a), the term "biofilm" was only introduced in the 20th century. For decades the term was characterised differently by different authors and in different contexts and a final definition is still elusive (Karatan and Watnick, 2009). Nowadays, biofilms are generally described as complex, coherent structures of cells and cellular products (extracellular polymeric substances, EPS) (Characklis and Marshall, 1990; Nicolella et al., 2000a) which appear as large, dense granules or grow on solid surfaces, either static (static biofilms) or suspended carrier material (particle-supported biofilms) (van Loosdrecht and Heijnen, 1993; Nicolella et al., 2000b). More specifically, biofilms are defined as surface-associated multicellular communities (Branda et al., 2005) or as thin coatings consisting of living material (Karatan and Watnick, 2009). Pointing out the complexity of the biofilm micro ecosystem, Watnick depicts the biofilm as a "city of microbes" (Watnick and Kolter, 2000).

COMPOSITION OF BIOFILM MATRICES. Biofilms can be built up by just a single bacterial species, but naturally occurring biofilms generally consist of an enormous mixture of different species of

bacteria, fungi, algae, yeasts, protozoa and other microorganisms (CBE, 2013a). Furthermore, biofilms incorporate inorganic particulate material like debris and the products of corrosion (CBE, 2013a). Sutherland (Sutherland, 2001) described the composition of biofilm matrices as shown in Table 2-21, revealing that biofilms are highly hydrated structures with the actual microbial cells only accounting for at most 15% of the matrix. Even with the exact composition depending greatly both on the microorganism community building the biofilm and on the environmental conditions, overall water is the main compound. It is bound within the microbial cells and is also present extracellularly, thus enabling diffusion in the biofilm (Sutherland, 2001).

EPS. The backbone of the gels which surround the cells in the biofilm is extracellular polymeric substances (EPS). These are biopolymers, namely exopolysaccharides, of microbial origin that are produced by archaeal, bacterial and eukaryotic microbes (Flemming et al., 2007). Further components of the biofilm matrix are cell materials (proteins, polysaccharides, DNA and RNA, peptidoglycan, lipids, phospholipids etc.), absorbed nutrients (e.g. ions like Ca^{2+}), membrane vesicles, metabolites, cell lysis products and particles of surrounding material (Sutherland, 2001; Karatan and Watnick, 2009).

The EPS matrix can be depicted as a "house of biofilm cells" which defines the architecture and structure of the biofilm in terms of mechanical stability, porosity, density, water content, charge, hydrophobicity and sorption properties, and ultimately characterises the immediate conditions for the microorganisms in the biofilm (Flemming et al., 2007). Thus, by nature, biofilms are extremely heterogeneous micro ecosystems, displaying chemical heterogeneity with gradients of substrate, nutrients and gases and different ecological microniches (see below) (Nadell et al., 2009).

The functional roles of EPS are numerous. Regarding biofilm-environment interactions, the biofilm matrix enables the sequestering of particulate and dissolved substances from the bulk liquid and thus provides nutrients for the biofilm community. Additionally, EPS ease the adherence of new microorganisms (Apilánez et al., 1998).

The EPS structure of multilayered biofilms is sponge-like (Karatan and Watnick, 2009) with interstitial gas-filled voids and channels between discrete aggregates of microbial cells. They enable liquid flow, molecular and possibly even cell transport deep into and through the biofilm matrix by convective mass transport (Beer et al., 1996; Sutherland, 2001; Karatan and Watnick, 2009). In the overall biofilm system, transport of substrates involves convection from the bulk liquid to the close vicinity of the cell clusters, diffusion through the mass transfer boundary layer and further diffusion from cell to cell. Since diffusion processes are generally slower than convective transport, cell-to-cell-diffusion is the energy limiting factor of biofilm growth (Beer et al., 1996; Nicolella et al., 2000b). Channels and voids in the matrix provide pathways for

convective transport in the biofilm areas at depths that would not be reached by diffusion in a channel-less biofilm (Stoodley et al., 1994). Furthermore, the biofilm provides a viscous or gelatinous shield against physical forces, it enables microorganisms to stay located instead of being washed out of a favourable micro environment, it influences predator-prey-interactions and it protects microorganisms against the effect of antimicrobials such as antibiotics in the surrounding liquid (Characklis, 1973; Watnick and Kolter, 2000; Stewart and Costerton, 2001; Sutherland, 2001; Flemming et al., 2007). Studies have shown that microbial biofilms can be up to 1,000 times more resilient to antibiotics than planktonic (free-floating) bacteria (CBE, 2013a). By keeping cells fixed in close proximity to each other, and by providing a transport medium, the biofilm matrix represents a unique ecological niche with all forms of inter- and intra-species competition, cooperation and coexistence (Sutherland, 2001; Nadell et al., 2009). It enables cell-cell communication by accumulation of signal molecules to coordinate gene expression within the biofilm (quorum sensing or "signalling") (Sauer et al., 2002; Karatan and Watnick, 2009; Kievit, 2009; Nadell et al., 2009; CBE, 2013c) as well as 'warfare' against competing neighbourhoods, for example by releasing bacteriocins, microcins or antibiotics (Sutherland, 2001).

BIOFILM DEVELOPMENT. Biofilms develop naturally on almost every surface which comes into lasting contact with an aqueous liquid, whether fresh or salty (Apilánez et al., 1998). The prerequisite initial step to biofilm development is the sorption of organic compounds on the submersed support surface (Figure 2-4). This surface is usually charged by adsorption of ions or by the ionisation of surface groups (Annachhatre and Bhamidimarri, 1992). The charged surface attracts oppositely charged ions from the feed liquid which are at the same time hindered by the thermal motion of the counter ions. Due to counterbalancing, this ultimately leads to the development of a diffuse electric double layer in close proximity to the charged surface where ions are accumulated in higher concentrations than in the surrounding liquid. By the subsequent sorption of dissolved organic material (glycoproteins, proteins etc.) the liquid-surface interface thus becomes a favourable nutrient-rich environment for microorganisms (Characklis, 1973; Annachhatre and Bhamidimarri, 1992).

Subsequently, planktonic bacteria attach to the surface in first reversible (transient attachment), then irreversible manner (permanent attachment) and change to a genetically diverse sessile phenotype (Karatan and Watnick, 2009; CBE, 2013a), as shown in Figure 2-4. The production of EPS enables a permanent hold on the surface and keeps developing microcolonies in close proximity. After initial adherence, the biofilm grows through cellular reproduction, production of EPS and adherence of new microbes.

Table 2-21: Composition of typical biofilm matrices (Sutherland, 2001)

Component	% of matrix
Water	Up to 97%
Microbial cells	2–15% (many species)
Polysaccharides (homo- and heteropolysaccharides)	1–2% (neutral and polyanionic)
Proteins (extracellular and resulting from lysis)	< 1–2% (many, including enzymes)
DNA and RNA	< 1–2% (from lysed cells)
Ions	? (bound and free)

Due to the consumption of substrates, nutrients and oxygen or electron donors by the microorganisms during biofilm growth, concentration gradients in the biofilm develop which cause these chemicals to diffuse into the biofilm, while metabolism by-products diffuse out of the biofilm (Annachhatre and Bhamidimarri, 1992). A typical example of chemical and ultimately physiological heterogeneity in biofilms is the depletion of oxygen with increasing distance from the biofilm-liquid-interface (Nadell et al., 2009), allowing microbes in the outer zone to use aerobic metabolism while anaerobic microorganisms occupy the oxygen-depleted zones (Xu et al., 1998). The spatial heterogeneities result in a stratification of the biofilm (
Figure 2-5) due to the development of microniches along gradients of nutrients, substrate or waste products (Nadell et al., 2009). These waste products can lead to a toxication of biofilm zones or they can be used by other types of microbes.
During the growth phase of the biofilm, total coverage of the available surface occurs (Apilánez et al., 1998).

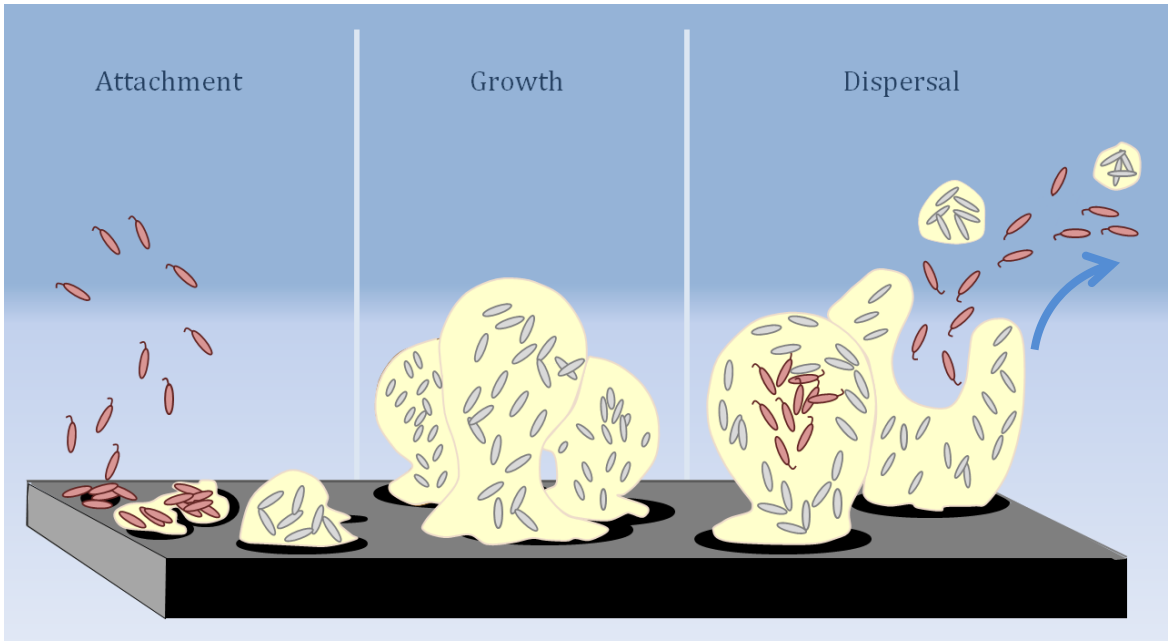


Figure 2-4: Schematic of the biofilm life-cycle (CBE, 2013a)

MORPHOLOGY AND TOPOLOGY. The morphology and surface topology of the biofilm is, in addition to the influence of shear forces, strongly determined by the nature of the feed liquid. It is suggested as a general rule that in nutrient-limited environments, biofilms are dense, sometimes even only of mono-layered structure, and feature a smooth surface, while in nutrient-rich surroundings, biofilms grow as thick multi-layers (Characklis, 1973; Nadell et al., 2009). However, studies have revealed that different microorganisms react in different ways to nutrient changes. While for some microbes an acceleration of biofilm growth was observed when nutrient supply increased, for other species growth is boosted by nutrient deprivation (Karatan and Watnick, 2009). The limitation of available nutrients is also discussed as a possible reason for the development of voids and channels in biofilms when only a thin layer of active cells on top of the biofilm is provided with nutrients like oxygen, which is rapidly depleted in the depth of the EPS matrix (Figure 2-6). Under these conditions, irregularities in the surface of the biofilm might become greatly amplified, with cells growing well at the top of irregularities due to sufficient access to nutrients from the bulk liquid, and cells growing poorly in the irregulation's troughs, resulting in fast-growing towers and growth-reduced bay-like areas which develop into channels when overgrown (Nadell et al., 2009). In contrast to the theory that cells on top of the towers grow faster because of better access to nutrients, some authors discuss surface depressions as spots of highly turbulent liquid flow that, depending on the flow rate, can be better provided with nutrients than smooth zones experiencing laminar flow (Beer et al., 1996).

BIOFILM SLOUGHING. The detachment of cells from the biofilm ("biofilm sloughing") is a natural phenomenon occurring either by the dispersal of single cells of planktonic, motile phenotype (so-called "swarmers") or by the detachment of whole clumps of biofilm (Annachhatre and Bhamidimarri, 1992; Sauer et al., 2002; CBE, 2013b). Studies imply that the release from the biofilm is not only a result of a weakening biofilm matrix, either due to cell lysis under unfavourable environmental conditions or due to pure force of flow shear, but it can be actively driven by the microbes through quorum-sensing induced enzymatic breakdown of the surrounding biofilm structure (Annachhatre and Bhamidimarri, 1992; Watnick and Kolter, 2000; Karatan and Watnick, 2009; Nadell et al., 2009). Thus, microorganisms are provided with an exit strategy from surface association (Watnick and Kolter, 2000; Karatan and Watnick, 2009) and subsequently can propagate to new areas downstream of the original community (CBE, 2013b). Depending on the environmental conditions, mature biofilms can reach a steady state where biofilm growth is balanced by detachment (Sauer et al., 2002).

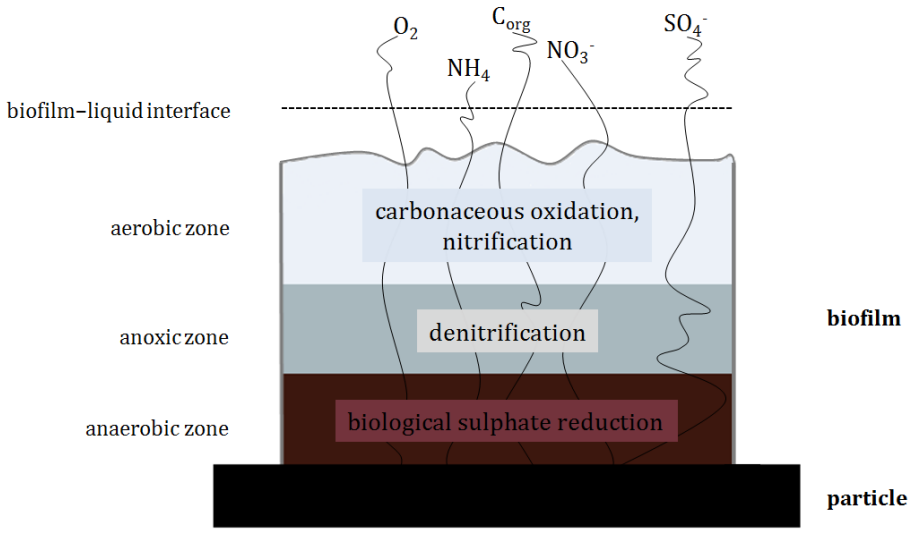


Figure 2-5: Stratification of biofilm (schematic diagram, Characklis and Marshall, 1990; Anton et al., 2002; Nadell et al., 2009)

STATE OF RESEARCH. To date, biofilms are far from being fully understood and their multiple roles have only partly been discovered. Even the function and precise construction principles of EPS are not fully comprehended (Flemming et al., 2007), let alone the extremely complex ecosystems that biofilms represent. The intensive research that has been carried out in recent years and is still underway constantly reveals more details, and new findings are frequently reported (CBE, 2013a).

BIOFILMS IN WASTEWATER TREATMENT. Biofilms are used in various applications for wastewater treatment (Figure 2-7). The biofilm is either fixed on a large, static support structure (fixed bed reactors or rotating biological contactors (RBC), also named rotating biological reactors) or it is attached to suspended particles (carriers). Typical carriers reported in literature are sand, activated carbon, pulverised rock, glass beads, diatomite earth material as well as plastic and ceramic materials (Koch et al., 1991; Apilánez et al., 1998; Garcia-Calderon et al., 1998; Welander et al., 1998; Nicolella et al., 2000a; Zhou et al., 2006). A significantly higher biofilm surface is achievable by attachment to small particles than with fixed-bed applications (Nicolella et al., 2000b). Carriers made of different material differ regarding their physical and chemical properties, which results in different start-up times and, according to some studies, even variations in the microbial community that will be developed (Apilánez et al., 1998; Anton et al., 2002). Several studies stated that porous carriers like foamed polyurethane or polystyrene are preferable since they provide a greater (inner) surface than mineral materials, and biofilms developing in these carriers are protected from shear stress (Loukidou and Zouboulis, 2001; Anton et al., 2002). On the other hand, shear forces are specifically used for the control of biofilm

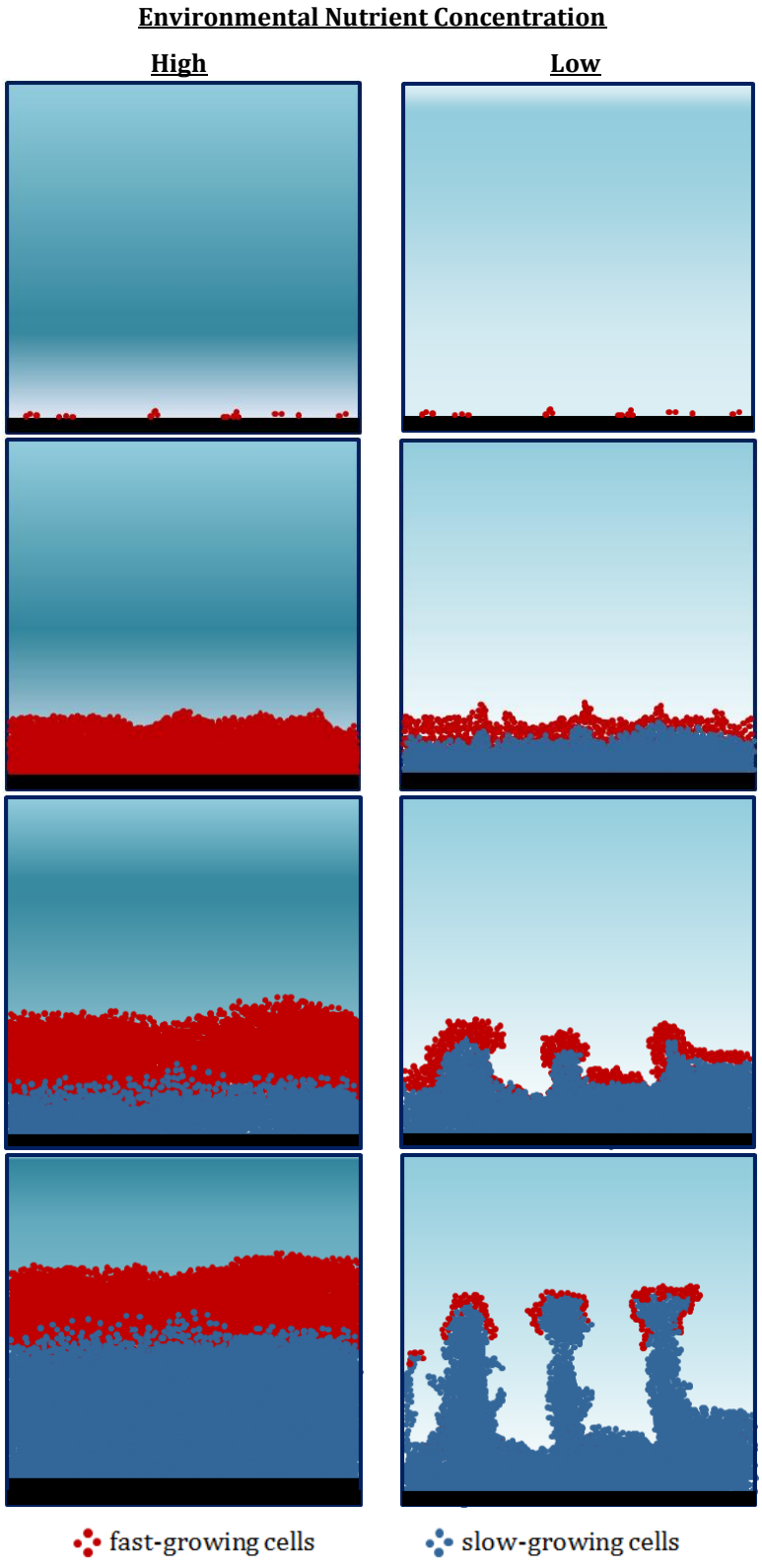


Figure 2-6: Influence of nutrient availability on the biofilm topology. In nutrient-rich environments (left column), smooth biofilms grow due to a high diffusion rate reaching deep into the biofilm, allowing for a thick layer of actively growing cells, while in nutrient-poor environments (right column) only a thin layer of cells is growing fast. Irregularities in the biofilm surface lead to the development of towers with actively growing cells on top and growth-inhabited cells in the troughs, resulting in a rough biofilm surface and channels (Computational simulations; nutrient: oxygen; Nadell et al., 2009).

thickness on the carriers. In wastewater treatment, thin, smooth biofilms are preferable to ensure high mass transfer rates into the biofilm. In addition, thick biofilms are prone to being randomly detached, resulting in a patchy, irregular and less effective biofilm and high biomass loads in the effluent (Nicolella et al., 2000a). Thick biofilm layers change the hydrodynamic properties of the carriers, seriously decreasing their particle density. This results in carriers being swept out of the system, leading to the need for an additional separation step (Nicolella et al., 2000a).

The application of fixed or particle-supported BFRs as the reactor of choice depends very much on the substrate load of the feed water and the flow rate (Figure 2-8).

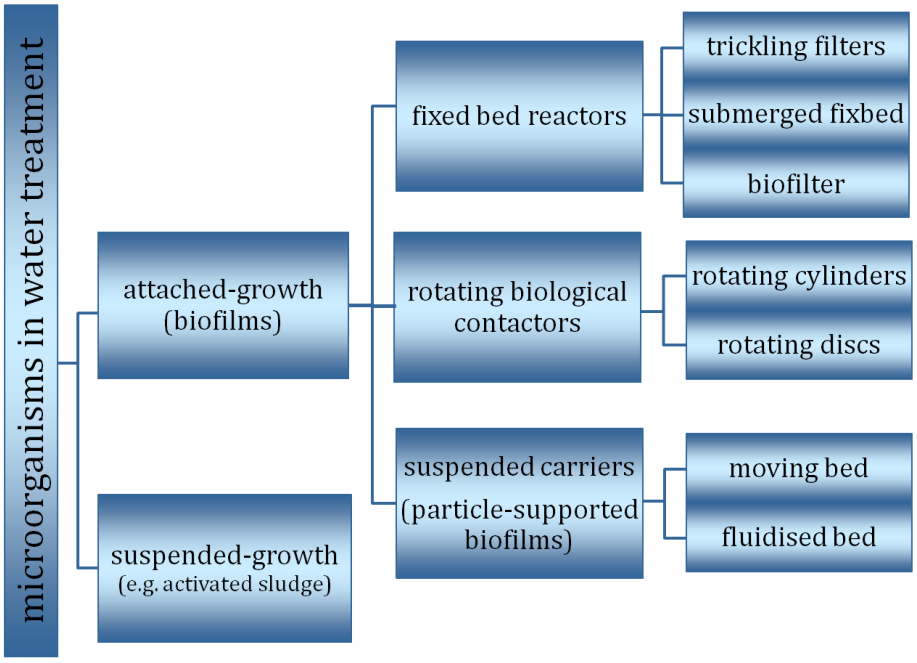


Figure 2-7: Biofilm principles and techniques for water treatment (Nicolella et al., 2000a; ATV-DVK-Arbeitsgruppe IG-5.6)

PARTICLE-BASED BFRS. As already mentioned, particle-based BFRs offer numerous advantages when compared to suspended-growth systems (Table 2-22). Thus, the settling velocity and the reactor concentration of particle-based biofilm reactors are described as being ten times higher. With greater biofilm surface area, higher biomass concentration and subsequently a higher mass transfer area, particle-supported BFRs provide higher conversion capacities. High SRTs lead to less excess sludge. Furthermore, biofilm reactors have a considerably smaller spatial footprint. Drawbacks include long start-up phases, the previously described difficulty of controlling the biofilm thickness and the overgrowth of carriers, as well as costs for liquid distribution in

fluidised bed reactors, since mechanical stirring is not feasible due to the possibility of damaging carriers and biofilm (see Table 2-22, Nicolella et al., 2000a).

FIXED-BED BFRS. Biofilms have been used in fixed-bed applications for wastewater treatment for more than a century (Tchobanoglous and Burton, 1991; Mathure and Patwardhan, 2005). In contrast, particle-based biofilm reactors did not come into use until the 1970s (Nicolella et al., 2000a). In 1976, a Biofilm Fluidized Bed (BFB) configuration was patented in the USA (Jeris et al., 1976). At about the same time, the first commercial-scale application of the BFB process was installed in the form of a denitrification system for municipal wastewater, quickly followed by industrial applications as well, where it was soon applied to different treatment processes like nitrification, denitrification, anaerobic treatment and carbonaceous oxidation (Nicolella et al., 2000a). In BFB reactors, the particles are kept fluidised by the up-flowing influent. Later developments of the same basic principle (Figure 2-9) are the Expanded Granular Sludge Blanket (EGSB) and the Biofilm Upflow Sludge Blanket (USB). In contrast, the more recently developed Biofilm Airlift Suspension (BAS) and Internal Circulation (IC) reactors use gas for mixing particles with liquid. In the case of BAS reactors, air is pumped into the system, while IC reactors use the gas that is produced in the system itself (Nicolella et al., 2000a).

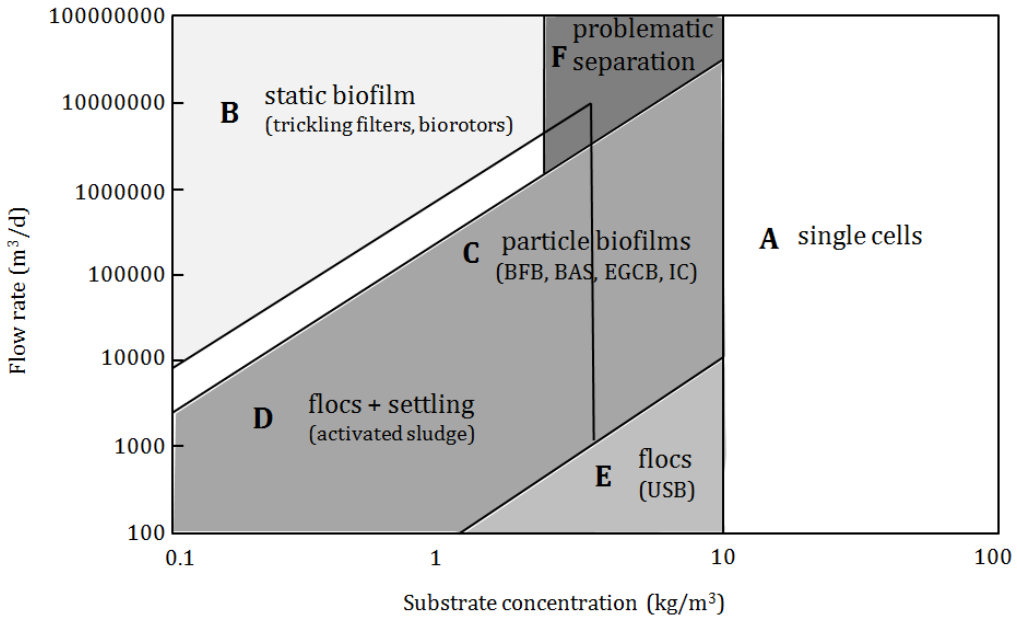


Figure 2-8: Diagram of concentration-flow rate phases for the application of floc and biofilm reactors. A: microbes grow in suspension due to long retention times; B: high flow rates only allow the retention of static biofilms unless the reactor is of an extremely flat and extended shape; C: conditions are suitable for the application of particle-supported biofilm reactors; D: conditions are suitable for the application of microbial flocs as long as liquid-solid separation and biomass recycling are used (e.g. activated sludge treatment); Note that regions C and D are partly overlapping. E: under conditions of high load and low flow, upflow sludge blanket reactors can be utilised (Nicolella et al., 2000a).

Table 2-22: Characteristics of particle-supported biofilm reactors (adapted from Nicolella et al., 2000a)

Advantages	**Disadvantages**
High terminal settling velocity of solids (50 m h^{-1}; for flocculated sludge: 5 m h^{-1}), leading to possible elimination of external clarification/separation stages	Biofilm formation on carriers poses problems leading to long start-up times
High reactor concentration (30 kg m^{-3}; for flocculated sludge systems: 3 kg m^{-3})	Control of biofilm thickness is difficult (depending on reactor type)
High biofilm surface area (3000 m^2 m^{-3}; in trickling filters: 300 m^2 m^{-3})	Overgrowth of biofilms can lead to elutriation of particles
High biomass concentration and mass transfer area result in high conversion capacities (for oxygen, 20 kg m^{-3} day^{-1}; in activated sludge and trickling filter processes: 3 kg m^{-3} day^{-1})	Liquid distributors for fluidised systems are costly for large-scale reactors and pose problems with respect to clogging and uniform fluidisation
Compact reactor with small area requirements High biomass age (several weeks); and minimisation of excess sludge production	

COMMERCIAL IMPLEMENTATIONS AND THE STATE OF AFFAIRS OF BFRS. Over the last two decades, biofilm reactors have been put to use for aerobic and anaerobic water treatment, nitrification and denitrification or a combination of both, as well as for more specialised applications like the removal of persistent compounds such as dichloromethane, chlorophenols, phenols and naphthalensulfonic acid. Commercial full-scale systems, treating both industrial and municipal wastewater, have been developed and installed in, e.g., the USA, France and the Netherlands since the middle of the 1990s (Nicolella et al., 2000a).

To summarise, it can be said that biofilm technology is nowadays regarded as a fully developed wastewater treatment technology with the majority of parameters (biofilm formation, mass transfer, hydrodynamics etc.) well investigated and understood (Nicolella et al., 2000b).

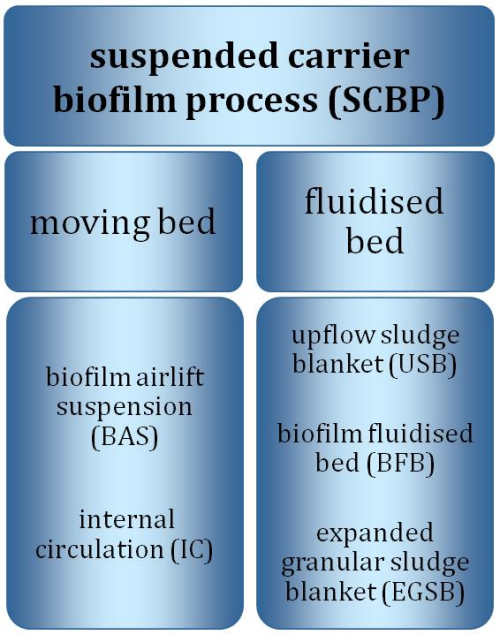


Figure 2-9: Main types of biofilm reactors operating with suspended carriers (Welander et al., 1998; Nicolella et al., 2000b)

2.2.3 NANOFILTRATION (NF)

Membrane filtration processes have become increasingly important in water and wastewater technology over the last decades (Radjenović et al., 2008). Their first introduction in water treatment processes was for treating secondary or tertiary effluent as a polishing step to achieve higher quality in excess waters (Wintgens et al., 2005).

MEMBRANE SEPARATION. Membranes, in the simplest terms, are two-dimensional materials which separate the constituents of fluids according to their relative size or their electrical charge in a purely physical process, leaving all compounds chemically unchanged (Semião and Schäfer, 2010). During membrane separation, feed water passes over the membrane surface dividing it into two very different streams by solid-liquid separation based on semipermeability. The rejected portion is called concentrate or retentate, the cleared water is named permeate (Kunst and Košutić, 2008).

MATERIALS AND PROPERTIES. In general, membranes are made of plastic or ceramic materials, while metallic membranes are rare. The most common materials are celluloses, polyamides, polysulphone, charged polysulphone and other polymeric materials (e.g. polyacrylonitrile (PAN), polyvinylidene difluoride (PVDF), polyethylsulphone (PES), polyethylene (PE) or polypropylene (PP) (Radjenović et al., 2008). While all of these materials possess a favourable chemical and physical resistance (Radjenović et al., 2008), they have the drawback of being hydrophobic, which is known to promote membrane fouling (Choi et al., 2002). Therefore, commercially available membrane surfaces are modified to reach a more hydrophilic finish (Radjenović et al., 2008). Besides the method of fabrication, this finish is the distinguishing element between different membranes (Radjenović et al., 2008).

Sophisticated separation membranes have an asymmetric structure, being anisotropic in vertical cross section, with a thin dense "skin" (selective layer) as the surface on top of a supporting, much thicker, porous layer (Kunst and Košutić, 2008).

Filtration membranes are based on either planar or cylindrical geometry. Three different configurations are commonly used: hollow fibre (HF), spiral-wound and flat sheet (FS); to a lesser extent, tubular membranes and pleated filter cartridges are employed (Radjenović et al., 2008). FS are mostly used as "plate-and-frame" configurations in modular forms with a number of FS mounted to a supporting construction (Radjenović et al., 2008). Equally, hollow fibre and spiral-wound fibres are usually connected by manifolds and bundled into units for easy maintenance and changing (EPA, 2008b).

MEMBRANE TYPES. The most common pressure-driven membrane separation processes are microfiltration (MF, separation 100-1,000 nm), ultrafiltration (UF, separation 5-100 nm), nanofiltration (NF, 1-5 nm; Radjenović et al., 2008), also given with 0.5-2 nm (Kunst and Košutić, 2008) and reverse osmosis (RO, 0.1 – 1 nm; Radjenović et al., 2008), also given with 0.2-1 nm (Kunst and Košutić, 2008),). All of these produce permeate and concentrate (retentate). Since NF and RO share similar pore size ranges, their rejection properties mostly differ in relation to ions. NF membranes typically reject di- and multivalent ions almost completely, whilst the rejection of univalent ions is less than 70%. The retention by RO is less variant and mostly driven by the size of the hydrated ion. Since in the case of organic molecules the rejection behaviour of NF and RO is very similar, they are often discussed together as NF/RO membranes (Kunst and Košutić, 2008).

RETENTION MECHANISMS. Several basic retention mechanisms determine the retention of organic solutes by filtration membranes. The most important ones identified are: size exclusion, charge repulsion and physico-chemical interactions such as sorption on the membrane surface (Kunst and Košutić, 2008; Radjenović et al., 2008; Wu et al., 2010).

SIZE EXCLUSION. Size exclusion, defined as the retention of solutes by sieving through steric hindrance, is the essential mechanism in the retention of organic solutes by NF/RO membranes (Kunst and Košutić, 2008). To determine the size of molecules, a number of parameters are used (molecular weight, molecular diameter, effective molecular size taking into account the molecular shape etc.) and while it was proved to not be completely accurate and predictive regarding the retention performance (Kunst and Košutić, 2008), the most common parameter used to describe the retention behaviour based on size exclusion in commercially available membranes is molecular weight cut-off (MWCO), the manufacturer's rating of the membrane's ability to reject an uncharged molecule based on its weight. Membrane pore size is another helpful parameter to estimate retention behaviour based on size exclusion, see e.g. Kimura et al., 2004 who found a better correlation of the rejection of antibiotics with pore size than with the MWCO.

CHARGE REPULSION. Charge repulsion (or charge exclusion) is based on the electric charge of the filtration membrane created by the manufacturer by integrating sulfonic, carboxylic or ammonium groups into its surface. Depending on pH, the groups dissociate resulting in a charged membrane. Typically, commercial membranes are negatively charged under neutral conditions (Kunst and Košutić, 2008; Semião and Schäfer, 2010). This results in the retention of

charged molecules at the membrane, an effect which is especially important for molecules smaller than the membrane pore size (Semião and Schäfer, 2010).

PHYSICOCHEMICAL INTERACTIONS. Physicochemical interactions between membrane material, solutes and water molecules, namely solute sorption on and in the membrane, are of importance for many substances. These mechanisms are mainly based on hydrophobic nonspecific interactions or on hydrogen bonding between solute and membrane, on the solute molecular polarity described by its dipole moment and on the equilibrium of ionised and non-ionised species of a solute in the water, expressed by the solute dissociation constant value, pK_a (Kunst and Košutić, 2008).

STATE OF RESEARCH. It is acknowledged that all mechanisms mentioned above, as well as a large number of additional parameters, are involved and interact in membrane separation. Subsequently, Semião and Schäfer, 2010 stated that the prediction of retention of a certain compound is difficult and more investigations of the still not fully understood mechanisms are required. Among other topics, the rejection of xenobiotic micropollutants by membranes is of special interest and has recently been comprehensively studied. Bellona et al., 2004 developed a rejection diagram for micropollutants during membrane treatment (Figure 2-10).

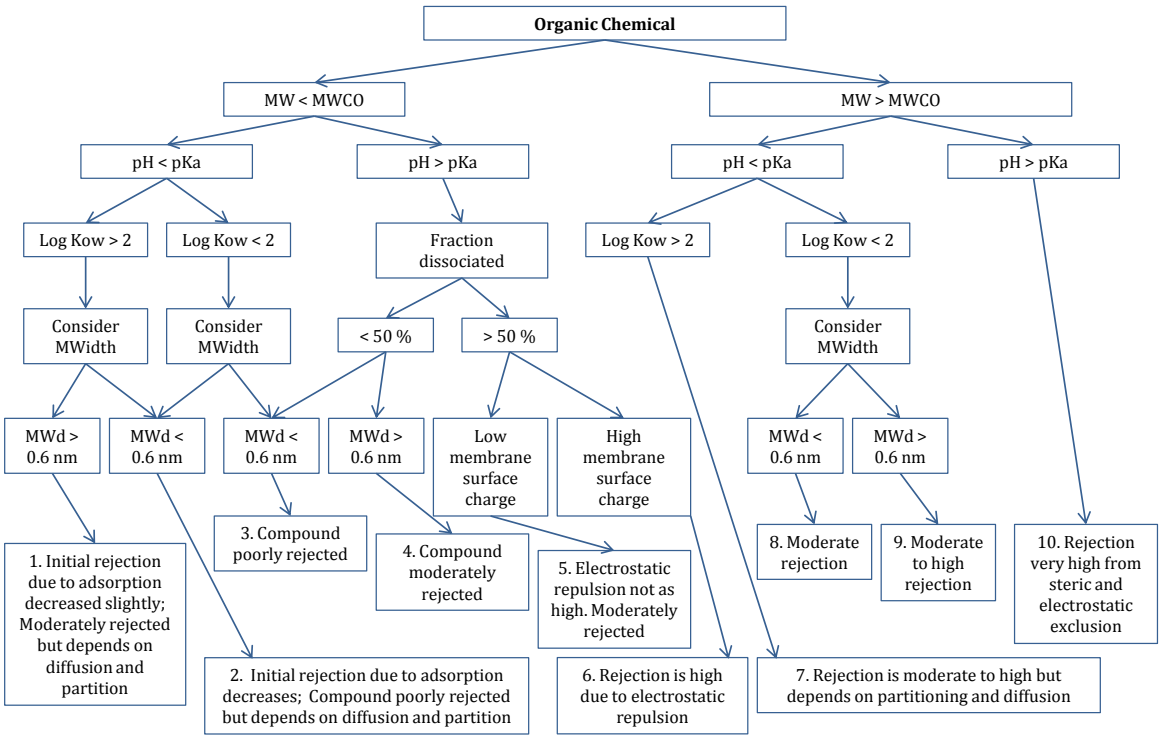


Figure 2-10: Rejection of organic micropollutants by membrane treatment (Bellona et al., 2004).

NF/RO FILTRATION. Mulder, 1996 defines NF/RO membranes as being intermediate between porous and nonporous type barriers. Depending on the description of NF as either homogeneous or porous, the theoretical description and modelling of the performance of membranes was carried out by various researchers with diverse approaches, e.g. solution-diffusion models, surface force–pore flow models (Smith et al., 1969; Chang and Kim, 2005) and hydrodynamic approaches (Cho and Fane, 2002). For the transport of charged particles through charged membranes (especially narrow pore NF), space-charge models (e.g. Wang et al., 1995; Bowen et al., 1997; Combe et al., 1997; Wang et al., 1997) have been applied, while van der Bruggen and Vandecasteele, 2002 promoted a porous membrane model to describe the passage of uncharged organics through NF membranes. Bowen and Welfoot, 2002 developed a two-parameter model based on pore radius and membrane charge for electrolyte rejection. Still, in order to develop models which provide a successful prediction of NF/RO membrane filtration behaviour, further research is necessary to gain a comprehensive understanding of the factors driving the mass transfer of solutes and regulating the retention mechanisms of said solutes (Bellona et al., 2004; Kunst and Košutić, 2008).

MEMBRANE PROCESSES. Nonetheless, some basic membrane processes can be described by the following governing equations (Radjenović et al., 2008):

The **mass balance** of solutes is generally given as:

$$Q_f c_f = Q_p c_p + Q_c c_c$$

with Q_f being the flow rate of the feed, c_f being the solute concentration in the feed, Q_p the flow rate of the permeate, c_p the solute concentration in the permeate, Q_c the flow rate of the concentrate and c_c the solute concentration in the concentrate.

The **membrane rejection of solutes** (R) is calculated as:

$$R = \frac{Cf - Cp}{Cf}$$

with c_f representing the concentration of solute in the feed and c_p the concentration in the permeate, respectively.

The **yield** (Y, also called recovery or water recovery) is the fraction of feed flow converted to permeate:

$$Y = \frac{Qp}{Qf}$$

with Qp representing the permeate flow and Qf the feed flow.

The permeate flux (J) is determined as the volume of water passing through a unit area of the membrane per unit of time, commonly given in [Lm^{-2} day^{-1}]. J is often normalised to a standard temperature. The driving force of J is the **transmembrane pressure** (TMP or ΔP). The **membrane permeability** (K) as a description of the membrane performance is calculated as the permeate flux per unit of TMP and mostly given in [Lm^{-2} h^{-1} bar^{-1}].

The limiting factor of membrane use is so-called **membrane fouling** which results in the increase of TMP or the decrease of permeate flux through the membrane. Though a lot of research was aimed at this multi-factorial phenomenon, it is only partly understood at this time (Radjenović et al., 2008) and its complexity presents a challenge to mathematical descriptions and models (Kunst and Košutić, 2008).

Among the main causes for membrane fouling are: a) the sorption of macromolecular/colloidal matter, b) the growth of microorganisms ("biofilms") on the membrane surface, c) the precipitation on the membrane. All these factors become progressively more severe with membrane aging.

Fouling is usually described by the **resistance** (R):

$$R = \frac{\Delta P}{\eta J}$$

with η being the permeate viscosity [kg m^{-1} s^{-2}].

This **total filtration resistance** can be divided into three parts (Chang and Lee, 1998; Chang et al., 1999; Jiang et al., 2003; Chang and Kim, 2005):

$$R = Rm + Rc + Rf$$

with R_m being the membrane resistance, R_c being the cake resistance and R_f being the fouling resistance.

MEMBRANE FOULING. The complex interactions of membrane fouling are far from being fully understood but it is generally agreed that it is caused by suspended particles present in the feed that form a cake layer in the membrane surface (cake layer resistance) as well as by soluble organic and colloid materials (organic fouling and colloidal fouling) that are responsible for e.g. blocking of membrane pores (Radjenović et al., 2008; Simon et al., 2011). Extracellular polymeric substances (EPS), produced by microorganisms, play a vital role in membrane fouling (Chang and Lee, 1998; Cho and Fane, 2002; Rosenberger et al., 2002). Besides being used as protective layers around bacteria cells and in the formation of flocs and microbial aggregates they are also present freely dissolved in the water and can block membranes in the form of a hydrated gel layer (Radjenović et al., 2008). Fouling changes the physico-chemical properties of the membrane surface considerably (e.g. morphology and charge), thus changing the separation mechanisms (size exclusion, electrostatic interactions) significantly. Whilst fouling is generally seen as a drawback reducing the performance of the fouled membrane, several studies showed increased retention of various compounds due to membrane fouling (Kunst and Košutić, 2008; Le-Minh et al., 2010; Semião and Schäfer, 2010). However, since fouling shortens the life-span of the membrane, therefore requiring shorter maintenance cycles and resulting in higher energy costs, it is undesirable in membrane usage.

FOULING CONTROL AND MEMBRANE CLEANING. The fouling rate increases with the flux. To control and minimize fouling, the operational flux of membrane systems is normally kept below the critical flux which is determined as the highest flux under which a prolonged filtration with constant permeability is possible (Radjenović et al., 2008). Another controlling mechanism is the suppression of concentration polarisation (the tendency of solutes to accumulate within the boundary layer of the membrane surface) by providing a mechanical shear stress over the membrane surface by cross-flow velocity or aeration (Radjenović et al., 2008).

Still, fouling is inevitable even with zero flow (Radjenović et al., 2008). To clean fouled membranes, various forms of cleaning are available. Physical cleaning uses back-flushing or relaxation of the membrane. It only takes minutes, but it does not remove all adsorbed material from the membrane. More effective is chemical membrane cleaning using e.g. sodium hypochlorite, sodium hydroxide or acidic solutions (Kunst and Košutić, 2008; Radjenović et al., 2008). The filtration system down-time required is greater than for physical cleaning. However, neither form of cleaning removes fouling completely, and this ultimately determines the life-span of the membrane.

COMMERCIAL IMPLEMENTATIONS AND THE STATE OF AFFAIRS. Today, membrane filtration is considered to be a fully developed water treatment technology. In recent years, a number of full-size treatment plants have been equipped with membrane filtration technology, e.g. the Méry-sur-Oise water purification plant in Paris, France (potable usage of river water and secondary effluent; maximum daily production: 340,000 m^3; treatment with MF followed by NF) or NEWater facilities in Singapore (water reclamation from secondary effluent of CAS treatment by MF and followed by RO: 75,000 m^3/day) (Semião and Schäfer, 2010).

2.2.4 MEMBRANE BIOREACTORS (MBR)

INTRODUCTION. The idea of membrane bioreactors (MBR) as a combination of the activated sludge process (see 3.2.2) and membrane separation (see 3.2.3) was introduced as long ago as the 1960s and 1970s (Smith et al., 1969; Sammon, 1974). The early use of MBRs was mainly in the treatment of industrial wastewater, to enhance the quality of final effluent water by tertiary treatment with MBR (Radjenović et al., 2008). At the end of the 1980s, MBRs with submerged membranes were developed, where vacuum-driven membranes are directly immersed into the bioreactor (Yamamoto et al., 1989). This led to a significant drop in energy costs and subsequently to a rapid increase in both the overall use of MBRs and their implementation in the treatment of municipal wastewater. Judd and Judd, 2006 described a doubling of the global MBR market between 2000 and 2005 to a value of over $215 million in 2005. With wider use, followed by decreasing production costs (Fatone, 2010) and the availability of different technical variations, MBRs have developed into an alternative secondary treatment technology, mainly applying ultrafiltration or microfiltration (Radjenović et al., 2008).

ADVANTAGES AND DRAWBACKS. In MBRs, the solid-liquid separation of activated sludge and water purely by gravity in the secondary clarifiers employed in CAS treatment is replaced by filtration, thus providing complete sludge retention. Furthermore, depending on the chosen membrane, complete disinfection of the effluent can be achieved (EPA, 2008b).
Since sludge settling characteristics are of less importance, MBR systems can be operated at considerably prolonged SRTs where biomass growth is not restricted to fast-growing and floc-forming microorganisms but where the development of specialised organisms and dispersed bacteria is possible (Radjenović et al., 2009b). Additionally, MBRs can be operated with higher sludge concentrations (typically up to 20 g/L instead of max. 6 g/L in conventional systems) which leads to a considerable reduction in required footprint, reactor volume and production of excess sludge (Cornel and Krause, 2006; Radjenović et al., 2009b). Drawbacks of the technique are higher total life costs, mainly due to high energy costs.

CONFIGURATIONS. MBRs are constructed either in side-stream configuration (Figure 2-11) or with submerged membranes (Figure 2-12). In side-stream the membrane separation is pressure-driven and carried out in an external sludge recirculation loop, primarily by in-to-out flow through tubular membranes (Lesjean and Judd, 2007). Aeration of the bioreactor is carried out by fine bubble aerators. The necessary shear over the membrane to prevent fouling is provided by pumping (Radjenović et al., 2008). With the development of submerged MBRs, side-stream reactors lost importance. Today, they are mainly applied in industrial contexts and for the treatment of landfill leachates (Radjenović et al., 2008).

SUBMERGED MEMBRANES. Submerged MBRs became known in the 1990s (Lesjean and Judd, 2007). Here, the filtration module is immersed directly in the bioreactor. Turbulent cross-flow aeration is supplied both to scour the membrane and to provide oxygen for the biomass (Radjenović et al., 2008). For the filtration process, low negative pressure (out-to-in permeate suction) is applied (Lesjean and Judd, 2007; Kunst and Košutić, 2008; Radjenović et al., 2008).
Without the energy-eating sludge recirculation loop, submerged MBRs are less energy demanding than side-stream MBRs. Additionally, in MBRs with submerged membranes, both permeate flux and trans-membrane pressure (TMP) are considerably lower than in side-stream configuration, leading to significantly lower costs for cleaning and maintenance. Even with the additional aeration, the energy costs for submerged MBRs are considered to be two magnitudes lower than for side-stream MBRs (Radjenović et al., 2008). Thus, submerged MBRs are the leading configuration nowadays.
Because the membranes are in direct contact with the activated sludge, the problem of fouling is very prominent in MBR systems. As well as the fouling preventions already mentioned (flux reduction or cleaning (physical/chemical) of the membrane), in submerged MBRs increasing the crossflow is a third possibility (Cornel and Krause, 2006). The energy demand for oxygen supply in a submerged MBR is calculated to be approximately 0.3 kWh/m^3 for the treatment of municipal wastewater, while the pumping energy for fouling prevention by coarse bubble aeration accounts for around 0.4 – 1 kWh/m^3 (Cornel and Krause, 2006).

SOLID RETENTION TIME (SRT). In MBR microbial communities, new activated sludge is continuously generated while at the same time parts of the sludge are decomposed by so-called endogenous respiration, which describes all forms of biomass loss and loss of energy for requirements that are not involved in growth (Radjenović et al., 2008). As the energy available for these processes stems from the substrate in the feed water, with long SRT (which results in increased biomass concentration), the limited nutrient supply ultimately limits the growth of new biomass. A higher biomass concentration reduces the food-to-microorganism ratio (F/M),

leading to a decrease in excess sludge production (Yamamoto et al., 1989; Muller et al., 1995; Stephenson et al., 2000). On the other hand, with higher concentration of activated sludge (mixed-liquor suspended solids, MLSS) the viscosity of the sludge increases, resulting in reduced oxygen transfers (Cornel and Krause, 2006), which demands greater amounts of aeration. Additionally, long SRTs can lead to the accumulation of inorganic and non-biodegradable compounds in the reactor which could lead to toxic effects. Furthermore, in sludge of greater age the enzymatic activity is lower, while cell dormancy and death reduce the viability of the biomass population (Cicek et al., 2001). Thus, at short SRTs, the adaptation and response of the microbial community to xenobiotic exposure should be faster (Cicek et al., 2001), which partly contradicts findings that long SRTs lead to a better adapted community. Long SRTs are one of the major advantages of MBR, with the MLSS concentrations in MBR (10–25 g MLSS/L) being much higher than in CAS (1.5–5 mg MLSS/L) (Rosenberger et al., 2000; Cicek et al., 2001). The control of sludge growth can be achieved not only by nutrition supply, but also by artificial sludge decay through e.g. chemical agents or heat. The dead biomass then acts as a nutrient supply for the active sludge. Additionally, temperature and pH affect biomass growth. Temperatures between 15 and 25 C have been found to be optimal for sludge growth, while reduced treatment efficiency was reported at lower temperatures (Marsili-Libelli and Tabani, 2002).

COMMERCIAL IMPLEMENTATIONS AND THE STATE OF AFFAIRS OF MBRS. At present, microbial and physiological characteristics are in many aspects not fully understood (Radjenović et al., 2008). Recent studies show somewhat contradicting results about the optimal operation of MBRs, revealing the high need for further research and a larger data base. Still, the technology has shown its feasibility for not only industrial but also municipal application. In recent years, submerged MBR treatment systems have been installed in a rapidly rising number of municipal wastewater treatment plants. In Europe, the first pilot-scale submerged MBR for municipal wastewater treatment was built in 1996 in Kingston Seymour (UK), soon followed by full-scale plants in Porlock (UK) in 1998 (3,800 p.e.), Büchel (Germany, 1999, 1,000 p.e.), Rödingen (Germany, 1999, 3000 p.e.) and Perthes-en-Gâtinais (France, 1999, 4,500 p.e.). In 2004, the largest MBR plant worldwide at the time was commissioned in Kaarst (Germany, 80,000 p.e.). In Europe in 2006, around 100 full-scale plants with a capacity of more than 500 p.e. were used in the treatment of municipal wastewater, complemented by approximately 300 large industrial plants (capacity > 20 m^3/d) (Lesjean and Judd, 2007). Due to the spread of MBR use, the technology became increasingly competitive regarding capital and operating costs and in the municipal sector it is considered a comparable technology to CAS. Still, the energy costs are

estimated to be 30% higher with MBR than CAS, preventing MBR from becoming the process of choice for municipal wastewater treatment (Lesjean and Judd, 2007).

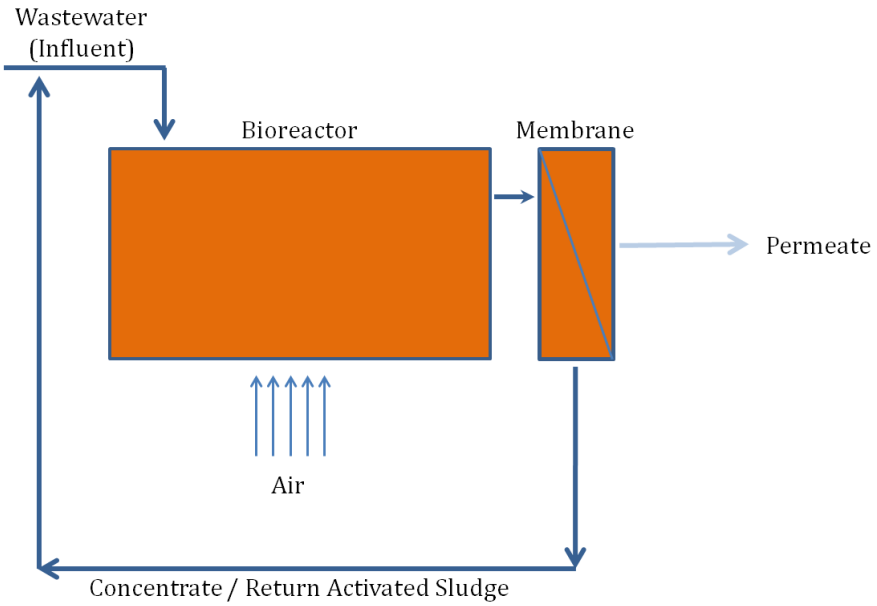


Figure 2-11: MBR in side-stream configuration with external pressure-driven membrane filtration module.

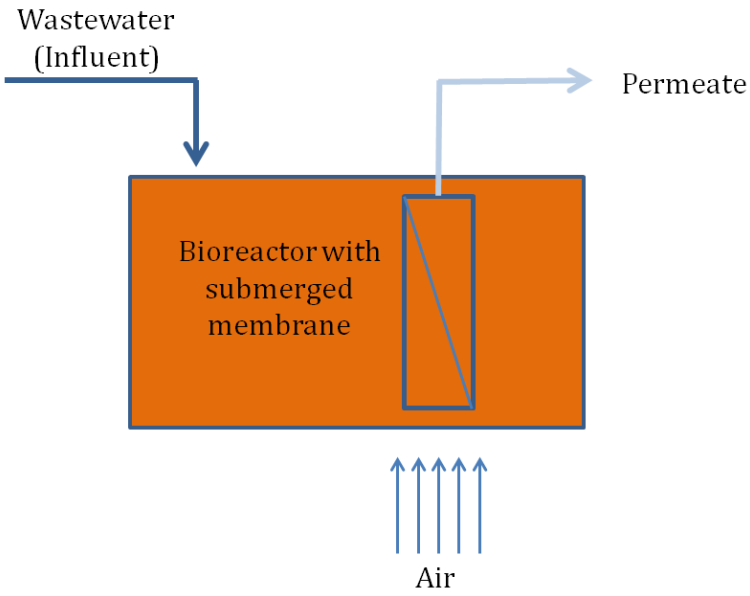


Figure 2-12: MBR with submerged, vacuum-driven filtration membrane.

2.3 Legal aspects, regulatory directives, tools and guidelines

Substances of environmental or toxicological concern may be subject to various legal regulations at different stages of their life cycle, such as the regulation of their production and marketing on one side and, on the other, the control of their disposal and their fate in the environment after consumption.

Pharmaceuticals. In general, in the EU, the USA and other industrial countries, the acute and chronic toxicological potential of pharmaceuticals in animals and humans are reviewed before their release onto the market, e.g. in the EU by the Directive 2001/82/EC (relating to veterinary medicinal products), the Directive 2001/83/EC (relating to medical products for human use) and the amending Directive 2004/27/EC. Consequently, only substances newly introduced to the market are investigated whereas older ones are not examined.

Regarding their occurrence in wastewater and environmental water bodies, pharmaceuticals are rarely considered by national or international bodies of law at all. Thus, to date, they are not targeted by European law, but the awareness of their importance as a possible environmental risk is growing. After two decades of intensive scientific effort, concerns about the widespread presence of pharmaceutically active compounds and their transformation products in water resources are starting to take hold in legislation. In 2011, the EU commission launched a proposal for an amendment to the Directives 2000/60/EC (EU Water Framework Directive) and 2008/105/EC (Directive on environmental quality standards in the field of water policy) with regard to priority substances in the field of water policy (European Commission, 2012b), in which three pharmaceutical substances (diclofenac, 17-alpha-ethinylestradiol and 17-beta-estradiol) were introduced as possible priority substances for the first time. While the two hormones are considered because of their endocrine potential, diclofenac is listed among the potential additional substances because of its direct and indirect toxicity to vertebrates, as proved in scientific studies (European Commission, 2012a). Legislation on maritime water bodies like the Directive 2008/56/EG (maritime strategy framework directive (MSFD); European Commission, 2008) and the HELCOM project "Development of HELCOM Core Set indicators (HELCOM CORESET)" (HELCOM, 2010) are considering the inclusion of pharmaceuticals in routine monitoring programs, while the OSPAR Commission has already identified a few pharmaceuticals as "substances for priority action" (OSPAR, 2011) and a wider range as "substances of possible concern" (OSPAR). In a unique national effort, Sweden developed an environmental classification of pharmaceuticals which is available online (www.fass.se) which characterises pharmaceuticals according to the potential environmental risk and hazard they pose, on the basis of PEC/PNEC ratios (environmental risk) and their biodegradation and bioaccumulation (environmental hazard).

ANTIBIOTICS. Despite the fact that by the early 1970s antibiotic resistance was clearly identified as a risk (WHO, 2011a) and in 1984 the World Health Assembly issued a resolution demanding a rational use of pharmaceuticals (WHO, 1984), antibiotics in the aquatic environment have not been taken into legislative account. In 2001, the WHO published a global strategy for the containment of antimicrobial resistance (WHO, 2001) focusing on efforts in surveillance, prevention and control of resistance, followed by a resolution demanding the enhancement of efforts in 2005 (WHO, 2005b). Yet the consumption rate of antibiotics is still rising, with no sign of more reasonable use (Schwabe and Paffrath, 2007). In light of the fact that in the last 30 years only two new antibacterial classes have been released onto the market, both of them targeting Gram-positive bacteria (WHO, 2011a), the European Centre for Disease Prevention and Control (ECDC) recently reported the severe gap in the development of new drugs against multi-drug-resistant bacteria. In the few cases where research is under way, the development is in its early stages and rarely targets Gram-negative bacteria (ECDC, 2009). Preventing the failure of existing antibiotics is, therefore, a major issue in WHO efforts (WHO, 1998b; WHO, 2001; WHO, 2005b; WHO, 2011a) which to date is not mirrored in legislative tools or guidelines. They neither encourage the reduction of the application of antibiotics in human and veterinary medicine nor prevent their entrance into the (aquatic) environment.

ORGANOPHOSPHATE ESTERS. Being high production volume (HPV) compounds, organophosphate esters had to be registered under the EU REACH procedure before the first deadline of 1st December 2010 (registration of substances with production or import amounts of more than 1,000 t per year in the EU). Comprehensive ecotoxicological information is currently not available for most compounds; however, the aquatic toxicity of some OPs is seen as being unlikely (e.g. tris(2-ethylhexyl) which is in contradiction to scientific opinion (DiGangi et al., 2010).

3. OCCURRENCE OF XENOBIOTIC ORGANIC MICROPOLLUTANTS IN RAW MUNICIPAL WASTEWATER AND THEIR REMOVAL BY A MEMBRANE BIOREACTOR EQUIPPED WITH NANOFILTRATION (NF-MBR)

{Water is} the one substance from which the earth can conceal nothing; it sucks out its innermost secrets and brings them to our very lips.
– Jean Giraudoux (1882-1944), The Madwomen of Chaillot, 1946 –

3.1 INTRODUCTION

Xenobiotics such as pharmaceuticals and industrial chemicals have been detected in raw and treated wastewater, surface water, and ground water (see Chapter 2.1) since they are not effectively removed by conventional biological wastewater treatment with activated sludge (CAS, see Chapter 2.2.1).

MEMBRANE BIOREACTORS. Advanced treatment technologies are discussed with regard to their removal capacities for xenobiotic micropollutants. Membrane bioreactors (MBRs) combine activated sludge treatment with liquid-solid separation by membrane filtration (for a detailed description of the characteristics and underlying principles, see Chapter 2.2.4). A major advantage of MBR systems with regard to xenobiotic micropollutants is that they produce no artificial transformation products, in contrast to other advanced treatment techniques such as oxidation (Semião and Schäfer, 2010). MBRs utilised for wastewater treatment are usually equipped with microfiltration (MF, separation 100–1,000 nm) or ultrafiltration (UF, separation 5–100 nm) membranes (Radjenović et al., 2008). Such MBRs showed enhanced removal rates for a range of micropollutants compared to CAS, while for others no improved elimination was found (Kimura et al., 2005; Bernhard et al., 2006; Radjenović et al., 2007; Reif et al., 2008; Bo et al., 2009; Tambosi et al., 2010). With increasing demand for high quality effluents in terms of xenobiotics, nanofiltration (NF, separation 1–5 nm) could possibly become a membrane filtration technique of choice in MBR technology. However, tight NF membranes with characteristics bordering on those of reverse osmosis (0.1–1 nm, Radjenović et al., 2008) are difficult to maintain permanently in the complex, heavily matrix-loaded environment of a MBR

operating in raw wastewater. A workable solution could be the use of a loose NF treatment, which would be closer to ultrafiltration and e.g. characterised by low salt retention (Nghiem et al., 2006), in combination with MBR treatment.

SCOPE OF THE STUDY. In this study, the occurrence of 52 xenobiotics – pharmaceuticals, metabolites, transformation products and OPs – in raw wastewater was investigated over the course of one week, including both weekdays and the weekend. The fate of these micropollutants during wastewater treatment by a NF-MBR pilot plant equipped with a loose NF and situated at the influent of a municipal WWTP (population equivalent: 300,000) was investigated by analysing hydraulic retention time-related daily samples of influent and effluent. Furthermore, these results were compared to the elimination performance of pure NF-treatment without biological treatment.

3.2 MATERIALS AND METHODS

3.2.1 REFERENCE COMPOUNDS, CHEMICALS AND STANDARDS

The following compounds (all analytical grade > 98% purity) were analysed:
roxithromycin (ROX), carbamazepine (CBZ), oxazepam (OZP), temazepam (TZP), oxycodone (OCN), doxepin (DXP), primidone (PMD), diazepam (DZP), nordiazepam (NZP), methadone (MTD), sulfamerazine (SMA), sulfisoxazole (SSX), sulfadimidine (SDI), sulfadimethoxine (SMI), sulfamethoxazole (SMX), trimethoprim (TMP), tri-iso-butyl phosphate (TiBP) (all provided by Sigma-Aldrich, Seelze, Germany); clarithromycin (CLA), diatrizoic acid (DTZ), iopromide (IMI) (provided by LGC Promochem Wesel, Germany); bezafibrate (BZF) diclofenac (DCF), ibuprofen (IBP), naproxen (NPX), tiamulin (TAM), tributyl phosphate (TnBP), tris(2-butoxyethyl) phosphate (TBEP), triphenyl phosphate (TPP), tris(1,3-dichloro-2-propyl) phosphate (TDCPP), tris(2-chloroethyl) phosphate (TCEP), tris(2-ethylhexyl) phosphate (TEHP), tris(chloropropyl) phosphate (TCPP) (all purchased from Dr. Ehrenstorfer, Augsburg, Germany); iopamidol (IPM), iomeprol (IOP) (Bayer-Schering Pharma, Berlin, Germany); codeine (CDN), dihydrocodeine (DHC, Th. Geyer, Renningen, Germany); 10,11-dihydrocarbamazepine (DH-CBZ) (Alltech, USA); 10,11-Dihydro-10,11-dihydroxycarbamazepine (DHH) (µ-Mol, Luckenwalde, Germany); morphine (MPN, Cambridge Isotopes Lab., Saarbrücken, Germany); N4-acetylsulfamethoxazole (N-Ac-SMX, EAWAG self-synthesis, Dübendorf, Switzerland); iopromide TP 805 A (IMI-TP 805 A), iopromide TP 805 B (IMI-TP 805 B), iopromide TP 819 (IMI-TP 819), iopromide TP 729 A (IMI-TP 729 A), iopromide TP 817 A (IMI-TP 817 A), iopromide TP 787 A (IMI-TP 787 A), iopromide TP 731 A (IMI-TP 731 A), iopromide TP 731 B (IMI-TP 731 B), iopromide TP 759

(IMI-TP 759), iopromide TP 701 B (IMI-TP 701 B), iopromide TP 701 A (IMI-TP 701 A), iopromide TP 643 (IMI-TP 643) were laboratory-prepared as described by Kormos et al., 2009.
The internal standards (IS, analytical grade > 98% purity) were purchased from the following suppliers: codeine-d6, diazepam-d5, methadone-d9, morphine-d6, nordiazepam-d5, tributylphosphate-d27 (Cambridge Isotopes Lab., Saarbrücken, Germany); oxazepam-d5 (Sigma, Deisenhofen, Germany); 13C-15N-carbamazepine, diatrizoic acid-d6 , diclofenac-d4, ibuprofen-d3, iomeprol-d3, iopamidol-d8 (Campro Scientific, Berlin, Germany); bezafibrate-d4, sulfadimethoxine-d_4, sulfadimidine-d_4, sulfamerazine-d_4, sulfamethoxazole-d4, and N4-acetylsulfamethoxazole-d4 (Toronto Research Chemicals, North York, ON, Canada); desmethoxy-iopromide (DMI) (Bayer-Schering Pharma, Berlin, Germany); (E)-9-[O-(2-methyloxime)]-erythromycin (EM-ERY) was self-synthesised according to Schlüsener et al., 2003; triphenylphosphate-d15 (TPP-d15) was self-synthesised according to Andresen et al., 2004.
All organic solvents (n-heptane, n-hexane, acetone, methanol, acetonitrile, ethyl acetate) were picograde and purchased from Merck (Darmstadt, Germany). Pure water was obtained from a Milli-Q system (Integral 3/5/10/15, Millipore, Billericis, MA, USA). Formic acid (98–100%) was ACS grade and purchased from Merck (Darmstadt, Germany).
For each analytical compound group (antibiotics, psycho-active drugs and organophosphorus compounds) a standard solution of all target analytes and an internal standard mix at a concentration of 10 µg/mL (OPs: 5 µg/mL) and 1 µg/mL respectively were prepared in methanol and stored in the dark at 4 °C.

3.2.2 The NF-MBR pilot plant

The NF-MBR pilot plant (Weise Water System GmbH & Co. KG (WWS), Giessen, Germany; Figure 3-1) was installed at the influent of a municipal WWTP, with a population equivalent (p.e.) of 300,000. It consisted of two tanks (total volume: 1.34 m^3) of which the first was an anaerobic denitrification tank while the second tank holding the submerged nanofiltration module was aerobic. Aeration was continuous. Two submerged polyethersulfone NF modules of flat sheet design were used for filtration, each of 7 m^2 (NAPIR® NP010, Microdyn-Nadir, Wiesbaden, Germany, polyethersulfone, nominal retention Na_2SO_4: 35–75% (40 bar); nominal retention NaCl: 10% (40 bar), water flux: > 200 L/(m^2*h)). Wastewater from the effluent of the primary clarifier was fed into the MBR discontinuously at intervals of < 20 min, with filling levels automatically controlled by sensors. The biocenosis of the MBR was developed from return activated sludge from the WWTP. The MBR was allowed to reach stable biological conditions for 158 days before the start of the sampling campaign, with the NF membranes being changed 86

days prior to the sampling campaign. Sludge concentration (SC, MLSS) was 7.2 g/L and 13.4 g/L in the denitrification tank and the NF module respectively. The solid retention time (SRT) was infinite since no sludge was discharged from the reactor, and the average hydraulic retention time (HRT) was 44.4 h. Permeation was intermittent, in response to the water volume pumped into the system. The average transmembrane pressure (TMP) was 0.7 (± 0.01) bar, the average permeate flux (J) was 26.2 (± 3.4) L/(m x day). The average membrane permeability (K) was 1.6 (± 0.2) L m^{-2} h^{-1} bar^{-1}. The average pH values over the period of the sampling campaign were 7.8 (± 0.15), 7.9 (± 0.06) and 7.8 (± 0.12) for influent, permeate and concentrate respectively. The amount of dissolved oxygen was 0.21 mg/L and 9.9 mg/L for the denitrification tank and the NF module respectively. The mean liquid temperature was 12.3 °C in the denitrification tank and 11.8 °C in the NF tank. The sampling campaign was carried out during a period of dry weather with an average maximum air temperature of 11.5 °C and an average minimum air temperature of 2.9 °C (Figure 3-2).

3.2.3 The NF pilot installation (bench-scale)

The bench-scale NF pilot system consisted of a 55 L HDPE tank, which was filled with 40 L of water from the WWTP influent (the feed water of the MBR). The tank's inner surface was allowed to saturate before the installation of a submerged NF module of flat sheet design (NAPIR® NP010, Microdyn-Nadir, Wiesbaden, Germany, polyethersulfone, 3.5 m^2 membrane surface; nominal retention Na_2SO_4: 35–75% (40 bar); nominal retention NaCl: 10% (40 bar), Water flux: > 200 [l/(m^2h)]). The system was pre-conditioned with an intermitted flow (15 min duration every 15-75 min) for five days. Feed water in the tank was replenished daily. The surrounding room temperature was kept at 20 °C. Sampling of feed water from the tank and permeate was carried out on day five. Sampling procedure, preparation and analytical procedures followed the same protocols as described for MBR samples (see below).

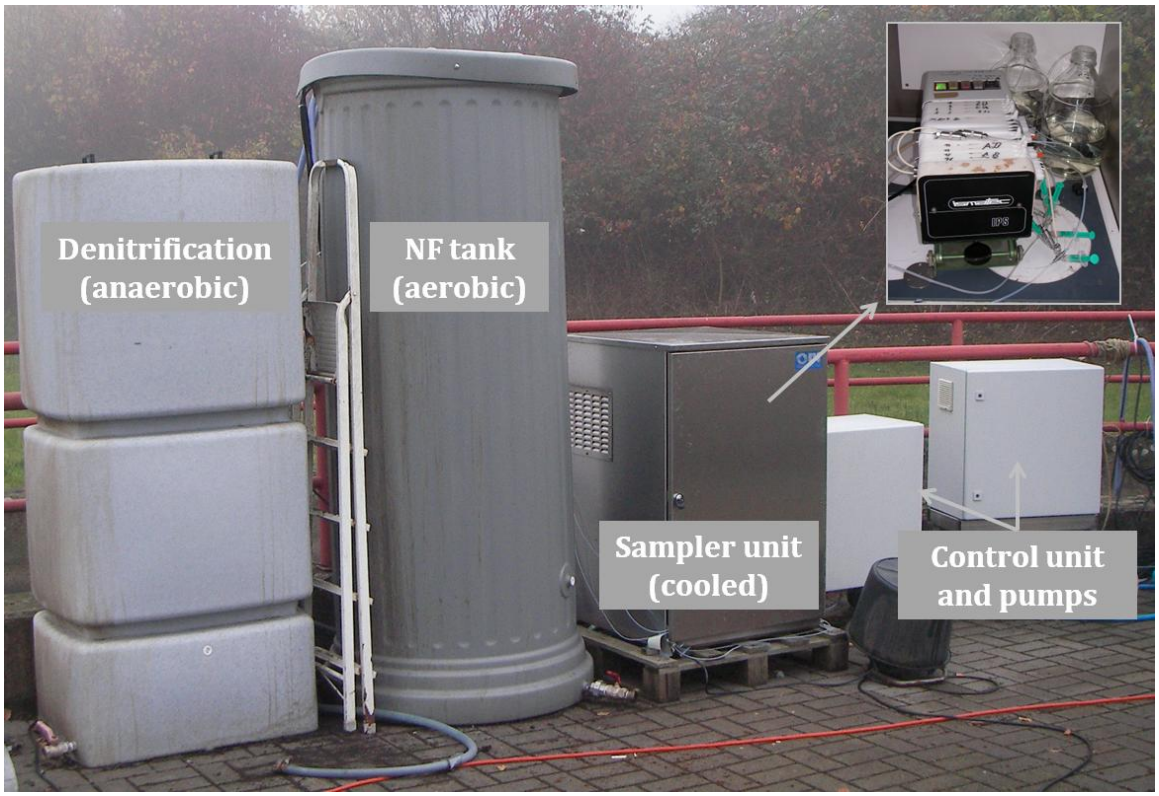


Figure 3-1: NF-MBR pilot plant at the influent of a municipal WWTP.

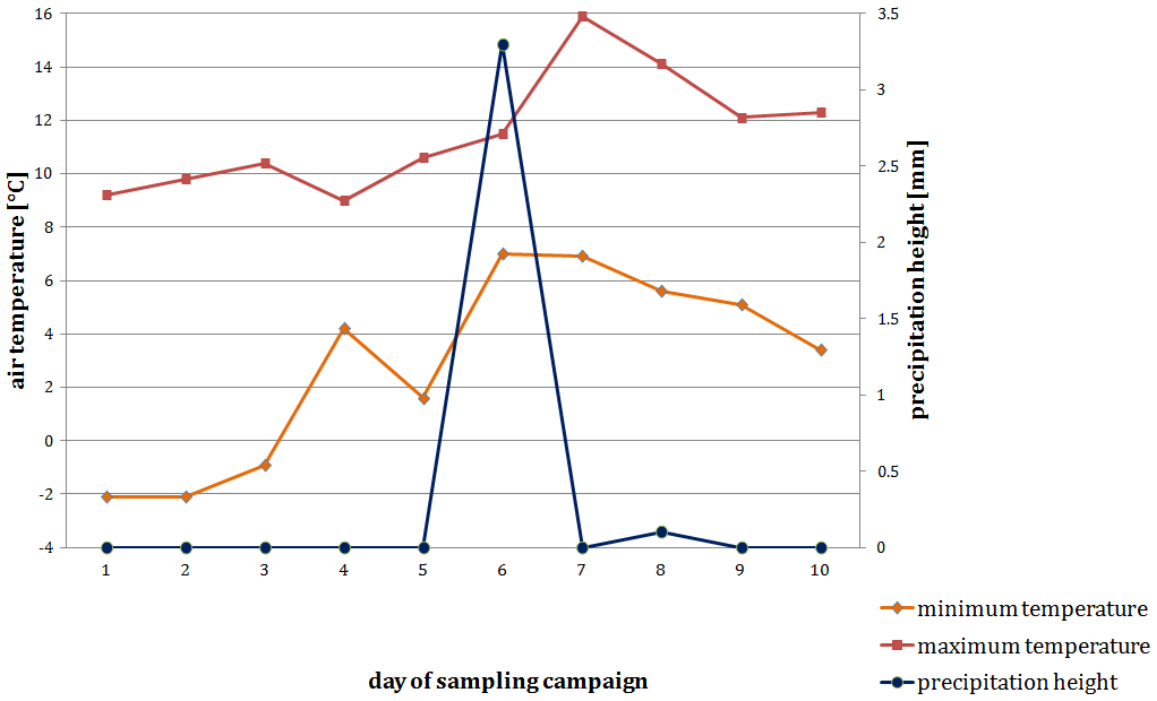


Figure 3-2: Meteorologic conditions during the sampling campaign (data: Deutscher Wetterdienst (DWD), Germany's National Meteorological Service)

3.2.4 SAMPLING AND SAMPLE PREPARATION

Samples of influent and permeate from the MBR were continuously collected as 24-h-composite samples by a peristaltic pump over the course of ten days. Since permeate samples were taken with a delay of two days to match them with the corresponding influent samples according to the HRT, the sampling period represents an actual time series of eight days. Grab samples of the concentrate with mixed-liquor suspended solids were collected three times during the study. All samples were taken in clean glass bottles rinsed with ultra pure water, heptane and acetone and subsequently heated overnight at > 240 °C. Samples were kept in the dark and transported on ice. Extraction was carried out within six hours of sampling.

Regarding acidic compounds and ICMs, the influence of direct acidification during sampling (by providing acid in the sampling bottles beforehand) and acidification in the laboratory after sampling before extraction was investigated (data not shown). For the majority of analytes, the different procedures yielded results which differed only slightly (< 10%), with no clear preference for one method, so subsequently samples were acidified after collection.

The liquid phase of the concentrate was separated from the MLSS by centrifugation directly after arrival at the laboratory. Samples were centrifuged for 60 min with 3500 r/min in a temperature-controlled centrifuge (Rotanta 460R, Hettich Lab Technology, Tuttlingen, Germany).

Samples of influent and supernatant of the concentrate were passed through binder-free glass fibre filters (MN 85/70 BF, Machery-Nagel, Düren, Germany; average retention capacity: 0.6 µm; diameter: 55 mm) which were conditioned prior to filtration by sequentially soaking them in heptane, acetone and methanol for 15 min each, rinsing with Milli-Q water twice and storing in Milli-Q water overnight before use.

Before extraction, samples were separated into aliquots for the determination of neutral analytes and the analysis of acidic drugs, bezafibrate and ICMs. The latter aliquots were acidified with H_2SO_4 to a pH of 2.3–2.8, while the native samples of the first aliquot were controlled to have a pH of 7–8. All samples were augmented with internal standards before SPE (see below).

3.2.5 ANALYTICAL PROCEDURES

The analytical protocols used in this study followed in large parts procedures previously described by Hirsch et al., 2000, Hummel et al., 2006, Magdeburg et al., 2014 (antibiotics, acidic compounds: NSAIDs and bezafibrate, psycho-active compounds, ICMs), Schulz et al., 2008 and Kormos et al., 2009 (TP of iopromide).

3.2.5.1 Solid phase extraction and clean-up

Neutral analytes: antibiotics, psycho-active compounds, OPs. Neutral analytes were extracted with Oasis HLB cartridges (500 mg, 6 mL, Waters, Milfort, U.S.). Prior to extraction, the cartridges were conditioned with 1 x 5 mL heptane, 1 x 5 mL acetone, 2 x 5 mL methanol and 3 x5 mL Milli-Q water (Figure 3-3). Samples were passed through the cartridges with a flow rate of approximately 5 mL/min. Following SPE, the HLB material was dried completely under a steady nitrogen stream for approximately 60–90 min. Elution was accomplished with 5 x 2 mL acetone. Extracts were evaporated to approximately 100 µL by a gentle nitrogen stream before the vials were rinsed with 300 µL of methanol, followed by a second reduction to 100 µL and a final addition of 400 µL Milli-Q water resulting in a final sample volume of 500 µL. Samples were kept at 4 °C in the dark until LC-MS/MS analysis.

Acidic analytes: NSAIDs and bezafibrate. For the extraction of acidic compounds, Oasis MCX cartridges (500 mg, 6 mL, Waters, Milfort, U.S.) were used. The procedure was in principle as described above except for the final dilution, which was carried out with formic acid (Figure 3-3).

ICMs and TP. ICMs and their TP were extracted with Isolute® ENV+ cartridges (200 mg; 3 mL, Biotage, Uppsala, Sweden) following the scheme depicted in Figure 3-3.

Since the SPE procedure for ICMs is highly sensitive to matrix material in the water sample, after conditioning the MCX cartridges were mounted on top of the ENV+ cartridges and the water extraction was performed in one step to shield the ENV+ cartridges from matrix material (Figure 3-4).

3.2.5.2 LC-MS/MS-Analysis

For chromatographic separation, an Agilent 1200 Series (Agilent Technologies, Waldbronn, Germany) liquid chromatographic system equipped with membrane degasser, binary high-pressure gradient pump, autosampler and column thermostat was used. The detection was carried out on a tandem mass spectrometer (Applied Biosystems/MDS Sciex 4000 Q Trap Qq-LIT-MS; Applied Biosystems, Langen, Germany) using multiple reaction monitoring (MRM). For the analysis, electrospray ionization (ESI) was used except for organophosphorus compounds, which were analysed by atmospheric pressure chemical ionization (APCI). The specific modes are detailed below. For each target compound two MRM transitions were monitored for quantification (transition 1) and confirmation (transition 2). Instrument control, peak detection and integration and quantification were carried out using Analyst 1.4 software.

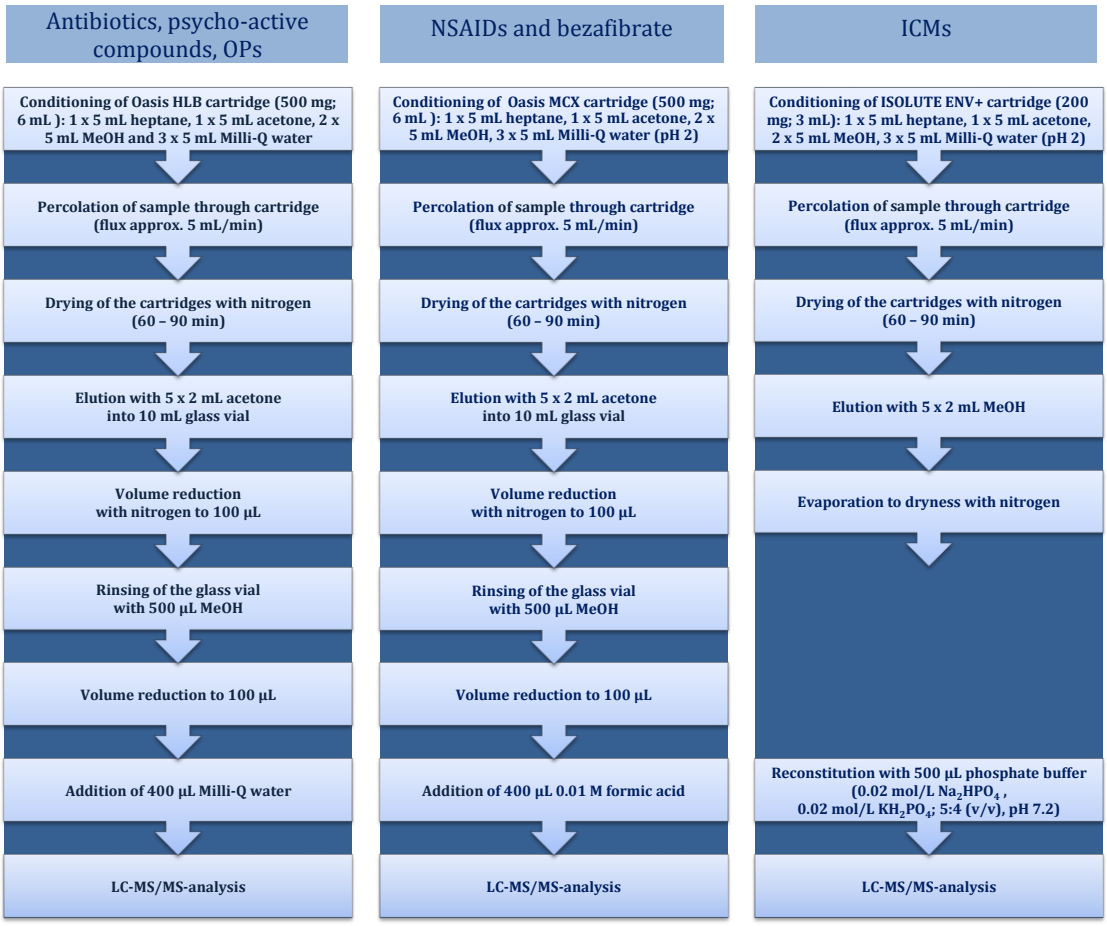


Figure 3-3: Overview of the SPE procedures for antibiotics, psycho-active compounds, OPs (left), NSAIDs and lipid regulators (middle) and ICMs (right).

ACIDIC ANALYTES: NSAIDS AND BEZAFIBRATE. Chromatography was performed with a Zorbax Eclipse XDB-C8 (150 x 4.6 mm; 5 µm) equipped with a Zorbax XDB-C8 guard column (4.6 x 12.5 mm, 5 µm, both purchased from Agilent Technologies (Waldbronn, Germany). The solvents used were acetonitrile (A) and 10 mM formic acid (B). Separation started with 60% A changing to 5% A within 6 min, was kept isocratic for 8 min before it was returned to the starting conditions within 1 min and was held isocratic for the final 5 min of the chromatographic run. Flow rate was set to 400 µL/min, injection volume was 15 µL. Mass spectrometry was carried out with ESI in negative mode with the following conditions: CAD: 5 psi; curtain gas: 30 psi; ion source gas 1: 45 psi; ion source gas 2: 50 psi; source temperature: 650 °C, entrance potential (EP): -10 V. Other parameters are shown in Table 3-1.

ANTIBIOTICS. Chromatography was carried out with a Chromolith® Performance RP-18e column (100 x 4.6 mm, 130 Å, 2 µm) preceded by a Chromolith® RP-18e (5 x 4.6mm) guard column, both supplied by Merck, Darmstadt, Germany. Mobile phase A consisted of a 10 mM ammonium formiate buffer adjusted to pH 4 with formic acid. Acetonitrile served as mobile phase B.

Table 3-1: Target compounds and the corresponding ESI-MS/MS parameters for the analysis of acidic analytes (RT: retention time; DP: declustering potential; CE: collision energy; CXP: collision cell exit potential).

Compound	CAS No.	RT [min]	Transition 1[1] (T1) [m/z]	Transition 2[1] (T2) [m/z]	Dwell time [msec]	DP [V]	CE [V]	CXP (T1/T2) [V]	related IS
DCF	15307-86-5	10.8	293.85/ 249.9	293.85/ 213.90	50	-50	-10	-10/-26	DCF-d4
IBP	15687-27-1	11.2	204.85/ 160.90	204.85/ 158.80	50	-30	-10	-10/-6	IBP-d3
NPX	22204-53-1	9.5	228.82/ 184.70	228.82/ 169.90	50	-30	-10	-8/ -20	IBP-d3
BZF	41859-67-0	9.4	359.89/ 273.80	359.89/ 153.90	50	-65	-10	-22/ -36	IBP-d4
				IS					
IBP-d3	121662-14-4	11.2	208.01/ 164.00		50	-55	-10	-10	
DCF-d4	153466-65-0	10.8	298.91/ 254.88		50	-50	-10	-16	
BZF-d4	1189452-53-6	9.3	364.89/ 158.00		50	-65	-10	-38	

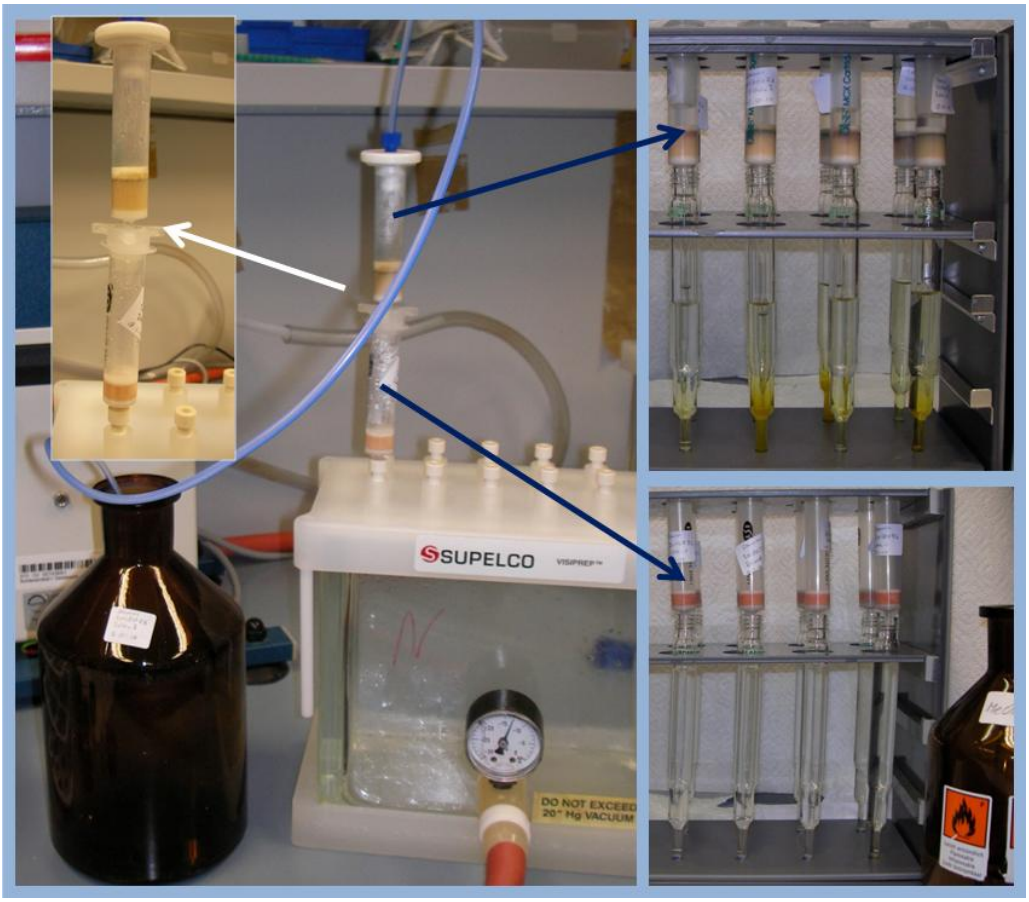


Figure 3-4: SPE installation for acidic analytes and ICMs.

Chromatography started with 0% B, held for 10 min, followed by an increase of B to 26% in 5 min, further increase to 38% within 2 min and held isocratic for 6 min before increasing B to 100% within 6 min, holding isocratic for 4 min before returning to initial conditions within 2 min which were held for 4 min. The flow rate was 400 µL/min. Injection volume was 5 µL; the temperature of the column oven was set to 25 °C.

Electrospray ionization (ESI) was executed in positive mode. Conditions for ESI were set as followed: collision gas: 5 psi; curtain gas: 35 psi; ion source gas 1: 45 psi; ion source gas 2: 40 psi; source temperature: 650 °C; entrance potential: 10 V; ionspray voltage: 5.5 kV. Further details of the MS/MS analysis are listed in Table 3-2.

Table 3-2: Target compounds and the corresponding ESI-MS/MS parameters for the analysis of antibiotics (RT: retention time; DP: declustering potential; CE: collision energy; CXP: collision cell exit potential).

Compound	CAS No.	RT [min]	Transition 1[1] (T1) [m/z]	Transition 2[1] (T2) [m/z]	Dwell time [msec]	DP [V]	CE (T1/T2) [V]	CXP (T1/T2) [V]	related IS
Macrolide antibiotics									
CLA	81103-11-9	26.5	748.4/158.1	748.4/590.3	25	86	39/27	14/12	EM-ERY
ROX	80214-83-1	26.9	837.5/158.1	837.5/679.2	25	106	47/29	12/26	EM-ERY
Sulfonamides									
SMX	723-46-6	23.7	253.94/155.8	253.94/188	25	66	23/21	12/14	SMX-d4
N-Ac-SMX	21312-10-7	23.7	296.06/134.0	296.06/197.9	25	81	35/25	12/14	N-Ac-SMX-d4
SMI	122-11-2	24.9	310.96/156	310.96/245	25	71	29/27	12/8	SMI-d4
SSX	127-69-5	23.7	268.0/156.0	268.0/113.0	25	66	21/23	4/10	SMX-d4
SMA	127-79-7	21.7	265.0/155.9	265.0/171.9	25	56	25/23	14/12	SMA-d4
SDI	57-68-1	22.3	279.0/185.9	279.0/124.0	25	71	25/33	16/10	SMA-d4
		23.7	296.1/134.0	296.1/197.9	25	81	35/25	12/14	N-Ac-SMX-d4
Other antibiotics									
TAM	55297-95-5	26.3	494.2/192.1	494.2/118.9	25	81	31/55	6/10	EM-ERY
TMP	738-70-5	21.1	291.0/229.9	291.0/261.0	25	86	33/35	8/10	EM-ERY
EM-ERY		27.3	763.5/605.5		25	86	27/--	28/--	
SMX-d4	1020719-86-1	23.7	258.0/160.0		25	66	23/--	12/--	
N-Ac-SMX-d4	21312-10-7	23.7	301.1/202.7		25	81	27/--	18/--	
SMA-d4	---	21.6	267.0/160.0		25	66	25/--	12/--	
SDI-d4	1020719-82-7	22.2	282.9/186.0		25	71	25/--	12/--	
CBZ-13C15N	---	26.0	239.0/191.9		25	61	41/--	8/--	
SMI-d4	---	24.8	315.0/160.0		25	71	53/--	6/--	

[1] Precursor ion/product ion

ICMs AND TRANSFORMATION PRODUCTS OF IOPROMIDE. A Chromolith® Performance RP-18e (100 x 4.6 mm, 130 Å, 2 µm) column preceded by a Chromolith® RP-18e (5 x 4.6 mm) guard column (both supplied by Merck, Darmstadt, Germany) was used for chromatographic separation. Mobile phase A was produced by mixing 980 mL Milli-Q water (additioned with 20 mmol/L NH_3 and adjusted to pH 5.7 with acetic acid) with 20 mL acetonitrile. Mobile phase B was produced by mixing 40% of mobile phase A with 60% acetonitrile. The gradient started with 100% A for six minutes, changed to 20% A within further 6 min and was held for 3 min before returning to 100% A within 0.5 min and held isocratic at these conditions for the final 4.5 min. Flow rate was 600 µL/min, injection volume was 25 µL and the temperature of the column oven was adjusted to 30 °C.

Detection was performed with electrospray ionization (ESI) in positive mode (collision gas: medium; curtain gas: 30 psi; ion source gas 1 and 2: 40 psi; source temperature: 600 °C; entrance potential: 10 V; see Table 3-3).

The transformation products of iopromide were analysed according to Schulz et al., 2008, where all details of the procedure are described.

Table 3-3: Target compounds and the corresponding ESI-MS/MS parameters for the analysis of ICMs (RT: retention time; DP: declustering potential; CE: collision energy; CXP: collision cell exit potential).

Compound	CAS No.	RT [min]	Transition 1[1] (T1) [m/z]	Transition 2[1] (T2) [m/z]	Dwell time [msec]	DP [V]	CE (T1/T2) [V]	CXP (T1/T2) [V]	related IS
DTZ	117-96-4	7.00	614.72/ 147.9	614.72/ 233.1	120	51	79/63	4/8	DTZ-d6
IOP	78649-41-9	12.70	777.87/ 405.2	777.87/ 531.8	120	106	59/41	14/18	IOP-d3
IPM	60166-93-0	6.70	777.8/ 558.7	777.88/ 386.9	120	106	31/53	20/14	IPM-d8
IMI	73334-07-3	15.1/ 14.8	791.87/ 572.7	791.87/ 558.7	120	101	33/39	20/18	DMI
IPM-d8	---	12.50	781.1/ 562	781.1/ 689.9	120	120	29/21	6/6	
IOP-d3	1185146-41-1	12.50	781.1/ 408.3	781.1/ 689.9	120	115	55/29	6/6	
DTZ-d6	---	6.70	620.9/ 343.1	620.9/ 367.1	120	92	21/25	6/6	
DMI	76350-28-2	13.10	761.89/ 528.8	---	120	106	47/---	18/---	

[1] Precursor ion/product ion

PSYCHO-ACTIVE COMPOUNDS. Chromatographic separation was accomplished with a Synergi Polar-RP 80 Å column (150 x 3 mm, 4 µm) with a SecurityGuard column (Polar-RP, 4 mm × 3 mm) (both purchased from Phenomenex, Aschaffenburg, Germany). Two mobile phases were used: (A) 10 mM ammonium formiate buffer adjusted to pH 4 with formic acid and (B) acetonitrile. Chromatography started with 10% B, held isocratic for 5 min, then increased

within 13 min to 80% B, which was kept for 7 min before returning to starting conditions within 1 min. Starting conditions were held for 10 min for re-equilibration. The flow rate was 500 µL/min, injection volume was 25 µL, and the temperature of the column oven was kept at 25 °C. Electrospray ionization (ESI) was executed in positive mode with the following parameters: collision gas: 6 psi; curtain gas: 25 psi; ion source gas 1 and ion source gas 2: 40 psi; source temperature: 450 °C; entrance potential: 10 V; ionspray voltage: 5.5 kV. Further details of the MS/MS analysis are shown in Table 3-4.

Table 3-4: Target compounds and the corresponding ESI-MS/MS parameters for the analysis of psycho-active compounds (RT: retention time; DP: declustering potential; CE: collision energy; CXP: collision cell exit potential).

Compound	CAS No.	RT [min]	Transition 1[1] (T1) [m/z]	Transition 2[1] (T2) [m/z]	Dwell time [msec]	DP [V]	CE (T1/T2) [V]	CXP (T1/T2) [V]	related IS
CBZ	298-46-4	15.3	237.0/ 193.9	237.0/ 179.1	25	71	27/49	16/12	CBZ-$^{13}C^{15}N$
DH-CBZ	3564-73-6	15.4	238.9/ 196.0	238.9/ 180.0	25	66	31/55	14/14	CBZ-$^{13}C^{15}N$
DHH	35079-97-1	12.3	271/ 236	---	25	41	19/--	6/--	CBZ-$^{13}C^{15}N$
PMD	125-33-7	11.4	218.9/ 162.1	218.9/ 90.9	25	46	19/39	14/8	CBZ-$^{13}C^{15}N$
DXP	1668-19-5	18.9	280.0/ 107.0	280.0/ 90.9	25	46	37/59	10/6	CBZ-$^{13}C^{15}N$
CDN	76-57-3	10.7	300.1/ 215.0	300.1/ 164.9	25	71	37/53	16/12	CDN-d6
DHC	125-28-0	9.6	302.1/ 128.0	302.1/ 200.9	25	71	85/39	12/16	CDN-d6
MTD	76-99-3	18.8	310.0/ 105.0	310.0/ 76.9	25	51	37/75	6/6	MTD-d9
MPN	57-27-2	4.26	286.1/ 201.0	286.1/ 152.0	25	86	35/77	6/14	MPN-d6
OCN	76-42-6	11.7	316.0/ 256.0	316.0/ 212.0	25	56	35/59	8/14	CDN-d6
DZP	439-14-5	17.8	284.9/ 221.9	284.9/ 193.0	25	76	35/45	20/14	DZP-d5
NZP	1088-11-5	16.6	271.0/ 140.0	271.0/ 208.0	25	71	41/39	12/6	NZP-d5
OZP	604-75-1	15.6	287.0/ 103.9	287.0/ 76.9	25	61	47/81	8/6	OZP-d5
TZP	846-50-4	16.8	301.0/ 254.8	302.9/ 256.9	25	56	31/31	18/6	NZP-d5
CBZ-$^{13}C^{15}N$	---	15.3	239.0/ 191.9		25	61	29/--	12/--	
CDN-d6	1007844-34-9	10.4	306.0/ 165.0		25	91	57/--	12/--	
MTD-d9	---	18.9	319.0/ 105.0		25	61	41/--	8/--	
MPN-d6	1334606-17-5	4.22	293.0/ 152.0		25	91	45/--	14/--	
DZP-d5	65854-76-4	17.7	290.0/ 198.0		25	86	43/--	12/--	
NZP-d5	65891-80-7	16.5	276.0/ 164.9		25	81	31/--	20/--	
OZP-d5	65854-78-6	15.5	291.9/ 235.9		25	61	29/--	12/--	

[1] precursor ion/product ion

OPs. Chromatographic separation was achieved with a Synergi Polar-RP 80 Å column (150 x 3 mm, 4 µm) with a SecurityGuard guard column (Polar-RP, 4 mm × 3 mm) (both purchased from Phenomenex, Aschaffenburg, Germany). The used mobile phases were Milli-Q water (A) and MeOH (B). Chromatography started with 64% B, was increased to 76% B within 0.5 min and further increased to 100% B within 15.5 min, held isocratic for 4 min and returned to initial conditions with 64% B within 0.1 min and then was kept isocratic for 4.9 min. The flow rate was set to 400 µL/min, injection volume was 10 µL, and the temperature of the column oven was 25 °C. Mass-spectrometric analysis was carried out in positive mode, utilizing atmospheric pressure chemical ionization (APCI), using the following parameters: collision gas, medium; curtain gas: 30 psi, nebulizer current, 3 µA; ion source gas 1: 60 psi; source temperature: 500 °C; entrance potential: 10 V. Declustering potential was 80 V and collision cell exit potential 10 V. Further details are given in Table 3-5.

Table 3-5: Target compounds and the corresponding APCI-MS/MS parameters for the analysis of OPs (RT: retention time; DP: Declustering potential; CE: Collision energy; CXP: Collision cell exit potential).

Compound	CAS No.	RT [min]	Transition 1[1] (T1) [m/z]	Transition 2[1] (T2) [m/z]	Dwell time [msec]	CE (T1/T2) [V]	related IS
TBEP	78-51-3	8.3	399/299	399/199	60	20/23	TPP-d15
TnBP	126-73-8	7.2	267/211	267/155	60	13/17	TnBP-d27
TiBP	126-71-6	6.8	267/211	267/155	60	13/17	TnBP-d27
TEHP	78-42-2	14.0	435/323	435/99	60	12/43	TnBP-d27
TPP	115-86-6	7.9	327/152	327/77	60	55/65	TnBP-d27
TCEP	13674-84-5	4.7	285/223	287/225	60	19/20	TnBP-d27
TCPP	13674-84-5	6.0	329/253	329/175	60	15/19	TnBP-d27
TDCPP	13674-87-8	7.6	431/321	431/209	60	19/24	TnBP-d27
TnBP-d27	---	7.01	294/102		60	27/--	
TPP-d15	---	7.81	342/82		60	65/--	

for all analytes: DP [V]:80, CXP [V]: 10; [1] Precursor ion/product ion;

3.2.6 Method validation

Calibration curves with 12, 12, 13, 10 and 8 calibration points ranging from 0.2 to 2,000 ng/L, 0.5 to 2,000 ng/L, 0.5 to 4,000 ng/L, 1 to 1,000 ng/L and 5 to 3,000 ng/L for acidic drugs, antibiotics, ICMs, psycho-active compounds and organophosphorus compounds respectively were prepared by spiking the eluent mixtures used for LC-MS/MS analysis.

A fixed amount of the associated internal standard was added (see Table 3-1 to Table 3-5). The linearity range of the calibration lay generally between 0.2 to 10 ng/L, 0.5 to 200 ng/L, 0.5 to 200 ng/L, 1 to 200 ng/L and 5 to 300 ng/L for acidic drugs, antibiotics, ICMs, psycho-active

compounds and organophosphorus compounds respectively. A quadratic fit (y=ax2+bx+c) with the weighing 1/x was used for the exceeded range of the calibration. Correlation coefficients higher than 0.998 were accepted. Concentrations of the target compounds were calculated by plotting the peak area ratio of analyte and internal standard in the samples against the same ratio in the calibration samples. The limit of quantification (LOQ) was set as the second lowest calibration point so long as the signal/noise ratio (S/N) for this calibration point exceeded 10 for the quantifying transition (T1) and 3 for the confirming transition (T2). Considering both the enriched sample volume and the difficult matrix, the calculated LOQ was multiplied by 10 for concentrate and influent samples, while the permeate samples were processed with a fivefold LOQ. Still, the required S/N ratios for quantification (T1) and confirmation (T2) had to be fulfilled. In some cases LOQ was individually adapted to fit the required S/R ratio. The stability of the analytical acquisition and any possible fluctuation in signal intensity and retention time was supervised by injecting a standard solution after every 5 to 7 native samples. Carryover from sample to sample was checked for by injecting a mixture of pure eluent at an interval of 5 samples.

Instrumental precision was determined by repeated injection of a standard solution during analysis at the same day (intraday: n = 4–8) and over the whole period of analysis (interday: n = 8 - 12) and is indicated by the relative standard deviation of the measurements (% RSD).

Quality assurance and quality control (QA/QC) of the applied instrumental methods have already been reported elsewhere (Hirsch et al., 2000; Hummel et al., 2006; Schulz et al., 2008; Kormos et al., 2009; Magdeburg et al., 2014). In addition to this, the accuracy of the full analytical procedure was assessed by studying the relative recovery in fortified native samples of all matrices (raw influent wastewater, concentrate and permeate of the MBR) over the complete analytical protocol. The accuracy was determined by spiking native samples with analyte standards (100 ng/100 mL for influent and concentrate, 50 ng/100 mL for effluent samples) and internal standards before extraction and analysis (n = 2–6 per sample type). The background amounts present in unfortified native samples were subtracted from the results of the spiked samples and the latter were subsequently related to standard solutions containing the same amount of analytes as was spiked into the fortified native samples. Blank values (Milli-Q water spiked with internal standards undergoing the whole analytical procedure) were determined for every set of 10–15 environmental samples to assess possible sample contamination during the laboratory process. Eventual blank contamination was not subtracted from environmental results.

3.3 Results and discussion

3.3.1 Method validation

Quantification, based on peak areas, was carried out by internal standard calibration. Calibration curves all showed a correlation coefficient (r^2) of at least 0.998. Validation data are given in Table 3-6. LOQ in samples of influent and concentrate ranged from 5 ng/L to 50 ng/L for pharmaceuticals and from 14 ng/L to 150 ng/L for OPs, while LOQ for the permeate ranged from 2.5 ng/L to 25 ng/L and from 12.5 ng/L to 75 ng/L for pharmaceutical compounds and OPs respectively. The accuracy of the method was determined by estimating relative recoveries in native samples of all matrices, which were spiked with the target compounds and internal standards before extraction. Accuracy was then estimated by subtracting the analyte amount measured in the native samples from the amounts measured in the spiked ones and subsequently relating the amounts to standards of the same concentration (Table 3-6). Accuracy was satisfactory, especially since for most analytes, precision was < 20% RSD. For ICMs, spiked samples of influent and concentrate yielded concentrations outside the calibration range, therefore accuracy for ICMs in these sample types was not determinable. In some cases, the accuracy of the method is low (Table 3-6). Since sometimes multi-compound analytical methods do not provide perfect conditions for every analyte, this is considered to be no shortcoming as long as at the same time satisfactory LOQs and high instrumental precision are achieved (Gros et al., 2006b; Gros et al., 2008). For the majority of the studied compounds, blank values in all sample types were below LOQ. For temazepam, iomeprol and TiBP, the LOQ for effluent samples was exceeded. Blanks values of trimethoprim, doxepin, ibuprofen, iopromide, TBEP and TCEP surpassed LOQ in all matrixes but were not greater than 5% of environmental samples in influent samples expect for TCEP and temazepam (Table 3-7). The latter showed relatively high blank values throughout, thus, results for this substance should be considered semi-quantitative. For iomeprol and iopromide, blank samples showed high varieties (ranging from not detected to 322 ng/L and 33 ng/L to 363 ng/L for iomeprol and iopamidol, respectively), more likely suggesting singular interferences during mass-spectrometric analysis than high ubiquitous contamination.

3.3.2 Occurrence of xenobiotic micropollutants in municipal wastewater

Out of 52 investigated substances, 6 pharmaceuticals and metabolites as well as one OP were found in neither raw municipal wastewater nor the concentrate or permeate of the NF-MBR. The absence of the antibiotic compounds sulfadimidine, sulfisoxazole, sulfamerazine and tiamulin can be explained by their exclusive use as veterinary drugs in Germany; as such they are less

prone to be discharged in wastewater but are largely released into the environment by diverse sources like runoff, leakage or drift from agricultural soils fertilized with manure (Christian et al., 2003; Sarmah et al., 2006). Furthermore, the psycho-active compounds diazepam and nordiazepam were not detected in quantifiable concentrations, while oxycodone was found in concentrations either very near or below LOQ. These results are in good accordance with earlier reports from studies in Germany, Spain and the USA (Carballa et al., 2004; Hummel et al., 2006; Wick et al., 2009; Hass et al., 2012; Du et al., 2014). Additionally, the organophosphorus compound TEHP was not quantifiably detected.

3.3.2.1 Untreated wastewater

In raw municipal wastewater, 39 pharmaceuticals, metabolites, transformation products and OPs were quantified (Table 3-8). Figure 3-5 shows the maximum concentrations in the influent from the NF-MBR ($c_{max\ influent}$) found for each compound during the sampling campaign. The highest concentrations were found for the ICMs iopamidol (150,602 ng/L) and iomeprol (20,829 ng/L). 18 substances, which equals 45% of the quantified xenobiotics, were present in maximum concentrations of more than 1,000 ng/L: five ICMs (iopromide, iomeprol, diatrizoic acid, iopamidol, iopromide TP 819), four NSAIDs (diclofenac, ibuprofen, bezafibrate, naproxen), four psycho-active drugs (10,11-dihydro-10,11-dihydroxycarbamazepine, primidone, carbamazepine, oxazepam), three OPs (TBEP, TiBP, TCPP) and the antibiotic sulfamethoxazole as well as its metabolite N4-acetylsulfamethoxazole. Table 3-8 shows that, with the exception of naproxen, the average concentrations of these substances also exceeded 1,000 ng/L, revealing that these substances are present in raw wastewater at constantly high levels and are not introduced in isolated peak events. However, in some cases high SD values indicated considerable fluctuations over the course of the sampling period. This was especially true for ICMs, which indicates a heterogeneous, variant input over the course of the sampling campaign (see Chapter 3.3.2.2). In addition, wide variations in SD were found for substances with values close to LOQ, such as sulfadimethoxine, methadone and oxycodone.

Table 3-6: LOQ [ng/L] and accuracy of the analytical procedure [%] for the three investigated matrices (influent, permeate, concentrate) and instrumental precision (intraday/interday) for the five analytical compound groups.

	LOQ [ng/L]		**Accuracy [%]**						**Instrumental precision [RSD, %]**	
	Influent/ Concentrate	Permeate	Influent[1] Average	*SD*	Permeate[2] Average	*SD*	Concentrate[3] Average	*SD*	Intraday[7]	Interday[8]
Antibiotics										
CLA	5	2.5	102	12	100	1	100	9	7	7
ROX	5	2.5	96	6	102	5	103	6	3	21
SMI	5	2.5	119	23	95	1	90	16	7	18
SDI	5	2.5	99	5	94	0	95	5	3	17
SSX	5	2.5	95	7	77	6	75	3	5	8
SMA	5	2.5	106	4	99	4	101	0	3	13
SMX	5	2.5	100	8	94	4	97	0	4	11
N-Ac-SMX	20	10	79	38	103	5	130	29	5	6
TAM	10	5	92	14	120	5	120	6	3	11
TMP	5	2.5	31	5	105	6	110	10	8	15
min	---	---	31	4	77	0	75	0	3	6
max	---	---	131	38	134	7	130	29	17	21
ICMs										
DTZ	50	25	a.r.[6]	a.r.[6]	99	---[5]	a.r.[6]	a.r.[6]	8	8
IOP	50	25	a.r.[6]	a.r.[6]	100	14	a.r.[6]	a.r.[6]	8	12
IPM	50	25	a.r.[6]	a.r.[6]	106	1	a.r.[6]	a.r.[6]	10	12
IMI	50	25	a.r.[6]	a.r.[6]	88	19	a.r.[6]	a.r.[6]	4	9
min	---	---	---	---	88	1	---	---	4	8
max	---	---	---	---	106	19	---	---	10	12
NSAIDs and bezafibrate										
BZF	10	5	73	10	78	1	76	23	4	26
DCF	10	5	52	12	87	17	56	0	4	13
IBP	10	5	120	3	50	1	64	8	5	4
NPX	10	5	86	22	45	4	41	7	9	12
min	---	---	52	3	45	1	41	0	4	4
max	---	---	120	22	87	17	77	23	9	26
Psycho-active compounds										
CBZ	10	5	113	8	107	1	113	9	2	3
DH-CBZ	10	5	99	5	99	2	100	1	1	8
DHH	20	10	123	24	86	12	131	---[5]	8	8
PMD	25	12.5	n.d.[4]	n.d.[4]	122	12	135	---[5]	8	11
DXP	10	5	119	33	75	23	86	15	18	20
CDN	50	25	93	8	99	8	100	4	3	6
DHC	20	10	101	16	88	9	98	5	6	7
MTD	10	5	88	3	84	0	88	2	3	8
MPN	30	15	62	5	29	4	32	5	12	n.d.
OCN	20	10	39	4	82	10	79	4	6	17
DZP	20	10	111	10	99	1	101	1	3	6
NZP	10	5	103	6	101	3	106	7	3	3
OZP	50	25	182	13	115	11	109	0	10	9
TZP	10	5	89	6	81	1	78	2	4	6
min	---	---	39	3	29	0	32	0	1	3
max	---	---	182	33	122	23	135	15	18	20
OPs										
TBEP	25	12.5	113	7	164	---[5]	n.d.[4]	n.d.[4]	1	7
TnBP	50	25	62	30	24	2	91	3	2	5
TiBP	150	75	57	18	28	0	n.d.	n.d.	4	6
TEHP	50	25	46	13	46	8	127	---[5]	2	10
TPP	25	12.5	50	2	35	2	49	3	1	9
TCEP	14	7	79	9	56	2	56	1	3	9
TCPP	110	55	77	8	63	---[5]	112	3	3	8
TDCPP	60	30	75	5	45	2	46	8	2	7
min	---	---	46	2	5	0	46	1	1	5
max	---	---	113	30	164	8	127	8	4	10

[1]n=3-6; [2]n=2; [3]n=2-3; [4]n.d. = not determined; [5]n=1; [6]a.r. = above concentration range; [7]n=4-8; [8]n=8-12

Table 3-7: QA/QC results for substances with blank values > LOQ. All blank values for substances not shown: < LOQ.

	Blank values[1] [ng/L]		% of LOQ		% of environmental values[2]			
			Influent/ Concentrate	Permeate	Influent		Permeate	
TMP	4	±5	85	170	3	±0	8	±2
TZP	11	±0	227	453	29	±3	47	±5
DXP	6	±0	383	767	5	±9	45	±19
IBP	12	±22	121	242	0	±0	56	±9
IOP	113	±228	57	115	2	±4	46	±91
IMI	192	±130	105	210	1	±1	58	±87
TBEP	36		142	284	1	±0	23	±8
TiBP	94		63	125	4	±4	69	---
TCEP	16		116	232	10	±2	12	±2

[1] n=3; n=1 for TBEP, TiBP, TCEP; [2] given as the average value per sample type

DIVERSE USE PATTERNS. Different consumption habits might lead to diverse occurrence patterns in wastewater in different countries (Behera et al., 2011). Consequently, comparing pharmaceutical concentrations between countries can be difficult (Lindqvist et al., 2005). Additionally, pharmaceutical values can vary during different seasons. For example, Göbel et al., 2005b, Gao et al., 2012a, Yu et al., 2013 and Du et al., 2014 described variant occurrence of NSAIDs, psycho-active compounds and antibiotics around the year for WWTP influent waters, while on the other hand Alexy et al., 2006 found only minor seasonal variations in the occurrence of antibiotics in influent wastewater.

According to Petrović et al., 2009, the highest concentrations of pharmaceuticals in WWTP influents are commonly those of NSAIDs since they are sold in large amounts as OTC drugs in many countries. This is not confirmed in this study, where the two highest concentrations in the influent are two ICMs with average concentrations 8 times (iopamidol) and 1.7 times (iomeprol) higher than those of the highest concentrated NSAID, ibuprofen. A possible reason for this is the presence of a large university hospital with several institutes providing ICM-related diagnostics in the service area of the WWTP. This is confirmed by the fact that when comparing the occurrence of ICMs in this study with earlier reports, highly specific, diverse use patterns for ICMs seem to be reflected in the wastewater. For example, Ternes and Hirsch, 2000 reported ICM values in WWTP influent from near Frankfurt (Germany) that were comparable with the findings in this study with regard to iopamidol and diatrizoic acid, while the reported values of iomeprol and iopromide were about eight times lower than the values found in this study. On the other hand, Carballa et al., 2004 reported equally high values of iopromide (6,000 – 7,000 ng/L) in the influent of a WWTP in Galicia (NW Spain), which correspond well with the data presented here.

Table 3-8: Mean concentration of analytes in influent, effluent and concentrate of the MBR over the investigation period [ng/L]. For the calculation of average values, single values < LOQ were accounted for as 0.5*LOQ and single values which not yielded a signal (not detected = n.d.) were taken into account as 0.

	NF-MBR						NF	
	Influent [ng/L][1]		Effluent [ng/L] [1]		Concentrate [ng/L]		Influent[2]	Effluent[2]
	Average	SD	Average	SD	Average	SD	[ng/L]	[ng/L]
Antibiotics								
CLA	544	67.7	103	31.2	103	57.7		11.7
ROX	123	20.4	65.9	21.5	82.7	18.8	19.8	n.d.
SMI	28.1	43.1	3.78	2.14	6.60	3.80	n.d.	n.d.
SDI	< LOQ		< LOQ		< LOQ		44.6	n.d.
SSX	n.d.		n.d.		< LOQ		n.d.	4.00
SMA	< LOQ		n.d.		< LOQ		n.d.	n.d.
SMX	1,297	126	679	156	626	172	887	257
N-Ac-SMX	2,573	334	< LOQ		< LOQ		807	250
TAM	< LOQ		< LOQ		< LOQ		n.d.	n.d.
TMP	136	14.4	59.2	16.1	65.3	25.6	12.3	n.d.
ICMs								
DTZ	7,331	3,760	7,549	1,970	9,346	2,250	9,705	2,781
IOP	14,131	7,164	716	402	416	130	31,818	7,325
IPM	1,727	2,000	1,564	695	1,248	1,075	1,214	310
IMI	67,244	56,260	1,950	1,640	1,291	1,074	94,505	22,613
IMI-TP 805 A	123	80.0	2,749	2,295	2,824	2,396	n.a.	n.a.
IMI-TP 805 B	< LOQ		1,325	1,074	1,390	1,177	n.a.	n.a.
IMI-TP 819	791	603	1,657	1,334	2,462	2,006	n.a.	n.a.
IMI-TP 817 A	< LOQ		2,023	1,560	4,735	3,382	n.a.	n.a.
IMI-TP 787 A	< LOQ		5,200	2,265	14,404	4,509	n.a.	n.a.
IMI-TP 731 A	< LOQ		4,918	2,955	4,300	3,398	n.a.	n.a.
IMI-TP 731 B	< LOQ		201	116	202	135	n.a.	n.a.
IMI-TP 729 A	n.d.		17,187	9,735	20,247	12,930	n.a.	n.a.
IMI-TP 759	476	202	8,754	3,621	19,007	8,421	n.a.	n.a.
IMI-TP 701 A	116	81.3	18,263	9,819	20,084	14,480	n.a.	n.a.
IMI-TP 701 B	< LOQ		756	383	1,102	638	n.a.	n.a.
IMI-TP 643	< LOQ		2,254	1,309	1,975	1,692	n.a.	n.a.
NSAIDs and bezafibrate								
BZF	1,270	204	16.6	5.17	< LOQ		n.a.	n.a.
DCF	1,322	245	866	77.6	911	16.2	n.a.	n.a.
IBP	8,135	1,352	21.9	3.68	< LOQ		n.a.	n.a.
NPX	898	261	20.1	4.68	18.1	11.4	n.a.	n.a.
Psycho-active drugs								
CBZ	1,165	288	1,255	267	1,156	18.0	1,214	355
DH-CBZ	52.8	6.66	57.8	1.56	59.2	2.88	76.8	23.9
DHH	7,652	1,458	2,189	291	2,608	542	4,382	1,524
DXP	291	82.9	14.8	5.69	24.8	1.78	245	66
PMD	2,362	455	543	100	578	125	647	306
CDN	231	27.9	< LOQ		< LOQ		376	100
DHC	32.7	16.5	< LOQ		n.d.		125	83.0
MTD	59.1	31.4	41.9	2.50	52.2	2.49	13.8	n.d.
MPN	396	70.8	< LOQ		n.d.	0.00	n.a.	n.a.
OCN	< LOQ		< LOQ		< LOQ		n.d.	n.d.
DZP	n.d.		n.d.		n.d.		41.0	n.d.
NZP	n.d.		n.d.		n.d.		22.7	8.79
OZP	1,024	125	295	40.6	244	5.85	< LOQ	<LOQ
TZP	36.8	3.91	22.8	2.71	32.3	4.88	92.3	40.6
OPs								
TBEP	7,447	2,274	182	105	250	131	3,873	699
TnBP	123	40.7	< LOQ		n.d.		n.d.	n.d.
TiBP	4,293	2,515	< LOQ		< LOQ		n.d.	n.d.
TEHP	< LOQ		n.d.		n.d.		n.d.	n.d.
TPP	27.7	6.33	n.d.		n.d.		n.d.	n.d.
TCEP	166	33.4	144	23.2	195	89.8	435	101
TCPP	1,088	224	753	112	1,091	670	1,750	302
TDCPP	222	53.5	150	19.6	245	101	<LOQ	<LOQ

[1] n=8; [2] n=1; < LOQ = below the limit of quantification; n.d. = not detected; n.a. = not analysed

NSAIDs AND BEZAFIBRATE. An example of the considerable diversity in reported values in wastewater for even globally used pharmaceuticals is ibuprofen. In many countries ibuprofen is available as an OTC drug and, consequently, high consumption rates are reported from around the globe (Alder et al., 2006). Additionally, it is listed as a core drug for a basic healthcare system in the WHO's List of Essential Medicines (WHO, 2011c). Nonetheless, very diverse data about its occurrence in raw wastewater can be found in the literature. Lindqvist et al., 2005 and Hedgespeth et al., 2012 reported concentrations of over 19,000 ng/L and 24,000 ng/L found in influents to WWTPs in Finland and the USA respectively. Other researchers found ibuprofen in concentrations closer to those presented here (e.g. 385–1,260 ng/L in the area of Bangkok, Thailand (Tewari et al., 2013), 2,265 ng/L in influents in South Korea (Behera et al., 2011). Carballa et al., 2004 reported concentrations between 2,600–5,700 ng/L in a WWTP in Galicia (NW Spain), while in Sevilla (southern Spain) influent concentrations no higher than 373.11 ng/L were found (Santos et al., 2007). Kosma et al., 2014 found highly diverse ibuprofen concentrations in several WWTPs in Greece, ranging from 2,634 ng/L to the complete absence of the substance in the wastewater. Such profound differences in the concentrations reported for a widespread drug can be explained by differences in the composition of the wastewater. Lindqvist et al., 2005 found that in the WTTPs of smaller municipalities, concentrations of NSAIDs were higher than in the WWTPs of big cities since they were less diluted by industrial wastewater.

Taking this into consideration, the values of diclofenac and naproxen found in this study accorded well with previous studies from Croatia (Gros et al., 2006a), Greece (Kosma et al., 2014), Japan (Kimura et al., 2007), Korea (Behera et al., 2011) and Spain (Celiz et al., 2009; Carballa et al., 2004). In contrast, in another raw wastewater study from Spain, diclofenac values of below the limit of detection were reported, while naproxen values were found that were more than 30 times higher than in this study (Santos et al., 2007).

Bezafibrate concentrations presented here are considerably higher than those found by researchers in Croatia (Gros et al., 2006a), Greece (Kosma et al., 2014) and Spain (Rosal et al., 2010) and lower than values reported from Finland (Lindqvist et al., 2005).

ANTIBIOTICS. Regarding antibiotics, the results are in good agreement with several studies from Europe and Asia (Carballa et al., 2004; Göbel et al., 2005b; Gros et al., 2006a; Behera et al., 2011; Gao et al., 2012a; Kosma et al., 2014). In contrast, Tewari et al., 2013 reported a very dissimilar antibiotic pattern in raw wastewater from Bangkok (Thailand) with considerably lower values of roxithromycin (over tenfold less) and concentrations of sulfamethoxazole over 40 times lower than in this study, while the trimethoprim values are comparable. For raw wastewater from two plants in Japan, low antibiotic values are reported, being 10-fold, 20-fold and 48-fold lower for

trimethoprim, sulfadimethoxine and sulfamethoxazole, respectively, than the concentrations reported here (Tewari et al., 2013). Overall, veterinary antibiotics are detected in raw wastewater only in small quantities or not at all, confirming the results presented here (Göbel et al., 2005b; Gao et al., 2012a; Gao et al., 2012b; Tewari et al., 2013).

PSYCHO-ACTIVE COMPOUNDS. In a previous study analysing the same cluster of psycho-active compounds as in this study, similar results were found for twelve WWTPs in Germany (Hummel et al., 2006). Carbamazepine, the most extensively studied psycho-active drug in wastewater, was reported in similar concentrations in wastewater in Canada, Croatia, Germany and Spain (Miao and Metcalfe, 2003; Zuehlke et al., 2004; Gros et al., 2006a; Celiz et al., 2009; Leclercq et al., 2009). Concentrations about tenfold lower than reported here were found in wastewater in studies in Greece, Korea and the USA (Behera et al., 2011; Gao et al., 2012b; Kosma et al., 2014).

OPS. TBEP and TiBP were the highest concentrated OPs in influent wastewater. This is in agreement with data given in studies from Spain (Rodil et al., 2009: TBEP: 1,004 ± 63 ng/L; TnBP 1,246 ± 123 ng/L; TiBP 664 ± 144 ng/L) and Sweden (Marklund et al., 2005: TBEP: 5,200–35,000 ng/L, TBP: 6,600–52,000 ng/L). However, the ratio of about 1:0.5 for TiBP and TnBP reported by Rodil et al., 2009 is fundamentally different from what was found in the presented study (1:34). The influent concentrations of TPP were low, which concurs with the average value of data given by other researchers (Marklund et al., 2005, Rodil et al., 2009).

TCEP influent concentrations reported from Norway (Green et al., 2008, cited in van der Veen and Boer, 2012) are over ten times higher than those found in the presented study. Other researchers found concentrations closer to those presented here (Marklund et al., 2005; Rodil et al., 2009). Average TCPP concentrations of 500–600 ng/L – i.e. lower than the concentrations found in the presented study – were reported from WWTPs in Germany and Spain (Bester, 2005; Rodil et al., 2009), while concentrations found in Norway and Sweden were usually comparable or higher (Marklund et al., 2005; Green et al., 2008, cited in van der Veen and Boer, 2012). TDCP concentrations reported are consistent with those found here (Marklund et al., 2005; Rodil et al., 2009).

Equally, as reported here, previous studies of OPs did not find TEHP in wastewater (e.g. Marklund et al., 2005; Rodil et al., 2009). According to van der Veen and Boer, 2012, this is based on analytical failure of sample extraction by liquid-liquid extraction or SPE with Oasis HLB material, rather than the absence of the substances. However, QA/QS for TEHP both in the presented study and in the literature data given above show acceptable results.

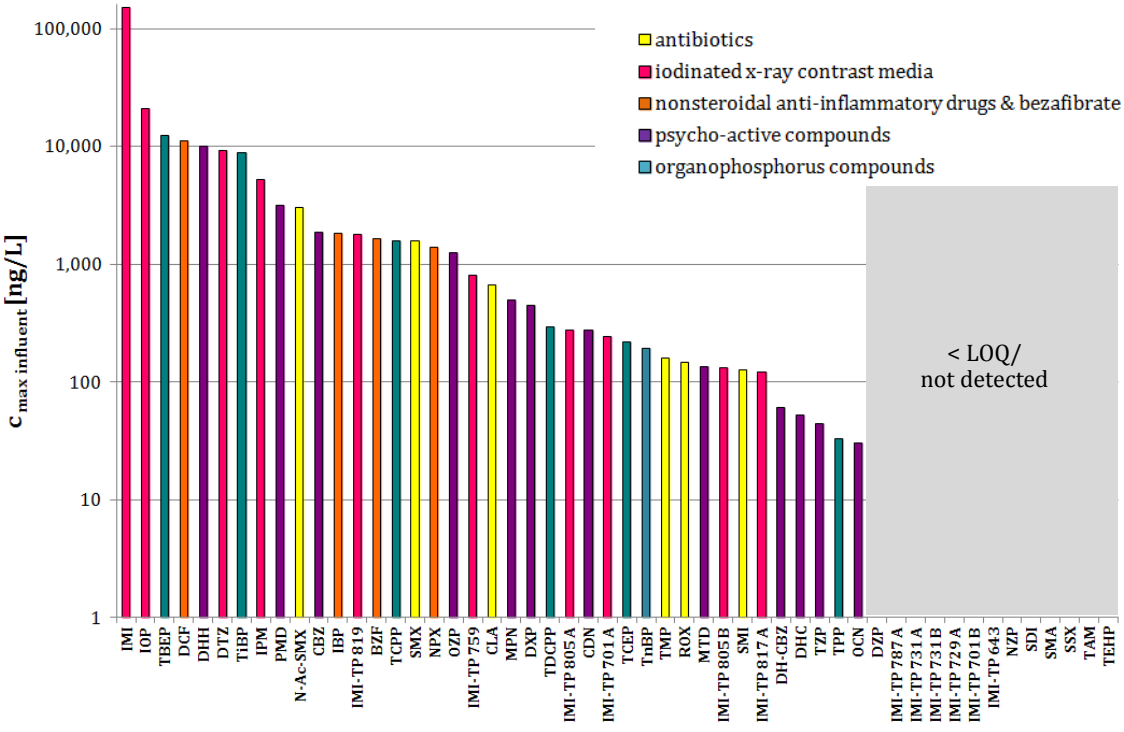


Figure 3-5: Maximum concentrations ($c_{max\ influent}$ [ng/L]) of each target compound in the influent of the NF-MBR. Colour codes show the compound group (including metabolites).

3.3.2.2 Weekly concentration profiles

Concentration profiles of the investigated xenobiotics for influent, concentrate and effluent as well as the removal rates over the course of the sampling campaign are shown in Figures 3-6 to 3-11. Removal rates were calculated by the equation: $(c_{inf}-c_{effl})\times 100/c_{inf,}$ where c_{inf} is the substance's concentration measured in the influent and c_{eff} the concentration found in the effluent (Santos et al., 2007). For reasons of clarification, values below LOQ were set to zero for the illustration of the removal rates in the graphs. Details regarding removal are discussed in Chapter 3.3.3.

ANTIBIOTICS. The amounts of antibiotics (Figure 3-6) used for the treatment of humans generally do not show a significant change in raw wastewater over the course of the week, whereas the veterinary antibiotic sulfadimethoxine shows a concentration profile suggesting isolated input events on particular days, such as run-off from agricultural land freshly top-dressed with manure.

ICMs. The ICMs show reduced concentrations at weekends with low inputs at Saturday and Sunday, which reflects their exclusive utilisation for diagnostics in radiological practices on working days (Figure 3-7). This pattern of occurrence was reported in previous studies (e.g. Ternes and Hirsch, 2000; Seitz et al., 2006). Moreover, whilst diatrizoic acid and iomeprol display high concentrations in raw wastewater throughout the week, with an evident drop only at the weekend, iopamidol shows distinctly elevated influent concentrations between Wednesday and Friday, which could possibly be explained by different temporal local use patterns due to specialised medical centres only being open certain days per week. The same can be assumed for iopromide, which shows elevated influent concentrations on Monday, Tuesday and Friday and concentrations similar to those at weekends on Wednesday and Thursday.

NSAIDs, BEZAFIBRATE AND PSYCHO-ACTIVE SUBSTANCES. As with antibiotics, the variations in the concentrations of NSAIDs and bezafibrate in raw wastewater show no distinct differences between weekdays and the weekend (Figure 3-8). Among the psycho-active drugs, only doxepin shows a distinct weekly pattern, with pointedly elevated concentrations at weekends (Figure 3-9, Figure 3-10).

OPs. The weekly distribution patterns of OPs are heterogeneous and do not show any particular trend (Figure 3-11). While most of the substances are present in the wastewater at stable levels, TBEP and TiBP show fluctuating amounts during the investigation period. It could be assumed that they are introduced to the wastewater through distinct events, by periodic activities or by periodically active point sources. Wide variations in the wastewater inflow were previously reported for TCPP (Bester, 2005), which is not in accordance with the presented data but emphasises possible inconsistencies in OP occurence in wastewater.

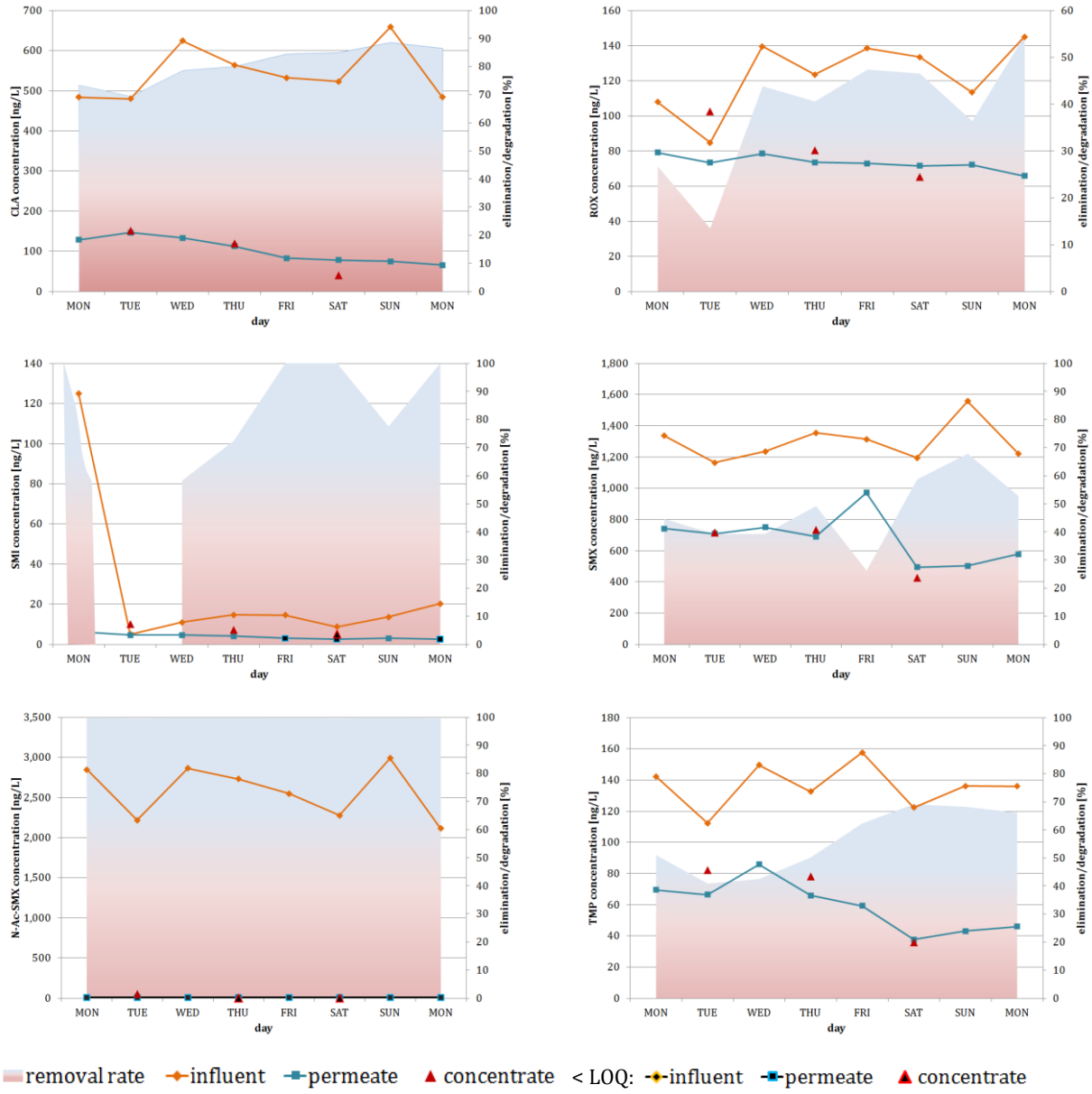


Figure 3-6: Concentrations [ng/L] of antibiotics in influent, permeate and concentrate from the NF-MBR pilot plant and correlating removal [%] over the course of the sampling campaign. Concentrations below LOQ are indicated by black-filled data point symbols.

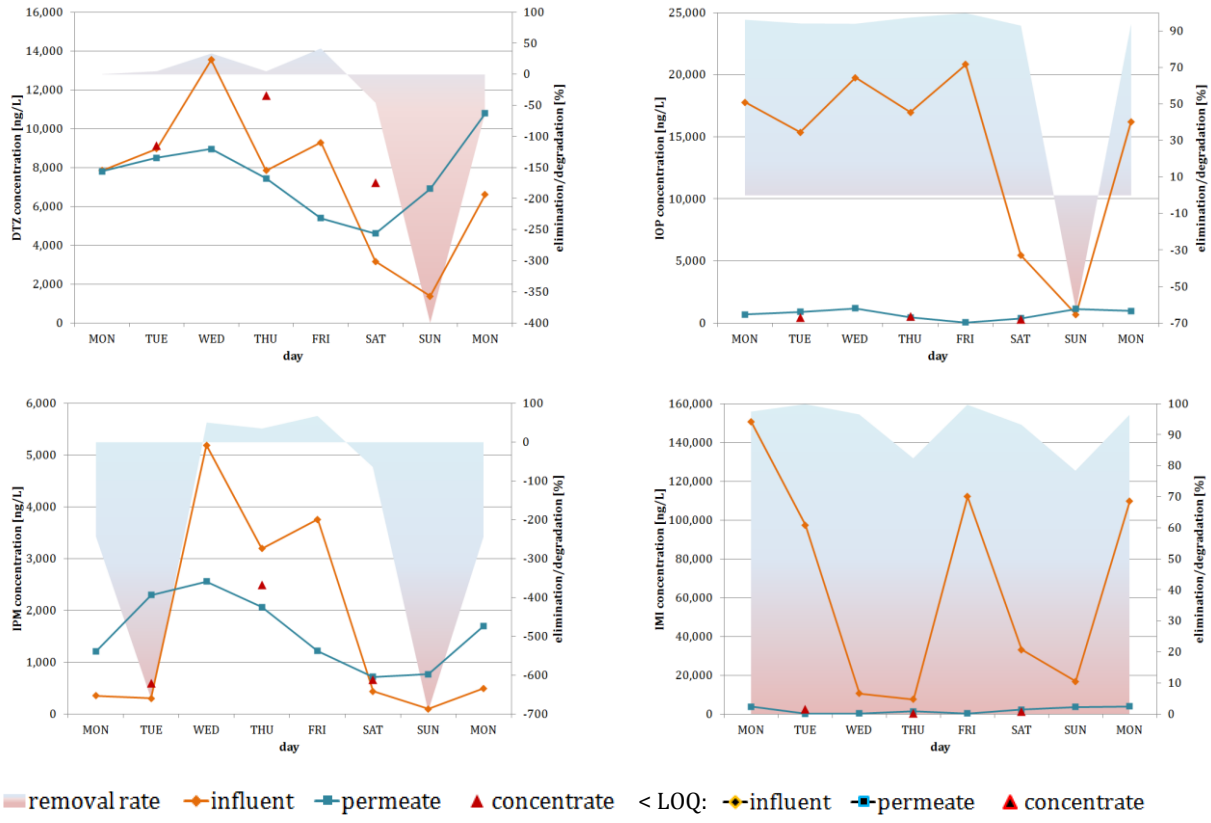


Figure 3-7: Concentrations [ng/L] of ICMs in influent, permeate and concentrate from the NF-MBR pilot plant and correlating removal [%] over the course of the sampling campaign. Concentrations below LOQ are indicated by black-filled data point symbols.

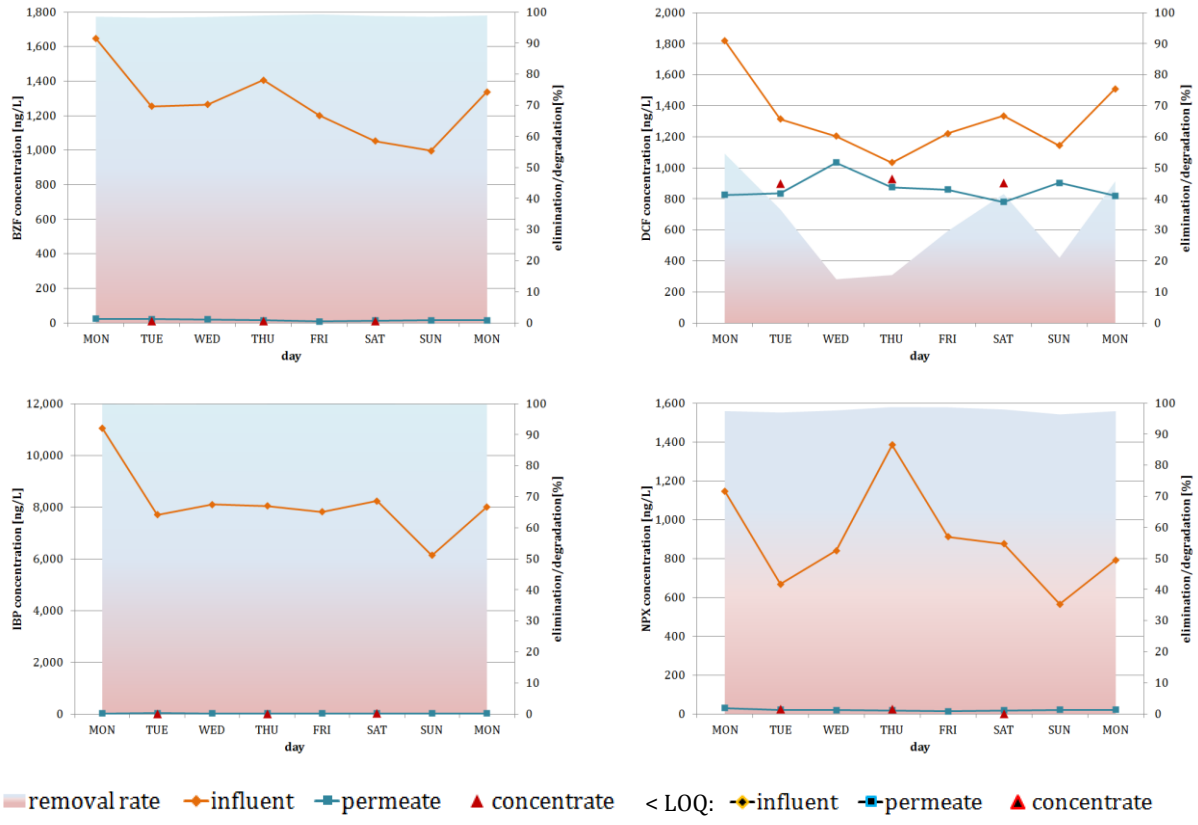


Figure 3-8: Concentrations [ng/L] of NSAIDs and bezafibrate in influent, permeate and concentrate from the NF-MBR pilot plant and correlating removal [%] over the course of the sampling campaign. Concentrations below LOQ are in indicated by black-filled data point symbols.

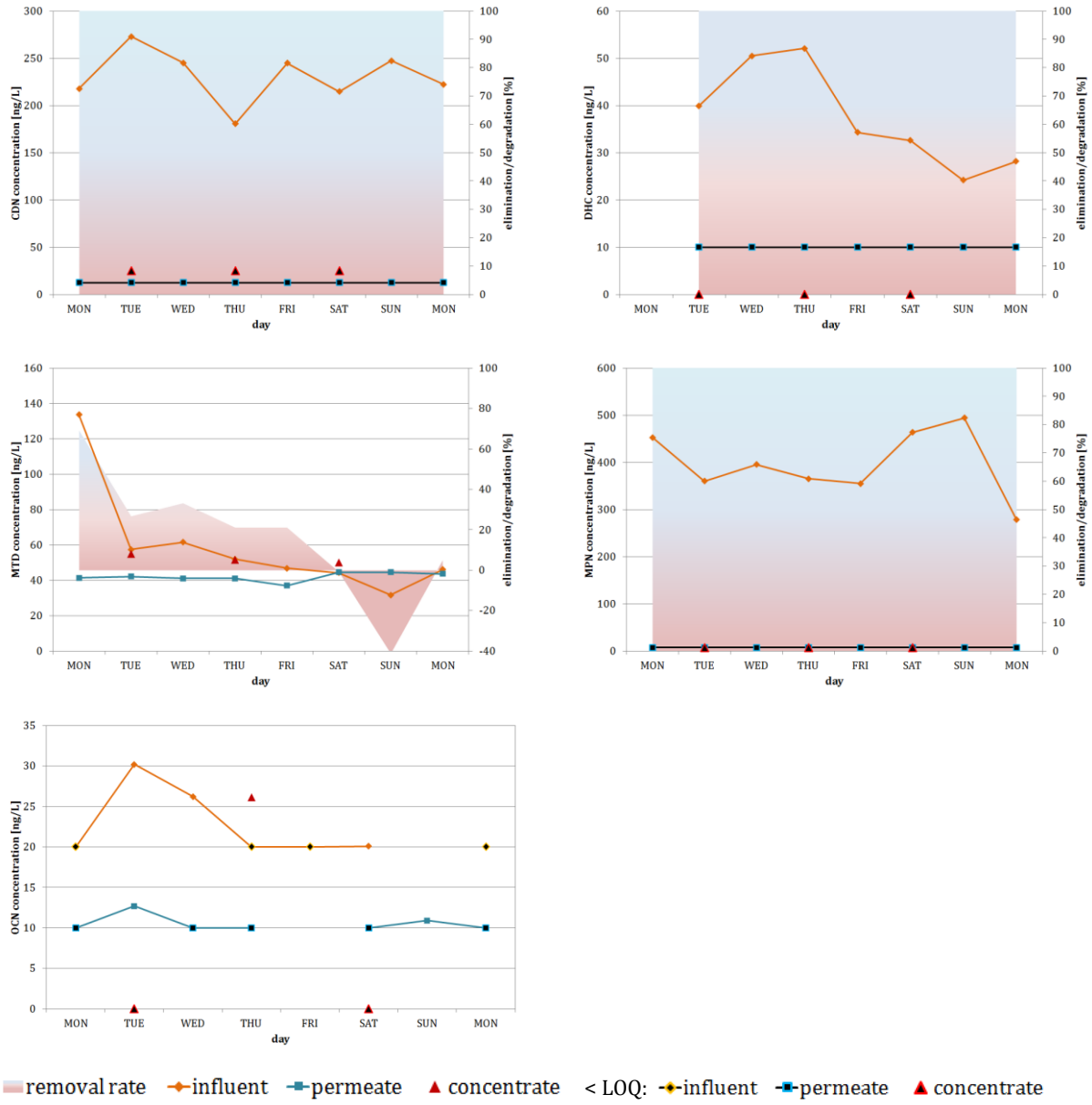


Figure 3-9: Concentrations [ng/L] of psycho-active drugs (opioids) in influent, permeate and concentrate from the MBR pilot plant and correlating removal [%] over the course of the sampling campaign. Concentrations below LOQ are indicated by black-filled data point symbols. For oxycodone no removal rates are given since most concentrations are below LOQ.

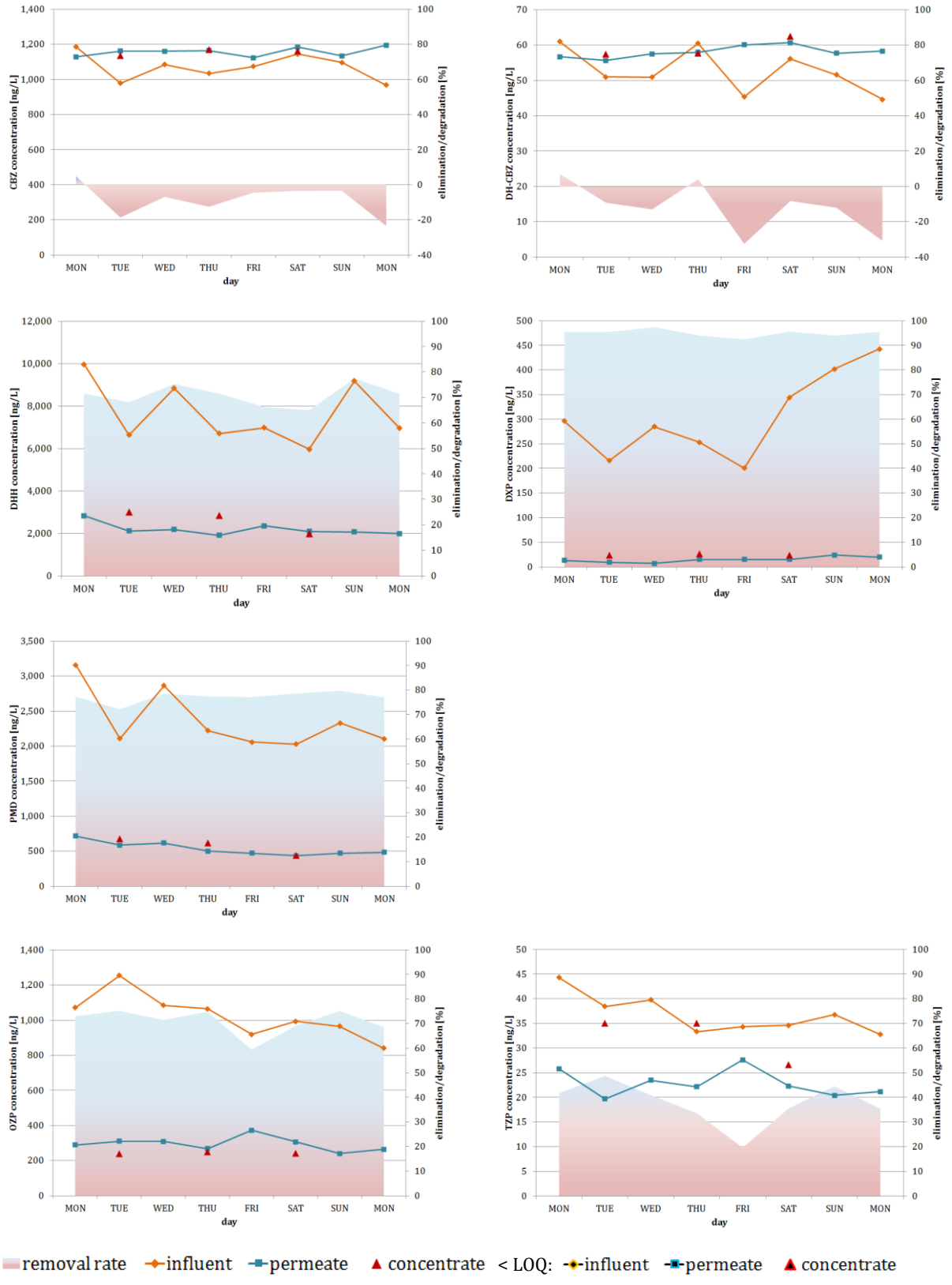


Figure 3-10: Concentrations [ng/L] of psycho-active drugs (carbamazepine and carbamazepine metabolites, doxepin, primidone and benzodiazepines) in influent, permeate and concentrate from the MBR pilot plant and correlating removal [%] over the course of the sampling campaign. Concentrations below LOQ are indicated by black-filled data point symbols.

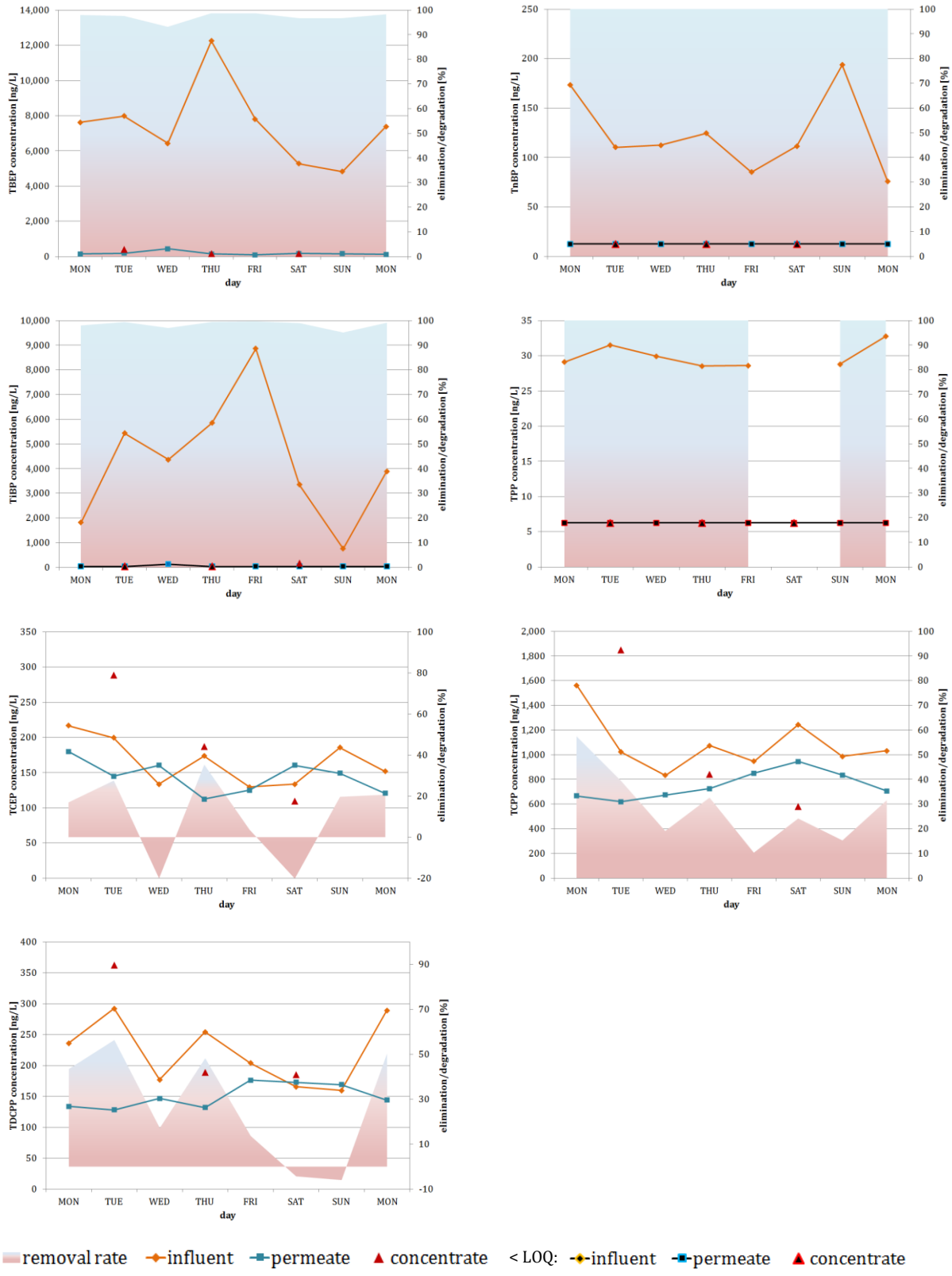


Figure 3-11: Concentrations [ng/L] of organophosphorous substances in influent, permeate and concentrate from the MBR pilot plant and correlating removal [%] over the course of the sampling campaign. Concentrations below LOQ are indicated by black-filled data point symbols.

3.3.3 Removal of xenobiotics from the aqueous phase by the NF-MBR

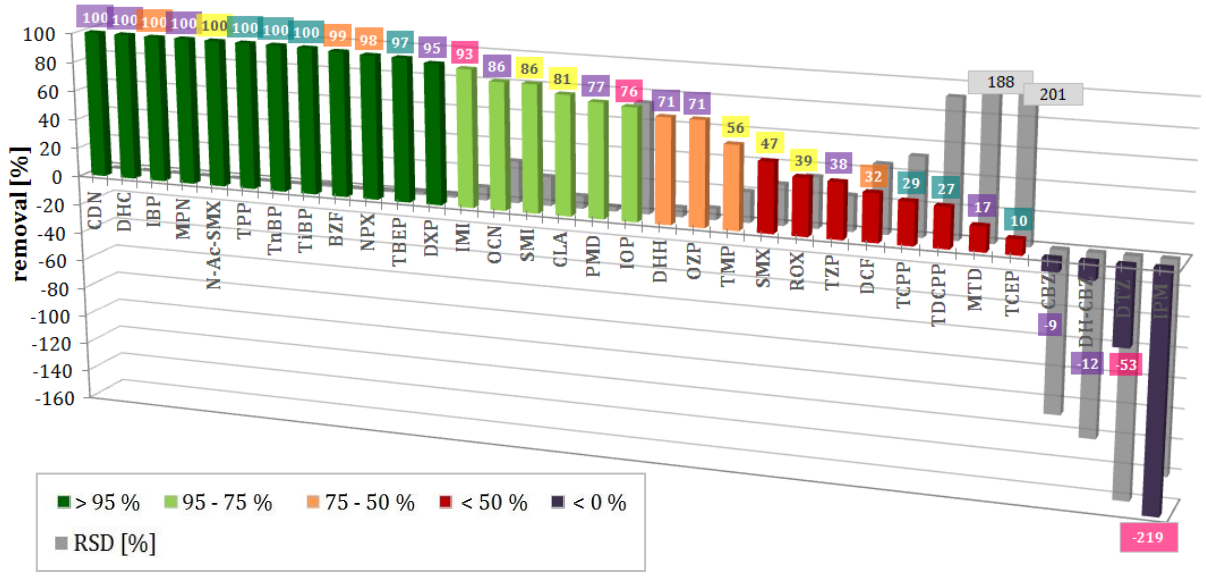


Figure 3-12: Average removal of xenobiotic compounds in the NF-MBR pilot plant. Colours of columns show very high (> 95% - dark green), high (75–95% - light green), moderate (50–75% - orange), poor (0–50% - red) and negative (< 0% - lilac) removal. Numbers above the columns depict the percentage of average removal and by colour code the compound group (colour code see Figure 3-5). The grey columns show the RSD [%].

Removal rates of the xenobiotics from the aqueous phase were estimated as mean values over the length of the studied period and classified into five categories (very high, high, moderate, poor, negative) as presented in Figure 3-12. As described before, removal in this context is to be understood as a summary of degradation and (bio-) transformation processes as well as elimination by NF filtration during treatment. Removal rates reaching the limit of quantification are given as 100%.

Overview. Very high removal rates were found for 12 compounds (4 psycho-active compounds, 1 antibiotic metabolite, 4 OPs, 3 NSAIDs). 6 substances showed high removal rates (2 ICMs, 2 NSAIDs, 2 psycho-active compounds). For 4 compounds moderate removal rates were found (1 psycho-active compound and 1 metabolite of a psycho-active compound, 2 antibiotics). 8 analytes were found to be poorly removed by the NF-MBR (2 antibiotics, 2 psycho-active drugs, 3 OPs, 1 NSAID). For 4 substances, carbamazepine and its metabolite DH-CBZ as well as for diatrizoic acid and iopamidol, negative removal was found. Possible errors in the determination of removal efficiency could have occurred by the exclusive use of filtered water without the consideration of pharmaceuticals possibly bound to particulate matter (for more details see Chapter 5). No correlations were found between removal and the physico-chemical properties of

the substances (see Chapter 2.1) (data not shown) except for a strong negative correlation with water solubility (r = –0.8).

NSAIDs. Earlier investigations studied the fate of xenobiotics in both laboratory-scale and pilot-plant MBRs typically equipped with MF- or UF-membranes. Radjenović et al., 2007 found high removal rates comparable to those presented here for ibuprofen (100%), naproxen (99%) and bezafibrate (96%) for a flat sheet MF-MBR (SRT indefinite) in which the effective porosity was that of an UF-membrane (0.01 µm), due to fouling processes on the membrane surface. In contrast, diclofenac removal was higher (88%) than in this study. In a later study (Radjenović et al., 2009b), the same author described similar results for ibuprofen (99%), naproxen (91%), bezafibrate (91%) and carbamazepine (no elimination) for a flat sheet MF-MBR (nominal porosity of 0.4 µm, fouling effect unknown), while the removal of diclofenac (66%) differed from the earlier study. Celiz et al., 2009 reported diclofenac reduction of 78% in a MF-MBR with indefinite SRT. In contrast to this, diclofenac removal in CAS treatment is reported to be very poor (approx. 20%) (Radjenović et al., 2009b; Rosal et al., 2010). A probable explanation is the presence of chlorine in the structure of this drug, which results in enhanced persistence and slow microbial degradation (Kimura et al., 2005; Reif et al., 2008; Tadkaew et al., 2011). MBR treatment offers longer SRTs than CAS treatment which allow for higher removal of a number of xenobiotic compounds, including diclofenac, due to longer contact time between microbes and xenobiotics, as well as the development of a more diverse microbial population (Kimura et al., 2007; Tambosi et al., 2009; for details about microbial specification, see Chapters 2.2.1, 2.2.2 and 2.2.4). In addition, not only sludge volume and sludge characteristics but also the pH at which the reactor is operated influence the removal efficiency of MBR treatment (Joss et al., 2006). Better removal of acidic compounds was found for pH values below the substances' pKa values (Urase et al., 2005) and put down to higher sorption to sludge when the substances change from their hydrophilic ionic forms to more hydrophobic forms. The high number of variants influencing MBR processes may explain why various studies reported no or very poor removal of diclofenac in MBR, despite SRTs over 70 days (Reif et al., 2008; Tadkaew et al., 2011).

The high removal rates (> 95%) for ibuprofen in MBR treatment consistently reported in the literature (Radjenović et al., 2007; Reif et al., 2008; Radjenović et al., 2009b; Tadkaew et al., 2011) are explained by the drug's readily biodegradable structure, with neither chlorine nor double aromatic rings (Kimura et al., 2005).

Previously reported naproxen removal rates in MBR treatment range from 40% (Tadkaew et al., 2011) to over 80% (Reif et al., 2008; Tambosi et al., 2010). No improvement in naproxen removal was found for prolonged SRTs by Tambosi et al., 2010, while Kimura et al., 2007 reported distinct improvements in removal with increasing SRT.

MACROLIDE ANTIBIOTICS. Regarding antibiotics, high removal rates (> 80%) for clarithromycin and sulfadimethoxine found in the presented study are contrasted by removal rates < 60% for sulfadoxine, trimethoprim, sulfamethoxazole and roxithromycin. While no literature data regarding MBR treatment are available for sulfadimethoxine, up to 90% removal of clarithromycin at SRTs of 60-80 days has previously been reported (Göbel et al., 2007). Under the conditions given in the presented study (pH-value in the MBR: 7.8–7.9), macrolides (pKa > 8.9) are positively charged through the protonation of the tertiary amino group (Göbel et al., 2005b) and removal is expected to be based largely on sorption to the predominantly negatively charged surface of activated sludge and only to a lesser extent on biodegradation (Abegglen et al., 2009). However, Göbel et al., 2005b found ionic interactions being of minor importance for the sorption of macrolides to sludge and pointed out that sorption must be influenced by other parameters which can vary in sewage, the latter being naturally a highly heterogeneous medium. The poor removal rate (39%) of the second macrolide investigated, roxithromycin, seems to emphasize the hypothesis that sludge sorption in the MBR studied is not strong. Additionally, low removal efficiency for roxithromycin can be explained by its complex chemical structure (see Chapter 2.1.1.1), which protects the drug against biological attacks (Tambosi et al., 2009). However, much higher removal rates of 57–82% were previously reported (Reif et al., 2008; Tambosi et al., 2010). Göbel et al., 2007 found removal rates rising from 39% for SRTs of 16 days to 60% for SRTs of over 33 days. This does not concur with the presented study, in which SRTs were well beyond 33 days. For a more detailed review of sorption behaviour of xenobiotics in activated sludge in the studied MBR, refer to Chapter 5.

INFLUENCE OF TEMPERATURE ON BIODEGRADATION. A possible explanation for the comparably low removal rates for compounds otherwise described as biodegradable can be found in the low temperatures the NF-MBR worked at: water temperatures of about 10 °C and a mean air temperature of 6.9 °C. These are conditions very unlike those normally used, especially for laboratory studies and, to a lesser degree, for indoor pilot plants. Besides the higher temperatures in indoor environments, small laboratory installations can additionally experience increases in water temperature resulting from the use of the filtration equipment when not actively cooled (Comerton et al., 2008). Biological processes in the MBR are temperature dependent (Zuehlke et al., 2006). Clara et al., 2005 showed that microbial activity in activated sludge doubles with every 10 °C increase in temperature, leading to a higher biodegradation of xenobiotics. Consequently, since the majority of MBR studies work at the laboratory scale or with indoor pilot plants, discrepancies between the results given in the literature and those found in the presented study are to be expected.

SULFONAMIDES & TRIMETHOPRIM. Unlike macrolides, the sulfonamide sulfamethoxazole is negatively charged at pH-values above 5.8, which renders sorption to sludge as a removal path negligible (Tambosi et al., 2009). At the same time, biodegradation is deterred by the antimicrobial properties of the compound (Tambosi et al., 2009). Thus, moderate to poor removal of about 50–60%, as was found in the presented study as well as in other investigations (Radjenović et al., 2007; Reif et al., 2008; Tambosi et al., 2010) seems plausible. However, higher removal rates of > 80% have also been reported (Radjenović et al., 2009b; Tadkaew et al., 2011). Increased SRTs seem to elevate removal (Tambosi et al., 2010). The apparently low removal rates found in several studies can be explained by the cleavage of the human metabolite N4-acetylsulfamethoxazole in wastewater, which is subsequently detected as the parent compound again (Radjenović et al., 2009b). In the presented study, the metabolite is amongst the ten highest concentrated substances in raw wastewater (see Chapter 3.3.2 and Figure 3-5), but practically completely absent in MBR permeate (see Figure 3-6), which points to the retransformation of N4-acetylsulfamethoxazole to sulfamethoxazole (Göbel et al., 2005b).
In the presented study, trimethoprim values were found to be halved by MBR treatment. Removal rates for the drug reported in the literature differ widely (e.g. 17% (Tadkaew et al., 2011), 36% (Reif et al., 2008), 67% (Radjenović et al., 2009b), 86%, 94% (Tambosi et al., 2010) and 97% (Celiz et al., 2009)). Enhanced removal with increasing SRTs was reported. Göbel et al., 2007 found 30% removal with SRTs of 33 days and less, while SRTs of 60–80 days resulted in removal rates of 87%. Similar results were reported by Tambosi et al., 2009. However, they are not consistent with the results of the presented study, where comparatively low removal was achieved at SRTs > 80 days.

PSYCHO-ACTIVE COMPOUNDS. Frequently, no or very poor elimination was reported for the psycho-active drug carbamazepine in MBR treatment (Clara et al., 2004b; Reif et al., 2008; Celiz et al., 2009; Radjenović et al., 2009b; Tadkaew et al., 2011), which is consistent with the results presented here. Changes in SRT have no influence on the elimination rate (Clara et al., 2004b). Possible reasons for the failing removal are retransformation processes in which human metabolites present in the raw wastewater are cleaved back into the parent compound (Miao et al., 2005). This could be supported by the fact that DHH is reduced considerably during treatment.
Removal rates of 12% were reported for primidone (Tadkaew et al., 2011), which is similar to the zero removal found for CAS treatment (Hummel et al., 2006). This is contradictory to the results of the presented study (77%). The reason for this could be the very high SRT in this study, which enables greater biodegradation.

ICMs. In accordance with the presented study, other researchers found that iopamidol and diatrizoic acid were not biologically degradable by MBR treatment (Abegglen et al., 2009). For iopromide and iomeprol, however, minor biodegradation was found, which is not supported by the presented study. A possible reason for different removal rates might lie in the details of the MBR treatment, since e.g. iopromide biodegradation is reported to be dependent of aerobic conditions (Abegglen et al., 2009).

THE MEMBRANE'S ROLE. To my knowledge, to date no data regarding the use of a NF-MBR for the treatment of raw wastewater have been published. In studies using the permeate of conventional MBRs as feed for downstream nanofiltration, profound improvement in the removal of xenobiotics was reported (Kim et al., 2007; Chon et al., 2011). While the elimination capacity of the MBR treatment in these studies was comparable to – or for some substances considerably less than – the elimination capacity in the presented study (e.g. Kim et al., 2007, removal of naproxen: 36%; trimethoprim, diclofenac: negative removal rates), concentrations for a range of compounds with moderate, poor or no removal in the NF-MBR of the presented study were reported to drop below the limit of detection after NF filtration, and the flame retardant TCEP was 85% removed (Kim et al., 2007; Chon et al., 2011).

These higher removal rates reported from downstream NF filtration of MBR permeate are likely to have been achieved by the use of tighter NF membranes than those applied in the presented study. Tighter membranes, however, would be difficult to use in direct contact with raw wastewater which has not been pre-treated. The combination of a full MBR treatment with a connected NF treatment downstream would, on the other hand, increase energy and maintenance costs drastically (see Chapter 2.2.4).

To summarise, the performance of the NF-MBR studied was for most compounds in the range reported for conventional MBR treatment. This suggests that a loose NF membrane as used here does not add greatly to the removal efficiency compared to UF, since many of the compounds not detained in UF filtration pass through loose NF membranes as well. Fouling processes seem not to improve their performance with regard to the substances in question.

3.3.4 Fate of metabolites and transformation products during MBR treatment

In this study, TPs of iopromide and metabolites of carbamazepine and sulfamethoxazole were investigated. As depicted in Figure 3-5 and Table 3-8, 6 out of 12 TPs of iopromide were found in influent samples as well as all three studied metabolites. In particular, the existence of environmental TPs in raw municipal wastewater indicates the likelihood of transformation processes taking place in the sewer system even before it reaches the WWTP.

TPs of iopromide. When comparing the maximum concentrations in the permeate from the MBR (Figure 3-13) with those in the influent (Figure 3-5), a drastic change is visible, with 9 out of the 10 highest concentrated analytes being TPs of iopromide in the permeate. Overall, all studied TPs are found in the permeate samples, 10 out of 12 with average concentrations higher than 1,000 ng/L (Table 3-8). This clearly demonstrates a) the formation of these substances within the MBR and b) the inability of the NF to retain them. The removal of the parent compound iopromide of over 90% (see above chapter and Figure 3-12) can be assumed to be completely caused by transformation, even though Figure 3-14 shows that the stacked concentrations of the TPs in the effluent do not equal the amount of the parent substance in the influent. This probably indicates more existing TPs which were not investigated in this study.

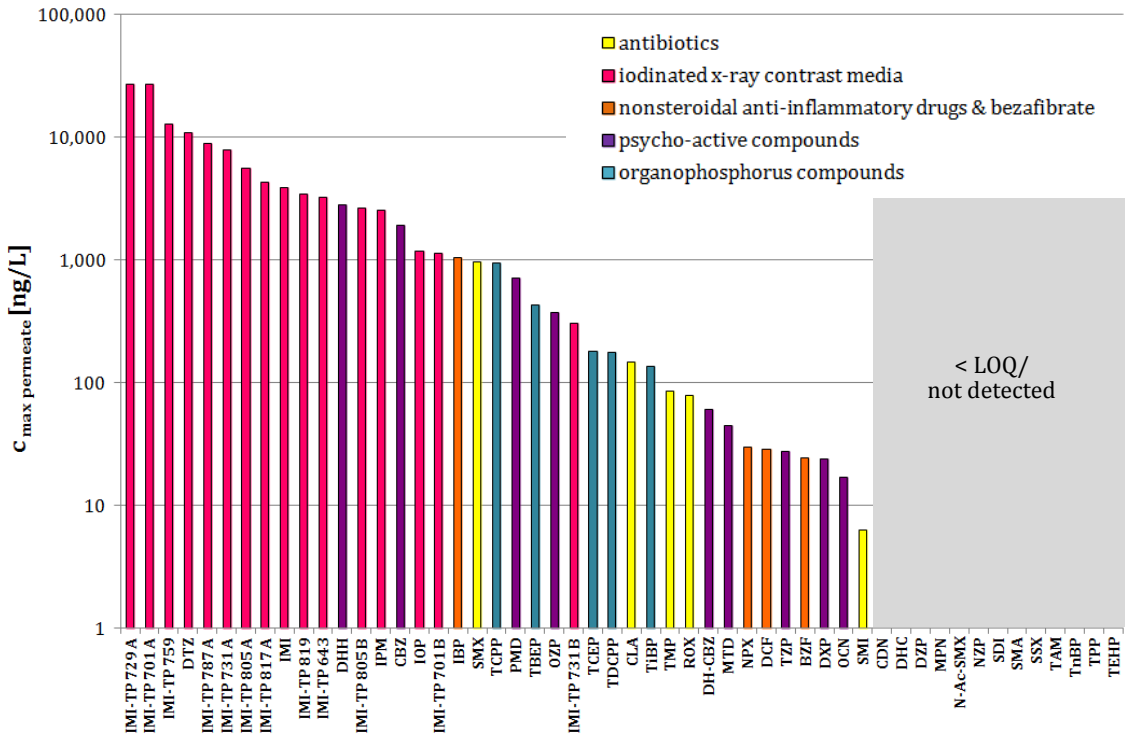


Figure 3-13: Maximum concentrations ($c_{max\ permeate}$ [ng/L]) of each target compound in the permeate from the NF-MBR. Colour codes show the compound group.

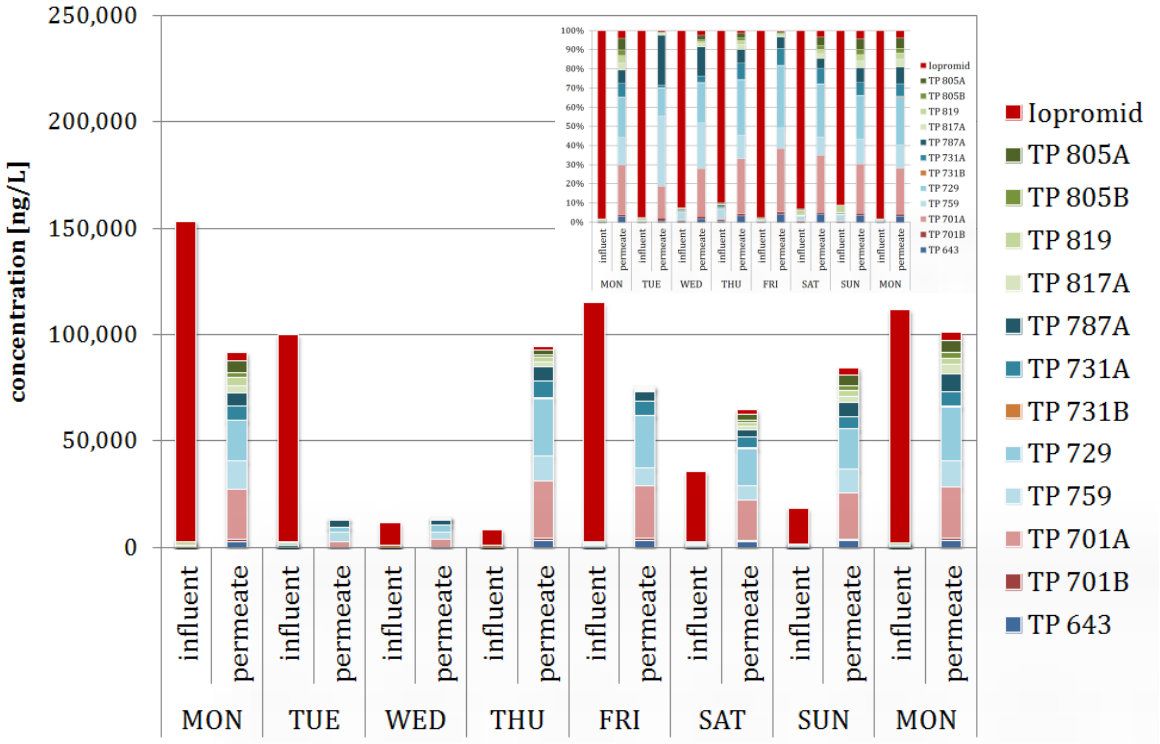


Figure 3-14: Concentration patterns of iopromide and its TPs in influent and effluent samples from the NF-MBR over the course of the study in [ng/L] and in [%] (small graph at the top right).

SULFAMETHOXAZOLE METABOLITE. The human metabolites investigated show a different pattern. While the amount of the parent drug, sulfamethoxazole, is approximately halved during NF-MBR treatment, its metabolite N4-acetylsulfamethoxazole is removed completely from the permeate water (Table 3-8; Figure 3-15). In previous studies, higher effluent values of sulfamethoxazole compared to corresponding influent values were described for CAS (Göbel et al., 2005b; Chang et al., 2008a), suggesting that a large amount of the metabolite present in the wastewater is cleaved, and re-transformation of the parent compound occurs. This mechanism is likely to occur in the NF-MBR as well; thus the estimated removal rate of 47% for sulfamethoxazole (Figure 3-12) is probably lower than the "true" elimination.

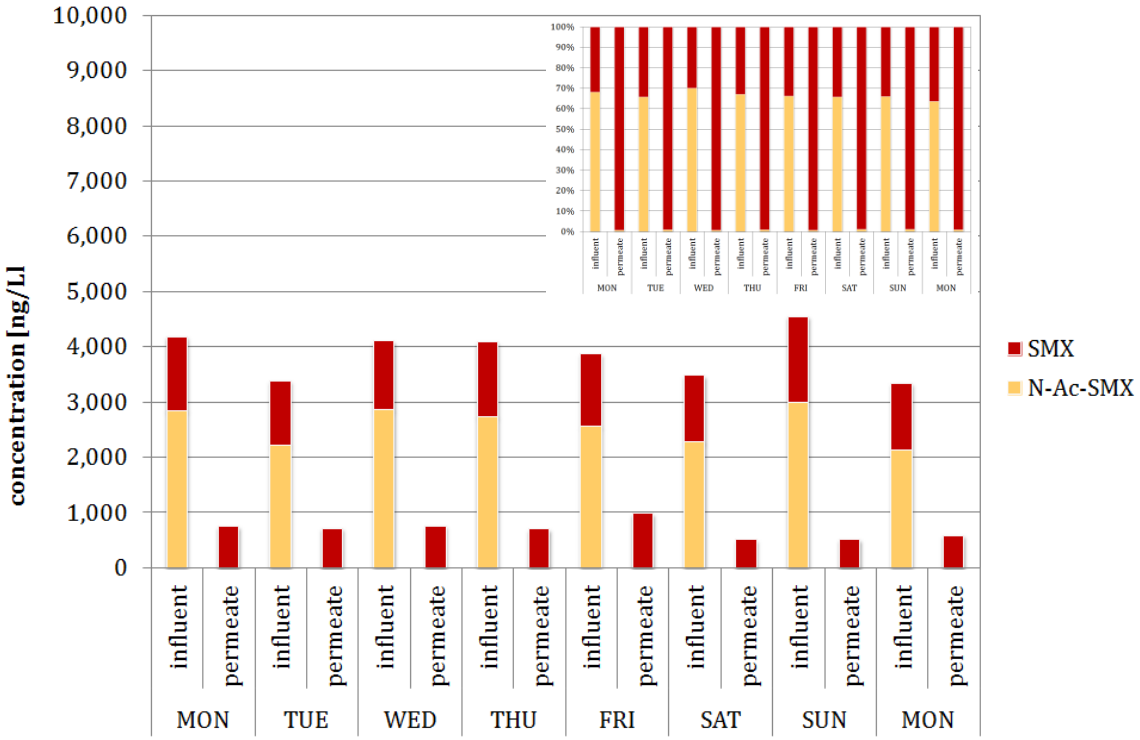


Figure 3-15: Concentration patterns of sulfamethoxazole and its metabolite N4-acetylsulfamethoxazole in influent and effluent samples from the NF-MBR over the course of the study in [ng/L] and in [%] (small graph at the top right).

CARBAMAZEPINE METABOLITES. The amounts of carbamazepine do not show any decrease during NF-MBR treatment (Figure 3-10, Table 3-8), which tallies well with earlier findings in both CAS and various MBR systems (Öllers et al., 2001; Clara et al., 2004b; Kreuzinger et al., 2004; Zuehlke et al., 2004; Bernhard et al., 2006; Gros et al., 2006a; Vieno et al., 2006; Radjenović et al., 2007; Santos et al., 2007; Vieno et al., 2007; Zhang and Zhou, 2007; Bo et al., 2008; Reif et al., 2008; Bo et al., 2009; Radjenović et al., 2009b; Ying et al., 2009; Pedrouzo et al., 2011; Ryu et al., 2011). In a study by Miao et al., 2005, amounts of the metabolite 10,11-Dihydro-10,11-dihydroxycarbamazepine (DHH) in wastewater were found to be up to three times higher than the parent compound. This is less than found in this study, where the DHH amount was 6.5 times higher than the parent substance in the influent of the system. During NF-MBR treatment, the DHH values were reduced by a factor of three. The second metabolite studied, DH-CBZ, shows low concentrations in the same range in both influent and effluent. Overall, the distribution pattern of carbamazepine and its metabolites is relatively stable during MBR treatment (Figure 3-16).

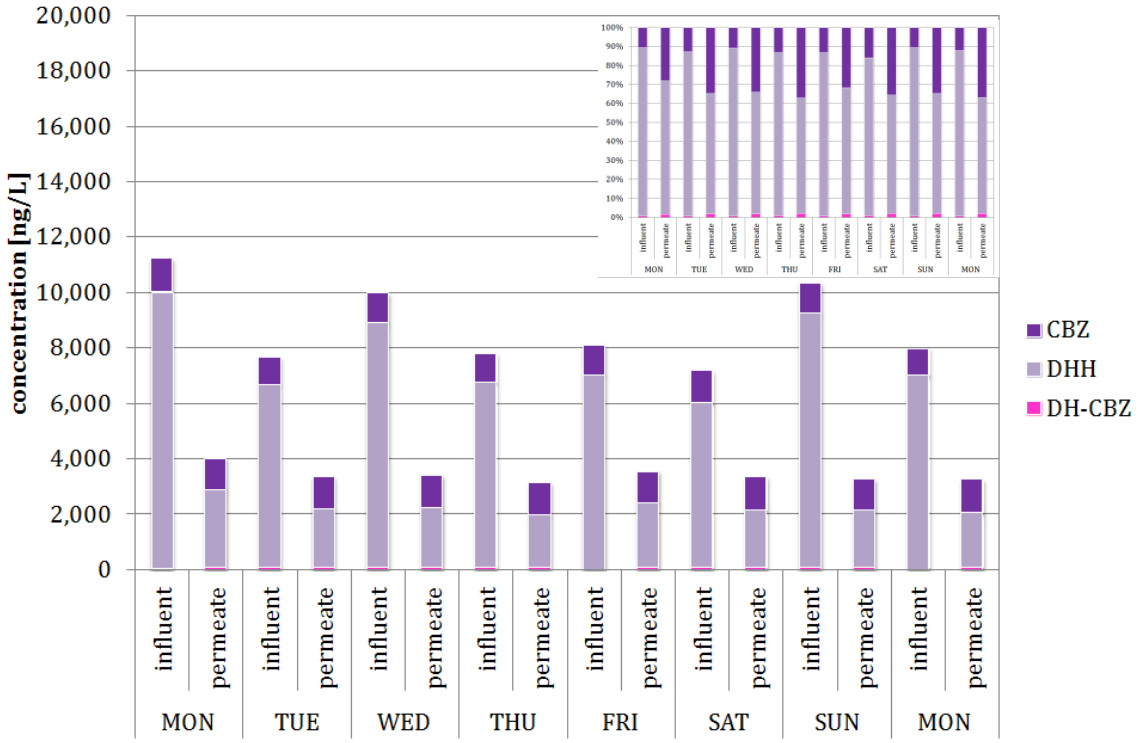


Figure 3-16: Concentration patterns of carbamazepine and its metabolites DHH and DH-CBZ in influent and effluent samples from the NF-MBR over the course of the study in [ng/L] and in [%] (small graph at the top right)

3.3.5 Removal of xenobiotics from the aqueous phase by pure NF

Overview. To compare the performance of NF-MBR technology with that of a pure NF process without biological treatment, feed water of the NF-MBR plant was treated with a NF lab-scale module and analysed for selected antibiotics, psycho-active compounds, ICMs and OPs. The module was operated for 5 days before sampling (for operation details see Chapter 3.2.3), and lost water was replaced daily with fresh influent water from the treatment plant. It was found that the feed water, despite the fact it came from the same treatment plant, showed a slightly different pattern of xenobiotic substances than the feed water of the NF-MBR: Ten substances which were regularly detected in the influent of the NF-MBR were not detected in the feed of the NF (sulfadimethoxine, morphine, oxycodone, oxazepam, TnBP, TiBP, TEHP, TPP, TCEP, TCPP). On the other hand, three substances (sulfadimidine, diazepam and nordiazepam) were detected in the NF feed which were not found even once in the feed of the NF-MBR (Figure 3-17). The nonappearance of a number of substances suggests sorption processes in the NF system were occurring despite the equilibration time of 5 days which was allowed before sampling, which should be sufficient for the establishment of membrane saturation according to results found in the literature (Verliefde et al., 2006; Comerton et al., 2008; Botton et al., 2012).

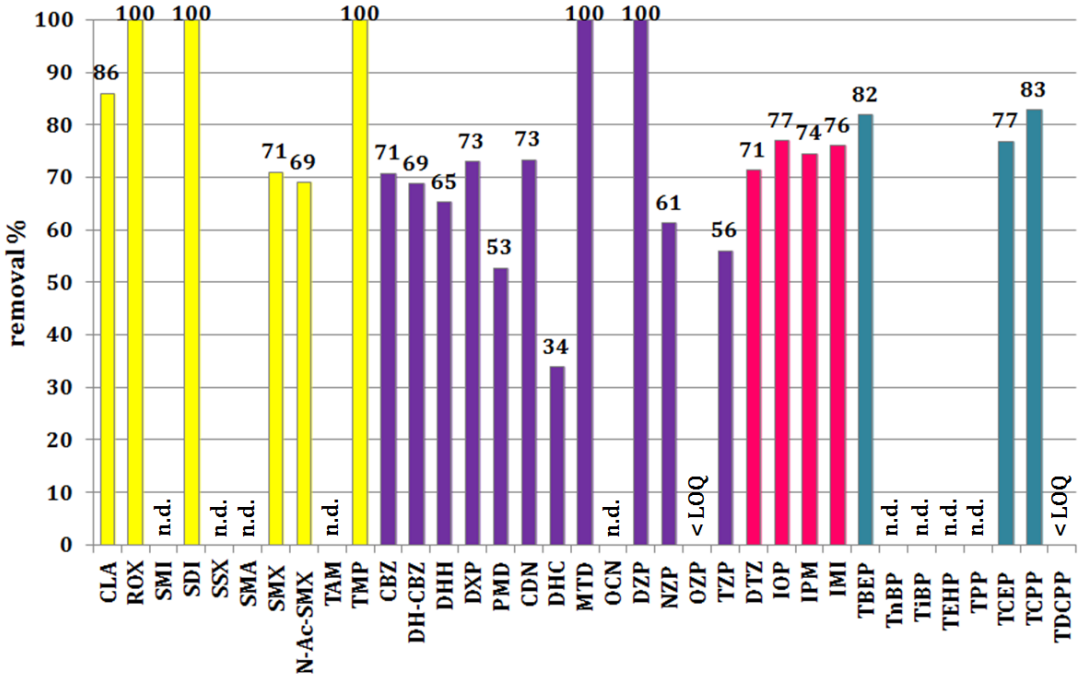


Figure 3-17: Removal of xenobiotics in pure NF treatment [%]. n.d. = not detected; < LOQ = below the limit of detection.

COMPARISON WITH NF-MBR. As illustrated in Figure 3-18, several xenobiotics show lower removal rates in pure NF treatment compared to NF-MBR treatment ($removal_{NF\text{-}MBR}/removal_{NF} > 1$), while for other compounds, higher removal rates were found during NF filtration ($removal_{NF\text{-}MBR}/removal_{NF} < 1$). For the substances showing less removal during NF treatment, biological degradation can be assumed to be the essential elimination path. Although it can be supposed that biological activity was not totally absent in the NF reactor due to the test conditions (surrounding temperature of 20 °C, presence of light and nutrients in the raw wastewater), it was certainly very low compared to that in the NF-MBR reactor, which accounts for the insufficient removal of dihydrocodeine, primidone, N4-acetylsulfamethoxazole, codeine, doxepin, iopromide and TBEP. The exclusive use of NF filtration is clearly not effective for their elimination in wastewater. Likely reasons lie in their physico-chemical properties, such as molecular weight, and their water solubility. Nonetheless, no correlations were found between retention by pure NF and physico-chemical properties of the xenobiotics (data not shown), which concurs with results reported in the literature (Comerton et al., 2008). For 10,11-Dihydro-10,11-dihydroxycarbamazepine and iomeprol, comparable removal between 70 and 80% was found for NF-MBR and NF treatment. This could suggest either that the bioprocesses necessary for the degradation are active even in the NF reactor or that their removal is governed by different processes during NF treatment. These processes are of especial interest with regard

to substances which show higher removal rates in pure NF treatment compared to NF-MBR treatment. For the positively charged antibiotics clarithromycin, roxithromycin and trimethoprim, sorption processes to the negatively charged membrane are the most likely reason for removal from the water phase. The properties of a new membrane are not yet altered by biofouling, thus the sorption could be greater than in a fouled membrane (Semião and Schäfer, 2010). For negatively charged compounds like sulfamethoxazole and sulfadimidine, high removal by a loose NF membrane can be put down to charge repulsion between the xenobiotic and the equally negatively charged membrane surface. Accordingly, Comerton et al., 2008 described 90% removal of sulfamethoxazole by a loose membrane from WWTP effluent water after a short membrane pre-treatment time (48 h). The repulsion effect as well as sorption processes are pronounced in unfouled membranes and are likely to change significantly with biofilm development over the course of the usage (Semião and Schäfer, 2010).

The seemingly higher removal of temazepam and 10,11-dihydrocarbamazepine in pure NF treatment could be explained by the missing microbial degradation of other benzodiazepines or benzodiazepine metabolites and carbamazepine and carbamazepine metabolites, respectively, in the NF reactor. As written before (see Chapter 3.3.3), microbial activity can lead to apparently increasing amounts of a substance during biological treatment, masking removal by continuous "production" of the substance by e.g. cleavage processes. Therefore, in the absence of considerable bioactivity, a seemingly higher removal rate is possible.

A similar process might be supposed to cause the occurrence of diazepam and nordiazepam in the feed water of the NF. Water refills of the feed water tank were carried out exclusively in the early mornings. Considering the low night temperatures during the sampling campaign, it could be assumed that microbial activity in the first clarifier (a relatively shallow, wide, open water basin) was low during the night. This might have prevented the biodegradation of diazepam and nordiazepam, which during the day was higher, and therefore the substances were not present in 24 h composite samples at concentrations above LOQ, whereas the concentration of the substances in the feed tank (very slightly) exceeded LOQ. This would be consistent with the fact that oxazepam, which is at the end of the metabolic pathway of a number of benzodiazepines (Hummel et al., 2006), was not found in the feed water of the NF. The complete removal by NF treatment of the small amounts of diazepam and nordiazepam cannot with certainty be attributed to true filtration elimination, but could be due to sorption – which is especially significant for compounds in low concentrations, where practically the whole amount of the substance finds sorption sites on the membrane and is temporarily removed from the aquatic phase (Semião and Schäfer, 2010).

Elimination of carbamazepine of about 70% by treatment with a loose NF membrane, as found in the presented study, has been reported before for pre-treated wastewater (Comerton et al.,

2008). Such high removal rates are not explainable by the physico-chemical properties of the substance. Due to its small molecular size, carbamazepine is not filtered out by loose NF membranes, and since the compound is neutral, neither charge repulsion nor sorption are expected to play any role. Several studies have suggested, though, that the dipole moment of a substance might be important for retention processes (Semião and Schäfer, 2010). As with other substance-membrane interactions, these processes change with the fouling of the membrane.

In the same way, the high removal rate of several further substances by pure NF treatment (methadone, TCEP, TCEP, diatrizoic acid and iopamidol), as well as the absence of substances from the feed water (sulfadimethoxine, morphine, TnBP, TiBP, TPP, TCPP), are probably also based on the above-mentioned mechanisms. Since specifications of the properties of the fouling layers of the membranes from NF- and NF-MBR reactors are beyond the scope of this study, the contradictory results cannot be conclusively explained. The fact that, even for well studied substances, removal mechanisms are neither completely clear nor safely predictable, illustrates the complexity of membrane related treatment.

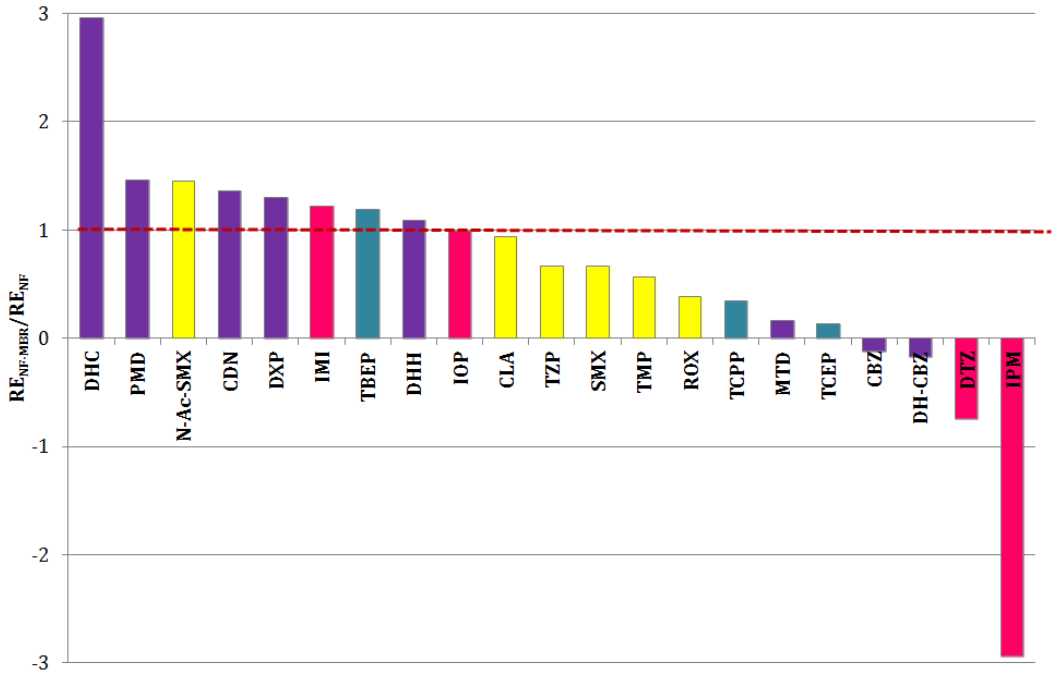


Figure 3-18: Removal comparison of the effectiveness of NF-MBR and pure NF treatment described as the ratio ($RE_{NF\text{-}MBR}/RE_{NF}$) with RE = removal [%]; colour code see Figure 3-5. Note that the negative values for CBZ, DH-CBZ, DTZ and IPM are based on negative removals (average removal rates from -9 to -219%) during NF-MBR treatment (see Figure 3-12) while NF treatment of these substances yielded positive removal rates of 69 to 74% (see Figure 3-17).

3.4 Conclusions

In this study, the presence and fate of 52 xenobiotics in municipal wastewater was studied. Their removal from raw wastewater by an NF-MBR equipped with a loose NF membrane was investigated. NF is thought to increase xenobiotic retention in comparison to conventional MBR treatment. At the same time, a loose NF is less cost- and maintenance-intensive than a tight membrane, which is prone to clogging up rapidly. However, despite higher removal capacities for some compounds, the utilisation of a loose membrane in a MBR reactor did not overall yield better results than have been reported for MBRs equipped with UF- or MF membranes. This was especially true for substances known to be biodegradable. This was caused by low microbial activity in the NF-MBR operated outdoors under winter conditions and reveals general restrictions to be taken into account in the use of MBRs.

The performance of the NF-MBR was compared to pure NF treatment. In the latter, biodegradable substances were removed less effectively, while comparably high removal of several substances could be assigned to physico-chemical xenobiotic-membrane interactions. Since these interactions are bound to change with membrane fouling, the positive results have to be assumed to be temporary and reversible.

In recent years there have been several attempts to provide schemes for the prediction of xenobiotic elimination in wastewater treatment. Thus, Joss et al., 2006 proposed a classification scheme for biological degradation in wastewater treatment which in principle could be applied to MBR treatment as well because of fundamental similarities (see Chapter 2.2). Tadkaew et al., 2011 proposed a qualitative framework for the prediction of trace organic removal by MBR treatment based on the molecular features hydrophobicity and molecular weight, and chemical structure. Bellona et al., 2004 developed a rejection scheme for organic micropollutants in membrane treatment. However, to date, the mechanisms contributing to the removal of xenobiotics in wastewater are far from being fully understood and are in some areas still unpredictable, thus obstructing the prediction of xenobiotic removal (Semião and Schäfer, 2010), as the results of this study have shown.

4. OCCURRENCE OF XENOBIOTIC ORGANIC MICROPOLLUTANTS IN RAW HOSPITAL WASTEWATER AND THEIR REMOVAL BY USE OF A PARTICLE-SUPPORTED BIOFILM REACTOR (PS-BFR)

Water has no taste, no colour, no odour; it cannot be defined, art relished while ever mysterious. Not necessary to life, but rather life itself.
– Antoine de Saint-Exupery (1900-1944), Wind, Sand, and Stars, 1939 –

4.1. INTRODUCTION

DECENTRALISED WASTEWATER TREATMENT: HOSPITALS. In recent years, hospitals have been discussed as possible point sources of large amounts of pharmaceuticals which normally reach the municipal wastewater stream untreated, where they are diluted and finally add to the pharmaceutical load of municipal WWTPs (for details, see Chapters 1 and 2.1). One possible way to reduce xenobiotic loads in both wastewater and receiving waters is source treatment, meaning the decentralised treatment of specific wastewater sources. Joss et al., 2006 identified this procedure as favourable since (biological) treatment is more promising in high-strength, undiluted raw wastewater at a point source.

PARTICLE-SUPPORTED BIOFILM. For numerous reasons, one being the high spatial footprint, conventional activated sludge treatment (CAS) is poorly suited to decentralised wastewater treatment at point sources (see Chapter 2.2.1). Suspended carrier biofilm processes (SCBPs), also referred to as particle-supported biofilm reactors (PS-BFRs), present a possible alternative. The PS-BFR set-up allows for a higher SRT resulting in a different composition of the microbial community, including slow growing microorganisms that are not present in suspended-growth systems involved in activated sludge treatment (Hall, 1987; van Loosdrecht and Heijnen, 1993; Nicolella et al., 2000b; for a detailed description of the principles of biofilm reactor and of the characteristic features of PS-BFRs, see Chapter 2.2.2).

XENOBIOTIC REMOVAL. PS-BFRs have proven their general suitability for wastewater treatment (Nicolella et al., 2000a,). However, to date very few studies have been published on the subject of the xenobiotic removal capacity of PS-BFRs. Results given in the literature are mainly based on

bench-scale installations, and are inconclusive. Thus, in a study utilising a fixed-bed reactor for the removal of diclofenac from wastewater, no removal was found to have taken place (González et al., 2006). This indicates that PS-BFRs are less efficient than CAS treatment, which usually achieves removal rates for diclofenac of some 10–60% (Verlicchi et al., 2012b). In contrast, other researchers reported that PS-BFRs are more effective than CAS treatment at removing several pharmaceuticals, including diclofenac (Falås et al., 2012; Falås et al., 2013). To my knowledge there are no studies reporting the use of particle-supported biofilm reactors for the treatment of xenobiotics in hospital wastewater.

AIM OF THIS STUDY. However, PS-BFRs seem to hold promise for this application since the undiluted raw wastewater is suspected to be sufficiently nutrient-rich to support a dense particle-supported microbial community. By providing a favourable environment for specialised, slow-growing microorganisms, the PS-BFR allows for the adaptation of the biocenosis to the specific environmental conditions in raw hospital wastewater (e.g. infrequent inflow, the possible presence of large amounts of disinfectants etc.). In turn, these specialised microorganisms might facilitate increased removal of xenobiotics e.g. by cometabolic degradation. Thus, a PS-BFR pilot plant was installed at the effluent of a municipal hospital. After a start-up phase of 199 days, concentration profiles of 47 xenobiotics – pharmaceuticals and organophosphorus compounds (OPs) – in influent and effluent were created over a period of one week. These profiles were used for characterising xenobiotic occurrence in hospital wastewater and estimating removal rates. The aim of this study was to investigate a) the temporal distribution patterns of pharmaceuticals (including metabolites and transformation products) and OPs in raw wastewater from a general hospital, and b) the removal capacity of a PS-BFR pilot plant (anoxic-oxic) with regard to these compounds in raw hospital wastewater.

4.2 MATERIALS AND METHODS

4.2.1 REFERENCE COMPOUNDS, CHEMICALS AND STANDARDS

Roxithromycin (ROX), carbamazepine (CBZ), oxazepam (OZP), temazepam (TZP), oxycodone (OCN), doxepin (DXP), primidone (PMD), diazepam (DZP), nordiazepam (NZP), methadone (MTD), sulfamethoxazole (SMX), trimethoprim (TMP) and tri-iso-butyl phosphate (TiBP) were purchased from Sigma-Aldrich, Seelze, Germany; clarithromycin (CLA), diatrizoic acid (DTZ), iopromide (IMI) were purchased from LGC Promochem Wesel, Germany; bezafibrate (BZF) diclofenac (DCF), ibuprofen (IBP), naproxen (NPX), tributyl phosphate (TnBP), tris(2-butoxyethyl) phosphate (TBEP), triphenyl phosphate (TPP), tris(1,3-dichloro-2-propyl)

phosphate (TDCPP), tris(2-chloroethyl) phosphate (TCEP), tris(2-ethylhexyl) phosphate (TEHP), tris(chloropropyl) phosphate (TCPP) were purchased from Dr. Ehrenstorfer, Augsburg, Germany; iopamidol (IPM), iomeprol (IOP) were provided from Bayer-Schering Pharma, Berlin, Germany; codeine (CDN), dihydrocodeine purchased from DHC, Th. Geyer, Renningen, Germany; 10,11-dihydrocarbamazepine (DH-CBZ) (Alltech, USA); 10,11-Dihydro-10,11-dihydroxycarbamazepine (DHH) bought from µ-Mol, Luckenwalde, Germany; morphine was purchased from MPN, Cambridge Isotopes Lab., Saarbrücken, Germany; N4-acetylsulfamethoxazole (N-Ac-SMX) was a self-synthesis by EAWAG, Dübendorf, Switzerland); iopromide TP 805 A (IMI-TP 805 A), iopromide TP 805 B (IMI-TP 805 B), iopromide TP 819 (IMI-TP 819), iopromide TP 729 A (IMI-TP 729 A), iopromide TP 817 A (IMI-TP 817 A), iopromide TP 787 A (IMI-TP 787 A), iopromide TP 731 A (IMI-TP 731 A), iopromide TP 731 B (IMI-TP 731 B), iopromide TP 759 (IMI-TP 759), iopromide TP 701 B (IMI-TP 701 B), iopromide TP 701 A (IMI-TP 701 A), iopromide TP 643 (IMI-TP 643) were laboratory-prepared as described by Kormos et al., 2009. (E)-9-[O-(2-methyloxime)]-erythromycin (EM-ERY) was self-synthesised according to Schlüsener et al., 2003.

From the following suppliers the internal standards (IS, analytical grade > 98% purity) were purchased: codeine-d6, diazepam-d5, methadone-d9, morphine-d6, nordiazepam-d5, tributylphosphate-d27 (Cambridge Isotopes Lab., Saarbrücken, Germany); oxazepam-d5 (Sigma, Deisenhofen, Germany); 13C-15N-carbamazepine, diatrizoic acid-d6, diclofenac-d4, ibuprofen-d3, iomeprol-d3, iopamidol-d8 (Campro Scientific, Berlin, Germany); bezafibrate-d4, sulfadimethoxine-d_4, sulfadimidine-d_4, sulfamerazine-d_4, sulfamethoxazole-d4, and N4-acetylsulfamethoxazole-d4 (Toronto Research Chemicals, North York, ON, Canada), desmethoxy-iopromide (DMI) (Bayer-Schering Pharma, Berlin, Germany); triphenylphosphate-d15 (TPP-d15) was self synthesised according to Andresen et al., 2004.

All organic solvents (n-heptane, n-hexane, acetone, methanol, acetonitrile, ethyl acetate) were picograde and purchased from Merck (Darmstadt, Germany). Pure water obtained from a Milli-Q system (Integral 3/5/10/15, Millipore, Billericis, MA, USA) was used. Formic acid (98–100%) was ACS grade and purchased from Merck (Darmstadt, Germany).

For each analytical compound group (antibiotics, psycho-active drugs and organophosphorus compounds) a standard solution of all target analytes and an internal standard mix at a concentration of 10 µg/mL (OPs: 5 µg/mL) and 1 µg/mL respectively were prepared in methanol and stored in the dark at 4 °C.

4.2.2 THE PS-BFR PILOT PLANT

FACILITY. The PS-BFR pilot plant (EMW Filtertechnik GmBH, Diez, Germany and Aquadetox International GmBH, Altmannshofen, Germany) was installed at the main effluent of the municipal hospital in Giessen. The hospital has 186 beds and about 200 medical staff. The hospital treats roughly 10,000 inpatients as well as 17,250 outpatients per year (Agaplesion Evangelisches Krankenhaus, 2013). Additionally, the hospital hosts a range of specialised healthcare centres exclusively for the treatment of outpatients. These include a group radiology practice where some 10,000 computer tomographies (CT) and 10,000 magnetic resonance tomographies (MRT) are carried out per year, the majority of them using X-ray contrast media (Evangelisches Krankenhaus Mittelhessen, 2009). The hospital and clinics together offer the whole range of medical services (e.g. surgery, internal medicine including cardiology and angiology, maternity clinic and geriatric medicine). The hospital's annual wastewater discharge has been estimated as some 20,000m^3. The main sewer receives water from the different wards, clinics and departments as well as from the hospital kitchen, but not water from the laundry or rainwater, which are discharged via a separate sewer.

REACTOR SETUP. Water from the main sewer was pumped into the pilot plant approximately every 15-30 min between 7 a.m. and 7 p.m., depending on the wastewater volume in the sewer, with filling levels automatically controlled by sensors. At night, no wastewater was fed into the pilot plant. The pilot plant was housed in a 6 metre overseas shipping container and consisted of a primary settling tank, two sequential biofilm reactors and a secondary settling tank. All tanks and reactors were made of stainless steel. The biofilm reactors were constructed to hold a volume of 2 m^3 each. 30% of the volume of each reactor was filled with polyurethane carriers (polyurethane foam in the form of 30 x 30 x 30cm cubes, bulk density: 22.5–27.5 kg/m^3, specific surface area: 2,000 m^2/m^3, EMW Filtertechnik GmBH, Diez, Germany, Figure 4-1), which equals 0.7m^3 of carrier material per reactor. One third of the carrier material used consisted of PORET®aqua carriers pre-inoculated by the manufacturer with bacteria cultures typically used for wastewater treatment (EMW Filtertechnik GmBH, Diez, Germany), before being installed in the reactors.

MODE OF OPERATION. The pilot plant was allowed a start-up phase of 199 days before the beginning of the sampling campaign to reach a stable state. 35 days prior to the sampling a final adjustment was made to the aeration, in which the first biofilm reactor was set up as a denitrification unit. The mean amount of dissolved oxygen in the first reactor during the sampling period was 0.355 mg/L, while the second biofilm reactor displayed a higher mean value of 3.41 mg/L. Gas sparging was used to keep the biofilm carriers in suspension. The

reactors incorporated 6 aerator candles each. By operating only the two middle aerators in reactor 1 and the two outer ones in reactor 2, liquid circulation in the reactors was achieved due to the gas hold-up difference between sparged and unsparged zones with different fluid densities, thus creating in principle a biofilm airlift suspension reactor (BAS, see Chapter 2.2.2). However, aeration was discontinuous, with aeration periods of 15 sec and 100 sec followed by aeration-free intervals of 750 sec and 380 sec in reactor 1 and 2 respectively. During the unsparged intervals the particles did not sink to the bottom of the reactors but created a fluidised bed of biofilm carriers in the upper half of the reactors. The pilot plant thus combined in one application the two typical types of suspended carrier biofilm processes (SCBP, see Chapter 2.2.2).

The wastewater feed volume into the pilot plant was $2 m^3/d$. $8 m^3/d$ of treated wastewater was recirculated from the secondary settling tank back into biofilm reactor 1 (denitrification). The HRT of the pilot plant during the sampling time ranged from 44 to 51 h (average HRT: 47.5 h). The chemical oxygen demand (COD) was 327 mg/L and 69.4 mg/L in influent and effluent, respectively.

The average water temperature during the sampling period was 22 $\pm$ 1.5 °C, 18 $\pm$ 0.8 °C and 19 $\pm$ 0.9 °C in the influent, the first and second biofilm reactor, respectively. The average pH values of influent and effluent were 7.95 $\pm$ 0.247 and 7.44 $\pm$ 0.058, respectively.

4.2.3 Sampling and sample preparation

24-h-composite samples of influent (primary clarifier tank) and effluent (secondary clarifier tank) of the biofilm reactor were continuously collected by a peristaltic pump over a period of 9 days with an offset of 48 h between influent and effluent samples to account for the HRT (see Chapter 4.2.2). Thus, an actual time series over 7 days was accomplished. All glassware used during sampling and extraction was pre-cleaned with ultra pure water, heptane and acetone, and subsequently heated overnight at > 240 °C. All samples were cooled during sampling, transported on ice in the dark to the laboratory directly after sampling was terminated, and were extracted within 4 h after arriving.

4.2.4 Analytical procedures and method validation

The details of the entire analytical procedure are described elsewhere (see Chapter 3.2.5). In short, samples were filtered, subsequently diverted in aliquots for the analysis of various compound groups (neutral analytes, acidic analytes, iodinated x-ray contrast media and their TP), cleaned, and pre-concentrated via off-line solid phase extraction. Analysis was then carried

out by multiple LC-MS/MS runs for acidic analytes, antibiotics, ICMs and TPs of iopromide, psycho-active compounds and OPs.

Figure 4-1: Biofilm carriers. Left: 1st generation, unused and after biofilm development. Middle: Carriers applied in PS-BFR 1. Right: 2nd generation of carrier material (PORET®aqua). Photo: J. Krisam (left), U. Kraus (middle), EMW (right)

4.3 Results and discussion

4.3.1 Method validation

Quality assurance and quality control (QA/QC) of the applied analytical procedures as well as calibration data, LOQ and blank values have already been described elsewhere (see Chapter 3.3.1).

The accuracy of the analytical protocols for the matrix of the studied hospital wastewater was determined by estimating the relative recovery of target compounds spiked into native influent and effluent samples before extraction, and subsequently subtracting the amount of native samples from the results found in the spiked samples (Table 4-1, see also Chapters 3.2.6 and 3.3.1).

For ICMs, spiked samples yielded concentrations above the calibration range, and therefore the accuracy of ICM measurement was not determinable for the hospital wastewater. However, the accuracy of ICM measurement in other wastewater types has been described previously and was found to be satisfactory (see Chapter 3.3.1).

Table 4-1: Accuracy of the analytical procedure [%] for influent and effluent and instrumental precision (intraday/interday) for the five analytical compound groups.

	Accuracy [%]				Instrumental precision [RSD, %]	
	Influent [1]		Effluent [2]		intraday[6]	interday[7]
	Average	*SD*	Average	*SD*		
			Antibiotics			
CLA	89	9	113	23	7	7
ROX	93	10	100	16	3	21
SMX	125	40	89	3	4	11
N-Ac-SMX	93	36	83	---[3]	5	6
TAM	76	23	84	31	3	11
TMP	38	6	90	20	8	15
min	38	6	83	3	3	6
max	125	40	113	31	8	21
			ICMs			
DTZ	a.r.[5]	a.r.[5]	a.r.[5]	a.r.[5]	8	8
IOP	a.r.[5]	a.r.[5]	a.r.[5]	a.r.[5]	8	12
IPM	a.r.[5]	a.r.[5]	a.r.[5]	a.r.[5]	10	12
IMI	a.r.[5]	a.r.[5]	a.r.[5]	a.r.[5]	4	9
min	a.r.[5]	a.r.[5]	a.r.[5]	a.r.[5]	4	8
max	a.r.[5]	a.r.[5]	a.r.[5]	a.r.[5]	10	12
			NSAIDs and bezafibrate			
BZF	86	18	148	---[3]	4	26
DCF	48	6	93	---[3]	4	13
FNP	90	15	68	---[3]	8	12
IBP	87	---[3]	38	---[3]	5	4
NPX	57	9	43	---[3]	9	12
min	48	6	38	---[3]	4	4
max	90	18	148	---[3]	9	26
			Pycho-active compounds			
CBZ	103	8	108	0	2	3.4
DH-CBZ	88	5	99	1	1	8.2
DHH	103	18	152	---[3]	8	8.2
PMD	175	12	169	---[3]	8	10.8
DXP	39	7	40	---[3]	18	19.6
CDN	85	10	90	10	3	5.9
DHC	127	18	97	17	6	6.8
MTD	84	3	79	9	3	8.5
MPN	71	10	46	---[3]	12	n.d.[4]
OCN	65	9	108	---[3]	6	16.6
DZP	107	6	109	1	3	5.8
NZP	90	5	98	1	3	2.7
OZP	127	23	131	3	10	9.0
TZP	64	5	76	2	4	5.7
min	39	3	40	0	1	3
max	175	23	169	17	18	20
			OPs			
TBEP	94	23	n.d.[4]	n.d.[4]	1.45	7.3
TnBP	51	3	48	4	2.01	4.9
TiBP	49	12	47	7	3.50	5.9
TEHP	43	3	62	8	1.68	10.3
TPP	67	7	20	3	0.92	8.5
TCEP	96	11	97	11	3.36	8.7
TCPP	96	8	91	14	3.00	8.0
TDCPP	100	11	86	18	1.76	6.8
min	43	3	20	3	1	5
max	100	23	97	18	4	10

[1]n=3-6; [2]n=2; [3]n=1; [4]n.d. = not detected; [5]a.r. = above concentration range; [6]n=4-8; [7]n=8-12

4.3.2 Occurrence of xenobiotic micropollutants in hospital wastewater

Table 4-2 shows the average concentrations of the target substances over the course of the study. Out of 47 analytes in total, 3 were not found in any samples (methadone, diazepam, TEHP) while 7 more were only detected in concentrations below LOQ: the ICM iopamidol, 5 transformation products of the ICM iopromide (IMI-805 b, IMI-787 a, IMI-731 b, IMI-729 a, IMI-701 b) and the OP TPP. OPs are generally regarded as being ubiquitously distributed. However, the most common applications of TPP (e.g. use in automobiles, electronic housings, varnishes, lubricant and hydraulic fluids, see Chapter 2.1.2) seem to be of minor importance in the case of hospital wastewater. The absence of ICM transformation products in raw hospital wastewater is to be expected, since no environmental degradation can have taken place since excretion after being administered.

4.3.2.1 Untreated hospital wastewater

While one of the ICMs investigated was not found in any samples at all (see above), by far the most highly concentrated target substances in raw hospital wastewater were also substances in this compound class (Figure 4-2): diatrizoic acid, for which a maximum concentration of over 1.6 mg/L (1,605,781 ng/L) was found, and iomeprol, at over 0.6 mg/L (656,846 ng/L). While the other target analytes were considerably less concentrated, still 20 substances were found in maximum concentrations of over 1,000 ng/L: nine ICMs and TPs of ICMs (diatrizoic acid, iomeprol, IMI-TP 805 A, IMI-TP 819, iopromide, IMI-TP 731 A, IMI-TP 701 A, IMI-TP 759, IMI-TP 817 A), two antibiotics (sulfamethoxazole, clarithromycin) and an antibiotic metabolite (N4-acetylsulfamethoxazole), two NSAIDs (ibuprofen, diclofenac), one lipid regulator (bezafibrate), three psycho-active substances and one of their metabolites (Oxazepam, Primidone, Carbamazepine, 10,11-dihydro-10,11-dihydroxycarbamazepine) as well as the organophosphate TBEP.

Antibiotics. Amounts of antibiotics found in raw hospital wastewater have been reported as differing widely, both over time at the same hospital and also between hospitals with different specifications and medical services (Lindberg et al., 2004; Chang et al., 2010; Gros et al., 2013; Santos et al., 2013). Taking this into account, the concentrations of antibiotics found in the presented study largely corroborate those previously reported (e.g. Gómez et al., 2006; Sim et al., 2010). Considerably higher roxithromycin values, with average values exceeding 2,000 ng/L, were reported from hospital effluents in China (Chang et al., 2010), while various European studies showed roxithromycin concentrations similar to those found in the presented study (Kovalova et al., 2012; Gros et al., 2013). Verlicchi et al., 2012a, investigating the composition of the same Italian hospital wastewater in summer and winter times, only found roxithromycin

during the winter season (Verlicchi et al., 2012a). Remarkably higher trimethoprim concentrations than found in other studies – and tenfold higher than found in the presented study – were reported from two hospitals in Oslo, Sweden (Thomas et al., 2007).

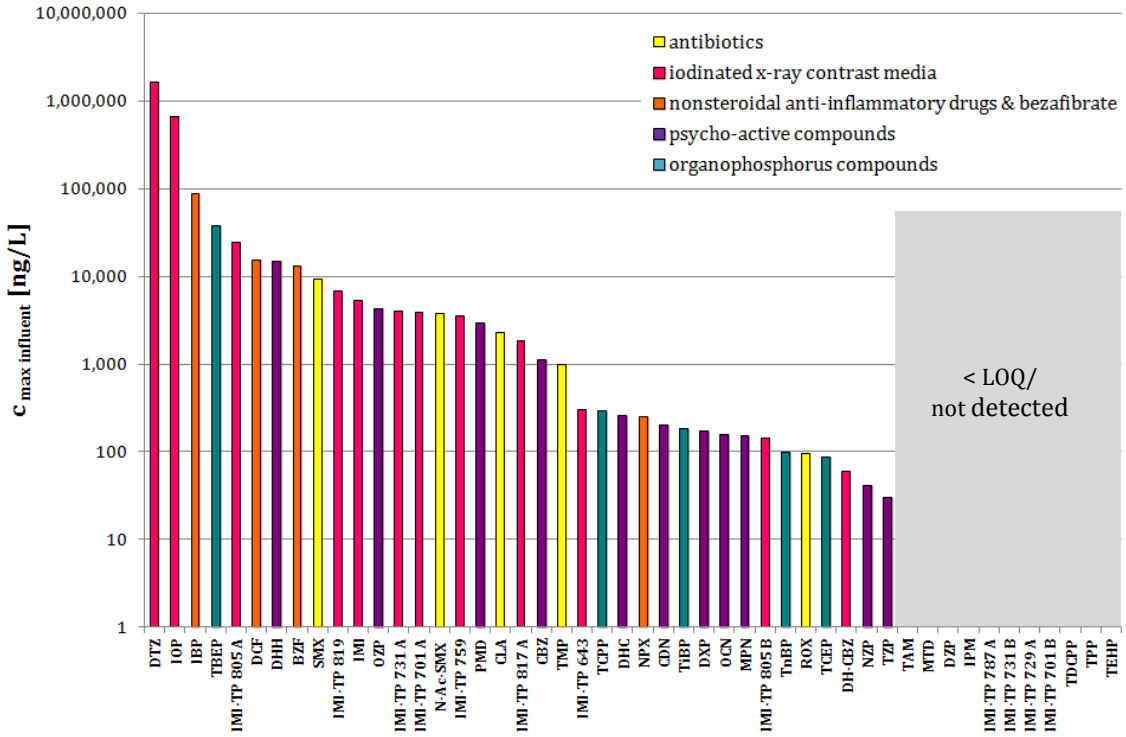


Figure 4-2: Maximum concentrations (c_{max} influent [ng/L]) of each target compound in the influent of the PS-BFR. Colour codes show the compound group (including metabolites).

ICMs. Diatrizoic acid concentrations found in raw wastewater from a Swiss hospital (Kovalova et al., 2012) were only a third of those in the presented study. On the other hand, the same study reported iopamidol concentrations of 2,599,000 ng/L, whereas this ICM was not quantifiably detected in the presented study. An investigation of raw wastewater from a university hospital and a general hospital in Portugal reported considerably higher average concentrations of iopromide (195,683 ng/L and 260,908 ng/L, respectively) than were found in the presented study; on the other hand fluctuations in the ICM concentrations were similarly high (Santos et al., 2013). For a hospital in Switzerland, iopromide concentrations up to 40 times higher than presented here were reported (Weissbrodt et al., 2009). Average iomeprol concentrations reported in literature corroborate those found in this study (Weissbrodt et al., 2009; Kovalova et al., 2012). Overall, it can be assumed that very specific application patterns are reflected in the concentrations found in raw hospital wastewater from different sources.

As mentioned before, the transformation products of ICMs were not expected to be found in raw hospital wastewater because – as presumably inert substances – they have been described as being excreted completely unchanged after treatment (Pérez and Barceló, 2007; see Chapter 2.1.1.2). However, no biodegradation could have taken place after excretion due to the extremely short time the wastewater was in the main hospital sewer before reaching the sampling point (less than 30 min).

Likewise, photodegradation was impossible since the wastewater ran through an underground sewer. Thus, another degradation pathway had to be responsible for the occurrence of the ICM transformation products. A possible reason for the rapid degradation of iopromide could have been the presence of high concentrations of aggressive substances (e.g. diagnostic agents, disinfectants) in the undiluted hospital effluent water, but the investigation of such cross-interactions lies beyond the scope of this study.

NSAIDs AND BEZAFIBRATE. Ibuprofen amounts reported in hospital wastewater differ widely (Thomas et al., 2007; Lin and Tsai, 2009; Verlicchi et al., 2012a; Santos et al., 2013). While the substance was not detected at all in wastewater of 4 Korean hospitals (Sim et al., 2011), mean values of about 20,000 ng/L were found in composite samples from a private health care centre in Spain (Gómez et al., 2006), which concurs with the results of presented study.

Comparable diclofenac concentrations in hospital wastewater have been reported in various studies from Spain (Gómez et al., 2006), Korea (Sim et al., 2011), and Sweden (Thomas et al., 2007), while Santos et al., 2013 reported average concentrations of roughly one tenth of that for various hospitals in Portugal.

The observed naproxen concentrations are about 10 to 100 times lower than those reported in hospital wastewaters in Taiwan (Lin and Tsai, 2009), Portugal (Santos et al., 2013), Korea (Sim et al., 2011) and Italy (Verlicchi et al., 2012a), reflecting very specific use patterns in different countries (Behera et al., 2011).

Bezafibrate concentrations in wastewaters of different types of hospitals in Italy (Verlicchi et al., 2012a) and Portugal (Santos et al., 2013) revealed mean values from one-fifth to less than one-twentieth of those in the presented study.

Table 4-2: Mean concentration of analytes in influent and effluent of the PS-BFR over the investigation period [ng/L]. For the calculation of average values, single values < LOQ were accounted for as 0.5*LOQ and single values which not yielded a signal (not detected = n.d.) were taken into account as 0.

	Influent [ng/L][1]		**Effluent [ng/L] [1]**	
	Average	SD	Average	SD
		Antibiotics		
CLA	1,168	718	1,407	468
ROX	29.7	30.0	44.6	21.4
SMX	2,717	3,099	1,812	1,518
N-Ac-SMX	1,262	1,217	751	478
TMP	402	280	687	292
		ICMs		
DTZ	1,058,819	450,313	890,270	297,836
IOP	497,530	77,557	483,037	14,717
IPM	< LOQ		< LOQ	
IMI	3,449	1,290	10,688	20,258
IMI-TP 805 A	6,908	7,809	20,540	11,730
IMI-TP 805 B	< LOQ		< LOQ	
IMI-TP 819	2,282	2,084	7,260	3,389
IMI-TP 817 A	570	606	2,139	1,117
IMI-TP 787 A	< LOQ		< LOQ	
IMI-TP 731 A	1,147	1,318	3,247	1,936
IMI-TP 731 B	< LOQ		< LOQ	
IMI-TP 729 A	< LOQ		< LOQ	
IMI-TP 759	1,311	1,007	3,652	1,263
IMI-TP 701 A	1,261	1,158	3,830	1,632
IMI-TP 701 B	< LOQ		< LOQ	
IMI-TP 643	197	72.3	199	27.3
		NSAIDs and bezafibrate		
BZF	5,538	4,402	4,730	4,900
DCF	3,518	5,284	3,803	4,306
IBP	26,567	27,075	3,192	3,705
NPX	54.4	90.3	39.3	80.6
		Psycho-active drugs		
CBZ	561	286	478	41.1
DH-CBZ	27.7	16.0	24.9	3.51
DHH	11,642	2,335	6,487	1,319
DXP	101	50.1	20.7	11.1
PMD	2,061	642	79.8	46.3
CDN	116	41.5	167	29.9
DHC	137	71.4	164	69.3
MTD	n.d.		n.d.	
MPN	77.7	42.7	< LOQ	
OCN	116	36.9	111	26.8
DZP	n.d.		n.d.	
NZP	19.4	11.2	20.1	27.1
OZP	3,837	395	3,144	906
TZP	19.7	5.52	20.7	4.61
		OPs		
TBEP	12,857	11,677	5,529	3,950
TnBP	68.9	23.9	37.1	25.2
TiBP	< LOQ		78.7	57.6
TEHP	n.d.		n.d.	
TPP	< LOQ		< LOQ	
TCEP	67.8	12.7	111	27.8
TCPP	< LOQ		86.5	67.6
TDCPP	< LOQ		43.0	35.5

[1] n=7

PSYCHO-ACTIVE COMPOUNDS. Carbamazepine has been investigated repeatedly in raw hospital wastewater all over the world (e.g. Brazil: 461–590 ng/L (Almeida et al., 2013), China: 88–161 ng/L (Yuan et al., 2013), Italy: 640–1,200 ng/L (Verlicchi et al., 2012a); Korea: 827 ng/L (Sim et al., 2011), Portugal: 64.5–771 ng/L (Santos et al., 2013), Switzerland: 222 ng/L (Kovalova et al., 2012)) and shows far less variation in reported concentrations than other pharmaceuticals. The reported concentrations concur with those found in the presented study. It might be assumed that, in most cases, carbamazepine in hospital wastewater stems from its most common application as an antiepileptic drug (see Chapter 2.1.1.5), for which purpose it is typically administered to the patient at unvarying doses for life. The number of epilepsy patients per head of population can be expected to be roughly the same. Other uses of the drug, e.g. as an anti-anxiety drug or for the treatment of ADHD, personality disorders or alcohol withdrawal, are assumed to be negligible as far as general hospitals are concerned. Thus, comparable dosages of the drug in long-term treatment could result in less variant concentrations in wastewater from different hospitals.

While diazepam was not detected in the presented study, several researchers have found the drug in hospital wastewater in concentrations of up to 645 ng/L (Kovalova et al., 2012; Almeida et al., 2013; Santos et al., 2013). Codeine in similar concentrations to those in the presented study was found in several Portuguese hospitals of different types (university, general, paediatric and maternity hospitals, Santos et al., 2013). Lin et al., 2010 reported comparable morphine concentrations to those in the presented study for hospitals in Taiwan, while 66 times higher concentrations were found in the wastewater of a hospital in Switzerland (Kovalova et al., 2012). In contrast, the same study reported oxazepam and primidone concentrations that were one third and one fifth, respectively, of those presented here.

OPS. While OPs are not expected to be discharged in especially large amounts from hospitals, it is nonetheless fruitful to take them into account when looking at distribution patterns as a hospital provides a chance to investigate a single, defined wastewater source. A highly selective pattern of these assumedly ubiquitous substances was discovered in raw hospital wastewater, with only 3 out of the 8 OPs found in quantifiable concentrations. While TBEP (average amount 12,857 ng/L) was among the highest concentrated target compounds, TnBP and TCPP were found in concentrations lower than 70 ng/L. Yet the wide standard deviation of TBEP points to heterogeneous influent amounts over the course of the study (see the following chapter).

4.3.2.2 Weekly Concentration profiles

Concentration profiles of the investigated xenobiotics for influent and effluent as well as the removal rates over the course of the sampling campaign are shown in the Figure 4-3 to Figure 4-8. The equation for the calculation of removal rates is given elsewhere (see Chapter 3.3.2.2). For reasons of clarity, values below LOQ were set to zero for the illustration of the removal rates in the graphs. Details regarding removal are discussed in Chapter 4.3.3.
The amounts of pharmaceuticals in the raw hospital wastewater were found to vary, in some cases profoundly, which is consistent with other studies of hospital effluent (Verlicchi et al., 2012a; Santos et al., 2013).

Antibiotics. The antibiotics roxithromycin, sulfamethoxazole, N4-acetylsulfamethoxazole and trimethoprim show roughly comparable influent patterns, with elevated influent concentrations at the beginning of the working week. Clarithromycin displays a dissimilar pattern with very large influent amounts on Wednesday and Thursday.

ICMs. For the ICMs iomeprol and iopromide, unexpectedly even inputs were found over the course of the study with no general steep drop at the weekend. Even with iomeprol showing lower concentrations on Sunday, the input concentration on Saturday was not significantly lower than for most of the workdays. Since ICMs are rapidly excreted after ingestion, it had to be assumed that they occurred in the wastewater at about the same time as application, which for weekends does not fit with the regular office hours of the hospital's radiological unit. A possible explanation for the occurrence of these substances in raw hospital wastewater on weekends could be their administration as part of an emergency treatment (angiographic diagnosis of e.g. strokes and heart-attacks). It can be assumed that fewer emergency patients are treated than regular patients on weekdays – however, since outpatients often undergo radiological examinations, it can be suggested that most of the ICMs these patients ingest are excreted away from the hospital. Thus, the lower number of emergency patients who are kept in for observation at the hospital could excrete equal amounts of ICMs into the wastewater as the higher number of outpatients. For diatrizoic acid, a dissimilar pattern was found with a peak concentration on Thursday followed by declining amounts.

NSAIDs. Diclofenac and ibuprofen show fairly constant high concentrations over the course of the week with extraordinarily high peak values on Monday. In contrast, the third NSAID investigated, naproxen, was overall much less concentrated and only quantifiable in the influent on four days (Tuesday, and Thursday to Saturday) with a single peak of about a 10-fold higher concentration on Friday than on the other days. Since naproxen is comparatively rarely used in

Germany, this could indicate a special use, e.g. as a substitute painkiller used in pain management, and might be linked to the office hours of a specialised practice. Bezafibrate shows elevated amounts on Monday, Wednesday and Friday, which could probably be connected to the office hours of specialised practices as well.

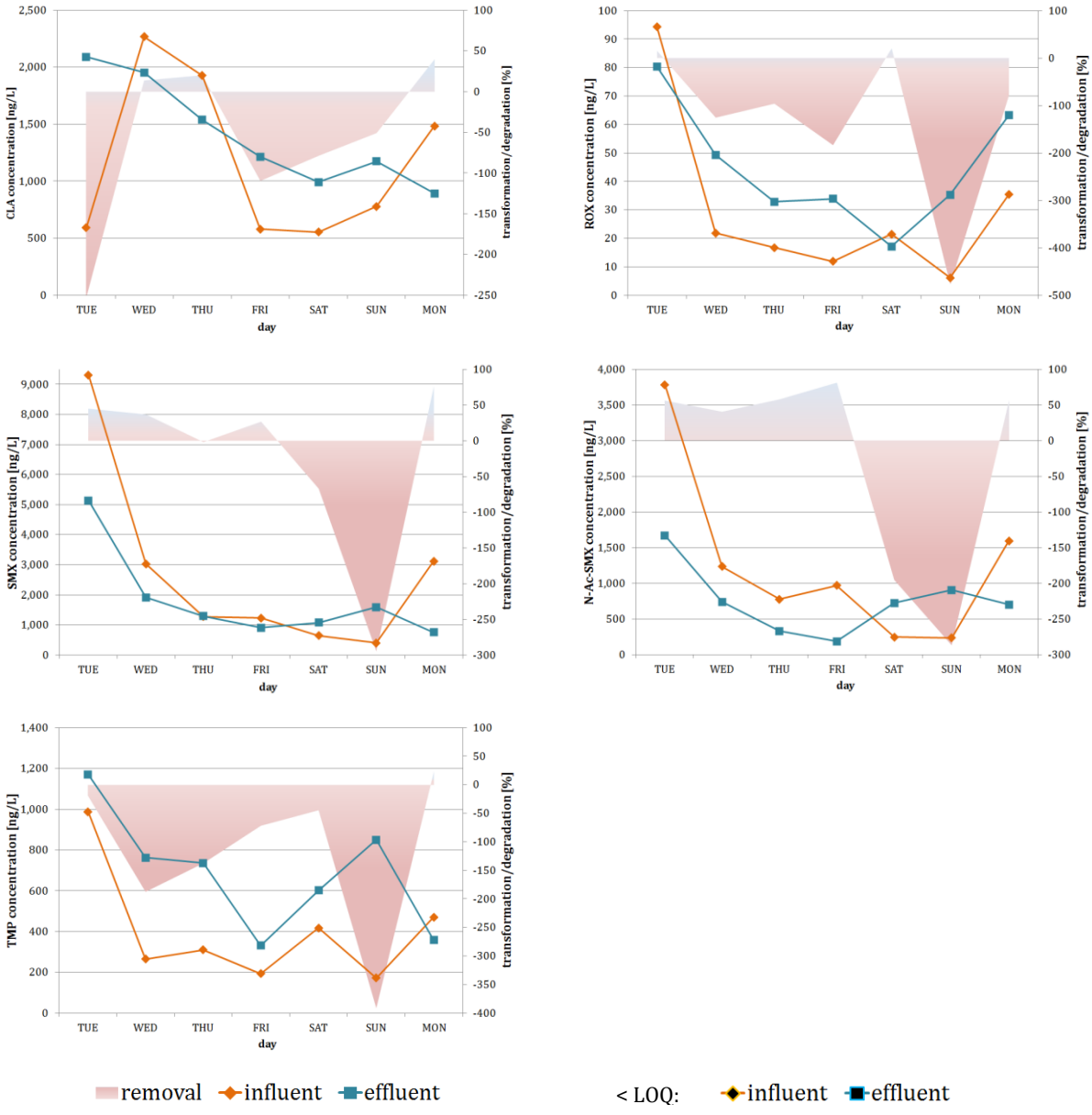


Figure 4-3: Concentrations [ng/L] of antibiotics in influent and effluent from the PS-BFR pilot plant and correlating removal [%] over the course of the sampling campaign. Concentrations below LOQ are indicated by black-filled data point symbols.

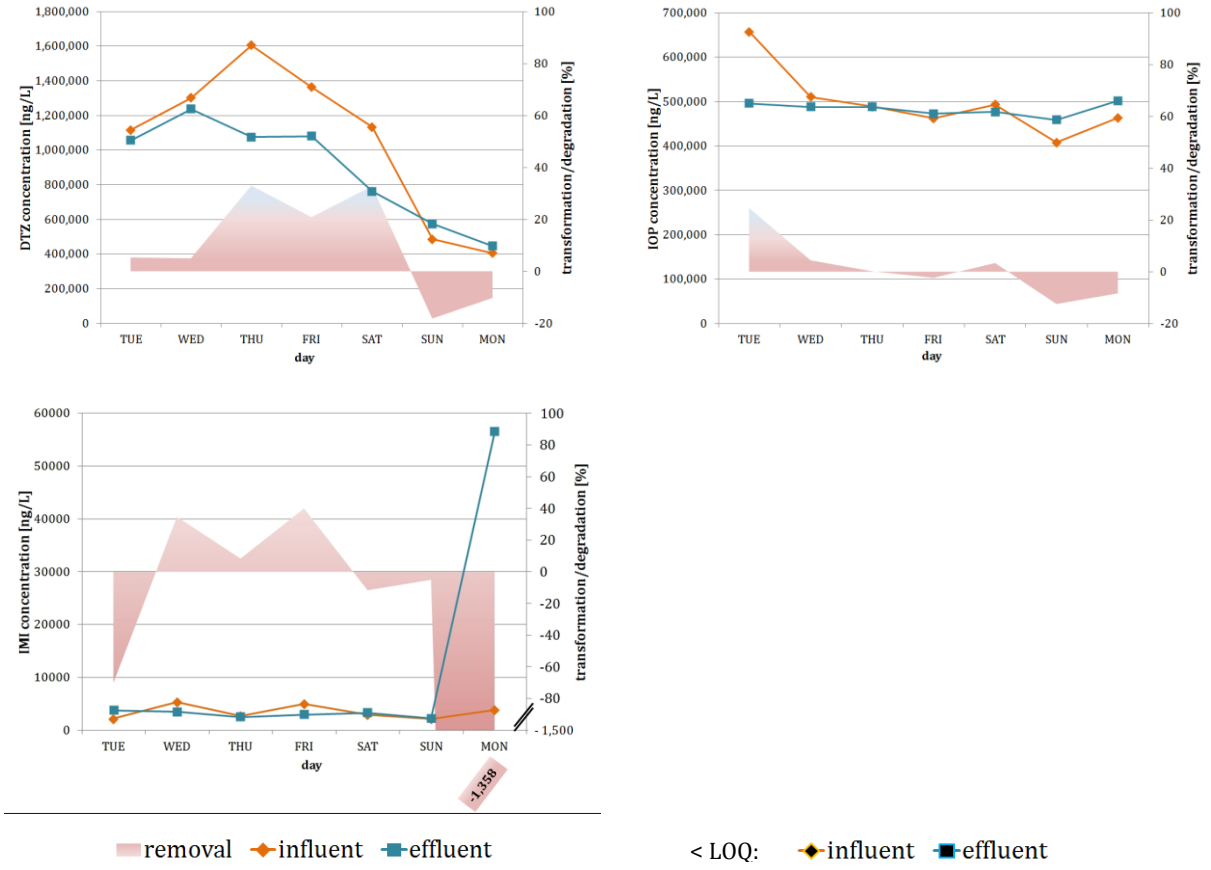


Figure 4-4: Concentrations [ng/L] of ICMs in influent and effluent from the PS-BFR pilot plant and correlating removal [%] over the course of the sampling campaign. Concentrations below LOQ are indicated by black-filled data point symbols.

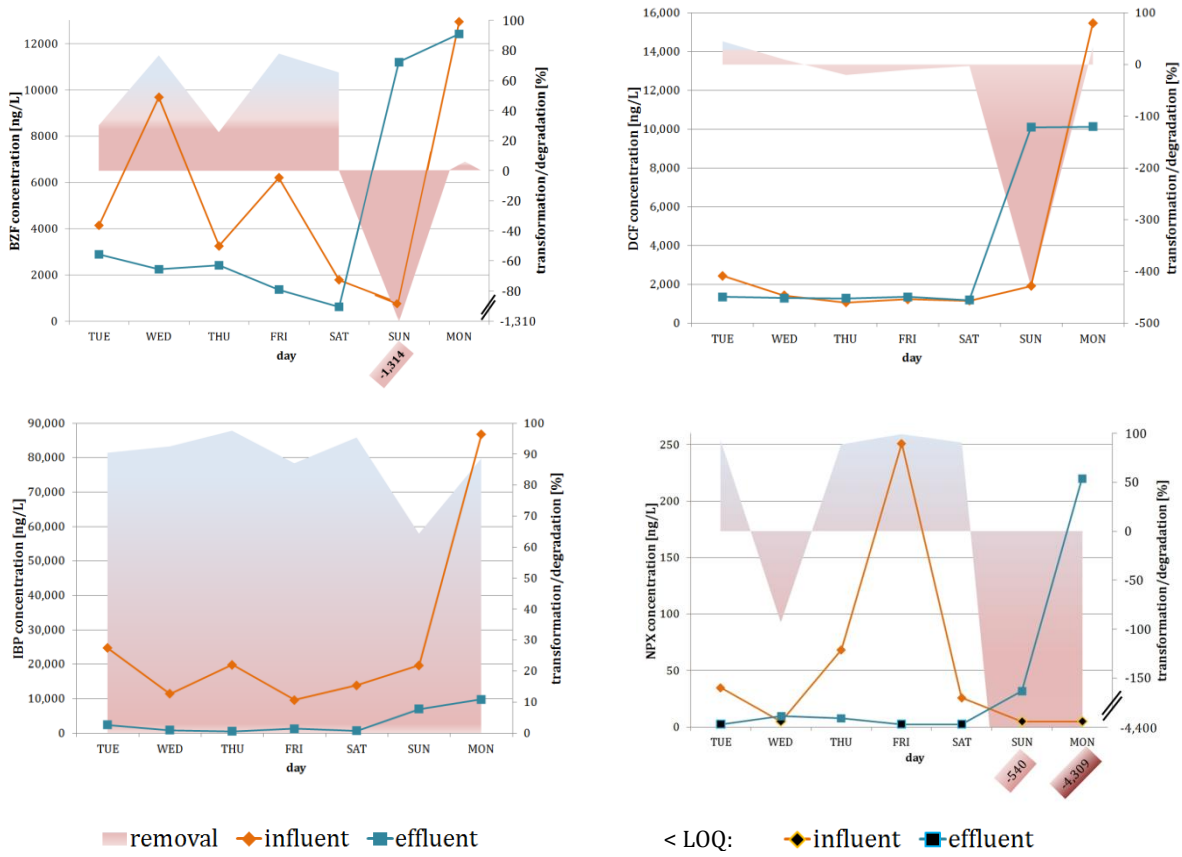


Figure 4-5: Concentrations [ng/L] of NSAIDs and bezafibrate in influent and effluent from the PS-BFR pilot plant and correlating removal [%] over the course of the sampling campaign. Concentrations below LOQ are indicated by black-filled data point symbols.

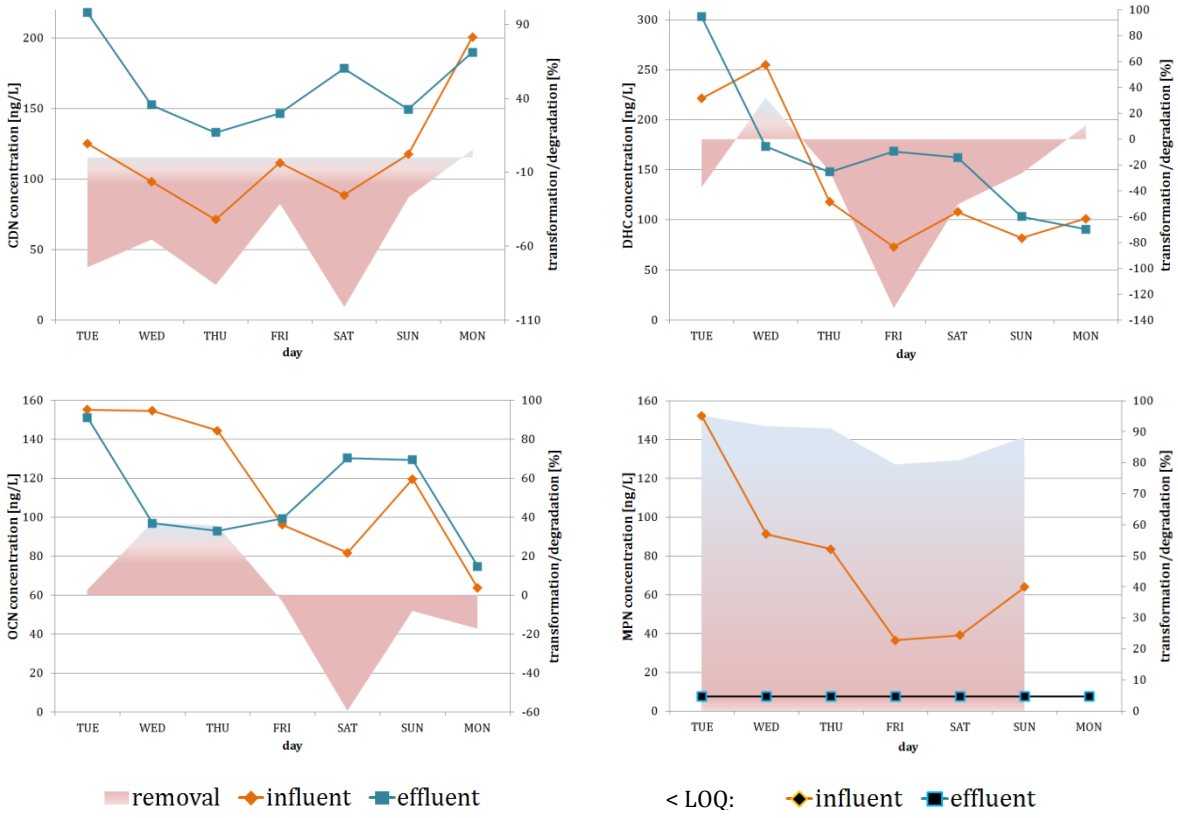


Figure 4-6: Concentrations [ng/L] of psycho-active drugs (opioids) in influent and effluent from the PS-BFR pilot plant and correlating removal [%] over the course of the sampling campaign. Concentrations below LOQ are indicated by black-filled data point symbols.

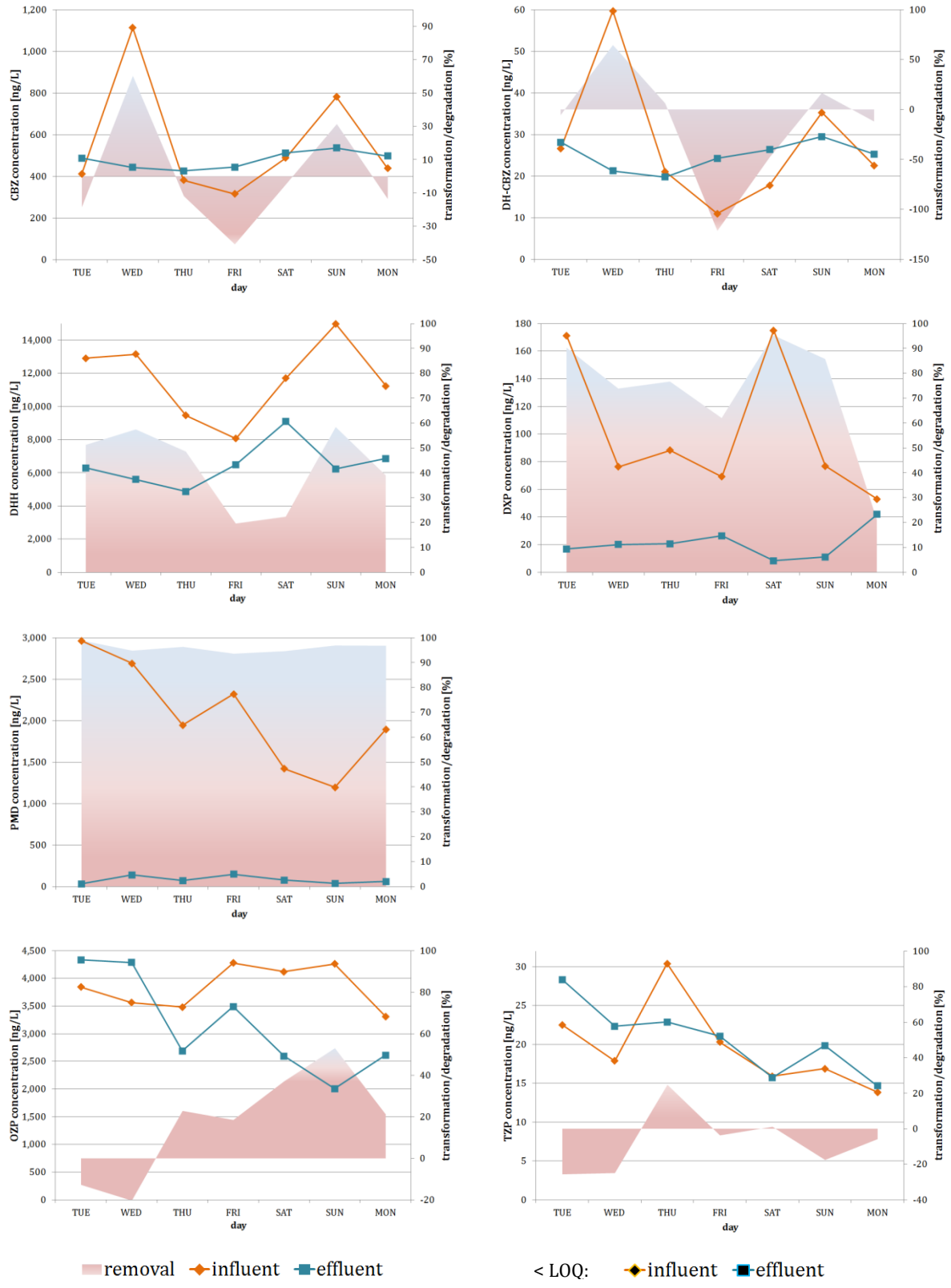


Figure 4-7: Concentrations [ng/L] of psycho-active drugs (carbamazepine and carbamazepine metabolites, doxepin, primidone and benzodiazepines) in influent and effluent from the PS-BFR pilot plant and correlating removal [%] over the course of the sampling campaign. Concentrations below LOQ are indicated by black-filled data point symbols.

Figure 4-8: Concentrations [ng/L] of organophosphorous substances in influent and effluent from the PS-BFR pilot plant and correlating removal [%] over the course of the sampling campaign. Concentrations below LOQ are indicated by black-filled data point symbols.

PSYCHO-ACTIVE COMPOUNDS. With the exception of Sunday, oxycodone and dihydrocodeine show similar profiles, with higher concentrations in the raw wastewater at the beginning of the studied period and declining amounts in the following days. Codeine shows relatively even concentrations over the course of the week apart from a very much higher concentration on Monday.

Carbamazepine and its metabolite 10,11-dihydrocarbamazepine show exactly matching weekly profiles, while the profile of the second metabolite 10,11-Dihydro-10,11-dihydroxycarbamazepine is merely similar. The second anticonvulsant, primidone, shows a different profile from carbamazepine, which suggests a different application pattern. Doxepin shows two levels of input amounts with lower, comparable concentrations on Wednesday to Friday and Sunday to Monday and equivalent peak concentrations on Tuesday and Saturday. The concentrations of oxazepam are relatively constant over the course of the week. Oxazepam is a highly effective benzodiazepine which is commonly used in premedication for medical procedures and has a wide range of applications as tranquilizer, anticonvulsant and muscle relaxant. In contrast to diazepam and nordiazepam (which were not detected in the hospital wastewater), it is relatively easy to control, which may explain the consistent distribution pattern (see Chapter 2.1.1.5). The low concentrations of temazepam seem to point to its occurrence merely as a metabolite of other benzodiazepines, presumably oxazepam.

OPS. The weekly profiles of OPs are inconsistent. While TnBP and TiBP show highly fluctuating inputs over the course of the week, TBEP and TCPP are found in higher concentrations on Sunday and Monday, while the latter is not quantifiable in the influent between Wednesday and Saturday. TDCPP, which is not quantifiable in the influent at all, and to a lesser degree TCEP, are obviously introduced into the wastewater during treatment in the pilot plant. Details of removals are given in the following chapter.

4.3.3 Removal of xenobiotics from the aqueous phase by the PS-BFR

Removal of the xenobiotics from the aqueous phase was estimated as mean values over the length of the studied period and classified into five categories (very high, high, moderate, poor, negative) as presented in Figure 4-9. Removal in this context is to be understood as a summary of degradation and (bio-)transformation processes during treatment. Removals reaching the limit of quantification are given as 100%. As shown in Figure 4-3 to Figure 4-8, removals over the course of the week are highly heterogeneous for the majority of substances. The determination of average removal rates, despite being a helpful tool for the assessment of the efficiency of the system, has to take these heterogeneities into account.

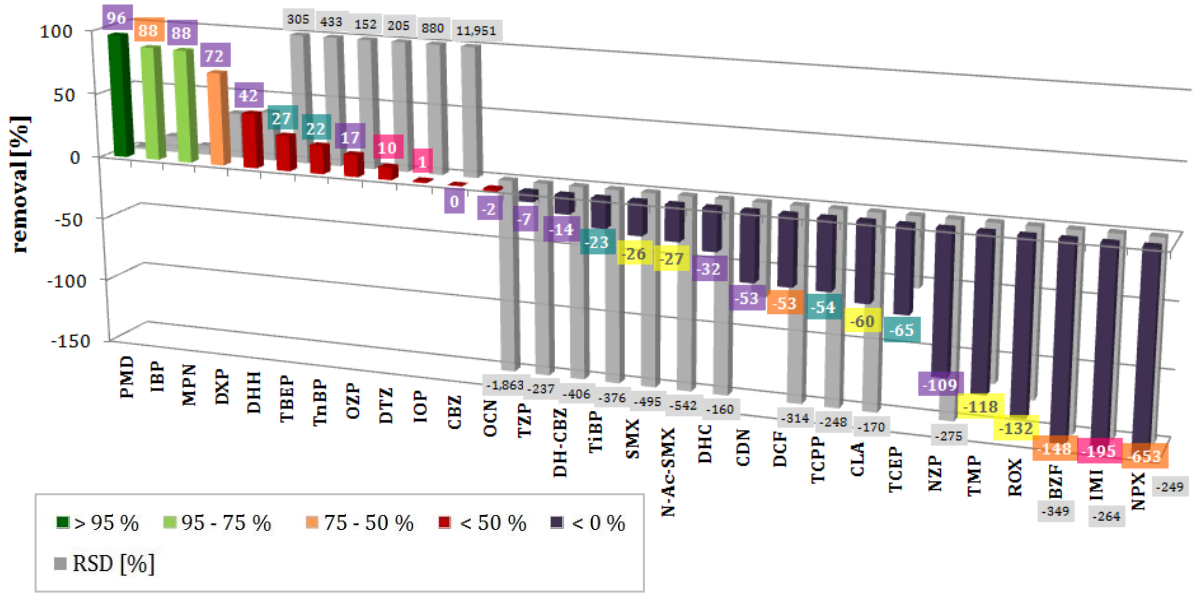


Figure 4-9: Average removal of xenobiotic compounds in the PS-BFR pilot plant. Colours of columns show very high (> 95% - dark green), high (75–95% - light green), moderate (50–75% - orange), poor (0–50% - red) and negative (< 0% - lilac) removal. Numbers above the columns depict the percentage of average removal and by colour code the compound group (colour code see Figure 4-2). The grey columns show the RSD [%].

Very high removal rates were found for primidone throughout the week. Likewise, ibuprofen and morphine showed high removal rates with small variations over the course of the study. One substance, doxepin, was moderately removed, and six more showed poor removal (10,11-dihydro-10,11-dihydroxycarbamazepine, TBEP, TnBP, oxazepam, diatrizoic acid, iomeprol). The two OPs among them displayed considerable variations in removal during the sampling period. Carbamazepine showed overall no removal. For 17 substances, average removal was found to be negative. The physico-chemical properties of these compounds vary widely (MW: 238 – 837 g/mol; log K_{OW} -2.05 – 4.51, pKa 1.83 – 9.20, HLC: $1.73x10^{-29}$ – $3.29x10^{-06}$ $atm\text{-}m^3$/mole, Ws: 0.0189 – 9000 mg/L, see also Chapter 2.1), which makes it unlikely that a single physical or

chemical attribute would be the cause. Neither does the form of excretion seem to be the reason for this removal pattern, since both substances which are excreted completely via urine (e.g. iopromide) and drugs which are to a great extent faeces-bound (e.g. roxithromycin), fall into this group. Some of these substances displayed overall uniform behaviour over the course of the study. With the exception of one or two days, codeine, roxithromycin and TCEP showed relatively regular patterns, with a constant offset between influent and effluent leading to a consistent negative removal. In contrast, bezafibrate showed the opposite behaviour with positive removals over the course of the study apart from only one day where there was a substantial negative removal, masking the otherwise noteworthy removal capacity of about 50%. Other substances showed greatly varying removal patterns, often with changes between positive and negative removal (e.g. carbamazepine and 10,11-dihydrocarbamazepine, oxycodone, temazepam, clarithromycin, sulfamethoxazole, nordiazepam, naproxen). The high variability of removal rates was not caused by any factor monitored in this study (e.g. no pH changes were found). For many pharmaceuticals, the cleavage of conjugated metabolites present in wastewater resulting in higher parent drug concentrations, and seemingly low or negative removal during wastewater treatment has previously been reported (Lindqvist et al., 2005; Radjenović et al., 2009b; Kovalova et al., 2012). Taking the high variation regarding the influent concentrations themselves into account, such processes are likely to be the reason for the varying removal rates found in the presented study. Furthermore, the ICM iopromide seems to suggest that similar processes occur between TPs and parent compounds, too, with removal rates for the parent substance generally being from 40% to –70% with a maximum removal of –1,358% (see Figure 4-4 and also Chapter 4.3.4).

COMPLEX MATRIX. Overall, a generally very similar xenobiotic pattern was found in the effluent (Figure 4-10) compared to the influent (Figure 4-2) with only minor changes regarding both the compound composition and concentrations, indicating the generally low removal efficiency of the PS-BFR. With the exception of ibuprofen and carbamazepine, the PS-BFR treatment is less efficient at removal than conventional CAS treatment (see e.g. reviews by Onesios et al., 2009; Verlicchi et al., 2012b). However, as mentioned above, for most of the substances displaying unsatisfactory overall removal, drastic changes in removals over the course of the week were found. Possible reasons could derive from the extreme environment that highly concentrated hospital wastewater represents. For example, Zwiener and Frimmel, 2003 showed that organic solvents like acetone, that are expected to be found in hospital wastewater, can greatly inhibit the capacity of biofilm systems to remove pharmaceuticals from wastewater. However, a constantly changing composition of the feed water is expected to influence the microbiological community, thus leading to the development of a possibly highly specified microbial population

that can withstand temporarily unfavourable conditions. This, on the other hand, might eliminate fast-growers (see Chapter 2.2.2) and therefore degradation processes in this aquatic environment could be expected to take longer than in less demanding milieus.

LITERATURE DATA. Very few studies have been published on the subject of the capacity of SCBPs to remove xenobiotics, and those available mostly operated on bench-scale. To my knowledge there are no studies that report using particle supported biofilm reactors for the treatment of pharmaceuticals in hospital wastewater. In a laboratory study, González et al., 2006 investigated the usefulness of a fixed-bed reactor for the removal of diclofenac from effluent water from a municipal WWTP and found no removal, regardless of whether the acclimatisation of the microorganisms to the target compound was brief or prolonged. In contrast, Falås et al., 2012 reported higher SCBP removal of 5 pharmaceuticals, including diclofenac, than was found for activated sludge treatment, and postulates that moving bed biofilm reactors have superior removal potential compared to CAS treatment. However, the same study found that SCBP and CAS treatment removed ibuprofen and naproxen comparably, which is similar to the findings of the presented study (that is, in the case of naproxen, when excluding the days when influent concentrations were below LOQ, resulting in a removal rate of 97%).

Anoxic conditions seem to be preferable for the removal of pharmaceuticals in SCBPs. Thus, Zwiener and Frimmel, 2003 found no removal of diclofenac under oxic conditions, while under anoxic conditions at least slight removal was achieved in a biofilm reactor. Accordingly, Zhou et al., 2006 reported only anaerobic conditions to be successful in the removal of two antibiotics (ampicillin and aureomycin) in high-strength pharmaceutical wastewater, while a BAS reactor showed no removal. However, other researchers found that for several pharmaceuticals, removal by oxic SCBP treatment were still higher than anoxic and oxic activated sludge processes (Falås et al., 2013).

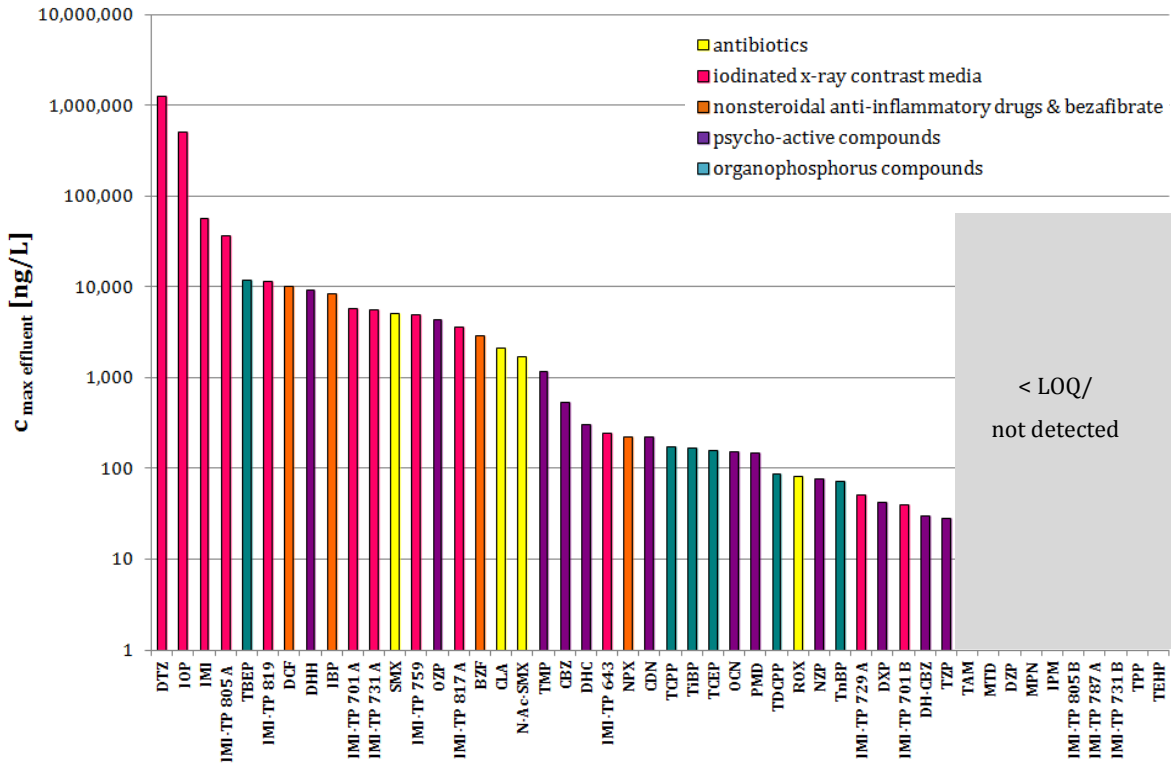


Figure 4-10: Maximum concentrations ($c_{max\ effluent}$ [ng/L]) of each target compound in the effluent from the PS-BFR. Colour codes show the compound group.

4.3.4 Fate of metabolites and transformation products during PS-BFR treatment

Transformation products of iopromide and metabolites of carbamazepine and sulfamethoxazole were investigated in raw hospital wastewater and in the PS-BFR effluent. As depicted in Figure 4-2 and Table 4-2, all three studied metabolites were found in raw hospital wastewater samples as well as 7 out of 12 TPs of iopromide.

TPs of iopromide. While the presence of human metabolites in raw hospital wastewater was expected, the existence of environmental ICM-TPs was not (see also Chapter 4.3.2). Although in laboratory studies iopromide had been found to be degradable during activated sludge treatment as well as in river water (see Chapter 2.1.1.2), the breakdown times were described as being in the range of days or weeks (Kalsch, 1999; Steger-Hartmann et al., 2002; Löffler et al., 2005). However, in case of the raw wastewater investigated in the presented study, only minutes elapsed between its entering the drains and reaching the sampling point, preventing practically all biodegradation activities that might take place in sewer systems. In contrast, the results might point towards very fast, unknown transformation processes taking place in the hospital sewer, probably by interactions with aggressive xenobiotics present in the hospital

effluent, degrading a supposedly stable ICM during extremely short contact times (see also Chapter 4.3.2). This would account for the fact that already in the influent to the pilot plant, the amount of the parent drug compared to the investigated TPs is only about 20% (Figure 4-11). The fact that the sum of parent drug and TPs in the effluent of the pilot plant is constantly higher than the sum estimated for raw wastewater could point to the presence of further, unknown TPs in the raw water that are (re-)transformed into compounds (both the parent substance and its TPs) which are being measured. This suggestion is supported by previous studies which reported different sets of TPs resulting from iopromide breakdown in different environmental settings (Kalsch, 1999). Additional transformation processes in the PS-BFR might be caused by the co-discharge of reactive substances in the hospital water. However, no explanation could be found for the high concentration of the parent compound in the effluent at the end of the sampling period.

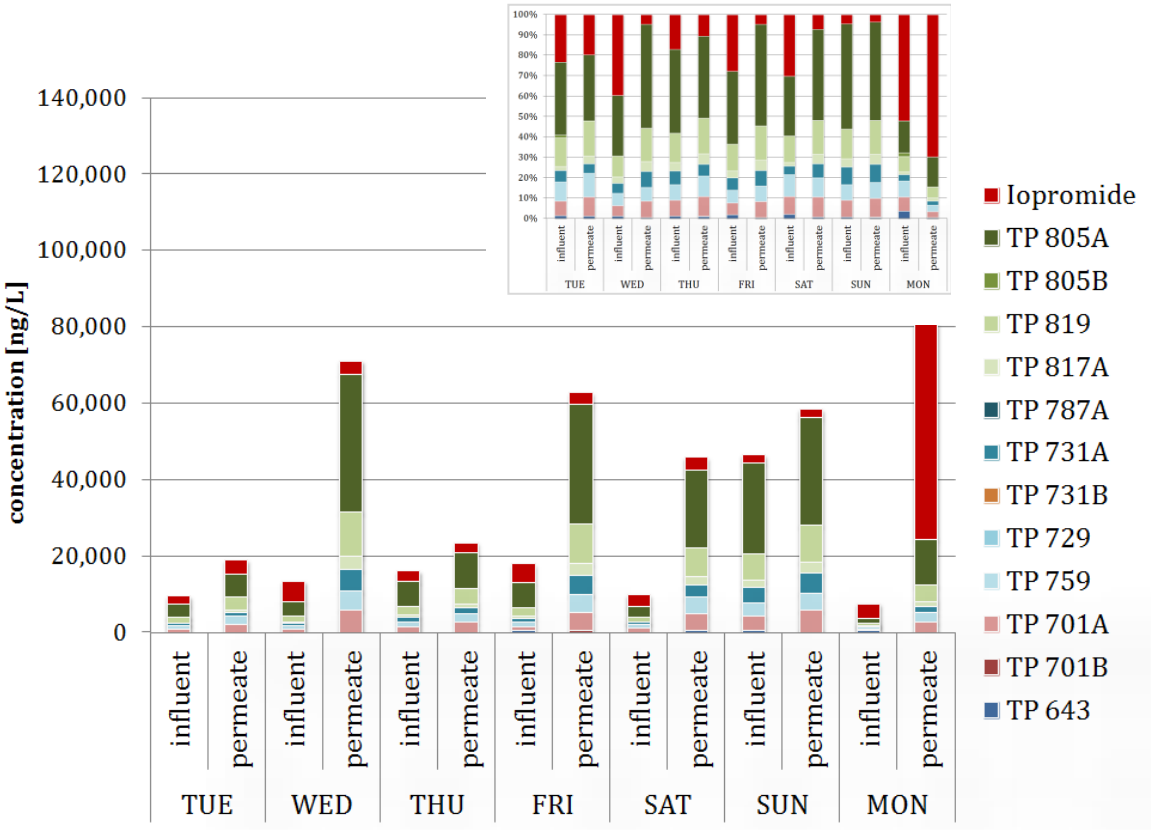


Figure 4-11: Concentration patterns of iopromide and its TPs in influent and effluent samples from the PS-BFR pilot plant over the course of the study in [ng/L] and in [%] (small graph at the top right).

SULFAMETHOXAZOLE METABOLITE. For CAS treatment, sulfamethoxazole removal rates between 10 and 100% have been reported (Verlicchi et al., 2012b). Some studies, however, found higher effluent values compared to corresponding influent values (Göbel et al., 2005b). The reason for this seemingly negative removal of sulfamethoxazole, which is often accompanied by the disappearance of its human metabolite N4-acetylsulfamethoxazole, is the cleavage of the metabolite and the retransformation of the parent compound during wastewater treatment (see Chapter 2.1.1). In the presented study, sulfamethoxazole showed a highly diverse removal pattern with considerable removal rates from 27 to 76% on four days of the sampling campaign, which contrasted with high negative removal rates (up to –295%) on two other days (see Figure 4-3). The metabolite, however, was found to follow a similar pattern, with high removals between 40 and 81% contrasted by two days of negative removals up to –287%. Thus, negative removal of the parent compound cannot have been caused by a cleavage of the metabolite since it was present in the samples at the times in question (Figure 4-12). For four days there was substantial removal of SMX, while at the same time the metabolite was not greatly reduced. This suggests that the parent drug was subjected to a different transformation process. The cause for this could be a specialised, adapted microbial community in the PS-BFR which might be different from that in conventional suspended activated sludge treatment. The reverse removal rates on two days might be explained by temporarily dissimilar wastewater composition, e.g. the introduction of aggressive chemical substances, which could cut into the biological performance of the system and additionally might cause unknown transformation reactions.

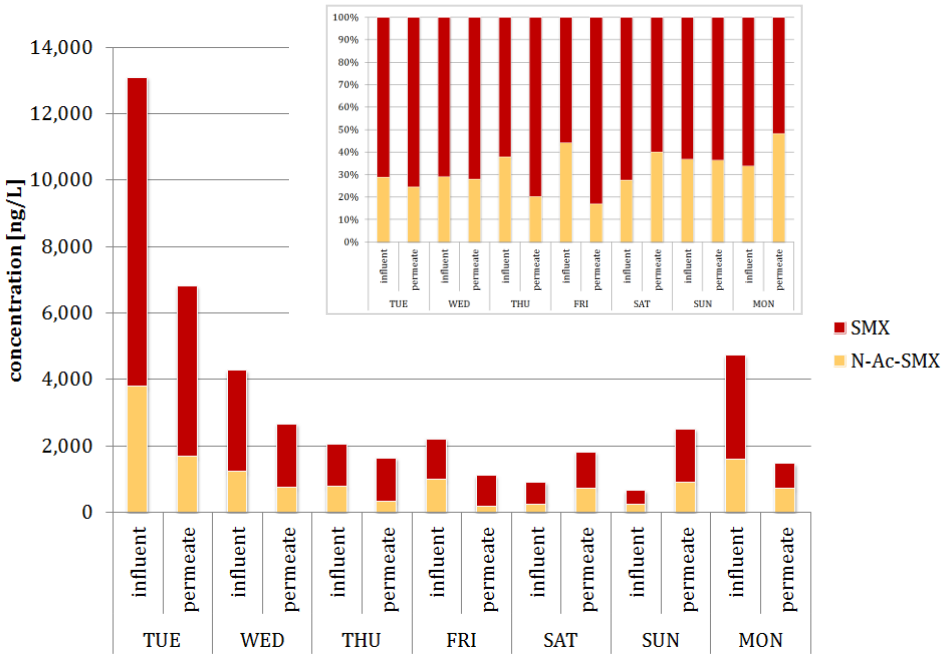


Figure 4-12: Concentration patterns of sulfamethoxazole and its metabolite N4-acetylsulfamethoxazole in influent and effluent samples from the PS-BFR pilot plant over the course of the study in [ng/L] and in [%](small graph at the top right).

CARBAMAZEPINE METABOLITES. Carbamazepine and its metabolites 10,11-Dihydro-carbamazepine and 10,11-Dihydro-10,11-dihydroxycarbamazepine seemed to pass through PS-BFR treatment without major changes in concentration patterns, and with no sign of retransformation to the parent drug (Figure 4-13). Regarding the total amount of parent compound and metabolites in the influent and effluent of the PS-BFR, a clear reduction was found over the whole course of the study, which was mainly due to the reduction of 10,11-Dihydro-10,11-dihydroxycarbamazepine (see Figure 4-7).

UNKNOWN TRANSFORMATION MECHANISMS. To summarise, the transformation of pharmaceuticals as demonstrated for the three substances iopromide, sulfamethoxazole and carbamazepine, suggest the existence of unknown transformation mechanisms in hospital wastewater which seem to differ from the reported processes. This might be caused by a) the operation of a seasoned suspended particle biofilm reactor which allows a highly specified microbial community to develop on and within the carriers and b) by the presence of a variety of reactive substances in the raw wastewater which might induce unknown degradation processes of the studied compounds.

4.4 CONCLUSIONS

Out of 47 xenobiotics studied, 37 were quantified in raw and 38 in treated hospital wastewater. In raw wastewater, the highest amounts found were of ICMs, with maximum concentrations exceeding 1.5 mg/L. For many substances, temporal distribution patterns showed widely varying concentrations over the course of the study. Surprisingly, despite an extremely short time of 30 min elapsing between the discharge of the wastewater and its reaching the sampling point, environmental TPs of the ICM iopromide were found in raw hospital wastewater. This might point to the rapid degradation of this compound in undiluted hospital effluent water by highly reactive substances not included in the present study (e.g. diagnostic agents, disinfectants, etc). A further particularity of the hospitals' discharge water is the absence of many of the OPs investigated in this study. OPs are regarded as being ubiquitous, but only 3 out of 8 were found in the raw hospital wastewater. A fourth OP was introduced at low levels (43.0 $\pm$35.5 ng/L) during PS-BFR treatment, which might be attributed to abrasion of the carriers used in the reactors. In general, when comparing the results of xenobiotic occurrence in raw hospital wastewater found in this study with data from the literature, highly specific application patterns seem to be reflected in the concentrations found for different hospitals.

PS-BFR treatment of the raw wastewater resulted in stable removal rates for only a small number of substances, while for many compounds wide variations in removal rates were found over the course of the study. This might be owing to wide variations in the composition of the

hospital wastewater and the irregular introduction of substances which temporarily impaired the performance of the biological treatment. However, high, stable removal rates for primidone, ibuprofen and morphine prove the effectiveness of the biological treatment and attest to the presence of an active microbial community throughout the study. It can be assumed that the biocenosis in the seasoned PS-BFR (start-up time prior to the study: 199 days) has successfully adapted to the wastewater matrix. Since specialised microbes often are slow-growers, biodegradation in the PS-BFR can be expected to be slower than in comparable systems in less demanding milieus. Between the presence of a specialised biocenosis on one side and a seemingly reactive wastewater matrix on the other, the uncharacteristic degradation patterns found for sulfamethoxazole and iopromide in this study could be explained.

Overall, hospital wastewater presents a highly dynamic system with interactions that are only partly understood so far. Substances not subjects of the presented study should be taken into consideration to further investigate the processes taking place in this matrix, for example to explain the presence of ICM-TPs in raw wastewater, which could be a result of degradation by aggressive co-eluents in the waste stream. Further research on this subject is essential to better understand the fate of xenobiotics in urban water cycles. The same is true for gaining more detailed insight into the performance of biofilm systems operating in wastewaters from specific point sources.

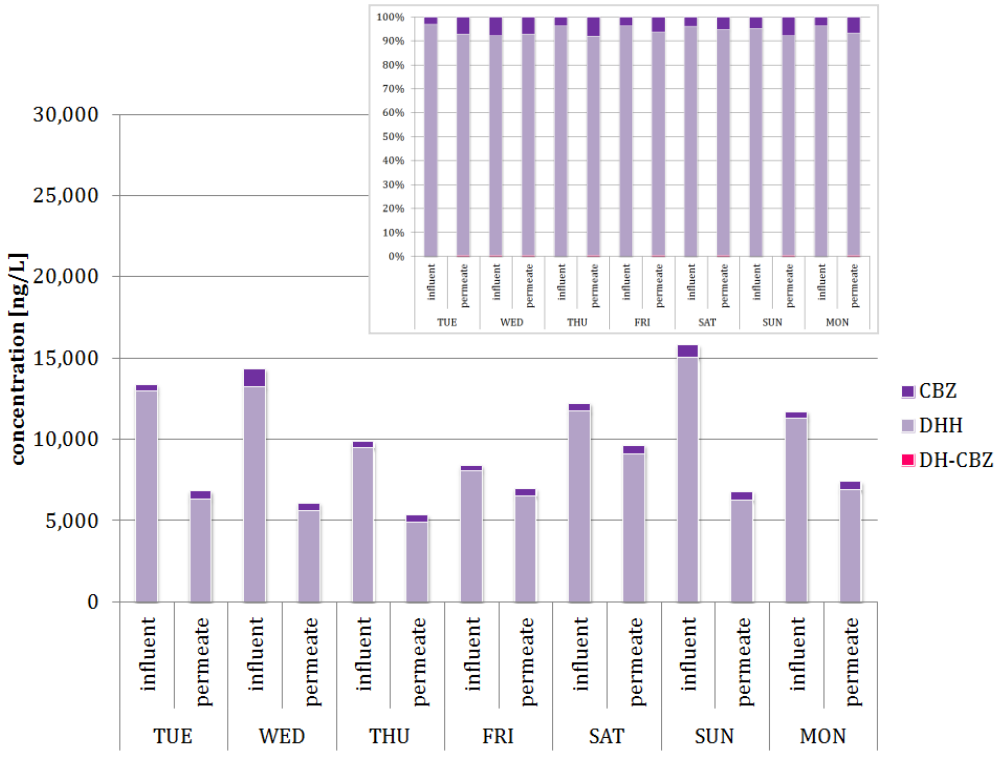


Figure 4-13: Concentration patterns of carbamazepine and its metabolites DHH and DH-CBZ in influent and effluent samples from the PS-BFR pilot plant over the course of the study in [ng/L] and in [%] (small graph at the top right).

5. DETERMINATION OF XENOBIOTIC ORGANIC MICROPOLLUTANTS IN BIOSOLIDS BY PRESSURISED LIQUID EXTRACTION (PLE) FOLLOWED BY LC-MS/MS AND THEIR OCCURRENCE IN SEWAGE SLUDGE FROM A NF-MBR AND IN SLUDGE AND CARRIER MATERIAL FROM A PS-BFR

If you could tomorrow morning make water clean in the world, you would have done, in one fell swoop, the best thing you could have done for improving human health by improving environmental quality.
– *William C. Clark (*1948- *), speech, Racine, Wisconsin, April 1988* –

5.1 INTRODUCTION

Xenobiotic substances such as pharmaceuticals and industrial chemicals can reach the environment after passing through treatment plants either in the water phase or sorbed to biosolids like sewage sludge. While recently much research has been directed at the fate of xenobiotics in the water phase (see Chapters 2, 3, and 4), their occurrence in sewage sludge has been much less investigated (Díaz-Cruz et al., 2009; Le-Minh et al., 2010). In many countries sewage sludge is still used as fertiliser, and there is concern about the amount and distribution of xenobiotics in soils treated with it (Kümmerer, 2004). Legislation has only very recently started to take this issue into account and, to date, there are no regulations in place in the EU for sewage sludge management with regard to organic xenobiotics (Díaz-Cruz et al., 2009, Lillenberg et al., 2009).

COMPOUNDS OF CONCERN. Among the wide range of xenobiotics present in the environment, pharmaceuticals raise particular concerns regarding public health implications (see Chapters 2.1.1). Among them, antibiotics are of special interest because of the unresolved issue of bacterial resistance (see Chapter 2.3), which is exacerbated by the presence of antibiotic compounds in the environment (Diwan et al., 2010; Bouki et al., 2013; Marti and Balcázar, 2013). Industrial substances are often regarded with concern because of the high volumes at which they enter wastewater (EPA, 2012a).

ANALYTICAL AND TECHNICAL IMPROVEMENTS. Recent improvements in analytical techniques and equipment allow for increasingly detailed studies of xenobiotics in environmental samples in lower and lower concentrations (Buchberger, 2007). Subsequently, numerous studies have been published investigating xenobiotics in sewage sludge. Göbel et al. developed sludge analysis procedures based on pressurised liquid extraction (PLE) and ultrasonic extraction followed by a solid phase extraction (SPE) clean-up step, and analysis by reversed-phase liquid chromatography-tandem mass spectrometry (LC-MS/MS) using positive electrospray ionisation (ESI) (Göbel et al., 2005a) to investigate antibiotics in sludge from municipal wastewater treatment plants (WWTPs) in Switzerland (Göbel et al., 2005b). Lillenberg et al., 2009 identified several antibiotics in digested sewage sludge from various Estonian WWTPs, similarly using pressurised liquid extraction (PLE) and ESI-LC-MS/MS. Other researchers (Radjenović et al., 2009a; Radjenović et al., 2009b; Wick et al., 2009; Jelic et al., 2011) investigated a wider range of pharmaceuticals, likewise using PLE and LC-MS/MS. Marklund et al., 2005 used PLE followed by a clean-up step with gel permeation chromatography (GPC) and GC-MS analysis to investigate the occurrence of OPs in sewage sludge from various WWTPs in Sweden.
Recently, alternative wastewater treatment technologies such as membrane bioreactors or biofilm reactors are increasingly being used (see Chapters 2.2.2 and 2.2.4). Thus, sludge from bioreactors and biofilms are biosolids related to wastewater treatment that have to be taken into account.

THE SCOPE OF THE STUDY. The aim of the presented study was to develop a multi-residue PLE method for subsequent LC-MS/MS analysis of 31 xenobiotics from different compound classes (pharmaceuticals, metabolites and OPs), from different kinds of biosolids. Antibiotics and psycho-active substances were chosen as model substance groups for pharmaceuticals and OPs as representative of industrial substances. The developed method was subsequently used for identifying these xenobiotics in a) sewage sludge from a nanofiltration membrane bioreactor pilot plant located at the influent of a WWTP ($NF\text{-}MBR_{WWTP}$, see Chapter 3) and b) in sludge and carrier material from a particle-supported biofilm reactor pilot plant situated at the effluent of a municipal hospital ($PS\text{-}BFR_{hospital}$, see Chapter 4). To my knowledge, this is the first study reporting the occurrence of organic xenobiotics in wastewater-related biofilms.

5.2 MATERIALS AND METHODS

5.2.1 REFERENCE COMPOUNDS, CHEMICALS AND STANDARDS

The following compounds were studied (all standards were of analytical grade with > 98% purity; information regarding the distributers of the standard materials is given elsewhere (Chapter 3)): *Antibiotics*: clarithromycin (CLA), roxithromycin (ROX), sulfadimethoxine (SMI), sulfadimidine (SDI) , sulfisoxazole (SSX), sulfamerazine (SMA), sulfamethoxazole (SMX), N4-acetylsulfamethoxazole (N-Ac-SMX), tiamulin (TAM), trimethoprim (TMP). *Psycho-active compounds*: carbamazepine (CBZ), 10,11-dihydrocarbamazepine (DH-CBZ), 10,11-Dihydro-10,11-dihydroxycarbamazepine (DHH) doxepin (DXP), primidone (PMD), codeine (CDN), dihydrocodeine (DHC), methadone (MTD), oxycodone (OCN), diazepam (DZP), nordiazepam (NZP), temazepam (TZP). *OPs:* tris(2-butoxyethyl) phosphate (TBEP), tributyl phosphate (T*n*BP), tri-iso-butyl phosphate (T*i*BP), triphenyl phosphate (TPP), tris(2-chloroethyl) phosphate (TCEP), tris(chloropropyl) phosphate (TCPP), tris(1,3-dichloro-2-propyl) phosphate (TDCPP).
For analysis the following internal standards (analytical grade > 98% purity) were used: (E)-9-[O-(2-methyloxime)]-erythromycin, sulfadimethoxine-d4, sulfadimidine-d4, sulfamerazine-d4, sulfamethoxazole-d4, N4-acetylsulfamethoxazole-d4 13C-15N-carbamazepine, primidone-d5, codeine-d6, methadone-d9, diazepam-d5, nordiazepam-d5, tri-butyl phosphate-d27, triphenyl phosphate-d15.

All organic solvents used (n-heptane, n-hexane, acetone, methanol, acetonitrile, ethyl acetate) were picograde and purchased from Merck (Darmstadt, Germany). Pure water obtained from a Milli-Q system (Integral 3/5/10/15, Millipore, Billericis, MA, USA) was used. Formic acid (98–100%) was ACS grade and purchased from Merck (Darmstadt, Germany).
For each compound group (antibiotics, psycho-active drugs and organophosphorus compounds) a standard solution of all target analytes and an internal standard mix at a concentration of 10 µg/mL (OPs: 5µg/mL) and 1µg/mL respectively were prepared in methanol and stored in the dark at 4 °C.

5.2.2 SAMPLING AND SAMPLE PREPARATION

SLUDGE. Grab samples of sludge were taken from (1) a membrane bioreactor pilot plant equipped with nanofiltration operated at the influent of a municipal WWTP with 300,000 inhabitant equivalents (NF-MBR$_{WWTP}$) and (2) a particle-supported biofilm reactor pilot plant fed with the raw wastewater from a municipal hospital (PS-BFR$_{hospital}$). Details about the pilot plants were given elsewhere (see Chapters 3.2.2 for NF-MBR$_{WWTP}$ and 4.2.2 for PS-BFR$_{hospital}$). For

sampling, clean glassware was used, which had been rinsed with MilliQ water, heptane and acetone and subsequently heated overnight at > 240 °C. Samples from the NF-MBR_{WWTP}, consisting of a water-solid-suspension, were directly taken from the pilot plant by sinking glass bottles into the tank. Sludge from the PS-$BFR_{hospital}$ was collected from the bottom of the secondary settling tank via a pre-cleaned silicone tube. All samples were transported on ice in the dark and immediately after arrival at the laboratory centrifuged for 90 min at 3500 r/min in a temperature-controlled centrifuge (Rotanta 460R, Hettich Lab Technology, Tuttlingen, Germany) within these same glass bottles.

The centrifuged sludge was freeze-dried and pestled before extraction. Since the sludge was not completely water-free, analytes in the remaining water were expected to dry onto the sludge during freeze-drying. However, the amount of these analytes originating from the water was calculated and generally found to be below 5% of the amount sorbed to the sludge (Table 5A-1). Exceptions were found for three substances with greater water-originating amounts (NF-MBR_{WWTP}-sludge: sulfamethoxazole (10%), primidone (16%) and 10,11-Dihydro-10,11-dihydroxycarbamazepine (17%); PS-$BFR_{hospital}$ sludge: 10,11-Dihydro-10,11-dihydroxycarbamazepine (9%), sulfamethoxazole (10%)).

PS-$BFR_{hospital}$ CARRIERS. In the case of the PS-$BFR_{hospital}$, in addition to the sludge samples, samples of the carriers were taken. They were collected with a coarse sieve and were transferred to perforated polyethylene bags to allow a gentle drying of the carriers by gravimetric drainage of the remaining water without disturbing the biofilm attached to the carriers. Afterwards, the carrier samples were freeze-dried and extracted in the same way as the sludge samples. To prevent possible trouble with the extraction cells blocking, the carrier material was not pulverised but was left in its natural form.

Since mechanical stress was to be avoided during the process of drying the carrier material, so as not to dislodge the attached biofilm, it was expected that there would be more residual water in the carrier samples put up for freeze-drying than in the sludge samples (Table 5A-1). However, the water-originating amounts were only up to 2% of the amounts in the carriers for most compounds, with only three exceptions (13%, 26% and 58% for sulfamethoxazole, 10,11-Dihydro-10,11-dihydroxycarbamazepine and primidone, respectively).

5.2.3 PRESSURISED LIQUID EXTRACTION (PLE)

Sludge samples were extracted by PLE with a Dionex ASE 200 instrument (Sunnyvale, CA, USA) (Figure 5-1). For PLE, 0.5 g and 1 g of sludge and approximately 3.5 g of carrier material were weighed into pre-cleaned 22 mL stainless steel extraction cells half-filled with baked out sea

sand. Internal standard was added (10 µL, i.e. 100 ng absolute) and the cells filled up with baked out sea sand (Riedel-de Haen, Seelze, Germany) previous to extraction.

PHARMACEUTICALS. For the extraction of pharmaceuticals, a number of PLE protocols are already described in detail in the literature (Göbel et al., 2005a; Lillenberg et al., 2009; Radjenović et al., 2009a; Wick et al., 2009; Jelic et al., 2011). Following the results of these studies, pharmaceuticals were extracted with methanol:water (1:1, v/v) using 3 cycles of 5 min (without preheating), 100 °C temperature and 100 bar. Flush volume was 140%, nitrogen purge time was 60 sec. The set parameters were tested by spiking suspended matter from a river (see next paragraph for details) with 20 µL of a 10ng/µL standard solution, i.e. 200 ng of analytes before being applied to the native samples.

OPS. For OPs fewer references are found in literature so a range of solvents was tested for extraction (Table 5-1). Since for time and work efficiency it would be best if the extraction method applied to pharmaceuticals could simultaneously be used for OPs, the mixture of methanol:water (1:1, v/v) was investigated. Furthermore, various solvents of different polarity were chosen: ethyl acetate, methanol, acetone and mixtures of the latter two (20:80; 50:50; 80:20 v/v) as well as a mixture of hexane:acetone:heptane (9:5:1). Extraction conditions were set in the same way as described above for the extraction of pharmaceuticals. Additionally, for ethyl acetate and the mixture of hexane, acetone and heptane, different parameter settings were tested, taking into account the higher temperature stability of OPs compared to pharmaceuticals (Table 5-2). To introduce the impact of organic matrix as early as during the method development phase, the experiments were carried out by spiking 0.5 g dried suspended matter from the river Rhine with 20 – 100 µL of standard and 10 µL of internal standard (Table 5-1) instead of spiking the analyte in pure sea sand. Blanks of the suspended matter were determined and amounts of the spiked samples were corrected with the amounts found in the blank samples.

Absolute recoveries were calculated from absolute peak areas without the correction of internal standards; they therefore revealed either analyte losses during preparation or extraction, or matrix effects during the analysis. In contrast, relative recoveries, calculated by using the analyte/standard ratios, demonstrate the accuracy of the analytical procedure as a whole.

Figure 5-1: Pressurised liquid extraction of sludge and biofilm carriers. The higher amount of matrix in samples from the carrier material (extract nos. 19 and 24) is reflected in a darker colour of the extract.

5.2.4 Solid phase extraction (SPE)

Final PLE extracts (approximately 40 mL) were diluted with 900 mL Milli-Q water to reduce the organic content below 5%. Subsequently, they were purified and enriched by SPE using Oasis HLB cartridges (500 mg, 6 mL, Waters, Milfort, U.S.). Prior to extraction, the cartridges were conditioned with 1 x 5 mL heptane, 1 x 5 mL acetone, 2 x 5 mL methanol and 3 x 5 mL Milli-Q water. The diluted extracts were passed through the cartridges with a flow rate of approximately 5 mL/min. Extracts containing non-polar solvents were reduced in a water bath

(40 °C) to < 2 mL, re-established with 20 mL of acetone and diluted with 400 mL Milli-Q water prior to SPE.

Following SPE, the HLB material was completely dried under a steady nitrogen stream for approximately 90–120 min. Elution was accomplished with 5 x 2 mL acetone. The extracts were evaporated to approximately 100 µL by a gentle nitrogen stream before the vial was rinsed with 300 µL of methanol, followed by a second reduction to 100 µL and a final addition of 400 µL Milli-Q water, resulting in a final sample volume of 500 µL (Figure 5-2). Samples were kept at 4 °C in the dark until LC-MS/MS analysis.

Table 5-1: Tested extraction procedures for organophosphorus compounds. For all schemes applied: temperature: 100 °C, pressure: 100 bar, cycles: 3, heat: 5 min, static: 5 min, flush: 140%, purge: 60 sec. Experiments were carried out by spiking dried suspended matter.

Test scheme No.	**Solvent**	**Mixing ratio (v/v)**	**Concentration of analytes spiked [ng]**	**Concentration of internal standard spiked**
1	methanol:water	1:1	100, 500	100
2	methanol		200	100
3	acetone		200	100
4	methanol:acetone	1:4	200	100
5	methanol:acetone	1:1	200	100
6	methanol:acetone	4:1	200	100
7[1]	ethyl acetate		200	100
8[1]	hexan:aceton:heptan	9:5:1	200	100

[1] additional PLE parameter tested, see Table 5-2.

Table 5-2: PLE parameters tested for organophosphorus compounds.

	Test schemes 1-8	**Additional schemes 7b/8b**
Temperature (°C)	100	120
Pressure (bar)	100	160
Cycles	3	3
Heat (min)	5	5
Static (min)	5	5
Flush (%)	140	100
Purge (sec)	60	60

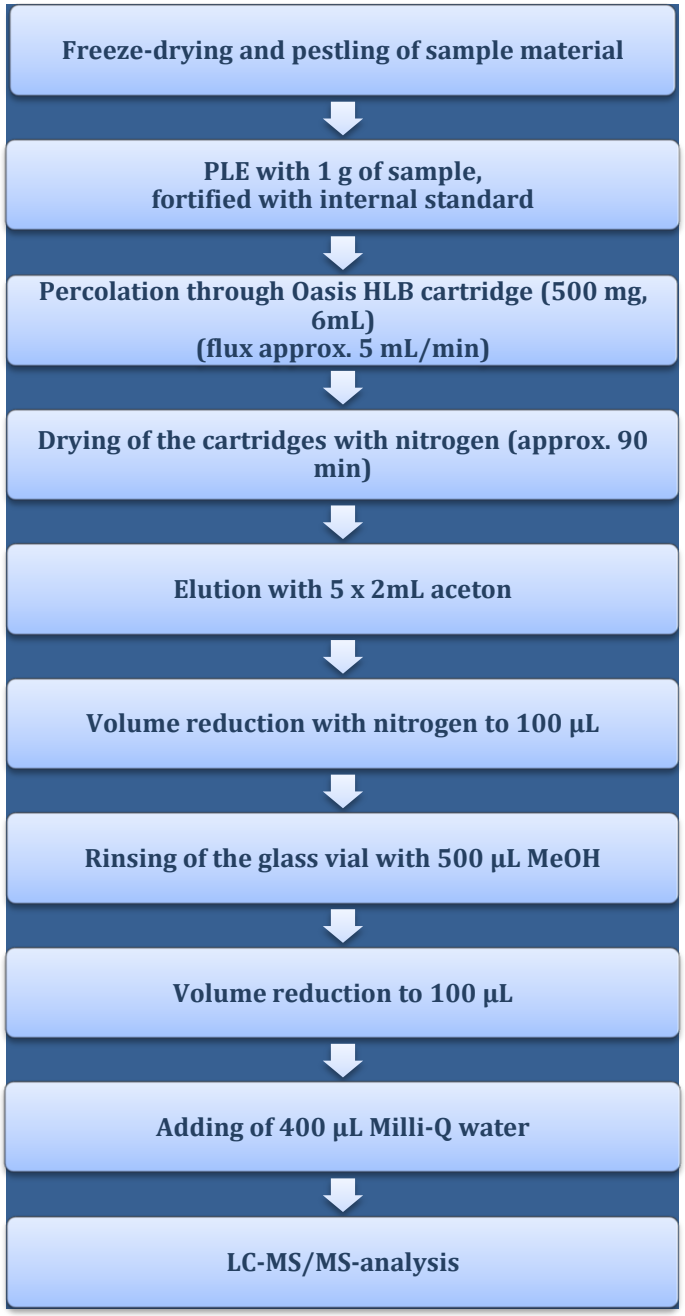


Figure 5-2: Flowchart of the laboratory procedure.

5.2.5 LC-MS/MS-Analysis

The analytical procedures used were described elsewhere (see Chapter 3.2.5).

5.2.6 Method validation

Details regarding quantification (calibration, linearity range etc.) have been given above (Chapter 3). Instrumental precision was determined by repeated injection of a standard solution during analysis (n = 2–6) and is indicated by the relative standard deviation of the results (%RSD). The accuracy of the method was assessed by studying the relative recovery in fortified native sampling material. Absolute and relative recoveries were determined in samples of suspended matter during method development (see Chapter 5.2.3). To account for matrix effects resulting from the different compositions of native samples, recoveries were determined for all investigated matrices (sludge from the NF-MBR_{WWTP}, sludge and carriers from the PS-$BFR_{hospital}$) by spiking native samples with 30 µL and 60 µL of analyte standard for pharmaceuticals and OPs (equalling 300 ng), respectively, and 10 µL of internal standard before extraction. The background amounts present in native samples were subtracted from the results of the spiked samples and the latter were subsequently related to standard solutions. To investigate how the sample mass used for PLE influences sensitivity and matrix effects, PLE of NF-MBR_{WWTP} sludge was carried out with both 0.5 g and 1 g of sample material.

5.3 Results and discussion

5.3.1 PLE methods

Pharmaceuticals. Based on various published studies, an extraction method for pharmaceuticals was chosen and tested. Table 5-3 shows the absolute and relative recoveries. Generally, absolute recoveries in suspended matter were low, probably caused by ion suppression, resulting in averages ranging from 1 to 37% for antibiotics and 5 to 32% for psycho-active drugs (n = 3), with a maximal RSD of 30%. However, correction by internal standards was successful for most analytes, while two antibiotics (roxithromycin and sulfisoxazole) were excluded from further investigations due to insufficient relative recoveries. The relative recoveries for N4-acetylsulfamethoxazole, sulfadimethoxine, trimethoprim, dihydrocodeine, codeine, methadone, diazepam, nordiazepam, carbamazepine, 10,11-dihydrocarbamazepine ranged between 69 and 109%, higher recoveries were accepted for clarithromycin, sulfamerazine, sulfadimidine, sulfamethoxazole, tiamulin, temazepam and oxycodone (131–156%), while for 10,11-dihydro-10,11-dihydroxycarbamazepine, primidone and doxepin lower recoveries were found (31–58%). RSD was below 15% for all compounds (Table 5-3).

The results show that literature data can provide a reliable basis for a fast method development with only slight adjustments. Nevertheless, the results clearly identify the need to verify published methods for each individual purpose. Reasons for problems with applying one method to different sample types can be found in the high variation within native sludge samples from different sampling sites. For native sludge samples, Ternes et al., 2005 showed large variations between activated and digested sludge, even from a single WWTP.

Table 5-3: Recoveries of pharmaceuticals in spiked suspended matter. Substances in grey – excluded from further investigations because of unsatisfactory recovery.

	methanol:water 50:50, 500 ng (n=3)					
	Absolute recovery			**Relative recovery**		
	%			%		
Sample Name	Median	Average	RSD	Median	Average	RSD
			Antibiotics			
CLA	33.2	36.5	17.8	150	156	7.40
ROX	35.3	37.3	16.1	214	218	3.65
SMI	14.0	13.7	9.93	101	109	14.4
SDI	4.76	4.52	9.32	154	155	2.82
SDX	7.32	8.18	25.1	342	339	4.76
SSX	0.820	0.914	21.3	19.1	19.2	1.38
SMA	3.03	3.01	12.6	138	137	5.87
SMX	4.01	4.35	25.6	132	132	3.42
N-Ac-SMX	6.46	7.42	30.3	105	105	8.07
TAM	18.1	18.1	15.8	129	131	3.04
TMP	10.8	11.2	21.8	68.5	69.8	10.2
min	3.03	3.01	9.32	68.5	69.8	2.82
max	33.2	36.5	30.3	153.5	156.2	14.4
			Psycho-active compounds			
CBZ	14.4	14.2	5.88	110	109	4.67
DH-CBZ	11.0	11.0	6.60	95.8	97.1	3.31
DHH	7.77	7.93	3.39	55.8	53.8	10.7
DXP	6.21	6.33	5.69	29.1	30.6	8.50
PMD	6.45	6.62	13.0	60.0	57.5	9.88
CDN	7.86	7.43	12.3	103	102	4.51
DHC	5.29	5.12	8.91	69.0	67.8	6.44
MTD	32.3	32.3	9.50	103	102	2.24
OCN	8.96	8.98	5.16	138	139	14.1
DZP	9.56	9.61	7.35	109	109	4.36
NZP	9.66	9.76	4.07	100	102	4.18
TZP	16.7	16.8	6.40	154	153	5.94
min	5.29	5.12	4.07	29.05	30.55	2.24
max	32.3	32.3	13.0	154.1	152.7	14.1

OPs. For the PLE of OPs, eight different solvents or solvent mixtures were tested through spiking experiments and were evaluated by absolute and relative recoveries (selected data see Table 5-4; all other results are shown in Annex 5-A).
For estimation of the method quality, in general recoveries between 70 and 140% were accepted. In some cases, deviating amounts were accepted as long as RSD was less than 20%.

The test schemes 3 (acetone), 4 (methanol:acetone 1:4), 7 b (ethyl acetate with elevated temperature and pressure) and 8 a and b (hexan:acetone:heptanes in two different temperature and pressure sets) produced unsatisfying absolute recoveries for most analytes, revealing massive losses during extraction and measurement, which for these settings were not corrected by internal standards, resulting in equally low relative recoveries. In detail, scheme 3 for all analytes yielded very low absolute recoveries and while relative recoveries for some OPs were satisfactory, RSD was high with only one value below 20% (n = 2). Test scheme 4 equally led to unsatisfying absolute recoveries for all analytes which were only corrected by the use of IS for TBEP, leading to a relative recovery of 80% with RSD of 5% (n = 3). Within test scheme 7 b absolute recoveries which failed were only corrected for TnBP, leading to relative recovery of 98% with RSD of 5% (n = 3).

The test schemes 2 (methanol) and 6 (methanol:acetone 4:1) showed highly heterogeneous absolute recoveries for different substances, but within these experimental set-ups the correction by internal standards led to acceptable relative recoveries for most of the analytes. For test scheme 2, TDCPP, TPP, TnBP, and TiBP yielded relative recoveries of 92 and 125% with RSD of 6–16% (n = 2). Test scheme 6 yielded relative recoveries between 90 and 140% for five of the seven OPs (TDCPP, TPP, TCPP, TnBP, TiBP, TCEP). RSD ranged between 8 and 15% for four of these analytes while the RSD of TCPP was higher (28%) (n = 3).

Best results for both absolute and relative recoveries were obtained using test schemes 1 (methanol:water 1:1) and 5 (methanol:acetone 1:1). Test scheme 1 yielded absolute recoveries between 78 and 132% for five analytes, four of these with RSD below 13% (n = 3); relative recoveries showed a suitable correction by the use of IS, leading to relative recoveries between 82 and 141% for five of the seven analytes with RSD below 10.5% except for TiBP (RSD 18%). Scheme 5 led to absolute recoveries between 77 and 88% (RSD: 4–20%) for five analytes; IS correction improved the method to achieve relative recoveries between 86 and 118% for the six analytes TDCPP, TPP, TCPP, TnBP, TiBP, TCEP, with RSD between 4 and 10% except for TCPP (RSD 31%) (n = 3).

Table 5-4: Recoveries of OPs in spiked suspended matter (only selected test schemes shown; for all other test schemes see Annex 5-A): spiking levels: * 100 ng, ** 500 ng.

Test scheme No.	**1a***					
	methanol:water 50:50 (n=3)					
	Absolute recovery %			Relative recovery %		
Sample Name	Median	Average	RSD	Median	Average	RSD
TBEP	159	151	10.4	228	224	8.78
TnBP	117	116	9.18	103	103	2.31
TiBP	155	152	13.2	141	134	18.0
TCEP	132	132	1.40	121	121	10.3
TPP	88.7	92.6	9.62	81.7	80.2	8.05
TCPP	78.3	70.0	21.3	-1.33	1.17	1773
TDCPP	94.0	92.2	12.6	82.2	81.7	1.91
min	78.3	70.0	1.40	-1.33	1.17	1.91
max	159	152	21.3	228	224	1773
Test scheme No.	**1b****					
	methanol:water 50:50 500 ng (n=3)					
	Absolute recovery %			Relative recovery %		
Sample Name	Median	Average	RSD	Median	Average	RSD
TBEP	132	134	3.05	175	174	2.45
TnBP	123	121	2.62	102	103	3.24
TiBP	123	126	7.49	111	106	7.51
TCEP	150	151	1.45	125	128	5.28
TPP	88.0	88.2	3.13	75.2	74.9	4.28
TCPP	119	108	21.1	90.1	85.5	10.3
TDCPP	95.5	96.0	3.29	81.4	80.9	6.95
min	88.0	88.2	1.45	75.2	74.9	2.45
max	150	151	21.1	175	174	10.3
Test scheme No.	**5***					
	methanol:acetone 50:50 (n=3)					
	Absolute recovery %			Relative recovery %		
Sample Name	Median	Average	RSD	Median	Average	RSD
TBEP	77.5	84.5	20.1	169	159	13.5
TnBP	76.9	80.1	6.91	102	99.0	7.07
TiBP	83.4	80.0	13.5	85.5	83.8	6.78
TCEP	88.5	90.6	7.97	118	113	9.79
TPP	78.2	79.0	4.21	117	119	4.44
TCPP	22.1	23.5	47.3	92.0	101	31.2
TDCPP	64.0	64.7	7.35	86.2	85.5	5.88
min	22.1	23.5	4.21	85.5	83.8	4.44
max	88.5	90.6	47.3	169	159	31.2

Since it was the goal of the method development not only to find an extraction method for the investigated OPs but also to combine the analysis of OPs with the PLE of pharmaceuticals, test scheme 1 (methanol:water (50:50, v/v)) was chosen for further investigation. In a second test with this set up, the spiking concentration in the samples was increased in expectation of relatively high concentrations in real sludge samples. In this test, absolute recoveries for six analytes lay within the required range from 70 to 140%. RSD were below 10% except for TCPP (21%). Correction by IS led to satisfactory relative recoveries (75–125%) for six out of seven analytes with RSD of 3–10% (n = 3). However, TBEP yielded in both spiking tests too high absolute recoveries (159 and 132% with RSD of 10 and 3%, respectively). Calculation against the internal standard TPP-d15 did not lead to a correction of the overestimation; relative recovery was 175% (RSD 3%; n = 3). The second available IS for OPs (TnBP-d27) did not yield better results (data not shown). However, since the main aim of the study was the development of a screening method for several compound groups, test scheme 1, which is also utilisable for pharmaceuticals, was kept for further analysis.

5.3.2 METHOD VALIDATION

Quantification, based on peak areas, was carried out by internal standard calibration. The calibration curves all showed a correlation coefficient (r^2) of at least 0.999. The instrumental precision yielded less than 20% RSD except for trimethoprim. LOQ in sludge ranged from 6 ng/g, d.w. to 50 ng/g, d.w. for pharmaceuticals and from 14 ng/g, d.w. to 150 ng/g, d.w. for OPs, while LOQ for the PS-$BFR_{hospital}$ carriers was lower due to the greater sample mass used for analysis (Table 5-7, Table 5-6). The accuracy of the method was determined by estimating relative recoveries in pre-spiked native samples of sludge from both PS-$BFR_{hospital}$ and NF-MBR_{WWTP}, as well as PS-$BFR_{hospital}$ carriers. Accuracy then was estimated by subtracting the analyte amount measured in native samples from the amount measured in the spiked ones (Table 5-5). Due to an irreplaceable loss of finished samples, the following results for the PS-$BFR_{hospital}$ stem from one set of spiked and native samples for sludge and carriers each. Accuracy for sulfamethoxazole, sulfamerazine, sulfadimidine, oxycodone, doxepin and TCEP ranged between 55 and 143% in all three matrices. Other compounds as sulfadimethoxine, tiamulin, trimethoprim, carbamazepine, codeine, nordiazepam, TDCPP, TPP, TnBP and TiBP showed acceptable accuracy in one or two matrices but less good results for the third. Generally, a tendency to too high recoveries is observed throughout, which could indicate an incomplete homogenisation of the sample material during pestling of the freeze-dried sludge. OPs in samples from the PS-$BFR_{hospital}$ show an especially poor accuracy. The reason for this can be found in the material of the polyurethane carriers which are assumed to emit OPs themselves (see Chapter 4.3), though the exact

composition of the carrier material is confidential. The carrier material was constantly moving and being rubbed, and the abraded matter was occasionally visible in the sludge samples as a fine dust, explaining insufficient accuracy results for OPs not only in carriers but in sludge as well. Severe inaccuracy was observed for TBEP and TCPP, which therefore are reported in Table 5-7 with their analysed environmental concentrations, but excluded from further discussion regarding the PS-$BFR_{hospital}$ samples.

To evaluate what influence the sample quantity used for PLE has on sensitivity (i.e., greater enrichment compared to the possibly higher matrix effect when using larger sample mass), sludge from the NF-MBR_{WWTP} was extracted using sample masses of 0.5 g and 1 g (Table 5-6). The differences in compound concentrations observed for the different sample quantities ranged from 1.28 to 64.4%. Nine substances show higher concentrations in samples of 0.5 g (10,11-dihydrocarbamazepine, 10,11-dihydro-10,11-dihydroxycarbamazepine, primidone, temazepam, doxepin, TDCPP, TPP, TiBP, TCEP), while six were higher concentrated in samples of 1 g (clarithromycin, sulfamethoxazole, carbamazepine, methadone, TBEP, TCPP). Since no definite tendency was found to prefer a sample mass of 0.5 g or 1 g, and in most cases the differences were small, a sample quantity for PLE of 1 g was chosen for further investigations.

Table 5-5: Accuracy and instrumental precision of the analytical procedure

	Accuracy [%]			Instrumental precision [RSD, %][3]
	Sludge BFR[1]	Carrier BFR[1]	Sludge MBR[2]	
		Antibiotics		
CLA	a.r.	a.r.	210 ; 228	8.46
SMI	145	470	132 ; 157	7.63
SDI	112	117	118 ; 119	4.35
SMA	122	127	117 ; 123	3.86
SMX	99.3	102	96.9 ; 102	15.6
N-Ac-SMX	90.9	155	101 ; 104	7.52
TAM	137	75.7	197 ; 204	0.989
TMP	243	a.r.	59.3 ; 72.3	46.9
		Psycho-active compounds		
CBZ	197	213	188 ; 197	1.34
DH-CBZ	192	182	180 ; 180	2.77
DHH	344	531	331 ; 392	1.39
DXP	103	55.0	67.4 ; 101	17.5
PMD	396	527	302 ; 385	5.29
CDN	177	186	192 ; 195	1.49
DHC	309	219	291 ; 297	4.03
MTD	207	151	232 ; 237	7.80
OCN	110	95.0	128 ; 153	10.6
DZP	222	201	197 ; 208	0.916
NZP	193	182	195 ; 216	1.63
TZP	222	200	262 ; 306	2.20

Table 5-5: continued.

	Accuracy [%]			Instrumental precision [RSD, %][3]
	Sludge BFR[1]	Carrier BFR[1]	Sludge MBR[2]	
		OPs		
TBEP	1293	-1,900	155 ; 174	6.21
TnBP	190	158	102 ; 117	5.97
TiBP	150	164	50.0 ; 146	10.4
TPP	190	23.7	111 ; 115	3.56
TCEP	143	113	94.1 ; 114	9.39
TCPP	410	466	267 ; 287	11.2
TDCPP	194	13.0	121 ; 130	4.47

[1]n=1; [2]n=2; [3]n=2-4.

Table 5-6: Comparison of compound amounts [ng/g, d.w.] found in aliquot samples of NF-MBR$_{WWTP}$ sludge with different extraction quantities during PLE. Print in bold: the greater average amount per compared pair.

Sample weight [g]		**0.5 g**			**1 g**			**Difference[1]**
ng/g , d.w.	**LOQ**	**Median**	**Average**	**SD**	**Median**	**Average**	**SD**	**[%]**
				Antibiotics				
CLA	10	35.9	35.9	1.84	47.6	**47.6**	2.19	24.5
SMI	10	< LOQ	< LOQ	---	< LOQ	< LOQ	---	---
SDI	10	< LOQ	< LOQ	---	< LOQ	< LOQ	---	---
SMA	10	< LOQ	< LOQ	---	< LOQ	< LOQ	---	---
SMX	10	46.0	46.0	1.13	50.0	**50.0**	2.05	7.91
N-Ac-SMX	40	< LOQ	< LOQ	---	< LOQ	< LOQ	---	---
TAM	10	< LOQ	< LOQ	---	< LOQ	< LOQ	---	---
TMP	10	< LOQ	< LOQ	---	< LOQ	< LOQ	---	---
				Psycho-active compounds				
CBZ	10	101	101	6.79	109	**109**	3.54	6.73
DH-CBZ	6	8.10	**8.1**	1.24	6.88	6.88	0.042	15.1
DHH	20	187	**187**	7.78	142	142	3.54	31.8
DXP	10	71.0	**71.0**	2.83	62.1	62.1	1.27	12.5
PMD	40	71.0	**71.0**	0.000	42.8	42.8	0.495	39.8
CDN	50	< LOQ	< LOQ	---	< LOQ	< LOQ	---	---
DHC	20	< LOQ	< LOQ	---	< LOQ	< LOQ	---	---
MTD	20	64.8	64.8	3.39	70.0	**70.0**	9.83	7.36
OCN	20	< LOQ	< LOQ	---	< LOQ	< LOQ	---	---
DZP	20	< LOQ	< LOQ	---	< LOQ	< LOQ	---	---
NZP	20	< LOQ	< LOQ	---	< LOQ	< LOQ	---	---
TZP	10	16.7	**16.7**	0.240	12.3	12.3	0.283	26.2
				OPs				
TBEP	25	484	484	56.57	552	**552**	22.63	12.3
TnBP	50	< LOQ	< LOQ	---	< LOQ	< LOQ	---	---
TiBP	150	652	**652**	45.25	232	232	16.97	64.4
TPP	25	232	**232**	28.28	187.4	187	13.29	19.2
TCEP	14	39.2	**39.2**	9.56	17.2	17.2	1.12	56.1
TCPP	110	5,000	5,000	849	5,330	**5,330**	891	6.19
TDCPP	60	98.2	**98.2**	0.849	78.2	78.2	3.68	20.4

[1] difference between the average amount in corresponding samples with 0.5 g and 1g sample mass for PLE

5.3.3 METHOD APPLICATION

5.3.3.1 Sludge

In sludge samples from the two pilot plants, the overall maximum concentrations found for single compounds of antibiotics, psycho-active drugs and OPs were 342 ng/g, d.w., 537 ng/g, d.w. and 5330 ng/g, d.w., respectively (Table 5-7). The antibiotics sulfadimethoxine, sulfamerazine, sulfadimidine and tiamulin as well as the antibiotic metabolite N4-acetylsulfamethoxazole were not quantified in any of the samples. In contrast, eleven analytes (sulfamethoxazole, carbamazepine, 10,11-dihydrocarbamazepine, 10,11-dihydro-10,11-dihydroxycarbamazepine, dihydrocodeine, temazepam, doxepin, TDCPP, TPP, TiBP, TCEP) were found in sludge from both pilot plants. Primidone could not be quantified in PS-$BFR_{hospital}$ sludge but was quantified in sludge from the NF-MBR_{WWTP}. Trimethoprim, codeine, nordiazepam and dihydrocodeine were only quantified in samples from the PS-$BFR_{hospital}$, while methadone was only found in sludge from the NF-MBR_{WWTP}.

UBIQUITOUS SUBSTANCES. In the group of compounds found in sludge both from the PS-$BFR_{hospital}$ and the NF-MBR_{WWTP}, clarithromycin, sulfamethoxazole, 10,11-Dihydro-10,11-dihydroxycarbamazepine and doxepin showed higher concentrations at the hospital sampling site. All other substances in this omnipresent group showed very similar concentrations in both sludge types, indicating that despite being different in terms of feed water and treatment method, the sludges from the PS-$BFR_{hospital}$ and NF-MBR_{WWTP} are comparable regarding their overall load of pharmaceuticals and OPs. This basic similarity between the xenobiotic composition in the sludge samples from PS-$BFR_{hospital}$ and NF-MBR_{WWTP} is also reflected in the sum of concentrations for each compound group (Figure 5-3). The overall amount of antibiotics was more than eight times higher in sludge at the hospital (PS-$BFR_{hospital}$) than at the WWTP (NF-MBR_{WWTP}), which is probably caused by high usage of these pharmaceuticals in the hospital. Yet the total of psycho-active compounds was no more than roughly twice as high in the PS-$BFR_{hospital}$ sludge, reflecting widespread use elsewhere than at the hospital site, and the amount of OPs was almost identical at both sampling sites, which reflects the ubiquitous character of this compound group.

ANTIBIOTICS. When comparing the results for antibiotics in the presented study with data given in the literature, similar amounts for sulfamethoxazole and clarithromycin at the WWTP were reported previously, while the amount of clarithromycin in the sludge from the hospital is higher than reported in other studies. On the other hand, trimethoprim, which was not found in the sludge from the NF-MBR_{WWTP}, was frequently detected in sludge from WWTPs by other researchers. In activated sludge from several WWTPs in Germany and Switzerland with

conventional activated sludge treatment (CAS), Göbel et al. (Göbel et al., 2005a; Göbel et al., 2005b) found concentrations of sulfamethoxazole, trimethoprim and clarithromycin ranging from 34 – 100 ng/g d.w., 13 – 133 ng/g d.w. and 16 – 95 ng/g d.w., respectively. They also investigated sulfadimidine but detected none in any samples, which is in agreement with the results of my study. In secondary sludge from a CAS-WWTP in Spain, Radjenović et al., 2009a found concentrations of 21.0 ± 7.2 ng/g d.w. and 42.6 ± 7.2 ng/g d.w. for sulfamethoxazole and trimethoprim, respectively. In the same study, sludge from two different types of NF-MBR_{WWTP} was investigated. One was equipped with hollow-fibre (HF) ultra-filtration membranes and the other with micro-filtration flat-sheet (FS) membranes. The sludge yielded concentrations of 27.9 ± 6.5 ng/g d.w. (HF) and 17.0 ± 9.6 ng/g d.w. (FS) for sulfamethoxazole, and 34.2 ± 13.1 ng/g d.w. (HS) and 22.4 ± 10.4 ng/g d.w. (FS) for trimethoprim.

PSYCHO-ACTIVE COMPOUNDS. Not many studies are available regarding the occurrence of psycho-active compounds in sewage sludge. Ternes et al., 2005 used ultrasonication and LC-MS/MS analysis to determine carbamazepine and diazepam in both activated and digested sludge from Swiss and German WWTPs. Neither substance was detected in any samples above the limit of quantification, which was 20 ng/g d.w., and it was assumed that sorption onto sludge was negligible for these substances, which in the case of carbamazepine is not in accordance with my data. In contrast, and essentially in agreement with the results presented here, Miao et al., 2005 reported the amounts of carbamazepine and 10,11-Dihydro-10,11-dihydroxycarbamazepine in activated sludge from a Canadian CAS-WWTP to be 69.6 ng/g d.w. and 7.5 ng/g d.w., respectively. Radjenović et al., 2009a found carbamazepine concentrations in activated sludge from a CAS-WWTP, in sludge from a MBR equipped with hollow-fibre ultra-filtration membrane and from another MBR with micro-filtration flat-sheet membranes to be 34.1 ± 6.3 ng/g d.w., 45.0 ± 11.9 ng/g d.w. and 41.6 ± 20.3 ng/g d.w., respectively.

OPS. To date, OPs in sludge have rarely been investigated. To my knowledge, no data regarding activated sludge have been published. Marklund et al., 2005 investigated digested sludge from 11 Swedish WWTPs of different sizes and using different treatment technology for the occurrence of eight OPs, finding TBEP, TCPP and TiBP to be the most abundant ones (< 5.1–1900 ng/g d.w., 61–1900 ng/g d.w. and 27–2700 ng/g d.w, respectively), while TnBP (39–850 ng/g d.w.), TPP (52–320 ng/g d.w.), TDCPP (3.0–260 ng/g d.w.) and TCEP (6.6–110 ng/g d.w.) were found at lower concentrations. In regard of TnBP, these results differ from the results presented here, where the substance is only found above the limit of quantification in the PS-$BFR_{hospital}$ carrier material, probably originating from the polyurethane foam itself.

Table 5-7: Concentrations of xenobiotics in sludge and carriers from PS-$BFR_{hospital}$ and in NF-MBR_{WWTP} sludge.

Sample weight [g]			**1.023**[1]	**3.522**[1]	**1.015±0.004**[2]
	LOQ		**BFR**		**MBR**
Compound	**Sludge**	**Carrier**	**Sludge**	**Carrier**	**Sludge**
	ng/g d.w.		**ng/g d.w.**		**ng/g d.w.**
		Antibiotics			
CLA	10	2.86	342	> 2,000[3]	47.6 ± 2.19
SMI	10	2.86	< LOQ	< LOQ	< LOQ
SDI	10	2.86	< LOQ	< LOQ	< LOQ
SMA	10	2.86	< LOQ	< LOQ	< LOQ
SMX	10	2.86	112	92.0	50.0 ± 2.05
N-Ac-SMX	40	11.4	< LOQ	< LOQ	< LOQ
TAM	10	2.86	< LOQ	< LOQ	< LOQ
TMP	10	2.86	291	240	< LOQ
		Psycho-active compounds			
CBZ	10	2.86	88.0	96.3	109 ± 3.54
DH-CBZ	6	1.71	6.77	5.76	6.88 ± 0.042
DHH	20	5.71	537	188	142 ± 3.54
DXP	10	2.86	143	90.9	62.1 ± 1.27
PMD	40	11.4	< LOQ	15.0	43.0 ± 0.495
CDN	50	14.3	47.9	70.7	< LOQ
DHC	20	5.71	93.9	47.1	n.d.
MTD	20	5.71	< LOQ	< LOQ	70.0 ± 9.83
OCN	20	5.71	< LOQ	9.54	< LOQ
DZP	20	5.71	< LOQ	10.6	< LOQ
NZP	20	5.71	20.0	34.9	< LOQ
TZP	10	2.86	12.5	8.77	12.3 ± 0.283
		OPs			
TBEP	25	7.14	4,920[4]	11,016[4]	552 ± 22.6
TnBP	50	14.3	< LOQ	69.8	< LOQ
TiBP	150	42.9	350	341	232 ± 17.0
TPP	25	7.14	33.0	123	188 ± 13.3
TCEP	14	4.00	21.6	14.7	17.23 ± 1.12
TCPP	110	31.4	830[2]	166[2]	5,330 ± 891
TDCPP	60	17.1	70.8	315	78.2 ± 3.68

[1] n=1; [2] n=2; [3] above concentration range; [4] semi-quantitative data due to poor quality assurance results

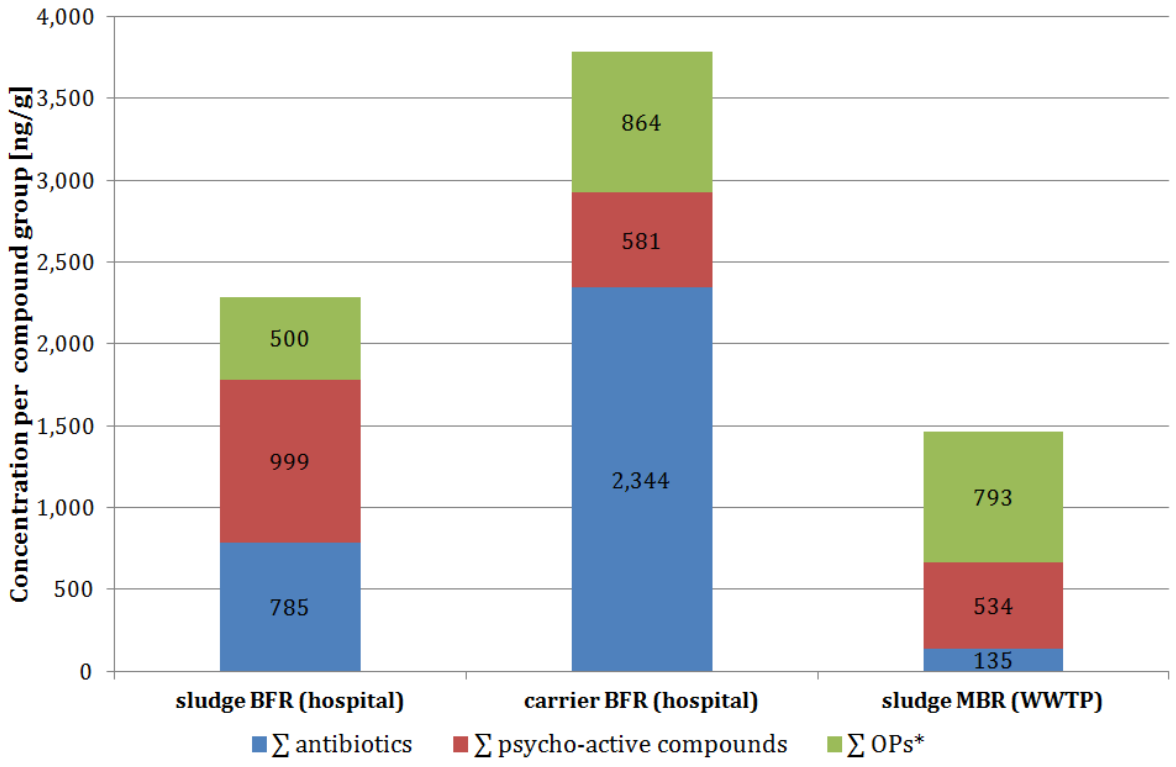


Figure 5-3: Sum of concentrations per investigated compound group in sludge and carrier material from the PS-BFR$_{hospital}$ and in the sludge from the NF-MBR$_{WWTP}$. Concentrations below LOQ were taken into account with LOQ/2; concentrations above the calibration range were calculated with the amount of the highest calibration point; *for the sum of OPs, concentrations of TBEP and TCPP were not taken into account (see Chapter 5.3.2).

5.3.3.2 Carriers

When comparing the concentrations detected in sludge and carrier material from the PS-BFR$_{hospital}$, for most compounds very similar amounts were found (Table 5-7). This could be a result of co-extracted sludge particles which remained attached to the carrier material throughout drying and freeze-drying or, more likely, it could be caused by the sludge and the biofilm having similar surface characteristics. So the exceedingly large amounts of clarithromycin found in the carrier material are unexpected at first. However, the extracellular polymeric substances (EPS), which hold flocs of activated sludge together as well as being the backbone of the cell-surrounding gels of biofilms, are negatively charged at pH > 7 (Bryers, 2000 cited in Wunder et al., 2011; Stewart and Costerton, 2001).

Ionic interactions. Since clarithromycin was, in contrast to the other xenobiotics investigated in this study, positively charged under the pH conditions present in the PS-BFR$_{hospital}$ pilot plant, higher sorption of clarithromycin can be expected. Wunder et al., 2011 found that ionic interactions are more essential for the sorption of antibiotics to biofilm polymers than

hydrophobic interactions. Accordingly, Abegglen et al., 2009 reported strong sorption processes for clarithromycin in the sludge from an MBR with approximately 40% of the originally spiked amount still being bound in the sludge after two weeks. As was shown elsewhere (see Chapter 4.3), clarithromycin concentrations in the feed water of the PS-$BFR_{hospital}$ were high (1.2 $\pm$ 0.7 µg/L). The large surface provided by the porous polyurethane foam carriers and the constant movement of the carriers in the feed water allow the substances easy access to the biofilm surface. In this way, the problem of restricted mass transfer to and through the biofilm is circumvented, which was found to inhibit sorption of the comparably large macrolide molecules (molecular weight of clarithromycin: 747.95; see Chapter 2.1.1.1) reported from bench-scale laboratory studies using biofilms grown on compact media (Wunder et al., 2011). Taking the strong sorption tendency of the substance and the large surface area provided by the porous polyurethane foam carriers into account, the large amounts of clarithromycin found in the carrier material seem realistic.

5.4 Conclusions

A PLE method was developed which allowed the simultaneous extraction of 27 substances of different xenobiotic compound groups (antibiotics and metabolites, psycho-active substances and metabolites, OPs) from biosolids. LOQs ranged from 1.71 ng/g d.w. to 150 ng/g d.w. depending on the extracted biosolids and the compound. While matrix effects were an issue in case of several substances, no general differences were found when varying sample quantities were used for PLE. The PLE method was applied to sewage sludge from a nanofiltration membrane bioreactor pilot plant located at the influent of a WWTP, and to sludge and carrier material from a particle-supported biofilm reactor pilot plant situated at the effluent of a municipal hospital. The highest concentrations found in the sludge were 342 ng/g d.w., 537 ng/g d.w. and 5330 ng/g d.w., for antibiotics, psycho-active drugs and OPs, respectively. These results underline the presence of xenobiotics in sludge. In many countries sludge is later used as fertiliser in agriculture, and therefore provides a possible gateway for sludge-bound xenobiotics to enter the environment. Xenobiotic concentrations found in biofilm carriers suggested bleeding of the carrier material during PLE, which in some cases led to inaccuracies regarding OP concentrations. Furthermore, indications were found that particle-supported biofilms have advanced sorption capacities.

5-A Annex

Table 5A-1: Amounts of water-originating xenobiotics in the biosolids investigated.

	PS-BFR$_{hospital}$		NF-MBR$_{WWTP}$
Compound	Sludge	Carrier	Sludge
	Antibiotics		
CLA	1.45		4.91
SMX	10.3	12.6	10.2
N-Ac-SMX	---	---	---
SMI	---	---	---
SMA	---	---	---
SDI	---	---	---
TAM	---	---	---
TMP	---	---	---
	Psycho-active compounds		
CBZ	2.71	2.48	4.19
DH-CBZ	1.74	2.04	2.66
DHH	9.21	26.3	17.6
PMD	---	58.2	15.7
CDN	---	---	---
DHC	---	---	---
MTD	---	---	0.331
OCN	---	---	---
DZP	---	---	---
NZP	0.411	0.236	---
TZP	0.669	0.953	0.958
DXP	n.w.	n.w.	1.65
	OPs		
TBEP	1.11	0.496	5.42
TDCPP	n.w.	n.w.	0.949
TPP	n.w.	n.w.	0.050
TCPP	n.w.	n.w.	0.079
TnBP	---	0.419	---
TiBP	n.w.	n.w.	3.66
TCEP	1.34	1.96	2.21

--- = not determinable due to sludge concentrations < LOQ; n.w. = not determinable due to water concentrations < LOQ.

Table 5A-2: Recoveries of OPs in spiked suspended matter (trial test schemes).

Test scheme No.	**2**					
	methanol					
	Absolute recovery (n=2) %			Relative recovery (n=3) %		
Sample Name	Median	Average	RSD	Median	Average	RSD
TBEP	71.9	71.9	35.1	185	173	13.9
TnBP	66.8	66.8	11.9	104	101	5.7
TiBP	75.0	75.0	0.4	92.0	94.3	15.6
TCEP	117	117	12.2	182	186	3.6
TPP	64.5	64.5	15.1	125	133	12.9
TCPP	266	266	124.9	348	451	85.8
TDCPP	58.3	58.3	5.1	102	100	5.4
min	58.3	58.3	0.356	92.0	94.3	3.56
max	266	266	125	348	451	85.8

Test scheme No.	**3**					
	acetone (n=2)					
	Absolute recovery %			Relative recovery %		
Sample Name	Median	Average	RSD	Median	Average	RSD
TBEP	11.5	11.5	421.5	875	875	110.1
TnBP	19.7	19.7	112.4	97.7	97.7	8.0
TiBP	16.2	16.2	104.3	83.2	83.2	21.2
TCEP	61.7	61.7	3.3	573	573	95.2
TPP	12.1	12.1	221.8	62.0	62.0	117.5
TCPP	-6.38	-6.38	-1431	711	711	85.5
TDCPP	14.2	14.2	174.5	68.8	68.8	66.4
min	-6.38	-6.38	-1431	62.0	62.0	7.96
max	61.7	61.7	421.5	875	875	117

Test scheme No.	**4**					
	methanol:acetone 20:80 (n=3)					
	Absolute recovery %			Relative recovery %		
Sample Name	Median	Average	RSD	Median	Average	RSD
TBEP	34.3	42.7	78.4	79.7	79.7	5.07
TnBP	18.2	21.4	71.7	33.8	33.8	6.84
TiBP	8.11	15.2	119	20.1	20.1	17.5
TCEP	26.7	19.7	72.0	30.7	30.7	47.3
TPP	22.7	24.7	81.1	53.3	53.3	19.0
TCPP	-79.3	-54.5	-105	-38.7	-38.7	-28.4
TDCPP	23.6	25.0	45.7	39.1	39.1	13.3
min	-79.3	-54.5	-105	-38.7	-38.7	-28.4
max	34.3	42.7	119	79.7	79.7	47.3

Test scheme No.	**6**					
	methanol:acetone 80:20 (n=3)					
	Absolute recovery %			Relative recovery %		
Sample Name	Median	Average	RSD	Median	Average	RSD
TBEP	65.1	65.5	9.73	187	186	6.98
TnBP	71.1	67.2	11.0	104	106	7.71
TiBP	81.4	76.1	13.8	101	100	9.57
TCEP	85.2	86.5	6.85	140	139	9.36
TPP	62.1	62.7	16.0	113	123	15.3
TCPP	-15.8	-14.1	-116	98.0	100	27.5
TDCPP	56.2	54.8	11.3	89.8	94.8	10.1
min	-15.8	-14.1	-116	89.8	94.8	6.98
max	85.2	86.5	16.0	187	186	27.5

Test scheme No.	**7a**					
	ethyl acetate (n=2)					
	Absolute recovery %			Relative recovery %		
Sample Name	Median	Average	RSD	Median	Average	RSD
TBEP	23.1	23.1	21.7	82.0	82.0	48.3
TnBP	49.9	49.9	16.6	113	113	1.88
TiBP	-190	-190	-5.09	-126	-126	-3.37
TCEP	88.0	88.0	83.9	202	202	92.0
TPP	39.3	39.3	26.8	106	106	8.64
TCPP	-282	-282	-39.6	51.5	51.5	625
TDCPP	47.2	47.2	18.2	108	108	1.96
min	-282	-282	-39.6	-126	-126	-3.37
max	88.0	88.0	83.9	202	202	625

Test scheme No.	**7b**					
	ethylacetate b (n=3)					
	Absolute recovery %			Relative recovery %		
Sample Name	Median	Average	RSD	Median	Average	RSD
TBEP	-54.4	-54.8	-1.97	300	382	43.8
TnBP	0.519	0.534	85.6	95.5	97.5	4.47
TiBP	-224	-224	-0.702	-41.0	-44.0	-22.4
TCEP	95.7	90.1	19.1	1335	1372	24.5
TPP	-9.65	-9.93	-7.59	47.7	48.3	15.4
TCPP	-213	-212	-2.76	1149	1106	15.3
TDCPP	-6.45	-6.88	-10.8	61.6	61.5	7.16
min	-224	-224	-10.8	-41.0	-44.0	-22.4
max	95.7	90.1	85.6	1335	1372	43.8

Test scheme No.	**8a**					
	hexan:aceton:heptan a (n=2)					
	Absolute recovery %			Relative recovery %		
Sample Name	Median	Average	RSD	Median	Average	RSD
TBEP	-15.6	-15.6	-15.4	142	142	22.9
TnBP	15.2	15.2	24.2	110	110	8.36
TiBP	-60.0	-60.0	-26.2	57.5	57.5	67.6
TCEP	1.51	1.51	46.4	12.7	12.7	2.23
TPP	-1.58	-1.58	-201	102	102	58.6
TCPP	-145	-145	-63.6	169	169	247
TDCPP	13.2	13.2	10.3	121	121	36.7
min	-145	-145	-201	12.7	12.7	2.23
max	15.2	15.2	46.4	169	169	247

Test scheme No.	**8b**					
	hexan:aceton:heptan b (n=2)					
	Absolute recovery %			Relative recovery %		
Sample Name	Median	Average	RSD	Median	Average	RSD
TBEP	13.4	13.4	69.5	486	486	34.9
TnBP	8.96	8.96	57.4	112	112	10.1
TiBP	22.0	22.0	52.5	255	255	17.2
TCEP	97.4	97.4	25.6	1331	1331	25.0
TPP	2.38	2.38	47.0	32.9	32.9	15.5
TCPP	52.7	52.7	133	375	375	13.2
TDCPP	5.48	5.48	42.2	70.1	70.1	10.4
min	2.38	2.38	25.6	32.9	32.9	10.1
max	97.4	97.4	133	1331	1331	34.9

6. SUMMARISING DISCUSSION

You have learnt something. That always feels at first as if you had lost something.
– Georg Bernard Shaw (1856-1950), Major Barbara, Act III, 1905 –

In this chapter, the results of the individual studies presented in Chapters 3 to 5 are contrasted and related to each other. Thus, the properties found for the investigated wastewater types are compared, the xenobiotic loads of different wastewater streams are investigated and the studied wastewater treatment methods are compared regarding their capacity for xenobiotic removal.

6.1 CHARACTERISATION OF WASTEWATER FROM DIFFERENT SOURCES

The cumulative concentrations of all investigated xenobiotic compound groups found for the hospital effluent and the influent of the municipal WWTP are shown in Figure 6-1. The total concentration is more than ten times higher in the hospital effluent (1,647 µg/L) than in raw municipal wastewater (135 µg/L). In both cases, ICMs are the xenobiotic compound group with the highest total concentration (WWTP influent: 91.9 µg/L, hospital effluent: 1,573 µg/L) and they are mainly responsible for the difference between the total concentrations in the two wastewater types: The concentration for ∑ NSAIDs at the hospital is only slightly higher than at the WWTP (35.7 µg/L vs. 11.6 µg/L), and all other compound groups show very similar cumulative concentrations at the hospital and at the WWTP (∑ antibiotics being 4.76 µg/L (WWTP) and 5.58 µg/L (hospital), ∑ psycho-active compounds being 13.3 µg/L (WWTP) and 18.7 µg/L (hospital), ∑ OPs 13.4 µg/L (WWTP) and 13.0 µg/L (hospital)). These results reflect the extensive use of ICMs and, to a lesser degree, NSAIDs in the hospital, while the incidence of antibiotics and psycho-active compounds seems not to be primarily hospital-bound. The similarity of total concentrations of OPs in both wastewater types seems to mirror the ubiquitous use of these industrial chemicals. However, it is noteworthy that while the total concentration is similar, the number of OPs contributing to it is not: in the case of the WWTP, six out of the eight OPs studied were quantified and amounted to roughly the same cumulative concentration as only three OPs quantified in raw hospital wastewater (for details, see Chapters 3.3.2 and 4.3.2).

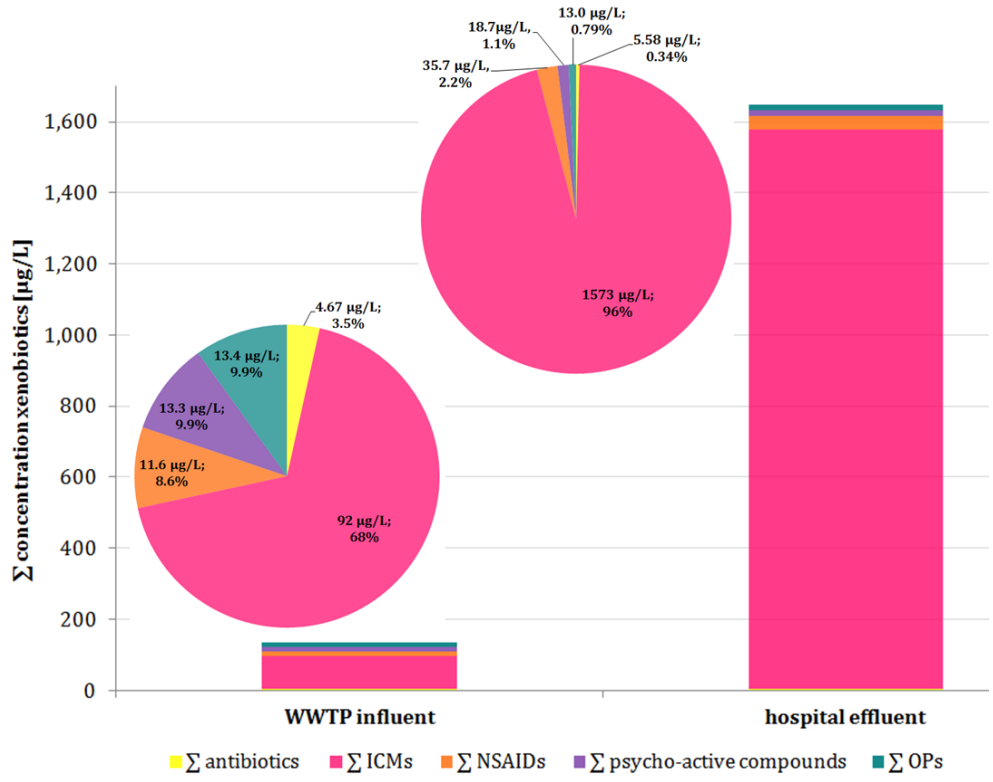


Figure 6-1: Cumulative concentrations per compound group [ng/L] in raw municipal wastewater (WWTP influent) and in raw hospital wastewater (hospital effluent).

For ICMs, too, distinct differences between the hospital and the municipal wastewater were found (Figure 6-2). In raw hospital wastewater, two substances, diatrizoic acid and iomeprol, are responsible for practically the whole concentration of ICMs. On the other hand, these two compounds are quite insignificant in the case of municipal wastewater, where iopromide is the most highly concentrated ICM. It can be assumed that diatrizoic acid and iomeprol reflect a very specific use at the hospital, with other sources (other hospitals and households) seemingly not contributing in major ways. The opposite seems to be true for iopromide, for which sources other than the investigated hospital seem to be important (see also Chapter 6.2).

In summary, it can be said that there are basic differences between wastewater streams originating from the hospital and from mixed municipal sources regarding concentrations for ICMs, while other pharmaceuticals are more evenly distributed. For OPs, similar overall concentrations in the two wastewater types derived from a very different set of single substances. The results regarding ICMs are in contrast to the often postulated opinion that ICMs are applied at hospitals to outpatients but excreted mainly away from the hospital after return to home (e.g. Chèvre et al., 2013). Indeed, the rapid passage of the substances through the human body, with highest excretion rates occurring within an hour after application (Sprehe et al.,

2000, see also Chapter 2.1.1.2), make it very plausible that ICMs are excreted in high concentrations at the hospital before outpatients leave the facilities, and thus reflect specialised use patterns.

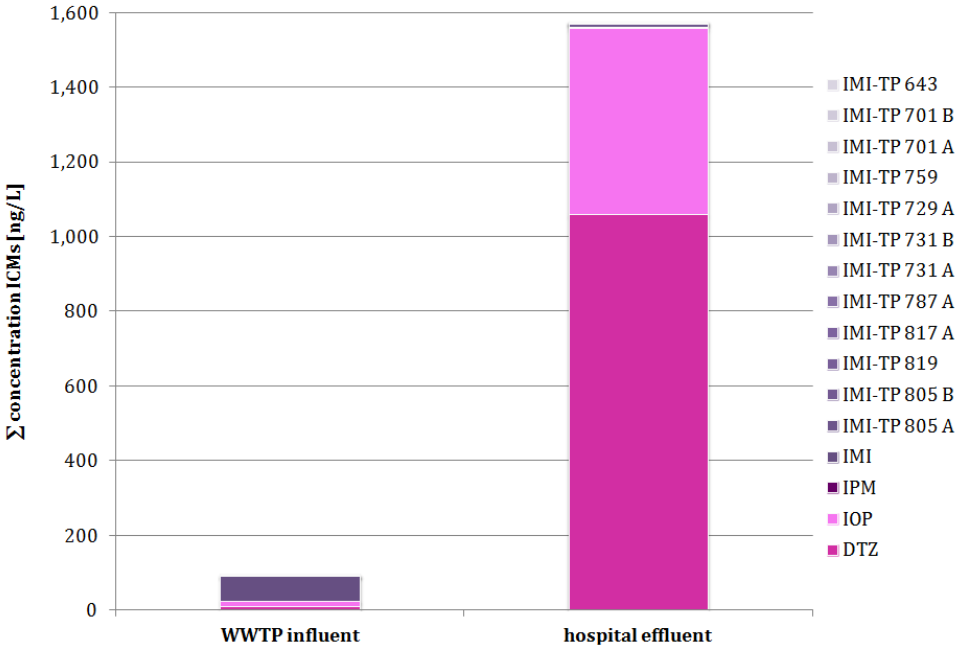


Figure 6-2: ICMs in raw hospital (left) and municipal (right) wastewater.

6.2 Contribution of hospital wastewater to the xenobiotic load in municipal wastewater

The annual loads of xenobiotics in hospital and municipal wastewater (normalised to the number of inpatients in the case of the hospital and expressed on a per capita basis for the WWTP) are given in Table 6-1 and Table 6-2. The hospital investigated is relatively small (see Chapter 4.2.2) and only 0.15% of the annual municipal WWTP water inflow originates from it. Subsequently, the hospital-derived load of most of the xenobiotics found in the WWTP is less than 1% (Figure 6-3), which concurs with results from other studies (Thomas et al., 2007; Langford and Thomas, 2009). Overall, for only four substances – all of them ICMs or ICM-TPs – was it found that the estimated load originating from the hospital exceeds 1% of the overall WWTP influent load. However, 21.5% of the annual load of diatrizoic acid reaching the WWTP is estimated to originate from the hospital. The high contribution of diatrizoic acid is surprising, especially when taking into account that a) ICMs are often assumed to be excreted away from

the hospital (Chèvre et al., 2013) and b) because of the presence of several further radiological facilities connected to a large university clinic within the service area of the WWTP. It could therefore be expected that these larger facilities exceed the smaller hospital in regard of administering ICMs. These results might point to a specific use pattern of ICMs between different medical facilities and underlines the assumption given above that wastewater streams from different sources can be dissimilar (see Chapter 6.1). Hospital effluent and municipal wastewater might have comparable patterns for some therapeutic groups and not for others, hospital wastewater thus having a different finger print from municipal wastewater.

When calculating the contribution of hospital wastewater to municipal loads, it has to be kept in mind that for substances known to retransform from metabolites into parent compounds in wastewater, the results have to be assumed to contain increased uncertainties.

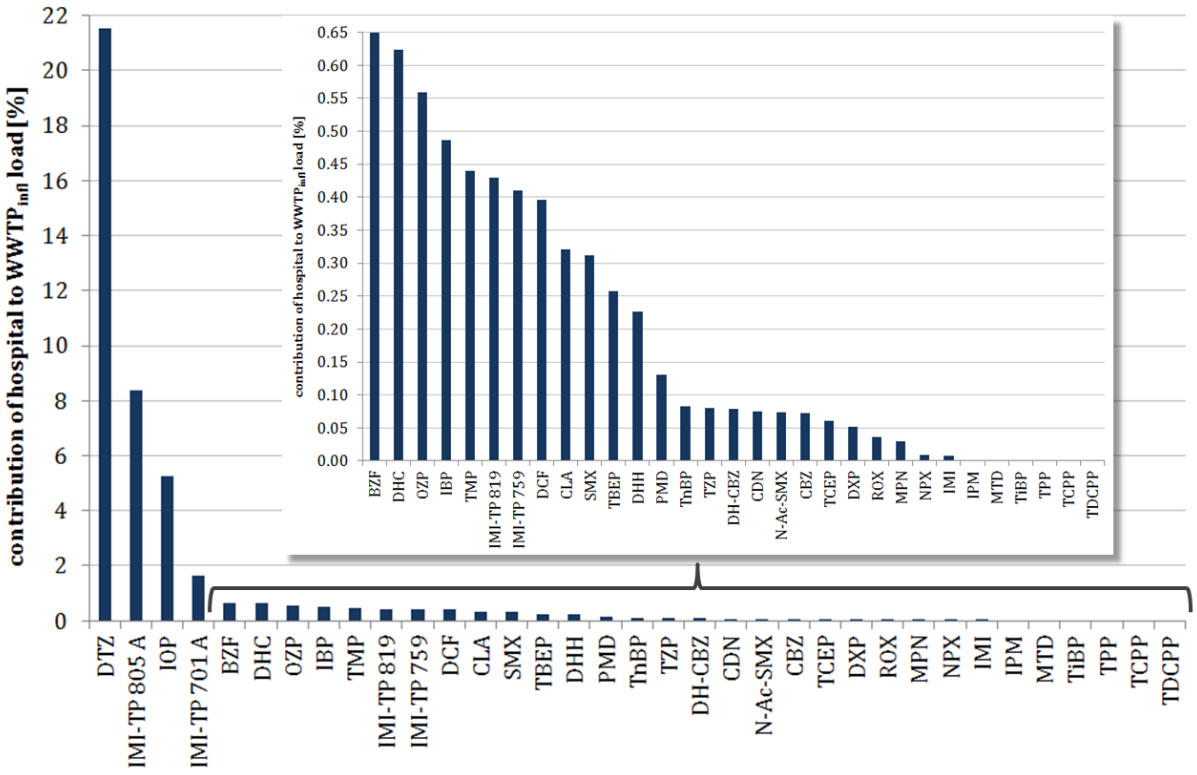


Figure 6-3: Estimated contribution of the effluent of the investigated hospital to the input load of the municipal WWTP [%].

Table 6-1: Estimated normalised annual mass loads of the investigated xenobiotics in raw municipal wastewater (influent WWTP). n.a.: not analysed; ---: < LOQ/not detected

	WWTP influent [mg/year/person]		**WWTP influent [mg/year/person]**
	Antibiotics		*NSAIDs and bezafibrate*
CLA	48.0	BZF	112
ROX	10.9	DCF	117
SMI	2.48	IBP	718
SDI	---	NPX	79.2
SSX	---		*Psycho-active compounds*
SMA	---	CBZ	103
SMX	114	DH-CBZ	4.66
N-Ac-SMX	227	DHH	675
TAM	---	DXP	25.7
TMP	12.0	PMD	208
	ICM	CDN	20.4
DTZ	647	DHC	2.89
IOP	1,247	MTD	5.22
IPM	152	MPN	34.9
IMI	5,933	OCN	---
IMI-TP 805 A	10.8	DZP	---
IMI-TP 805 B	---	NZP	---
IMI-TP 819	69.8	OZP	90.3
IMI-TP 817 A	---	TZP	3.25
IMI-TP 787 A	---		*OPs*
IMI-TP 731 A	---	TBEP	657
IMI-TP 731 B	---	TnBP	10.9
IMI-TP 729 A	---	TiBP	379
IMI-TP 759	42.0	TEHP	---
IMI-TP 701 A	10.2	TPP	2.45
IMI-TP 701 B	---	TCEP	14.6
IMI-TP 643	---	TCPP	96.0
		TDCPP	19.6

[a] Loads were calculated and normalised using average concentrations (see Table 3-8) and the following data: WWTP: influent flow rate: 15,000,000 m^3/year; persons connected to the sewage system: 170,000 (MWB, 2013).

Table 6-2: Estimated normalised* annual mass loads of the investigated xenobiotics in raw hospital wastewater (hospital effluent). n.a.: not analysed; ---: < LOQ/not detected

	Hospital effluent [mg/year/patient]		**Hospital effluent [mg/year/patient]**
	Antibiotics		*NSAIDs and bezafibrate*
CLA	2.61	BZF	12.4
ROX	0.066	DCF	7.86
SMI	n.a.	IBP	59.4
SDI	n.a.	NPX	0.122
SSX	n.a.		*Psycho-active compounds*
SMA	n.a.	CBZ	1.25
SMX	6.07	DH-CBZ	0.062
N-Ac-SMX	2.82	DHH	26.0
TAM	n.a.	DXP	0.227
TMP	0.899	PMD	4.61
	ICM#	CDN	0.260
DTZ	2,366	DHC	0.306
IOP	1,112	MTD	---
IPM	---	MPN	0.174
IMI	7.71	OCN	0.260
IMI-TP 805 A	15.4	DZP	---
IMI-TP 805 B	---	NZP	0.043
IMI-TP 819	5.10	OZP	8.57
IMI-TP 817 A	1.27	TZP	0.044
IMI-TP 787 A	---		*OPs*
IMI-TP 731 A	2.56	TBEP	28.7
IMI-TP 731 B	---	TnBP	0.154
IMI-TP 729 A	---	TiBP	---
IMI-TP 759	2.93	TEHP	---
IMI-TP 701 A	2.82	TPP	---
IMI-TP 701 B	---	TCEP	0.152
IMI-TP 643	0.441	TCPP	---
		TDCPP	---

* Loads were calculated and normalised using average concentrations (see Table 4-2) and the following data: hospital: effluent water flow per year estimated from measurements during sampling campaign: 22,350 m^3; estimated number of inpatients per year: 10,000 (Agaplesion Evangelisches Krankenhaus, 2013) – outpatients are not taken into account); #for ICMs, a larger uncertainty for the normalisation has to be considered since ICMs are administered to outpatients on a large scale but it is assumed that due to rapid excretion rates large amounts are actually excreted at the hospital before the patients leave (see Chapter 4).

6.3 Distribution patterns of metabolites and transformation products in raw and treated wastewater

Human metabolites of two pharmaceuticals – carbamazepine and sulfamethoxazole – and the transformation products (TPs) of the ICM iopromide were analysed in raw hospital and municipal wastewater as well as in the effluents from the NF-MBR_{WWTP} and PS-$BFR_{hospital}$.

RAW WASTEWATER. The distribution patterns of the three pharmaceuticals and their metabolites and TPs, respectively in raw hospital and municipal wastewater are shown in Figure 6-4.
In the case of iopromide, a profound difference between the two types of raw wastewater can be seen. Whilst in raw municipal wastewater, 98% of the substance was found in the form of the parent drug, it only accounts for 22% in raw hospital wastewater, whereas six of the twelve investigated transformation products amount to 78%. Since ICMs are designed to pass through the human body without being metabolised, it can be assumed that no degradation of the iopromide has taken place before the substance reaches the sewage system. Therefore, the transformation must have occurred in the wastewater almost instantaneously since only minutes elapsed between the water reaching the sewage system and its arrival at the sampling point. It can be assumed that highly reactive compounds in the undiluted hospital wastewater, such as disinfectants, or reactions between organic compounds and the chlorine present in the water (Orias and Perrodin, 2013) trigger immediate degradation processes of the ICM. For ICMs which are excreted away from the hospital by outpatients leaving the facility after treatment, these processes might take place with less intensity since the less concentrated (municipal) wastewater receiving the excreted substance is less reactive. The same might hold true for wastewater from larger clinic complexes where raw wastewater is considerably diluted on site by wastewater from e.g. laundry. The total load of iopromide and its investigated TPs ($load_{IMI+TPs}$) originating from the investigated hospital only accounts for less than 0.04% of the anual $load_{IMI+TPs}$ reaching the WWTP (see Chapter 6.2). This could explain why in raw municipal wastewater, by far the main substance found is iopromide, while the TPs are practically absent.
In contrast to iopromide, the distribution patterns of carbamazepine in the two types of raw wastewater were found to be largely similar. In both cases, the metabolite 10,11-dihydro-10,11-dihydroxycarbamazepine is, at about 90%, the most abundant form (95 and 86% in hospital effluent and WWTP influent samples, respectively) while the percentage of the parent drug is less than 15% and the second metabolite 10,11-Dihydrocarbamazepine does not exceed 1%.
The distribution found for sulfamethoxazole and its metabolite N4-acetylsulfamethoxazole is practically inverted for hospital and municipal wastewater: in hospital wastewater, the percentages of parent drug and metabolite are 68 and 32%, while for municipal wastewater they are 34 and 66% (Figure 6-4). According to Göbel et al., 2005b, about 50% of administered

sulfamethoxazole is excreted in form of N4-acetylsulfamethoxazole. A reason for the lower amounts of the metabolite being detected in the raw hospital wastewater could be the highly reactive nature of the wastewater, as suggested above for iopromide. This might facilitate very rapid cleavage of the sulfamethoxazole metabolite and retransformation of the parent compound, leading to a higher percentage of sulfamethoxazole and a lower percentage of N4-acetylsulfamethoxazole than would be expected from metabolism rates. For the lower percentage of the parent compound (34%) in raw municipal wastewater compared to the expected 50%, no explanation was found.

TREATED WASTEWATER. When comparing the occurrence of the three compounds in the effluent of the PS-BFR$_{hospital}$ (Figure 6-5) with those in the untreated wastewater (Figure 6-4), almost identical patterns were found, making it evident that this form of treatment was not having much impact on these three substances and that no transformation processes seemed to take place. On the other hand, wastewater treated by the NF-MBR$_{WWTP}$ was very different from the corresponding raw wastewater (Figure 6-4 and Figure 6-5). While in raw municipal wastewater only about 2% of the iopromide was found in the form of (four different) TPs, in the effluent of the NF-MBR$_{WWTP}$, 97% was found as TPs and only 3% as the parent compound. Furthermore, it should be pointed out that although all twelve investigated TPs were present in the effluent, many of them were detected exclusively in only this one matrix of all the four[1] investigated, which points to dynamic biodegradation processes during the water treatment in the NF-MBR$_{WWTP}$. Biodegradation also explains why the percentage of carbamazepine in the water treated by the NF-MBR$_{WWTP}$ was higher (36%) than in the raw influent (13%), leading to the cleavage of the metabolites and the retransformation of the parent drug. The complete absence of the sulfamethoxazole metabolite N4-acetylsulfamethoxazole in the effluent of the NF-MBR$_{WWTP}$ also can be related to the same reason. It can be assumed that under the influence of intense microbial activity as it occurs in the membrane reactor, the metabolite was entirely deconjugated back into the parent compound (Göbel et al., 2005b, see also Chapter 2.1.1).
To determine whether removal in the NF-MBR was caused by biodegradation processes or by the NF-membrane, pure nanofiltration was investigated in comparison (see Chapter 3.3.5). The distribution patterns of carbamazepine and sulfamethoxazole along with their metabolites (Figure 6-6) revealed that the pure NF treatment does not alter the distribution of these substances in the wastewater in a profound way. Since the influent of the NF module was raw wastewater from the WWTP, the differences between the distribution patterns of sulfamethoxazole in the influent of the WWTP and the NF are unexpected, and no obvious

[1] the four are raw hospital and municipal wastewater, PS-BFR effluent and NF-MBR effluent

reason can be found. However, they might be explained by different sampling strategies and sampling times.

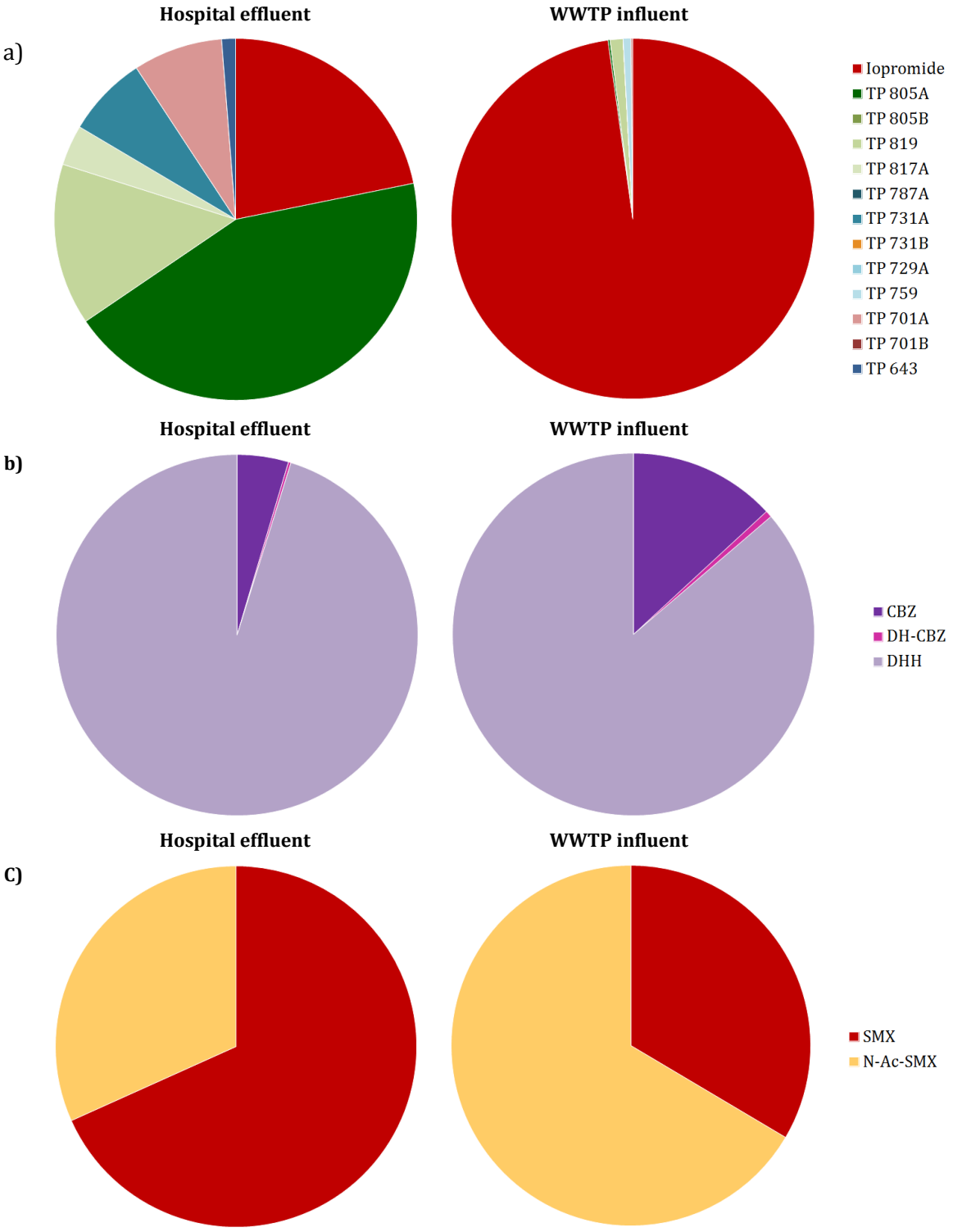


Figure 6-4: Distribution patterns [%] of a) iopromide and its transformation products, b) carbamazepine and its metabolites and c) sulfamethoxazole and its metabolite in raw hospital wastewater (hospital effluent) and raw municipal wastewater (WWTP influent). Data are based on average concentrations (see Tables 3-8 and 4-2) and colour codes refer to figures in these same chapters.

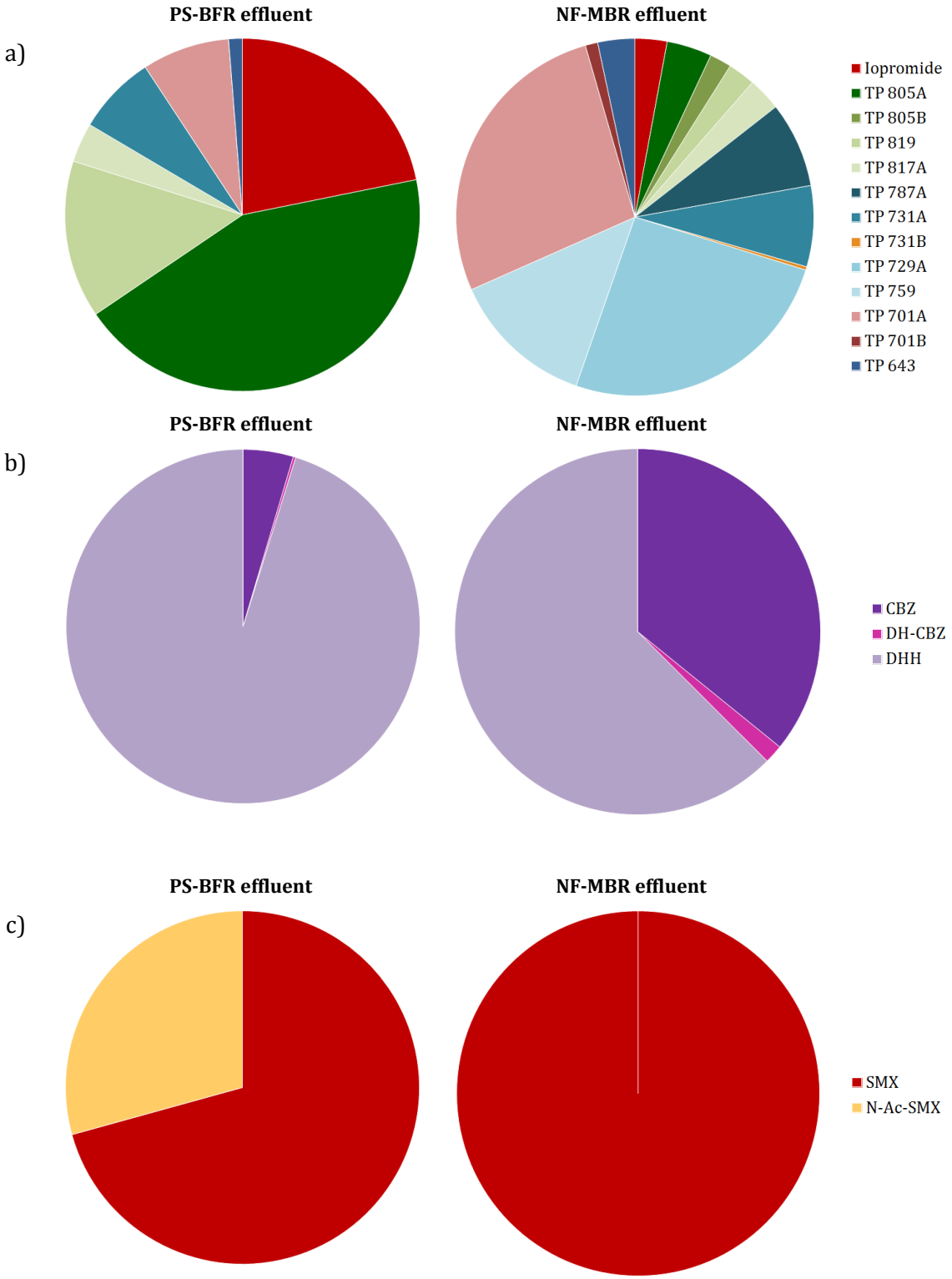


Figure 6-5: Distribution patterns [%] of a) iopromide and its transformation products, b) carbamazepine and its metabolites and c) sulfamethoxazole and its metabolite in the effluent from the BFR (left column) and the effluent from the NF-MBR. Data are based on average concentrations (see Tables 3-8 and 4-2) and colour codes refer to figures in these same chapters.

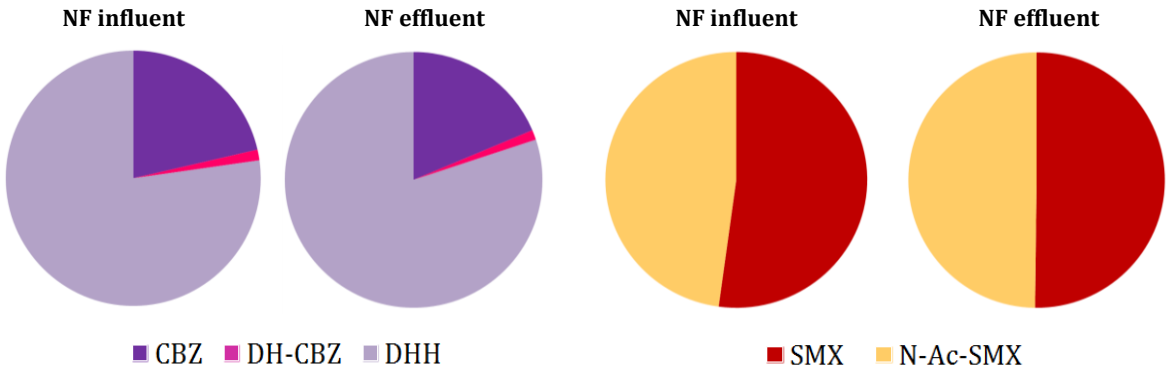


Figure 6-6: Distribution patterns [%] of carbamazepine and sulfamethoxazole and their metabolites in the influent and effluent of the lab-scale NF module (see Chapter 3). Colour codes refer to figures in this chapter.

6.4 Removal efficiency of NF-MBR and PS-BFR

When comparing the removal efficiency found for the NF-MBR and the PS-BFR (see Chapters 3.3 and 4.3), the former yields better results for the majority of the xenobiotics (Figure 6-7): For eight substances (circled in green), both treatment systems investigated yielded positive removal with the NF removal always exceeding that found for PS-BFR, while for a group twice as large (yellow circle), positive removal rates found for the NF-MBR contrasted with negative removal rates during BFR treatment. Only for two substances (orange circle) was negative removal in the NF found, while BFR treatment yielded zero or very slightly (< 10%) positive removal. Two substances were found to be removable by neither of the investigated systems (circled in red). On the whole, the NF-MBR treatment shows principally a much higher removal capacity for the very diverse group of investigated compound (sum of yellow- and green-circled compounds) than the PS-BFR treatment (sum of the green- and orange-circled compounds). This translated into a level of highly or very highly efficient treatment (meaning > 75% removal) for only four substances in the case of the PS-BFR, while this effectiveness was found for 18 substances in NF-MBR treatment.

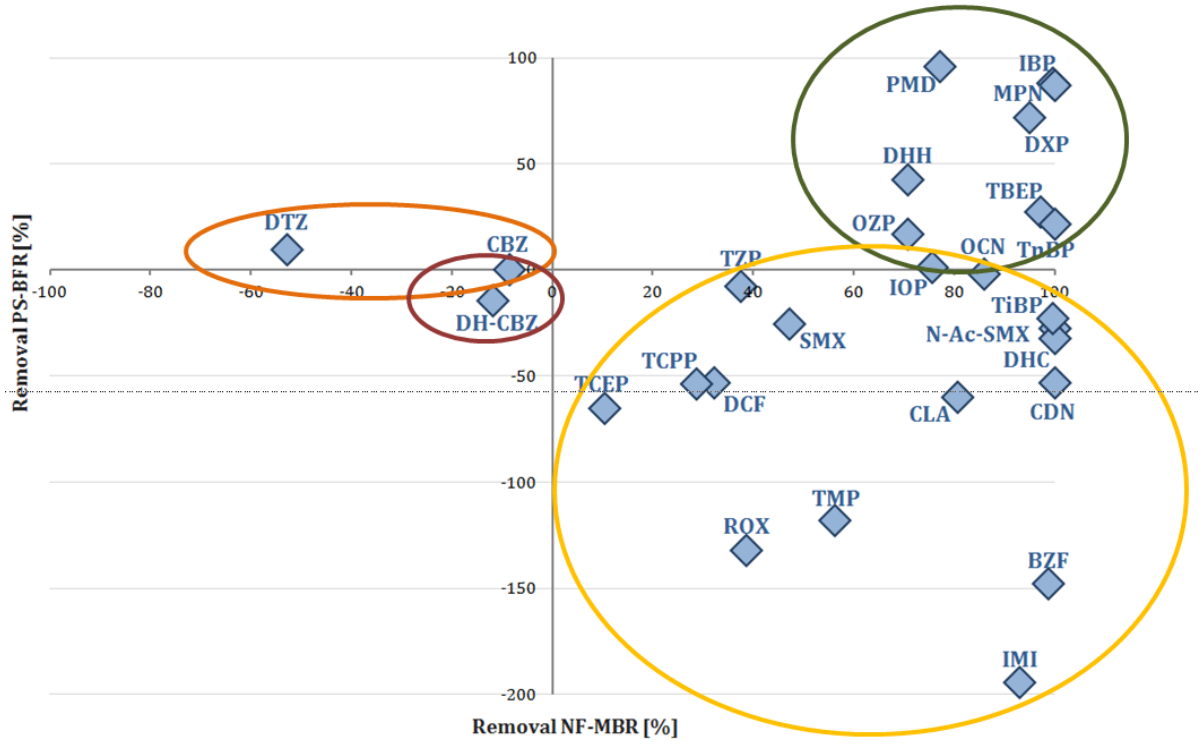


Figure 6-7: Xenobiotic removal in the PS-BFR (y-axis) vs. the NF-MBR (x-axis) based on average concentrations (see Tables 3-8 and 4-2). Explanation for coloured markers: see text.

6.5 AQUATIC ENVIRONMENTAL ASSESSMENT

To evaluate the risk posed by xenobiotics in the aquatic environment, environmental risk assessment (ERA) schemes are used. In the EU, ERA was implemented for, among other purposes, the evaluation of substances during the REACH registration (European Chemicals Bureau, 2003) and for medicinal products for human use (EMEA, 2006). This ERA approach consists of a multi-tiered process of which the exposure assessment is the first part. For exposure assessment, the ratio of PEC (predicted environmental concentration) to PNEC (predicted no effect concentration) is determined for the substance in question. A PEC/PNEC ratio of < 0.1 equals minimal risk to aquatic organisms, PEC/PNEC ratios between 0.1 and 1 indicate medium risk, while substances with ratios > 1 are interpreted as posing high risk.
The proper calculation of PEC according to European Chemicals Bureau, 2003 is often difficult since many required data are not commonly available, and highly diverse data may be reported for a single parameter (e.g. metabolism and excretion rates, production volume or market volume) (Letzel et al., 2009). Furthermore, PEC does not represent the situation at a certain sampling point but rather in generalised, hypothetic circumstances. Thus MEC (measured environmental concentration) is increasingly used instead of PEC to provide more realistic risk scenarios (e.g. Grung et al., 2008; Zhao et al., 2010; Santos et al., 2013). To be valid for ERA, PNECs must be derived from tests with organisms of three different trophic levels, of which the results of the most sensitive one are used in order to guarantee that the chosen PNEC is an appropriate indicator for the protection of the ecosystem.

6.5.1 WORST CASE SCENARIO

The ratio between the highest concentration measured (MEC_{max}) and the PNEC represents a worst-case scenario for the exposure assessment at a specific sampling site, assuming that no wastewater treatment takes place. It describes the impact of raw wastewater at the infusion point before dilution, sorption or degradation in the receiving surface water can take place (European Chemicals Bureau, 2003). Such a scenario is also common during heavy rain events that overwhelm the capacity of the WWTP and lead to untreated water being discharged. From the data obtained in the presented study, the evaluation of risk posed by the municipal wastewater stream was evaluated, a site-specific assessment for the hospital was carried out and the effluents of the two pilot plants were considered.

PHARMACEUTICALS. As shown in Table 6-3, in *raw municipal wastewater*, the MEC_{max}/PNEC ratios revealed high environmental risk for eleven substances (four psycho-active substances, four

antibiotics, two NSAIDs and one lipid regulator) with oxazepam and diclofenac showing MECmax/PNEC ratios as high as over 500. For carbamazepine, iopromide, naproxen and sulfadimethoxine, medium risk is indicated, but it has to be pointed out that the MEC_{max}/PNEC ratio of carbamazepine is bordering on factor 1 (0.9).

In the *effluent of the NF-MBR*, generally reduced MEC_{max}/PNEC ratios were found, pointing to effective reduction of these compounds during removal. However, the MEC_{max}/PNEC ratio of oxazepam was still close to 200, and overall nine pharmaceuticals (four antibiotics, three psycho-active compounds and two NSAIDs including ibuprofen) exceeded factor 1. These results emphasise that environmental risk can be caused by a) substances that are not degraded in wastewater treatment (e.g. carbamazepine), of which the risk factor is slightly higher for the effluent of the NF-MBR than for untreated wastewater and by b) so called "pseudo-persistent" substances like ibuprofen, that are indeed degraded by wastewater treatment (see Chapters 2, 3 and 4) but pose a risk nonetheless due to the sheer volume of application and subsequent permanent, high infusion into the environment, despite efficient removal (Dietrich et al., 2006).

In *raw hospital wastewater*, the MEC_{max}/PNEC ratio of oxazepam exceeded 2,000, and the ratios for diclofenac, ibuprofen, sulfamethoxazole and trimethoprim were over 100. Overall, a MEC_{max}/PNEC ratio of more than 1 (high risk) was found for eleven substances (four antibiotics, four psycho-active compounds, two NSAIDs and one lipid-regulator).

In *hospital wastewater treated by a PS-BFR,* for three substances a MEC_{max}/PNEC ratio of more than 200 was still found, and overall ten substances showed high environmental risk (four antibiotics, three psycho-active substances, two NSAIDs and one lipid-regulator). Thus, while for some substances like ibuprofen and primidone, considerable reductions in the risk factor are found compared to in untreated wastewater, it is suggested that the BFR treatment does not greatly reduce the overall impact in the wastewater. In the case of the ICM iopromide, the MEC_{max}/PNEC ratio is even higher after PS-BFR treatment than in raw hospital wastewater. This is due to complex transformation processes of the substances in raw hospital wastewater what seems to lead to retransformation of the parent compound during BFR treatment (see Chapters 4.3.3 and 4.3.4).

OPs. As industrial chemicals, OPs are subject to the REACH process in which risk assessment is mandatory. Thus, PNEC values for several aquatic media (for fresh water, meaning inland surface water, for marine waters and also for the microorganisms used in biological wastewater treatment) are to be found within the ECHA dossiers (ECHA). For TBEP, however, to date no PNECs have been given, rendering risk assessment unfeasible. When applying the PNECs from the REACH dossiers for the other OPs to the MECs of this study, medium risk is indicated for TiBP in raw municipal wastewater when compared with $PNEC_{freshwater}$, suggesting a possible

minor threat when untreated wastewater reaches surface water. For all other OPs, the MEC/PNEC ratios do not imply any risk either for microorganisms during wastewater treatment or for receiving surface water.

Table 6-3: PNECs and $MEC_{max}/PNEC_{aquatic}$ ratios for pharmaceuticals found in the PS-$BFR_{hospital}$ and the NF-MBR_{WWTP}. bold: RQ > 1; unterlined: 0.1 ≤ RQ ≤ 1; --- not listed in the literature or databases consulted.

		$MEC_{max}/PNEC_{aquatic}$*			
		PS-$BFR_{hospital}$		**NF-MBR_{WWTP}**	
Compound	**PNEC [µg/L]**	**Influent**	**Effluent max**	**Influent**	**Effluent max**
		Antibiotics			
CLA	0.04[a,b,n]	**57**	**52**	**16**	**4**
ROX	0.01[a], 0.15[n]; 4[p]	**9**	**8**	**14**	**8**
SMI	0.248[d,e], 3.5[p]			0.5	0.0257
SDI	12.77[b,d,e]	n.d.	n.d.	n.d.	n.d.
SSX	0.62[f,g]	n.d.	n.d.	n.d.	n.d.
SMA	0.68[f,b,g], 116[n]	n.d.	n.d.	n.d.	n.d.
SMX	0.027[p], 0.59[a,b,h], 20[n]	**16**	**9**	**3**	**2**
TAM	23[i]	n.d.	n.d.	n.d.	n.d.
TMP	0.0058[a,b], 1[n], 2.6[p]	**170**	**202**	**27**	**15**
		ICMs			
IPM	5363[o]			0.001	0.0005
IMI	256[b], 370,000[p]	0.0206	0.2	**1**	0.0151
		NSAIDs and bezafibrate			
BZF	0.46[a], 5.3[p]	**28**	**6**	**4**	0.1
DCF	0.02[a], 13.5[j], 138.74[k]	**772**	**506**	**552**	**1**
IBP	9.06[l], 0.2[a], 1.65[r]	**434**	**42**	**9**	**5**
NPX	2.62[r] , 6.6[a], 21.2[j], 128[m]	0.038	0.0334	0.2	0.0045
		Psycho-active substances			
CBZ	2[a,b], 6.36[k]	**1**	0.3	0.9	**1.0**
PMD	0.069[o]	**43**	**2**	**46**	**10**
CDN	0.06[o], 16[p]	**3**	**4**	**5**	
MPN	0.09[a]	**2**		**5**	
DZP	0.01[a,b], 2[p], 4.2[l]	n.d.	n.d.	n.d.	n.d.
OZP	0.0019[o]	**2,252**	**2,280**	**660**	**196**

*calculated with the most sensitive PNEC found in literature; n.d. = not determined due to measured environmental concentration was < LOQ or the substance was not detected; [a] Molander et al., 2009 cited in Orias and Perrodin, 2013; [b] EPA, 2012b; [c] Isidori et al., 2005b; [d] García-Galán et al., 2012 cited in Orias and Perrodin, 2013; [e] Park and Choi, 2008 cited in Orias and Perrodin, 2013; [f] Białk-Bielińska et al., 2011 cited in Orias and Perrodin, 2013; [g] Park, 2005 cited in Orias and Perrodin, 2013; [h] Grung et al., 2008; [i] Boxall et al., 2000 – PNEC for soil pore water and groundwater; [j] Farré et al., 2001 cited in Santos et al., 2007; [k] Jones et al., 2002; [l] Stuer-Lauridsen et al., 2000; [m] Webb, 2000 cited in Jones et al., 2002; [n] Kümmerer and Henninger, 2003; [o] Orias and Perrodin, 2013; [p] Sanderson et al., 2003; [q] Ferrari et al., 2004; [r] Quinn et al., 2008 cited in Verlicchi et al., 2012b

Table 6-4: PNECs, $MEC_{max}/PNEC_{freshwater}$ and $MEC_{max}/PNEC_{WWTP}$ ratios for pharmaceuticals found in the PS-$BFR_{hospital}$ and the NF-MBR_{WWTP}. bold: RQ > 1; unlinked: 0.1 ≤ RQ ≤ 1; *PNEC for soil pore water and groundwater; --- not listed in the literature or databases consulted.

	PNEC [µg/L]		**$MEC_{max}/PNEC_{freshwater}$**				**$MEC_{max}/PNEC_{WWP}$**			
			PS-$BFR_{hospital}$		**NF-MBR_{WWTP}**		**PS-$BFR_{hospital}$**		**NF-MBR_{WWTP}**	
Compound	Freshwater	WWTP	Influent	Effluent	Influent	Effluent	Influent	Effluent	Influent	Effluent
TBEP	no PNEC	no PNEC	---	---	---	---	---	---	---	---
TnBP	35[a]	no PNEC	0.003	0.002	0.006	n.d.	n.d.	n.d.	n.d.	n.d.
TiBP	11[a]	3720[a]	0.017	0.015	0.806	0.012	$4.91x10^{-5}$	$4.45x10^{-5}$	0.0024	$3.64x10^{-5}$
TEHP	"no hazard	1000[a]	---	---	---	---	n.d.	n.d.	n.d.	n.d.
TPP	3.7[a]	5000[a]	n.d.	n.d.	0.009	n.d.	n.d.	n.d.	$6.55x10^{-6}$	n.d.
TCEP	65[b]	32000[b]	0.001	0.002	0.003	0.003	$2.68x10^{-6}$	$4.92x10^{-06}$	$6.77x10^{-6}$	$5.62x10^{-6}$
TCPP	420[a]	no PNEC	0.001	0.000	0.004	0.002	n.d.	n.d.	n.d.	n.d.
TDCPP	10[a,c]	100000[a,c]	n.d.	0.009	0.029	0.018	n.d.	$8.79x10^{-7}$	$2.92x10^{-6}$	$1.76x10^{-6}$

[a] ECHA; [b] European Chemicals Bureau, 2009; [c] European Chemicals Bureau, 2008

6.5.2 Environmental risk quotient (ERQ)

When wastewater (treated or not) reaches the receiving surface water, dilution takes place, reducing the impact of the discharged xenobiotics. In exposure assessment, this can be accounted for by applying a suggested default factor of 10 (European Chemicals Bureau, 2003). Thus, Figure 6-8 and Figure 6-9 show the environmental risk quotient ((MEC_{max}/10)/PNEC) for the four investigated media (raw municipal and hospital wastewater, effluent NF-MBR, effluent PS-BFR).

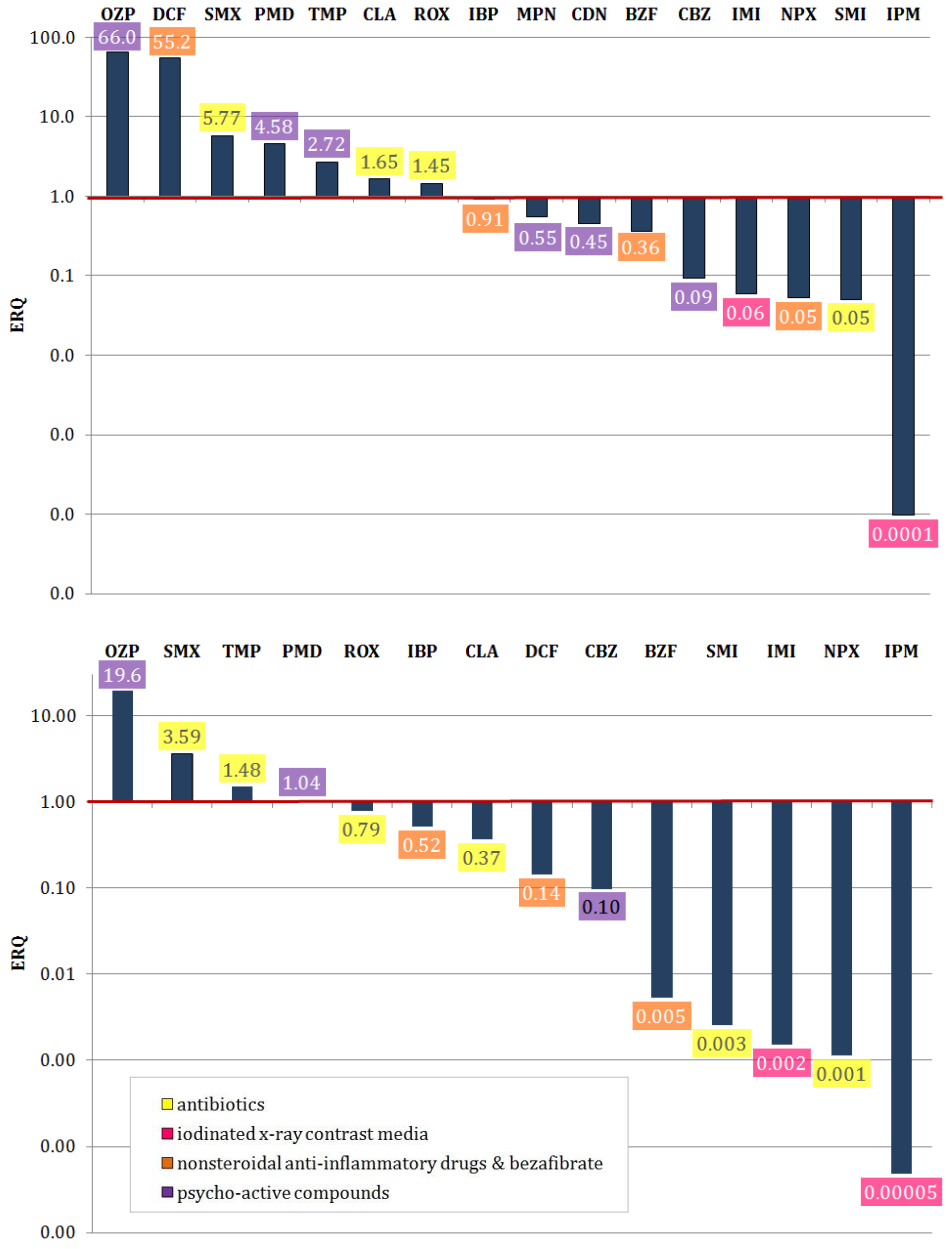


Figure 6-8: ERQs of raw municipal wastewater (above) and the effluent of the NF-MBR (below).

MUNICIPAL WASTEWATER. In raw municipal wastewater, seven substances yielded ERQs > 1 (four antibiotics, two psycho-active compounds and one NSAID) with the maxima being oxazepam (ERQ: 66) and diclofenac (ERQ: 55). For four substances, ERQs indicated medium risk for the aquatic environment. In the effluent of the NF-MBR, four substances, the psycho-active compounds primidone and oxazepam as well as the antibiotics sulfamethoxazole and trimethoprim, still showed ERQs of > 1, the highest being oxazepam (ERQ: 19.6). Five more substances yielded ERQs suggesting medium risk.

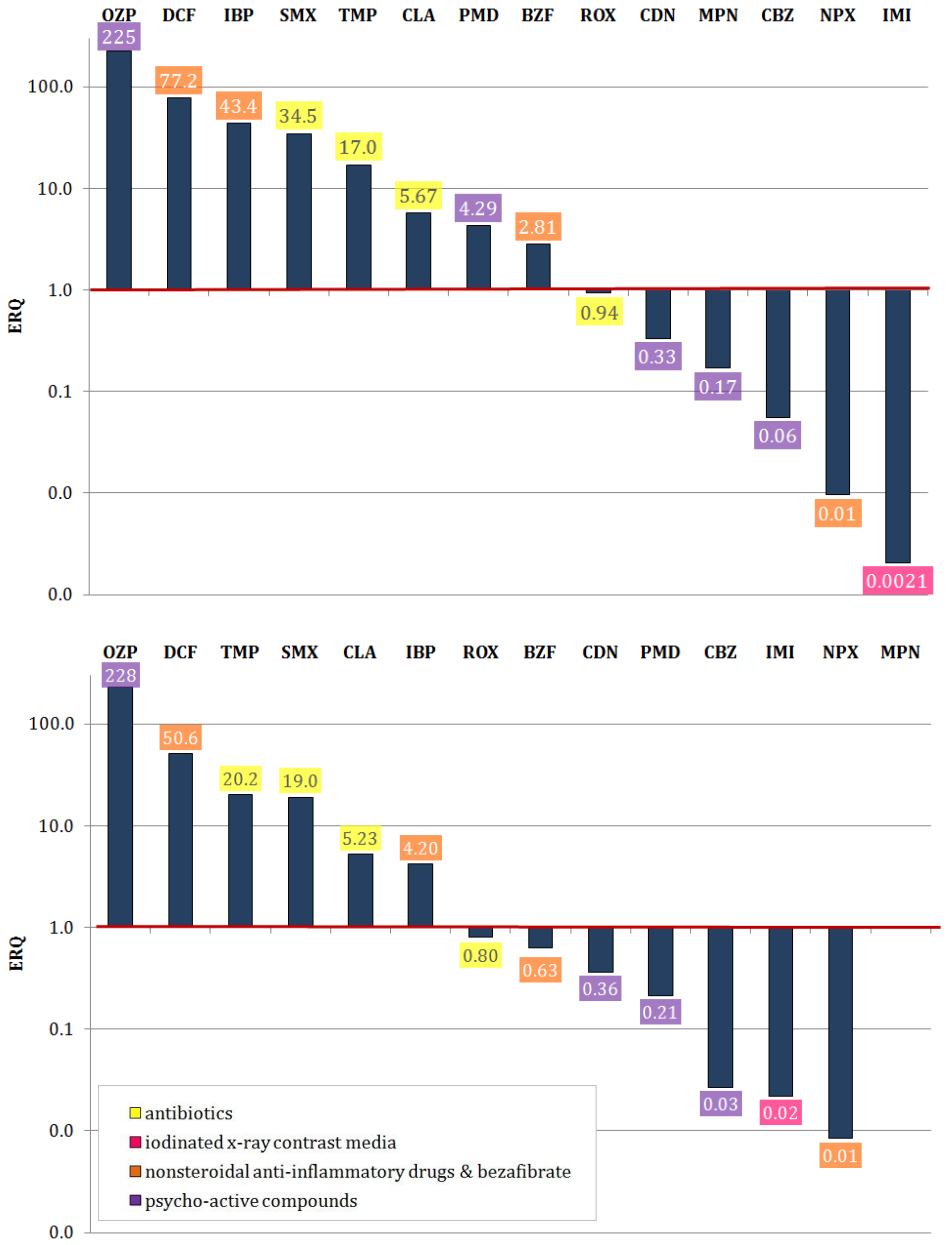


Figure 6-9: ERQs of raw hospital wastewater (above) and the effluent of the PS-BFR (below).

HOSPITAL WASTEWATER. The ERQs for raw hospital wastewater were > 1 for nine substances (three antibiotics, 2 NSAIDs, 2 psycho-active compounds and one lipid-regulator), with again, oxazepam yielding the highest risk quotient (ERQ: 225). For three more pharmaceuticals, the ERQs revealed medium risk (roxithromycin, codeine, morphine). In the effluent of the PS-BFR, five substances were ranked as high risk (the antibiotics trimethoprim, sulfamethoxazole and clarithromycin, the NSAIDs diclofenac and ibuprofen as well as the psycho-active compound oxazepam), while four more are of medium risk to the aquatic environment.

COMPARISON WITH ERQS FROM LITERATURE. Because of the great diversities in wastewater streams, a comparison of MEC_{max}/PNEC ratios or ERQs between different studies is difficult. This is even truer for a comparison between calculated PEC/PNEC ratios with studies using measured data (MECs). So, it is not surprising, that various studies describing ERAs for pharmaceuticals reported widely varying risk levels for certain substances. Based on data from Germany, Kümmerer and Henninger, 2003 determined PEC/PNEC ratios for several antibiotics in untreated wastewater, defining clarithromycin (PEC/PNEC: 13.6), sulfamethoxazole (11.13) and trimethoprim (1.53) as the highest risks, which is in general agreement with the results presented here, while for roxithromycin only minor risk was calculated (0.2), which is not supported by the result of this study. In a study describing the environmental risk posed by the top 25 prescription pharmaceuticals in the UK, Jones et al., 2002 found ibuprofen and carbamazepine posing minor risk to the environment, while no risk was identified for the environmental levels of naproxen and diclofenac (both 0.01). With the exception of naproxen, this is not congruent with the results of this study. Santos et al., 2007 described the MEC/PNEC for influent and effluent of a CAS-WWTP for carbamazepine as being 0.34 und 0.20, respectively, which is generally similar to the results presented here. For ibuprofen and naproxen, considerably higher MEC/PNEC ratios than in this study were found for the influent (41.00 and 1.28, respectively), while the effluent ratios (5.30 and 0.20) were close to what we found.
In a risk assessment for wastewater from a hospital in Brazil, Souza et al., 2009 calculated PEC/PNEC ratios according to consumption data and metabolisation rates, and found no risk in connection with sulfamethoxazole (PEC/PNEC: 0.0536) and high risk for trimethoprim (PEC/PNEC: 16.78). Thomas et al., 2007 determined ERQs for hospital wastewater in Norway and found diclofenac and sulfamethoxazole being of minor risk (ERQs 0.32 and 0.18 respectively), while ibuprofen ERQ: 3.1×10^{-5}) and trimethoprim (7.9×10^{-4}) posed no risk, which does not concur with this study.

IN CONCLUSION. The results of the presented study demonstrate that wastewater, both raw and treated, represents a risk when reaching receiving waters. High risk levels are being caused by

substances of not a single therapeutic group but several (psycho-active compounds, antibiotics, NSAIDs). However, in all wastewater types investigated, the highest risk was found for oxazepam.

THE VALUE AND LIMITS OF RISK ASSESSMENTS. Comparison with literature data shows that exposure assessments are in essence only valid as a site-specific description, and generalising from the conclusions provided by a specific case seems highly problematic. Furthermore, it has to be kept in mind that this form of assessment only evaluates the risk of a single compound and does not account for any synergetic or cumulative effects of the random mixture of thousands of substances that are present in natural waters today. So the results of exposure assessment are inclined to underestimate the real risk pharmaceuticals pose to the aquatic environment. Exposure assessments revealing no or minor risk should therefore not be seen as a "card blanche" declaring a substance free of any notion of being environmentally relevant. More so, since for many substances toxicity data are profoundly incomplete or completely missing (which is, apparently, even true for substances regulated and supposedly assessed in depth under REACH). So, no PNEC values were available for 24 substances (23 pharmaceuticals and one OP) out of a total of 52, which equals 46% of the overall number of xenobiotics investigated in this study. Subsequently, for this 46% not even an attempt at a risk assessment, with whatever short-comings, can be made, which makes them "orphan" compounds in terms of ecotoxicity data (Orias and Perrodin, 2013). Another limitation of the current risk assessment process is that for both pharmaceutical metabolites and transformation products practically no ecotoxicological data exist (Orias and Perrodin, 2013).

A basic flaw of the exposure assessment is the central role of PNEC values. The whole assessment is based on the determination of the PNEC, and therefore on the initial quality of the study data used to derive these values and the relevance of the endpoint used (Dietrich et al., 2006). Since data regarding ecosystem effects for xenobiotics are to date generally very scarce, in the absence of more reliable data describing long-term, chronic toxicity, data about short term toxicity have in many cases been used to determine the PNEC and adequate assessment factors are applied (European Chemicals Bureau, 2003). Nonetheless, the somewhat questionable quality of many PNEC data is in stark contrast to the emphasis given to the PEC/PNEC calculations in recent (scientific) use of this quotient to arrive at a plausible risk estimate for pharmaceuticals in the aquatic environment. This becomes especially evident when observing the highly diverse PNECs reported in literature for estimating PEC/PNEC (see Table 6-3, e.g. PNEC for diclofenac ranging from 0.02 to 138 µg/L and varying between 256 µg/L and 370,000 µg/L for iopromide). In the light of these considerations, exposure assessments can only be seen as a rough pointer to possible risks.

7. OVERALL CONCLUSIONS AND FURTHER PERSPECTIVES

Till taught by pain,
Men really know not what good water's worth.
*– Lord Byron (*1788 –1824*), Don Juan, Canto II, Stanza 84, 1818-1824 –*

If we were logical, the future would be bleak, indeed. But we are more than logical.
We are human beings, and we have faith, and we have hope, and we can work.
– Jacques-Yves Cousteau (1910-1997) –

THERE IS REASON FOR CONCERN. The results of this study show that a wide range of xenobiotics is present in both municipal and hospital wastewater. While the fingerprints of the single point source and the accumulated mixture are for some compound groups different, the compositions of both wastewater streams give reason for environmental concern. This concern stems not only from the results of exposure assessments, which reveal high environmental risks in both raw and treated wastewater, but even more from the profound lack of knowledge due to missing, unreliable or questionable ecotoxicological data. For about 50% of the substances investigated in this study, the ecotoxicological data needed for attempting a risk assessment were unavailable in the literature, which, it could be argued, makes toxicological risk assessment a discipline of faith more than knowledge. This is bad enough in the case of the parent compounds, but is even worse for metabolites and environmental transformation products, the latter being for most pharmaceuticals completely unknown to date.

A group of xenobiotics which serves to illustrate these problems is ICMs. In raw wastewater, they are found in concentrations up to mg/L which is 10 to 100 times higher than other micropollutants. As substances which are both highly polar and inert by design, they mostly bypass wastewater treatment, meaning they are constantly discharged into the aquatic environment in very considerable amounts, where they can be expected to be transported over long distances and timespans. As long ago as 2001, Kümmerer, 2001 pointed out that this is reconcilable with neither drinking water safety nor a precautionary principle approach. In the meantime, it was found that ICMs might be not as non-toxic to aquatic organisms as was postulated, with PNEC values for iopromide found in the literature dropping from 370,000 µg/L (Sanderson et al., 2003, estimated by Quantitative Structure Activity Relationship, QSAR) by a factor of about 1500 to 256 µg/L (EPA, 2012b). Subsequently, the MEC_{max}/PNEC ratio for raw

municipal wastewater found in this study exceeded 1 (=high risk). Furthermore, my results revealed a highly dynamic transformation of the ICM iopromide in raw hospital wastewater (see Figure 4-11 and Figure 6-4), challenging the assumption of high stability. While this could be positive with regard to possible (bio)degradation in the environment, it makes clear that very large amounts of transformed ICMs are present in the environment. Moreover, while ecotoxicological information on the parent compounds is scarce (see Table 6-3), it can be considered to be completely absent for transformation products. Many of them have probably not even been identified yet. Thus, it must be assumed that the environmental behaviour of ICMs is currently unsatisfactorily described at best.

PRECAUTIONARY PRINCIPLES. Taking all of the above mentioned into account, it is impossible to say with certainty that pharmaceuticals and other xenobiotics in (waste)water are not a threat to the aquatic environment. To compensate for the lack of knowledge, precautionary principles must be applied, and these xenobiotics should be kept from reaching the aquatic environment. Several international guidelines, such as the Convention for the Protection of the Marine Environment of the North-East Atlantic (OSPAR) and as a result the European Water Framework Directive (European Commission, 2000), too, set the ultimate target concentrations for man-made synthetic pollutants "close to zero" (since the natural background is zero), rendering any amount of the substance in question found in the environment unfavourable. This should be the goal for "emerging compounds" such as pharmaceuticals and OPs as well.

NON-APPLICATION. One possible way to achieve this would be the non-application of the substances, which in the case of pharmaceuticals is highly problematic. While attempts are being made to develop "green drugs" which pose less environmental threat (Daughton, 2002a; Daughton, 2002b; Zhang and Geißen, 2010), this approach to the problem seems to have limits. For example, the widely used painkiller ibuprofen, which is regularly named as one of the drugs posing risks to aquatic environment, is identified as a core drug for basic healthcare systems and part of the WHO's List of Essential Medicines (WHO, 2011c) and therefore not likely to be substituted any time soon.

SOURCE CONTROL. Another approach, recently discussed favourably, especially for pharmaceuticals, is source control by decentralised treatment of e.g. hospital wastewater streams. The treatment of high-strength, undiluted wastewater is described as being more efficient for biological removal and favourable for the elimination of persistent micropollutants (Joss et al., 2006). However, the results of the presented study suggest that highly concentrated wastewater, such as raw hospital wastewaters, favours the development of transformation

products and completely uncontrolled degradation processes. Treating such wastewaters on site instead of allowing them to enter the municipal wastewater system would increase the contact time of a seemingly highly reactive wastewater matrix with the pharmaceuticals (and other xenobiotics which might be affected in the same way), which could lead to the increased development of transformation products of unknown nature and toxicity (Orias and Perrodin, 2013). With no hope of identifying all the individual TPs that could possibly develop under such conditions (which in themselves are highly variable both spatially and timewise), research into decentralised wastewater treatment should be closely connected to investigations of ecotoxicity effects.

ADVANCED END-OF-PIPE-TREATMENT. Since organic micropollutants are not exclusively introduced into the wastewater stream by special point sources but by practically every sewage pipe entering the municipal sewers, decentralised source treatment is not the answer for overall reduction of xenobiotics in wastewater. Ultimately, advanced end-of-pipe-treatment is the only realisable way at present, since the currently used CAS treatment is mostly insufficient for micropollutant removal (see e.g. Joss et al., 2006). In the presented study, two advanced treatment systems were investigated for this purpose. While the NF-MBR reveals a much greater potential for micropollutant removal than the PS-BFR, even this system showed unsatisfactory results (< 75% removal) for 45% of the investigated substances. The results showed that the NF-MBR was especially efficient in regard of biodegradation, while the removal of non-biodegradable substances like carbamazepine was insufficient. The filtration capacity of the loose NF membrane did not yield better retention results than previously described for wider membrane types. The use of a tighter NF membrane would enhance removal, but such a system would not be suitable for the treatment of larger wastewater volumes and its use is therefore limited to small-scale applications (Fane, 2011).

The PS-BFR demonstrated basic potential for biodegradation by removal rates of over 75% found for primidone, ibuprofen and morphine, but failed in regard to increased overall xenobiotic degradation. However, high concentrations (ranging up to over 2,000 ng/g d.w.) of clarithromycin found in the biosolids (sludge and biofilm of the carrier material) indicate that the system has a high sorption potential for ionic compounds such as macrolides that are positively charged under pH conditions found in hospital wastewater (pH 7.4 – 8.0). These sorption processes can be assigned to the extracellular polymeric substances (EPS), which hold flocs of activated sludge together as well as being the backbone of the cell-surrounding gels of biofilms, and which are described as being negatively charged at pH > 7 (Bryers, 2000 cited in Wunder et al., 2011; Stewart and Costerton, 2001). Recent studies indeed suggest the use of biofilms as adsorbents of pollutant ions in the treatment of water contamination (Kurniawan

and Yamamoto, 2013). Thus, it might be promising to implement this technology as a sorption step in decentralised hospital wastewater treatment to reduce the risk potential of micropollutants prone to sorption, such as macrolide antibiotics (Escher et al., 2011). This might be especially beneficial in the case of antibiotics since the biofilms attached to carrier material can be disposed of in a controlled fashion, thus permanently removing the antibiotics from the aquatic system. Furthermore, this would prevent the spreading of possibly changed genetic information ensuing from contact with antibiotics and contributing to bacterial antibiotic-resistance.

COMBINATION OF TREATMENT METHODS. None of the advanced treatment methods investigated in this study could remove the whole range of the studied xenobiotic micropollutants. Recent research shows that the same is true of virtually all realistic advanced treatment processes such as advanced oxidation or treatment with powdered activated carbon (PAC). Thus, a combination of treatment methods is increasingly becoming the centre of attention as the preferred strategy and is subsequently subject to intense research. For example, moving biofilm MBRs combine MBR treatment with the advantages of particle-supported biofilms (Fane, 2011). Other approaches are the combination of PAC with NF (Meier and Melin, 2005), with microorganisms ("biological *[sic]* activated carbon", Reungoat et al., 2011) or as a follow-up to ozonisation (Hollender, 2013).

OUTLOOK. Even after almost two decades of intensive research, numerous aspects of the fate of xenobiotic micropollutants in the water cycle are not even partly understood and in many cases the answers found just lead to more questions. However, newly developed high resolution mass spectrometry allows a much closer look at xenobiotics, their transformational pathways and their impact on the aquatic system, and holds the promise of more much-needed knowledge to be gained in this area.

Nonetheless, the information that is already available today gives reason for concern. The transfer of scientific knowledge into legislative action is, however, only slowly evolving. To my knowledge, as the first country ever, Switzerland is currently implementing a fourth treatment step for large WWTPs (aiming at load reduction in surface waters), for WWTPs discharging into small receiving waters (where only low dilution of infused micropollutants is accomplished) or waters used for drinking water production (Hollender, 2013). These criteria apply to about one seventh of the country's WWTPs. The goal is 80% removal of micropollutants in final effluents, which is to be achieved by a combination of treatment techniques (ozonisation followed by PAC treatment).

In the EU, on the other hand, the application of precautionary principles was neglected when the water frame work directive (European Commission, 2013) was amended. In a commission

proposal (European Commission, 2012b), it was recommended that three pharmaceuticals (diclofenac and two estradiols) should be newly included in the list of priority substances. However, this was shelved and they were pushed down onto a so-called 'watch list' to be investigated and monitored further. At present no mandatory action is required to be taken by the member states with regard to these micropollutants. Since legislative regulation is often a necessary driver for both research and development, this is an unfortunate signal.

If there is magic on this planet, it is contained in water.

- Loren Eiseley (1907–1977), The Immense Journey, 1957 –

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