018530 - SWITCH

Sustainable Water Management in the City of the Future

Integrated Project
Global Change and Ecosystems

Deliverable 4.1.2

Listing the most important pharmaceutical compounds from various therapeutic groups and the testing and validation of analytical methods for the selected compounds

4.1.2 A. Pre-selection of representative compounds for laboratory degradation tests
4.1.2 B Survey of the mostly used pharmaceutical compounds and hormones in the West Bank/Palestine
4.1.2 C. Estrogens in aquatic environment: A review.

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SWITCH Deliverable Briefing Note (months 0-12)

SWITCH Document Deliverable 4.1.2 consists of three parts:
(A) Pharmaceutical compounds in environment
Pre-selection of representative compounds for laboratory degradation tests
(B) Survey of the mostly used pharmaceutical compounds and hormones in the West Bank/ Palestine
(C) Estrogens in aquatic environment: A review

Audience This document is targeted at scientists, engineers and policy makers.

Purpose

The purpose of this document was to give:
- a general overview on a variety and nature of human pharmaceutical compounds and their pathways into the environment;
- a overview of the characteristics of the compounds in relation to their possible behaviour in a wastewater treatment system;
- to make a pre-selection of a few representative compounds deserving special attention in further SWITCH laboratory research aiming at the removal potential of pharmaceutical compounds and their metabolites from concentrated domestic wastewater streams using proven biological, chemical-physical treatment technologies.

Background

Human pharmaceuticals are consumed in high quantities world wide; the consumption is in the range of tons per year per one pharmaceutical compound depending on the size of a country. The expectations are that these amounts will only keep increasing because of improving health care systems worldwide and longer life expectations of people.

Conventional, sewer-based, sanitation systems are characterised by a high degree of dilution and many pharmaceutical compounds are not sufficiently removed. Discharge of these compound to surface water may form a threat to aquatic life and in the worst case may re-enter the water cycle through raw water intake from surface water or ground water. To minimise these risks source control is an interesting option. Applying separation of wastewater streams of different origin (black water, grey water) and their target treatment enables to keep pharmaceuticals concentrated in black water and urine and provide for effective removal.

The reason that pharmaceutical compounds in the environment receive much attention is that they have been developed to perform a specific biological effect in human (and other) organisms. Next to, they possessed several common features like e.g. polarity or persistence to prevent their inactivation before they pose a therapeutic effect. This already indicates that if these substances are not eliminated prior to discharge they may enter aquatic and terrestrial ecosystems resulting in bioaccumulation and provoking environmental effects.

The knowledge on the fate of pharmaceuticals during a variety of treatment technologies is, despite of significant scientific efforts, limited. To design an optimal treatment system that is able to eliminate the majority, if not all, of entering pharmaceutical micro-pollutants has not been possible yet.

Potential Impact

Retrofit of existing treatment plants- or source control by implementation of source separated based sanitation system to remove human pharmaceutical would eliminate or minimize the potential risk of these compounds entering essential water resources, the risk of exposure of aquatic organisms to these micro-pollutants and the potential effect and accumulation of specific compounds in environment. Examples of proven effects have been already reported in different parts of the world.

Since retrofit of the whole sanitation is not possible on a short term, the attention can focus in the first instance on significant point sources of emissions such as hospitals, nursery houses etc. For instance target treatment of (whole) hospital pharmaceuticals containing wastewater (black water = toilet water) would reduce total emission of certain, specific antibiotics even up to 80% (examples: Ampicillin, Penicillin G., Vancomycin).

Issues

- although the issue of the pharmaceuticals in the environment attracts a lot of attention, especially, of the scientific world. Within the EU there are no policies and standards yet that define which compounds should be removed and to which level.
- analytical methods to determine pharmaceuticals in a complex matrix are complex, time-consuming and costly; for many pharmaceutical compounds they have not even been developed/validated yet
- the fate of excretion, next to the parent compound, of (active) metabolites/conjugates in wastewater treatment systems is generally unclear.

Recommendations

The problem of pharmaceuticals in the environment requires wider recognition.
Adequate measures are needed to minimize the emissions of human pharmaceuticals to the environment, such as:

- technical (upgrading of existing treatment systems,
- introduction of source separation and application of appropriate techniques for degradation of persistent pharmaceutical compounds,
- on-site treatment for significant point sources like hospitals, elderly houses and
- non-technical (increase public awareness, justified use of certain compound (e.g. avoid excessive use of anti-biotics and of very persistent pharmaceuticals), limit sell over the counter, etc.)

For technical measures comprehensive knowledge on degradation and removal pathways (biological, chemical, physical) is necessary to establish.

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Sixth Framework Programme (2002-2006)

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Preface

Human pharmaceuticals are consumed in high quantities world wide; the consumption is in the range of tons per year per one pharmaceutical compound depending on the size of a country. The expectations are that these amounts will only keep increasing because of a improving health care system and longer life expectations of people.
In current sanitation systems characterised by a high degree of dilution, pharmaceutical compounds are not removed to a sufficient degree. Discharged to surface water form a threat to aquatic life and in the worse case may re-enter water cycle. To minimise these phenomena source control is required; source separation based sanitation approach, applying separation of wastewater streams of different origin (black water, grey water) and their target treatment may enable to minimise the emission of human pharmaceuticals to the environment.

A general overview was given on a variety and nature of human pharmaceutical compounds. Attention was paid on characteristics of the compounds in relation to their possible behaviour in a wastewater treatment system. A pre-selection was made for few compounds deserving a special attention in further study within the SWITCH project. The compounds: diazepam, oxazepam, temazepam, metoprolol, gemfibrozil, diclofenac, naproxen, ibuprofen, carbamazepine ´represent´ 4 therapeutic groups. A brief overview of a found behaviour of selected compounds in physical systems (STP, batch experiments) is given.
A validation of this selection will take place in laboratory pre-tests.
The laboratory activity will start with fate of selected compounds in biological systems (biodegradability, 2nd year of a project) followed by physical-chemical systems (3rd year of the project). Attention will be especially paid on source separation wastewater streams containing pharmaceuticals and their metabolites, urine and feaces.
Analytical methods will apply solid phase extraction, possibly followed by cleanup and detection using LC-MS(MS).
Information included in this report are far from being complete mainly because the matter is of a complex nature. In the course of the project relevant information will be supplemented.

Complementary, to the main body of this report, additional information is to be found in two appendices:

- Appendix 1: survey of the mostly used pharmaceutical compounds and hormones in the West Bank/Palestine , and
- Appendix 2: Estrogens in aquatic environment: A review.

1 Introduction

Human pharmaceuticals are consumed in high quantities world wide. The consumption is in the range of tons per year per one pharmaceutical compound depending on the size of a country. The expectations are that these amounts will only keep increasing because of a improving health care system and longer life expectations of people.
The diversity of the human pharmaceuticals is large. In the Netherlands, for instance, there are 12000 human pharmaceuticals approved (authorised). There are 850 active compounds in human pharmaceuticals, important fact from environmental point of view (Derksen 2004).
Pharmaceuticals administered (it is a medical term, in other words consumed) by humans after required action in the body get excreted with urine and feaces as a parent (original) compound and usually as a number of metabolites. The toilet wastewater (consisting of urine and faeces flushed with clean water; often called black water) is mixed with other wastewater streams forming finally a sewage that enter the municipal sewer. In a sewage treatment plant (STP) effluents many pharmaceuticals compounds do not get removed to a sufficient degree. This is because of the configurations of the current STPs that are not efficient enough to remove these micropolllutants. Consequently they are present in the effluents of STPs, enter the surface water where they may pose effects onto aquatic life (Figure 1.1). There are evidences that they do so.

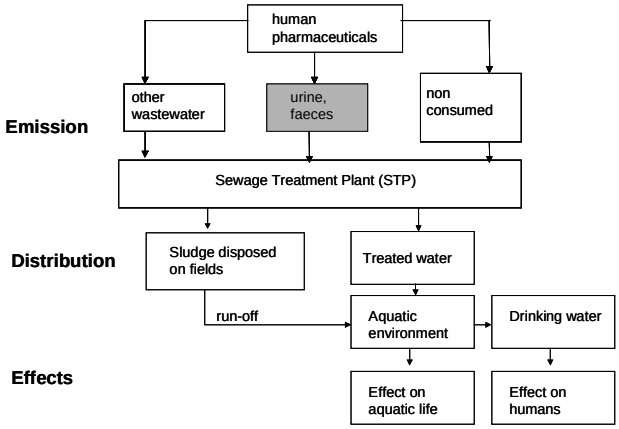


Figure 1.1: Exposure routes of human pharmaceuticals in the environment

The reason that pharmaceutical compounds in the environment has been recently deserved so much attention is that they have been developed to perform a specific biological effect in human (and other) organisms. Next to, they possessed several common features like e.g. polarity or persistence to prevent their inactivation before they posed a therapeutic effect. This already implicate that these substances will enter the aquatic and terrestrial ecosystems to bioaccumulate and provoke environmental effects (Halling-Sorensen 1998).
In this report diverse information was gathered on fate of pharmaceutical compounds in various environmental compartment and especially in wastewater treatment system. A pre-selection of representative compounds to be investigated in the following, laboratory part of the project was made.

2 General characteristics of pharmaceuticals

According to EU definition, a drug (medicinal product, pharmaceutical) is:

- any substance or combination of substances presented as having properties for treating or preventing disease in human beings; or

- any substance or combination of substances which may be used in or administered to human beings either with a view to restoring, correcting or modifying physiological functions by exerting a pharmacological, immunological or metabolic action, or to making a medical diagnosis (EU 2004).

According to U.S. Food and Drug Administration Centre for Drug Evaluation and Research (F.D.A. 2004) a drug is defined as:

- A substance recognized by an official pharmacopoeia or formulary.
- A substance intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease.

- A substance (other than food) intended to affect the structure or any function of the body.
- A substance intended for use as a component of a medicine but not a device or a component, part or accessory of a device.
- Biologic products are included within this definition and are generally covered by the same laws and regulations, but differences exist regarding their manufacturing processes (chemical process vs. biological process.)

Human pharmaceuticals comprise a wide array of chemical structures answering a wide array of medical needs. Classification of pharmaceuticals is complex because different groups have different preferences for the base for classification. The following is taken into account for classification of pharmaceuticals:

- **chemical structure** – chemical structure may but usually does not overlap with biological activity of the compounds;
- **pharmacological activity** – based on biological activity therapeutic groups of compounds are distinguished containing a wide range of chemicals, usually, of different chemical structures;
- **physiological classification** – based on the targeted physiological system – like e.g. central nervous system.
- **receptor interaction** – based on specific receptor with which they interact (e.g. beta-blockers) (Williams 2005).

In order to measure drug use classification system and a unit of measurement were developed. Norwegian researchers developed a system known as the Anatomical Therapeutic Chemical (ATC) classification and a technical unit Defined Daily Dosis (DDD) used for the first time in 1976 (WHO 2006). In the ATC classification system, the drugs are divided into different groups according to the organ or system on which they act and their chemical, pharmacological and therapeutic properties. Drugs are classified in groups at five different levels as presented in Table 2.1.

Table 2.1: Five levels of drug classification to illustrate the structure of the code based on example drug ibuprofen

Group/level	Group, subgroup	ATC code	Group
1	Main	M	Musculo-skeletal system
2	Pharmacological/therapeutic	M01	Antiinflammatory and antirheumatic products
3	Chemical/pharmacological/ therapeutic	M01A	Antiinflammatory and antirheumatic products, non steroids
4		M01AE	Propionic acid derivatives
5	Chemical substance	M01AE01	Ibuprofen

The DDD is the assumed average maintenance dose per day for a drug used for its main indication in adults. A DDD is only assigned for drugs that already have an ATC code. The DDD does not necessarily reflect the recommended or prescribed daily dose. Doses for individual patients will often differ from the DDD and will necessary have to be based on individual characteristics (e.g. age, weight) and pharmacokinetic considerations (WHO 2006).
Each pharmaceutical consists of an active pharmacological compound (usually in small quantity) and a number of help compounds to allow for medicine handling and dosing. From environmental points of view only active compounds are important.

3 Pharmaceutical metabolism and excretion

Drug metabolism is the metabolism of drugs, their biochemical modification or degradation, usually through specialized enzymatic systems. Drug metabolism often converts **lipophilic chemical compounds** into **more readily excreted polar products**. Its rate is an important determinant of the duration and intensity of the pharmacological action of drugs.
Drug metabolism can result in toxication or detoxication - the activation or deactivation of the chemical. While both occur, the major metabolites of most drugs are detoxication products.
Drugs are almost all xenobiotics.
Phase I and Phase II reactions are biotransformations of chemicals that occur during drug metabolism. Phase I metabolism usually precedes Phase II, though not necessarily (Figure 3.1 and 3.2). During these reactions, polar bodies are either introduced or unmasked, which results in (more) polar metabolites of the original chemicals. Phase I reactions may occur by oxidation, reduction or hydrolysis reactions. If the metabolites of phase I reactions are sufficiently polar, they may be readily excreted at this point. However, many phase I products are not eliminated rapidly and undergo a subsequent reaction in which an endogenous substrate combines with the newly incorporated functional group to form a highly polar conjugate. Phase II reactions — usually known as conjugation reactions (e.g., with glucuronic acid, sulfonates (commonly known as sulfation), glutathione or amino acids) — are usually detoxication in nature, and involve the interactions of the polar functional groups of phase I metabolites (Wikipedia).

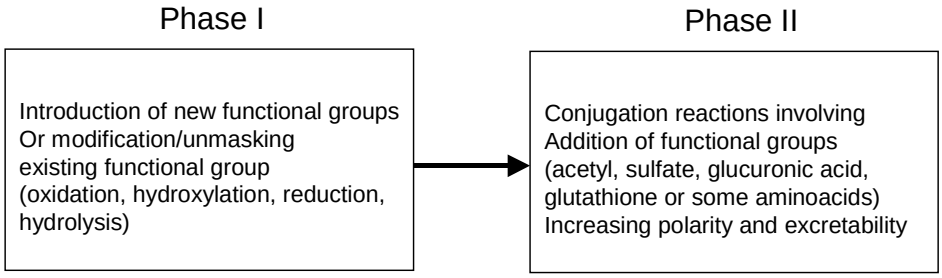


Figure 3.1: Two phases of drug metabolism; all drugs undergo both phases; conjugation reactions can be reversed.

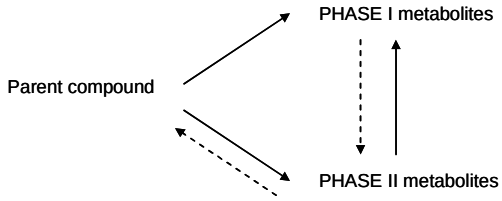


Figure 3.2: Metabolism of pharmaceutical compounds ; solid line transformation into a more water soluble compound; doted line – reactivation of the phase II metabolites

Most of the pharmaceutical substances are metabolised to phase I or II metabolites before being excreted. Products of phase I are often more toxic than the parent drug. Conjugates from phase II are normally inactive. Both phases change the chemical-physical behaviour of substance; metabolites are more soluble than parent compounds (Halling-Sorensen 1998). Attention needs to be paid therefore in any studies on both, parent compound and metabolites.

Pharmaceuticals undergo a number of enzymatic transformations (metabolism) in human tissues including liver, intestine, kidney and lung. The main part of metabolism occurs in liver. Every drug is metabolised to different degree resulting in more polar metabolites with loss of some or all pharmacological activity of the parent substance (Williams 2005).

More polar character of transformed pharmaceuticals enables their excretion, although unmetabolised compounds leave also human body. Urine and feaces are two excretion routes of pharmaceuticals. Feaces contains usually unabsorbed drugs (oral administration) or drugs metabolites excreted in the bile (Williams 2005).
In Figure 3.3 a distribution of excreted 40 pharmaceutical compounds between urine and faeces is shown (Moffat et al, 2004). It can be stated generally that 30% of the compounds are excreted in faeces and 70% in the urine.

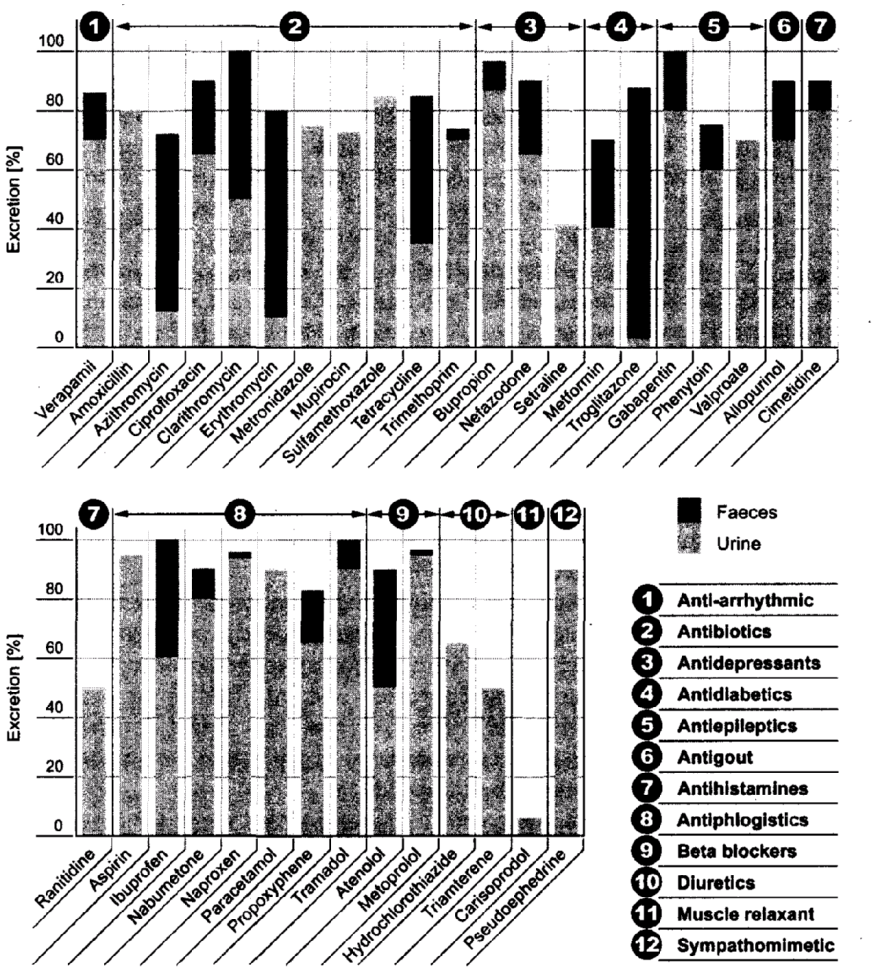


Figure 3.3: Fraction of excreted pharmaceuticals (parent compounds and metabolites) in urine and faeces for selected compounds (Moffat et al., 2004).

Commonly, glucuronide and sulphate conjugates of the parent drugs are the major excreted metabolites. It is supposed that glucuronide and sulphate conjugates may be at least partially hydrolysed in sewage, thus effectively increasing the excreted contribution to sewage concentrations of the parent drugs (Ternes 1998).

4 Sources of emission of pharmaceutical compounds

Emission routes of pharmaceuticals to water environment have a very diffuse character:

- production (cleaning processes in pharmaceutical industry)
- consumption in household or hospital
- not-consumed pharmaceuticals flushed in toilet
- effluent of a WWTP

Patients excretion

It is generally accepted that the principal source of human pharmaceuticals detected in the aquatic environment is patient excretion. The most important is on-house consumption. Humans excrete pharmaceuticals via urine and faeces. Pharmaceuticals are often excreted only slightly transformed or even unchanged mostly conjugated to polar molecules (e.g. as glucoronides. These conjugates can be easily cleaved during sewage treatment and the original PhAC will then be released into the aquatic environment (Heberer 2002).

The 'typical' wastewater from a residential area contains, conform domestic consumption, pain killers, beta-blockers, cholesterol lowering agents and anti-epileptics in concentrations up to tens of µg/L. Antibiotics, anastatics and X-ray contrast media were also detected but in much lower concentrations. Detected concentrations in the effluents from pharmaceutical industry and hospitals differ a lot concerning a type of pharmaceutical as well as its concentration. In general wastewater from hospital contains high concentrations of X-ray contrast media (in mg/L) and antibiotics, different than those used in a household (µg/L).

Disposal from pre-patient supply and unused pharmaceuticals

Handling pharmaceutical products to be disposed, because of, e.g., the expiration date has passed, is controlled. Expired products are commonly returned to the manufacturing company. Expired products are then usually destructed via incineration. In this way disposal of undistributed or outdated products is unlikely to be a source of pharmaceuticals detected in the environment (Williams 2005).

Patient disposal of unused, outdated or sold over-the-counter pharmaceuticals can be into either domestic wastewater or solid waste. Only limited data is available on the magnitude of this emission source. According to (Kummerer, 2004) between 25% (in Germany) and 33% (Austria) sold drugs are disposed to household waste(water) streams.

Disposal to wastewater is believed to be an emission form but is not dominant (Heberer 2002), (Williams 2005). Disposed pharmaceuticals are not modified by human metabolism prior entering wastewater.

Disposal to solid waste. Residential solid waste is either incinerated or disposed via landfill. It is not clear whether biodegradation of pharmaceutical compounds occurs in bioreactive landfills. Excess leachate from landfills that may contain pharmaceuticals may be disposed to WWTP.

Pharmaceutical industry

Discharges from manufacturing facilities are not believed to contribute significantly to the overall emission. Discharge of active pharmaceutical ingredient via waste stream is generally avoided since it constitutes a valuable product. A common practice in pharmaceutical industry is recovery and reuse of active ingredients, otherwise treatment and disposal via incineration is applied. A number of manufacturing sites is relatively small in the world and they are usually concentrated in specific regions. Also there are very few facilities for a specific active ingredient. When active ingredients are blended with some help substances (starch, lactose) some solid waste stream may be produced, that is commonly incinerated (Williams 2005).

5 Variety of pharmaceutical compounds

There are 14 main groups of human pharmaceutical substances as shown in Table5.1.

Table 5.1: Main groups of human pharmaceuticals (WHO 2006)

ATC group	Number of pharmacological therapeutic subgroups	Remark
A Alimentary tract and metabolism	16 (A01-A16)	
B Blood and blood forming organs	5 (B01-B06) no B04	
C Cardiovascular system	9 (C01-C10) no C06	
D Dermatologicals	11 (D01-D11)	
G Genito urinary system and sex hormones	4 (G01-G04)	
H Systematic hormonal preparations	5 (H01-H05)	Excl. sex hormones and insulines
J Antiinfectives for systematic use	6 (J01-J07) no J03	
L Antineoplastic and immunomodulating agents	4 (L01-L04)	
M Musculo-skeletal system	6 (M01-M09) no M6,7,8	
N Nervous system	7 (N01-N07)	
P Antiparasitic agents, insecticides, repellents	3 (P01-P03)	
R Respiratory system	6 (R01-R07) no R4	
S Sensory organs	3 (S01S03)	
V Various	9 (V01, 03,04,06,07-09,10,20)	

5.1 Group A: alimentary tract and metabolism

This group comprises the largest number of the subgroups: A01 - stomatological preparations; A02 Drugs for acid related disorders, A03 Drugs for functional gastrointestinal disorders, A04 Antiemetics and antinauseants, A05 Bile and liver therapy, A06 Laxatives , A07 Antidiarrheals, intestinal anti-inflammatory/anti-infective agents, A08 Antiobesity preparations, excluding diet products, A09 Digestives, including enzymes, A10 Drugs used in diabetes, A11 Vitamins, A12 Mineral supplements, A13 Tonics, A13A tonics, A14 Anabolic agents for systemic use, A15 Appetite stimulants, A16 Other alimentary tract and metabolism products. Pharmaceuticals belonging to this ATC group will be further not subjected to analysis within this part of the project.

5.2 Group B: blood and blood forming organs

Medicines from this group are applied to fight against diseases of blood and blood forming organs, like iron deficiency anemias, other deficiency anemias, hereditary hemolytic anemias, acquired hemolytic anemias, aplastic anemia, other and unspecified anemias, coagulation defects, purpura and other hemorrhagic conditions, diseases of white blood cells, other diseases of blood and blood-forming organs. The pharmaceuticals from this group will be further not investigated within this project.

5.3 Group C: cardiovascular system

Beta blockers (β-blockers) are a class of drugs used in the highest quantities within ATC group C used for various indications, but particularly for the management of cardiac arrhythmias and

cardioprotection after myocardial infarction. Beta blockers are pharmaceuticals designed to block the β1–receptor from stimulating the higher hart rate and the cardiac output in humans with mainly cardiovascular diseases, like hypertension and angina pectoris, but also some other diseases like migraine, thyrotoxicoses and the control of tremors. Some beta blockers have a high first pass metabolism, while others are excreted unchanged in the urine. Most of the generic names for beta blockers end with *"olol"*(e.g. sotalol, timolol, esmolol, carteolol, carvedilol, nadolol, propranolol, propranolol, betaxolol, penbutolol, metoprolol, acebutolol, atenolol, metoprolol, labetalol, pindolol, bisoprolol).

Table 5.2: Examples of selective and non-selective beta-blockers and their metabolism

Selective beta blockers	
Metoprolol	Extensively metabolised in the liver, so that only 5 % is excreted by the kidney as the parent compound. The plasma half live is 3-4 hours and to prolong the plasma half-life, extended release tablets are developed. It has a first pass effect of 50 %. In the liver metoprolol is metabolised by the cytochrome P450 isoenzyme cyp2D6. It also undergoes oxidative deamination, O-dealkylation followed by oxidation and aliphatic hydroxylation. The metabolites are also excreted in urine.
Atenolol	Metabolism in the body is different than of metoprolol. Only 50 % is absorbed in the intestine and with food this decreases with 20 %. From the 50 %, which is absorbed only 10 % is metabolised in the liver. The drug is excreted in the urine. It has a plasma half life of 7-8 hours. And the maximum plasma concentration is reached after 2-4 hours.
Bisprolol	After oral intake it is absorbed in the gastro intestinal tract and approximately 90 % is bio available in the body. It is metabolised in the liver. 50 % is excreted as parent compound in the urine and the other 50 % as inactive metabolites.
Selective beta blockers	
Propranolol	It binds to both α- and β-adrenoreceptors. It has a high first pass metabolism and is subject to hepatic tissue binding. The maximum concentration in the plasma is reached after 1-2 hours. In the blood 80-90 % is bound to plasma proteins, so only 10-20 % of the absorbed and metabolised drug can cause effects. The plasma half live is 3-6 hours . Propranolol has high lipophilic solubility and passes the blood-brain barrier, placenta and is distributed in milk (EU 2004). Less than 1 % is excreted as the parent compound and 90 % is excreted as a metabolite in urine. Three primary pathways of metabolism of propranolol are described. 41 % is metabolised through a side-chain oxidation, 17 % through glucuronic acid conjugation and the other route is ring oxidation. Another route of metabolism, which is of little importance, is the O-dealkylation. Recently it was discovered that the cytochrome P450 isoenzymes, CYP1A2 catalyses the oxidative metabolism of propranolol. 4-hydroxypropranolol is a ring hydroxylated metabolite and is biologically active. 4-hydroxypropranolol and propranolol are formed in the same amount in the liver after oral administration, but this metabolite isn't excreted in urine and has a lower plasma half live as propranolol.
Sotalol	It has low lipid solubility, but is absorbed almost for 100 % in the intestine. A very little amount is metabolised and all is excreted unchanged in the urine. It is given in racemic mixtures of two stereoisomers *d*-sobatol and *l*-sobatol. Unlike the *d*isomers of the other beta blocking drugs, *d*-sotalol has arrhythmic properties. The plasma half life is 10-20 hours.
Nadolol	Incompletely absorbed in the gastro-intestinal system after oral administration. It does appear not to be metabolised and is excreted in urine. It has a plasma half-life of 12-24 h. It has a low lipid solubility.

Lipid-lowering drugs reduce serum cholesterol levels by inhibiting a key enzyme involved in the biosynthesis of cholesterol; examples:

- Resins cholestyramine (Cholybar, Questran) colestipol (Colestid)
- HMG CoA Reductase Inhibitors lovastatin (Mevacor) pravastatin (Pravochol) simvastatin (Zocor)
- Fibric Acid Derivatives gemfibrozil (Lobid) clofibrate (Atromid-S)
- Miscellaneous nicotinic acid (Niacin) probucol (Lorelco)

5.4 Group J: antibiotics

Antibiotic are widely used to treat many bacterial infections. Bacteria are classified as either Gram-positive or Gram-negative. They differ in several respects, especially in the structure of the cell wall, which has implication for the action of the antibiotics. The cell wall of the Gram-positive bacteria is a relatively simple structure, while the cell wall of Gram-negative organisms is more complex. Some of the antibiotic classes, such as macrolides, show difficulty in penetrating the complex outer layer of Gram-negative bacteria. There are three proven targets for the main antibacterial drugs: (1) bacterial cell-wall biosynthesis; (2) bacterial protein synthesis; and (3) bacterial DNA replication and repair. Phenoxymethylpenicillin and amoxicillin inhibits the cell wall synthesis; Tetracycline, erythromycin and clarithromycin inhibits the protein synthesis and trimetoprim and ciprofloxacin inhibits the nuclei acid synthesis. The antibiotic compounds will be further not subject of this study.

5.5 Group N: nervous system

Sedatives, tranquilizers, depressants, anxiolytics, soporifics, sleeping pills, downers, or sedative-hypnotics these are different names for substances, which depress the central nervous system (CNS), resulting in calmness, relaxation, reduction of anxiety, sleepiness, slowed breathing, slurred speech, staggering gait, poor judgment, and slow, uncertain reflexes. At high doses or when they are abused, many of these drugs can cause unconsciousness and death (Wikipedia).
A depressant is a chemical agent that diminishes the function or activity of a specific part of the body. The term is used in particular with regard to the CNS. In that case these chemicals are known as neurotransmitters. Depressants intended to act on the CNS do so by increasing the activity of a particular neurotransmitter known as gamma-aminobutyric acid (GABA).
GABA's task is to calm the CNS and to promote sleep. Drugs that stimulate the production of this amino acid produce slowed brain activity and a drowsy or calm feeling, and so depressants are generally prescribed to relieve symptoms of anxiety or insomnia. Internal systems regulate the body's production of GABA, but when medication is taken to stimulate GABA production, it is possible to induce hazardously high levels, which can dangerously slow breathing and heart rates, and may result in death.
An **antidepressant** is a medication designed to treat or alleviate the symptoms of clinical depression. Some antidepressants are also used to help one sleep, to treat anxiety, and to relieve certain types of pain. Other antidepressants, notably the tricyclics, are commonly used off-label in the treatment of neuropathic pain, whether or not the patient is depressed. Smaller doses are generally used for this purpose, and they often take effect more quickly. Many antidepressants also are used for the treatment of anxiety disorders and tricyclic antidepressants are used in the treatment of chronic pain disorders such as chronic functional abdominal pain (CFAP), myofascial pain syndrome, and post-herpetic neuralgia.

Like many psychiatric drugs, antidepressants were discovered by accident. The first useful antidepressants belonged to a group called MAOIs (MonoAmine Oxidase Inhibitors) and were discovered in the early 1950s. The original member of this group was iproniazid, which was originally developed to treat tuberculosis. The next group were the tricyclic antidepressants. The first

was imipramine. They were effective and safer than the MAOI but still quite dangerous in overdose. They are still used today but have been largely replaced by another group: SSRIs (Selective Serotonin Reuptake Inhibitors). The first SSRI was fluoxetine (better known as Prozac). Drugs from all three groups have been found to improve the mood of depressed patients. The SSRI antidepressants were early examples of rational drug design.
TCA's have increasingly been replaced by selective serotonin reuptake inhibitors (SSRI's), serotonin and noradrenaline reuptake inhibitors (SNRI's) and other newer antidepressants. SSRIs are prescribed for anxiety disorders, obsessive-compulsive disorder, and eating disorders. They are also sometimes prescribed to treat irritable bowel syndrome. SNRI's are used in the treatment of depression and other affective disorders. They are also sometimes used to treat anxiety disorders, obsessive-compulsive disorder, attention deficit hyperactivity disorder (ADHD) and chronic neuropathic pain. Some 'well-known' antidepressants examples are given in Table 5.3:

Table 5.3: Examples of anti-depressants

Compound	**class**	**Brand names**
Fluoxetine	SSRI	Prozac, Sarafem, Fluctin, Fontex, Prodep, Fludep, Lovan
Sertraline	SSRI	Zoloft, Lustral, Apo-Sertral, Asentra, Gladem, Serlift, Stimuloton
Venlafaxine	SNRI	Effexor, Efexor
Citalopram	SSRI	Celexa, Cipramil, Talohexane
Paroxetine	SSRI	Paxil, Seroxat, Aropax
Escitalopram	SSRI	Lexapro, Cipralex
Fluvoxamine	SSRI	Luvox, Faverin
Duloxetine	SNRI	Cymbalta
Bupropion	DRI and NRI	Wellbutrin, Zyban
Amitriptyline	TCA	Elavil
Dothiepin (Dosulepin)	TCA	Prothiaden, Dothapax

Depressants generally fall into two classes, barbiturates and benzodiazepines, but also include narcotics (or opioids) and sedative-hypnotics. Also there are tranquilizers.
Barbiturates are effective in relieving the conditions they are designed to address; they are also readily abused, and when, in the late 1960s, it became clear that the social cost of barbiturates was beginning to outweigh the medical benefit, a serious search began for a replacement drug. Most people still using barbiturates do so to prevent seizures.
Benzodiazepines mediate the same symptoms as barbiturates, but without the same degree of toxic hazard. This is not to say they are not without their own risks; where barbiturates pose a greater "front-end" risk in that overdose or drug/alcohol interactions may result in fatality, benzodiazepines pose a greater "back-end" risk in the possibility of addiction and serious physical and psychological withdrawal symptoms. Even so, any suggestion that it is safe to consume alcohol while using benzodiazepines, or to attempt to stop barbiturate use "cold turkey" is foolish in the extreme.
Barbiturates are drugs that act as central nervous system (CNS) depressants, and by virtue of this they produce a wide spectrum of effects, from mild sedation to anesthesia. Some are also used as anticonvulsants. Barbiturates are believed to be GABA (gamma-aminobutyric acid) agonists, acting on the GABA-A receptor. GABA is the principal inhibitory neurotransmitter in the mammalian CNS. Barbiturates are derivatives of barbituric acid.

The benzodiazepines as minor tranquilizers are a class of drugs with sedative, hypnotic, anxiolytic, anticonvulsant, amnestic and muscle relaxant properties. Benzodiazepines are often used for short-term relief of severe, disabling anxiety or insomnia. Long-term use can be problematic due to the development of tolerance and dependency. They are believed to act on the GABA receptor GABAA,

the activation of which dampens higher neuronal activity. They began to be widely prescribed for stress-related ailments in the 1960s and 1970s. Their chemical structure is based upon diazepine and phenyl groups. Examples: Alprazolam, Bromazepam, Chlordiazepoxide, Cinolazepam, Clonazepam Clorazepate Diazepam Flunitrazepam Flurazepam Halazepam Ketazolam Loprazolam Lorazepam Lormetazepam Medazepam Nobrium Midazolam Nitrazepam Mogadon Nordazepam Oxazepam Prazepam Quazepam Temazepam Tetrazepam Triazolam DMCM.

The term **antipsychotic** is applied to a group of drugs used to treat psychosis. Common conditions with which antipsychotics might be used include schizophrenia, mania and delusional disorder, although antipsychotics might be used to counter psychosis associated with a wide range of other diagnoses. Antipsychotics also have some effects as mood stabilizers, leading to their frequent use in treating mood disorder (particularly bipolar disorder) even when no signs of psychosis are present. Some antipsychotics (haloperidol, pimozide) are used to treat Tourette syndrome.
Antipsychotics are also referred to as neuroleptic drugs, or simply neuroleptics. There are currently two main types of antipsychotics in use, the typical antipsychotics and atypical antipsychotics. A new class of antipsychotic drugs has recently been discovered, known as dopamine partial agonists. Clinical development has progressed rapidly on partial dopamine agonists, and one drug in this class (aripiprazole) has already been approved by the Food and Drug Administration. Although the underlying mechanism of this new class is different from all previous typical and atypical antipsychotics, dopamine partial agonists are often categorized as atypicals.
Typical antipsychotics are sometimes referred to as major tranquilizers, because some of them can tranquilise and sedate. This term is increasingly disused because many newer antipsychotics do not have strong sedating properties and the terminology implies a connection with benzodiazepines, whereas none exists.
Further there are within this group - **Atypical antipsychotic** (also known as second generation antipsychotics) are a class of prescription medications used to treat psychiatric conditions; All atypical antipsychotics are FDA approved for use in the treatment of schizophrenia. Some carry FDA approved indications for acute mania, bipolar mania, psychotic agitation, bipolar maintenance, and other indications; clozapine (Clozaril), quetiapine (Seroquel) , Risperidone (Risperdal) , Ziprasidone (Geodon). It may make some people tired, while making others unable to sleep olanzapine (Zyprexa)

5.6 Group pain relievers, antiphlogistics, analgesics, anti-inflammatories, non-steroidal drugs

An **analgesic** (colloquially known as a painkiller) is any member of the diverse group of drugs used to relieve pain and to achieve analgesia. Analgesic drugs act in various ways on the peripheral and central nervous system; they include paracetamol (acetaminophen), the nonsteroidal anti-inflammatory drugs (NSAIDs) such as the salicylates, narcotic drugs such as morphine, synthetic drugs with narcotic properties such as tramadol, and various others. Some other classes of drugs not normally considered analgesics are used to treat neuropathic pain syndromes; these include tricyclic antidepressants and anticonvulsants.
Antiphlogistic drugs and pain killers are agents, which are applied in medical therapy for relieving pains, fevers and against inflammatory caused by various diseases. They are substances used in many non-prescription drugs in the primary health sector. In the current context, antiphlogistic drugs and pain killers are chosen for a separate section because of their widespread application in high doses make up the largest tonnage of one group of pharmaceuticals.
Non-steroidal anti-inflammatory drugs, usually abbreviated to **NSAIDs**, are drugs with analgesic, antipyretic and anti-inflammatory effects - they reduce pain, fever and inflammation. The term "non-

steroidal" is used to distinguish these drugs from steroids, which (amongst a broad range of other effects) have a similar eicosanoid-depressing, anti-inflammatory action. NSAIDs are sometimes also referred to as non-steroidal anti-inflammatory agents/analgesics (NSAIAs). The most prominent members of this group of drugs are aspirin and ibuprofen. Paracetamol (acetaminophen) has negligible anti-inflammatory activity, and is strictly speaking not an NSAID.
Beginning in 1829, with the isolation of salicylic acid from the folk remedy willow bark, NSAIDs have become an important part of the pharmaceutical treatment of pain (at low doses) and inflammation (at higher doses). Part of the popularity of NSAIDs is that, unlike opioids, they do not produce sedation, respiratory depression, or addiction. NSAIDs, however, are not without their own problems. Certain NSAIDs, including ibuprofen and aspirin, have become accepted as relatively safe and are available over-the-counter without prescription.

5.7 Group V: contrast media

Radiocontrast agents (or simply contrast agents) are compounds used to improve the visibility of internal bodily structures in an X-ray image. Iodinated contrast agents contain iodine, which enhances the visibility of vascular structures and organs during radiographic procedures.
Iodinated contrast media may either be oil-based or water-soluble, the former of which is slowly absorbed by body tissue and is usually only used in sialographic and hysterosalpingographic examinations. Water-soluble iodinated medium, which is more quickly absorbed, may be used in place of barium sulfate for gastrointestinal studies that are contraindicated by the use of barium for that reason. Contrast media are highly persistent. They will be further not subjected to further study within this project

6 Properties of pharmaceuticals

Pharmaceuticals are compounds characterised by complex chemical structures. Most pharmaceuticals are charged and hydrophilic. Many pharmaceuticals have multiple ionisable functional groups. The hydrophobic reactions dominating partitioning neutral organic compounds to sediments and suspended solids (limited sorption properties) are relatively unimportant for most of the pharmaceuticals. Many pharmaceutical are chiral and often administered as racemic mixtures (Williams 2005).
Properties of few pharmaceutical groups relevant for their behaviour in environment are given below.

Antibiotics
(Al-Ahmad 1999)investigated biodegradability of some clinically important antibiotics, Cefotiam, Ciprofloxacin, Meropenem, Penicillin G, and Sulfamethoxazole, in the closed bottle test (CBT). These drugs possessed different chemical structures and mode of action (= antibiotic spectra). None of the investigated antibiotics was readily biodegradable (Table 6.1). Low biodegradation rates were also reported in soil. Adaptation of microorganisms was not concluded. Penicillin seems to be easier biodegradable than the rest of tested antibiotic compounds. These finding were in agreement with reported poor biodegradability in soils. These on the other hand could have been caused by adsorption resulting in poor bioavailability. Authors concluded that biodegradation of antibiotics in STPs might not be a reliable expectation for the removal of antibiotic substances. The CBT was a screening test using low bacteria density. In tests with higher bacteria density (biodegradation tests) or a higher degree of simulating an STP, higher biodegradability and nonbiotic elimination processes like adsorption, hydrolysis, or partial degradation of active moieties may take place in a higher extension, but not necessarily.

Tabel 6.1: Results of the closed bottle test (OECD 301 D) (Al-Ahmad 1999)

Test Compound	Supplied By	Test Concentration (µg/ml)	Biodegradation After 28 Days (%)	Biodegradation After 40 Days (%)
Cefotiam dihydrochloride	Takeda Pharma GmbH	4.8	7	10
Ciprofloxacin	Bayer MG, Lever Kusen	3.5	0	0
Meropenem	Zeneca-Grünenthal, GmbH Stolberg	2.5	7	7
Penicillin G	Zeneca-Grünenthal, GmbH Stolberg	3.0	27	36
Sulfamethoxazole	Sigma Aldrich Chemie GmbH, Steinheim	3.8	0	0

Group β-blockers, lipid lowering agents
High solubility (metoprolol >1000ppm) or moderate solubility (atenolol, propranolol 10-1000ppm) and low logK_{ow} (<3) correspond to a high affinity of β-blockers to water. The presence of beta blockers in the gaseous compartment is neglectable due to its low vapour pressure.

Antidepressants
In a study by (Black 2004), three selective serotonin reuptake inhibitors (SSRI's): paroxetine, sertraline and fluvoxamine, were tested for biodegradability using activated sludge inoculum from a waste water treatment plant. No degradation was observed during a test period of 28 days. It was concluded that none of the compounds could be labelled readily biodegradable in waste water treatment plants. (Cunningham, Constable et al. 2004) found in a preliminary biodegradation study with sewage biomass a rapid depletion of paroxetine from solution over the first day of the studies followed by no further depletion despite culture acclimations and enrichments. In extensive aerobic

biodegradability studies no depletion of paroxetine was observed in the period after sorption had reached equilibrium.

The antidepressants are not volatile from water or they have a very slow rate of volatilisation as Henry constants are low, K_H < 1.3·10-7 atm·m^3 ·$mole^{-1}$. They have rather little mobility or are immobile in soil and will most likely sorb to soil and sludge.

It is expected that the compounds tend to bioaccumulate as logK_{OW} values are around 3 and higher. In a study performed by (Brooks, Turner et al. 2003) fluoxetine, sertraline and the metabolites norfluoxetine and desmethylsertraline were detected at levels greater than 0.1 ng/g in all tissues examined from fish residing in a municipal effluent-dominated stream.

Pain relievers, antiphlogistics, analgesics, anti-inflammatories, non-steroidal drugs

Salicilic acid found to be easily biodegraded. Higher degradability of paracetamol can be assumed (Henschel, 1997). Zwiener et al, (2000) found a high degree of degradation for ibuprofen in the oxic biofilm reactor, which was attributed to adaptation of the biofilm to the residue (Zwiener, 2000). Two metabolites could be identified on the basis of their mass spectra and comparison with literature data, viz. hydroxyibuprofen and carboxyibuprofen.

7 Quantities of pharmaceuticals used (Dutch situation)

Pharmaceuticals for human treatment are used in high quantities. The consumption and abundance of pharmaceutical compounds differ per country. The global consumption of pharmaceuticals used by humans is predicted as 100,000 tons per year. This number corresponds to a worldwide average pro capita consumption of 15 $g.cap^{-1}.a^{-1}$ (Ternes 2006), (Kummerer 2004).
The consumption of all therapeutic groups of pharmaceuticals in the Netherlands in years 2001 till 2005 expressed in number of users is given in Table 7.1.

Table 7.1: Users per ATC group of pharmaceuticals (* 1000) in the Netherlands (CVZ 2006)

ATC group	2001	2002	2003	2004	2005
A Alimentary tract and metabolism	2 831	2 899	3 002	2 767	3032
B Blood and blood forming organs	1 641	1 655	1 663	1 667	1 720
C Cardiovascular system	2 606	2 684	2 759	2 910	3 080
D Dermatologicals	3 412	3 421	3 465	3 192	3 200
G Genito urinary system and sex hormones	2 824	2 784	2 703	1 418	1 437
H Systematic hormonal preparations	787	828	854	890	947
J Antiinfectives for systematic use	3 884	3 840	3 826	3 775	3 978
L Antineoplastic and immunomodulating agents	134	145	157	169	184
M Musculo-skeletal system	3 442	3 403	3 423	3 322	3 182
N Nervous system	3 590	3 605	3 597	3 344	3 385
P Antiparasitic agents, insecticides, repellents	137	144	148	160	163
R Respiratory system	3 094	3 158	3 064	3 033	3 155
S Sensory organs	1 777	1 786	1 802	1 759	1 787
V Various	33	34	36	40	43

Group A, C, D, G, J, M, N and R are characterised by the highest number of users, between 2,5 and 4 mln people per ATC group. In Table 7.2 number of DDDs are listed per ATC main group. The prevailing groups are then A, B, C, D, N and R.

Table 7.2: Amount of DDDs (* 1000) used in The Netherlands in years 2001-2006 (CVZ 2006)

ATC group	2001	2002	2003	2004	2005
A Alimentary tract and metabolism	792.040	839.970	897.320	828.640	924.510
B Blood and blood forming organs	398.190	416.550	441.640	433.180	448.140
C Cardiovascular system	1.592.100	1.713.300	1.870.900	2.047.900	2.190.500
D Dermatologicals	477.450	495.190	522.350	472.220	486.990
G Genito urinary system and sex hormones	798.290	790.670	799.100	277.720	284.000
H Systematic hormonal preparations	107.930	113.920	120.190	125.290	129.590
J Antiinfectives for systematic use	63.940	63.646	64.441	65.000	69.333
L Antineoplastic and immunomodulating agents	30.681	34.981	40.856	47.140	52.409
M Musculo-skeletal system	235.650	241.730	256.170	250.080	238.240
N Nervous system	642.170	670.150	699.110	686.140	691.350
P Antiparasitic agents, insecticides, repellents	4.098	4.249	4.502	5.207	5.052
R Respiratory system	586.760	592.720	582.280	565.320	568.760
S Sensory organs	198.390	208.450	220.610	220.300	222.910
V Various	2.117	2.979	3.706	4.647	5.753

In Table 7.3 ten of the most often prescribed specific compounds in year 2005 is given.

Tabel 7.3: Top 10 prescribed pharmaceuticals 2005 (CVZ 2006)

	ATC code, active compound	branch		Number prescribed
1	C07AB02 Metoprolol	Lopresor® Selokeen®	angina pectoris and high blood pressure	2.984.000
2	N05BA04 Oxazepam	Seresta®	tranquilliser	2.860.000
3	N05CD07 Temazepam	Normison®	sedative	2.487.000
4	M01AB05 Diclofenac	Voltaren®	Pain killer	2.307.000
5	B01AC06 Acetylsalicilic acid	Aspirine®	Blood plasma, inhibition aggregation	2.294.000
6	A02BC01 Omeprazol	Losec®	Stomach acid	2.185.000
7	B01AC08 Carbasalaatcalcium	Ascal®	Blood plasma, inhibition aggregation	1.893.000
8	C10AA01 Simvastatine	Zocor®	Decreasing cholesterol	1.815.000
9	A10BA02 Metformine	Glucophage®	Diabetes	1.693.000
10	H03AA01 Levothyroxine	Thyrax®	To enhance thyroid hormone	1.577.000

In the following an example of a procedure is shown of selecting pharmaceutical compounds for the further study (laboratory phase). The C group (cardiovascular system) was chosen as the pharmaceuticals used for cardiovascular system are consumed by a large part of population in high quantities. Within this group there are 9 subgroups (Table 7.4); and pharmaceuticals from 6 subgroups are used in the highest quantities – above 0.5 mln of users.

Table 7.4: Subgroups of ATC C group and their consumption between 2001 and 2005 (in number of users)

	2001	2002	2003	2004	2005
C01 Cardiac therapy	526.670	515.080	503.020	507.670	489.670
C02 Antihypertensives	58.971	57.932	57.079	58.971	57.753
C03 Diuretics	907.360	912.300	942.730	990.220	1.024.000
C04 Peripheral vasodilators	12.981	10.746	8.533	7.485	6.456
C05 Vasoprotectives	206.290	213.890	222.720	206.650	204.830
C07 Beta-blocking agents	1.122.000	1.172.000	1.228.000	1.327.000	1.368.000
C08 Calcium channel blockers	513.070	532.810	548.130	576.150	585.100
C09 ace inhibitors	881.940	967.700	1.056.000	1.177.000	1.268.000
C10 Lipid modifying agents	710.500	795.170	900.180	1.046.000	1.160.000

In Table 7.5 specific pharmaceuticals used in the highest quantities in numbers of users and number of DDDs consumed (2005) (CVZ 2006)

Table 7.5: Consumption and environmental load (mass of the sold products) of 5 pharmaceuticals from ATC group C

	Users	DDD	DDD, mg	Env. load, t/year
C07AB02 Metoprolol	706.090	129.929.300	150	19.5
C10AA01 Simvastatine	462.830	213.647.200	15	3.2
C10AA05 Atorvastatine	382.390	251.456.500	10	2.5
C03AA03 Hydrochloorthiazide	366.310	85.303.600	25	2.1
C03CA01 Furosemide	360.500	101.434.400	40	4.0

Among the pharmaceutical compounds used for cardiovascular system, metroprolol, simvastatine, atorvastatine, hydrochlorothiazide and simvastatine are the compounds used by a largest number of people. Considering the total number of Daily Defined Doses (DDD) sold as well as an individual

DDD (mg.person^{-1}.d^{-1}), the total load of sold compounds was calculated (Figure 7.1). Metroprolol is consumed in the highest quantity.

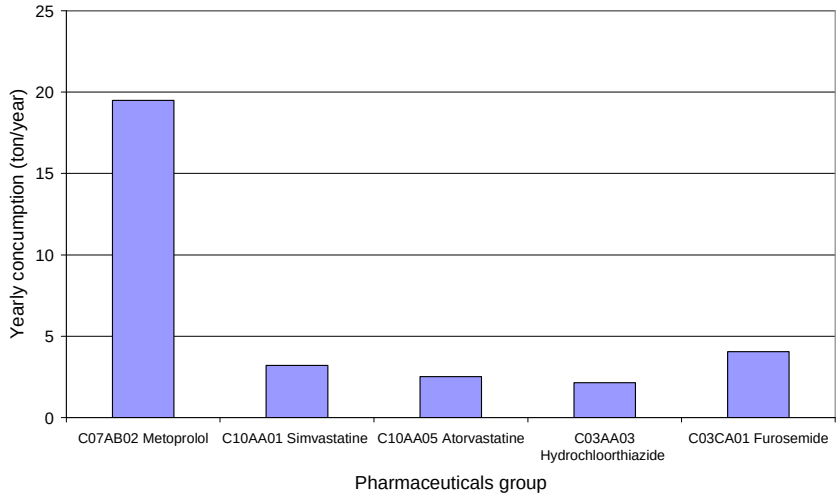


Figure 7.1: Yearly Consumption of mostly used pharmaceuticals for cardiovascular system in The Netherlands in 2005 (CVZ 2006)

In another pharmaceuticals group of musculo-skeletal system (group M), anti-inflamatories and anti-rheumatic agents are used by the largest number of people (almost 3 mln in 2005 (CVZ 2006)). Diclofenac (acetic acid derivative) was used by 1.4 mln of people, followed by ibuprofen and naproxen (propionic acid derivatives). The yearly consumption of mentioned compounds in shown in Table 7.6 and Figure 7.2.

Table 7.6: Specific pharmaceuticals used in the highest quantities in numbers of users and number of DDDs consumed (2005); ATC group M (CVZ 2006)

	Users	DDDs	DDD, mg.p^{-1}.d^{-1}	Predicted max environmental. load, t/year[1)]
M01AB05 Diclofenac	1.386.000	51.072.400	100	5,1
M01AE01Ibuprofen	848.610	24.782.600	1200	29.7
M01AE02 Naproxen	537.330	27.480.400	500	13.7

[1)] Assumption: administered is excreted for 100% as a parent compound

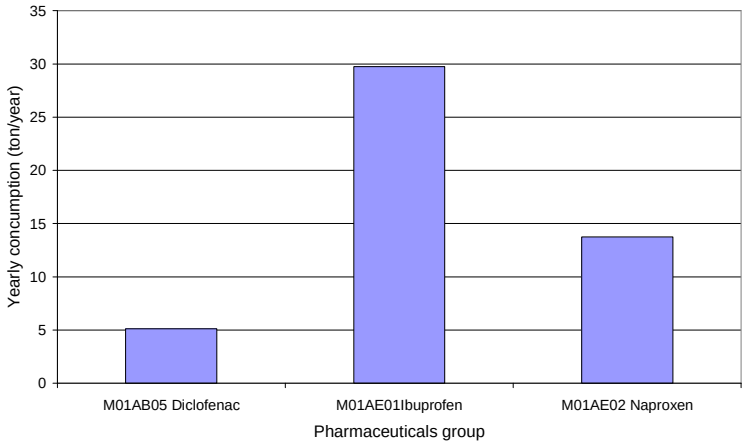


Figure 7.2: Yearly Consumption of mostly used pharmaceuticals for musculo-skeletal system in The Nethelands in 2005 (CVZ 2006)

For comparison consumption of certain specific pharmaceutical compounds in Denmark is given in Table 7.7.

Tabel 7.7: Consumption of specific substances and therapeutic groups in Denmark (5.2 mln inhabitants in 1995) of some pharmaceutical substances (Halling-Sorensen 1998)

substances	DDD per jaar (millions)	DDD grams	Applied weight, tones
Single substances			
Ibuprofen	27.7	1.2	33.2
Furosemid	91.9	0.040	3.7
Estrogens	58,3	6.5.10-5	3.8.10-3
Estradiol	24,3	0.002	0.049
Therapeutic groups			
Antibiotics	25.1	1.5	37.7
Analgesics (NSAID)	56.6	0.5	28.3
hypotensiva	41.0	0.010	0.41
diuretica	95.3	0.040	3.8
antiasthmatic	110.5	0.015	1.7
psycholeptics	147.5	0.050	7.4

8 Occurrence in aquatic environment

8.1 Wastewater

The presence of several pharmaceuticals in STP effluents has been confirmed in Germany, The Netherlands, Switzerland, United Kingdom, France, Greece, Sweden and Italy, Spain the United States, Canada, Brazil, and Australia (Castiglioni 2006), Table 8.1.

Table 8.1: Occurrence of pharmaceutical residues in STP effluents (Castiglioni 2006)

Occurrence of pharmaceutical residues in the STP effluents

Compounds	Concentrations (μg/l) median (maximum)			
Antiphlogistics/anti-inflammatory drugs				
Ibuprofen	0.05 (7.11)[a]	0.37 (3.4)[b]	3.09 (27.3)[c]	4.0 (24.6)[d]
Naproxen	1.12 (5.22)	0.30 (0.52)	–	12.5 (33.9)
Ketoprofen	n.d (1.62)	0.2 (0.38)	–	n.d.
Diclofenac	0.68 (5.45)	0.81 (2.1)	0.42 (2.35)	n.d.
β-Blockers				
Propanolol	0.01 (0.09)	0.17 (0.29)	0.08 (0.28)	–
Metoprolol	0.08 (0.39)	0.73 (2.2)	–	–
Acebutolol	0.06 (0.13)	–	–	–
Oxprenolol	0.02 (0.05)	–	–	–
Lipid regulators				
Gemofibrozil	0.84 (4.76)	0.40 (1.5)	–	1.3 (1.3)
Fenofibrate	0.14 (0.16)	n.d. (0.03)	–	–
Bezafibrate	n.d. (1.07)	2.2 (4.6)	–	–
Clofibric acid	n.d. (0.68)	0.36 (1.6)	–	n.d.
Antiepileptic				
Carbamazepine	0.87 (1.20)	2.1 (6.3)	–	0.7 (2.3)
Antibiotics				
Trimetroprim	0.04 (0.13)	–	0.07 (1.29)	–
Sulfamethoxazole	0.05 (0.09)	–	<0.05 (0.13)	0.24 (0.87)
Erythromycin	–	–	<0.01 (1.84)	0.08 (0.84)
Reference	[22]	[23]	[24]	[25,26]

[a] Seven STP in France, Greece, Italy and Sweden.
[b] Forty-nine STP in Germany.
[c] Five STP in the UK.
[d] Fourteen STP in Canada (eight STP for antibiotics).

8.2 Hospital wastewater

Hospital wastewater is a significant source of pharmaceuticals such as antibiotics, anti-cancer agents and iodinated contrast media. The share of specific antibiotics used in hospitals may vary between few percent up to 90% of total emission (BLAC, 2003). Most hospitals are directly connected to a sewer and no pre-treatment takes place. Also nursing homes are significant point sources of some specific pharmaceuticals.

8.3 Surface water

To be able to describe negative effects of pharmaceuticals on the on the aquatic organisms a lot of monitoring studies are being performed to determine the concentrations of different pharmaceutical compounds found in various aquatic compartments. Pharmaceuticals are present in surface water in measurable concentrations. Concentrations depend on a type of pharmaceutical and its active compound and aquatic environment compartment and vary roughly from tens to hundreds of nanograms per liter (surface water) to tens of micrograms per liter in raw influent.
Compounds found most often in surface water are:

- almost all X-ray contrast media,
- (few) pain killers
- (few) beta-blockers
- antiepileptics (carbamazepine and primidon)
- antibiotics
- anaesthetics

Prevalence and concentrations found in the Netherlands are not different than those found in German (or other European) studies.
In Table 8.2 the maximum concentrations of human pharmaceuticals are given in ng/l (Boxall, 2004).

Table 8.2. Pharmaceuticals detected in surface water monitoring studies; (Boxall 2004), (Daughton, 1999), (Kolpin DW 2002), (Boxall, 2004a).

Pharmaceutical group	**Substance**	**Max concentration (ng/l)**
Antibiotics	Chloramphenicol	355
	Chlortetracycline	690
	Ciprofloxacin	30
	Lincomycin	730
	Norfloxacin	120
	Oxytetracycline	340
	Roxithromycin	180
	Sulphadimethoxine	60
	Sulphamethazine	220
	Sulphamethizole	130
	Sulphamethoxazole	1,900
	Tetracycline	110
	Trimethoprim	710
	Tylosin	280
Analgesic	Codeine	1,000
	Acetylsalicylic acid	340
	Carbamazepine	1,100
	Diclofenac	1,200
	Aminopyrine	340
	Indomethacine	200
	Ketoprofen	120
	Naproxen	390
	Phenazone	950
Antianginal	Dehydronifedipine	30
Antihypertensive	Diltiazem	49
Antidepressant	Fluoxetine	12
Antihyperlipidemic	Gemfibrozil	790
Antidiabetic	Metformin	150
Antipyretic	Acetaminophen (Paracetamol)	10,000
Anti-inflammatory	Ibuprofen	3,400
Beta blockers	Betaxolol	28
	Bisoprolol	2,900
	Carazolol	110
	Metoprolol	2,200
	Propanolol	590
	Timolol	10
Bronchodilator	Clenbuterol	50
	Fenoterol	61
	Salbutamol	35

Contraceptive	17a-Ethinylestradiol	4.3
Lipid regulator	Bezafibrate Clofibrate Gemfibrozil	3,100 40 510
X-ray contrast media	Diatrizoate	100,000

8.4 Ground water

Sacher (2001) analysed 105 ground water wells and in one third of tested ground water samples (39) pharmaceuticals from groups beta-blockers, analgesics, antiepileptics, antirheumatics, antibiotics, iodinated X-ray contrast media could be detected. Carbamazepine was detected in ground water sample up to 1,1 µg.l^{-1} (Ternes, 2001) . In a German monitoring program 32 bank filtration samples from 22 surface water were measured; sulfamethoxazole was found at concentrations up to 0.079 µg.l^{-1} and diatrizoate up to 1.4 µg.l^{-1} (BLAC, 2003). The highest concentrations for ground water samples were found for iodinated contrast media iopamidol, up to 2,4 µg.l^{-1} (Ternes and Hirsch 2000).Of other pharmaceutical compounds, analgesics phenanzone, propyphenanzone and dimethylaminophenazone (Reddersen, Heberer et al. 2002), lipid regulators gemfibrozil were detected in ng range (Daughton 2001).

8.5 Drinking water

Due to a specific situation with water resources around of city Berlin, some pharmaceutical compounds were detected in drinking water samples: clofibric acid (270 ng.l^{-1}), diclofenac, propylphenazone, ibuprofen. Several compounds were detected in raw drinking water samples in San Diego county, California – clofibric acid, ibuprofen, ibuprofen methyl ester (Loraine and Pettigrove 2006).

8.6 Sewage sludge

Some antibiotics were detected in sewage sludge, fluoroquinolones, ciprofloxacin, norfloxacin (Golet, Strehler et al. 2002). Recently other antimicrobials, sulfapyridine, sulfamethoxazole, trimethoprim, azithromycin, clarithromycin and roxithromycin in sewage sludge were detected in activated sludge up to 0.20 mg.kg^{-1} of dry matter (Gobel, Thomsen et al. 2005). From neutral and acidic drugs only diclofenac was quantified above the limit of quantification (0.2 – 0.45 mg.kg^{-1}) (Ternes 2005).

8.7 Predicted environmental concentrations

Predicted (environmental) concentrations is often calculated under the following assumptions:

- all sold pharmaceuticals are used in the same year
- the pharmaceuticals are released to the sewer
- there is no elimination in man or the sewerage system
- the use pattern is evenly distributed temporally and spatially.

This is a worst-case estimate of predicted environmental concentration (PEC) for the surface water where removal in man is not encountered, is calculated as follows:

$$PEC_{surfacewater} = \frac{Consumption(g/1000inh./day)}{V_{ww}(m^3/1000inhab.day)\cdot D} \ (g/m^3)$$

Predicted environmental concentration in surface water, taking into account human metabolism:

$$PEC_w = \frac{A \cdot (100 - R)}{365 \cdot P \cdot V \cdot D \cdot 100}$$

where:
A – the amount of pharmaceutical (active substance) used per year (kg/y)
R – removal (%) in man and sewer
P – numbers of inhabitants
V – volume of wastewater per capita (m^3/d)
D – dilution factor in the environment (10 often used) (Stuer-Lauridsena 2000).

Examples
Fibrates/β-blockers: concentration of metoprolol in concentrated wastewater streams: DDD = 200 mg/person/d (Ruiz 1997), excretion of a parent compound E = 5%, Volume undiluted urine: 1.5 L, **EC_{urine} = 6,67mg/L,** in black water collected with vacuum: V_{BW}=7.5 L, **PEC_{BW} = 1.3 mg/L.**

Table 8.3: Calculated concentration of selected pharmaceutical compounds in concentrated wastewater (undiluted urine, black water collected with vacuum toilets)

	DDD (Ruiz 1997), mg (E, %)	Concentration parent compound undiluted urine (V=1.5 L), mg/L	Concentration concentrated black water (V=7.5L)	Quantities used in the Netherlands, kg/y
metoprolol	200 (5%)	6,67	1.3	16200 (RECETO) 2.354.000 prescriptions
propanolol	160 (1% parent compound, 90% metabolite)	1,06	0.21	
atenolol	100			
sotalol	300 (95% receto)	190	38	74,28

9 Selected pharmaceutical compounds

From the highlighted groups in Table 7.1 a number of specific compounds was selected mainly based on their consumption, occurrence in the environment and behaviour in a STP. These are: diazepam, oxazepam, temazepam, metoprolol, gemfibrozil, diclofenac, naproxen, ibuprofen, carbamazepine (CVZ 2006). In Figure 9.1 a consumption of these specific compounds is shown.

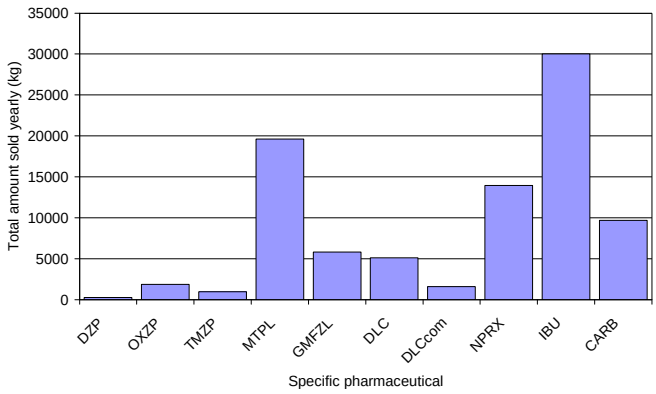
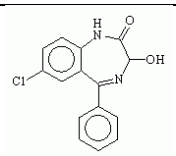
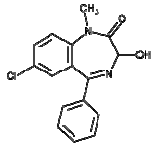
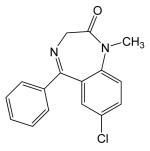
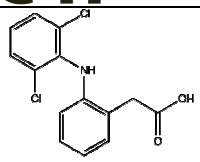
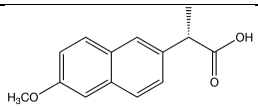
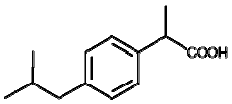
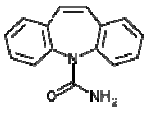
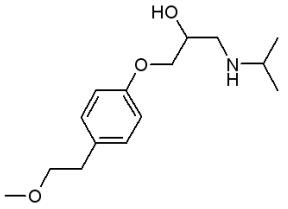
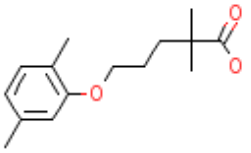


Figure 9.1: Total consumption (assumed that sold is consumed) of some specific pharmaceuticals in the Netherlands (source CVZ, 2006). DZP – diazepam, OXZP – oxazepam, TMZP – temazepam, MTPL – metoprolol, GMFZL – gemfibrozil, DLC – diclofenac, DLCcom – diclofenac combined, NPRX – naproxen, IBU – ibuprofen, CARB – carbamazepine (CVZ 2006)

Detailed chemical-physical characteristics of selected compounds is given in Table 9.1.

Table 9.1: Characteristic of selected compounds (CVZ 2006), (WHO 2006)

No, ATC group	**Structure**	**Compound, formula, molecular weight, logKow value, excretion**
1 N05BA04 anxiolytic		Oxazepam $C_{15}H_{11}ClN_2O_2$ 286,713 g/m **DDD = 50 mg/p/d** Elimination with urine as glucuronide conjugate
2 N05CD07 Hypnotic sedative		Temazepam, $C_{16}H_{13}ClN_2O_2$ 300,7 g/m **DDD = 20 mg/p/d** Elimination 80% with urine as metabolite, 12% with faeces.
3 N05BA04 anxiolytic		Diazepam $C_{16}H_{13}ClN_2O$ 284.7 g/m Log Kow = 2.82 (2.7) **DDD = 10 mg/p/day** Elimination as oxazepam

4 M01AB05 Antiinflamatory antirheumatic non steroids		Diclofenac $C_{14}H_{11}ClN_2O_2$ 286,713 g/mol logKow = 0.7 or 4,5 (acidic pH) **DDD = 100 mg/p/d** Elimination as metabolites, ca. 60% with urine, the rest with faeces.
5 M01AE02 Antiinflamatory antirheumatic non steroids		Naproxen $C_{14}H_{14}O_3$ 230,259 g/mol **DDD = 500 mg/p/d** Elimination with urine; 95%, mainly conjugated, 10% as a parent compound
6 M01AE01 Antiinflamatory antirheumatic non steroids		Ibuprofen $C_{16}H_{13}ClN_2O$ 206.3 g/mol **DDD = 1200 mg/p/d** Elimination with urine mainly as metabolites.
7 N03AF01 antiepileptic		Carbamazepine $C_{15}H_{12}N_2O$ 236.27 g/mol **DDD = 1000 mg/p/d** Elimination mainly as metabolites; ca. 70% with urine and 30% with faeces
8 C07AB02 Beta blocker		Metoprolol $C_{15}H_{25}NO_3$ beta1 receptor blocker 267,364 g/mol **DDD = 150 mg/p/d** Elimination with urine, 5% as a parent compound.
9 C10AB04 Lipid modifying agent plain		Gemfibrozil $C_{16}H_{13}ClN_2O_2$ 250,333 g/mol **DDD=1200 mg/p/d** Elimination with urine, 70% of which 5% as a parent compound

10 Transformation of pharmaceuticals

In this chapter general aspects of various transformation routes of organic (micro)pollutants is described. Further attention is mainly paid on four representative compounds: carbamazepine, diclofenac, ibuprofen and metoprolol.

10.1 Biodegradation

Biodegradation (in STP, natural aquatic systems, soil, sediments) is the most important process resulting in transformation (structural changes) of organic compounds. Biotransformation can vary from partial transformation to complete mineralisation. Organic compounds are used as energy source and for growth of microorganisms. Biodegradation of some compounds takes place without any gain of energy. In this case presence of another compounds providing energy is necessary (co-metabolism). Co-metabolism is important for biodegradation of pharmaceuticals in STP or another environmental compartment because they are present in relatively low concentrations when compared to other organic compounds.
During biotransformation metabolites are produced being more stable that the parent compound. An example can be clofibric acid, often described in literature, metabolite of clofibrate – lipid lowering agent. Character of metabolites may be different from the original compounds in terms of toxicity and fate in the environment. The same may count for conjugates. It is expected that the conjugated compounds present in the environment are usually converted back into the original compound (Williams 2005).

There is a potential for biological degradation of the pharmaceutical parent compounds and their metabolites. Some biodegradation may already occur during in-pipe transport to the STP but most will probably occur in the secondary stage of treatment when the compound is exposed to large concentration of microorganisms.
Biological degradation rates show big differences from one to another compound. These differences seem not to be dependent only on the molecular structure or quantitative structure. (This is why degradation rate of each compound should be determined by experiments). According to (Ternes and von Gunten 2005) the degradation rate of pharmaceutical compounds can be identified by pseudo first order reaction (eq 10.1):

$$\frac{dC_i}{dt} = k_{i,biol} \cdot SS \cdot C_i \quad (\text{L.gSS}^{-1}.\text{d}^{-1}) \qquad \text{(eq 10.1)}$$

where:
C_i soluble substance concentration of the compound i inside the reactor [µg/L]
$k_{i,biol}$ kinetic constant for pseudo first order degradation [L/gSS.d]
SS suspended solids (biomass) concentration [gSS/L]

An attempt was undertaken to find a relation between removal capacity and kinetic degradation constant, $k_{i,biol}$, of the pharmaceuticals based on aerobic batch experiments, leading to the following (Ternes and von Gunten 2005):

- $k_{i,biol} < 0.1$ [L gSS^{-1} d^{-1}]: no substantial removal due to biological degradation
- $0{,}1 < k_{i,biol} < 10$: degree of removal strongly dependent on reactor configuration
- $k_{i,biol} > 10$: more than 95% removal by biological degradation

Estimated, based on batch test, degradation constants, $k_{i,biol}$, for many pharmaceutical compounds are shown in Figure 10.1. The thick horizontal line emphasizes the minimum $k_{i,biol}$ (0.1 L/gSS.d) required for any degree of degradation to occur. From selected previously compounds ibuprofen is the only one which can be potentially significantly degraded in a STP. No data for $k_{i,biol}$ of metoprolol is available in literature.

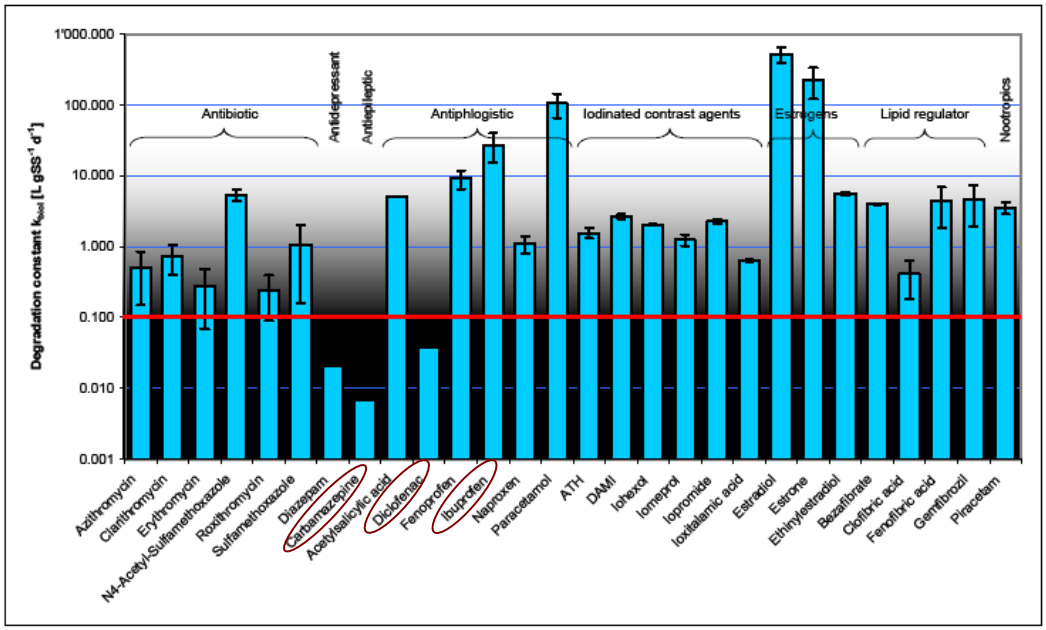


Figure 10.1: Biological pseudo first order degradation rate constants k_{biol} for a number of pharmaceutical compounds estimated based on aerobic batch experiments with activated sludge, SRT≥8d. Thick horizontal line shows the limit below which there is no significant biodegradation is expected; after (Ternes and von Gunten 2005).

In literature there is often suggested that SRT may be a crucial parameter determining efficiency of pharmaceuticals removal (Ternes and von Gunten 2005). The expected effluent concentration of a given compound can be modelled in a following way:

$$\frac{C_{i,out}}{C_{i,in}} = e^{-k_{i,biol} \cdot SS \cdot HRT} = e^{-k_{i,biol} \cdot SP \cdot SRT} \qquad \text{(eq 10.2)}$$

where:

$C_{i,in}$: influent substance concentration of the compound i [µg L^{-1}]

$C_{i,out}$: final substance concentration of the compound i [µg L^{-1}]

HRT: hydraulic retention time of the whole reactor or duration of the batch [d]

SP: specific sludge production per amount of wastewater treated [gSS $m^{-3}_{wastewater}$]

SRT: sludge age [d]

Based on above considerations the following behaviour of the selected compounds can be expected in a biological part of a STP system:

Carbamazepine:	$k_{i,biol}$ < 0.1 [L gSS^{-1} d^{-1}],	no substantial removal is expected
Diclofenac:	$k_{i,biol}$: (0.25±0.2)	15-40% removal, mainly in aerated compartment, at ≥ 2 days SRT
Ibuprofen:	$k_{i,biol}$: (23±10)	>90% biological removal, mainly in aerobic reactor at ≥ 5 days SRT.
Metoprolol:		No data was found in the available literature about $k_{i,biol}$ for Metoprolol.

10.2 Sorption onto sludge

Sorption onto particulate matter is an important removal mechanism when the tendency of organic compounds to partition onto the primary or secondary sludge is high (Ternes 2006). For sorption of the organic compounds onto particulate matter two mechanisms are assumed to be relevant for sorption onto particulate matter:

- absorption – hydrophobic interactions of the aliphatic and aromatic groups of the compounds with the lipophilic cell membrane of the microorganisms and lipid fraction of the sludge
- adsorption – electrostatic interaction of positively charged groups of chemicals with the negatively charged surfaces of the micro-organisms.

Removal by sorption onto suspended solids is an important mechanism for hydrophobic and positively charged compounds (Figure 10.2).

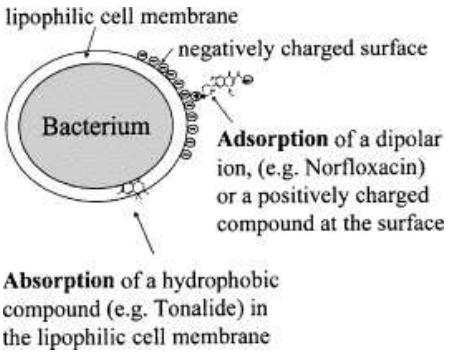


Figure 10.2: Absorption and adsorption mechanisms of organic compounds on the sludge; (Larsen et al, 2004, Schwarzenbach et al., 2003) after Golet et al, 2002). (Tonalide; personal care products, Norfloxacin; antibacterial)

A sorption coefficient, K_d, describes the solid liquid partitioning characteristics of a compound in sorption mechanism. The concentration of a compound sorbed onto the sludge during wastewater treatment is assumed to be proportional to the concentration of the same compound in the solution (equation 10.3 (Ternes and von Gunten 2005)):

$$C_{i,sorbed} = K_{d,i} \cdot SS \cdot C_{i,soluble} \qquad \text{(eq 10.3)}$$

where:
$C_{i,sorbed}$ the particulate concentration of a compound *i*;

$K_{d,i}$ the sorption constant of a compound *i*;
SS suspended solids concentration in wastewater or production suspended solids in primary or secondary treatment, per L of treated wastewater [kg $L^{-1}_{wastewater}$]
S_i the soluble concentration of a compound *i*;

Only compounds having K_d values higher than 500 L.kgSS^{-1} will be sorbed significantly onto the sludge. Hydrophobic compounds and positively charged ionic substances have high K_d values enough to be sorbed onto the sludge (Ternes et al, 2005).
Consequently concerning the elimination of pollutants from the water phase of municipal wastewater, sorption can be neglected for compounds with a $K_d \leq 500$ L.kgSS^{-1} . Above this value substances can partition significantly (> 10%) onto the sludge. From pharmaceutical group of micropollutants diclofenac (hydrophobic substances) and norfloxacin (antibiotic from fluoroquinolones group; positively charged ionic substances) are examples of compounds, which should sorb to the particles during the STP process.

In Table 10.1, the K_d values of the compounds are given for primary and secondary sludge. It can be stated that very low K_d values for Carbamazepine and Ibuprofen show that sorption plays no significant role for removal of these compounds in WWTPs. Only Diclofenac has higher K_d values (almost 500 LkgSS^{-1}) in primary sludge. The sorption potential of Diclofenac is relevantly higher than the Carbamazepine and Ibuprofen (Ternes et al, 2004a).

Table 10.1: Kd, solid-water distribution coefficient of the selected compounds (Ternes et al, 2004a)

Compounds	Kd (Primary Sludge) (L/kg)	Kd (Secondary Sludge) (L/kg)
Carbamazepine	-(<20)	1.2±0.5
Diclofenac	459±32	16±3
Ibuprofen	-(<20)	7.1±2.0
Metoprolol		

Regarding mainly hydrophobic interactions, the octanol-water partition coefficient, K_{ow}, or the partitioning coefficient to particulate organic matter, K_{oc} can be used to estimate the sorption constant $K_{d,i}$ ($1g_{ss}^{-1}$) (Ternes 2005).
Analagously to K_d value, high K_{ow} values for compounds show that they can sorb to the sludge while low values indicate that the compounds generally stay in the aquatic phase (Jones et al, 2005, Rogers et al, 1996):

Log K_{ow} < 2.5	Low sorption potential
Log K_{ow} > 2.5 but < 4.0	Medium sorption potential
Log Kow > 4.0	High sorption potential

There are ongoing studies to prove the relation between K_{ow} and K_d for the pharmaceutical compounds (Tolls, 2001). The relation between the Log K_{ow} value of some example compounds and their sorption affinity is given in Table 10.2.

Table 10.2: Log Kow values of the representative compounds and their sorption potential

Pharmaceutical	Log K_{ow} value	Sorption affinty
Carbamazepine	2.45 [a]	Low
Diclofenac	0.70 [a], 4.6 [b]	Low, High
Ibuprofen	3.97 [a]	Medium
Metoprolol	1.88	Low

[a] Yoon et al, 2006, [b] Hansch et al, 1995

For diclofenac conflicting K_{ow} values were found in literature. Considering the high K_d value and high K_{ow} values from some of the literature, it can be concluded that Diclofenac is considered as a hydrophobic and positively charged compound and has the highest ability to be sorped onto suspended solids when comparing to other compounds. Regarding the K_d and K_{ow} values, the sorption mechanism is not relevant for carbamazepine. For Ibuprofen K_d and K_{ow} values are conflicting with each other. K_d value shows that Ibuprofen is not a compound which has sorption potential whereas Kow value indicates medium sorption potential in sludge. Only available value for Metoprolol is K_{ow} and it shows that sorption is not a removal mechanism for metoprolol.

10.3 Stripping

Stripping (volatilisation) during wastewater treatment is the transferring a compound from the aqueous to gaseous phase (Ternes et al, 2006). Stripping process is dominant in the aerobic part of the treatment plant where there is an intensive aeration of the activated sludge mixture (Ternes et al, 2005, 2006). Volitisation depends on several factors according to the equation 10.4 [Ternes et al, 2005]:

$$K_{i,H} = \frac{p_i}{C_{i,soluble} \cdot R \cdot T} \qquad \text{(eq 10.4)}$$

where:
$K_{i,H}$: Henry or air water partitioning coefficient of the compound i
p_i : partial pressure in the gas phase [Pa]
R: universal gas constant; 8.314 [J /Mol.K]
T: temperature [K]
$C_{i,\ soluble}$: soluble concentration of the compound i [µg/L]

When H> 0.003 a observe significant amount of a compound will be stripped in a bioreactor with a fine bubble aeration (Ternes et al, 2006). The values of Henry coefficient for representative compounds are listed in Table 10.3.

Table 10.3: Henry coefficients of the representative compounds and their stripping (volatilisation) potential

Pharmaceutical	Henry Coefficient	Stripping potential
Carbamazepine	1.08E-10 [a]	no
Diclofenac	4.73E-12 [a]	no
Ibuprofen	1.5E-07 [a]	no
Metoprolol	1.4E-13 [a]	no

[a] http://chem.sis.nlm.nih.gov/chemidplus/

Pharmaceuticals expose a fairly good solubility and therefore low gas-water-partitioning coefficient. This is confirmed by generally very small Henry coefficients (below 0.005) (Larsen et al, 2004, Schwarzenbach et al, 2003) showing that stripping process is not relevant for their removal from the wastewater (Larsen et al, 2004).

10.4 Chemical oxidation

Chemical oxidation seems to be very an efficient mechanism to remove (transform) pharmaceuticals from the biologically treated wastewater. Ozonation and Advanced Oxidation Processes (AOPs) seem to be two of the promising techniques (Ternes et al, 2005, Ternes et al, 2003, Zwiener et al, 2000, Huber et al, 2003, Ternes et al, 2002).

Second order rate constants (k_{O3}) of the ozonation process for representative pharmaceutical compounds were determined in bench-scale experiments. The k_{O3} values above $5x10^4$ $M^{-1}s^{-1}$ indicate that the respective compound can be **transformed** during the ozonation process very efficiently. Next to the reaction with ozone, reaction with hydroxyl radicals (OH) also plays a role in oxidation of micropollutants. This is why a second order rate constants (k_{OH}) for the reaction of OH radicals with the representative pharmaceuticals were calculated as well (Ternes et al, 2004 (POSEIDON), Huber et al, 2003). The k_{O3} and k_{OH} values are given in Table 10.4 for the representative compounds.
Chlorination with chloride dioxide is an alternative chemical oxidation method for pharmaceutical compounds. To be able to predict the ability of the pharmaceutical compounds to be oxidized, second order rate constants for the reaction of chlorine dioxide (k_{ClO2}) were determined in bench-scale experiments.

Table 10.4: Second order rate constants for the reaction of ozone and hydroxyl radicals and chlorine dioxide (Ternes et al, 2004, Huber et al, 2003).

Compound	k_{O3} (T=20°C) ($M^{-1}s^{-1}$)	Reactive Species	OH Generation	k_{OH} (10^9 $M^{-1}s^{-1}$)	k_{ClO2} ($M^{-1}s^{-1}$)
Carbamazepine	$3E10^5$	Neutral	UV/H_2O_2	8.8±1.2	<0.015
Diclofenac	$1E10^6$	Dissociated	γ - radiolysis	7.5±1.5	$1.05E10^4$
Ibuprofen	9.6±1	Dissociated	UV/H_2O_2	7.4±1.2	<0.01
Beta Blockers (Metoprolol)	$1\text{-}10E10^3$ [a]				

[a] estimated value

Considering the k_{O3} values of the representative compounds, it can be concluded that Carbamazepine and Diclofenac can be easily chemically oxidized by the reaction of ozone. On the other hand it can be concluded that Ibuprofen in addition to carbamazepine and diclofenac can be oxidized by the reaction of hydroxyl radicals.

11 Transformation of pharmaceuticals during treatment

11.1 General in a STP

For many of the pharmaceuticals removal by conventional biological treatments seems inefficient, since they are found in significant amounts in STP effluents and surface water. For instance, carbamazepine is described as a persistent compound, not degraded or adsorbed during wastewater treatments (Clara 2004) or only barely degraded (Ternes 1998). However, for other compounds such as clofibric acid and bezafibrate, removal can reach 34-51% and 50-83%, respectively (Ternes 1998) , (Clara 2004), while up to 90% of ibuprofen is apparently removed (Ternes 1998). The removal rate of pharmaceuticals in STPs can therefore vary and is potentially affected by several factors, such as the nature of the pharmaceutical, the treatment process employed, the age of the activated sludge, the environmental conditions such as the temperature and the light intensity, and the characteristics of the influent (Carballa and Carmen Garcıa-Jares 2004) , (O'Brien E. and Dietrich 2004).
In literature there are discussions taking place on possible importance of several parameters of configurations within biological system having possible impact on removal rate of pharmaceutical compounds. The most important are:

- **sludge age,** supposed to have an impact on the specific degradation activity in three independent ways, influencing: (1) the biodiversity, (2) inert material content in the sludge, (3) sludge production;

  For several compounds it was found that a minimal sludge age exists, beyond which a partial of total removal by degradation occurs. The palette of chemical structures broadens with increasing sludge age. Significant difference is seen between COD and nutrient removing plants. Highly loaded plants (SRT – 1-4 d) none or only slight removal of pharmaceuticals is observed.

- **hydraulic retention time and wastewater dilution;** dilution should be avoided (in the sewer, infiltration) and biological treatment should be located as close as possible to the source of emission.
- **reactor configuration; e.g. cascades** – number of compartments in series significantly increases the biological removal for all compounds; **MBR** – unclear whether it is advantegous above conventional systems, possibly comparable to conventional activated sludge (AS); performs better when higher ages are required (MBR = 20-50 d). On the other hand smaller sludge particles characterising MBR are in advantage of better k_{biol} – less diffusion limitation; Many authors postulate that MBR should not be associated with better removal; b**iofilter** – lower HRT compensates for higher bioactivity, since sludge has ´infinite´ SRT (higher biodiversity).

RIZA (2002) formulated a sequence of removal of pharmaceuticals in the conventional treatment:
- pain killers are considered to be well removed (up to 95%)
- anti-epileptics, beta-blockers, cholesterol lowering agents (vary between 10-80%)
- antibiotics (< 25%)
- X-ray contrast media as the most persistent (< 10%).

To give an idea on different behaviour of different pharmaceutical compounds in biological system examples are given in Table 11.1.

Table 11.1: Examples of good and poor degradable pharmaceutical compounds

Well (better) removed	Bad (only little) or not removed	Source, methods, comments
penicillin G (27% after 28 days, 35% after 40 days)	Antibiotics (cefotiam, ciprofloxacin, meropenem, sulfamethoxazole	(Al-Ahmad 1999) Closed Bottle Test with low bacterial density and low (relative) carbon concentrations (high in comparison with real (sewage) conditions (µg/mL)
5-fluorouracil (not in any tests),	Anti-tumor agents:, cytarabine (50% CBT, 50% in ZWT after adaptation of 20 days, 95% after , gemcitabine (42% CBT, 80% after 40 days)	Kummerer et al., 1997 CBT and Zahn-Wellens test (ZWT)
Paracetamol biodegradable but to lesser extent than salicylic acid	methotrexate (neoplastic diseases, severe psoriasis, and adult rheumatoid arthritis) clofibric acid (not biodegradable)	Henschel et al., 1997
Acetaminophen (paracetamol), acetylsalycilic acid (ASA)	0.22 µg/L in sewage effluent (AS) as a pro-drug is easily degraded into its more active form salicylic acid and two metabolites ortho-hydroxyhippuric acid and the hydroxylated metabolite gentisic acid.	(Heberer 2002) (Ternes 1998)
salicylic acid and two metabolites ortho-hydroxyhippuric acid and the hydroxylated metabolite gentisic acid.	Detected in influent STP 54, 6.8 and 4,6 µg/L resp. all three compounds efficiently removed, in MSTP only salicylic acid detected (low) in effluent and rivers (salicylic acid comes also from other sources)	(Ternes 1998b)
	Clinically important antibiotics: ciprofloxacin, ofloxacin, metronidazole	(Kümmerer 2000)
	X-ray contrast media not significantly removed in a STP	Ternes et al., 2000
Ibuprofen	66-93% (mean 79%) PS = 32% Retention time 15-20 days (AS+PS)	(Tauxe-Wuersch 2006 (in press))
Some degradation of iopamidol, 85% transformed to two metabolites		(Kalsch 1999)
Clofibric acid	None removal in short retention AS and/or BF[1)]	(Tauxe-Wuersch 2006 (in press))
Dichlofenac	A significant sorption and an efficient attenuation of dichlofenac residues in the subsoil	(Heberer)
	One of the most important PhAC present in the water-cycle; generally reported as persistent Possible photodegradation	(Heberer, Reddersen et al. 2002) A lot of information on occurrence in environment
	Removed (from drinking water) by ozonation	Zwiener and Frimmel (2000)
	Efficiently removed from surface or municipal sewage effluent by membrane filtration	(Heberer, Reddersen et al. 2002)

[1)] Biological filtration=BF; PS = primary sedimentation; AS = activated sludge;

The fate of the pharmaceutical compounds in STPs are being investigated since the last decade. In a STP near Frankfurt/Main in Germany, the elimination of different pharmaceuticals was investigated during passage through a conventional (pre-settling, aeration with simultaneous P precipitation and

secondary settling). Since sorption was neglected, a differentiation could not be made between fraction absorbed and mineralised (or transformed). The removal efficiencies of a number of compounds is given in Figure 11.1. Ibuprofen, diclofenac and metoprolol were eliminated from the water phase in a relatively high rates of 90, 69 and 83 %, respectively while carbamazepine was removed only 7% (Ternes 1998).

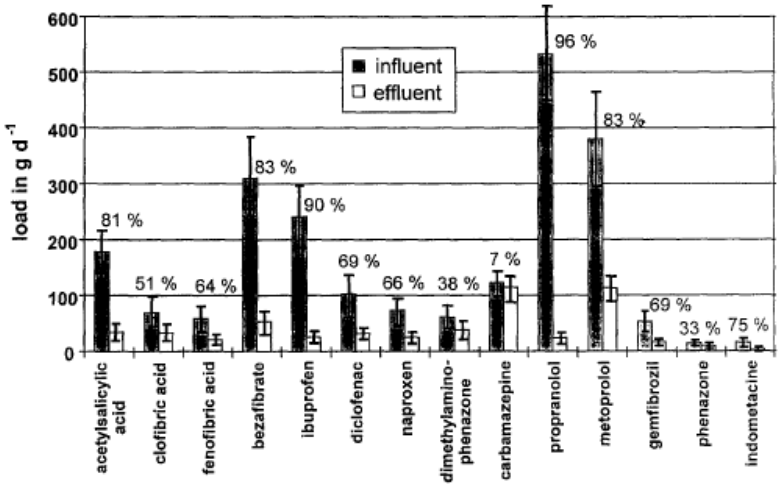
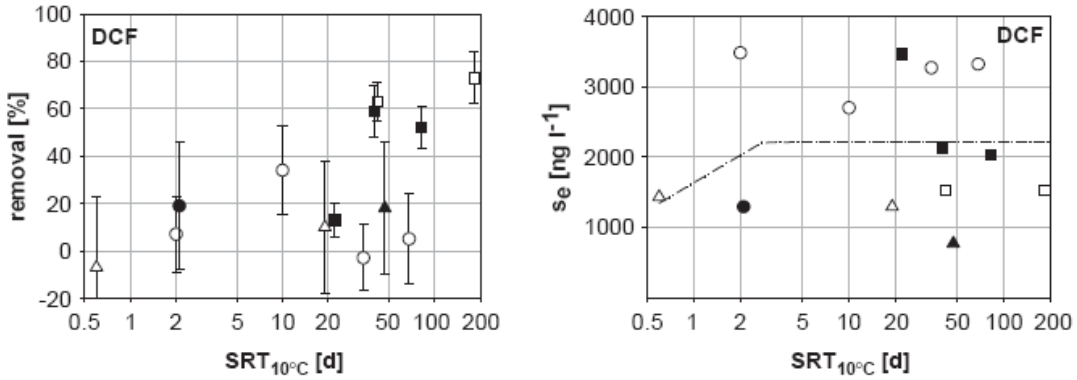


Figure 11.1: Elimination of different pharmaceuticals in a German conventional municipal STP (Ternes 1998)

Higher removal efficiencies for carbamazepine were found by (Miao 2005) – 29% over different treatment units of the wastewater treatment plant in Canada. No significant removal for its metabolites was detected.
In Italy, removal efficiencies of different pharmaceuticals in six different STPs were investigated. No significant removal of carbamazepine was detected in all STPs. Ibuprofen removal differed between winter and summer period between 38% and 93 % respectively (Castiglioni 2006).
In other research performed at six full scale STPs from different countries (activated sludge secondary treatment with chlorination, HRT 24-48 h) 87 % removal efficiency for metoprolol was obtained as average (Huggett, Khan et al. 2003).
Studies on the influence of SRT on the removal capacity of pharmaceuticals in full scale STPs indicated that SRT does not determine the biodegradation. Different plant configurations with primary treatment, activated sludge, anaerobic sludge digestion and trickling filter were operated (Clara, Strenn et al. 2004). In other study performed by (Clara 2005) again differently configured full scale wastewater treatment plants were operated and the effect of SRT on the removal rates of different pharmaceuticals was investigated. Only the results for the representative compounds are given in this section.

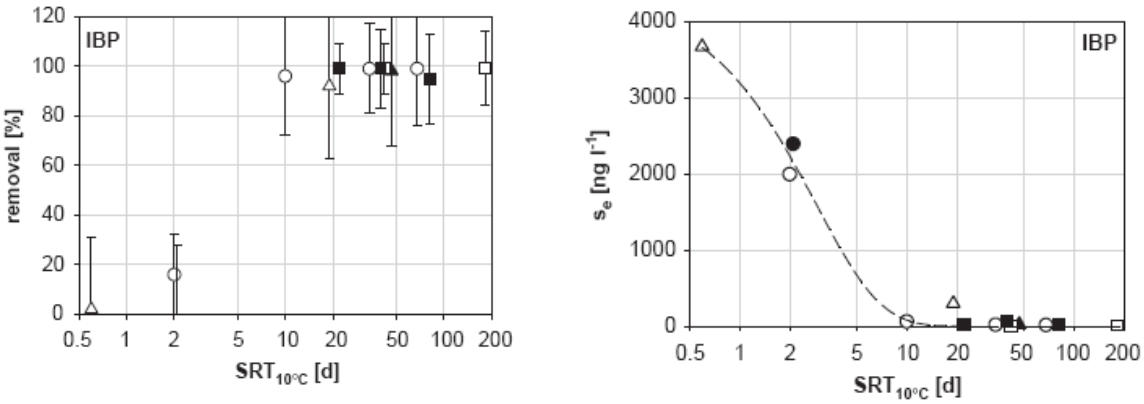
Carbamazepine removal was unaffected by SRT during the treatment and no significant removal for carbamazepine was observed in none of the treatment configurations. For diclofenac SRT was an important factor influencing the removal rate as it is shown in Figure 11.2, where different removal rates were obtained in different treatment facilities. No clear correlation could be derived between the removal efficiency and the SRT values and no critical SRT value was identified.



A B

Figure 11.2: (A) Removal efficiencies for diclofenac with different SRT values. (B) The changes in effluent concentration of diclofenac according to the different SRT values (● LP1-LP4; LP: Lab-scale experiments, ○ WWTP 1, ▲ WWTP 2, Δ WWTP 3, ■ WWTP 4, □ WWTP 5) (Clara 2005)

In the same study high removal of Ibuprofen was observed, more than 95 % (Figure 11.3). Critical SRT value for ibuprofen was stated as 5 days. No significant differences in removal rates could be observed between conventional activated sludge and membrane bioreactor systems.



A B

Figure 11.3: (A) Removal efficiencies for Ibuprofen in relation to SRT; (B) Changes in effluent concentration of Ibuprofen in relation to SRT values (Clara 2005) (● LP1-LP4; LP: Lab-scale experiments, ○ WWTP 1, ▲ WWTP 2, Δ WWTP 3, ■ WWTP 4, □ WWTP 5)

Conventional activated sludge, membrane bioreactor and fixed-bed reactor were compared according to their removal efficiencies for some selected compounds (Joss 2005). Similar performances were obtained for carbamazepine, diclofenac and ibuprofen in all three treatment systems. Again no significant removal for carbamazepine was for any applied SRT, temperature and configuration (Figure 11.4).

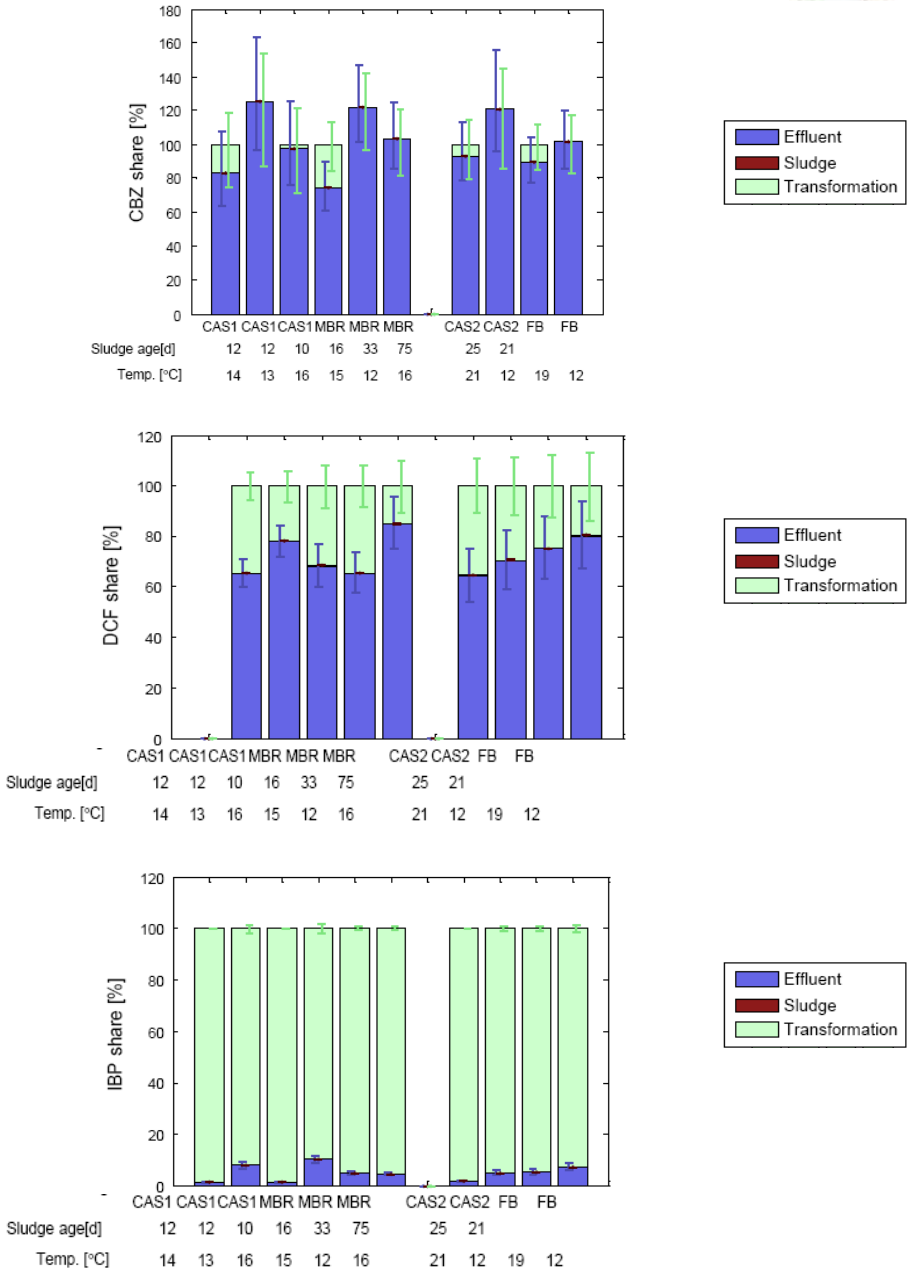


Figure 11.4: Removal of pharmaceutical compounds in full scale conventional activated sludge, membrane bioreactor and fixed bed reactor systems (Joss 2005); CBZ; carbamazepine, DCF; diclofenac, IBP; ibuprofen.

For diclofenac, 20-40% removal capacity was measured; no clear correlation between removal and operational factors could be concluded. Ibuprofen was removed for more than 95%. In the same research, it was also observed that the amount of the pharmaceutical compounds in the effluent of the treatment processes can be sometimes higher than the amount in the influent. Reconjugation of the metabolites into the original compounds during the treatment is suggested to be one of the reasons (Joss 2005).

A conceptual model for Australian sewage was developed by Khan and Ongerth (2003) (Khan 2002) to be able to predict the removal efficiencies of pharmaceuticals in different stages of a WWTP. Fate of 50 pharmaceuticals including the representative compounds was modelled (Table 11.2). The main focus was on a distribution between two removal mechanisms, sorption and biodegradation. According to the model predictions, it was found out that there was no significant removal of the representative compounds either in primary or secondary sedimentation. Aeration tank is the treatment unit where the removal rates are the highest. It should be also added that biodegradation ratios can be lower than the ones presented in the table because of the limited available data gathered from the batch scale studies (Mohle, Kempter et al. 1999).

Table 11.2: Predicted concentrations and removal rates for some pharmaceuticals as obtained in a model of (Khan 2002).

Compound	Influent (µg/l)	Primary Effluent (µg/l)	Aeration tank Effluent (µg/l)	Clarifier effluent (µg/l)	Removal to sludge (%)	Bio Degradation (%)	Total removal (%)
Carbamazepine	2	2	1	1	6	33	39
Diclofenac	0.4	0.4	0.3	0.3	7	24	30
Ibuprofen	1	1	0.6	0.6	4	49	52
Metoprolol (tartrate)	0.09	0.09	0.06	0.05	4	39	42

Summarizing all gathered information, the conventional STPs are not efficient enough to remove many pharmaceutical compounds from the wastewater. Modification in current configuration by e.g. addition of further treatment steps is probably necessary to achieve better removal or even elimination of the pharmaceuticals.

11.2 Per process unit

11.2.1 Pre-treatment:

Screening, grit removal,
Due to a generally poor sorption affinity no significant removal of pharmaceuticals is expected during primary screening. This was confirmed by study of in a study of (Carballa and Carmen Garcıa-Jares 2004) where no significant reduction of ibuprofen was observed during screening process in a STP in Spain (Carballa and Carmen Garcıa-Jares 2004). In the same study there was also no significant reduction of ibuprofen during grit removal process.

Primary Sedimentation
Carballa and Carmen Garcıa-Jares (2004) found also no significant reduction of ibuprofen during primary sedimentation process in a full scale STP. In another study, (Miao 2005) found an unexpected decrease in the concentration of carbamazepine and its main metabolite, CBZ-DiOH after primary treatment (Figure 11.3). The removal efficiency of the primary treatment was stated as high as 46%. This result is contradictory to other studies and the lab-scale experiments performed on the sorption potential of the carbamazepine as mentioned earlier.

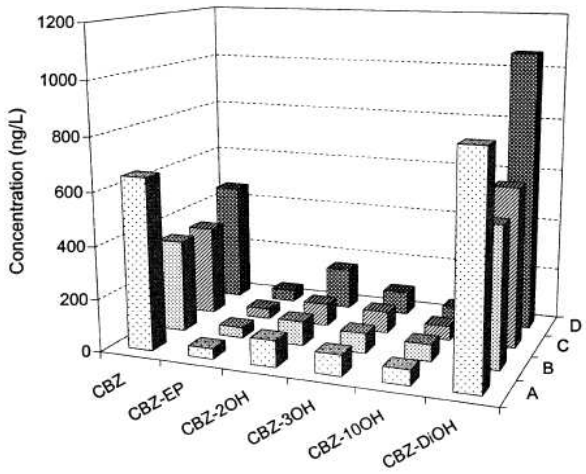


Figure 11.3: Mean concentrations of carbamazepine (CBZ) and its metabolites in the aqueous phase of wastewater in different sampling sites of STP; sampling sites A-raw sewage, B – primary sewage, C – biologically treated sewage, D – tertiary treated sewage (UV) (Miao 2005) .

Based on the K_{ow} value and the sorption potential of the 4 priority compounds, only diclofenac and ibuprofen are expected to be sorbed to the suspended particles. However, there is no literature data available showing any significant removal of representative compounds by sorption mechanism in primary sedimentation.

11.2.2 Physico-chemical processes

Coagulation- Flocculation

Addition of Coagulation-Flocculation and Flotation units was considered in some research as a possibility of a primary treatment to enhance the removal of pharmaceuticals in existing STP. In the research performed by (Carballa 2003), it was stated that the behaviour of acidic and neutral compounds was different during the coagulation-flocculation processes. The higher removal efficiencies (50-70%) of diclofenac were obtained when applying ferric chloride ($FeCl_3$) and aluminium sulphate (Al (SO_4)) as coagulants at 25°. The removal efficiency was not dependent on the dose of the coagulant. No significant removal of Ibuprofen and no effect on carbamazepine were detected in the experiments

Lab-scale coagulation-flocculation process experiment performed within POSEIDON project (Ternes 2004) did not result in significant (if any) removal of carbamazepine, diclofenac and ibuprofen. Similar results were obtained in another pilot scale experiments where it was concluded that flocculation with iron chloride is very inefficient for carbamazepine and diclofenac reduction. The high polarity of the compounds result in nonappreciable sorption quantities (Ternes, Meisenheimer et al. 2002).

Flotation

With flotation process no significant removal of pharmaceutical compounds was obtained in lab scale experiments. A 20 % removal of carbamazepine was stated (Carballa 2003).

11.2.3 Main Treatment

Conventional Activated Sludge

In activated sludge processes in a German municipal STP, 83% of metoprolol was removed (Miege et al, 2006, Ternes, 1998). On the other hand Paxeus, (2005) claims only 10% removal efficiency of metoprolol in activated sludge process. According to Carballa et al (2004), 60-70 % removal efficiency for ibuprofen was obtained. In their study it was suggested that suitable SRT and combining various redox conditions (the anoxic/aerobic) could improve the removal efficiencies. Many investigation have been performed to determine the effects of SRT, HRT and the reactor configuration on the removal capacity of the pharmaceutical compounds.

No significant improvement of the removal capacity was observed by changing HRT by factors of more than 10 for amongst others compounds, carbamazepine, diclofenac and ibuprofen (Joss et al, 2005). The same was observed for different SRT values: although the sludge age was changed between 10 and 60-80, no significant impact is observed on the conversion efficiency of the mentioned compounds. Similar results were gained for diclofenac in the research of Clara (2005). Although in one of the investigated STP, 70% of removal rate for diclofenac was measured, no removal rate was observed in all of the other STPs.

In a test done in a pilot plant by Zwiener and Frimmel (2003), 60% removal was achieved for Ibuprofen; a small fraction of 5% was sorbed to the sludge. Similar to other studies, diclofenac was neither degraded in aeration reactor nor sorbed in the settling tank as expected.

In lab scale tests on the influence of SRT on pharmaceutical compounds removal in activated sludge units no relation could be derived (Figure 11.4). No significant removal of carbamazepine either by degradation or by adsorption was observed (Clara et al, 2004, Clara et al, 2005)). In another study carried out in Berlin, a similar result was reported where there was only 8% removal of carbamazepine (Heberer, 2002).

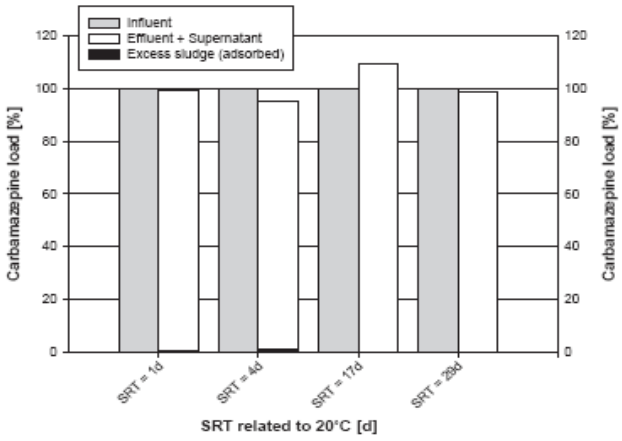


Figure 11.4: STP influent and effluent loading of carbamazepine in relation to SRT (Clara et al, 2004)

In a study made in Brazil, the removal efficiencies pharmaceutical compounds and their metabolites in activated sludge process and biological (trickling) filter were compared. Activated sludge turned out to be more effective process in removing pharmaceuticals and their metabolites than biological filter. A 75 % removal efficiency was obtained in activated sludge process for both ibuprofen and diclofenac whereas the efficiencies in biological filter were only 22% and 9% respectively; Figure 11.5 (Stumpf et al, 1999).

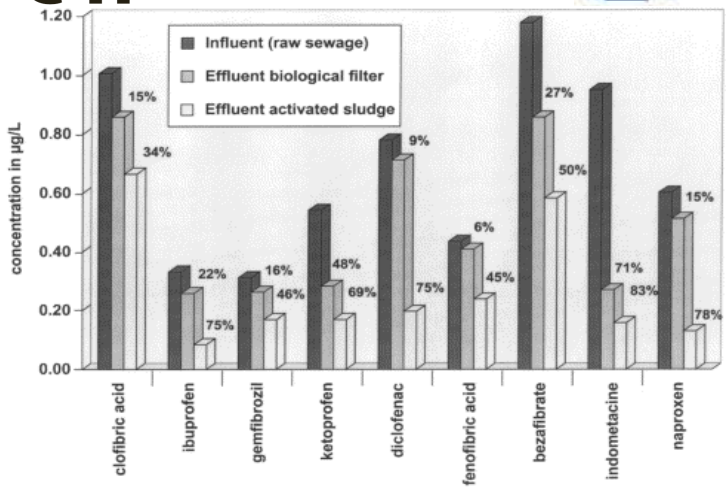


Figure 11.5: Removal of pharmaceutical compounds in STP in Brazil. Removal efficiencies activated sludge of and biological trickling filter (Stumpf et al, 1999).

Summarising activated sludge system is an efficient process unit for ibuprofen characterised by a high degradation constant and medium sorption potential. Diclofenac is removed only partially as a result of biodegradation mechanism while sorption potential of the compound is low. As expected based on the low biodegradation constant and low sorption potential, no significant removal of carbamazepine was observed in all of the activated sludge systems.

Membrane Bioreactor

In the study where the removal efficiencies of activated sludge, MBR and fixed bed reactor were compared no significant differences were observed. Since the molecular size of the compounds are at least 100 times smaller than the pore size of the membranes, it was concluded that micro and ultra filtration membranes can not remove pharmaceutical compounds by sieving. No significant change was observed in the removal capacity by changing HRT by factors of more than 10 for the compounds, carbamazepine, diclofenac and ibuprofen. No significant impact was observed on the removal efficiency of the compounds, carbamazepine, diclofenac and ibuprofen when changing SRT between 10 and 60-80 (Joss et al, 2005).

A study made on the behaviour of ibuprofen during the membrane bioreactor process. During the conversion of ibuprofen in MBR process, two isomers of hydroxyl-ibuprofen were detected. In the effluent of the membrane bioreactor none of these metabolites were detected, and the removal efficiency of ibuprofen and its metabolites was stated as approximately 99% (Quintana et al, 2005). Similar results, >90 % removal efficiency of ibuprofen in MBR were achieved in several studies (Quintana and Reemtsma, 2004, Ternes, 1998, Buser at al, 1999).

Clara (2004) found no influence of SRT on the removal rates of carbamazepine in MBR process; no significant retention of carbamazepine was detected in MBR even when SRT was changed in a range of 10-100 days (Figure 11.6).

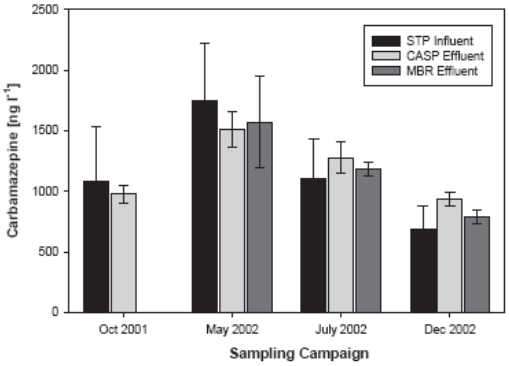


Figure 11.6: Comparison of the concentrations of carbamazepine in the influent and the effluent of the conventional activated sludge plant (CASP) and membrane bioreactor (MBR); SRT was increased between May to Dec 2002, from 10 to 100 days (Clara et al, 2004).

No removal of diclofenac was observed in MBR operated with SRT of approximately 10 days. Partial removal of diclofenac was measured only in one STP among ten investigated; presumably due to the higher SRT value (Clara et al, 2005).
Based on above it can be stated that MBR is not more advantageous over the conventional systems in removing the pharmaceuticals.

SBR
In lab scale SBR tests, the biodegradability and toxicity of atenolol were investigated. Different SRTs were applied to study the influence of the SRT on the removal rates. According to the results, 96 % removal of atenolol was obtained with the highest SRT values. Nitrification inhibition was observed in the presence of atenolol while there is was no negative impact on the removal of other organic substances (Cappai et al, 2004). This study was continued by Carucci&Cappai&Piredda (2006) where contradictory results were obtained: 33.5% removal efficiency was detected in aerobic conditions where in anoxic/aerobic mode the efficiency was slightly higher, 36%. This slight difference shows that addition of anoxic phase at the beginning of the treatment cycle may increase the removal efficiency of atenolol. According to the results shown in Figure 11.7, it can be stated that atenolol removal efficiency increases directly proportional to the increase in sludge age and inversely proportional to the concentration of the compound.

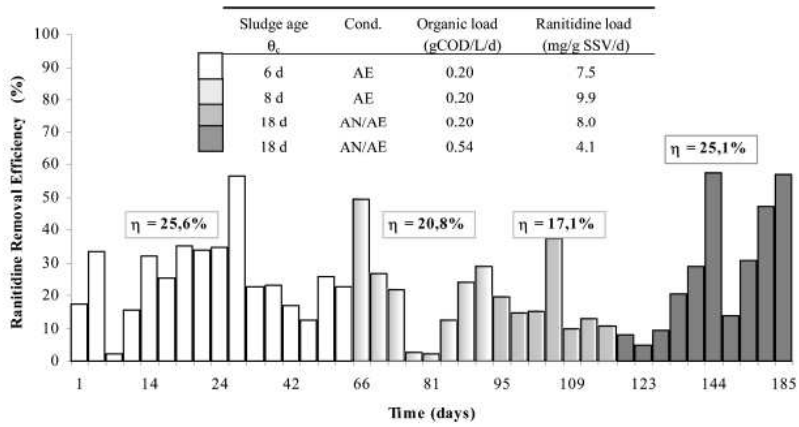


Figure 11.7: Atenolol removal efficiencies during the SBR in lab scale.

Biofilm Reactor

When biofilm reactor was compared with activated sludge, it was found that removal of ibuprofen was higher in oxic biofilm reactor (70%). In biofilm, hydroxyibuprofen was detected as the major metabolite of ibuprofen (Zweiner et al, 2002) but in this study, its concentrations were below 10 % of the degraded amount of ibuprofen. In anoxic conditions, no significant removal was obtained for ibuprofen whereas the removal efficiency of diclofenac was 35% - better than activated sludge process (Zwiener et al, 2003).

11.3 Anaerobic sludge digestion

Little information is in general available on fate of pharmaceuticals during anaerobic digestion. In a research within POSEIDON project, two anaerobic pilot scale reactors, mesophilic and thermophilic were operated. Influents containing different pharmaceutical compounds were fed into the reactors to determine the removal efficiencies. Since the data were considered no to be accurate, it was not sure whether carbamazepine was removed partially or not removed at all. A range for removal was given as high as 0-60 %. Removal of diclofenac could not generally be quantified but in the cases it could be measured the efficiency was changing between 25 and 75 %. For ibuprofen, both reactors gave medium elimination capacity, 20-45 % (Ternes et al, 2005).
In the study of Carballa et al, (submitted), some of the representative compounds were removed in some extent whereas no elimination was observed in carbamazepine (Table 11.3).

Table 11.3: Removal of pharmaceuticals in anaerobic digestion of sludge. (Carballa et al, (submitted).

Compound	Mesophilic	Thermophilic
Carbamazepine	No removal	No removal
Diclofenac	0-75%	25-75%
Ibuprofen	45±15%	47±10%

In another study much higher removal rate for diclofenac was obtained after the adaptation of the sludge Carballa et al, (2006))Table 10.4).

Table 11.4: Removal of pharmaceuticals in anaerobic digestion of sludge (Carballa et al, (2006).

Compound	Mesophilic	Thermophilic
Carbamazepine	No removal	No removal
Diclofenac	60±18 %	73±9 %
Ibuprofen	40±15 %	47±10 %

11.4 Tertiary Treatment

Ozonation

Ozone is an oxidant which is used widely for a treatment of drinking water but also wastewater. It is used for disinfection and oxidation purposes to control taste and odour, decolouration and removal of micropollutants including pharmaceuticals (Ternes et al, 2006; von Gunten, 2003a, Huber et al, 2003). Direct reaction of ozone or $OH^{\cdot}$ radicals is required for the ozonation to occur. OH represents the strongest oxidants in water formed during spontaneous ozone decomposition. Ozone is a very selective oxidant reacting mainly with double bonds, activated aromatic systems and non-protonated amines. Electron donating groups such as amines, conjugated double bonds accelerate the reaction

rate. On the other hand electron withdrawing groups such as alcohol, aldehydes, ketones, iodine and chloride reduce the reaction rates of ozonation.
Chemical oxygen demand of a treated wastewater (generally 15-50 mg COD.l^{-1}) is another important parameter in ozonation process. It is significantly higher than the oxidation equivalents required for pharmaceuticals (Ternes et al, 2006, von Gunten 2003b).

(Ternes et al, 2003, Zwiener et al, 2000, Huber et al, 2003, Ternes et al, 2002) stated that ozonation is a very efficient technique to remove pharmaceuticals in biologically treated wastewater. According to Andreozzi (2002), ozonation is a proper method to eliminate carbamazepine. As a result of a research done in a pilot plant by Ternes et al (2003), ozone dose range between 10-15 mg l^{-1} and 18 minute contact time were sufficient to eliminate pharmaceuticals including carbamazepine, diclofenac, metoprolol and ibuprofen in 90-99% from wastewater (Figure 11.8). Higher ozone requirement compared to other researches can be explained by the presence of high bulk organics in the effluent, 30 mg.l^{-1} COD.

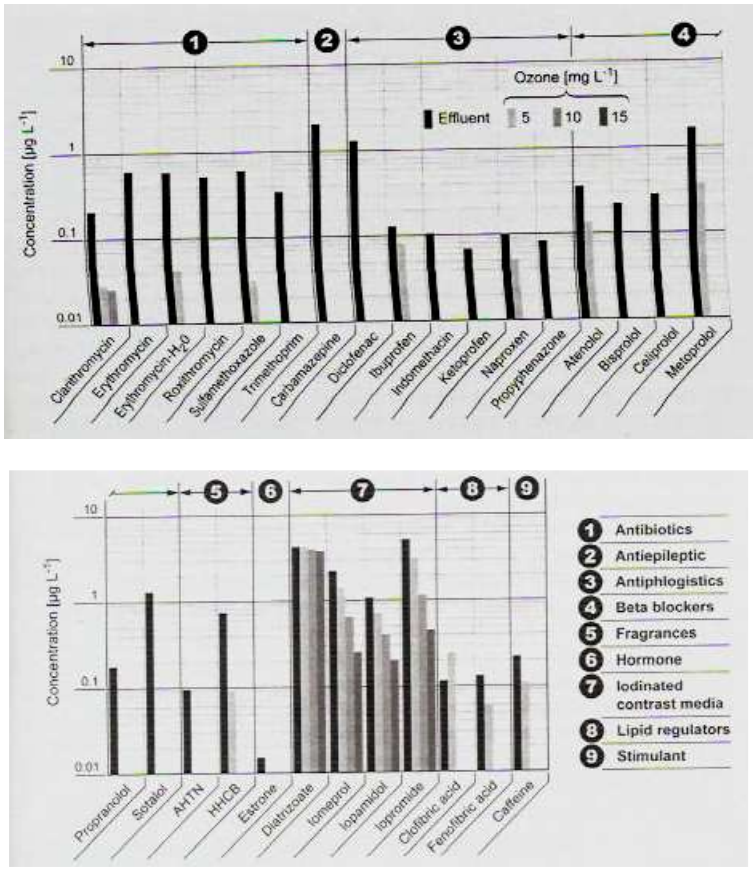


Figure 11.8: Removal of pharmaceuticals in the effluent of a municipal STP by ozonation (Ternes et la, 2006, 2003)

According to Huber et al, 2003, complete ozonation does not occur after the reaction of the pharmaceutical compounds with ozone; formation of some toxic metabolites can cause further problems.
The removal efficiency of pharmaceutical compounds from (untreated) urine by ozonation process was investigated. It was found out that 0.6-0.8 g.l^{-1} ozone dose is enough to decrease propranolol,

diclofenac and carbamazepine concentrations below the Limit of Detection (LOD), whereas for Ibuprofen, 1.3 $g.l^{-1}$ ozone dose was required. Despite all (parent) compounds disappeared, toxicity was still manifested since the compounds were transformed to other toxic compounds and not mineralized (Escher et al, 2006). On the other hand, another study proves that ozonation is an appropriate study to decrease the toxicitiy after 2-3 minutes application of advanced oxidation processes (AOPs) to the synthetic aqueous solution (Andreozzi et al, 2004). However full scale studies need to be performed to confirm it.
Beside ozonation is the most promising treatment process for removing pharmaceuticals from wastewater it is an energy intensive technology. 15-20 kWh/kg ozone is needed for the ozone production and depending on the energy price it costs 0.8-1.6 €/kg (Ternes et al, 2006). Approximately 0.1 $kWh.m^{-1}$ is needed for the ozonation process and this cause a 40-50 % increase in the energy demand of normal WWTPs (Larsen et al, 2004).

Advanced Oxidation Process (AOP)
AOP is considered as a good choice in order to treat the hazardous non-biodegradable pollutants including pharmaceuticals. Hydroxyl radicals ($OH^{\bullet}$) which are produced in AOP process are very reactive and play an important role in the mineralization of the pharmaceutical compounds in the final stage (Perez-Estrada et al, 2005).
There are different AOPs; one of which is photo-Fenton treatment. In photo-Fenton treatment a complete mineralization of diclofenac was obtained (Perez-Estrada et al, 2005, Ravina et al, 2002) in 100 minutes, while total degradation required 60 minutes (Perez-Estrada et al, 2005).

UV-Treatment
According to Miao (2005), the carbamazepine concentration in the treated water was higher than the water entering the UV-treatment unit. It may be a reason that UV radiation causes the metabolites of carbamazepine to be converted to the free form or release the analytes from the bound form to the dissolved phase.

Membrane Filtration
Membrane filtration is a treatment process where the pollutants and the carrier liquid are separated by forcing the liquid through a permeable or semi permeable membrane. With membrane process, specific pollutants can be removed according to the size of the compounds and the pore size of the membrane. Nanofiltration (NF) and Reverse Osmosis (RO) are the tight membrane filtration processes which allow the retention of the pharmaceutical compounds by molecular sieving (Ternes 2006).
Regarding the investigations conducted, it was found out that in membrane systems, compound rejection depends on the molecular width, size and the hydrophobicity of the compound which describes the charge and the polarity (Yoon 2006). Less polar, more volatile and more hydrophobic compounds have more ability to be retained by ultra and nanofiltration membranes.
According to the results of a study made in a membrane testing unit, carbamazepine, diclofenac and Ibuprofen were retained from the surface water in 100 % with nanofiltration. The same removal efficiencies were obtained for diclofenac and ibuprofen with ultrafiltration, whereas the removal efficiency of the carbamazepine was <80 %. In overall, the results for the selected pharmaceuticals in the study nanofiltration showed better removal efficiencies than ultrafiltration (Yoon et al, 2006).

In an experimental research using nanofiltration for treatment of urine, it was found that 74%, 96%, 96% and 59% removal efficiencies were achieved for carbamazepine, diclofenac, ibuprofen and propranolol (beta-blocker, same group with metoprolol), respectively. In the same study it was also stated that nanofiltration removed also metabolites from the urine. Toxicity from metabolites was reduced 80-90% by nanofiltration as found in bioassays with algae (Escher et al 2006).

Pronk et al, (2006) stated that in his laboratory research with non-hydrolysed urine that more than 90 % retention was achieved for all micropollutants investigated including carbamazepine, diclofenac, ibuprofen and propranolol (Figure 11.9).

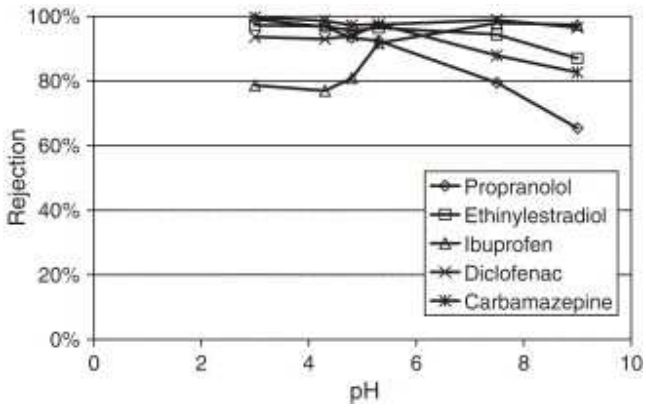


Figure 11.9: Rejection of micropollutants depending on the pH for natural urine using NF270 nanofiltration membrane (Pronk et al, 2006).

Nanofiltration and reverse osmosis seem to be more also promising membrane processes for removal of pharmaceuticals. However little studies were performed on the efficiencies of these tight membrane technologies.

Activated Carbon Adsorption

Activated Carbon is a common used process used for elimination of micropollutants. In a lab scale experiment the removal efficiencies of pharmaceutical compounds by adsorption on a Powdered Activated Carbon (PAC) was investigated. A 99 % removal of carbamazepine could be achieved with < 0.2 mg.l^{-1} PAC dose. As it is shown in Figure 11.10 higher doses of PAC were required (<1.0 mg/L) to to remove ibuprofen (Ternes et al, 2004 (POSEIDON). Also in other studies activated carbon filtration was efficient for carbamazepine and diclofenac removal (Ternes et al, 2002).

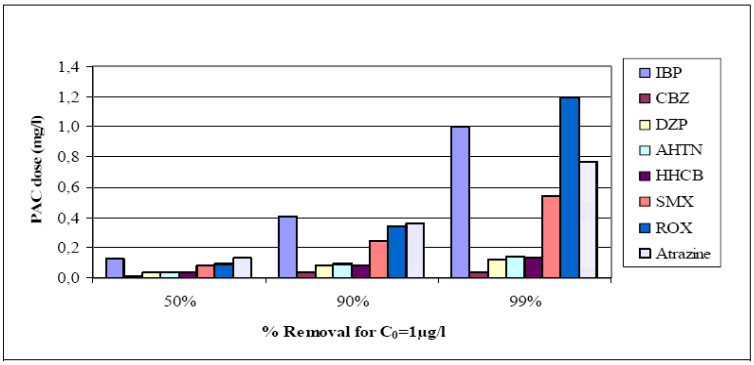


Figure 11.10: PAC doses calculated for removal efficiencies of 50, 90 and 99% for different pharmaceutical compounds (IBP - ibuprofen, CBZ – carbamazepine, DZP – diazepam).

Infiltration

In unsaturated zones approximately 40% elimination of diclofenac was measured whereas carbamazepine showed no removal efficiency. Ibuprofen was removed with approximately 55% (Table 11.5) (Scheytt et al, 2006).

Table 11.5: Comparison of removal efficiencies of pharmaceuticals during infiltration in saturated and unsaturated zones together with estimated K_{oc} values (Scheytt et al, 2006)

		Carbamazepine	Diclofenac	Ibuprofen
Sat [a]	Recovery	93-105%	97-106%	9-46%
Unsat [b]	Recovery	102%	63%	46%
Log Koc [c]	Column [b]	2.00		
Log Koc [c]	Batch [d]	2.00-2.21	2.43-3.87	2.94-3.13

[a] Sat = column experiments under water saturated conditions; DOC = 0.2%, pH = 6.7, medium sand (Scheytt et al., 2004 and Mersmann et al., 2002).
[b] Unsat = column experiments under unsaturated conditions (this publication).
[c] log K_{OC} = organic carbon normalized sorption coefficient; log K_{OC} = log(K_d/f_{OC}).
[d] Batch = results from batch experiments utilizing the same sediment as in the unsaturated sand column experiments (Scheytt et al., 2005).

In another study performed by Ternes et al (2004) (POSEIDON project) the concentration of Diclofenac decreased below limit of Detection (LOD) in unsaturated zone after a flow time of 75 days. On the other hand only 30 % removal was achieved for carbamazepine after a flow time of100 days. Also no significant removal of carbamazepine was detected during groundwater infiltration; the lower concentrations in the groundwater were only because of the dilution processes (Clara et al, 2004). The behaviour of the carbamazepine during the soil passage was also studied by Preuβ (2001) where also poor removal was reported.

11.5 Nutrient recovery

Struvite ($MgNH_4PO_4$), MAP Precipitation
In the Struvite Precipitation process, Magnesium ammonium phosphate ($MgNH_4PO_4 \cdot 6H_2O$) which is called MAP, AMP or struvite is precipitated in the process tank. This precipitate which is an important product containing two dominant wastewater nutrients (N, P) can be used as a slow release fertilizer (Maurer et al, 2006, Bridger et al, 1961; Johnston and Richards, 2003).
Addition of magnesium, in the form of MgO, $Mg(OH)_2$, $MgCl_2$ or bittern (the magnesium-rich brine from table-salt production) is necessary for the precipitation to occur.

In an experimental research of (Escher et al, 2006), removal efficiencies of pharmaceutical compounds from urine by struvite precipitation were investigated. After the filtration process, in filtrate 99% removal efficiency was stated achieved for carbamazepine, diclofenac, ibuprofen and proponalol. Similar results were obtained in another study by Ronteltap et al, (2006).

12 Analytical methods

12.1 General

Quantification of pharmaceutical compounds in aquatic environment requires sensitive and reliable analytical methods with detection limits down to the lower ng.l^{-} range. In the past the analytical determination has been mainly limited to biological samples such as blood, tissues or urine. A simple adaptation of these methods to environmental samples was not possible because the therapeutic dose of pharmaceutical is much higher than the concentrations found in environment.
It is different for new sanitation concepts, where separate collection of wastewater streams is being implemented sometimes in a very concentrated form (pure of slightly diluted urine, concentrated black water).
The three important difficulties playing the role in establishing of a reliable method for detection of pharmaceutical compounds and their metabolites are:

- elevated polarity
- low concentration
- complex matrix (concentrated wastewater (black water), sludge, wastewater, sediment, soil, biota).

Until few years ago most of the analytical methods reported in literature were based on Gas chromatography – mass spectroscopy (GC-MS), which often requires derivatization of (acidic) compounds. In the last years LC-MS and LC-MS-MS was indicated as the technique of choice to assay polar pharmaceuticals and their metabolites, and is especially suitable for environmental analysis because of its selectivity. Analytical procedures are proceeded by extraction and clean-up procedures (Figure 12.1 and 12.2).

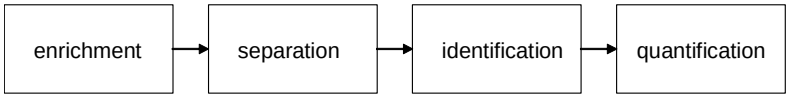


Figure 12.1: General steps in analytical techniques to determine pharmaceutical compounds and their metabolites in various environmental matrices.

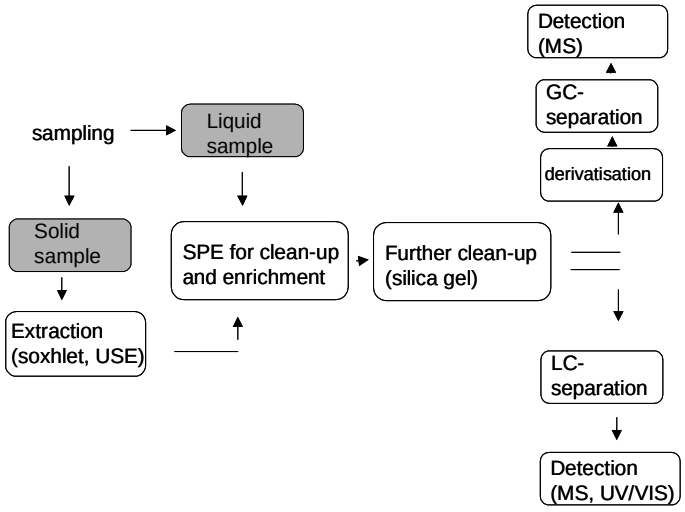


Figure 12.2: Analytical multiple step approach determine pharmaceutical compounds and their metabolites in various environmental matrices.

Sampling constitutes the first step in multi step approach a is essential to obtain representative data and to calculate mass fluxes in various systems. To extract substances from solid matrix Soxhlet method was historically used; ultrasonic solvent extraction (USE) and pressurized liquid extraction (PLE) have been used recently. In the following steps compounds from aqueous matrix need to be separated and preconcentrated since they are usually present in very low concentrations. Solid phase extraction (SPE) combines both requirements. If there are still disturbing compounds in extracted solution, further clean up is required; in, e.g., silica gel columns. The individual analytes are quantified with chromatographic techniques. GC can be directly implemented when compounds are volatile; molecules with charged groups require derivatisation preceding detection. To avoid this additional step, polar and charged pharmaceuticals are analysed with High Performance Gas Chromatography (HPLC). Among different detection techniques, such as, electron capture (GC), UV/visible absorption or fluorescence detection (LC), MS became a method of choice for both quantification techniques, LC and GC. MS being highly selective and sensitive, provides structural weight and structural information. GC/MS has been applied for decades for volatile and semi-volatile compounds. LC/MS has been used since 90´s after development of robust interfaces, such as electrospray ionization (ESI), atmospheric pressure photo ionization (APPI). High resolution or tandem mass spectrometry (MS/MS) provide detailed structural information, selectivity and sensitivity to determine organic pollutants at trace concentrations.

12.2 Sampling

To assess fate on pharmaceutical compounds in environment, some general issues should be taken into account when sampling: resolution in time, distribution in space, storage and collection.
Occurrence of any substances in urban water cycle varies at different time scales; seasonally, weakly, diurnal, daily. It also depends on the weather – rain events. Ideally high frequent sampling should be performed and analysed to determine the occurrence pattern – usually not feasible activity. Composite samples are recommended when mass balances are needed to be calculated. Grab samples enable to determine peak concentrations or short time distribution.

Measurements from a larger geographic area allows for a better general interpretation than measurements from the single sites (point sources, specific locations). Close to the point sources and areas of uneven morphology, denser sampling pattern should be applied, than from more uniform areas.

After sampling stability of the target compounds should be ensured. Storage of samples should be performed in such a way that transformation of a compound is avoided. Liquid samples should be filtered first and treated according to the compound property (e.g. acidification). Another way is freezing the sample in amber glass bottles in horizontal position. Adding disinfectants (sodium azide) or adjusting pH to 2 efficiently prevents microbial activity.
Direct SPE is however considered as a best practice. The dried SPE cartridges can be then stored and sent to laboratory for analysis. Amber glass is recommended to prevent photolytic degradation. For easily oxidizable and olatile compounds sampling vials should be filled completely. Argon or nitrogen sparging can also reduce oxidation by oxygen. To avoid losses by sorption Teflon (PTFE) or polypropylene are recommended as materials for sapling and analysis. Sometime complexing agents (EDTA) are added to prevent precipitation and complexation of complex compounds.
Sludge samples are usually filtered through glass fibre filters or are centrifuged and subsequently frozen for storage. For analysis sludge samples are freeze-dried and ground in a ball mill or mortar.

12.3 Extraction, enrichment, clean-up

Sample preparation is necessary due to low concentration of analytes in interaction due to matrix. Appropriate internal or surrogate standard is crucial because of high interference with matrix.
They should be added to the sample at an early stage, prior extraction and enrichment. A surrogate standard should behave exactly in the same way as an analyte and compensate for all losses during all analytical steps. Similar substances are used or isotope labelled standards (for MS).

Solid samples

Interactions between analyte and matrix are in solid extremely complex due to the heterogenous character of the latter. Chemicals interact with organic and inorganic sites and in addition they may be located in micropores of particles covered by bulk of organics or covered by water layers. Common four extraction methods for solid samples are:

- ultrasonic solvent extraction (USE)
- microwave assisted solvent extraction (MASE)
- pressurised liquid extraction (PLE)
- supercritical fluid extraction (SFE).

Liquid samples

In aqueous samples analytes need to be extracted and concentrated. The following techniques are applied:

- liquid/liquid extraction (LLE)
- solid phase extraction (SPE)
- solid phase microextraction (SPME)
-

SPE is widely used as seletive method for sample preparation and has replaced many others classical LLE methods. The extraction is performed by passing the samples through preconditioned sorbent materials. Commonly used SPE sorbents are±

- silica based sorbents (e.g. RP-C_{18})
- graphitised (Carbopack B)
- copolymers (Lichrolut EN)
- mixed phases (OASIS MCX)

In many cases after implementation of an appropriate extraction method, there are still disturbing matrix components in the sample. Further clean-up steps are then used:

- adsorption in silica gel columns or aluminium oxides columns
- gel permeation chromatography (GPC) where molecules are separated according to their size.

12.4 Chromatography and mass spectrometry

12.4.1 GC(MS)

A GC enables to separate chemicals in a complex sample. A GC uses a flow-through the column, through which different chemical constituents of a vaporised sample pass in a gas stream (carrier gas, mobile phase) at different rates depending on their various chemical and physical properties and their interaction with a specific column filling, called the stationary phase. As the chemicals exit the end of the column, they are detected and identified electronically. The function of the stationary phase in the column is to separate different components, causing each one to exit the column at a different time (retention time). Other parameters that can be used to alter the order or time of retention are the carrier gas flow rate, and the temperature.

Gas chromatography – mass spectroscopy (GC-MS) combines two techniques to a one method to analyse mixture of chemical compounds. Gas chromatography separates the components of the mixture and mass spectroscopy characterises each of the components individually. Combination of two techniques enables to evaluate a solution containing a number of chemicals qualitatively and quantitatively.
GC separation of many pharmaceutical compounds can only be performed after derivatisation. This converts protonic functional groups into thermally stable non-polar groups. There are 4 main derivatisation methods (based on reagents used and the reaction achieved): silylation, acylation, esterification, alkylation

12.4.2 HPLC, LC, LC MS/MS

In HPLC the analyte is forced through a column of the stationary phase by pumping a liquid (mobile phase) at high pressure through the column. The sample to be analyzed is introduced in a small volume to the stream of mobile phase and is retarded by specific chemical or physical interactions with the stationary phase as it traverses the length of the column. The amount of retardation depends on the nature of the analyte, stationary phase and mobile phase composition. The time at which a specific analyte elutes (comes out of the end of the column) is called the retention time and is considered a reasonably unique identifying characteristic of a given analyte. The use of pressure increases the speed giving the components less time to diffuse within the column, leading to improved resolution in the resulting chromatogram. Common solvents used include any miscible combinations of water or various organic liquids (the most common are methanol and acetonitrile).

LC-MS is used for non volatile polar compounds with medium to high polarity.
To cope with complex sample composition and not fully resolved chromatographic peaks, MS-MS are used applying triple quadropole mass spectrometers. LC-MS with single quadropole mass spectrometers can also be used to produce the fragmented spectra.
The application of advanced LC-MS/MS techniques allows:

- the determination of broader range of compounds
- offers improvement over GC-MS since derivatisation is avoided
- the limits of detection (LOD) less than 1 ng/L can still be achieved (comparable to GC-MS).
- Versatility and less complicated sample preparation.

LC-MS is nowadays a method of choice for determination and quantification of polar compounds

12.4.3 Quality assurance

The high polarity and low concentration of analytes in environmental matrices require comprehensive quality assurance. To report and compare the results and evaluate them a complete and detailed description of the analytical method and the applied quality assurance program is needed:

Description of analytical method includes the following elements:

- listing of used analytes, solvent and chemicals
- information on sampling (sample volumes), transport and storage
- pH adjustment, filtration, filter material, extraction, solvent evaporation technique, derivatisation, method of detection,
- use of surrogates and instrumental standards (which point of method added and in which amounts).

In a **quality control program** the following aspects are important to be described in detail:

- use of surrogate standard for each analyte and analyte group
- use of instrumental standards

- method of quantification
- determination of recoveries for method validation and quantification
- limit of quantification
- limitation of the method with regard to matrix effect
- employment of procedural and instrumental blank (Terner and Joss, 2006).

13 Conclusions

Human pharmaceuticals are consumed in high quantities world wide; the consumption is in the range of tons per year per one pharmaceutical compound depending on the size of a country. The expectations are that these amounts will only keep increasing because of a improving health care system and longer life expectations of people.

In current sanitation systems characterised by a high degree of dilution, pharmaceutical compounds are not removed to a sufficient degree. Discharged to surface water form a threat to aquatic life and in the worse case may re-enter water cycle.

A general overview was given on a variety and nature of human pharmaceutical compounds. Attention was paid on characteristics of the compounds in relation to their possible behaviour in a wastewater treatment system.

A pre-selection was made for few compounds deserving a special attention in further study within the SWITCH project. The compounds: diazepam, oxazepam, temazepam, metoprolol, gemfibrozil, diclofenac, naproxen, ibuprofen, carbamazepine ´represent´ 4 therapeutic groups.

A validation of this selection will take place in laboratory pre-tests.

The laboratory activity will start with fate of selected compounds in biological systems (biodegradability, 2nd year of a project) followed by physical-chemical systems (3rd year of the project).

Analytical methods will apply solid phase extraction, possibly followed by cleanup and detection using LC-M(MS).

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SWITCH

Sixth Framework Programme (2002-2006)

Appendix 1:

Survey of the mostly used pharmaceutical compounds and hormones in the West Bank/ Palestine

1 Preface

In Palestine, pharmaceuticals and hormones in the environment represent an emerging environmental issue, and recently a great interest has developed in the Water Studies Institute (WSI) of Birzeit University regarding the presence of drugs in the environment mainly through SWITCH project. So far, in Palestine nothing is known about the occurrence of pharmaceutical compounds in environmental compartments (surface water, ground water, soil). Continuous growth in Palestinian population leads to increased pressure on the scarcely available freshwater resources emphasizing the need for ensuring that any aggregate impacts on water supplies and resultant potential for human or ecological cumulative exposure should be minimized. The existence of pharmaceuticals residues in the Palestinian environment had not been investigated.

This study aims at identification and ranking the mostly used pharmaceuticals in the West Bank. The survey of the mostly used and distributed pharmaceutical compounds and hormones in the West Bank and particularly in Ramallah and Al Bireh cities are mainly related to the pharmacologic groups of: antibiotics (Amoxicillin HCl, Glibenclamide and cephalosporin), analgesic antipyretic (paracetamol and aspirin), non steroidal and anti-inflammatory (NSAID) (diclofenac sodium and ibuprofen), β blockers/ antihypertensive (atenolol), H_2 blockers (Ranitidine), antidiabetic/ biguanide (metformin), minerals and vitamins namely Multivitamins and hormones (clomiphenecitrate, allyestranol, menotrophin and human chorionic gonadotrophic).

2 Introduction

Up to date only few investigations have been published about the assessment of the environmental relevance of pharmaceuticals, although tons of those compounds are used every year since several decades. Pharmaceutical substances may be metabolized, and the active substance and any metabolite may then be excreted. Human medicines and hormones may usually be released to the sewer system and only partially removed by conventional biological treatment, this can be detected in sewage treatment plant (STP) effluents and in receiving waters. As more becomes known about the health impacts of these compounds, it is anticipated that discharge limits may be developed for a number of these compounds. Over 30 million organic compounds are known to exist. It is clear that the list of emerging compounds will continue to grow as analytical technique continues to improve (Tchobanogous *et al.*, 2003). According to Ternes (2001) the first results about the occurrence of pharmaceutical residues in the environment were published by *Garrison et al.* (1976) from the Environmental Protection Agency (US-EPA), who found clofibric acid and salicylic acid in a municipal STP of Athens (United States) with 1-2 µg/l. These two compounds were also found by Hignite and Azarnoff (1977) in a STP of Kansas City with relative high concentration levels of 1-95µg/l. In Canada the pharmaceuticals ibuprofen, clofibric acid and naproxen were identified in wastewater by *Rogers et al.* (1986). These investigations in the United Kingdom revealed that drugs

were present in the aquatic environment at concentrations up to approximately 1 microgram/l, whereas the exact concentrations for the individual drugs were not always determined. In Germany, clofibric acid has been identified in river and groundwater and even in drinking water with concentration levels ranging up to 0.165 µg/l reported by Stan *et al.* (1994).

In Palestine, pharmaceuticals and hormones in the environment represent an emerging environmental issue, and recently a great interest has developed in the Water Studies Institute (WSI) of Birzeit University regarding the presence of drugs in the environment mainly through SWITCH project. So far, in Palestine nothing is known about the occurrence of pharmaceutical compounds in environmental compartments (surface water, ground water, soil). Continuous growth in Palestinian population leads to increased pressure on the scarcely available freshwater resources emphasizing the need for ensuring that any aggregate impacts on water supplies and resultant potential for human or ecological cumulative exposure should be minimized. The existence of pharmaceuticals residue in the Palestinian environment had not been investigated. This study aims at identification and ranking the mostly used pharmaceuticals in the West Bank. Based on the results of this survey, later on the existence of the mostly used pharmaceutical compounds in raw wastewater and in the effluent of Al Bireh sewage treatment plant will be examined. Afterwards, biodegradability studies will be carried out.

3 Methodology

3.1 Study area

The West Bank/Palestine lies on the Western edge of the Asian continent and the eastern extremity of the Mediterranean Sea, in the heart of the Middle East (Map 1) (ARIJ, 1997). The study was mainly focused on Al-Bireh and Ramallah adjacent cities in the West Bank. Ramallah and Al Bireh cities are located in the central part of the West Bank and considered as one of the most important administrative centers in Palestine. Ramallah and Al Bireh are the main urban centers for commerce and services with small and medium scale industries. Al Bireh city is served with an extended aerations STP that was operated in the year 2000. This STP is the only well functioning STP in the West bank.

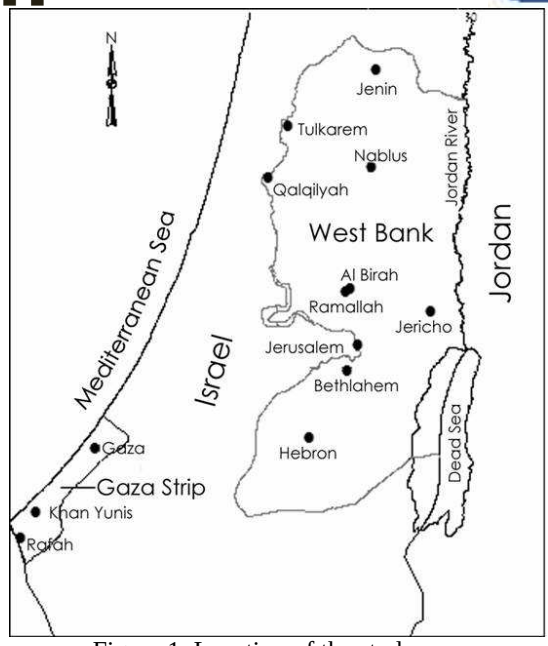


Figure 1: Location of the study area

3.2 Study approach

Data collection was conducted by visiting several privately owned pharmacies and pharmacies in main hospitals in five cities in the West Bank with main emphasis on Ramallah and Al Bireh cities as presented in Table 1. In addition to the private and hospital pharmacies, the central medical stores of the Ministry of Health (MoH) located in Ramallah city was consulted and a list showing the quantities of the mostly distributed medicines in the whole West Bank MoH hospitals and medical centers during the year 2005 was compiled. Besides the UNRWA central medical stores located in the city of Jerusalem was contacted by phone that provided a list of the medicines that they mostly distribute to their Palestinian refugee camps clinics during the year 2005. The medical services who serve the police and security sectors were also contacted, and a list of the mostly distributed medicines during the year 2005 was provided. All those medical centers were asked about the mostly sold and distributed medicines. In addition, several personal interviews were conducted with pharmacists, medicine doctors and process engineers in main pharmaceutical factories in the West Bank.

Table 1: Number and distribution of surveyed pharmacies of the mostly sold pharmaceuticals in the main cities in the West Bank/ Palestine

	Private pharmacies	Hospital pharmacies	Sub total
Ramallah and Al Bireh cities	26	2	28
Tulkarem City	4	2	6
Nablus City	1	2	3
Hebron City	1	0	1
Total	32	6	38

4 Results and discussion

4.1 Reports of the main medical centers in the West Bank

Table 2 shows the total quantity of the distributed pharmaceuticals in kg provided by the MoH, UNRWA clinics and the governmental medical services during the year 2005. The table shows all medicines distributed in the West Bank excluding the medicine sold by the private pharmacies. A clue about the medicine sold by private pharmacies is provided by the questionnaire results as presented in Tables 2 -12.

Table 2: Total quantities of the mostly distributed drugs in the whole West Bank during the year 2005

Product	MoH in kg	UNRWA Clinics in kg	Medical services in kg
Glibenclamide 5mg	24.64	20.84	1.25
Metformin 850	2'564	4'948.27	133
Ranitidine 150 mg	583	216.375	26.75
Furosemide 40 mg	135	37.132	8.6
Atenolol 50 mg	92.223	138.74	4.2
Captopril 25 mg	78.74		2.55
Diltiazem 60 mg	76	34.986	1.6
Enalapril 5 mg	7	20.48	0.2
Aspirin 100mg	16.7	500	25.4
Diclofen 50 mg	198.31	72.715	15
Paracetamol 500mg	3'353	2'149.55	166.13
Amoxicillin 500mg	835.3	593.15	94.10

4.2 Survey results

The results of the mostly sold and distributed medicines obtained from the conducted survey are presented in Table 3. The results analysis for each city and pharmacy type is presented in the table here after.

Table 3: All served pharmacies and medical centers including both private pharmacies and pharmacies in hospitals

Ranking	Percentage[†]	Name of medicine
1	95	Paracetamol 500 mg
2	89	Amoxicillin 500 mg
3	84	Ibuprofen 400 mg
4	53	Diclofenac sodium
5	42	Atenolol 50 mg Ranitidine 150 mg, & 50mg/2ml
6	37	Glibenclamide 5mg
7	32	Cephalexin 500 mg Aspirin/ baby Aspirin
8	24	Multi vitamin

[†]Percentage represents the percent of the number of the surveyed pharmacies that considered the specific medicine among the mostly sold pharmaceuticals out of the total interviewed pharmacies.

Table 4: All private served pharmacies

Ranking	Percentage	Name of medicine
1	100	Paracetamol 500 mg
2	91	Amoxicillin 500 mg
		Ibuprofen 400 mg
3	47	Diclofenac sodium
4	44	Rantidine 150 mg, & 50mg/2ml
5	41	Atenolol 50 mg
6	34	Glibenclamide 5mg
		Cephalexin 500 mg
7	31	Aaspirin/ baby Aspirin
8	22	Multi vitamin

Table 5: All served hospital pharmacies

Ranking	Percentage	Name of medicine
1	83	Amoxicillin 500 mg
		Diclofenac sodium
2	67	Paracetamol 500 mg
3	50	Atenolol 50 mg
		Glibenclamide 5mg
		Ibuprofen 400 mg
		Ceftriaxone 500 mg
		Cefuroxime as sod salt 1mg

Table 6: All Ramallah and Albireh cities served pharmacies and medical centers including both private pharmacies and pharmacies in hospitals

Ranking	Percentage	Name of medicine
1	100	Paracetamol 500 mg
2	96	Ibuprofen 400 mg
3	93	Amoxicillin 500 mg
4	54	Diclofenac sodium
5	50	Atenolol 50 mg
		Rantidine 150 mg, & 50mg/2ml
6	36	Glibenclamide 5mg
7	32	Aaspirin/ baby Aspirin
8	29	Cephalexin 500 mg
		Multi vitamin

Table 7: Private Ramallah and Albireh cities served pharmacies

Ranking	Percentage	Name of medicine
1	100	Paracetamol 500 mg
2	96	Ibuprofen 400 mg
3	92	Amoxicillin 500 mg
4	50	Rantidine 150 mg, & 50mg/2ml
		Diclofenac sodium
		Cephalexin 500 mg
5	46	Atenolol 50 mg
6	31	Aaspirin/ baby Aspirin
		Glibenclamide 5mg
7	27	Multi vitamin

Table 8: Ramallah and Albireh cities served hospital pharmacies

Ranking	Percentage	Name of medicine
1	100	Amoxicillin 500 mg Paracetamol 500 mg Atenolol 50 mg Glibenclamide 5mg Ibuprofen 400 mg Diclofenac sodium Ciprofloxacin 500 mg

Table 9: All Tulkarem city served pharmacies and medical centers including both private pharmacies and pharmacies in hospitals

Ranking	Percentage	Name of medicine
1	100	Amoxicillin 500 mg Paracetamol 500 mg
2	67	Cephalexin 500 mg Ethinylestradiol Levonorgestterol
3	50	Glibenclamide 5mg Ibuprofen 400 mg
4	33	Aaspirin/ baby Aspirin Diclofenac sodium Metformine HCL 850 mg Enalapril maleate Furosemide Omeprazole

Table 10: Tulkarem city served private pharmacies

Ranking	Percentage	Name of medicine
1	100	Amoxicillin 500 mg Paracetamol 500 mg Ethinylestradiol Levonorgestterol
2	75	Cephalexin 500 mg
3	50	Glibenclamide 5mg Ibuprofen 400 mg

Table 11: Tulkarem city served hospitals pharmacies

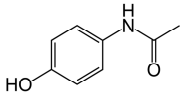
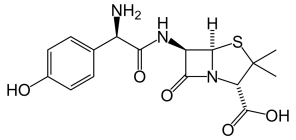
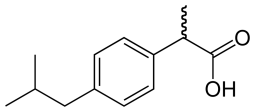
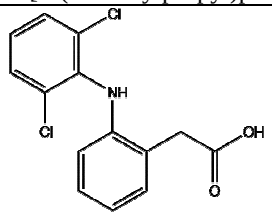
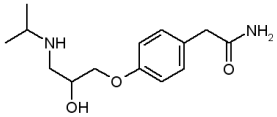
Ranking	Percentage	Name of medicine
1	100	Amoxicillin 500 mg Paracetamol 500 mg

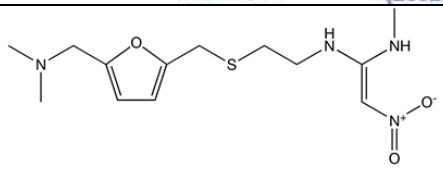
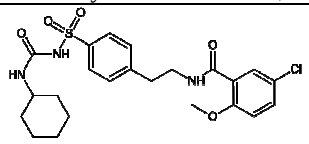
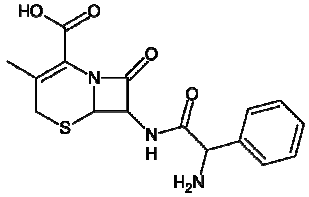
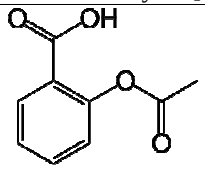
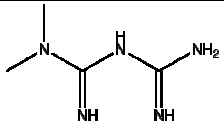
Table 12: Nablus city served hospitals pharmacies

Ranking	Percentage	Name of medicine
1	100	Diclofenac sodium Ceftriaxone 500 mg Cefuroxime as sod salt 1mg

By comparing the results presented in the previous table, especially Tables 2, 3 and 6, the mostly distributed and sold pharmaceuticals in the West Bank are presented in Table 13. The chemical formula and structure are also provided.

Table 13: All served pharmacies and medical centers including both private pharmacies and pharmacies in hospitals

Name of medicine	Pharmacological group	Chemical data
Paracetamol	Analgesic antipyretic	 $C_8H_9NO_2$ *N*-(4-hydroxyphenyl)acetamide
Amoxicillin	Antibiotic/ Penicillin	 $C_{16}H_{19}N_3O_5S$ 7-[2-amino-2-(4-hydroxyphenyl) -acetyl]amino-3,3-dimethyl-6-oxo -2-thia-5-azabicyclo[3.2.0]heptane -4-carboxylic acid
Ibuprofen	NSAID	 $C_{13}H_{18}O_2$ 2-[4-(2-methylpropyl)phenyl]propanoic acid
Diclofenac sodium	NSAID	 $C_{14}H_{11}NCl_2O_2$ 2-[2-(2,6-dichlorophenyl) aminophenyl]ethanoic acid
Atenolol	β blockers/ antihypertensive	 $C_{14}H_{22}N_2O_3$ 2-[4-[2-hydroxy-3-(1-methylethylamino)propoxy]phenyl]ethanamide

Ranitidine	H_2 blockers	 **$C_{13}H_{22}N_4O_3S$** (*E*)-N-(2-((5-((dimethylaminomethyl) furan-2-yl)methylthio)ethyl)-N'-methyl-2-nitroethene-1,1-diamine
Glibenclamide	Sulfonylurea/ Antidiabetic	 **$C_{23}H_{28}N_3ClO_5S$** 5-chloro-*N*-[2-[4-(cyclohexylcarbamoylsulfamoyl) phenyl]ethyl]-2-methoxy-benzamide
Cephalexin	Antibiotic/ Cephalosporin	 **$C_{16}H_{17}N_3O_4S$** 8-(2-amino-2-phenyl-acetyl)amino -4-methyl-7-oxo-2-thia-6-azabicyclo [4.2.0]oct-4-ene-5-carboxylic acid
Aspirin/ baby Aspirin	Analgesic, antipyretic, NSAID & antiplatelet/ Salicylate	 **$C_9H_8O_4$** **$C_6H_4(OCOCH_3)COOH$** 2-(acetyloxy)benzoic acid
Metformin	Antidiabetic/ Biguanide	 **$C_4H_{11}N_5$** 1-(diaminomethylidene)-3,3-dimethyl-guanidine
Multi vitamin	vitamin	

NSAID: non steroidal anti-inflammatory drug

Based on the interviewed pharmacies, medicine doctors, the mostly used hormones are presented in Table 14.

Table 14: Mostly used hormones in the West Bank as provided by Ramallah, Nablus and Tulkarem surveyed hospitals, as well as from two pharmacies in Tulkarem city

No	Name of hormone	Pharmacological group
1	Ethinylestradiol Levonorgestterol	Estrogen & progesterone
2	Northisterone acetate	Progestogen/premenstrual disorders; menorrhagia
3	Chorionic gonadotrophin	Trophic hormones
4	Clomiphene citrate	Steroid&sex hormones
5	Menotrophin	Ganadotrophin
6	Allyestranol	Steroid & sex hormones

For the sake of predicting the pharmaceutical compounds in the aquatic ebvironment the total quantity of selected mostly sold and distributed pharmaceutical compounds in the West Bank/ Palestine in kg during the year 2005, as well as basic Pharmacokinetic data of metabolism and excretion are presented in Tables 15 and 16.

Table 15: Total quantity of selected mostly sold and distributed pharmaceutical compounds in the West Bank/ Palestine in kg during the year 2005[+]

	Reference				
Pharmaceutical compound	MoH	UNRWA Clinics	Medical services	Private Pharmacies[†]	Total estimated quantity
	(kg)				
Glibenclamide	24.64	20.84	1.25	24.88	71.61
Metformin	2’564	4’948.27	133		7’645.27
Ranitidine	583	216.375	26.75	1’687.44	2514.565
Atenolol	92.223	138.74	4.2	137.223	13’850.65
Aspirin	16.7	500	25.4	284.40	826.5
Diclofe	198.31	72.715	15	708.63	994.655
Paracetamol	3’353	2’149.55	166.13	10’250.25	15’918.93
Amoxicillin	835.3	593.15	94.10	8’863.8	10386.35

[+]Note: available data about the quantity of some important pharmaceutical compounds like Ibuprofen is inadequate

[†] Estimated from the average yearly reports of three pharmacies multiplied by the total number of pharmacies in the West Bank of 711 pharmacies;

Table 16: Metabolism and excretion of the mostly sold and distributed pharmaceutical compounds in the West Bank/ Palestine in kg during the year 2005[+]

Pharmaceutical compound	Total estimated quantity	Metabolism and excretion
Glibenclamide	71.61	Excretion is renal and biliary (Wikipedia, 2007).
Metformin	7'645.27	excreted by the kidneys as the active compound (Katzung, 1997).
Ranitidine	2514.565	It is primarily eliminated by renal excretion of the unchanged drug (30%) of an oral dose, or by fecal elimination (Craig & Stitzel 1986).
Atenolol	13'850.65	Urinary excretion accounts for about 40% of elimination of Atenolol (Craig & Stitzel 1986).
Aspirin	826.5	Ingested salicylate and that generated by the hydrolysis of aspirin may excreted unchanged, but most is converted to weaker soluble conjugates that are rapidly cleared by the kidney alkalnization of the urine (pH 6.6) increases the rate of excretion of free salicylate. (Katzung 1997). Renal excretion (Wikipedia, 2007).
Diclofen	994.655	Excretion is biliary, only 1% in urine (Wikipedia, 2007).
Paracetamol	15'918.93	Less than 5% is excreted unchanged (Katzung, 1997). Little unchanged drug is excreted in the urine, but most metabolic products appear in the urine within 24 hours (Wikipedia, 2007).
Amoxicillin	10386.35	Most of the pencillins are rapidly excreted in the urine as the active drug, also in bile in high concentration (Craig and Stitzel 1986). Approximately one third of a dose appears in urine (Wikipedia, 2007).
Ibuprofen	[+]	It is extensively metabolized in the liver, and little is excreted unchanged (Katzung, 1997). Renal excretion (Wikipedia, 2007).

[+]Note: available data about the quantity of some important pharmaceutical compounds like Ibuprofen is inadequate

5 Conclusions

The survey of the mostly used and distributed pharmaceutical compounds and hormones in the West Bank and particularly in Ramallah and Al Bireh cities are mainly related to the following pharmacologic groups:

Pharmaceutical groups:

1. Antibiotics, namely Amoxicillin HCl, Glibenclamide and cephalosporin
2. Analgesic antipyretic namely Paracetamol and Aspirin
3. Non steroidal and anti-infilmmatory (NSAID) namely Diclofenac sodium and Ibuprofen
4. β blockers/ antihypertensive namely Atenolo
5. H_2 blockers namely Ranitidine
6. Antidiabetic/ Biguanide namely Metformin
7. Minerals and vitamins namely Multivitamins

Hormones:

1. Clomiphenecitrate, Allyestranol, Menotrophin and Human chorionic gonadotrophic.

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SWITCH

Sixth Framework Programme (2002-2006)

Appendix 2:

Estrogens in aquatic environment: A review

Preface

This document gives information on the fate of three estrogenic compounds estrone (E1), 17β-estradiol (E2), and synthetic hormone (17α-ethynylestradial, i.e. EE2) in aquatic environment. These three estrogens have been suggested to be the major compounds responsible for endocrine disruption in sewage wastewater (Onda, Yang et al. 2002). The estrogenic potencies of those three sterols are three or more orders of magnitude more than that of most EDCs such as BPA and nonylphenol, which are weakly estrogenic (Shi, Fujisawa et al. 2004). The properties of E1, E2 and EE2, the sources of emissions and occurrence in various aquatic compartments (wastewater, surface water, ground water) are described.

Urine separation might be one of the promising choices to deal with the problem of estrogen pollution in aquatic environment. Some urine treatment technologies such as filtration, ozonation, were found to be efficient with respect to toxicity reduction. The night soil treatment system with a biological process followed by a tertiary treatment also showed a high estrogens removal rate. Although there are already some researches on the safety of urine as fertilizer, further researches on risk assessment are still needed.

1 Introduction

An endocrine disrupter is defined as an exogenous agent that interferes with the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behaviour (USEPA 1997). Those chemicals which can interfere with the endocrine system in several ways to produce an undesired response or disruption are collectively referred to as endocrine disrupting chemicals (EDCs) (Birkett 2002). The European Union (EU) has produced a report containing a range of substances suspected of interfering with the hormone systems of humans and wildlife. The study identified 118 substances that were classed as endocrine disrupters or potential endocrine disrupters. Of these, 12 have been assigned priority for in-depth study. In broad terms, endocrine disrupting chemicals can be grouped into three types, namely: (1) synthetic hormones (chemicals designed to intentionally disrupt the endocrine system such as 17α-ethinylestradiol); (2) natural compounds, such as human hormones and their breakdown products, the phytoestrogens (e.g. genistein, coumestrol) found in a wide variety of plants; (3) some man-made chemicals include certain type of pesticides and herbicides such as o,p'-dichlorodiphenyl-trichloroethane (DDT) and its metabolites (e.g. p,p'-DDE), dieldrine, chlordane, methoxychlor, toxaphene and endosulfan; plastics and other industry related materials, such as bisphenol A (BPA), alkylphenols, some phthalates, alkylphenol ethoxylates (APEs), butyl and dibutylphthalates, hydroxy-polychlorinated biphenyls, polychlorinated biphenyls (PCBs) and dioxins (Baker 1988; Irmak, Erbatur et al. 2005)

The problem of EDCs has been evident since the early 1900s, but recently this phenomenon has emerged as a major environmental and human health issue, generating a vast amount of attention among scientific communities worldwide and considerable media interest. There are a number of ways in which EDCs may interfere with the endocrine system of wild population and human being. The main mechanisms of action used by EDCs include: (1) mimicking the effects of natural hormones by binding to the hormone receptor; (2) by stimulating the formation of more hormone receptors within cells, which will lead to the amplification of both natural and foreign hormones; (3) by antagonising the effects of natural hormones by blocking the binding to the hormone receptor; (4) by accelerating a hormone's breakdown and elimination form the body leads to depletion of the hormone; (5) by interfering with hormone synthesis; (6) by destroying the hormone or the hormone's ability to carry its function by acting directly/ indirectly to alter its structure(Baker 1988; Birkett 2002). It was generally accepted that the problem of endocrine disruption is of global importance in terms of effects on human health and wildlife (EUCommission 2001). In the case of wildlife, it appears that, since the 1950s, many species have been affected by EDCs in the environment. More recently, sewage water treatment plant effluents have been shown to have estrogenic activity which is causing feminization of river fish, and this may ultimately affect their population densities(Purdom, Hardiman et al. 1994). Study also shows that steroidal estrogen can bioaccumulate in fish, mainly in the bile and in both ovaries and testes of fishes exposed to contaminated water(Gibson, Smith et al. 2005). Masculinization has been observed in female snails exposed to tributyltin which was used as an antifouling agent in paints (Matthiessen, Gibbs et al. 1998), resulting in decline or extinction of local populations worldwide. In birds, there are known developmental problems (e.g. pesticide induced egg shell thinning) but contradictory population observations(EUCommission 2001). It also has been argued that endocrine disrupters may be responsible for decline in sperm counts, abnormalities in the male reproductive tract, slow development in infants and increases in the rate of testicular and breast cancer in human being(EUCommission 1996; Beard 2006). Since the Weybridge workshop in December 1996 there is more knowledge available on exposure and more findings indicating an association of endocrine disrupter exposure and human endocrine effects even at very

low doses. It is therefore considered more probable that endocrine disrupters' effects are causally linked with negative influences on human health(EUCommission 2001). Associations between reproductive and developmental effects and exposure to EDCs in wildlife populations have also been reported (Vos, Dybing et al. 2000; Waring and Harris 2005).

In this literature review, we will emphasize the sources and occurrences of estrogens including estrone (E1), 17β-estradiol (E2), and synthetic hormone (17α-ethynylestradial, i.e. EE2) in aquatic environment because estrogens have been suggested as the major compounds responsible for endocrine disruption in sewage wastewater(Onda, Yang et al. 2002). The estrogenic potencies of those three sterols are three or more orders of magnitude more than that of most EDCs such as BPA and nonylphenol, which are weakly estrogenic(Shi, Fujisawa et al. 2004). Among of the three sterols, EE2 showed the highest estrogenic potency *in vitro* test. The potency can be expressed as EE2>E2>E1(Larsson, Adolfsson-Erici et al. 1999). And also, EE2 is considerably more persistent in wastewater treatment plant compared to the other natural hormones(Murphy, Andersen et al. 2002). Due to the introduction of the ethynyl group, the ring of EE2 becomes extremely stabile against oxidation. Meanwhile, the fate of estrogens in some high strength media (e.g. source-separated urine) will also be mentioned.

2 Physicochemical properties of estrogens

Estrogens, like all steroids, share the same hydrocarbon ring nucleus as cholesterol, their parent compound. Their fate and behaviour are influenced by its physicochemical properties, which were summarized in Table 14.1(Newman, Lait et al. 1998). The log octano/water coefficient values (log) are 3.43 for E1, 3.94 for E2 and 4.15 for EE2, and thus, it is evident that these compounds are lipophilic and are only sparingly soluble in water. As a matter of fact, E1 and E2 have solubility of approximately 13mg/L, while EE2 has much lower solubility of 4.8mg/L. A high log K_{ow} also means that these estrogens may be rapidly removed from the aqueous phase as a result of binding to suspended solid or biota (Birkett 2002). All these steroids have very low vapour pressures ranging from 2.3×10^{-10} to 4.5×10^{-11}mm Hg, indicating low volatility of these compounds (Table 1).

Table 1: Physicochemical property of estrogens

Chemical name	Molecular weight	Water solubility/mg/L at 20°C	Vapor pressure/mmHg	Log K*ow*
E1	270.4	13	2.3×10^{-10}	3.43
E2	272.4	13	2.3×10^{-10}	3.94
EE2	296.4	4.8	4.5×10^{-11}	4.15

3 Sources of estrogens

EDCs, as well as other pollutants, have a variety of sources. The main sources arises from domestic sewage effluent, industrial wastewater effluent, industrial discharge, agricultural runoff for crops(pesticide and herbicide) and animals, atmospheric deposition, leachate of waste dumps, etc. For estrogens the discharged domestic effluents represent the most significant estrogenic input to the aquatic environment and serve as important point sources, especially in densely populated areas(Belfroid, Van der Horst et al. 1999).

a. Estrogens from human excretion

The presence of estrogens in wastewater arises from mammalian excretion, in particular females of reproductive age and those who are pregnant. Calculations for the percentage contribution to the total excretion of both conjugated and unconjugated natural estrogens and the synthetic EE2 show that pregnant women contribute the most estrogens (44%) to the total excreted amount, followed by 36% from women (not pregnant)(de Mes, Zeeman et al. 2005). The total daily excretion rate of natural estrogens ranges from 10 to 100 µg for women, depending on the phase of their cycle, 5–10 µg for women after the menopause and 2–25 µg for men(Ogunsola and Williams 1998). Average excretion values from a study amongst female inhabitants of a Roman condominium were 32 and 14 µg per day of conjugated E1 and E2 (D'Ascenzo, Di Corcia et al. 2003). Women can excrete with urine (0.9-1.2L/d per person) about 7 µg of E1 and 2.4 µg of E2 of unconjugated forms daily(Adlercreutz, Fotsis et al. 1986). Approximately 0.4 µg E2 and 0.5 µg E1 is eliminated in faeces (70-140g/d per person) (Adlercreutz, Gorbach et al. 1994). The amount of E2 used for pharmaceutical purposes contributes less than 5% compared with the natural E2 excretion(Christensen 1998). The majority of these estrogens are excreted from the human body within urine in a biologically inactive, conjugated form (predominantly as glucuronides and sulfates). The conjugates are more polar and water-soluble than free estrogens. And estrogenic activity decrease remarkably with the addition of conjugate group. However, because free estrogens have been observed in wastewater treatment plant effluent, this implies that deconjugation has occurred at some stage during or prior to sewage treatment(Sumpter 1998).

The synthetic EE2 is the main estrogen used in oral contraceptives, which is the most prescribed drug world-wide. In 2001, 43% of the female Dutch population in the age of 16–49 years used oral contraception(de Mes, Zeeman et al. 2005). The average daily dose of the synthetic hormone used in the contraceptive pill is 35 µg EE2, taken during 21 days of a 28 day period(Katzung 2000). Up to 80% of the EE2 digested is excreted as unmetabolized conjugates (Ranney 1977). Of the daily dose, 22–50% of EE2 is excreted in urine of which about 64% is conjugated and approximately 30% is excreted in faeces(Reed, Fotherby et al. 1972). The oral bioavailability of EE2 is about 42% due to an extensive first-pass metabolism in the intestinal wall and liver (Weber, Jager et al. 1996). More than 30% of EE2 is sulphated, which accounts for approximately 60% of the first-pass metabolism (Back, Breckenbridge et al. 1982). Only 1–2% of the administered EE2 has been found to be de-ethynylated and transformed to E1, E2 or E3(Ranney 1977). The contribution of EE2 to the total amount of excreted estrogens is only about 1%, but this compound is considerably more persistent in STPs compared to the natural hormones(Ternes, Kreckel et al. 1999; Ternes, Stumpf et al. 1999).

Estimations of the maximum concentration of natural estrogens present in wastewater are about 1 µg/l and for the synthetic EE2 about 13.4 ng/l(de Mes, Zeeman et al. 2005). This calculation is based on a wastewater production of about 200 L/d per person. In USA, another estimation of EE2 concentration (2.16ng/l) into the aquatic environment was produced based on the amount of pharmaceuticals in the United States(Arcand-Hoy, Nimrod et al. 1998). Measurements in municipal influents are generally lower than these estimated values, for example in the Netherlands, values were ranging from 20 to 130 ng/l for E1, from 17 to 150 ng/l for E2 and <0.3–5.9 ng/l for EE2 (Vethaak et al. 2002). The

samples were filtered first, so only the hormones in the liquid phase were measured, and no deconjugation step was applied, although a considerable amount of conjugated estrogens can be present in influents (58% of total E1 and E2 and 26% of EE2) (Adler 2001).

b. Estrogens from animals' excretion

Direct excretion of steroid hormones by animals into water courses, or discharges from farmyard drains, are likely to be more important sources of contamination rather than via normal agricultural scenarios. Different farm animals excrete estrogens by different routes: cattle mostly in their faeces (58%), whereas, swine excrete estrogens mostly (96%) in urine (Hanselman, Graetz et al. 2003). An individual normalized dairy cow excretes two orders of magnitude more, typically from 300 to 550 mg/animal per day(Sullivan and Lucas 1998), and a normalized pig excretes more than one order of magnitude more steroid estrogens than a normalized human. In terms of excretion, the combined farm animal population (including sheep and poultry) probably generates around four times more estrogens than the human population in the UK. The biggest contributor on the animal side is the relatively small dairy cow population (Johnson, Williams et al. 2006). A study which represents a search for evidence of steroid hormone contamination in streams associated with livestock farms also shows that fish in headwater streams on or near some livestock farms may be at risk of endocrine disruption(Matthiessen, Arnold et al. 2006). Even though some data indicate that E1 and E2 are not particularly persistent with lasting only a few days in soil amended with animal waste or in manure(Sullivan and Lucas 1998), the potential for estrogen loss to aquatic environmental is still large considering that cattle may produce 20–70 L of excreta/cow per day (de Boer, Smits et al. 2002)and that runoff or subsurface flow from agricultural land to freshwater frequently occurs during heavy rainfall events. Further, it is well documented that other components of animal faeces are regularly lost to freshwater (Baginski, Hale et al. 1988).

c. Estrogens from industrial wastewater and disposal

Industrial wastewater has been identified as the sources of many kinds of EDCs, such as BPA, PCBs, dioxins, surfactants and pesticides(Ferguson, Iden et al. 2001; Voutsa, Hartmann et al. 2006). There is so far no report found concerning steroid estrogens in industrial wastewater although it can be confirmed that there should be estrogen existence in wastewater discharged from some estrogen producers. BPA, alkylphenols, phthalic acid esters and nonylphenol have been detected in leachates from landfill for household waste, bulky waste, incombustible waste, and business waste(Wintgens, Gallenkemper et al. 2003; Asakura, Matsuto et al. 2004; Li, Seiffert et al. 2006). Again, there is no information available for estrogens in leachates from landfill.

d. Estimation of estrogen concentration in wastewater

Estimations of the maximum concentration of natural estrogens present in wastewater are about 1 µg/l and for the synthetic EE2 about 13.4 ng/l(de Mes, Zeeman et al. 2005). This calculation is based on a wastewater production of about 200 l per person per day. In USA, another estimation of EE2 concentration (2.16ng/l) into the aquatic environment was produced based on the amount of pharmaceuticals in the United States(Arcand-Hoy, Nimrod et al. 1998). Measurements in municipal influents are generally lower than these estimated values, for example in the Netherlands, values were ranging from 20 to 130 ng/l for E1, from 17 to 150 ng/l for E2 and <0.3–5.9 ng/l for EE2 (Vethaak et al. 2002). The samples were filtered first, so only the hormones in the liquid phase were measured, and no deconjugation step was applied, although a considerable amount of conjugated estrogens can be present in influents (58% of total E1 and E2 and 26% of EE2) (Adler 2001).

4 Occurrence of estrogens in aquatic environment

a. Estrogens in surface water

The occurrence of natural steroids hormones, including E1, E2, estriol (E3), EE2 and other EDCs in the aquatic environment has been documented in some reports(Ying, Kookana et al. 2002). One extensive survey of estrogenic steroids in 109 Japanese rivers and found E2 in 222 of 256 samples in summer with a mean concentration of 2.1 ng/l and in 189 of 261 samples in autumn with a mean concentration of 1.8 ng/l (Tabata, Kashiwa et al. 2001). One study from South Korea shows E1 was detected in some surface water sample with concentrations between 1.5 and 5.0 ng/L, while most other hormone compounds were observed consistently below detection limits in all sites(Kim, Cho et al. 2006). E1 was detected in 7 of 11 Dutch coastal/estuarine and freshwater samples with a median concentration of 0.3 ng/l, while E2 and EE2 were only detected in 4 and 3 of 11 samples, with most of the concentrations below the quantification limit of < 1 ng/l(Belfroid, Van der Horst et al. 1999). One recent report reveals that E1 was detected most frequently and at the highest concentrations in the water samples from Scheldt estuary, with concentrations ranged from the LOQ up to 10 ng/l (Noppea, Verslyckeb et al. 2007). Another integrated EDCs assessment in the Netherlands also shows that E1, E2, BPA and more than nine kinds of phthalates were detected in some surface water samples, suspended matter from surface water and sediment studied (Belfroid, Van der Horst et al. 1999). E3 was found in Tiber river water in Italy with a concentration of 0.33 ng/l, while E2 and E1 were 0.11 and 1.5 ng/l in the river water, respectively (Baronti, Curini et al. 2000). However, other investigators reported much higher concentrations of hormones in surface waters. For example, Kolpin reported E2, EE2, and testosterone to be present at averages of 9, 73 and 116 ng/L, respectively, in surface water in USA(Kolpin, Furlong et al. 2002). Bursch et al (2004) reported that endocrine effects on fish due to EE2 exposure cannot be excluded for majority of Austrian surface waters(Bursch, Fuerhacker et al. 2004).

Conventional drinking water treatment processes (e.g., coagulation and sand filtration) tested in this study was inefficient for the removal of micropollutants found in source water(Kim, Cho et al. 2006), even UV treatment (18000J/m^2) showed a low removal efficiencies(Bursch, Fuerhacker et al. 2004). Estrogenic steroids have been detected in some drinking water samples from southern Germany with an average concentration of 0.4, 0.7 and 0.35 ng/l, respectively(Kuch and Ballschmiter 2001).

b. Estrogens in groundwater

Recent studies have shown that disposal of animal manure to agricultural land could lead to movement of estrogenic steroids into surface and probably into ground water(Sullivan and Lucas 1998; Lange, Daxenberger et al. 2002). E2 has been found mobile and detected in runoff from manured land. From agricultural plots fertilised with animal excreta sex hormones can be washed out by rainwater. E2 in run-off from experimental plots treated with horse stall bedding (with E2 of 35µg/kg) from stalls that held mares between 12 and 16 weeks gestation at a rate of 9.1 t/ha and artificially watered at 64mm/h were 0.60µg/l E2, resulting in a calculated total run-off transport of 61 mg E2/ ha (Lange, Daxenberger et al. 2002). Ground water has been reported to be contaminated with E2(Shore, Correll et al. 1995; Peterson, Davis et al. 2001). Shore believed that a constant E2 concentration of about 5ng/l in spring waters was caused by infiltration of E2 through the soil profile to the ground water. Peterson measured E2 concentrations ranging from 6 to 66ng/l in mantled karsts aquifers in northwest Arkansas. The observed E2 concentration trends imitated the changes in stage over the recharge event. The contamination was associated with poultry litter and cattle manure waste applied on the area. Estrogens were also detected in groundwater in Austrain. E2 was detected in about one-half of the samples at concentrations higher than the LOQ. The maximum was 0.79 ng/l and the median was 0.07 ng/l. E1 exhibited the highest concentration of the hormones at 1.6 ng/l, but

it was detected in less than one-fifth of the samples. EE2 occurred above the LOQ in one of 112 samples at 0.94ng/l, which was probably contaminated by domestic runoff(Hohenblum, Gans et al. 2004). However, another study from Germany finds that estrogens were not detected in the groundwater samples (Ternes, Bonerz et al. 2007). In Braunschweig, wastewater has been irrigated continuously for more than 45 years. In the winter time only the effluent of the sewage treatment plant (STP) of Braunschweig is used for irrigation, while during summer digested sludge is mixed with the effluent. Groundwater samples were taken from wells in different locations to measure the concentration of estrogens and other micro-pollutants. The results indicates that there were no E1, E2 and EE2 found in any aqueous sample taken from groundwater under the irrigation area, although the loading in the summer period when sludge was mixed to the irrigation water, was 12.2 ng/l for the sum E1 + E2 and 1.1–1.6 ng/l for EE2. This indicates that steroid estrogens seem to be removed more than 90% when the irrigated water passes through the top horizon (upper 55 cm of topsoil).

EE2, E1 and E2 have been shown to rapidly adsorb irreversibly to agricultural topsoil and they are subsequently slowly mineralized in a model system without water flow , while EE2 has a tendency to be degraded slower than the natural estrogens by bacteria in both sludge and soil (Colucci, Bork et al. 2001; Colucci and Topp 2001). This could indicate the possibility that EE2 is leaking through the topsoil. Some laboratory experiments(Casey, Larsen et al. 2003; Das, Lee et al. 2004) exhibited that the natural estrogens were also able to penetrate through the packed topsoil in columns. However, the concentrations applied and the water flows are relative high in comparison to those found in some areas irrigated with STP effluent. For such low estrogens concentrations, only partial degradation or sorption occurred in the topsoil would already reduce the concentration below the LOQ.

5 Estrogens in source separated urine treatment system

The treatment efficiency of E2 in authentic activate sludge process is up to 80% and concluded that removal of the natural estrogen from municipal sewage is not enough under authentic activate sludge system. To avoid the problem of estrogen pollution, urine separation will be one of the promising choices(Matsuda, Matsui et al. 2001). And also because urine accounts for at least 50% of the phosphate and 80% of the nitrogen found in the wastewater although it accounts for only less than 1% of wastewater in volume(Wilsenach and van Loosdrecht 2006), urine is extremely burdensome for wastewater treatment plant. In fact, it is illogical to allow such a high strength wastewater flow to mix with other wastewater, as has occurred in sewers for more than a hundred years. There is growing support therefore for collecting and purifying urine separately. By separating urine, phosphate, nitrogen and other micro- pollutants are more effectively removed. Phosphate can even be reclaimed as a raw material.

In recent years, some urine-separating toilets have been installed in some eco-villages (i.e. Understenshöjden, Palsternackan Housing Estate), ordinary detached houses, apartment blocks and many schools in different parts of Sweden and the rest of Europe are now showing increasing interest in urine separation(Johansson 2004). The urine is collected in tanks in order to use it directly in agriculture. This strategy is useful not only for recovery of the nutrients and for prevention of water pollution, but also probably a solution for the problem of endocrine disruptor and other hazardous micro-pollutants in water environment. Considering of the safety of urine as fertilizer, a preliminary study published by the Swedish Environmental Protection Agency indicates that medication does not represent a significant environmental risk in connection with the use of urine as a fertilizer. Although there is no research concerning the behavior of hormone from human urine in agricultural environments, one study using different soils mixed with sheep urine, sheep manure and cattle manure reveals that E1 and E2 are not particularly persistent in soil especially when present in a natural matrix, lasting only a few days in most soils(Sullivan and Lucas 1998). However, another study indicates that both hormones have a high affinity to the organic fraction of the immobile phase leading to a high retardation within soil materials(Lange, Daxenberger et al. 2002).

Various urine treatment technologies for their performance to remove micropollutants such as pharmaceuticals, natural and synthetic steroid hormones, and their human biotransformation products had been assessed(Escher, Pronk et al. 2006). This research shows that filtration methods, such as nanofiltration and electrodialysis, were highly efficient with respect to toxicity reduction. Micropollutant degradation during biological treatment in a sequencing batch reactor (SBR) was very compound specific. Ozonation removed the target analytes and the estrogenicity completely, but the baseline toxicity was only reduced by 50-60% depending on the ozone doses. The results of this study present a method to assess the micropollutant removal efficiency, and therefore, support the choice of an appropriate urine processing technique for real-world applications.

Night soil is also one kind of source separated system for urine and faeces. Night soil treatment plant which collects a high amount of human urine and excreta is a very unique system in Japan. According to Takigami et.al (1998), night soil accepts quiet a high amount of human estrogens. Among them, E2 concentration in raw night soil showed a high level of 1200ng/L which was reduced remarkably to 0.12ng/l in the final effluent of a biological process followed by a tertiary treatment. E2 concentration in the sludge from raw night soil was 274ng/g-dry weight. The content of E2 in the activated sludge was almost constant (100ng/g) at four different tank, i.e. the denitrification tank, the nitrification tank, the second denitrification tank, and concentrated sludge. In the raw night soil, E2 occupied 16% of

the whole estrogenic activity. The calculated contribution of E2 became higher during the treatment despite the decrease in concentration. This suggested that E2 is relatively recalcitrant (or deconjugated from its conjugated form) and tend to remain in the aqueous phase compared with other estrogenic substance. The ration of E2 to the total estrogenic activity in the sludge samples tended to be higher (24.5%-63.5%) compared with the ratio of the aqueous samples (Takigami, Taniguchi et al. 1998).

6 Conclusions

Estrogens excreted by humans and animals enter the environment through the discharge of domestic sewage effluents and disposal of animal waste. Direct excretion of steroid hormones by animals is likely to be more important sources of contamination rather than via normal agricultural scenarios. Hormone steroids have been detected in wastewater effluents and surface water as well as ground water at various levels. The behavior and fate of these hormone steroids in the environment depend on their physiochemical properties and environmental media.

Urine separation might be one of the promising choices to deal with the problem of estrogen pollution in aquatic environment. Some urine treatment technologies such as filtration, ozonation, were highly efficient with respect to toxicity reduction. The night soil treatment system with a biological process followed by a tertiary treatment also showed a high estrogens removal rate. Although there are already some researches on the safety of urine as fertilizer, further researches on risk assessment are still needed.

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