First SWITCH Scientific Meeting
University of Birmingham, UK
9-10 Jan 2006

Elimination of pharmaceuticals from concentrated wastewater

K. Kujawa-Roeleveld, G. Zeeman

Sub-dpt. of Environmental Technology, Wageningen University, The Netherlands.

A. Mels

Urban Environment group., Wageningen University, The Netherlands

Abstract
Pharmaceuticals are consumed in high quantities world wide. The expectations are that these amounts will continue increasing because of a improving health care system and longer life expectations of people.
Administered pharmaceuticals are excreted by humans, as a parent compound or metabolite, with urine (mainly) and faeces. Combined with other components of sewage they enter a sewage treatment plant (STP) where they do not get removed to a satisfactory degree so finally they end up in the environment. The medical compounds detected in the environment (water, soil) have recently deserved much attention. As having specific properties they may provoke effects to the aquatic and terrestrial ecosystems even at very low concentrations. They also possess several common features like e.g. polarity or persistence that implicate poor removal and bioaccumulation. The retrofit of existing STPs to minimise the emission of pharmaceutical compounds to the aquatic environment is difficult: pharmaceuticals are present in trace concentrations (µg/L or ng/L) in large wastewater streams.
New sanitation concepts where wastewater streams are separated and treated according to their characteristics is an important source control strategy to avoid input of pharmaceuticals to the environment. Since pharmaceuticals are present mainly in a small wastewater stream (black water), a target treatment can be accomplished, when managing this stream separately. One of the advantages of this approach is that pharmaceuticals are present in the highest possible concentrations in a relatively small stream.
Knowledge on the fate of pharmaceutical compounds during different wastewater treatment units is very incomplete. On the removal of the pharmaceuticals from the concentrated, source separated wastewater streams knowledge is even more limited. This research has a main objective to investigate the fate of pharmaceuticals during biological degradation of concentrated wastewater streams followed by a physical-chemical post-treatment, if necessary. The most promising overall wastewater scheme for removal of pharmaceuticals will be formulated at the end of this 3-years research.

Keywords: human pharmaceuticals, urine, black water, biological treatment, physical-chemical treatment

* Corresponding Author: Katarzyna.Kujawa@wur.nl

1 Introduction

According to EU definition a pharmaceutical, or a drug or a medicinal product 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

Human pharmaceuticals comprise a large variety of chemical structures answering a wide array of medical needs; approximately 3000 active components are used in Europe. Pharmaceuticals are consumed in high quantities world wide. The expectations are that these amounts will continue increasing because of a improving health care system and longer life expectations of people.
Classification of pharmaceuticals is complex because different groups have different preferences for the base for classification. The following is taken usually into account for classification of pharmaceuticals: chemical structure, pharmacological activity, physiological classification and receptor interaction (Williams 2005)

Each pharmaceuticals 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. Administered by humans pharmaceuticals are excreted, as a parent compound or metabolite, with urine (mainly) and faeces. Combined with other components of sewage they enter a sewage treatment plant (STP) where they do not get removed to a satisfactory degree so finally they end up in the environment. The medical compounds detected in the environment (water, soil) have recently deserved much attention. As having specific properties they may provoke effects to the aquatic and terrestrial ecosystems even at very low concentrations. They also possess several common features like e.g. polarity or persistence that implicate poor removal and bioaccumulation. The retrofit of existing STPs to minimise the emission of pharmaceutical compounds to the aquatic environment is difficult: pharmaceuticals are present in trace concentrations (µg/L or ng/L) in large wastewater streams.

New sanitation concepts where wastewater streams are separated and treated according to their characteristics is an important source control strategy to avoid input of pharmaceuticals to the environment. Since pharmaceuticals are present mainly in a small wastewater stream (black water), a target treatment can be accomplished, when managing this stream separately. One of the advantages of this approach is that pharmaceuticals are present in the highest possible concentrations (Tabel 1) in a relatively small stream.

Table 1: Comparison between concentrations of excreted pharmaceuticals (examples) in source separated urine, black water (calculated, max possible) and in an influent to a STP from a combined sewer (measured) in µg/L as an argument to treat concentrated streams separately in order to remove pharmaceutical compounds

Example compound	Urine[1]	Black water[1]	Influent STP, combined sewer
Ibuprofen (analgesic)	80 000	16 000	27
Carbamazepine (anti-epileptic)	13 000	2 700	0.25-2.2

[1] the worst case scenario was calculated assumed that all people administer a given pharmaceutical compound at a max defined daily dose (WHO 2006) and a percentage excreted is a parent compound.

In this study a pre-selection was made for the compounds deserving an attention. The criteria taken into account were: yearly consumption, reported occurrence in the environment, potential risk for aquatic life, behaviour in a treatment situation and availability of analytical methods. Furthermore an

extended literature survey is being conducted on the fate of pharmaceuticals in the various compartments of environment with a special focus on wastewater treatment systems. This will serve as a strong base to design and perform a series of degradation experiments (biological, physical-chemical) for selected representative compounds in concentrated wastewater streams (urine, black water). This research will be completed with definition of the most promising treatment scenario to minimise (or eliminate) emissions of pharmaceuticals to the environment.

2 Selection representative compounds

2.1 Types

There are several major therapeutical groups of pharmaceuticals like antibiotics, analgesics, anti/inflamatories, anti-depressants, anti-epileptics, anti-astma agents, bronchitis, beta-blockers, cholesterol (lipid) lowering agents, cytostatics, X-ray contrast media.
Antibiotic are widely used to treat many bacterial infections. An analgesic (a painkiller) is any member of the diverse group of drugs used to relieve pain and to achieve analgesia. Anti-inflamatories (antiphlogistic) drugs and pain killers are agents, which are applied in medical therapy for relieving pains, fevers and against inflammatory caused by various diseases. Both belong to a group of a non-steroidal drugs, usually abbreviated to NSAIDs; drugs with analgesic, antipyretic and anti-inflammatory effects – to reduce pain, fever and inflammation. Anti-depressants (sedatives, tranquilizers, anti-depressants, anxiolytics, soporifics, sleeping pills, downers, or sedative-hypnotics) are substances, which depress the central nervous system, resulting in calmness, relaxation, reduction of anxiety, sleepiness, slowed breathing, slurred speech, staggering gait, poor judgment, and slow, uncertain reflexes. 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. Lipid-lowering drugs reduce serum cholesterol levels by inhibiting a key enzyme involved in the biosynthesis of cholesterol. Cytostatic drugs are used to treat cancer in a chemotherapy. Radiocontrast agents (or X–ray 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.

Pharmaceuticals are compounds characterised by more complex chemical structures. Most pharmaceuticals are charged and hydrophilic. To predict the rates of transformation in WWTP tests must consider the relevance of the inoculum as well as the potential for cometabolic processes.
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 (Williams 2005).

2.2 Drug metabolism in a human body

Pharmaceuticals undergo a number of enzymatic transformations (metabolism) in human tissues including liver, intestine, kidney and lung. The body responds to remove or detoxificate foreign substances by metabolising the pharmaceuticals. 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).
Thus the drugs are transformed to more polar compounds enabling their excretion. Usually water soluble metabolites are excreted but, in some cases, also unmetabolised compounds. 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). Attention needs to be paid therefore in any studies on both, parent compound and metabolites.

2.3 Consumption

Pharmaceuticals for human treatment are used in high quantities. Which volume will enter sewage depends on the amounts consumed and excretion rate of given substance. In Table 2 the consumption of all groups of pharmaceuticals (in number of users) in the Netherlands in the last 5 years is given.

Table 2: Users per ATC group of pharmaceuticals (* 1000) in the Netherlands (CVZ 2006)

	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

From the highlighted groups in Table 3 a number of specific compounds is 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 1 a consumption of these specific compounds is shown.

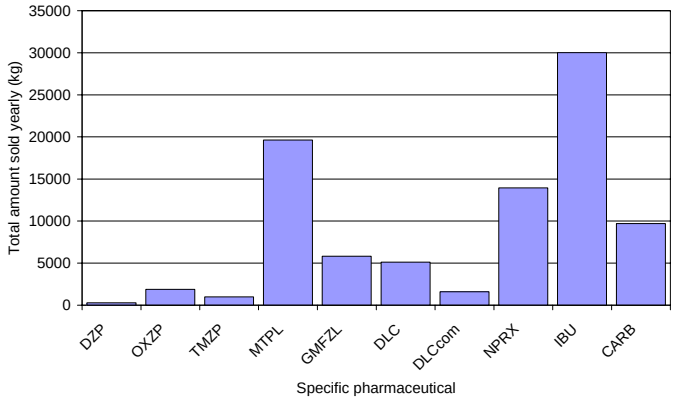
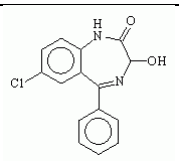
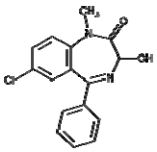
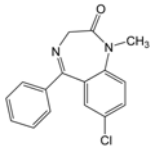
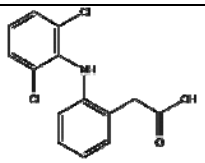
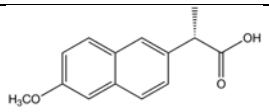
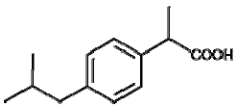


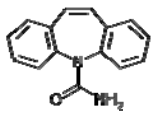
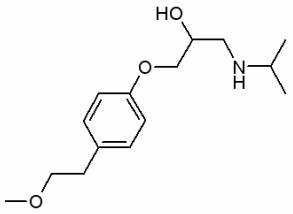
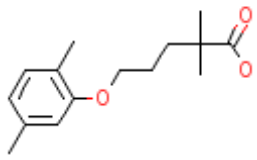
Figure 1: Total consumption (assumed that sold is consumed) of some specific pharmaceuticals in the Netherlands (source CVZ, 2006). DZP – diazepam, OXZP – oxazepam, TMZP – temazepam, MTPL –

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 3.

Table 3: 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

ATC – anatomical therapeutic chemical classification; Kow – octanol-water coefficient

3 Biological degradation tests

Biodegradation (in WWTP, natural aquatic systems, soil, sediments) is the most important process resulting in transformation (major structural changes) of pharmaceutical compounds. Biodegradation tests will be performed using inocula of bacteria isolated from activated sludge WWTP. The nutrients, pH and redox conditions affect activity of microbial community and the rate of transformation of the compound. Sludge age has an impact on the specific degradation activity by three independent ways, by influencing: (1) the biodiversity, (2) inert material content in the sludge, (3) sludge production.

There is diversity of species present within different inocula from different environmental compartments (also from different WWTPs of different configurations, receiving different type of wastewater). Inocula contain generalists (Williams 2005) able to convert easily degradable chemicals present usually in high quantities. These organisms grow fast and can adapt easily to a new test compound especially when this is present in high concentration (= not corresponding to real concentration in the environment). On the other hand inocula can contain specialists growing in a narrow range of environmental conditions. When test is performed with high concentration of a test substrate generalists will outcompete specialists. Based on above the biodiversity will vary in each test and will influence the rate of conversion of pharmaceuticals. The rate of conversion depends also on the concentration of biomass and environmental conditions such as T, pH, DO, substrate concentration.
These and other factors will be taken into account when designing biodegradation experiments.

4 Physical-Chemical degradability

It is expected that biodegradation of the selected compound will be insufficient and additional treatment step needs to be added.
Micropollutants, like pharmaceuticals can be oxidised with chlorine, chlorine dioxide, ozone (O_3), or OH radicals (advanced oxidation processes, AOPs,. In the case of ozone, the reaction can take place directly with ozone or with the secondary oxidants (e.g. OH-radicals) formed during ozonation (Maurer 2006). In the first instance oxidant reacting specifically with pharmaceuticals will be selected. Wastewater to be subjected to oxidation will probably contain still significant amount of COD (e.g. source separated urine, biologically treated black water).

As most of the compounds tested show enhanced reactivity towards ozone (Huber 2003), use of ozone seems to be preferable to advanced oxidation processes because a larger fraction of the oxidant (OH radical) is lost to the matrix in the latter. A series of oxidation batch tests will be performed applying various oxidation techniques (e.g. ozone, AOPs) to remove pharmaceuticals from different wastewater streams. Rate constants for the reaction of pharmaceuticals with given oxidant will be determined. Attention will be also paid on formation of oxidation products (toxicity, identification of the compounds).

5 Analytical methods

The three important difficulties playing the role in establishing of a reliable method for pharmaceuticals detection are: elevated polarity, low concentration, complex matrix (concentrated wastewater (black water), sludge, wastewater).
The problem of matrix is that target compounds interfere with other substances such as e.g. humic acids. In source separation sanitation concept, relevant wastewater streams are characterized by significantly higher initial concentration of pharmaceuticals than in conventional system (mg or µg per liter vs. µg or ng/L) but also more complex matrix.
One of the most difficult steps during analysis of micropollutants is the difficulty in separating and enriching water soluble, polar compounds from a complex matrix as wastewater. Solid phase extraction (SPE) methods are commonly used.
So far most of the analytical methods reported in literature were based on GC-MS, which often requires derivatization of pharmaceutical compounds. In the last years LC-MS and LC-MS-MS is indicated as the technique of choice to assay polar pharmaceuticals and their metabolites, and is especially suitable for environmental analysis because of its selectivity.
In this study LC-MS-MS method will be used to quantify fate of pharmaceuticals during various degradation steps.

References

[1] (EU 2004). Directive 2004/27/EC of the European Parliament and of the Council of 31 March 2004 amending Directive 2001/83/EC on the Community code relating to medicinal products for human use (Text with EEA relevance). Official Journal L 136, 30/04/2004 P. 0034 - 0057. 32004L0027.
[2] CVZ. (2006). "Farmacotherapeutisch Kompas."

[3] Huber, M. M., Canonica, S., Park, G.Y. and Gunten, von, U. (2003). "Oxidation of Pharmaceuticals during Ozonation and Advanced Oxidation Processes." Environ. Sci. Technol. **37**: 1016-1024.
[4] Maurer, M., Pronk, W. and Larsen, T.A. (2006). "Treatment processes for source-separated urine. A review." Water Research **40**: 3151 – 3166.
[5] Ternes, T. A., Hirsch, R., Mueller, J. and Haberer, K. (1998). "Methods for the determination of neutral drugs as well as betablockers and ,2-sympathomimetics in aqueous matrices using GC/MS and LC/MS/MS." Fresenius J Anal Chem (1998) **362**: 329–340.
[6] WHO, Ed. (2006). ATC classification index with DDDs. Oslo, Collaborating Centre for Drug Statistics Methodology WHO.
[7] Williams, R. T. e. (2005). Human pharmaceuticals. Assessing the impacts on aquatic ecosystems., Society of Environmental Toxicology and Chemistry (SETAC).