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Pharmaceutical Input and Elimination from Local Sources

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Final report of the European cooperation project PILLS

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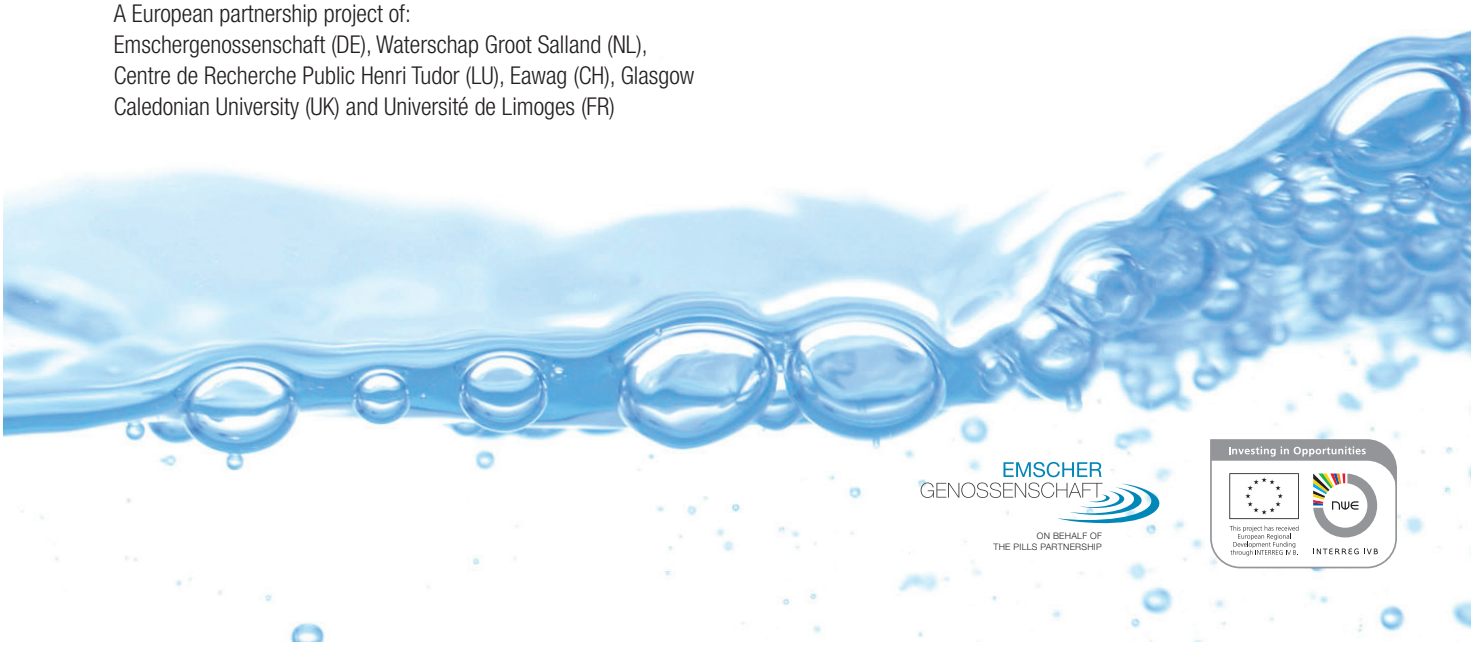

EMSCHER GENOSSENSCHAFT
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Chapter 1

Introduction and summary

1. Introduction and summary

To help to prevent or cure diseases or make our life more convenient l large quantities of various pharmaceutically active substances are manufactured today for the protection of humans and animals. As a result of improved medical care, rising life expectancy and the progressive industrialization of agriculture, an increasing amount of medicinal products are consumed. About 3.000 pharmaceutical active substances have permits in Europe. These products are, however, in many cases not completely absorbed and metabolized by the patient but partially excreted. Therefore traces of the products reach the water cycle.
It is not only the growing use of medicinal products that has led to an increased awareness of this topic however: Thanks to enormous advancements in chemical analysis technologies, many pharmaceutical residues can now be determined in water at extremely low concentrations, often many times lower than was possible several years ago. As a result, concentrations can now often be detected in the nanogram per litre range.
The concentrations of pharmaceutical residues, which are detected in the water, are very low and according to the current state of knowledge are not harmful to humans. However, for many substances it is unclear what effects these residues have on the water habitat – for example on micro organisms. Other micropollutants are already known as harmful for the environment.
The EU published the new draft annex for the Water Framework Directive in Jan. 2012 and if this WFD annex passes the Council and Parliament, all member states' surface waters have to meet the environmental quality standards for the priority substances by 2021 – for the first time including pharmaceutical substances as well. How the reduction of these substances in the environment shall be realized is currently an open question – it may happen by waste water treatment, prohibition of substances or other options

Regarding the PILLS project one approach to deal with pharmaceutical micropollutants was the investigation of point source treatment:

- Which impact may point source treatment have on the load of specific substances that are mainly consumed in hospitals?
- Which techniques are appropriate to reduce the concentrated discharge at hospitals and care homes?
- What is the effect of this cocktail of pharmaceuticals on the bacteria population in the waste water treatment plants, in terms of the spread of multi-resistant bacteria?

To find answers to these questions the work in the PILLS project focussed on the following project components:

- Characterisation of waste water from point sources (hospital waste water) including chemical analysis in order to investigate the concentration and load of selected pharmaceuticals in the waste water and bioassays for the evaluation of the ecotoxicological potential and the relevance of antibiotic resistance bacteria of the point source waste water.
- Design, construction and operation of waste water treatment plants at hospital locations which incorporate advanced treatment technologies. Two full-scale pilot plants, two small-scale built pilot plants and lab-experiments were implemented in which various advanced treatment techniques (activated carbon, ozone, UV/ozone, UV/H_2O_2, (UV)/ H_2O_2/Fe^{2+} or Fe^{3+}, UV/TiO_2, reversed osmosis, ferrate (Fe(VI)) were investigated after the biological waste water treatment in membrane bioreactors.
- Assessment of different advanced treatment technologies regarding the elimination efficiency of pharmaceuticals and the reduction of ecotoxicological effects and antibiotic resistant bacteria as well as the evaluation of the costs and the overall environmental impacts (using a life-cycle assessment methodology).
- Communication of the issues and of the results of the project through an exchange of information in the scientific and political field. The topic was also brought to the attention of the broader public to make them aware of the issues.

The findings of the PILLS project can be summarised as follows:

Characterisation of hospital wastewater

Hospitals can be seen as a "hot-spot" of pharmaceutical emission because here there is a high load of pharmaceuticals used and emitted through hospital waste water into the municipal sewerage. However, the fraction of the total pharmaceuticals distributed in hospitals compared to what is distributed in the communities is relatively low (and estimated by around 20%). But, certain pharmaceuticals (X-ray contrast media, cytostatics and some antibiotics) are distributed in much higher amounts in hospitals than at home.

The contribution of the hospital is different for each waste water treatment plant catchment, depending on the amount of beds and natural inhabitants connected to the facility. The range normally found varies between 5-50 beds per 1000 inhabitants. Geriatric hospitals do not emit the expected high load because of the use of liners/diapers/pampers.
The ecotoxicological risk of hospital waste water is higher than of municipal waste water. The proportion of multi-resistant bacteria (measured by integrons) in the bacterial community is higher in hospital waste water than in municipal waste water. Therefore risk potential is caused by pathogens and antibiotic resistant bacteria in hospital waste water, e.g. because sewer overflows from municipal sewer systems may lead to discharge of hospital waste water into the receiving waters with a potential risk of spreading the mentioned resistant bacteria and pathogens. Treating hospital waste water at the source reduces these risks for groundwater and surface water bodies.

Assessment of waste water treatment techniques

The biological waste water treatment in a membrane bioreactor (MBR) leads to a good wastewater quality in terms of COD, nutrients and bacteria. But, advanced treatment is necessary to eliminate most pharmaceuticals from waste water as biological treatment is not enough. Advanced treatment with ozone and/or activated carbon or UV/H2O2 or reverse osmosis is effective to achieve this elimination. With the advanced treatment with ozone or PAC an elimination of 80% can be achieved for most of the investigated compounds, but not for all of them. Activated carbon filtration with a fresh GAC filter and RO led to high elimination rates for all compounds.
Decreasing ecotoxicological effects are observed by the treatment with MBR and the advanced treatment techniques in various bioassays. But, also increasing toxicity is measured after oxidation processes, presumably due to transformation products. These negative effects could be reduced by a subsequent biofilter or GAC filter, but not it totally in the subsequent sand filter. Antibiotic resistant integrons are significantly reduced by MBR treatment (with ultrafiltration membranes). Additional advanced waste water treatment by ozonation or activated carbon after the MBR with ultrafiltration membranes had no significant influence on the reduction of the resistant integrons.
From life cycle analysis (LCA) point of view the toxicity impact of pharmaceuticals has been observed to be negligible compared to other impacts, like nutrient removal (with the effect of avoided eutrification). The Comparison of the advanced treatment technologies (from best to worst) considering the overall environmental impacts in a LCA results in the following order: ozone (by low energy consumption) > activated carbon > ozone (by high energy consumption) > UV. Besides the above described topics also criteria, e.g. like energy consumption, costs and local aspects and others may contribute to support decisions for decentralized hospital waste water treatment.

Further actions for a sustainable reduction

The PILLS cooperation leads to increase in understanding of the issue, both in scientific terms and amongst the various communities (political, operational and public). It is possible to eliminate pharmaceuticals at one important points of use, the hospitals, and it makes sense to do so from an ecotoxicological and multi-resistant bacteria point of view. However, at the current state of knowledge waste water treatment either centralized or decentralized will be expensive. And, waste water treatment is not able to reduce the burden to the environment sustainably. Once these micropollutants have reached the waste water, their complete elimination is hardly reasonable – even if, in many cases, a low enough concentration is achieved so that their appearance is below the detection limit, or they have no (measurable) effects.
Alternative approaches should be investigated taking into account the entire life-cycle of the sub-stances examined, from the production, to the points of use, to the disposal.

Possible measures to minimise pharmaceutical residues at the source could be:

- Legislative body: The creation of incentives which promote the use of more environmentally friendly substances in the manufacture of medicinal products. Furthermore, establishing a framework for the emissions of pharmaceutical substances to the environment would be a good first action.
- Pharmaceutical industry: Taking into consideration the possible environmental effects of individual active substances already in their development and performing targeted research in this field.
- Health professionals: Further training for health professionals concerning the long-term change of prescription practice so that overall, fewer or – where possible – “more environmentally friendly” medication is used.
- Medical centres, hospitals and nursing homes – so-called point sources: Waste water separation and local treatment of the waste water where high concentrations of pharmaceutical residues are encountered.
- Waste water management companies and drinking water providers: Advanced waste water treatment and improved drinking water purification helps to eliminate residues.

Chapter 2

Selection of pharmaceuticals and analysis of hospital wastewater

Contents

List of Abbreviations

A&E	Accident and Emergency
AF	Assessment Factor
BOD	Biological Oxygen Demand
BGH	Borders General Hospital
CAS	Conventional Activated Sludge
CH	Switzerland
CHEM	Centre Hospitalier Emile Mayrisch
CT	Computer Tomology
DE	Germany
EMEA	European Medicines Agency
GP	General Practitioner
FR	France
GWRC	Global Water Research Council
HH	Huntlyburn House
LU	Luxembourg
MBR	Membrane Bio Reactor
MHG	Marienhospital Gelsenkirchen
ML	Melburn Lodge
MRI	Magnetic Resonance Imaging
NL	The Netherlands
NOEC	No Effect Concentration
NSAID	Non Steroidal Anti-inflammatory Drugs
PNEC	Predicted No Effect Concentration
SPE	Solid Phase Extraction
SRM	Selected Reaction Mode
SS	Suspended Solids
WI	Western Infirmary
WWTP	Wastewater Treatment Plant

The partner hospitals

Germany: Marienhospital Gelsenkirchen (MHG)

The German pilot plant receives wastewater from Marienhospital Gelsenkirchen. With 580 beds, about 1,150 employees and up to 75,000 in-patients & out-patients per year, the Marienhospital Gelsenkirchen is one the biggest hospitals in the densely populated Ruhr area. The current building of the hospital was built in 1972. It is an university teaching hospital since 1978. The hospital offers the whole range of medicinal services, e.g. internal medicine (cardiology, hematology, gastro-enterology), oncology, surgery, gynecology, otolaryngology (ENT), pediatrics (neonatology), orthopedics, urology, nuclear medicine, and radiology, including computer tomography (CT) and magnetic resonance tomography (MRT). The fresh water consumption of the hospital was 73,000 m^3 in 2010 (200 $m^3 day^{-1}$). In a project parallel to the PILLS project about 12,000 m^2 impervious areas were disconnected from the combined sewer system. Thus rainwater inflow to the pilot plant is reduced to a minimum.

2.1.2 The Netherlands: Isala Clinics

The ISALA clinics in Zwolle are situated at two locations: Weezenlanden and Sophia.
Currently, the location Sophia is extending in order to concentrate all departments at one location while Weezenlanden will ultimately be closed. Isala is the 5th largest non-academic hospital in the Netherlands. It employs 5,700 people, has 1,076 beds, 470,000 polyclinic visits and 40,000 hospitalisations per year. Wastewater flow is estimated to be 87,600 m^3 per annum (240 $m^3 day^{-1}$).

2.1.3 Switzerland: Cantonal Hospital of Baden

The cantonal hospital of Baden is a typical, regionally important general hospital in Switzerland with 346 used beds serving more than 250,000 inhabitants. In 2009, there were 126,328 “days of care” and 17,163 patients leaving the hospital. The whole range of medical services is offered, e.g. internal medicine, oncology, surgery, maternity clinic, nuclear medicine, and radiology, including CT and MRI. In 2008, 11,767 CTs were carried out, of which 7,490 were with X-ray contrast media; and 3,154 MRIs (2,691 with X-ray contrast media). Around two thirds of these X-rays were carried out on out-patients.
In 2009, 203,368 m^3 of water was used in total and 84,987 m^3 (233 $m^3 day^{-1}$) in the main hospital wing that hosts patients, where pharmaceuticals are excreted. At this location, the wastewater from the restaurant is included, but not that from the laundry facility.

2.1.4 Luxembourg: Centre Hospitalier Emile Mayrisch (CHEM)

The Luxembourg partner hospital is the Centre Hospitalier Emile Mayrisch (CHEM) in Esch-sur-Alzette. CHEM has 360 beds. In 2010, total water consumption was around 6,100 73200m^3, equivalent to 201$m^3 day^{-1}$, of which 141$m^3 day^{-1}$ was used in the main clinical hospital. Effluent is discharged from the hospital via four manholes. Sampling took place in two of these, representing departments of surgery, maternity, oncology, psychiatry, urology, internal medicine, intensive care unit and addiction medicine, with a total of 211 available of which on average 177 beds (84%) were occupied during the sampling period.

2.1.5 United Kingdom

2.1.5.1 UK: Borders General Hospital (BGH)

Borders General Hospital (BGH) is the largest hospital complex in the Scottish Borders area. The main building houses medical, surgical, geriatric, paediatric, stroke, palliative care, gynaecological, and labour wards, as well as 5 theatres and A&E. There is also a restaurant in the building.

Furthermore, there are other separate facilities – ‘Melburn Lodge’ (geriatric unit), ‘Huntlyburn House’ (psychiatric unit), a crèche, a laundry, and 50 residential properties (mainly housing for nurses) on site.

The main hospital building has 300 beds and occupancy in 2009/10 was 77%. Water usage for the entire complex (including the lodges, laundry, crèche and residential properties) was 63,737 m^3 in that year, which gives an estimated effluent volume of (95% x supply) 60,550 m^3 (166 m^3day^{-1}). Sampler 1 (BGH) was located in a pumping station catching effluent from the entire complex described above.

2.1.5.2 UK: Melburn Lodge and Huntlyburn House (ML+HH)

Melburn Lodge, the geriatric facility, had 16 beds in 2009/10, an occupancy rate of 85%, and a water usage of 857 m^3, so an estimated effluent volume of 814 m^3 (2.2 m^3day^{-1}). Huntlyburn House had 26 beds at 80% occupancy and used 2,430 m^3, giving an annual effluent volume of 2,308 m^3 (or 6.3 m^3day^{-1}).

Sampler 2 (ML+HH) was located to catch combined effluent from Huntlyburn House and Melburn Lodge only, so it is a substream of the effluent arriving at Sampler 1.

2.1.5.3 Western Infirmary (WI)

The Western Infirmary is a general hospital with A&E facilities in the West End of the city of Glasgow.

The hospital complex comprises a number of buildings, housing clinical wards, workshops, a mortuary, an MRI scanning unit, labs and offices. There is no longer an oncology department at the Western Infirmary. The sampler catches the effluent from the main clinical building and an office building. The main building houses surgical, renal, vascular, orthopaedic, medical, and cardiac wards, an intensive therapy unit, a high dependency unit, an X-ray department, A&E, as well as a dining room, catering, a shop and an enquiries and administrative section. There are 318 beds in the building and occupancy is 100%.

In 2010-11, the total supplied volume of water was 78,238 m^3. To allow for boiler and staff losses, the effluent volume is calculated as 95% of supply, or an estimated 74,326 m^3. This is equivalent to 204 m^3day^{-1}.

2.1.5.4 UK: Drumchapel Hospital (DRUM)

Drumchapel Hospital, in the west of the city of Glasgow, has 120 beds. It provides stroke, general and ortho-geriatric rehabilitation services for older patients; all are in "long-stay" care. There are also day patients services for physiotherapy, podiatry and occupational therapy. A small internal laundrette and a canteen are included in the water supply and effluent figures.

Water consumption in 2010 was 9,944 m^3, giving an estimated effluent volume (95% x supply) of 9,447 m^3, or 79 m^3 per bed. This was slightly higher than normal due to a burst pipe in Nov-Dec 2010.

2.1.6 FR: Dupuytren Hospital

The Limoges university hospital centre is divided into 4 structures; this study was conducted at the Dupuytren Hospital which represents 70% of all Limoges' clinical activities. The Dupuytren Hospital provides 869 patient beds and the water consumption reaches 923 m^3day^{-1}. The hospital effluent samples analysed in this study were collected from the sewerage system which comprises only sewers from clinical activities of the hospital. During sampling the average raw inflow during the week and week-end was 813.6 m^3day^{-1} and 405.6 m^3day^{-1}, respectively.

2.1.7 A note on dilution

Expected concentrations in wastewater are influenced by the dilution factor provided by water usage in the hospital: higher water usage results in reduced concentrations of pollutants in the wastewater. High concentrations of pharmaceuticals may inhibit biodegradation in wastewater treatment (Kujawa-Roeleveld and Schuman, 2009). However, Joss (2006) expects dilution of municipal wastewater (e.g. by extraneous water) to reduce the degree of biological removal and states that "wastewater segregation and treatment at the source are therefore to be favoured for elimination of persistent micro-pollutants over centralized end-of-pipe treatment" (Joss et al., 2006).

As outlined above, some of partner hospitals had restaurant and / or laundry facilities and in some cases samples would necessarily contain some rain water ingress. The figures below refer to the wastewater stream at the point of sampling. In some cases (FR and CH) the hospital wastewater arriving at the wastewater treatment plant (WWTP) would be more dilute than at the point of sampling because of the laundry contribution. In the UK hospital, the drainage was designed so that no rain would ingress into the wastewater stream, however, larger flows were observed in wet periods.

2.1.8 Facilities overview

In summary, water usage and number of beds for each hospital are given in table 1. The wastewaters' wet chemistry is characterised in Ch 3.4.

Table 2-1: Water usage and number of beds in partner hospitals

Partner	Year	Water usage (m^3/a)	Number of beds	Comments
GE	2010	73,000	580	Including restaurant
NL	–	87,600	1,076	Assumed water flow
CH	2009	84,987	346	Including restaurant, excluding laundry. Bed number accounts for occupancy rate.
LU	2010	51,400	360	Excluding water used by cooling towers
UK- BGH	2010	63,767	265	(Bed number accounts for occupancy rate)
UK – ML+HH	2010	3,122	34	(Bed number accounts for occupancy rate)
UK- WI	2010	47,400	318	(Bed number accounts for occupancy rate)
UK- DRUM	2010	9,447	120	(Number of available beds)
FR	2008	157,183	863	Available beds. Excluding laundry, restaurant, hotel.

2.2 Selecting pharmaceuticals for monitoring in the PILLS project

2.2.1 Selection process

As there are thousands of different licensed pharmaceutical products, with many hundreds of active compounds, a selection for monitoring needed to be made. To achieve this, a 'common list' was established of compounds to be analysed by all partners to enable comparison.

Key criteria for selection were environmental risk and likelihood for each compound to occur in hospital wastewater. Partners were asked to suggest compounds, which resulted in a 'long list' containing 134 compounds. This list featured 97 pharmaceutically active compounds, 11 contrast media, 10 metabolites and also a number of other compounds such as personal care products. Partner suggestions were generally based on local hospital usage, national hospital usage, previous measurements, known high toxicity, national and international priority lists and specific research interests. Subsequently, the list was circulated amongst the partners and feedback, usage data and expressions of interest were received. Key parameters relevant to environmental risk were identified and a literature study was carried out. Commonality amongst the partners' choices, prior concern expressed by environmental organisations and researchers, and further information from literature were decisive to come to a selection of 15 pharmaceuticals for monitoring: atenolol, carbamazepine, diclofenac, naproxen, lidocaine, ifosfamide, cyclophosphamide, ciprofloxacin, erythromycin, clarithromycin, sulfamethoxazole (and its metabolite N-acetyl-sulfamethoxazole), iopromide, iopamidol, diatrizoate and bezafibrate. Four key parameters were identified, for which values were sought for all selected compounds: usage, excretion rate, removal in WWTP and Predicted No Effect Concentration (PNEC). Because of their relevance to behaviour in conventional WWTP processes, log K_{ow}, pK_a and K_d values were also sought. Individual partners monitored additional compounds according to local interest, with Switzerland monitoring in total 68 pharmaceuticals, the Netherlands 99, Germany 120 and Luxembourg 17.

In January 2012, diclofenac was proposed for inclusion as a Priority Substance in Annex II of the Priority Substances Directive EC/105/2008. An earlier proposal included diatrizoate, carbamazepine and iopamidol (European Parliament, 2007). All of these had been suggested for monitoring by several partners and were therefore included for monitoring on the basis of commonality.

Several other organisations and authors have prioritised compounds for environmental monitoring. The long list was cross referenced with the following studies:

- OSPAR List of Chemicals for Priority Action, Update 2007 (OSPAR, 2007)
- Report No. 161d, Stoffliste Rhein 2007 (Internationale Kommission zum Schutz des Rheins, 2007)
- Environment Agency Priority List (Environment Agency, 2003)
- (Hanisch et al., 2004)
- (Castiglioni et al., 2006)
- (Sattelberger, 1999)
- Federal state of Saxony, Germany: Engelmann et al. (2009)
- Germany: LANUV/IWW (Hembrock-Heger and Bergmann, 2007)
- GWRC Priority Setting (De Voogt, 2009)

Retrospectively, the PILLS selection was also cross referenced with a study commissioned by the Global Water Research Coalition GWRC (De Voogt, 2009). This study yields a representative and qualitative profile ('umbrella view') of priority pharmaceuticals based on an extensive set of criteria and data derived from 25 previous prioritisation studies. It identifies 10 High Priority Pharmaceuticals, 18 Priority Pharmaceuticals and 16 Lower Priority Pharmaceuticals. De Voogt selected the following criteria: regulation, consumption / sales, physico-chemical properties, degradability / persistency, human toxicity, ecotoxicity, occurrence in groundwater / surface water / drinking water, occurrence in wastewater. A high correlation with the GWRC priority setting is shown, validating the partners' choices. Eight out of the ten High Priority Pharmaceuticals feature in the PILLS selection; only ibuprofen and gemfibrocil do not. Both are widely used in the community. The PILLS project has a different focus, in that it primarily considers the hospital environment, that it has hospital contribution (to the total load for each compound) as a separate criterion and that the availability of an analytical method is also considered. All other compounds on the PILLS list were listed as High Priority Compounds in GWRC except lidocaine, iopamidol and ifosfamide. All of these are considered typical hospital drugs and therefore of special interest to PILLS.

In the following section, the letters CH (Switzerland), DE (Germany), NL (the Netherlands), FR (France), LU (Luxemburg) and UK (Scotland) indicate that specific reasons for inclusion were cited by the respective PILLS partners from these countries.

2.2.2 Selection by treatment group

Pharmaceuticals are usually classified according to their pharmaceutical purpose, e.g. antibacterials, analgesics, antineoplastics, etc. (Kümmerer, 2004). Although some treatment groups, such as cytostatics (Kümmerer, Steger-Hartmann & Meyer, 1997) are by design likely to affect non-target organisms, a similar pharmaceutical action does not always correspond with a similar chemical structure or a similar level of toxicity. Additionally, drugs may be indicated for more than one condition. The GWRC priority setting does not use representativeness of a drug group as a selection criteria, as "...from a scientific point of view there is no need for all different classes of pharmaceutical to be represented in the final priority list" (De Voogt, 2009). However, proposals for amendments to 'Directive 2001/83/EC with respect to Information to Patients, Pharmaco-vigilance and Counterfeit Medicines' include extension of the pharmaco-vigilance concept to include not only public health and patient health but also the environment (Wennmalm, Gunnarsson, 2009). In other words, medical professionals could be asked to take environmental effects into account alongside public and patient health when prescribing. Treatment class is then clearly relevant. Furthermore, pharmaceutical class might indicate relative hospital usage. Kümmerer (2008) notes cytostatics are mostly used in hospitals; contrast media and anaesthetics may also be considered typical 'hospital drugs' (although they may be administered to outpatients) whereas medication for chronic complaints is likely to be predominantly used in the community.

Although contrast media are technically not pharmaceutically active, they are considered relevant to the PILLS objectives as they are used in high quantities. Several other non-pharmaceutically active compounds on the 'long list' were excluded as these fell outside the scope of the project.

Based on the comments above and the pre-selections made by the partners, representation of the following classes was sought:

- Analgesics / anti-inflammatories
- Anticonvulsants / tranquilisers
- Beta blockers and anti-hypertensive
- Lipid regulators
- Anaesthetics
- Antibacterials
  - penicillins
  - cephalosporins
  - macrolides
  - fluoroquinolones
  - sulphonamides
  - tetracyclines
- Contrast media
- Cytostatics

The 'long list' also considered psychoactive drugs and anti-viral drugs but there was insufficient commonality amongst the partners to include drugs from these classes.

2.2.2.1 Analgesics and anti-inflammatories

Analgesics, or pain killers, are widely used and have been measured in high concentrations in sewage influent and effluent, as well as in surface waters (Derksen et al, 2001). For some analgesics the hospital contribution is relatively small, as these compounds can be bought over the counter. Anti-inflammatories are effective as pain killers by reducing swelling and inflammation. Most are non-steroidal.

The following compounds from this treatment group were selected from the "long" list for monitoring:

Compound	Reason for inclusion
Naproxen	• identified by 4 partners • MEC of 9300ng/l (Zorita, 2009) • Found in waste water and surface water (CH, NL, DE) • Up to 30% hospital contribution
Diclofenac	• selected by 5 partners • high consumption (CH, NL) • highest acute toxicity of Non-steroidal anti-inflammatory drug (NSAID) (Fent, 2006) • proposed as Priority Substance

Paracetamol and Ibuprofen were also considered, as they pose a high environmental risk; however as the hospital contribution for these is small compared to the community contribution they were not included.

2.2.2.2 Anticonvulsants / tranquilisers

Compound	Reason for inclusion
Carbamazepine	• selected by 4 partners • on 5 priority lists • persistent in standard waste water treatment (e.g. Ferrari, 2003)

Carbamazepine is not a typical hospital drug, but has been previously encountered in waste water and identified as a priority by several organisations. Diazepam was encountered in drinking water in Italy (Zuccato et al., 2000).

2.2.2.3 Betablockers / Anti-hypertensives

Betablockers are not typical hospital drugs, but may be used in above average amounts in care homes. Therefore, atenolol was selected as a representative.

Compound	Reason for inclusion
Atenolol	• Selected by 3 partners • Found in wastewater (CH) • on 3 priority lists

2.2.2.4 Lipid Regulators

Isidori et al (2007) studied the ecotoxicity of bezafibrate, fenofibrate and gemfibrocil and their photoproducts. Whereas acute toxicity was in the order of dozens of mg/L for all trophic levels utilised, chronic exposure to these compounds did show inhibition of growth population on rotifers and crustaceans at lower concentrations. Genotoxic and mutagenic effects were especially found for the gemfibrocil photoproduct. Isidori et al. suggest that by-products have to be considered. The PNEC value of 0.625 µg/L is based on a genotoxic effect (Isidori et al. 2007).

Clofibric acid has been encountered in Italian drinking water (Zuccato, 2000), albeit in quantities of only a few ng/L.

Compound	Reason for inclusion
Bezafibrate	• Selection by 4 partners • Found in wastewater (CH) • on 5 priority lists • on 5 priority lists

2.2.2.5 Anaesthetics

Lidocaine is a widely used anaesthetic and expected to have a high hospital contribution. It was therefore included in the list.

Compound	Reason for inclusion
Lidocaine	• Selection by 3 partners • Found in wastewater (CH) • Widely used anaesthetic • Expected high hospital contribution

2.2.2.6 Antibacterials

Antibacterials are a diverse group of chemicals that can be divided into subgroups such as β-lactams (including penicillins and cephalosporins), quinolones, tetracyclines, macrolides, sulphonamides and others. Their functionalities may be different and for the same compound they can differ under different environmental circumstances. In most countries, β-lactams account for approximately 50-70% of antibacterials. Some sorption to sludge occurs, but biodegradation is poor for most antibacterials (Kümmerer 2009).

Numerous papers on the occurrence of antibacterials in the aquatic system have been published. Concentrations are mostly in the same range; the higher µg/l range in hospital waste water, the lower µg/l range in municipal waste water and the ng/l range in surface water. Algae and of course bacteria can be highly sensitive to antibacterials; effects have been observed at low µg/l concentrations. Some fluoroquinolones are genotoxic (Derksen et al., 2001).

Compounds that have been analysed so far cover a number of classes, including macrolides, aminoglycosides, tetracyclines, sulfonamides, sulfanilamides and quinolones. β-lactams have not been covered despite accounting for the highest proportion of consumption. The concentrations found for β-lactams are low compared to othersthose expected given the extensive use. It is not clear whether that is because they are not present in the aquatic environment or due to possible analytical shortcomings and difficulties (Kümmerer 2004). β-lactams are not very stable and readily hydrolise (Hirsch et al., 1999; Längin et al., 2009). According to literature, the hospital contribution to total environmental load varies enormously depending on the compound, with only a few percent of erythromycin and roxithromycin contributed by hospitals compared to over 80% of ampicillin, penicillin G and vancomycin (Ternes 2006).

The following compounds were selected:

Compound	Reason for inclusion
Amoxicillin*1)	• selected by 4 partners • high concentrations expected • most used antibacterial (NL)
Ciprofloxacin	• selected by 4 partners • most used fluoroquinolone (NL, CH) • high concentrations expected in hospital • low PNEC
Clarithromycin	• selected by 4 partners • most frequently used macrolide (NL) • found in waste water (CH) • low PNEC

Erythromycin	• selected by 5 partners • on 5 priority lists
Sulfamethoxazole*2)	• selected by 5 partners • high concentrations expected (NL) • top sulfanomide (NL) • 4 priority lists (DE) • found in waste water (CH)

*1) It was agreed that Amoxicillin would be of interest, but there were concerns over the analyte stability. It was nonetheless planned to be monitored by 5 out of 6 partners and has therefore been left in the data tables accompanying this paper.

*2) The metabolite N-acetyl-sulfamethoxazole should also be measured. The excretion of sulfamethoxazole is mainly in the form of the metabolite, but there is a degree of conversion back to the parent compound during the wastewater treatment, therefore both were measured and reported as sulfamethoxazole after back calculation. Without the metabolite, it would not be possible to get a correct mass balance (Göbel et al., 2005)

Metronidazole is also known to have genotoxic effects (Kümmerer et al., 2004); however, as only one partner indicated an intention to monitor for this compound, it was not selected for the common list.

2.2.2.7 Contrast media

Radio-contrast agents are used to improve the visibility of internal body structures in X-ray based imaging techniques such as computerised tomography (CT) or radiography. Most contrast media are water soluble and are typically complex iodinated compounds. A barium sulphate mixture is another commonly used variety, which is based on a suspension of large insoluble particles. All tend to be excreted almost 100%, and all tend to be persistent.

The following compounds were selected:

Compound	Reason for inclusion
Diatrizoate	• selected by 5 partners • on 4 priority lists
Iopamidol	• selected by 4 partners • on 3 priority lists
Iopromide	• selected by 4 partners • on 3 priority lists

The Luxembourg hospital does not use any the above compounds and monitored iodixanol and iohexol instead.

2.2.2.8 Cytostatics

Cytostatics, or anti-neoplastics, are anti-cancer drugs and may have carcinogenic, mutagenic, fetotoxic and teratogenic effects (Kümmerer et al., 1997). They are generally persistent. However, Ferk et al., in experiments with cisplatin, carboplatin and 5-fluorouracil, showed that these cytostatics cause a significant induction of DNA damage only at concentrations that are substantially higher than those encountered (Ferk 2009). Ifosfamide is a widely used antineoplast and was encountered in hospital wastewater as well as communal treatment plant influent and effluent (Kümmerer, et al., 1997) and was found not to be eliminated by adsorption, biodegradation or otherwise during standard treatment.

Two compounds were selected:

Compound	Reason for inclusion
Cyclophosphamide	• selected by 4 partners • highly persistent in waste water (Buerge, et al., 2006) • 3 priority lists
Ifosfamide	• selected by 4 partners • highly persistent in waste water (Buerge,et al., 2006) • 3 priority lists

2.2.2.9 Other treatment groups

Psycho-active drugs are not typically hospital drugs, although a report by the Scottish Care Commission found that 75% of care home residents was taking one or more psychoactive drugs (Care Commission and Mental Welfare Commission, 2009). Fluoxetine, venlafaxin and ritalinic acid have been encountered in wastewater. The anti-viral drugs ritonavir and oseltamivir were also suggested. Ritonavir is an anti-HIV drug; oseltamivir is an antiviral and ingredient of tamiflu. However, as none of these drugs was selected by more than one partner, no psychoactive drugs or antivirals were included in the final selection. Other compounds suggested by the partners included preservatives, diuretics, anti-diabetics, anti-fungals, disinfectants, antacids and anti-worming agents. No representatives of these treatment groups were included in the final selection.

The anti-diabetic drug Metformin was suggested for monitoring. Following discussion it was however decided that Metformin is primarily used in the community and not so much in hospitals and was therefore less relevant to the project.

2.3 Compound parameters

The key parameter values toxicity, excretion and removal are discussed below. PNEC values and excretion rates, as well as physico-chemical characteristics pKa, log K_{ow} and speciation at pH7, are presented in table 2-2. Also included are ranking in the GWRC Priority Setting. Tables 2-3 and 2-4 give sorption and biodegradation constants and literature values for elimination.

2.3.1 Toxicity

Most data on environmental effects are on acute toxicity of a single compound to standard organisms. However, beside acute emissions of pharmaceuticals by combined sewer overflows in sewer systems a significant amount of these substances enter the environment continuously via WWTP effluents. Therefore aquatic organisms are exposed to low doses chronically downstream of WWTPs. Aside from lethal effects after long-term exposure, other effects, such as developmental, behavioural or reproductive effects are likely to occur as non-target organisms will be affected by the pharmaceutical compounds. Organisms at different trophic levels may be affected. Chronic effects and a range of end-points must be taken into account and effects on organisms on different trophic levels must be evaluated accordingly. Guidance is in place on determining toxicity values taking these three spectra (acute – chronic, range of end points and trophic level) into account in the European Medicines Agency (EMEA) guidelines on Environmental Risk Assessment (EMEA 2006).

Furthermore, organisms are rarely exposed to a single pharmaceutical but rather to a cocktail of pharmaceutical residues in varying concentrations. The toxicity of such a cocktail cannot be calculated from the individual toxicities of compounds in the mix and may well exceed their combined effects. Ecotox testing involves exposing organisms (of typically three trophic levels) to a real effluent sample. The toxicity of the total effluent can so be determined, with results being specific in time and place, regardless of whether the composition of the wastewater is known and regardless of whether cocktail effects are understood.

Derksen et al. reported in 2001 that there is a lack of data regarding toxicity, in particular chronic and specific toxicity (Derksen et al, 2001). Since then, numerous studies have started to fill this data gap. In the EMEA guidelines, toxicity is expressed as the Predicted No Effect Concentration or PNEC. This is derived from either EC50 tests for acute toxicity, or from No Effect Concentrations (NOECs) tests for chronic effects. An Assessment Factor (AF) is used to express a degree of uncertainty, with AF being 1000 for EC50 results and 10 or 50 for $NOEC_{chronic.}$. Significant progress has been made in the last decade and PNEC values based on the EMEA guidelines were found in the literature for most compounds on the 'Long List', although they can vary by several orders of magnitude between studies. Normally, the lowest value found should be used under the precautionary principle; however uncertainties should be taken into account when using the values in multi-criteria analysis where removal is offset against e.g. environmental impact of the plant.

As mentioned before, some information is available on specific treatment groups. Cytotoxic and cytostatic compounds, because of their specific therapeutic effect, may have genotoxic or mutagenic effects. In the Waste Framework Directive (Directive 2006/12/EC), these drugs are considered hazardous waste and consequently subject to special provisions for disposal. This does however not address excretion as a pathway.

As is to be expected, bacteria in particular, but also algae are very sensitive to antibacterials. Some fluoroquinolone antibacterials have been shown to have genotoxic effects. Kümmerer mentions that antibacterials are "…often complex molecules which may possess different functionalities within the same molecule. (...) Because of the different functionalities within a single molecule (...) toxicity may change with pH" (Kümmerer, 2009a). A particular issue can occur in hospital wastewater treatment using activated sludge or where Membrane Bioreactor (MBR) technology is used: if exposed to a high dosage of ecotoxic chemicals, the active bacteria in the treatment system can be affected and the treatment can become less effective as a result.

Information on PNEC values has been made available in an on-line database by the research group MistraPharma. Where available, values have been taken from the MistraPharma database.

2.3.2 Excretion

Most drugs are designed to be persistent e.g. during stomach transfer, and contrast media tend to be excreted 100% unchanged. Excretion rates for the same active compound may vary with the method of administration. Topical products such as creams and gels will have different excretion rates and furthermore, a certain amount of such products is washed off, or rubs off on sheets and enters the wastewater stream via laundry. Some nasal sprays etc. may be lost to surfaces and clothing. We have not attempted to predict how these fractions may account for some variation up or down.

A significant proportion of drugs may be issued to outpatients and not be excreted within the hospital compounds. Mass flow analysis in the hospitals can elucidate this fraction. The UK data analyst was able to exclude prescriptions for outpatients; however, as outpatients may spend several hours in the hospital grounds, this may, conversely, explain a slight under-representation. As residues can be excreted up to several days after administration, short stay in-patients also may excrete some of the non-metabolised fraction outside the hospital. Conversely, patients excrete residues of drugs prescribed by their GP in the hospital (and may indeed bring such drugs into the hospital with them). These issues apply less to permanent residents, such as in geriatric and psychiatric hospitals, who normally excrete all residue from medicines administered there, although they may still excrete residues of drugs administered during off-site treatment (e.g. anaesthetics or contrast media) in their residential hospital.

There is some variation in excretion rates found in the literature as different studies may use different end points. The method by which a drug is administered may affect the excretion rate.

Metabolites of the drugs will also be excreted and can also have environmental effects. One is included for monitoring.

Table 2-2: Selected compounds with key parameters

			EXCRETION		PHYSICOCHEMICAL			PNEC	
			u = unchanged in urine, f = unchanged in fecal, g = glucuronides of parent compound						
Compound	**CAS**	**GWRC Priority**	**Excretion rate (%) ***	**Reference**	**pKa**	**Log Kow**	**Speciation at pH7**	**µg/l**	**Reference**
Diclofenac	15307-86-5	I	50	Estimated average inc. wash-off	4.06	4.5	4.5	0.1	Jahnel et al., 2006
			<1 (u)	EMC, accessed 6/6/12	–		–	0.1	Grung et al., 2008
			15	Jjemba, 2006	–		–	10	www.fass.se
			1 (u) + 15 (f)	Lienert, 2007				10	Lin, 2009
					–		–	10	Carlsson, 2006
					–		–	100	Larsen, 2009
					–		–	138.74	Jones et al., 2002
					–		–	36-40	Hanisch, 2002
					–		–	0.01	Wikipharma
					–	–	–	4.33	Ecosar
Naproxen	22204-53-1	I	0 to 10	Carballa, 2008	4.15	3.18	anionic	31.00	Ecosar
								190.00	Larsen, 2009
								0.64	www.fass.se
								128.00	Jones et al., 2002
								28-30	Hanisch, 2002
								37.00	Lin, 2009
								37.00	Carlsson, 2006
								3.3	Wikipharma
Carbamazepine	298-46-4	I	1to 2	Jjemba, 2006	7	2.3	neutral	1.44	Ecosar
			1 to 31	Carballa, 2008				6.36	Fernand, 2009
			2	EMC, accessed 6/6/12				0.50	Jahnel et al., 2006
			2 (u) + 24 (f)	Lienert, 2007				17.00	www.fass.se
								17.00	Hanisch, 2002
								2.50	Larsen, 2009
								2.50	Ternes and Joss, 2006
								43.00	Fisk et al., no date
								6.36	Jones et al., 2002
								25.00	Lin, 2009
								2.50	Wikipharma
Atenolol	29122-68-7	I	37 (u) + 46 (f)	Lienert, 2007	9.48	0.16	cathionic	0.50	Ecosar
								77.70	Fernand, PhD thesis 2009
								77.70	Jones et al., 2002
								330.00	Larsen, 2009
								1.00	Wikipharma
								10000.00	Lin, 2009
Bezafibrate	41859-67-0	I	49 (u) + 2 (f)	Lienert, 2007	3.61	4.25	anionic	0.45	Ecosar
			45	Jjemba, 2006				0.63	Fernand, PhD thesis 2009
			50 (u), plus 20 (g)	EMC, accessed 6/6/12				2.30	Larsen, 2009
								100.00	Lin, 2009
								0.46	Wikipharma

Lidocaine	137-58-6	none	5 (u) + 3 (f) <10 <10 5	Lienert, 2007 Jjemba, 2006 EMC, accessed 6/6/12 estimated average inc. wash-off from creams	8.01	2.26	cathionic	0.30 106.00	Ecosar Hanisch, 2002.
Amoxicilin	26787-78-0	II	65 (u) + 10 (f) 80-90 80-90 60-80 (u, within 6hrs)	Lienert, 2007 Hirsch, 1999 Jjemba, 2006 EMC, accessed 6/6/12	3.39, 6.71, 9.41	0.9	Zwitterionic (neutral)	1.11 0.08 0.00 250.00	Ecosar www.fass.se Jones et al., 2002 Jones et al., 2002
Cipro-floxacin	85721-33-1	I	43 (u) + 24 (f) 83.7 44.7 (u), 25 (f)	Lienert, 2007 Jjemba, 2006 EMC, accessed 6/6/12	6.18, 8.80	-1.08	Zwitterionic (neutral)	6.17 0.01 1 to 10 0.02 0.005 0.02 0.02 0.05	Ecosar Fernand, 2009 Kummerer, 2004 Hanisch, 2002 Grung, 2008 Lin, 2009 Kummerer and Henninger, 2003 wikipharma
Clarithro-mycin	81103-11-9	II	>60 15-36 (u, depending on dosage)+ 5-10 (f)	Hirsch, 1999 EMC, accessed 6/6/12	8.99	1.6-3.1	cathionic	0.68 0.31 0.03 0.01 0.04	Ecosar Larsen, 2009 Yamashita et al., 2006 Hanisch, 2002 Lin, 2009
Sulfame-thoxazole	723-46-6	I	20 (u) 15 to 30 (u)	Lienert, 2007 EMC, accessed 6/6/12	1.39, 5.81	0.89	anionic	1.17 45.00 0.03 0.10 0.59 0.12 20.00 2.00 0.01	Ecosar Fernand, 2009 www.fass.se Ternes and Joss, 2006 Larsen, 2009 Grung, 2008 Lin, 2009 Kummerer and Henninger, 2003 wikipharma
Erythro-mycin	114-07-8	I	9 (u) + 90 (f) 4 to 10 2 to 5 (u), partly metabolised, remainder excreted by bile in active form	Lienert, 2007 Carballa, 2008 EMC	8.8	3.06	cathionic	0.78 74.00 74.00 2.00 0.20 121.00 22.70 0.04 0.22	 Fernand, 2009 Jones et al., 2002 www.fass.se Larsen, 2009 Fisk et al., no date Jones et al., 2002 Lin, 2009 wikipharma

Diatri-zoate (Amidotri-zoate)	117-96-4	II	100	EMC	nd	-1.04	anionic	102.57 11000	Ecosar Larsen, 2009
Iopamidol	62883-00-5		>99 (u), <1 (f)	EMC	nd	-2.42	neutral	12.98 380000	Ecosar Larsen, 2009
Iopromide	73334-07-3	II	92 (u) (24hrs)	EMC, accessed 6/6/12	nd	-2.05	neutral	13.22 100000	Ecosar Larsen, 2009
Cyclo-phospha-mide	50-18-0	II	5 to 25 (u)	EMC, accessed 6/6/12	nd	0.63	neutral	157.63 1968.00 1120.00 984.00	Ecosar Hanisch, 2002 Grung, 2008 Carlsson, 2006
Ifosfa-mide	3778-73-2		34		nd	0.86	neutral	157.63 200.00 162.00	Ecosar Hanisch, 2002 Carlsson, 2006
N4-Acetyl-Sulfame-thoxazole	21312-10-7		50 (u)	Vree 1987	0.38, 5.88	0.86	anionic		

Physico-chemical data from: Babi et al., 2007; SNIFFER, 2010; Suarez et al., 2008; http://logkow.cisti.nrc.ca/logkow/index.jsp
PNEC values from http://www.wikipharma.org/api_data.asp and others as listed; Excretions rates from www.medicines.org/EMC, accessed 6/6/12 and others as listed

2.4 Elimination in conventional WWTP

Sewage treatment plays an important role in the removal of pharmaceuticals from waste water (Nakada et al., 2006) . The extent to which pharmaceuticals are removed is compound-specific and depend in first instance on the treatment processes applied, secondly on process parameters. Removal rates show considerable variability between samples from the same plant and between plants (Tauxe-Wuersch et al., 2005), (Lin, Yu & Lateef, 2009).

In most EU countries, sewage is generally treated by mechanical and biological processes: at least, screening, sedimentation, aeration and activated sludge. A Membrane Bioreactor (MBR) may be used as well but this technology is not commonly used for general sewage treatment in EU countries.

Certain compounds are metabolised to glucuronides by the patient but then hydrolyse back to the parent compound during treatment processes. Excretion rate is based on total metabolism and doesn't account for this hydrolysis of the metabolite back to the parent compound in waste water. This may result in more compound being found than predicted.

Most substances that are not eliminated in wastewater treatment plant are also persistent in the environment and are not easily removed by degradation or sorption to soil or sediments (Sadezky, 2008).

Factors affecting removal rates include the residence time of wastewater in the treatment tanks (hydraulic retention), existence and size of anoxic and aerobic compartments, rainfall, composition and pH of the sludge, sludge retention time, light intensity and the season (Ternes, 2004; Ternes, 2005; Castiglioni, 2006; Cirja, 2008; Tauxe-Wuersch, 2005; Kovalova, 2012).

It would be of interest to further investigate the effects of these parameters and other factors that might affect removal efficiency, as their control may be a relatively simple way to improve removal rates.

During mechanical and biological processes, sorption and biodegradation are the most important removal mechanisms (Carballa, 2005), with chemical degradation playing a lesser role. Literature values for elimination in Conventional Activated Sludge (CAS and Membrane Bioreactor (MBR) are given in table 2-4.The main elimination mechanisms of sorption and biodegradation are discussed in more detail below.

2.4.1 Sorption

A solid-water distribution coefficient K_d is used to indicate total sorption affinity of the compound; the sorbed concentration can be assumed proportionate to the concentration of the compound in the sewage and the amount of suspended solids (SS) and is therefore described by Ternes (2004) as $C_{i,sorbed} = K_{d,i}\ SS\ C_{i,soluble}$. Typical values for sludge production in municipal WWTPs are in the range of 200–500 gSS m^{-3}. When sludge production is in this range, sorption in the WWTP can be neglected when the K_d value < 500 L/kgSS.

Wells (2006) remarks that "Because most water treatment is conducted between pH 7 and 8 and because D_{ow}, the pH-dependent n-octanol–water distribution ratio embodies simultaneously the concepts of hydrophobicity and ionogenicity, D_{ow} at pH 7–8 is presented as an appropriate physico-chemical parameter for understanding and regulating water treatment. Although the pH-dependent chemical character of hydrophobicity is not new science, this concept is insufficiently appreciated by scientists, engineers, and practitioners currently engaged in chemical assessment. The extremely hydrophilic character of many pharmaceuticals at pH 7–8, indicated by D_{ow} (the combination of K_{ow} and pK_a) not by K_{ow} of the neutral chemical, is proposed as an indicator of occurrence in surface water."

Ternes et al. (2004) report that where K_d values are derived from modelling based on K_{ow} or D_{ow} values and compared with measured values, discrepancies of at least an one order of magnitude are frequently found, due to the polar nature of most pharmaceuticals. Where available, K_d values from measurements should therefore be used to evaluate sorption. Table 2-3 shows literature values for K_d for the selected compounds, showing that only ciprofloxacin is clearly sorbing.

The Sewage Sludge Directive (86/278/EEC) seeks to encourage the use of sewage sludge in agriculture (with safeguards in place imposing a minimum time between application and harvest or grazing to avoid risk from pathogens), but legislation and practice display considerable variation in disposal methods across member states. In the Netherlands and Switzerland, all sludge must be incinerated. In the UK, 50% is applied to land, whereas in Sweden, most is applied to land. Sludge application to land may constitute a pathway for pharmaceutical pollution and deserves further study; this is however outside the scope of the PILLS project.

2.4.2 Biodegradation

The biodegradability of pharmaceuticals is substance specific (Abegglen et al., 2009). Kümmerer (2004) quotes a number of studies showing that most antibacterials are not biodegraded.

Ternes (Ternes 2006) describes the biodegradation rate of a certain pharmaceutical with a pseudo first order reaction:

$$\frac{dC_i}{dt} = k_{biol,i} \times SS \times C_i$$

where:

C_i total concentration of pharmaceutical i (µg/L)
t time (d)

$k_{biol,i}$ specific biological degradation rate constant of pharmaceutical i (L/gSS/d)
SS suspended solids concentration (g/L).

This first order parameter shows that degradation is proportional to the concentration of pharmaceuticals and the concentration of sludge. K_{biol} values from literature are given in Table 2-3.

Table 2-3: Literature on K_d and K_{biol} for municipal wastewaters (from Kovalova, 2012)		
LITERATURE (municipal wastewater)	**K_d (L/kg_{ss})**	**K_{biol} (L/day.g)**
Diclofenac	16[28], 20[38], 18-151[39], 710[37], <30[33], 118-321[7]	1.2[40], 0.04[27]
Naproxen	217[32], 20[38], 11-51[39], 630[37], <30[33]	9[40], 1[27]
Carbamazepine	1[28], 25[32], 17[10], 50[38],20-68[39], 150[37], 36-65[33], 135-314[7]	<0.06[40], 0.007[27]
Atenolol	38[30], 0[31], 0[32], <50[33], 10-95[7], 1600[34]	0.69[30] 1.9[10],1.5[35]
Bezafibrate	0[31], 170[37]	1.8[31], 3.3[27]
Lidocaine		
Amoxicillin		
Ciprofloxacin	20 000[41], 450[37]	
Clarithromycin	262[36], 280[37]	
Erythromycin	165[32], 11-309[7]	6[40]
Diatrizoate		<0.1[27]
Iopamidol		
Iopromide	11[28], 5-30[39]	1.8[27]
Cyclophosphamide	2[28], 1800[37]	
Ifosfamide	1[28]	

(xx): References see table 2-4

Table 2-4: Elimination in municipal WWTP, with reference to sorption and biodegradation (from Kovalova, 2012)

K_{biol}	K_d	Micropollutant	% elimination	
			Literature – municipal or synthetic waste water	
			CAS	MBR
		Diclofenac	22±29[7], 30-100[8], 12-52[9], 28-37[19], -30[11], ≈40[22],	[35, 40, 20][19], 45-81$_{(SRT >60d)}$[7], 17±4$_{(SRT 70d)}$[15], [40, 80]
		Naproxen	0-98[13], 66-93[14]	
		Carbamazepine	5-24[9], -25[11], 5[20], -25-18[19], -193-53[13], 0-30[14]	[25, -20, 0]$_{(SRT 16, 33, 60-80d)}$[19], -10-13[13]
		Atenolol	61±19[7], 59±50[8], 1-85[9], 50-60[10], 80[11], 78[12], <0-	57-90$_{(SRT >60d)}$[7], 66[13], 96.9±0.2$_{(SRT 70d)}$[15]
		Bezafibrate	81±21[7], 23-99[8], 12-75[9], 65[11], -11-100[13], 50-97[14]	73-100$_{(SRT >60d)}$[7], 76-97[13]
		Lidocaine	–	
		Amoxicillin	100[47], 100[48], >99[49]	
		Ciprofloxacin	37-99[8], 60-96[13], 78- >80[14]	
		Clarithromycin	18-40[9], -52-26[16], -45-54[13], 54[14]	[54, 40, 90]$_{(SRT 16, 33, 60-80d)}$[16]
		Sulfamethoxazole	74±13[7], 30-92[8], 75[11], -153-6[16], -280- >98[13], <0-	[38, 40, 37]$_{(SRT 16, 33, 60-80d)}$[16], 64-93$_{(SRT >60d)}$[7], 92±1$_{(SRT 70d)}$
		Erythromycin+ Eryt.-H2O	Eryt.: 50[11], 35±51[7], -22-67[16], 54[14] Eryt.-$H_2$0: -26-	[32, 26, 90]$_{(SRT 16, 33, 60-80d)}$[16], -84-134$_{(SRT >60d)}$[7]
		Diatrizoate	0[13]	
		Iopamidol	17[13]	
		Iopromide	30-90[19], 32-50[13]	[40, 68, 80]$_{(SRT 16, 33, 60-80d)}$[19]
		Cyclophosphamide	80[21]	
		Ifosfamide	<3[13]	

LEGEND:

	Biological elimination*	Sorption to activated sludge*
	(K_{biol} 0.1, spec. sludge prod. 500g/m^3 ≈ 20%	(K_d 500, spec. sludge production 200g/m^3 ≈ 10%
	K_{biol} < 0.1 L/day.g (all literature)	K_d < 500 L/kg$_{ss}$ (all literature)
	K_{biol} > 0.1 L/day.g (some literature)	K_d > 500 L/kg$_{ss}$ (some literature)
	K_{biol} > 0.1 L/day.g (all literature)	K_d > 500 L/kg$_{ss}$ (all literature)

*see Table 2-3 for K_{biol} and K_d values available from literature

(6) **Feldmann 2008**: one WWTP in Berlin, DE (nitrif., denitrif., HRT up to 23h), daily composite samples, 3 sampling campaigns ca. 1 week each, p 1761

(7) **Radjenovic 2009**: one WWTP near Barcelona, ES (CAS pre-denitrif., nitrif., HRT app. 11.5h, 277 000 ei, SRT app. 10d) and two MBRs in parallel with CAS (UF 0.05 µm, MF 0.4 µm, SRT >60d), 24-h flow-proportional composite samples (1 sample/h), n=9, elimination from aqueous phase only, Tab. 3 (possible error – all data for ranitidine and metoprolol, including RSDs, are identical: CAS 24.7±44.9, FS MBR 44.2±29.6, HF MBR 29.5±47.9)

(8)	**Gros 2010**: 7 WWTPs in Erbo river basin, ES (6 CAS, 1 biofilter), 24-h time-averaged composite samples, 4 sampling campaigns, Tab.4
(9)	**Jelic 2011**: 3 WWTPs in Catalonia, ES, (CAS), 24-h time-proportional composite samples (2 samples/h), effluent takent with HRT shift, 8 sampling campaigns within 2 years, sum of removed and sorbed (overall removal rate), Fig.4
(10)	**Wick 2009**: one WWTP near Frankfurt, DE (CAS, SRT 18d, 1 350 000 ei), 48 and 72 h composite samples, 3 sampling campaigns ca. 1 week each, Fig.5
(11)	**Kasprzyk-Hordern 2009**: one WWTP in UK: CAS WWPT 24-h composite samples, sampling at least twice a month for 5 months, Fig.2 (often excessive error bars, not included here)
(12)	**Alder 2010** 3 WWTPs in Glatt valley, CH, 20 000 – 37 000 ei, 1 week sampling, 24-h flow-proportional composite samples, Tab 3
(13)	**Onesios 2009**: (review paper) Tab. 2 "PPCP removal efficiencies attributed to biodegradation in addition to other removal mechanisms"
(14)	**Monteiro 2010**: (review paper) CAS Tab.12
(15)	**Tadkaew 2011**: lab MBR, synthetic waste water, SRT 70d, n=16, Tab.1 (additional comparison with literature)
(16)	**Gobel 2007**: 2 WWTPs in CH (CAS-K: 55 000 ei, SRT 10-12d, HRT ≈15h, MBR operating in parallel, 3 SRT tested [16d, 33d, 60-80d] Fig.4; CAS-A 52 000 ei, SRT 21-25d, HRT ≈31h) sampling campaigns of 1 week – 3 at WWTP-K and 2 at WWTP-A, 48 and 72 h composite flow-proportional samples, Tab.3
(17)	**Ternes 2001**: (analytical meth. paper) one WWTP near Frankfurt, DE, 24-h flow-proportional samples of 5 days, p 182 4-aminoantipyrine (semi-quant.)
(18)	**Reemtsma 2010**: 4 WWTPs in Berlin, DE (CAS), 24-h composite samples, p 599
(19)	**Joss 2005**: 2 WWTPs in CH (CAS-1: 55 000 ei, SRT 10-12d, MBR operating in parallel, 3 SRT tested [16d, 33d, 60-80d]; CAS-2: 80 000 ei, SRT 22-24d) sampling campaigns of 1 week – 3 at WWTP-1 and 2 at WWTP-2, 48 and 72 h composite flow-proportional samples, Fig. 5
(20)	**Letzel 2010**: one WWPT at Southern Bavaria, DE (CAS, 26 000 ei, effluent taken with HRT shift, 24-h composite samples, 4 days sampling) and one continuous lab-scale sewage plant with synthetic wastewater (HRT 11.4h, SRT 11.2d) n=2
(21)	**Delgado 2009**: lab scale MBR
(22)	**Kimura 2007** 1 WWPT (CAS, 200 000 ei, HRT 12h, SRT 7 days), 2 MBRs (MBR-1: SRT 15d, MBR-2: SRT 65d), grab samples were taken 11 times around 10:00 am
(23)	**Ternes 2000**: one WWTP near Frankfurt, DE, (preliminary clarification followed by an aeration tank with addition of Fe(II)chloride for phosphate elimination and a
(24)	final end point clarification) flow-proportional samples of ca. 1 week, Tab.4
(25)	**Prasse 2010**: : one WWTP in Dortmund, DE (CAS: 1 350 000 ei, HRT 5h, SRT 18d), 24-h composite samples, Tab.3
(26)	**Slater 2011**: lab scale MBR, synthetic waste water, SRT 24d
(27)	**Metcalfe 2010**: one WWTP in southern Ontario, CA (CAS followed by UV-disinfection, HRT 11.9h, 69 000 ei, SRT 8.1 – 10.4d), a single day 24-h composite sample
(28)	**Joss 2006**: batch, estimated from K_{biol}, Fig. 6
(29)	**Ternes 2004**: "A rapid method to measure the solid–water distribution coefficient (Kd) for pharmaceuticals and musk fragrances in sewage sludge"
(47)	**Chang 2007**: 7 WWTPs in Beijin, CN, 24-h composite samples, 4 weeks, Fig. 3
(48)	**Watkinson 2007:** Brisbane (Australia) CAS (SRT=12.5 days)
(49)	**Zuccato 2010:** Italy and Switzerland (Milan, Varese, Como, Lugano)
	Watkinson 2009: South-East Queensland (Australia)

2.5 Summary of analytical procedures

The scope of this section is to describe, in summary, the general analytical chemistry approach undertaken by the partners. Where possible, sharing of knowledge and expertise of the partners was encouraged. It is also important to note, that it was agreed that each partner develop methods that were appropriate to their available equipment and resources.

2.5.1 Sampling and general considerations

The raw hospital wastewater was sourced and sampled according to protocols that were designed to yield results that would show amounts of pharmaceutical loadings at various times during the sampling process. Other parameters were measured in addition to the trace quantities of pharmaceuticals and these were the macro-pollution indicators, such as phosphate, Biological Oxygen Demand (BOD) indicating organic matter loading, pH, and nitrate.

The subsequent sample processing and "clean-up" procedures, used by the different partners, were reflected by the varied matrix of the waste water sampled. For example, in cases where the waste water that was sampled was completely untreated and came directly from sewage outlets (as in the UK), an additional sample clean-up procedure was employed. This along with dilution reduced the matrix effect and was employed for all UK samples to maintain consistency.

Where appropriate, isotopically labelled internal standards were added in line with general quality assurance procedures.

2.5.2 Sample Preparation

This involved various processes with the general aim of yielding a concentrate or liquid sample that is suitably purified and enriched in order to be ready for LC- or GC-MS-MS analysis. The waste water samples (up to 1 litre) were filtered to remove any suspended particulate matter (typically with 0.2/0.1 micrometres filters) and from there the sample, (after addition of internal standards) proceeded to clean-up and enrichment. In most cases this included the use of Solid Phase Extraction (SPE) media, through which the waste water sample was passed at a particular pH. The SPE cartridge should retain the pharmaceuticals with the unwanted material passing through into the filtrate. Once dried the SPE cartridges were first washed with water, then treated with a small volume of organic solvent e.g. methanol or acetonitrile to elute the pharmaceuticals. The samples were then dried down under a stream of nitrogen and reconstituted in solvent. If starting from 1 litre and reconstituting in 1ml this gives a 1,000 fold enhancement in concentration. In samples from further sources this would require further clean-up by supported liquid extraction cartridges (SLE) and dilution to further reduce matrix effect.

Most partners apply SPE techniques, and the Swiss partner used an online-SPE followed directly by the separation and detection with LC-MS-MS (Kovalova et al., 2012).

2.5.3 Determination of concentration of pharmaceuticals in samples

The extracts were analysed by LC-MS-MS or GC-MS-MS. The majority of partners used LC-MS-MS with selected reaction monitoring mode (SRM mode) for specificity. Where available, isotopically labelled internal standards were used, to correct for losses during the extraction and interferences during analysis. Other quality assurance procedures such as the determination of recoveries and limit of quantitation, were employed in order to test the validity of the analysis. Calibration standards were prepared covering the expected concentrations for each analyte. The concentration of pharmaceuticals in waste water was calculated based on internal standard and recovery data as appropriate. Details on sample preparation, analytical methods and quality assurance are given in the individual reports of the partners (CH: McArdell et al., 2011, Kovalova et al., 2012; LU: Köhler et al., 2012 and the other partner reports to follow).

2.6 Predictions and measurements

2.6.1 Pharmaceutical consumption

Pharmaceutical usage data was mostly obtained as annual totals of prescribed medicine at the selected hospitals, and include pharmaceuticals that are prescribed but not consumed by patients. Table 2-5 shows predicted pharmaceutical loads per bed, based on annual dispensed amounts. The column UK-GALA gives annual amounts dispensed in the community for the UK Borders region, per head of population, for comparison.

Table 2-5: Annual pharmaceutical consumption (gbed^{-1}a^{-1}) and per head of population (UK-GALA column) (gh^{-1}a^{-1})

Compound	DE 2010	LU[1]	CH 2009	FR	NL	UK (WI) 2010	UK (DR)	UK (BGH)	UK (ML+HH)	UK (GALA)
Diclofenac	7.07	1.62	3.78	1.93	0.50	3.98	0.19	3.26	0.30	n.d.
Naproxen	0.00	8.73	0.00	2.18	0.07	1.85	0.44	3.98	8.87	1.34
Carbamaz.	1.64	1.00	0.34	2.91	0.00	4.49	1.87	4.34	3.78	0.72
Atenolol	0.27	0.15	0.46	0.64	0.00	1.59	0.47	2.20	1.19	0.63
Bezafibrate	0.84	0.00	0.00	0	0.01	0.41	0.00	0.78	0.23	0.17
Lidocaine	n.d.	2.21	8.30	46.95	2.36	8.45	0.36	17.21	0.06	n.d.
Amoxicillin	22.73	92.52	256.11	67.05	47.49	125.37	21.52	72.26	8.53	1.87
Ciprofloxacin	9.61	16.50	17.88	21.80	8.96	24.19	1.32	27.31	1.45	0.17
Clarithromycin	3.82	5.47	4.91	0.85	0.40	18.04	1.33	24.75	1.76	0.40
Sulfamethox.	3.32	0.33	2.89	13.39	5.37	15.07	1.33	0.00	0.00	0.03
Erythromycin	2.18	0.55	0.48	2.98	1.45	2.41	0.00	2.12	6.50	0.45
Diatrizoate	67.06	0.00	166.72	181.37	3.89	29.45	0.53	0.00	0.00	0
Iopamidol	13.49	0.00	918.81	4.36	0.00	9.63	0.00	687.67	0.00	0
Iopromide	0.00	0.00	71.2	0	248.42	0.00	0.00	4.26	0.00	0
Cyclophosph.	0.31	0.48	0.79	1.79	1.94	8.45	0.00	0.00	0.00	0
Ifosfamide	0.10	0.28	0.13	0.71	0.03	0.00	0.00	0.18	0.00	0

[1] For LU, topical products such as creams and sprays are not included in the annual consumption data above.

2.6.2 Daily variation

High variation in concentrations and loads were encountered when analysing hospital wastewater day by day. Short-term variation will exist where certain treatments are only carried out on certain days of the week e.g. less contrast media are found at weekends. Figure 2-1 illustrates the daily pharmaceutical concentration for BGH for one week (based on 24h flow proportionate samples). This week is a 'snapshot' and although it is interesting in that it shows a daily variation between different pharmaceuticals, without knowing the patient treatment pattern for this particular week no conclusions can be drawn from this data. More data would also be required over several weeks to determine if this pattern is consistent. The comparison of BGH and Galashiels WWTP waste water over the same period (Figure 2.2) similarly indicates some daily variation – interestingly also for the WWTP – and a significantly higher concentration of some compounds in the hospital effluent.

Figure 2-1 Daily variation in pharmaceutical concentrations in BGH effluent

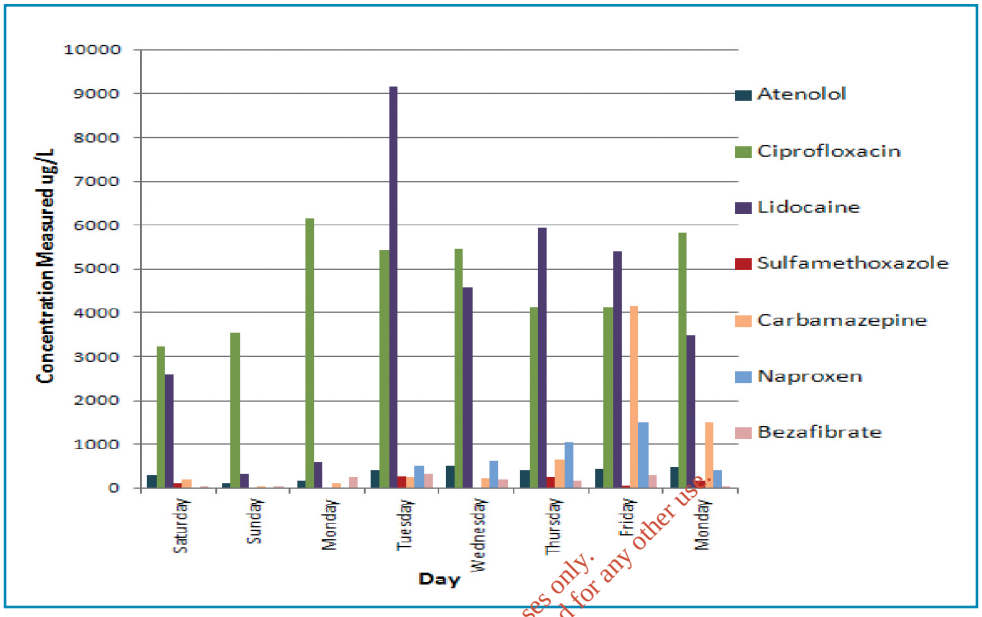
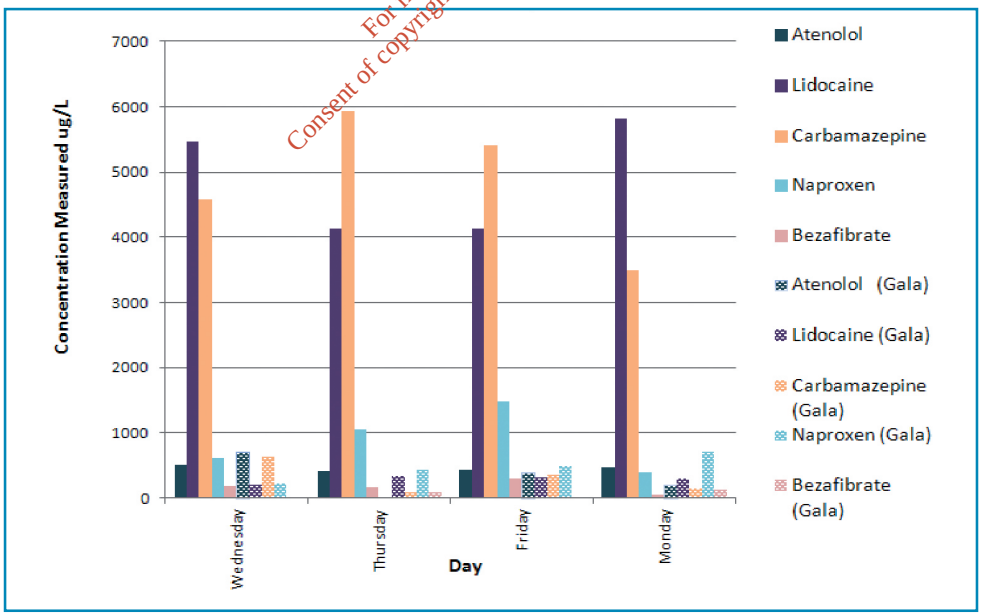


Figure 2-2 BGH and Gala WWTP based on 24hr composite samples



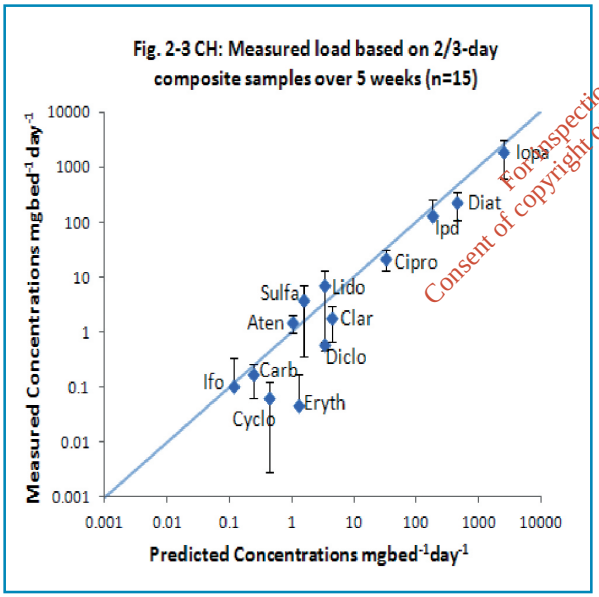
2.6.3 Comparison between predicted and measured values

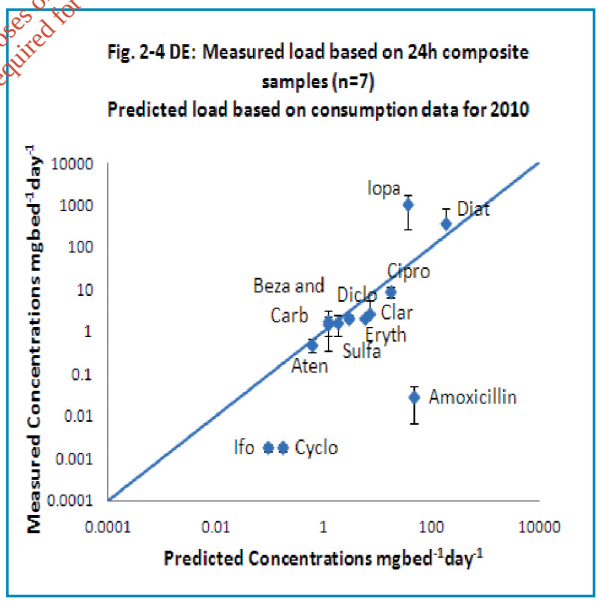
For calculating predicted loads in hospital wastewater from the consumption data, excretion was taken into account. Since each partner site used different products (capsules, suppositories, creams) with different excretion rates, each partner used an excretion taking the different products into account (see Table 2-2 for commonly used excretion rates).

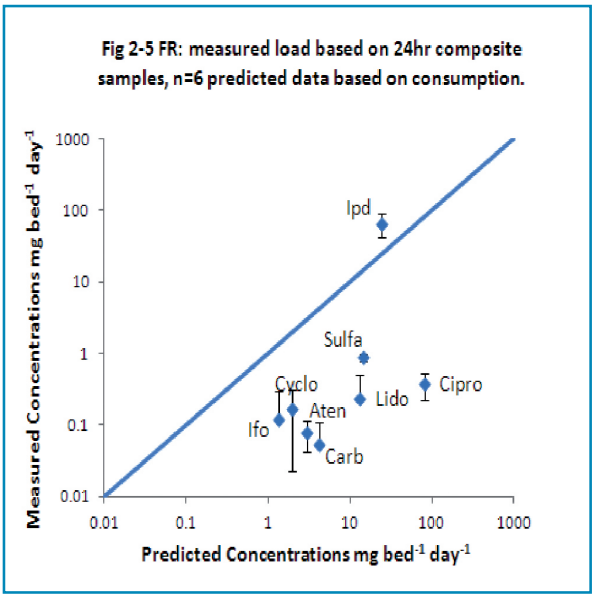
Predicted and measured daily loads per bed are compared, for each hospital, in figures 2-1 to 2.11. Discrepancies between measured and predicted values can occur for a number of reasons. Annual consumption numbers may not be representative for the measurement period since there might be seasonal variations in consumption, e.g. for antibacterials. Part of the consumed pharmaceuticals might also be excreted at home by outpatients, or pharmaceuticals can been prescribed in the community and brought into the hospital by patients, visitors and staff, in particular analgesics and antibiotics.

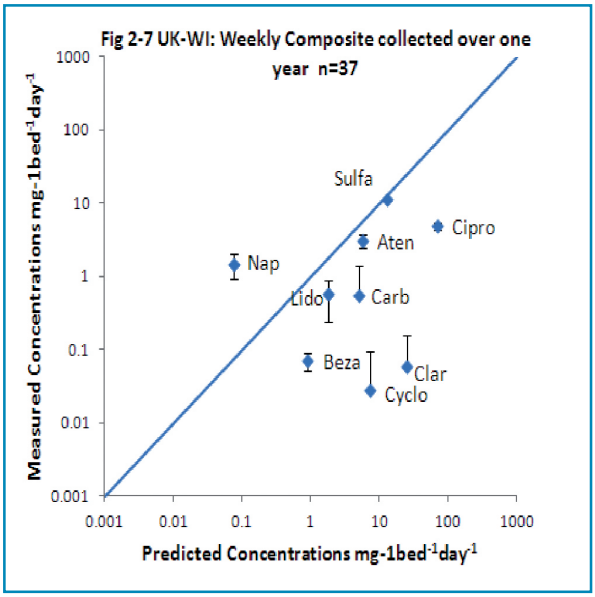
In the UK consumption data in Table 2-5, data analysts excluded pharmaceuticals prescribed by outpatient departments. For other partners, annual consumption includes prescriptions for outpatients which will be excreted in the community, resulting in measured values below predictions. Patients using incontinence pads will not excrete pharmaceuticals into the wastewater. Their excretions are normally disposed of via solid waste. In the UK, staff interviews indicated that 10-15% of patients in the main hospitals normally use incontinence pads. In a geriatric long-term care unit, this figure was as high as 60% on average and 90% during the period of intensive sampling in October 2011. This practice will prevent a similar percentage of prescribed drugs from entering the wastewater.

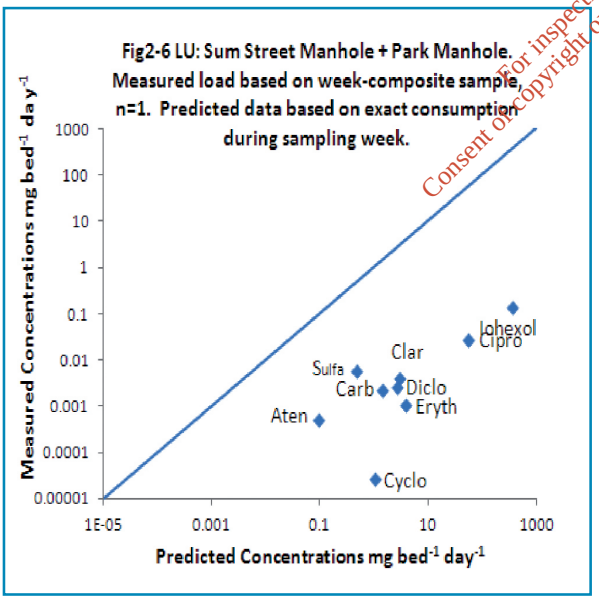
Figures 2-3 – 2-11 Comparisons between measured and predicted loads.

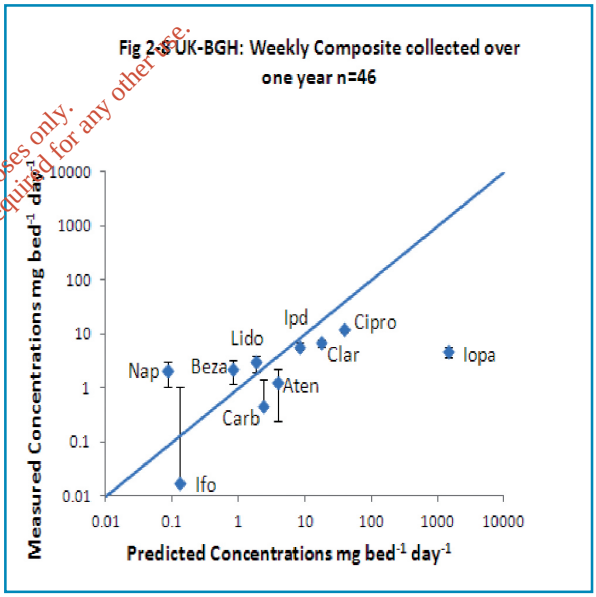


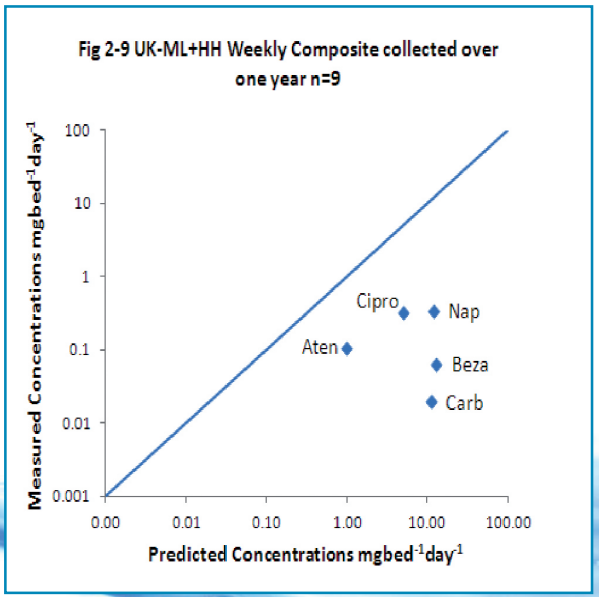


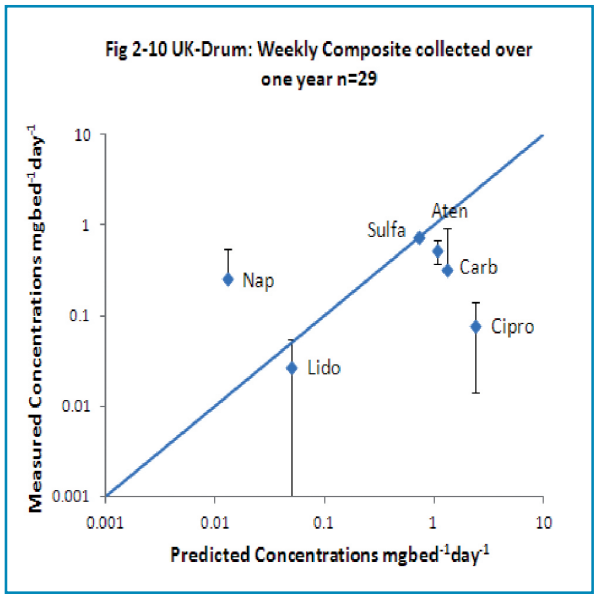


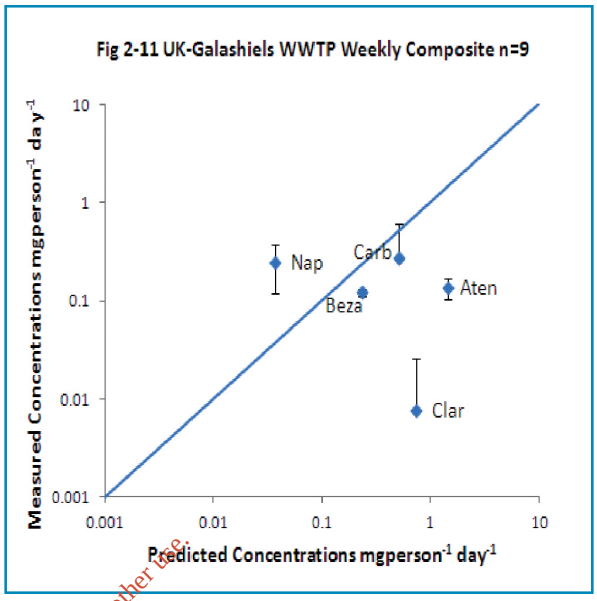












Abbreviations: Amox; Amoxicillin, Aten;Atenolol, Beza;Bezafibrate, Carb;Carbamazepine, Cipro;Ciprofloxacin, Clar;Clarithromycin, Cyclo;Cyclophosphamide, Diclo;Diclofenac, Eryth;Erythromycin, Ifo;Ifosfamide, Iopa;Iopamidol, Ipd;Ioprimide, Lido;Lidocaine, Nap;Naproxen, Sulph;Sulphamethoxazole, Diat;Diatrizoate, NACS;N-Acetyl sulphamethoxazole. 2.7 Hospital and care home contribution to total pharmaceutical load

2.7 Hospital and care home contribution to total pharmaceutical load

2.7.1 Hospital contribution

In the EU, the average number of hospital beds per 100000 heads of the population varies from 35 (Denmark and Portugal) to 83 (Germany), so an even pharmaceutical usage for hospital patients and non-hospital patients alike would account for 0.4-0.9% hospital contribution (WHO, 2010). Data from CH show that in Switzerland, most of the WWTPs treat a ratio of 5-50 beds per 1000 inhabitants.

Of course, the hospital fraction for some drugs is much larger and overall, the hospitals account for an estimated 20-25% of all human medicine (Schuster, Hädrich & Kümmerer 2008; Weissbrodt 2009). The hospital fraction varies per drug. In Germany, for total antibacterials it does not exceed 25% (Kümmerer 2009b), with cephalosporins and penicillins in particular showing a relatively high hospital fraction (Kümmerer 2004). For iodized contrast media (ICM) it is estimated to be about 50% (Kümmerer 2004); hospital consumption of the cytostatics is also relatively high. Weissbrodt et al. however note that 70% of cytostatics and 50% of ICM consumed in hospitals is administered to outpatients and therefore likely to be excreted in the community (Weissbrodt et al., 2009). Ort's (2010) measurements of residues in WWTP and hospital wastewater give low values for the hospital contributions for all drugs apart from trimethoprim and roxithromycin <15%. A study by Escher (2011) finds that for the WWTP considered, around 38% of the total pharmaceutical load in the WWTP stems from the regional hospital connected to the plant. Specialised (e.g. psychiatric and geriatric) hospitals are likely to use quite a different range of drugs than general hospitals.

Moreover, hospital fractions vary from country to country and catchment to catchment. In rural areas with no large population centres, large hospitals with a full range of treatment facilities may be encountered in smaller towns, where they will serve a population much greater than that of the town where the hospital is located.

Table 2-6: Hospital fraction of the selected compounds for 3 partners as relative load of pharmaceuticals in the hospital compared to the load in the respective catchment area or the connected municipal WWTP

	Hospital Fraction (%)			
Compound	**DE**	**CH**	**LU**	**UK Galashiels**
Diclofenac	2.9	0.7	5.5	n.d.
Naproxen	1.2	n.a.	11.2	5.3
Carbamazepine	4.4	0.8	21.4	10.1
Atenolol	1.0	1.7	1.9	6.1
Bezafibrate	2.1	0.2	n.a.	8
Lidocaine	n.a.	56	61.2	n.d.
Amoxicillin	1.0	n.a.	n.a.	42
Ciprofloxacin	11.8	40	154.6	74.8
Clarithromycin	59.1	12	18.2	36
Sulfamethoxazole	4.7	16	53	n.d
Acetyl-sulfamethoxazole	6.1	11	12.9	n.d
Erythromycin	82.4	<7.2	64.3	8.1
Diatrizoate (Amidotrizoate)	67.8	95	n.a.	n.d.
Iopamidol	77.8	112*	n.a.	100
Iopromide	0.1**	40	n.a.	100
Cyclophosphamide	< LOQ	<LOQ	12.7	n.d.
Ifosfamide	< LOQ	<LOQ	n.a.	100

DE: Load of pharmaceuticals in Marienhospital relative to load from catchment area measured over one week (n=7). The catchment area has about 77,235 inhabitants and an average flow of approx. 25,000 $m^3 day^{-1}$ over the sampling period of which 0.8 % is coming from the Marienhospital (7.25 beds per 1,000 inhabitants). ** There are two additional hospitals in the catchment area (with 295 and 180 beds).

CH: Load of pharmaceuticals in hospital Baden relative to load in the connected municipal WWTP Laufäcker measured over three weeks. Laufäcker treats water of 55,254 inhabitants and receives an average inflow of 23,751 $m^3 day^{-1}$, of which 1% is coming from the main wing of the hospital. 6.3 beds per 1,000 inhabitants is calculated for this site.

LU: Based on measured values in CHEM (Park and Street manholes) and the connected WWTP Schifflange. Schifflange treats water of about 47,000 PE and received an average inflow of 14,934 m^3 day^{-1} in the time of the measurement campaign. CHEM consumes around 225 m^3 day^{-1}, accounting for 1.5% of the influent at Schifflange.

UK: Based on the pharmaceuticals dispensed to the entire BGH complex (inc. the psychiatric and geriatric lodges), excluding outpatient prescriptions, and pharmaceuticals dispensed in the community in the Borders region per head of population, calculated for the population discharging to Galashiels WWTP. Water consumption for BGH in 2010 was 165,890 L day^{-1}, which, assuming 170 L $person^{-1} day^{-1}$ domestic water use for a population of 14,192, accounts for 6.9% of the influent at Galashiels (not accounting for rainwater).

A hospital's contribution to pharmaceutical load (total weight of pharmaceuticals) is not necessarily the same as its contribution to aquatic toxicity. Cytostatics are considered highly toxic at low concentrations, whereas the heavy contrast media are toxicologically not relevant (Escher, 2011).

Four partners collected data on the hospital fraction of their partner hospitals and the receiving WWTPs. DE, CH and LU did so by comparing measured pharmaceutical analytes, UK used pharmaceutical consumption data. The calculated hospital fractions for the selected compounds are given in table 2-6.

2.7.2 Residential care facilities

One of the PILLS research questions was how important residential care facilities are as point sources of pharmaceutical pollution. This was analysed for the Galashiels WWTP.

UK-DR (geriatric) and UK-ML+HH (geriatric + psychiatric), both residential facilities, have notably lower consumption of antibiotics per bed than the general hospitals, but similar or higher values for naproxen, carbamazepine and atenolol. Pharmaceutical usage per bed in ML+HH was considerably higher than average for people living in the community in the Borders.

Apart from the residential geriatric hospital, there are also 4 care homes for older people, with a total of 125 beds, connected to Galashiels WWTP. Pharmaceutical consumption in care homes for older people can be expected to be lower than in geriatric hospitals, as, over a range of residential facilities open to elderly people, the geriatric hospital is likely to cater for those with the most serious health issues. For the Galashiels catchment, the number of care home beds is considerably lower than the number of hospital beds connected to the WWTP. With pharmaceutical consumption also generally lower than in general hospitals, the care home contribution is expected to be less important than the (general) hospital contribution. This expectation is further supported by considering that a relatively high number of elderly people in residential care will be using incontinence pads.

However, in other WWTP catchments the number of care home beds may be far higher than the number of hospital beds and a different analysis may result.

2.8. Conclusions

2.8.1 The selected compounds

The following compounds were chosen to be investigated in detail within the PILLS project: diclofenac, naproxen, carbamazepine, atenolol, bezafibrate, lidocaine, ciprofloxacin, clarithromycin, sulfamethoxazole, erythromycin, diatrizoate, iopamidol, iopromide, cyclophosphamide, ifosfamide. The selection is in good agreement with most of the already existing priority lists. Slight discrepancies may be justified by the specific hospital focus of the project.

Literature values for the key parameters excretion, elimination and toxicity (PNEC), were sought for the selected compounds:

Excretion rates were low (<20%) for naproxen, carbamazepine, cyclophosphamide and lidocaine; moderate (20-80%) for all other compounds apart from contrast media, which have excretion rates close to 100% excretion. With regard to elimination in WWTP, literature on municipal wastewater treatment appeared to indicate that most pharmaceuticals were moderately (20-80%) removed. Carbamazepine, diatrizoate, iopamidol and ifosfamide were poorly removed (<20%) and only amoxicillin was well removed (>80%). It must be noted that elimination rates found in literature generally showed wide variation. The elimination observed in the MBR treatment of the hospital sites are shown and discussed in chapter xx. PNEC values in literature were generally low (0.01µg/l range) for antibacterials. Values found for cytostatic agents are relatively high (100µg/l range), however, observed genotoxic effects are not represented by these PNEC values. Values for contrast media indicated no real toxic significance for this group, as expected. The toxicity of hospital wastewater was investigated within PILLS and results are shown in chapter xx.

Consumption (by weight) of the pharmaceuticals under investigation in the partner hospitals is highest for contrast media (for single compounds up to 981 $gbed^{-1}a^{-1}$), while antibacterials generally also had relatively high consumption figures (typically 5-25 $gbed^{-1}a^{-1}$).

2.8.2 Mass-balance

Due to the variability between the considered hospitals, and also between different samples from the same hospital, a general characterization of hospital wastewaters is hard to achieve. However, with proper consumption data a good prediction of pharmaceutical loads in hospital effluents can be achieved. As would have been expected, there is a tendency for the reliability of mass balances (consumption vs measured values) to increase with a longer observation period. A more levelled-out consumption was observed for substances which are applied widely and/or in high loads like sulfamethoxazole or carbamazepine. For other substances like cyclophosphamide, which are often applied for individual therapies to single patients and in small dosages, it is much harder to get a reliable mass balance. However, there is still a lot of uncertainty and inaccuracy linked to the comparison of predicted and measured loads due to:

- Inaccurate excretion rates (e.g. for substances which may be administered via more than one route)
- Substances which were prescribed but not excreted in the hospital or vice versa
- Substances which were prescribed but not used at all
- Substances which are used infrequently and may not appear in the samples collected

Consequently, a reliable mass balance requires a detailed acquisition of data at hospital level.

2.8.3 Comparison of wastewaters

Comparing hospital and municipal wastewaters, no big differences were observed for the "classical" wastewater parameters, but higher concentrations of antibacterials and contrast media occurred in hospital wastewater. For the WWTPs, hospitals and specific compounds investigated, hospital contributions to the total load of pharmaceutical in the WWTP influent showed considerable variation – both between compounds and hospitals – but were highest for contrast media (40-100%), lidocaine (56-62%), and antibacterials, in particular ciprofloxacin (12-100%) and clarithromycin (12-60%). Hospital contributions for compounds for other treatment groups were below 20%.The research did not indicate that residential care facilities are likely to be a significant source of pharmaceutical pollution, but as only one partner investigated residential care facilities a general statement cannot be made.

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Chapter 3

Pilot plants investigating pharmaceutical removal

Contents

Abbreviations

EBCT Empty bed contact time
GAC Granular Activated Carbon
MBBR Moving Bed Bioreactor
MBR Membrane Bioreactor
MF Microfiltration
NF Nanofiltration
PAC Powdered Activated Carbon
RO Reversed Osmosis
UF Ultrafiltration
ww wastewater

3 Pilot plants investigating pharmaceutical removal

3.1 Introduction

This chapter gives an overview of the activities of all the PILLS partners who implemented a pilot-plant or demonstration plant. Two 'full scale' plants were implemented in Germany (DE) and the Netherlands (NL), while pilot scale plants were operated in Luxemburg (LU) and Switzerland (CH).

All the designs of the pilot plants are characterized by a relative high degree of complexity as the focus of this research is to eliminate (the majority) of pharmaceutical compounds, that are in general persistent. Characteristic of each design is that the core technology comprises a membrane bioreactor (MBR) followed by varying advanced physical-chemical (UV, Ozone, Activated Carbon) treatment methods. Also reverse osmosis (RO) and oxidation with ferrate, titanium oxide and hydrogen peroxide (TiO_2/H_2O_2) are described.

In this chapter, the performance of the pilot installations is evaluated in terms of removal efficiencies for pharmaceutical substances and the 'classical' parameters (COD, BOD, N, P) as well as the energy consumption. Further performance evaluation with regard to ecotoxicity and antibiotic resistance are described in the chapters 4 and 5.

The evaluation is carried out for the list of pharmaceuticals as described in chapter 2. As stated there, pharmaceuticals (or: pharmaceutical compounds) are defined as comprising all therapeutical sub-classes, used by patients within the hospital, including X-ray contrast media.
In order to enhance the removal of pharmaceuticals from wastewater, advanced treatment techniques after secondary biological treatment are required. Oxidation is a possible advanced treatment alternative. Several oxidation techniques exist. In the next sections the oxidation using ozone and other Advanced Oxidation Processes (AOP's) will be explained.

3.1.1 Ozone

Ozone is an oxidant used widely for the disinfection of drinking water but also for wastewater polishing. Ozone can react directly with dissolved organic substances or it can form secondary oxidants like OH• (Staehelin and Hoigne 1985). Decompositions of ozone via reactions with OH- and organic substances yields hydroxyl radicals. The produced OH radicals can oxidize the pollutants (alkyl groups of organic compounds) but they also can be scavenged by organic substrates or bicarbonates (Chen 1997).

The oxidative activity and selectivity of ozone is dependent on the organic compounds that have to be oxidized (Chen 1997). Ozone is a very selective oxidant, which reacts with specific functional groups ((Huber, Gobel et al. 2005). Ozone generally reacts faster with deprotonated species. For example, roxithromycin reacts much faster when its amine group is nonprotonated. Therefore, its reactivity with ozone is pH dependent (Huber, Canonica et al. 2003). Ozone reacts as well selectively with double bonds. Also phenolic groups can react with ozone (Huber, Canonica et al. 2003). Groups like alcohols, aldehydes, ketones, iodine and chloride only slowly react with ozone (Ternes, 2006). Pharmaceuticals like bezafibrate, diclofenac, sulfamethoxazole and carbamazepine posses these specific groups reacting with ozone (Huber, Canonica et al. 2003).

The ozone dosages applied in post treatment of wastewater will result in the formation of by-products and oxidation products. Ozonation of wastewater will lead to partial oxidation of the organic compounds and therefore organic oxidation products are expected in the effluent of the oxidation unit. These products can be toxic or persistent to biodegradation. However, research revealed reduced toxicity of wastewater after ozonation (Ternes and Joss 2006).

In addition to the formation of organic oxidation products, bromate formation from bromide is also possible during ozonation of wastewater (Huber, 2003).

In general, ozone dosages of 2-5 mg/L should be sufficient for the removal of 90-99% of the pharmaceuticals in wastewater containing <8 mg DOC/L. For DOC levels of 23 mg DOC/L, the ozone dosage will be in the range of 5-10 mg/L ozone (Ternes and Joss 2006).

3.1.2 Advanced Oxidation Processes

Advanced Oxidation Processes (AOPs) are combined processes aiming mainly at formation of the hydroxyl radical (OH•) (Kümmerer 2001). The OH-radicals are strong non-selective oxidants. They can oxidize pharmaceuticals but also other organic compounds. In addition, the hydroxyl radicals can be scavenged by the water matrix (Ternes and Joss 2006). With AOPs, partial oxidation of the pharmaceuticals will be achieved. Formation of oxidation products is expected and should be researched with respect to their toxicity and biodegradability. Main AOPs for pharmaceutical removal in wastewater and drinkwater treatment are UV/Ozone, UV/H_2O_2, Ozone/H_2O_2, Fenton reactions and UV/TiO_2.

The H_2O_2 can be used in combination with ozone to oxidize organic compounds (Chen 1997). The addition of H_2O_2 leads to the decomposition of ozone and the formation of OH• (Westerhoff, Rodriguez-Hernandez et al. 2005). The efficiency of the reactions when applying Ozone/H_2O_2 depends on the OH• scavenging of the water characteristics (Huber, Canonica et al. 2003). In general, it is expected that the addition of H_2O_2 to the ozonation unit will result in only a slightly higher removal of pharmaceuticals. The natural organic matter present in the wastewater can also catalyse the OH-radical formation from ozone (Ternes and Joss 2006).

3.1.3 UV/Ozone

The UV-light can photolyse compounds directly, but normally UV is used in combination with oxidants (Vogelpohl 2007). During UV/ozone processes, ozone absorbs UV-light and is subsequently photolyzed, resulting in the production of hydrogen peroxide (H_2O_2) and OH• (Chen 1997). This AOP is comparable to the ozone/H_2O_2 process. The UV-light should be emitted at a wavelength of 254 nm or shorter to effectively photolyse the ozone (Chen 1997).

3.1.4 UV/H_2O_2

Another technique to oxidize organic compounds is the application of UV and H_2O_2. The H_2O_2 can be photolyzed with UV to OH•. The wavelength of the UV should be short (eg. 185 nm) in order to effectively photolyse H_2O_2. At wavelengths of 254 nm, the photolyses of H_2O_2 is by far less effective than of ozone. The UV/H_2O_2 process is expected to yield comparable results as the ozone/H_2O_2 process. Therefore the costs of both process might be most important for the optimal choice (Ternes and Joss 2006).

3.1.5 Fenton reactions ((UV)/H_2O_2/Fe^{2+} or Fe^{3+})

To accelerate the decomposition of H_2O_2, a ferrous ion catalyst can be used. The oxidative reactions with Fe^{2+} or Fe^{3+} and H_2O_2 is called a Fenton reaction. When the H_2O_2 and the ferrous ion catalyst are used in combination with UV, then the reaction is called photo Fenton reaction (Chen 1997). A drawback of Fenton oxidation is that the optimal pH range for high process efficiency is low and narrow. It is between a pH of 2-4.

3.1.6 UV/TiO_2

The oxidation of pollutants using UV in combination of TiO_2 is an example of heterogenous photocatalysis: TiO_2 is an effective photocatalyst and is present in solid phase. When UV is illuminated on TiO_2, various organic compounds can be oxidized at the surface of TiO_2 or they can be oxidized in the solution because the illumination of TiO_2 results in strong oxidation and reduction sites at the surface of the photocatalyst (Chen 1997) Also OH• and other radicals can be formed at the surface of TiO_2 (Yang, Yu et al. 2008). This technique can be used for the oxidation of pharmaceuticals. Advantages of the technology is that it can be operated at ambient conditions and that the catalyst is inexpensive (Klavarioti, Mantzavinos et al. 2009). A potential disadvantage is that the separation of powdered TiO_2 from the wastewater can be a problem. Fixing the TiO_2 on carriers can be a solution for this but could also reduce the efficiency of the system (Chen 1997) because the surface area available for reactions will be reduced.

3.2 Characteristics of each pilot plant

3.2.1 Full scale plant in Germany

Influent quantity and quality
The pilot plant in Germany treats the whole effluent of the hospital. The hospital wastewater is coming from Marienhospital Gelsenkirchen. This hospital has 580 beds. The designed wastewater flowrate was 200 m^3/day.

Table 3-1: Wastewater characteristics of demonstration plant Gelsenkirchen (during the start-up period of 6 months) (Nafo et al., 2012)

Process Parameter	Unit	Median ± Standard deviation	Design
Flow rate hospital wastewater	m^3/d	90.0 ± 21.4	200
T in bioreactor	°C	26.8 ± 1.0	25
pH in bioreactor	–	6.8 ± 0.2	7
COD	mg/L	709 ± 280	1407
Total N	mg/L	64 ± 21	79

Pre-treatment

Pre-treatment includes a fine screen of about 1 mm mesh size. No further pre-treatment was applied at the German demonstration plant.

Main biological treatment

The main biological treatment consists of an aerobic MBR. The MBR tanks has a volume of 250 m^3. The designed sludge concentration was to 8-12 g/l with a design SRT of 20-25 d. The aim of the MBR is mainly organic removal, disinfection (through the use of membranes) and nutrients removal. Submerged flat-sheet UF-membranes are used (Biocel® from Microdyn Nadir, Germany, pore size of 0.04 µm). Three modules with a total membrane surface of 1,200 m^2 were directly immersed in the nitrification tank.

Advanced treatment

Advanced treatment consists of ozone, powdered activated carbon and sand filtration. Parallel operation of the advanced treatment steps and different combinations including recirculation loops, as well as serial connections were investigated.

The ozone unit can treat up to 100% of the permeate and has a volume of 3 m^3, enabling the dosage of 5-10 mg/L of ozone at a HRT of at least 15 min.

Ozone is produced locally from air oxygen. For this purpose ambient air is compressed by a compressor, dried and fed into the ozone generator. The ozone reactor consists of bubble columns in series DN that are about 5.0 m high. The number of columns in operation can be varied. Ozone is fed into the reactor through ceramic diffusers.

The PAC dosage is 20 mg/L. The PAC treatment process comprises a contact reactor with an HRT>30 min. The loaded PAC is finally separated from the treated wastewater by a sand filtration (filtration velocity, vF< 12m/h). If applying direct dosing into the bioreactor the PAC dose was adjusted to 40 mg/L.

Effluent quality and destination

- The effluent of the pilot plant is discharged into surface water.
- The sludge waste of the pilot plant is incinerated at a municipal WWTP.
- There is an extensive multi-step exhaust air treatment with filtration, UV, catalytic oxidation and ionization.

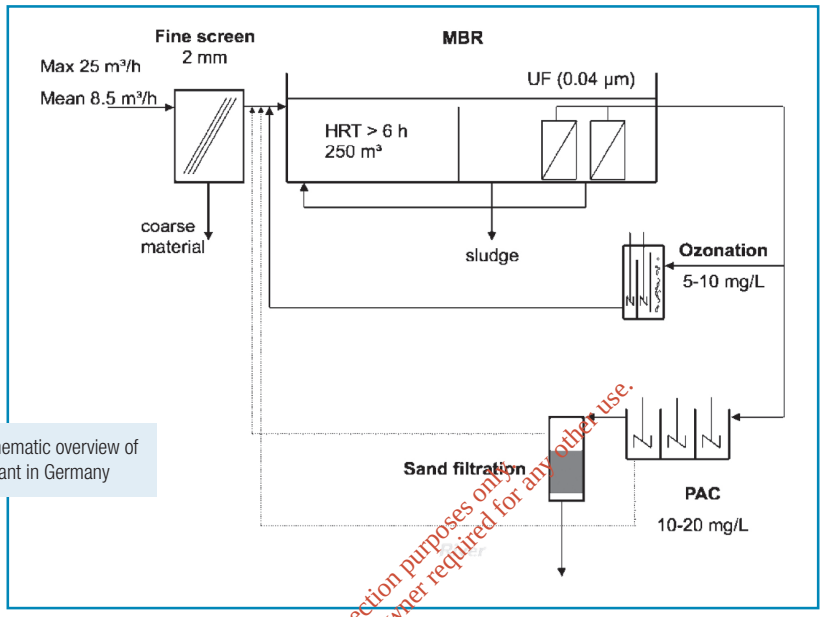


Figure 3-1: Schematic overview of the full scale plant in Germany

3.2.2 Full scale plant in the Netherlands

A simplified process flow diagram is presented in Figure 3-2. The wastewater is collected in a buffer tank at the hospital site, then transported to the demo-plant where it is treated using a coarse screen, drum sieve, biological reactor and ultrafiltration (UF) membranes followed by activated carbon filter. Post-treatment consisted of three units in parallel: ozone oxidation unit, a UV/H_2O_2 oxidation unit and/or reverse osmosis (RO) filtration unit. The treated effluent of the demo-plant was discharged to the sewer. The following paragraphs describe the different parts of the process flow diagram.

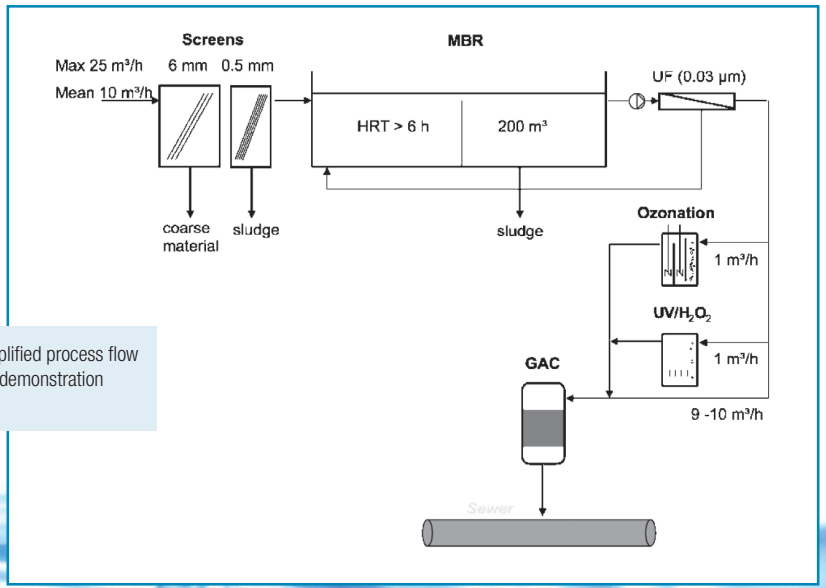


Figure 3-2 Simplified process flow diagram of the demonstration plant in NL.

Wastewater

The demonstration plant in the Netherlands was designed for the treatment of the hospital wastewater from Isala Clinics, Zwolle. The design wastewater characteristics of the plant, as given in Table 3-2, were mainly based on the hospital wastewater characteristics in 2007. In 2007 the average hourly flow of hospital wastewater was 8.5 m^3/h on weekdays and 5.5 m^3/h in the weekend. Taking into account the extension plans of Isala Clinics in the near future (a new-construction project of the hospital at this location) would result in an hourly average design flow of 10 m^3/h. The design composition of the wastewater was based on 7 measurements of the hospital flow taken in 2007 (Table 3-2). Temperature measurements were not available and this parameter was therefore assumed. The hospital wastewater was expected to have a higher strength in terms of COD and BOD than municipal sewage mainly because of the exclusion of rainwater. In addition to the values of several parameters in Table 3-2, it was assumed that the following ratios apply:

- COD/BOD 3
- COD/P-total 10
- COD/Suspended Solids 2

Table 3-2: Design wastewater characteristics of NL plant

Parameter	Unit	Minimum	Average	Maximum
Flow	m^3/h	0	10	25
pH	–			
Temperature	°C	13 (max 3 months)	17.5	24 (max 1 month)
COD	mg/L	490	576	690
N-Kjeldahl	mg/L	50	59	69

Buffering wastewater

The wastewater of Isala Clinics (influent) is first collected in a buffer tank at the hospital site and then pumped to the demonstration plant, close by. During the research phase, two buffer tanks were in use. The first tank (200 m^3) was the original buffer tank of the hospital used for discharge of sewage. This buffer tank was operated at approximately 50% of its capacity and thus 80 m^3 was available for buffering of the sewage. A second buffer was built during the new-construction project of the hospital, with an average hydraulic retention time of approximately 1-2 hours (volume is about 50 m^3). With an average discharge of hospital wastewater of about 10 m^3/h, the hydraulic retention time of the wastewater in these buffer tanks was estimated to be 0.75 day (about 18 h). The exact operational regime with regard to the transport of the wastewater from the buffer tanks to the demonstration plant is unknown. Generally, if there was sufficient wastewater it was pumped to the demonstration plant at a flowrate of 25 m^3/h (however, it is possible that during night time wastewater was collected in the buffers and during daytime pumped to the demo installation).

Pre-treatment

Pre-treatment of the wastewater comprised a rake screen of 6 mm for the removal of coarse material. In order to protect the membranes of the MBR, additionally a drum filter for the removal of fine particulates was applied with a filtration level of 0.5 mm (Micro Drum Filter, MDF -802, LWS).

Membrane bioreactor

Bioreactor

The main function of the aerobic bioreactor was the removal of organic pollutants and the nitrification of the wastewater. Because the focus of the research was mainly on the removal of pharmaceuticals, for operational ease, complete nitrogen and phosphate removal were considered to be of less priority and the plant was not explicitly designed to eliminate them. If required however, both could be achieved by intermittent aeration for nitrogen removal and the addition of chemicals (e.g. $FeCl_3$) for phosphorus removal. However, during the research phase these options were not used.

The influent flow of the bioreactor resulted from the discharge system of the hospital wastewater. If there was sufficient wastewater in the buffer tanks at the hospital site, it was pumped at 25 m^3/h to the plant. Because the membranes of the MBR had a maximum net average design capacity of 10 m^3/h, the water level in the bioreactor could increase to the maximum height of 5.6 m when there was a large supply of wastewater. When the bioreactor was completely filled, the excess hospital wastewater could be by-passed to the sewer. On the other hand, when there was a relatively low flow of wastewater the water level in the bioreactor could drop to a minimum water level of 4 m (corresponding to 200 m^3 reactor volume). In such a situation the permeate production was temporarily stopped. Therefore, in short, the bioreactor could co-function as a buffer tank because of the higher capacity of the pumps of the buffer tanks (25 m^3/h) compared to the membranes (10 m^3/h). The by-pass of wastewater to the sewer was possible at several locations of the demo-plant.

The design dimensions of the bioreactor are presented in Table 3-3. The air supply into the bioreactor was accomplished by the use of two compressors and fine bubble aeration (disc aerators).

Table 3.3: Design parameters of the bioreactor

Parameter	Unit	Value
Total volume	m^3	280 (incl. 80 m^3 buffer volume)
Minimum water level	m	4
Maximum water level	m	5.6
Sludge load	kg BOD/(kg TSS*d)	0.035
Sludge concentration	g TSS/L	8-12
Excess sludge	m^3/h	Min: 0 Average: 3 Max:10

Membrane unit

The membrane unit was placed outside the bioreactor (external tubular membranes). The characteristics of the membrane unit are given in Table 3-4 and the principle of the membrane extraction unit is shown in Figure 3-3. Through the use of circulation pumps and air, water and sludge from the bioreactor were transported through the membrane extraction unit. The filtration direction was from the inside to the outside of the tubular membranes. Permeate production was regulated with permeate pumps and the concentrate was fed back to the bioreactor. To prevent fouling of the membranes, back flushing with permeate and automated chemical cleaning was applied once a week with a 15% solution of Sodium Hypochlorite and a soak time of 40 minutes. The cleaning water was, after use, returned to the bioreactor. The maximum net design capacity of the membranes was 10 m^3/h. The produced permeate was collected in a 5 m^3 permeate buffer.

Table 3-4: Characteristics of membrane unit

Parameter	Unit	Value
Amount of modules	#	9
Module type	–	Hyperflux tubular module (MO 83G_I8LE BA, Berghof)
Module material	–	FRP (Fiber Reinforced Polymer), resin
Membrane area	m^2/module	27.2
Membrane type	–	66.03 I8 (Berghof)

Membrane pore size	nm	30
Membrane material	–	Polyvinylidene fluoride
Membrane inner diameter	mm	8
Membrane support	–	Polyester/Polyester

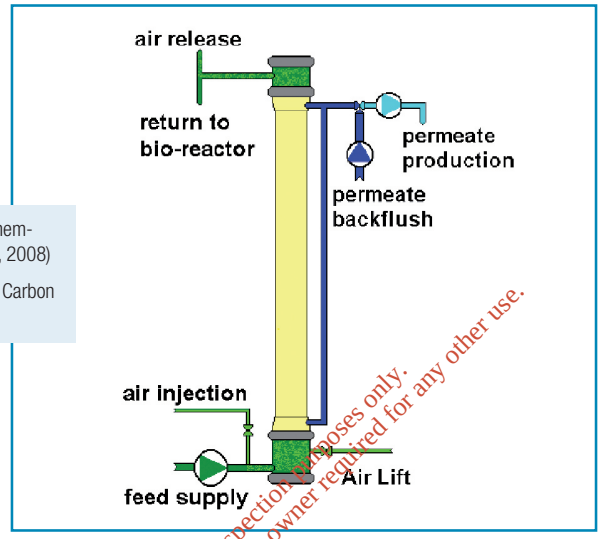


Figure 3-3: Principle of the membrane extraction unit (Koetse, 2008)

Full-scale Granular Activated Carbon Filter (GAC-1)

The main part of the collected permeate was treated in a full-scale Granular Activated Car-bon filter (GAC-1) of 8.5 m^3. The characteristics of this filter are presented in Table 3 5.

Table 3-5: Characteristics of full-scale activated carbon filter, GAC-1

Parameter	Unit	Value
Type of GAC	–	NORIT, ROW 0,8 SUPRA
Design flow	m^3/h	10
Volume	m^3	8.5
Diameter filter	m	1.9
Filter bed height	m	3.0
Empty Bed Contact Time (EBCT)	min	51

Ozone + GAC filter (pilot-scale)

One of the advanced treatment techniques investigated within the PILLS/SLIK project was the application of ozone oxidation followed by activated carbon treatment. The influent of this treatment system was the permeate from the MBR. The ozone and activated carbon installations were designed for treatment of permeate at pilot-scale.

Ozone unit

The characteristics of the ozone unit are presented in Table 3-6. The ozone unit had a plug-flow configuration with an effluent recirculation flow. The ozone dosing to the permeate took place in the influent tube. The minimum applicable ozone dose was 10 g/h and the maximum ozone dose was 40 g/h using compressed air. Approximately 5-10% of the ozone was not dissolved in the permeate, but left the ozone unit through the flue gas which was fed to the biological reactor.

Table 3-6: Characteristics of pilot-scale ozone unit

Parameter	Unit	Value
Type	–	Lenntech, ICT 80
Design flow	m^3/h	1.2
Ozone generation	g O_3/h	10-40
Max ozon supply	g O_3/h	40
Contact time	Min	40
Oxygen supply		From air
Contact tank	m^3	0.750
Energy consumption	W/h	580-840

Granular Activated Carbon Filter (GAC-2)

The effluent of the ozone unit was further treated in a pilot-scale activated carbon filter (GAC-2). Its characteristics are given in Table 3-7.

Table 3-7: Characteristics of pilot-scale Activated Carbon filter

Parameter	Unit	Value
Type of AC	–	NORIT, ROW 0,8 SUPRA
Design flow	m^3/hh	1.2
Volume	m^3	1.2
Diameter filter	mm	600
Filter bed height	m	2.0
Empy Bed Contact Time (EBCT) Time (EBCT)	min	60 min

UV/H_2O_2 + GAC filter (pilot-scale)

The second post-treatment line for the removal of pharmaceuticals investigated within this project was the treatment of permeate from the MBR by activated carbon filter followed by UV/H_2O_2 oxidation and, subsequently, a second activated carbon filter. The first filtration step was achieved using the full-scale activated carbon filter. The effluent of the full-scale AC filter was subsequently treated in the pilot UV/H_2O_2 installation followed by the second AC filter (GAC-3).

The GAC-1 was applied to reduce the dissolved organic matter concentration before the oxidation by UV/H_2O_2. However, it turned out during the research phase, that the concentration of pharmaceuticals in the effluent from this activated carbon filter was very low. Therefore, to be able to assess the performance of the UV-installation, permeate of the MBR was also used directly as influent for the UV/H_2O_2 unit.

UV/H_2O_2 unit

The UV-unit consisted of 3 modules each containing 2 medium pressure lamps (total 6 UV-lamps of 400-800 W each). The UV-unit had a plug-flow configuration. A 17.5% H_2O_2-stock solution was pumped to the influent tube of the UV-unit at either a low or a high flowrate (150 ml/h or 300 ml/h respectively).

The characteristics of UV/H_2O_2 installation are presented in Table 3-8.

Table 3-8: Characteristics UV/H_2O_2 installation

Parameter	Unit	Value
Design flow	m^3/h	3.5-5
Modules	#	3
Lamps	#/module	2
Medium pressure lamp	W	400-800
Wavelength of lamp	nm	200-300
Sleeve	nm	> 240 nm
Contact volume	L	5 per module; 15 total
Internal diameter	mm	125
Pipe length	m	1.3 (3 modules)
Length between sampling points influent and effluent	m	2.5 total
Brand	–	Berson

Granular activated carbon filter (GAC-3)

The characteristics of the activated carbon filter used to filter the effluent from the UV-unit (GAC-3) were identical as those of GAC-2 (Table 3-7).

RO+GAC filter (pilot-scale)

RO unit

The Reversed Osmosis (RO) system consisted of 1 membrane. The details of these membranes are given in Table 3-9.

Table 3-9: Details of the RO membrane

Parameter	Unit	
Membrane type	–	ACM-LP Fully Aromatic Polyamide Low Pressure Advanced Composite
Model	–	4040-ACM5-TWF (TRISEP Corporation)
Configuration	–	Spiral Wound, Fiberglass Outer Wrap
Active membrane area	m^2	8.2
NaCl rejection (average)	%	98.50

Activated carbon filter (GAC-4)

The effluent of the RO unit was further treated in a pilot-scale activated carbon filter (GAC-4). Its characteristics are given in Table 3-10.

Table 3-10: Characteristics of pilot-scale Activated Carbon filter

Parameter	Unit	Value
Type of AC	–	NORIT, ROW 0,8 SUPRA
Design flow	m^3/h	0.5
Volume	m^3	0.5
Diameter filter	mm	250
Filter bed height	m	2.0
Empy Bed Contact Time (EBCT)	Min	60 min

Buffer tanks

As described previously , during the research period two buffer tanks were operated for the collection of raw hospital wastewater (influent). To be able to operate the different process units within the PILLS/SLIK-plant as much as possible independently from each other, another three buffer tanks were applied to collect and store (treated) wastewater. An overview of the volumes of the buffer tanks and their location is in given in Table 3-11.

Table 3-11: Buffer tank volumes

Buffer tank	Retention time (h)	Volume (m^3)	Location
Hospital wastewater buffer tank I	10-15	200 (operated at 50% of capacity)	Hospital site
Hospital wastewater buffer tank II (new)	1-2	50	Hospital site
Permeate buffer tank		5	Directly after MBR
Buffer tank after O_3-unit	0.9	0.7	In between O_3-unit and pilot-scale GAC-2
Buffer after GAC-1		24	Directly after full-scale AC filter

3.2.3 Pilot plant in Switzerland

Influent quantity and quality

The pilot plant of Eawag treats wastewater from the Swiss cantonal hospital in Baden. Details are given in McArdell et al. 2011 and Kovalova et al. 2012. This hospital has 346 beds and produced 673 $L.bed^{-1}.day^{-1}$ of wastewater in 2009. The pilot plant treated wastewater excluding the wastewater from the laundry facility. The flow of wastewater for the main biological treatment was on average 1.2 m^3/d with a maximum of 1.7 m^3/d. The influent was pumped continuously and flow-proportionally from the sewer, based on real-time measurements of hospital drinking water consumption, which have been shown to be proportional to the wastewater level in the sewer. The suction tube was perforated with holes of 4 mm diameter for about 12 cm to keep bigger particles out of the pilot plant. A flushing system was installed to clean the suction tube with drinking water (once every hour). Two sprays were installed facing the perforated suction tube and were shown to be necessary in order to prevent clogging. The advanced treatment techniques were designed for different flows (0.100-24 L/d) and were run independently from the wastewater flow.

Pre-treatment

To remove settleable solids a primary clarifier was used with a volume of 0.5 m^3 and a long HRT of about 10 h.

Main biological treatment

The main biological treatment comprises an MBR unit with a pre-anoxic zone of 0.5 m^3 for pre-denitrification and an aerobic zone of 1 m^3 (Picatech Huber AG, Kriens, Switzerland) that was installed with the assistance of Holinger AG (Liestal, Switzerland). The total HRT was 32 h as measured by a tracer test. Submerged ultrafiltration flat sheet membrane plates (Huber MembraneClearBox®, PP carrier, PES membrane, 7m2, 15-30 $L.m^{-2}.h^{-1}$, 38 nm pore size, 150 kDa) were used. Wastewater from the aerobic compartment was recycled to the anoxic compartment (6-8 m3/day). The excess sludge volume was 20-50 L/day. The sludge concentration in the MBR was on average 2 g/L, the SRT 30-50 days (organic sludge load 0.06-0.1 $gCOD.gTSS^{-1}.d^{-1}$ corresponding to 0.03-0.05 $gBOD_5.gTSS^{-1}.d^{-1}$), the average operating temperature was 29°C (the temperature of the wastewater was 27-28°C), pH 7.8, and the conductivity was 1100 µS/cm. The oxygen concentration in the aerobic compartment was maintained by aeration at 3±2 mg/L. The permeate was discharged into a buffer tank with a HRT of approximately 4 h.

Advanced treatment

Three processes were investigated for the treatment of the permeate from the MBR: ozonation, Powdered Activated Carbon (PAC) and UV/TiO_2 photocatalyses. These post-treatments were run with a disconnected MBR to ensure a constant concentration of micropollutants and a constant flow in the influent to the units.

For the ozone treatment, 4-7 g /m^3 ozone (0.64 – 1.08 g O_3 / g DOC) was utilized in a counter current bubble column of 2 m height and 4.5 L volume. The ozone was produced from the oxygen in the air with the Chemodata 1.0 g/Hmax (Chemonorm AG, Altendorf, Switzerland) Generator. The water flow was adjusted to around 0.2-0.6 m^3/d to achieve the required ozone concentration in the column, resulting in a HRTs of 10-30 minutes. The ozone analyzer BMT 964 provided an online measurement (BMT Messtechnik Gmbh, Stahnsdorf, Germany). Since an excess of ozone was measured in the off gas of the column, the utilized ozone dosage was calculated from the applied dosage minus the lost ozone in the off-gas.

For the studies with powdered activated carbon (PAC), Norit SAE Super was used as an adsorbent (surface area 1300 m^2/g). The water flow into the 180-L PAC reactor was kept constant for all 3 set-ups (8, 24 and 43 mg PAC/L) at 0.18 m^3/d, resulting in a HRT of 1 day. The effluent of the PAC reactor was filtered through UF membrane flat sheets (Martin System, siClaro FM 611, pore size 0.04 micrometer, membrane surface 6.2 m^2). The PAC reactor retention time was kept at 2 days for most experiments. An increased temperature in the PAC-reactor (32°C) was caused by recirculation pumps ensuring mixing in the reactor. Samples were taken after an equilibration times of 3 or 4 days.

For the post-treatment with UV/TiO_2, a commercially available setup from UBE Industries (Model 3 m^3/h) was operated with a water flow of 14 m^3/d (HRT 11/18 sec. with/without photocatalytic fibres). The reaction column contained four cartridges consisting of a photocatalytic fiber with embedded TiO_2, positioned around a low pressure UV lamp (254 nm, 220 V, 100-400 W overall energy consumption (including 40 W lamp power consumption)). The effective fluence with the photocatalytic fiber was 1.7 mW/cm^2 (fluence of 306 J/m^2 after a single treatment), and 4.0 mW/cm^2 (fluence of 800 J/m^2 for a single treatment) in the UV system with removed photocatalytic fibres. The volume of the column was 3.0 L (3.34 L without the fibers). In order to achieve a higher elimination, the system was run with several cylces using a fluence of 2754 J/m^2 (UV/TiO_2) and 7200 J/m^2 (UV only), respectively, in 9 cycles.

As a final step, a biological post treatment step was included to reduce oxidation byproducts. This comprised a moving bed bioreactor (MBBR) with a volume of 0.2 m^3 and HRT of 0.3-1 days at a flow of 0.2-0.6 m^3/d. The MBBR was aerated and mixed. Figure 3-4 shows a schematic of the pilot plant.

Effluent quality and destination

The effluent of the pilot plant and the produced sludge were discharged into the sewer system.

The off-gas of the MBR was treated using a granular activated carbon (GAC) column to remove odor and possibly harmful substances. A self-regulating heating was installed (temperature always below 100ºC) to avoid condensation of the air and clogging of the activated carbon column.

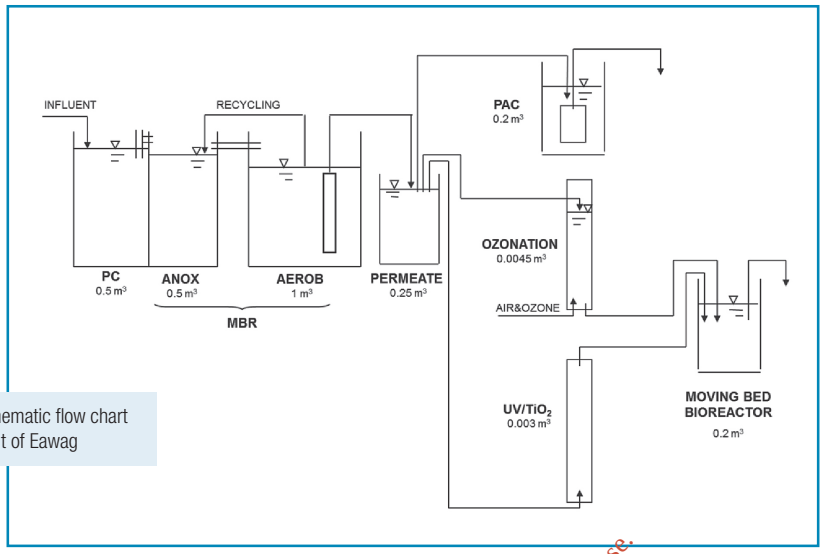


Figure 3-4: Schematic flow chart of the pilot plant of Eawag

Calculation of elimination rates

If the concentrations of the analyte in the effluent of a treatment system was lower than LOQ, a minimal elimination rate was calculated based on the LOQ. For calculating the elimination in the MBR, the total load over three weeks was analyzed from three 2- or 3-day flow-proportional composite samples per week and the variation calculated during the three weeks. For the investigations on the performance of the post-treatments a permeate that was collected, mixed and stored before use in the post treatments was used, resulting in constant influent concentrations. Two experiments were performed for each concentration of ozone, PAC or UV dosages, except for 20 mg/L PAC where four experiments were run.
Pilot plant in Luxembourg

3.2.4 Pilot plant in Luxembourg

Influent quantity and quality

The Public Research Centre (PRC) Henri Tudor built a pilot plant treating part of the hospital wastewater produced in the Centre Hospitalier Emile Mayrisch (CHEM). In total the CHEM has 640 beds and 360 beds are located at the hospital that was investigated in Esch-sure-Alzette. The total Inflow to the pre-treatment of the pilot plant was on average 9m³ per day. The hydraulic capacity of the MBR was 1-3 m^3/d. However, the average inflow to the biological treatment unit of 1,7m³ per day turned out to be optimal.

Pre-treatment

Pretreatment consists of a curved sieve, gap width 0,2 mm which was designed to be self-cleaning by hydraulic flushing. Due to the flushing requirement the inflow to the pre-treatment exceeded the hydraulic capacity of the subsequent treatment and the remainder was therefore discharged back to the sewer system. The sewage inflow passing the sieve was collected in a buffer tank which provided sufficient storage volume to feed the biological treatment.

Main biological treatment

The main biological treatment consists of an MBR including a denitrification tank and a subsequent aerobic treatment tank. The denitrification tank has a volume of 0.175 m^3. The biological aerobic treatment tank includes the membrane units and has a volume of 0.515 m^3. The denitrification tank was equipped with a stirrer whereas the aeration in the nitrification unit allowed for a complete mixing and cleaning of the membranes surface. Two microfiltration KUBOTA (London, United Kingdom) M-BOX membrane modules (pore size: 0.4 µm) are submerged in the aerobic reactor (concentration of O_2 around 6.1 mg L^{-1}). The poly-chlorinated polyethylene cartridges are vertically placed in the reactor and comprise a total filtration area of 9.6 m². A recirculation flow from the aerobic to the anoxic compartment provides nitrate nitrogen rich wastewater to the denitrification process. The recirculation flow equates to about four times the MBR influent flow. The system is designed to benefit from the deve-

lopment of a filtration layer on the membranes which reduces the effective pore size to around 0.1 µm allowing for an ultrafiltration mechanism. A schematic drawing of the main biological treatment is presented in Figure 3-5.

Figure 3-5: Schematic of the Luxemburg pilot plant.

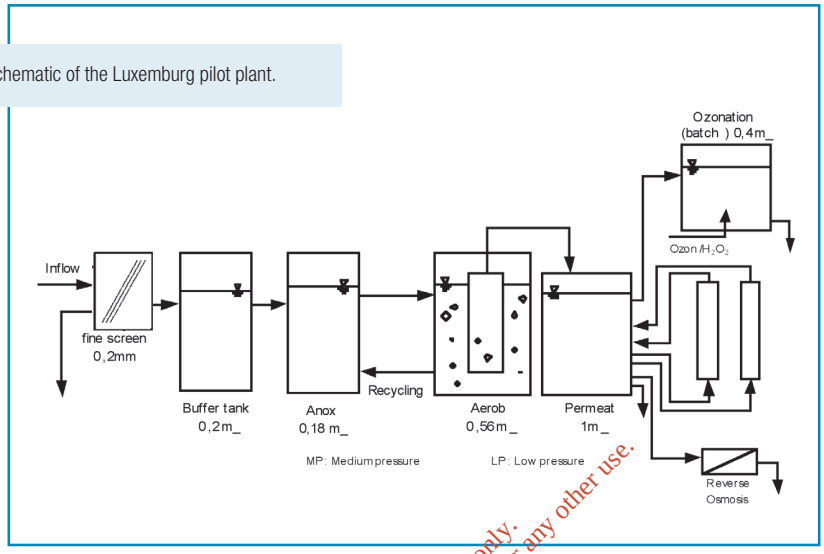


Table 3-12: MBR in Esch-sur-Alzette design and operation parameters

Parameter	Unit	Value
Design Parameter		
Design capacity	m^3 h-1	0.125
MBR volume	m^3	0.690
Denitrification tank volume	m^3	0.175
Max. design flow	m^3 d-1	3
Average flow	m^3 d-1	2
Max. TMP	bar	0.2
MBR Operation		
Hydraulic Retention Time (HRT)	h	8
Temperature in the aeration tank	°C	16 – 18
MLSS in the aeration tank	kg m^{-3}	10.0 – 13.2
Sludge loading	kgBOD_5 kg$MLSS^{-1}$ d^{-1}	0.05 – 0.15
Volume loading	kgBOD_5 m^{-3} d^{-1}	0.7 – 1.5
Instantaneous Flux	L m^{-2} h^{-1}	10.9
SRT	d	> 30

Advanced treatment

Three advanced treatments are used to treat the MBR permeate:

- a photocatalytic treatment with UV/H_2O_2
- treatment with O_3/H_2O_2
- treatment with Reverse Osmosis (RO)

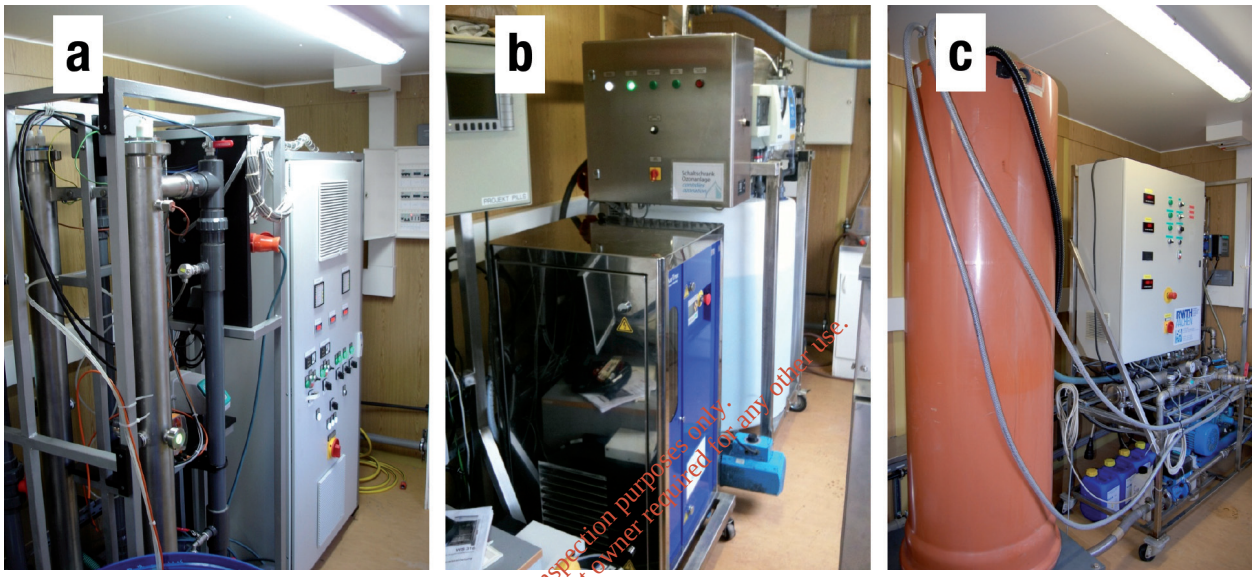


Figure 3-6: Picture of the advanced treatments. a: UV photocatalytic. b: ozone. c: Reverse Osmosis

Photocatalytic treatment with UV/H_2O_2

For the batch studies with UV photocatalytic treatment, two different UV lamps (IBL Umwelt- und Biotechnik GmbH, Heidelberg, Germany) were applied in the framework of this study. A medium pressure (MP) lamp with an adjustable power of 2 kW – 10 kW and a low pressure (LP) UV lamp with a fixed power of 0.25 kW (see Table 3-13). Beside the composition of the noble gases, their filling pressure influences the UV spectrum significantly. Consequently, the LP lamp offers two energy emission peaks at UV light wavelengths of 254 nm and 185 nm while the MP lamp has a polychromatic emission along the UV spectrum. Furthermore, the latter offers (besides direct photolysis) photochemical oxidation process, i.e. in-situ production of hydrogen and hydroxyl radicals that are formed because of the vacuum UV (VUV) spectrum (< 190 nm).

Table 3-13: UV treatment design data (IBL Umwelt- und Biotechnik GmbH, Heidelberg, Germany)

	UV low pressure lamp	UV medium pressure lamp
Name	uviblox® WPT 250	uviblox® WPT 10
Nominal Power	0.25 kWh	10 kWh
Effective radiated power	UVC (185 nm, 254 nm): 110 W	UVC (200 – 280 nm): 1500 W UVB (280 – 315 nm): 800 W UVA (315 – 400 nm): 700 W

For each test with the low and medium pressure UV reactor one cubic meter of MBR permeate collected in a buffer tank served as influent. Tests were always conducted by operating just one UV lamp. Before the water enters the reactor, H_2O_2 can be added to provide (besides the photochemical oxidation process) a combined, advanced oxidation processes. The hereby formed hydroxyl (OH) radicals are considered to oxidize organic material non-selectively and thereby enhancing the elimination of pharmaceuticals. In the comprehensive monitoring program several UV operation modes have been investigated. The operation modes were based on four different process conditions assumed to have significant effects on the degradation efficiency of pharmaceuticals and on the operation expenses: electrical energy needed to reduce the content of pharmaceuticals to a specific concentration, the power variation of the MP UV lamp, the difference between the MP and LP UV lamp and the dosage of H_2O_2.
The variation of the MP UV lamp power was chosen to investigate potential effects of changes in the UV spectrum when a different lamp power is applied. For each of the observed scenarios one cubic meter of pre-treated hospital sewage (permeate) was recirculated in the UV reactor until a total electrical energy input of 10 kWh was obtained.

Treatment with O_3/H_2O_2

For the batch ozone treatment, experiments were performed in a batch reactor (400 L capacity). A G-Sapphire P 20 model ozone generator (AirTree Ozone Technology Co., Ltd) was used for the production of ozone from oxygen-fed (at 4% by weight). The oxygen flow rate to the generator was maintained at 6 l min-1 and monitored with a rotameter incorporated into the ozone generator where ozone is formed between the surfaces of quartz tubes. Ozone production was kept constant for all the experiments (i.e. 23.1 gO_3 h^{-1}). Thus, the ozone dosage was set by the water residence time in the batch reactor. The equipment used offered no possibility to measure the transferred O_3 in the liquid phase. Consequently, ozone losses via the off-gas are not taken into account. All experiments were performed at ambient temperature (i.e. T=22 °C).

Treatment with Reverse Osmosis (RO)

The Nanofiltration/Reverse Osmosis (NF/RO) test rig was also operated as a batch reactor. From the storage tank, the feed solution (MBR permeate) is fed by a pump to the membrane module. The RO module is the DURASLICK 2540 type from Osmonics, having a membrane area of 2.5 m^2. The typical operating pressure is around 13.8 bar while the maximum pressure rises up to 41.37 bar. The typical operating flux is normally in the range of 15 and 25 L $m^{-2}h^{-1}$, at 13.8 bar. The pH ranged in between 4 and 7 during normal operation and between 2 and 10.5 during the cleaning procedure. To reduce fouling, the modules were cleaned using a basic cleaning agent with pH value of 13.8 for 1 h. To prevent scaling effects, the RO module was cleaned using a liquid acid and surfactant-free cleaning agent (pH value about 1), which consisted of orthophosphoric and nitric acid.

3.3 Discussion of the pilot plants

Differences in applied pre-treatment systems are shown in Table 3-14. Pretreatment is especially necessary to protect the membranes which are very sensitive. Installation properties in Switzerland resulted in higher HRT for the primary clarifier. The Luxemburgish and Dutch plants include fine screens as the pre-treatment.

Table 3-14: Characteristics of the pre-treatment of the pilot plants (- = not relevant).

Pre-treatment		DE	NL	CH	LU
Coarse screen	mm	–	6	4	5
Primary clarifier/sedimentation tank (HRT)	h	–	–	10	2
Fine screens (pore size)	mm	1	0.5	–	0.2

In Table 3-15 the characteristics of the main biological treatment are presented. All partners included an aerobic MBR as the main treatment. The main differences are in flow, location of the membrane, type of membranes and involvement of N-removal processes.

Luxembourg included a pre-denitrification tank. In the MBR of both DE and NL, partly anoxic conditions can be created. In the NL and D plants $FeCl_3$ could be dosed, possibly having an impact on the removal of certain pharmaceuticals. However this dosing option was not used during the investigations described in this report.

Many similarities can be identified between the operational parameters of the different MBRs. The HRT of the MBR of NL and CH are similar. Furthermore, differences occur in sludge loading between full and pilot-scale MBRs with significantly higher sludge loading in the smaller systems (see Table 3-15).

The membrane type was generally based on ultrafiltration, microfiltration was applied only in the LU pilot plant.

A significant difference in the membrane configuration is the sidestream configuration of NL and the submerged configuration of DE, CH and LU.

The advanced treatment steps are given in Table 3-16. CH included three advanced treatments (PAC, O_3 and UV/TiO_2) and after the oxidation steps a moving bed bioreactor. NL has two options for the advanced treatment of wastewater with a post treatment after the oxidation steps using GAC. DE investigated the most complete sequence using ozone, PAC, sedimentation and sand filtration. LU tested three advanced treatment steps (RO, UV/ H_2O_2 and O_3/H_2O_2).

The use of ozone for the removal of pharmaceuticals was considered in four pilot plants with different designs and different post-treatment steps. The ozone dosage tested in the four plants was roughly in the same range, 0.45-1.5 g/g DOC.

UV was used by LU, NL and CH. NL and CH both tested different advanced oxidation processes: UV/H_2O_2 and UV/TiO_2 respectively. The Scottish partner also tested Ferrate oxidation on lab scale.

Activated carbon was also applied in three pilot plants. The main differences were found in the use of powdered activated carbon (PAC) by CH and DE and the use of granular activated carbon (GAC) by NL. CH and DE were using different post-treatments to remove the PAC from the wastewater.

Table 3-15: Characteristics of the main biological treatment of the four different pilot plants. (n.a. = not available, - = not relevant)

Treatment		DE	NL	CH	LU
Pre-denitrifaction (HRT)	m^3/h		–		2
MBR	h				
Volume	m^3	250	200	1.5	0.7
Aerobic	%	50	100	67	75
Anoxic	%	50		33	25
HRT	h	60	20	32	8
SRT	d	249	>50	30-50	30-40
Sludge loading rate (F/M)	g BOD/ (gMLSS.d)	0.010	0.014	0.051	0.047
Sludge concentration	g/L	10-12	8-12	2.0	10-13
Membrane pore size	µm	0.04	0.03	0.038	<0.4
Membrane Configuration	–	submerged, integrated filtration tanks	sidestream	submerged, integrated in bioreactor	submerged, integrated in bioreactor
Buffer tank permeate (HRT)	H	n.a.	0.5	–	–

Table 3-16: Advanced treatment options, n.a. = not available, - = not relevant

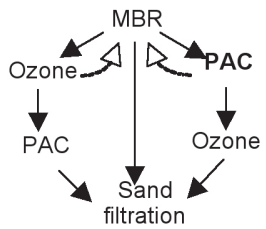
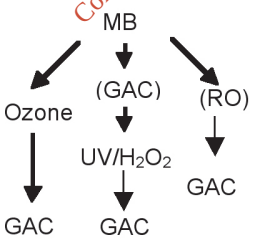
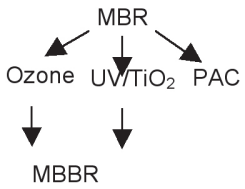
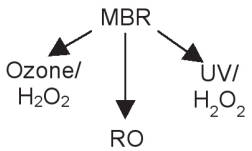
DE	NL	CH	LU
			

Table 3-17: Overview of the main process parameters influencing the elimination …

Tech-nology	MBR					Ozone			PAC	
	MLSS	SRT	HRT	F/M	T	Dose	HRT	Dose	Dose	HRT
Unit	g/L	d	h	kgCOD/ kgMLSS/d	°C	mg/L	min	g /gDOC	mg/L	min
DE	9.5	249	60	0.0396	26	5	30	0.45	20	30
NL	10	infinite	10	0.073	19	13	40	1.5	–	–
LU	10-13	30-40	8	0.08-0.10	18	6-15	6-16	0,24-1.28	–	–
CH	2.0	30-50	32	0.06-0.1	29	4-7	10-30	0.6-1.1	8-43	60

[1] Low pressure UV lamp [2] Medium pressure UV lamp [3] UV-C: 200nm – 280nm, UV-B: 280nm – 315nm, UV-A: 315nm – 400nm

3.4 Treatment results

3.4.1 Influent composition

The wastewater from the hospitals differ in concentrations in the 'normal' parameters COD, phosphorus and nitrogen. These concentrations are within the range of municipal wastewater, however the hospital wastewater at NL was more diluted than anticipated, probably due to buffering tanks in the sewer system, that acted like primary clarifiers.

Table 3-18: Influent characteristics of the wastewaters (mean values or range).

Influent		DE	NL	CH	LU
Flow	m^3/h	3.75	3.75	0.05	0.4-0.125
COD	mg/L	709	424	380	359
P_{tot}	mg/L	8.3	10		6.4
PO_4-P	mg/L			13	
N_{tot}	mg/L	64	66.2	30.1 (NH_4+NO_x)	59.1

… of micropollutants for the applied treatment technologies (mean values)							
UV (AOP)				GAC		RO	
Fluence	HRT	H_2O_2	TiO_2	EBCT	Size	TMP	Recovery
J/m²	Sec	mg/L		min	mm	bar	%
6440	15	5-15		60	0.5-1.5	12	50
7,400- 29,700 [1] UV-C: 10,125- 506,250 [2 3] UV-B: 5,400- 270,000 [2 3] UV-AC: 4,725- 236,250 [2 3]	18-71 [1] 1.3-64 [2]	0-1.1 [1] 0-1.1 [2]	– –	–	–	14	75
800-72001	18-162		fibre				

3.4.2 Effluent from MBR

Effluent from the MBR systems were more or less comparable, with the exception of nitrogen. The NL plant did not operate with denitrification, so total nitrogen concentration was much higher than forthose of the other plants.

Biological treatment was found to be very effective for 3 out of 16 PILLS compounds. Naproxen, Bezafibrate and Atenolol were removed by >80%. This is of specific interest since in Switzerland a 80% threshold is going to be prescribed for the removal of micropollutants from municipal wastewater (FOEN 2012). Half of the analyzed compounds were removed to less than 50% by the MBR. Only partially removed were Diclofenac, Sulfamethoxazole, Iopamidol, and Iopromide, while no removal was detectable for Carbamazepine, Cyclosphosphamide, Ifosfamide, and Diatrizoate. Variable degradation was observed for Erythroymcin, Clarithroymcin and Lidocaine. Ciprofloxacin is mainly eliminated by sorption to sludge and therefore exhibits lower elimination at a lower sludge production typical for MBRs. The elimination efficiencies are in good agreement with results for municipal MBRs (Kovalova et al. 2012).

Table 3-19: Effluent characteristics of biological treatment in MBR

Effluent		DE	NL	CH	LU
COD	mg/L	31	23±2	30	26.5
DOC	mg/L	11± 0,52	8.7 ±0.54*	6-8	
P_{tot}	mg/L	2.0	9.3 ±4		5.3
PO_4-P	mg/L			9	
N_{tot}	mg/L	3.3	53±16	3.8 (NH_4+NO_x)	17

*Measured as TOC

Table 3-20: Removal efficiencies (%) for pharmaceuticals from the mandatory list for MBR treatment

	DE	NL	CH	LU
	n=6	n=6		n=2
Diclofenac	18	35 ±21	-5 ± 3	44
Naproxen	>90	97 ±2	n.a.	34
Carbamazepine	-29	-63±73	-6 ± 12	10
Atenolol	>94	89±6	99 ± 1	80
Bezafibrate	95	98	>91	n.a.
Lidocaine	n.a.	79 ±21	56 ± 13	20
Ciprofloxacin	n.a.	79 ±7	51 ± 13	-3
Clarithromycin	98	34 ±43	50 ± 12	50
Sulfamethaxozole*2)	13	85 ±11	7 ± 57	-76
Sulfamethaxozole&N4-Acetylsulfamethoxazole	n.a.	n.a.	36 ± 28	91
Erythromycin*1)	90	-3 ±82	>60	>98
Diatrizoate	-12	-460±800	-5 ± 16	n.a.
Iopamidol	31	n.a.	-29 ± 218	n.a.
Iopromide	38	n.a.	31 ± 2	n.a.
Cyclosphosphamide	n.a.	26 ±19	>20	13
Ifosfamide	n.a.	-780±1200	<LOD	<LOQ

<LOD/LOQ: below limit of detection/quantification
> : when one or more effluent concentrations were lower than LOQ, and influent was higher than LOQ
*1) Erythromycin was analyzed by Eawag and EG as the sum of Erythromycin and Erythromycin-H_2O
*2) Sulfamethoxazole can be formed from the human metabolite N4-Acetylsulfamethoxazole during biological treatment.

3.4.3 Transformation efficiency by ozonation

Table 3-21 shows the removal efficiencies in percentages after ozonation of MBR permeate at the four plants. Differences can be found due to differences in ozone-dosages. Furthermore, some differences might be explained by differences in design of the contact tank.

The tendencies to remove the pharmaceuticals over the four pilot sites were quite consistent for similar ozone dosages. Eliminations of >80% were achieved for a dosage of 0.5 g O_3/g DOC for most pharmaceuticals. Only the cytostatics cyclosphosphamide and ifosfamide and the X-ray contrast media diatrizoate, iopamidol and iopromide cannot be removed effectively. With a higher dose of 1.1 g O_3/g DOC eliminations are better for these problematic compounds (>55%), except for the X-ray contrast media diatrizoate (16%). Higher concentrations of ozone should not be used due to the formation of potentially carcinogenic bromate from bromide during ozonation (Hutchinson 1997, von Gunten 2003).

For certain compounds like Naproxen, Erythromycin, Bezafibrate and Atenolol the elimination in MBR results in low concentrations at the inflow to the post ozonation. Therefore, concentrations below LOQ may occur more frequently.

The elimination efficiencies are in good agreement with results for municipal wastewater treatment (Hollender et al. 2009). Therefore, experiences from the advanced treatment of municipal wastewater seem to be transferable to the application in hospital wastewater treatment (e.g. HRT and ozone dose).

Table 3-21: Removal efficiencies (%) for pharmaceuticals from the mandatory list for ozonation of MBR permeate using Ozone

g O_3/g DOC	DE (n=6) 0.45	NL 1-3	CH (n=2) 1.08	CH (n=2) 0.64	LU 1.28
Diclofenac	>95	99.7	100	100	99
Naproxen	>60	90	n.a.	n.a.	>88
Carbamazepine	>88	99,7	>99	>99	>99
Atenolol	>17	97	>23	>23	>95
Bezafibrate	>57	n.a.	87	n.a.	n.a.
Lidocaine	n.a.	99	>98	>98	>99
Ciprofloxacin	n.a.	99.9	100	100	91
Clarithromycin	>80	n.a.	100	100	>97
Sulfamethaxozole	>97	99	99	96	>99
Erythromycin[*1)]	>90	n.a.	>93	>93	>33
Diatrizoate	21	45-60	16	7	n.a.
Iopamidol	43	n.a.	55	31	n.a.
Iopromide	0	n.a.	60	37	n.a.
Cyclosphosphamide	n.a.	78-85	57	33	58
Ifosfamide	n.a.	80-90	62	20	n.a.

n.a.: not analysed;
<LOD/LOQ: below limit of detection/quantification
> : when one or more effluent concentrations were lower than LOQ, and influent was higher than LOQ
[*1)] Erythromycin was analyzed by Eawag and EG as the sum of Erythromycin and Erythromycin-H_2O

3.4.4 Pharmaceutical removal with UV

Different combinations of UV with H_2O_2 and TiO_2 and other conditions (different lamps and fluences) were tested, as presented in Table 3-22.

At the Luxembourgish plant, the highest removal efficiencies were obtained with peroxide dosage of about 1 g H_2O_2/L. The NL plant performed less well, probably due to a lower fluence, but also because it was operated differently than the design had intended. The feed of the UV installation was meant to have passed an activated carbon filter, but it turned out that this filter would remove all substances to levels below detection limit. It was therefore decided to bypass the activated carbon filter, resulting in lower transmission of the feed water, leading to lower efficiency of the process.

Furthermore, the low pressure lamps (LP) seem to have a higher efficiency for pharmaceutical removal in combination with peroxide, as demonstrated by the tests in Luxembourg. UV alone was not very effective to eliminate the pharmaceuticals, nor was the treatment with UV/TiO_2 using a photocatalytic fibre. Only UV/H_2O_2 applying a fluence of more than 29700 J/m² with a LP lamp was found to be effective to remove >70% of all the analyzed pharmaceuticals. It has to be noted however, that the X-ray contrast media, which are not effectively removed by ozone nor PAC treatment, can be effectively eliminated with this treatment.

Table 3-22: Removal efficiencies (%) for pharmaceuticals from the mandatory list for treatment of MBR permeate with UV and addition of H_2O_2 or TiO_2

	NL	CH	CH	LU	LU	LU	LU
Medium Pressure/ Low Pressure	**MP**	**LP**	**LP**	**LP**	**LP**	**MP**	**MP**
	UV/H_2O_2	**UV/TiO_2**	**UV**	**UV**	**UV/H_2O_2**	**UV**	**UV/H_2O_2**
Fluence (J/m²)	**6440**	**2754**	**7200**	**29700**	**29700**	**A:47250 B:54000 C:101250**	**A:47250 B:54000 C:101250**
H_2O_2 (g/L)	**–**	**–**	**–**	**–**	**1**	**–**	**1**
Diclofenac	97	90	>98	>99	97	>99	>99
Naproxen	50	n.a.	n.a.	n.a.	>94	n.a.	n.a.
Carbamazepine	n.a.	2	1	21	94	81	97
Atenolol	23-50	0	0	<LOQ	89	>84	<LOQ
Bezafibrate	25	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
Lidocaine	-86	2	5	21	87	83	99
Ciprofloxacin	72	35	57	n.a.	93	n.a.	n.a.
Clarithromycin	17	7	14	7	85	84	>99
Sulfamethaxozole	48	50	85	79	82	>98	>99
Erythromycin[*1)]	21	0	10	n.a.	n.a.	n.a.	n.a.
Diatrizoate	32-58	73	96	n.a.	n.a.	n.a.	n.a.
Iopamidol	n.a.	41	92	n.a.	n.a.	n.a.	n.a.
Iopromide	n.a.	63	92	n.a.	n.a.	n.a.	n.a.
Cyclosphosphamide	9	3	0	-3	70	58	>75
Ifosfamide	3-15	0	n.a.	<LOQ	<LOQ	<LOQ	<LOQ

n.a.: not analysed;
< : LOD/LOQ: below limit of detection/quantification
> : when one or more effluent concentrations were lower than LOQ, and influent was higher than LOQ

The energy input for the Luxemburgish application of the MP UV lamp with and without H_2O_2 input were quite high (10 kWh/m³). Results indicate a significant increase of treatment efficiency for the scenario including H_2O_2 injection only for a few substances like Cyclophosphamide in this case.

3.4.5 Pharmaceutical removal with activated carbon

In some plants the MBR permeate was also treated with activated carbon, either by adding powdered activated carbon (PAC), or by activated carbon filtration (the filter consisting of granular activated carbon, GAC).

Addition of powdered activated carbon led to considerable removal of pharmaceuticals. Eliminations of >80% were calculated for most pharmaceuticals applying 20 mg/L PAC. Only sulfamethoxazole and the X-ray contrast media diatrizoate and iopamidol showed lower elimination. In the CH plant, at a dosage of 43 mg/L PAC the elimination for sulfamethoxazole was still only 62% and less than 20% for diatrizoate.

A comparision to the application of PAC in municipal wastewater is difficult because there are not many results published yet. In general, elimination seems to be similar. Better removal was obtained by recycling the PAC used in a post-treatment back to the activated sludge treatment in municipal WWTPs (Boehler et al. 2012). Compared to ozonation, PAC treatment is less effective for sulfamethoxazole, but slightly more efficient for X-ray contrast media removal.

Activated carbon filtration (GAC) reduced all pharmaceuticals to levels below detection limit, corresponding to removal efficiencies of more than 95%.

Table 3-23: Removal efficiencies (%) for pharmaceuticals from the mandatory list for treatment of MBR permeate with activated carbon

	DE (n=6)	NL (n=4)	CH	CH
	PAC-SF	GAC	PAC	PAC
dosage PAC (mg/L)	**20**	**–**	**23**	**43**
Naproxen	>54	>93	n.a.	n.a.
Diclofenac	59	98	98	99
Lidocaine	n.a.	>98	100	100
Cyclosphosphamide	n.a.	>97	73	>73
Ifosfamide	n.a.	>97	>60	>60
Ciprofloxacin	n.a.	>99	>99	>99
Clarithromycin	80	>99	100	100
Erythromycin*[1)]	90	>99	>88	>88
Sulfamethaxozole	12	>96	33	62
Diatrizoate	1	98	14	18
Iopamidol	23	n.a.	69	80
Iopromide	0	n.a.	85	91
Carbamazepine	>72	98	99	100
Bezafibrate	>59	n.a.	>86	>86
Atenolol	>7	>97	>88	>88

n.a.: not available;
> : when effluent concentration was lower than LOQ, and influent was higher than LOQ
*[1)] Erythromycin was analyzed by Eawag and EG as the sum of Erythromycin and Erythromycin-H_2O

3.4.6 Pharmaceutical removal with RO

LU and NL tested removal of pharmaceuticals with reversed osmosis (RO). The permeate of the MBR was used as feed for the reverse osmosis membrane installation in short term experiments. The applied recovery ranged from 50 to 75%.
Pharmaceutical concentrations in the permeate of RO were all below level of detection. TOC removal was >90%.

3.4.7 Lab scale experiments: oxidation with ferrate

The Scottish partner carried out lab scale experiments for the removal of ibuprofen and ciprofloxacin with ferrate(VI). Experiments were performed with model wastewater and real wastewater.

Model wastewater samples, see table, were prepared by the dilution of stock solutions to 1 L with tap water, with the test solution pH (6.8 – 7.3) unadjusted before dosing ferrate (VI).

The real waste water samples were taken from the effluents of secondary clarifier at Glasgow Shieldhall WWTP. A series of jar test experiments was carried out with a six-unit stirrer (Kemiraflocculator 2000, Kemwater) under the following protocol: fast mixing for 1 min at 400 rpm; slow mixing for 20 min at 40 rpm; and then sedimentation for 60 min. The ferrate dose applied was 0 – 4 mg/L as Fe, and pH of solutions was adjusted by H_2SO_4 or NaOH to 7.0-7.5 or to the required values. 10-100 µg/L ibuprofen or ciprofloxacin were spiked (n=2). Solid phase extraction was used and the analysis was done either with LC/MS in the lab of BWV (Germany) or with LC-UV.

The removal efficiency of ciprofloxacin by ferrate was at least 60% for very low ferrate doses (< 0.3 mg/L) and increased to greater than 80 % when ferrate dose was up to 1 mg/L. Ibuprofen can be removed by 30% at a ferrate dose of 2 mg/L. However, final solution pH affects the treating performance for both compounds. The maximum ciprofloxacin removal was achieved at pH 6 and low ferrate doses; when pH was greater than 8, the removal efficiency decreased significantly. Ibuprofen removal could reach 55% when the final solution pH was adjusted to pH 4. Ferrate performance was also affected by multi-compounds solutions; the overall treatment of each compound decreased. In treating real waste water effluents, it was observed that some drug removal by ferrate was encouraging (e.g., atenolol) but other removals were unsatisfactorily (e.g., lidocaine and carbamazepine). Full investigations are being carried out to comprehensively assess ferrate performance.

3.5 Energy consumption

The installations that were used to investigate the removal of pharmaceuticals from hospital wastewater were especially designed and operated with the aim of research. The performance indicators of these installations must therefore be evaluated with care, and cannot be considered normative for future installations. The obtained results were interpreted and adapted, resulting in estimated energy consumptions for the different treatment steps, as presented in Table 3-24. The figures for MBR are presented only for the German and Dutch situation, because these were full scale installations. The pilot scale MBR results in Luxemburg and Switzerland were considered to be too small to be used for this purpose.

The applied (advanced) oxidation processes require an energy intensive pretreatment, in these cases membrane bioreactors. The observed range for energy consumption of the Dutch MBR is caused by different operation regimes for the membrane aeration. During optimal operation figures as low as 0.9 kWh/m^3 were obtained whereas in other periods the membrane aeration caused a substantial higher energy consumption.

The subsequent oxidation steps require relatively less energy input. The differences between NL, CH and LU in energy consumption for ozonation are caused by different dosage rates and different ways to generate ozone (from liquid oxygen or from air on-site). These figures therefore represent different levels of removal efficiency, as described previously. Treatment with UV was found to be the most energy consuming treatment method.

Table 3-24: Predicted energy consumption (kWh/m³) based on the results from full scale and pilot installations and expert judgment

	Pre-treatment	Bioreactor	Membranes	Ozonation	Activated carbon	UV	Air treatment
NL	0.61	0.3	0.6	0.9 [1]	0.2 (GAC)	0.50 - 1.07	0.1
CH				0.1-0.2			
LU				0.5		1	
DE	0.3	0.9		0.5 *1)	0.45 (PAC-SF)		2 *2)

GAC: Granular activated carbon filtration; PAC-SF: Powdered activated carbon and sand filtration
*1) Oxygen generation on-site from ambient air; *2) Exhaust air treatment by Photoionisation incl. heating

3.6 Cost considerations

The investigations described in this report were carried out with installations especially designed for this purpose. In some case extra equipment was installed, solely for the purpose of the research. This means that if a new treatment facility were to be designed, exclusively for the treatment of hospital wastewater, it would be different and cheaper. The cost figures presented here are therefore not the actual figures, but indicate the cost level for construction and operation of a new on-site treatment installation of hospital wastewater.

Table 3-25: Optimised costs in euro / m³ for treatment of hospital wastewater with different treatment techniques, as calculated for the NL situation

	MBR	MBR + GAC	MBR+O_3+GAC	MBR+UV/H_2O_2+GAC
Investment cost	3.25	3.35	3.50	3.65
Variable cost	1.45	1.65	1.75	1.85
Total cost	4.70	5.00	5.30	5.50

In this table the assumption is made that there will be full scale treatment of all the wastewater by different techniques, being 10 m³/h or 240 m³/d. The ozone and UV/H_2O_2-installations were scaled up to the full-scale 10 m³/h based on their performances during the research. Based on the break-through pattern of the full-scale GAC filter, it is as assumed that the activated carbon has to be replaced after 270 days full hydraulic load when treating MBR effluent. For the GAC-filters treating effluents of the post treatment it is assumed that the activated carbon has to be replaced after three years.

In the variable cost are included maintenance, as percentage of the investments (civil works: 1%, pumps, buffers, etc.: 3%, membrane installation: 5%, ozone, UV and air treatment: 4%). The investments are amortised over ten years, without interest.

The costs of PAC-SF in DE were comparable to the mentioned costs of GAC.

3.7 Practical Experiences

The operation of a full scale system for on-site treatment of hospital wastewater led to some instructive experiences, which may be of use for further projects or installations.

Pilot plants

The pilot plants in this research were treating a part of the total flow out of a hospital. There were difficulties in measuring flow rates within the hospital sewer system, because of the fluctuations, as well as the solids that were present in the water. Debris tend to accumulate around measuring devices, disturbing accurate measurements. Altogether, the corrosive environment poses an issue for the operator of the plants.

Furthermore, the UV installation in the pilot in the LU plant suffered from precipitates at the sleeves from the UV lamps, reducing the efficiency of the lamps.

3.7.1 Design aspects

As there are no specific design requirements for the removal of micropollutants, the design and approval of the pilot plant in Germany was based on general requirements for municipal wastewater treatment. According to the wastewater flow rate and the associated size of the plant the required wastewater quality for direct discharge is met by stable COD and BOD removal. Due to requirements for a reliable operation of advanced treatment techniques a mechanical-biological treatment by a membrane bioreactor (MBR) was selected. Therefore, an excellent effluent quality was already produced by the MBR pretreatment including COD and BOD removal, nitrification, denitrification and biological phosphorus removal. The challenge of the design process lies in the absence of data on hospital wastewater flow rate and wastewater composition. Therefore, a special sampling and measurement campaign was conducted in advance. In conjunction with a conservative design procedure (typical for municipal WWTPs) the pilot plant can be characterized as follows, which seemed to be typical for full-scale hospital WWTPs:

- Low sludge loading (F/M ratio)
- High sludge age due to low sludge production
- High hydraulic retention time (HRT) due to low wastewater flow rate
-

3.7.2 Operational reliability

A stable operation can be secured by the educated staff of an experienced operator of WWTPs. This includes both the MBR pretreatment as well as the different full-scale advanced treatment techniques (ozonation, activated carbon adsorption). Even at high levels of disinfectants, antibiotics and surfactants a viable biocenosis (MLSS) was present at all times. Even with the high aeration demand of an MBR no significant foaming events were observed. The high load of fibers and other particles in the raw wastewater seem to be the main challenge regarding operational reliability. Occasions of higher wastewater burden (e.g. pH peaks) can be detected in time and subsequent treated by an adapted management of internal buffers. The logistics of the produced residues (screens and excess sludge) can be managed by a combination of internal and external customers. The sludge characteristics were comparable to municipal MBRs (e.g. sludge volume index SVI). The performance of the MBR including the membrane filtration was satisfying at all times.

Due to a high automation level of the ozonation plant a stable and reliable operation could be secured with minimum personal effort. For the operator of the plant the maintenance effort is limited to the control of the online sensors. Similar experiences were achieved for the PAC step. The procedure of hand-made stock solutions with PAC once every 14 days proved to be a reliable option for the German pilot plant.

The membrane aeration in the Dutch plant suffered sometimes from problems with the air distribution across the membranes, leading to pressure loss over the membrane tubes.

3.7.3 Legislation

Hospital wastewater treatment means that treatment installations must be built in non-industrial areas, since it is near by a hospital. This limitation has several consequences, for example for the appearance of the building. The Dutch project had to involve an architect to comply with the building regulations. The building had to be designed in such a way that it fitted in the street view. In NL and DE an exhaust air treatment were required.

Furthermore, in the NL case, there were uncertainties about the emissions to the air from the treatment facility. The authorities required a collection system for off gas and a disinfection unit. The efficiency of the disinfection unit has to be proved twice per year by measuring traces of E-coli in the off gas of the disinfection unit.

The challenging requirements (for the German plant) for noise (35 dB at night and 45 dB in the day) and odor protection can be satisfied by an associated design of the pilot plant (additional costs have to be considered for that issue).

Measurements indicate that the air treatment was necessary, the inflow of the air treatment contained more traces of pathogens than was permitted. The air treatment reduced the pathogen concentrations to the required level. However, the data can not be compared to off gas quality from municipal wastewater treatment, because no measurements are available. It must be kept in mind that the standards were not based on risk analysis, but on requirements for air quality in offices.

3.7.4 Health and safety

Both in the DE and NL case, the treatment facility was operated by personnel from the water boards. There were concerns about the risks connected to the operation and maintenance of the installation. For example, anti-cancer medicines as well as viruses and pathogens were considered a possible source of risk for personnel.

Having consulted several experts, it was concluded that contamination pathways are not different from what is normal during operation and maintenance of municipal wastewater treatment systems. However, it was estimated that the associated risks, in the case of a hospital plant, might be higher since concentrations of hazardous substances in hospital wastewater are reasonably expected to be higher when compared to municipal wastewater. This is based on the definition of risk as probability x effect. The effects do not change, but the probability of contamination by hospital wastewater increases, since concentration, for example for pathogens are higher when compared to municipal wastewater.

The outcome was that during maintenance activities FFP3-SL respiratory masks were used, to prevent contact with aerosols, as well as the usual gloves, glasses and disposable overalls.

Standard precautions were taken when sampling campaigns were carried out.

In France and Switzerland ozone has a MAC (maximum allowable concentration at the workplace) of 200 µg/m^3 (average over 8 hr) that should not be exceeded in the room. In the UK the MAC value is defined over 15 minutes and is 400 µg/m^3. In the Netherlands this value is 120 µg/m^3 (time weighted average over one hour). Therefore, a measuring device for ozone concentration in the room should be installed.

3.8 Conclusions

It was demonstrated that biological treatment of hospital wastewater is feasible as no inhibition of nitrification due to wastewater constituents was observed. A good wastewater quality was achieved by the MBR pretreatment in terms of COD, nutrients and bacteria removal which is attributed to both the biological treatment as well as the filtration performance of the membranes. An additional removal of these parameters cannot by observed for the advanced treatment techniques. Therefore, the biological treatment is important as a first step of wastewater treatment. However, the treatment with a MBR was found to be not sufficiently effective to eliminate the target pharmaceuticals. Half of the analyzed compounds were removed to less than 50% by the MBR.

Advanced treatment is mandatory if a significant elimination of pharmaceuticals is required. An elimination of 80% could be achieved for most compounds with the treatment with 0.5 g O_3/g DOC (except for cyclosphosphamide, ifosfamide and the X-ray contrast media diatrizoate, iopamidol and iopromide) and 20 mg/L PAC (except for sulfamethoxazole and the X-ray contrast media diatrizoate and iopamidol). Activated carbon filtration led to elimination rates of >95% for all compounds with a fresh GAC filter. During the research period 1200 bed volumes were treated with the GAC filter. UV/H_2O_2 applying a fluence of more than 47,250 J/m^2 was effective to remove >77% of all the analyzed pharmaceuticals.

Energy consumption for UV treatment is between 0.5 and 1.0 kWh/m³, this is higher than for the treatment with ozone (ranging from <0.2 to 0.9 kWh/m³). The range for ozonation is caused by differences in the origin of the applied oxygen, where on site production of oxygen is more energy intensive than storing pure oxygen on-site. A pure oxygen storage system requires higher investment costs and special safety efforts. Therefore, for smaller systems like hospital wastewater treatment plants a less effective on-site production often looks cost effective.

High elimination could also be achieved with RO, but energy consumption was more than 1.0 kWh/m³.

Of course, all technologies have some disadvantages. During ozonation and UV treatment, transformation products are formed from the micropollutants but also from the bulk organic matter in the wastewater (see also chapter 4). An additional biological treatment, e.g. a biofilter, after oxidative treatments is needed to eliminate these, mostly well degradable, by-products (Escher et al. 2008, Hollender et al. 2009, Stalter et al. 2010). With the addition of PAC as a treatment technology, the compounds are adsorbed onto the PAC surface and not transformed. The loaded PAC needs to be separated from the treated wastewater and disposed properly, e.g. by incineration. When applying RO, a highly contaminated concentrate is accumulated which needs to be responsibly discarded.

The standard parameters for wastewater effluents required to be allowed to directly discharge into a receiving water can be met with hospital wastewater treatment. A value to consider is AOX (adsorbable organic halogen compounds), which is high even in the treated wastewater due to high concentrations of remaining X-ray contrast media that contain the halogen iodide. This concentration may be higher than requirement values for effluents discharging into the receiving water (McArdell et al. 2011, Beier 2010).

The fee for wastewater discharge is often calculated based on the pollution load. Lower fees might be applicable if the wastewater is treated in-house before being discharged.

3.9 References

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Chapter 4

Ecotoxicity assessment

Contents

Abbreviations

DIN	Deutsches Institut für Normung (German Institute for Standardization)
ER-CALUX	Estrogen Receptor – Chemically Activated Luciferase expression bioassay
EC50	Effective Concentration (concentration causing an adverse effect of 50% on the respective endpoint, e.g. mortality, growth rate, etc.)
GAC	Granular Activated carbon
ISO	International Organization for Standardization
LC50	Lethal Concentration (concentration causing mortality to 50% of tested organisms)
LOD	Limit of Detection
MBR	Membrane Bio-Reactor
NOEC	No Observed Effect Concentration (the highest concentration causing no effect)
LOEC	Lowest Observed Effect /Concentration
PAC	Powdered activated carbon
OECD	Organisation for Economic Cooperation and Development
YES	Yeast Estrogenic Screen

4 Ecotoxicity assessment

4.1 Introduction – Ecotoxicological testing methods

Micropollutants, such as pharmaceutical residues, can today be detected in the aquatic environment at concentrations lower than a few nanogram per litre. However, the analytical detection of these aquatic chemicals by itself, does not allow any conclusions to be drawn about the effect of a mixture of compounds on the environment. The toxic effects involve mortality or sub-lethal such as non-specific effects as well as mechanism specific effects such as endocrine disruption, genotoxicity and mutagenicity effects or antibiotic resistance due to low antibiotic exposure levels. Therefore these ecotoxicological tests/bioassays are essential to assess the ecological risk of micropollutants in the tested water because these test systems integrate effects of all single chemical compounds together even if the substances appear at nanogram per litre concentration in the environment. Some chemicals in such low concentrations e.g. estrogens, for which the chemical analysis is often difficult (concentrations in the environment below available chemical detection limit), can nevertheless elicit biological effects. Therefore, ecotoxicological tests are a useful tool in addition to chemical analysis.

Ecotoxicology aims to measure the adverse effects of substances upon organisms, natural populations or ecosystems (e.g. Leblanc 2004). For this purpose ecotoxicity tests/bioassays are performed on cells or on living organisms in order to determine the toxicological effects of substances.

Different testing methods are used. They can be characterised by the following criteria:

- Test organisms used, representing different trophic levels, e.g. luminescent bacteria, algae, water flea, worm, fish
- Examined environmental compartments, e.g. soil, water, sediment
- Duration of the test: short-term or acute toxicity tests often with mortality as endpoint, long-term or chronic toxicity tests often with reproduction or growth endpoints, such as early life stage tests over several days or weeks (e.g. with fish), reproduction tests (e.g. with water flea), life-cycle-tests (covering a whole life-cycle of an organism) or generation tests (covering at least 2 life-cycles)
- Tests for general or specific toxicity
  - specific effect tests: aiming at the assessment of specific toxicity (e.g. endocrine disruption or mutagenicity)
  - non-specific effect tests: aiming at the assessment of the systemic effects on organisms without any specification of the toxicological mode of action leading to these effects e.g. mortality (LC_{50}), immobilisation, growth inhibition or reproduction inhibition

Many of these tests are standardised within ISO or OECD guidelines. But there are some tests which are already established but not yet standardised (e.g. the YES test (Routledge and Sumpter, 1996) or the ER-Calux test (van der Linden et al. 2008) for the assessment of estrogenic active substances and the waterscan test for the assessment of antibiotic effects (van der Grinten et al. 2010).

Toxicity screening test systems, based on single celled organisms like bacteria or yeast usually, have a high sensitivity and a good reproducibility. These tests require a short exposure period and can be used with low effort. But their relevance for an ecological risk assessment is relatively low compared to test systems with higher species (e.g. primary consumers, vertebrates), population tests or tests assessing effects on whole ecosystems. However, test systems with higher species are more complex, sometimes less sensitive, require a great effort, and are therefore often more expensive than bioassays evaluating specific effects. Furthermore, they raise ethical concerns and an animal welfare permit may be needed.

In acute toxicity tests, whole organisms (representing different trophic levels) are exposed to the test medium (water sample) for a certain time duration. The toxic effect of the test medium is assessed by measuring different endpoints (e.g. mortality rates or growth inhibition). These tests often use short exposure times and responses can only be observed at relatively high pollutant concentrations (Schweigert et al. 2002) after a pre-concentration step of the sample. These tests provide valuable information in general for the toxicity potential of the test medium and such tests may be used for the comparison of hospital effluent before and after the wastewater treatment processes, for example. However, they have only limited value for the ecotoxicological evaluation of substances which are continuously present, but only in low concentrations such as micropollutants in the aquatic system.

Long-term toxicity tests are usually performed without a sample preparation step and ideally on-site in a flow-through design. This results in a more realistic assessment of effects on organisms in the environment. But these test systems are often time consuming and costly and are therefore usually applied when negative effects in acute/short-term assay have already been observed (Schweigert et al. 2002).

For an environmental risk assessment of chemicals, test strategies are defined which combine short-term and long-term tests with different organisms representing different trophic levels in the environment. The acute hazard of substances is assumed to be associated with its short-term toxicity to organisms. Therefore, the criteria for setting acute hazard categories are based on acute assays measuring LC_{50} or EC_{50} data. From the considered organisms the classification is usually based on the lowest valid toxicity value. In the same way a battery of tests for chronic effects on organisms of different trophic levels is required to determine the lowest NOEC.

Short-term tests with organisms generally only assess direct toxicity, while specific effects of compounds, e.g. estrogenic effects are not detected. Long-term tests may include such specific effects, e.g. by assessing vitellogenin induction in fish as an indicator for the exposure to estrogenic substances (e.g. Vermeirssen et al. 2005). But due to their labour intensiveness, their high costs as well as the ethical concerns for in-vivo tests with vertebrates, these tests should only be performed if concerns are identified in screening tests, for example when estrogenic effects have already been observed in in-vitro tests (like YES- or ER-Calux tests).

In the last four years a range of ecotoxicity tests have been performed in studies investigating the removal of micropollutants by advanced treatment of wastewater. In these studies various bioassays assessing general and specific effects have been applied (e.g. Pinnekamp et al. 2009; Abegglen et al. 2009; Kienle et al. 2011). The tests strategies included short-term and long-term tests and covered different trophic levels and the effects of substances with different modes of action.

4.2 Toxicity tests systems used in PILLS

The following objectives were identified for toxicological tests in the PILLS project:

1) Characterisation of the hospital wastewater: Rough comparison between hospital wastewater and municipal wastewater.

2) Determination of the efficiency of the pilot plants: Comparison between raw hospital wastewater and treated hospital wastewater; comparison between the different treatment techniques of the pilot plants.

3) Assessment of possible toxic effects induced by the advanced treatment with ozone or UV (effects due to by-products after ozonation or UV-treatment).

The aim of objective 1 is to obtain initial information to characterize hospital wastewater. Objective 2 aims at demonstrating the effect of wastewater treatment. In objective 3 the aim was to investigate whether ozonation leads to „new“ specific toxicity effects (due to by-products in the ozonation or UV-treatment process).

The tests used to characterise potential toxicity in the PILLS project are listed in Table 4-1. The tests were performed by different institutions with samples from different pilot plants (see chapter 3) and municipal wastewater treatment plants (see Table 4-2). The test battery consists of various short-term and long-term toxicity tests considering different aquatic trophic levels and representing functions. It includes *in-vitro* screening tests for the assessment of specific effects (e.g. cytotoxicity or endocrine disrupting effects) and general toxicity to bacteria and algae as well as *in-vivo* tests on organisms like snails, worms, water fleas or fish.

The tests used in PILLS are shortly described below. A more detailed description of the test methods and the tests results are given in McArdell et al. (2011), Mulder et al. (2012), Pahl et al. (2012, in prep.), Nafo et al. (2012, in prep.).

Tests systems for the assessment of specific toxicity

In Germany cell viability (cytotoxicity) and irreparable damage to DNA (mutagenicity) were measured using the MMT test and the Ames test. To measure estrogenic effects the binding of estrogenic substances to the human estrogen receptor was assessed using the YES (Routledge and Sumpter 1996) and the ER-Calux assays (Van der Linden et al. 2008).

In the Netherlands antibiotic effects in wastewater of different treatment plants were measured using the waterscan test (van der Grinten et al. 2010). This test measures growth inhibition after 24 hours exposure of 5 bacterial strains (sensitive to specific antibiotic groups) using optical

density. The following antibiotic groups were tested: Tetracyclines, Quinolones, Sulfanomides, Macrolides/β-lactam and Aminoglycosides. Although these tests were designed to be as specific as possible, effects of other substances cannot be excluded.

Table 4-1: Overview of the applied test systems

Bioassays	Endpoints	PILLS Objectives	NL*	DE*	CH*	UK*
MTT test (with T47D cells)	Cytotoxicity (viability)	Characterisation, treatment efficiency, (oxidation by-products)		with original and pre-concentrated samples		
Ames test *(Salmonella thyphimurium, strain YG7108)* (Reifferscheid et al. 2011)	Mutagenicity (No. of histidine revertants)	Characterisation, treatment efficiency, (oxidation by-products)		with original and pre-concentrated samples		
Er-Calux (with T47D cells)	Estrogenicity (EE2 equivalent)	Characterisation, treatment efficiency, (oxidation by-products)		with original and pre-concentrated samples		
A-YES test (AQUA 1.0)	Estrogenicity (Phytase activity) (EE2 equivalent)	Characterisation, treatment efficiency, (oxidation by-products)		with original and pre-concentrated samples		
Waterscan (antibiotics test)	Inhibition of bacterial growth (concentration factor EC50)	Characterisation, treatment efficiency, (oxidation by-products)	with pre-concentrated samples; Inhibition after 24 hr			
Bacteria test *(Vibrio fischeri)*	Inhibition of luminescence (concentration factor EC50)	Characterisation, treatment efficiency	with pre-concentrated samples; Inhibition after 30 min		with pre-concentrated samples; Inhibition after 30 min	with pre-concentrated samples; Inhibition after 30 min
Algal photosynthesis test	Inhibition of photosynthetic efficiency (concentration factor EC50)	Characterisation, treatment efficiency, (oxidation by-products)	with *Selenastrum capricornutum*; with pre-concentrated samples; inhibition after 4.5 and 24 hr			with *Selenastrum capricornutum*; with pre-concentrated samples; inhibition after 4.5 hr
Algae growth test *(Desmodesmus subspicatus)* (ISO-NWIP)	Growth rate inhibition after 72 hr	Characterisation, treatment efficiency, oxidation by-products		with original and diluted samples		
Test with *Gammarus fossarum* (scuds)	Mortality after 4, 24, 48, 72 hr	Characterisation			with original samples	

Daphnia magna population test (water flea) (Hammers-Wirtz et Ratte 2003)	Population growth inhibition (population abundance and structure, ephippia)	Treatment efficiency, oxidation by- products		21 d, semistatic in laboratory with original samples		
Fish embryo test *(Danio rerio)* (DIN EN ISO 15088, 2009)	Mortality (coagulation of embryos, non-detachment of the tail, lack of heart-beat)	Treatment efficiency, oxidation by-products		48 hr, with original and diluted samples		
Potamopyrgus antipodarum (snail) (Duff et al. 2007)	Mortality, shell size, number of embryos per female	Oxidation by-products		28 d, flow through with original samples		
Lumbriculus variegatus test (worm) (OECD 2007	Reproduction, biomass, biomass per worm	Oxidation by-products		28 d, flow through with original samples		

*PILLS-partner countries: Germany (DE), The Netherlands (NL), Scotland (UK), Switzerland (CH)

The MTT tests measures the viability of cells determining the mitochondrial activity of human breast cancer cells (T47D) by measuring the color change after 24 hours of exposure. Only viable cells are able to transform the yellow MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) into a purple formazan by mitochondrial dehydrogenases. Therefore a direct correlation between the amount of transformed dye and the number of viable cells is possible.

The above mentioned tests were performed using enriched samples after an extraction procedure and a concentration step (different extraction methods and concentration factors). Additionally different dilution steps of the original samples were used for the Ames test. The same samples were used for the MMT test, the A-YES test and the ER-CALUX assay.

Table 4-2: Applied test systems and performing institutions

Bioassays	**Performing Laboratory**
In-vitro assays with native samples or sample pre-concentration	
MTT test (with T47D cells)	IWW Water Centre (DE) [1,2]
Ames test *(Salmonella thyphimurium, strain YG7108)* (Reifferscheid et al. 2011)	Biodiversity and Climate Research Centre (DE) [1,2]
Er-Calux (with T47D cells)	IWW Water Centre (DE) [1]
A-YES test (AQUA 1.0)	IUTA Institute of Energy and Environmental Technology (DE) [1,2]
Waterscan (antibiotics test)	RIVM National Institute for Public Health and the Environment (NL) [3]
Bacteria test *(Vibrio fischeri)*	Ecotox Centre, Eawag (CH) [4] RIVM National Institute for Public Health and the Environment (NL) [3] Glasgow Caledonian University (UK) [5]

Algal photosynthesis test (*Selenastrum capricornutum)*	RIVM National Institute for Public Health and the Environment (NL) [3] Glasgow Caledonian University (UK) [5]
In-vivo assays without sample pre-concentration conducted in the laboratory	
Algae growth test *(Desmodesmus subspicatus)* (ISO-NWIP)	gaiac Research Institute for Ecosystem Analysis and Assessment at the RWTH Aachen University (DE) [1,2]
Test with *Gammarus fossarum* (scuds)	Ecotox Centre, Eawag-EPFL (CH) [4]
Daphnia magna population test (water flea) (Hammers-Wirtz et Ratte 2003)	gaiac Research Institute for Ecosystem Analysis and Assessment at the RWTH Aachen University (DE) [1]
Fish embryo test *(Danio rerio)* (DIN EN ISO 15088, 2009)	gaiac Research Institute for Ecosystem Analysis and Assessment at the RWTH Aachen University (DE) [1]
In-vivo assays without sample pre-concentration conducted in flow through systems at the pilot plant	
Potamopyrgus antipodarum (snail) (Duft et al. 2007)	Biodiversity and Climate Research Centre (DE)[1]
Lumbriculus variegatus test (worm) (OECD 2007)	Biodiversity and Climate Research Centre (DE) [1]

[1] Investigations on samples from the pilot plant in Gelsenkirchen and [2] the municipal wwtp Bad Sassendorf, Germany; [3] Samples from the pilot plant in Zwolle and different municipal wwtp, The Netherlands; [4] Samples from the pilot plant in Baden and the municipal WWTP Laufäcker, Switzerland; [5] Samples from wwtp Galashiels, wwtp Shieldhall, Borders General Hospital and Western Infirmary in Scotland, UK

Tests systems with aquatic organisms

To assess the effects of wastewater on organisms representing different aquatic trophic levels and the performance of the treatment processes in the pilot plant tests with primary producers, primary consumers and secondary consumers were performed.

Decomposers: Tests with the luminescent bacteria *Vibrio fisheri* were performed in CH, NL, and UK using concentrations series of wastewater samples (different extraction methods and concentration factors). In general *V. fisheri* is exposed to dilution series of the pre-concentrated samples. When the bacteria are affected, the luminescence decreases in a dose-dependent manner. The effect concentrations (EC50 values) were determined after 30 minutes exposure to test samples.

Primary producers: To assess effects of wastewater on primary producers inhibition of the photosystem II of the green algae Selenastrum capricornutum and growth inhibition of the green algae *Desmodesmus subspicatus* were performed.

The algal photosynthesis inhibition test used in NL and UK measures photosynthetic efficiency of the algae S. capricornutum via assessment of chlorophyll fluorescence using the saturation pulse method (Genty et al. 1989). The EC50 values were calculated after 4.5 hours (in UK and NL) and 24 hours of exposure to dilution series of the pre-concentrated samples.

The algal growth inhibition test was conducted in DE with the green algae *D. subspicatus* according to the Draft of the ISO-NWIP. In this test procedure monospecies algal strains are incubated in microplates for 72 hours in a defined medium containing a range of concentrations of the test sample, prepared by mixing appropriate quantities of growth medium, test sample and an inoculum of exponentially growing algal cells. In the microplates the cell density of the algae in each test solution is measured as fluorescence every 24 hours. The test result is the inhibition as a reduction in growth rate, relative to control cultures grown under identical conditions. An optimised growth medium according to Altenburger et al. (2010) was used in order to minimise the promotion of algal growth by wastewater samples due to additional macro- and micronutrients in the wastewater samples compared to the control medium. Dilutions of the wastewater samples of 1:1.25; 1:2; 1:4 and 1:8 were tested which are equivalent to a wastewater proportion of 80%, 50%, 25% and 12.5% respectively. Each wastewater dilution was tested in three replicates and for the negative control six replicates were used.

Primary consumers: The effects of wastewater on primary consumers were measured using in-vivo tests: acute toxicity test with the freshwater amphipod *Gammarus fossarum*, population test with the water flea *Daphnia magna* and reproduction tests with the aquatic worm *Lumbriculus variegatus* and the New Zealand mud snail *Potamopyrgus antipodarum*.

In the *G. fossarum* test performed in CH the animals were exposed to original samples. . For assessing acute effects on freshwater crustaceans, adolescent *G. fossarum* (5-8 mm) from the river Bäntalbach in Bäntal (community Zell, ZH) were exposed in 200 mL of sample with three replicates per sample. Those amphipods occupy a key role in stream ecosystems regarding the structure and functioning of the ecosystem, as they shredder and exploit dead organic matter and make it available in the nutrient cycle again (e.g. Karaman and Pinkster, 1977, Kunz et al. 2010). If a dose response curve was available, toxicity was expressed as EC10 or EC50, the concentration at which 10 or 50% of the test organisms showed mortality after 4, 24, 48 and 72 hours exposure.

The *Daphnia* population test was performed in DE as a semi-static test according to the method described by Hammers-Wirtz and Ratte (2003). The animals used for the tests were all from a healthy stock culture. The experiment was started with a mixed-age population of five neonates and three adults. The control and the wastewater samples were replicated three times. In all experiments the original wastewater samples and the control medium were renewed at least three times per week. The growing populations were studied for a period of 21 days. Based on the measured number of living and dead animals, of aborted and ephippial eggs, different parameters were calculated to describe the effects on the population dynamics.

The *in-vivo* bioassays with the mudsnail *Potamopyrgus antipodarum* (Duft et al. 2007) and the worm *Lumbriculus variegatus* (OECD, 2007) were conducted in DE without sample enrichment or dilution in a flow through system online at the pilot treatment plant.

In the *Lumbriculus* test replicates were provided with ten synchronized annelids each (the animals were on the same developmental stage). A reference water was used to check if test conditions are appropriate to match validity criteria. As *Lumbriculus* is a sediment-dwelling organism, exposure vessels were equipped with quartz sand. The worms were fed every 2nd day. After 28 days exposure worms were counted and developmental stage as well as dry biomass per replicate recorded.

Potamopyrgus antipodarum reacts sensitive on estrogenically active compounds with an increased reproduction. Therefore, the reproduction assay was predominantly applied to analyse the test waters on *in vivo* estrogenic effects. 22 snails per replicate (two replicates per test water) were placed glass vessels and fed every 2nd day. A control group was exposed to a reference water to validate the tests results and a positive control group was exposed to 50 ng/L 17α-ethinylestradiol in reference water. After 28 days of exposure snails were frozen and stored at -80°C. For analysis, mortality was recorded, the shell sizes were measured, the shells were removed and each embryo in the brood pouch counted.

Secondary Consumers: The effects of wastewater on fish as the most important secondary consumers in aquatic were measured in DE in the fish embryo test with the zebrafish *Danio rerio* according to the standard DIN EN ISO 15088 (2009).

In this test zebrafish embryos are individually exposed in 24-well microtiter plates to a range of wastewater dilutions. The test is initiated immediately after fertilization and is continued for 48 hours. Lethal effects, as described by three apical endpoints, are determined by comparison with controls to identify the LC50 and the lowest ineffective dilution (LID). Each wastewater sample was replicated twice with independent samples of fish eggs to show the reproducibility of the results and allow statistical evaluation. After 24 and 48 hours exposure to samples the embryos were inspected using an inverse microscope. Coagulation of embryos, non-detachment of the tail as well as lack of heart-beat are recorded as apical endpoints for acute mortality. The validity criteria for each test were: a) the overall survival of fertilized eggs in the controls should be at least 90% and b) the mortality in the positive control should be higher than 10%. Long-term effects can not be evaluated with this method.

4.3 Ecotoxicity of hospital wastewater

Table 4-4 gives an overview of the tests results regarding the ecotoxicity potential of raw hospital wastewater in comparison to municipal wastewater. Wastewater samples from different treatment plants were analysed in the different PILLS partner countries. Information about the respective tested samples is summarized in Table 4-3.

Table 4-3: Samples investigated for the evaluation of the ecotoxicity potential of hospital wastewater and municipal wastewater

Bioassays	Raw municipal wastewater	Effluent of municipal wastewater treatment plant	Raw hospital wastewater
MTT test (with T47D cells) [DE] A-YES test (AQUA 1.0) [DE]	wwtp Bad Sassendorf, Germany, n=1 (24h-composite sample, both tests with the same sample)		pilot plant Marienhospital Gelsen-kirchen, Germany, n=1 (24h-composite sample, both tests with the same sample)
Ames test *(Salmonella thyphimurium, strain YG7108)*	wwtp Bad Sassendorf, Germany, n=1 (24h-composite sample)		pilot plant Marienhospital Gelsen-kirchen, Germany, n=4 (24h-composite samples)
Waterscan (antibiotics test) [NL]		wwtp Zwolle and wwtp Hessenpoort, The Netherlands, n=6 (grab samples)	–
Bacteria test *(Vibrio fischeri)* [NL]		wwtp Zwolle and wwtp Hessenpoort, The Netherlands, n=6 (grab samples)	–
Bacteria test *(Vibrio fischeri)* [CH]	wwtp Laufäcker, Switzerland, n=1 (1-week composite sample)	wwtp Laufäcker, Switzerland, n=1 (1-week composite sample)	pilot plant in Baden, Switzerland,
Bacteria test *(Vibrio fischeri)* [UK]	wwtp Galashiels n= 9 (7d-comp. sample); wwtp Shieldhall n=13 (7d-comp. sample)		Borders General Hospital n= 46 (7d-comp. sample); Western Infirmary n=35 (7d-comp. sample)
Algal photosynthesis test [UK]	wwtp Galashiels n= 9 (7d-comp. sample); wwtp Shield-hall n=13 (7d-comp. sample)		Borders General Hospital n= 46 (7d-comp. sample); Western Infirmary n=35 (7d-comp. sample)
Algal photosynthesis test (*Selenastrum capricornutum)* [NL]		wwtp Zwolle and wwtp Hessenpoort, The Netherlands, n=6 (grab samples)	–
est with *Gammarus fossarum* [CH]	wwtp Laufäcker, Switzerland n=1 (1-week composite sample)	wwtp Laufäcker, Switzerland n=1 (1-week composite sample)	pilot plant in Baden, Switzerland, n=1 (1-week composite sample)
Algae growth test with test *(Desmodesmus subspicatus)*	wwtp Bad Sassendorf, Germany, n=1 (24h-composite Sample)		pilot plant Marienhospital Gelsenkirchen, Germany, n=2 (24h-composite samples)

The tested raw municipal wastewater from the wwtp in DE was not cytotoxic to the human cell line (T47D) or mutagenic. Raw hospital wastewater samples showed an average moderate cytotoxic and mutagenic effects. In all other tests raw municipal wastewater, effluent of municipal wastewater and raw hospital wastewater from different locations had moderate or high toxic effects. According to the tests results in CH municipal wastewater had a higher toxic effect on *Gammarus fossarum* than hospital wastewater. In other tests hospital wastewater was in comparison to municipal wastewater more toxic to algae (tested with or without pre-concentration) and to bacteria (tests with pre-concentrated samples). Furthermore, hospital wastewater had a higher estrogenicity than the tested municipal wastewater.

Table 4-4: Test results on the ecotoxicity potential of hospital and municipal wastewater

Bioassays	Endpoints	Raw municipal wastewater	Effluent of municipal wastewater treatment plant	Raw hospital wastewater
MTT test (with T47D cells) [DE]	Cytotoxicity (viability)	88.3% (original sample) [1]		66.6% (original sample)[2]
A-YES test (AQUA 1.0) [DE]	Estrogenicity (EE2 equivalent)	19.7 ng/L (original sample) [1]		43 ng/L (original sample) [2]
Ames test *(Salmonella thyphimurium, strain YG7108)* [DE]	Mutagenicity (No. of histidine revertants)			[2]
Waterscan (antibiotics test) [NL]	Sulfanomides (concentration factor EC50)		[3]	
Waterscan (antibiotics test) [NL]	Macroliden/ß-lactam (concentration factor EC50)		[3]	
Bacteria test *(Vibrio fischeri)* [NL]	Inhibition of luminescence (concentration factor EC50)		[3]	
Bacteria test *(Vibrio fischeri)* [CH]	Inhibition of luminescence (concentration factor EC50)	1.26 fold [4]	33.85 fold [4]	0.84 fold [5]
Bacteria test *(Vibrio fischeri)* [UK]	Inhibition of luminescence (concentration factor EC50)	0.72 fold [6]		0.26 fold [7]
Algal photosynthesis test [UK]	Inhibition of photosynthetic efficiency (concentration factor EC50)	12.07 fold [6]		1.97 fold [7]
Algal photosynthesis test (*Selenastrum capricornutum)* [NL]	Inhibition of photosynthetic efficiency (concentration factor EC50)		[3]	
Algae growth test with test *(Desmodesmus subspicatus)* [DE]	growth rate inhibition, average for dilutions 80% and 50% wastewater	34 % [1]		64-88 % [2]
Test with *Gammarus fossarum* [CH]	Mortality after 48hr	100% [4]	100% [4]	> 50% [5]

Origin of the samples: [1] Influent of the municipal wwtp Bad Sassendorf, Germany (n=1, 24h-composite sample); [2] Raw wastewater of Marienhospital in Gelsenkirchen, Germany (n=1-4, 24h-composite sample); [3] wwtp Zwolle and wwtp Hessenpoort, The Netherlands (n=6, grab samples); [4] Influent/effluent of the municipal wwtp Laufäcker, Switzerland (n=1, 1-week composite sample); [5] Raw wastewater of hospital Baden, Switzerland (n=1, 1-week composite sample); [6] Average of influents of wwtp Galashiels (n= 9, 7d-comp. sample) and wwtp Shieldhall (n=13, 7d-comp. sample), UK; [7] Average of raw wastewater of Borders General Hospital (n= 46, 7d-comp. sample) and Western Infirmary (n=35, 7d-comp. sample), UK

Evaluation is performed on average values. Color codes:

	Cell viability in the cytotox-icity test (according DIN EN ISO 10993-5)	EC50 values based on expert judgment	Other values (change of effect com-pared to negative control)
no negative effects	81–100%	EC50 > 100	< 5%
weak or moderate effects	61–81%	20 < EC50 < 100	5 - 20%
strong effects	0–60%	EC50 < 20	> 20%

4.4 Effects of wastewater treatment on toxicity

The effects of the wastewater treatment processes on the removal of toxicity in raw wastewater were investigated by different institutions (see Table 4-2) based on samples from different pilot plants. Table 4-5 gives an overview of the respective tested samples. The sampling points for the performance investigations of the treatment processes are shown in Figure 4-1.

Figure 4-1: Sampling points for the ecotoxicological investigations of pilot plants (simplified schemes)

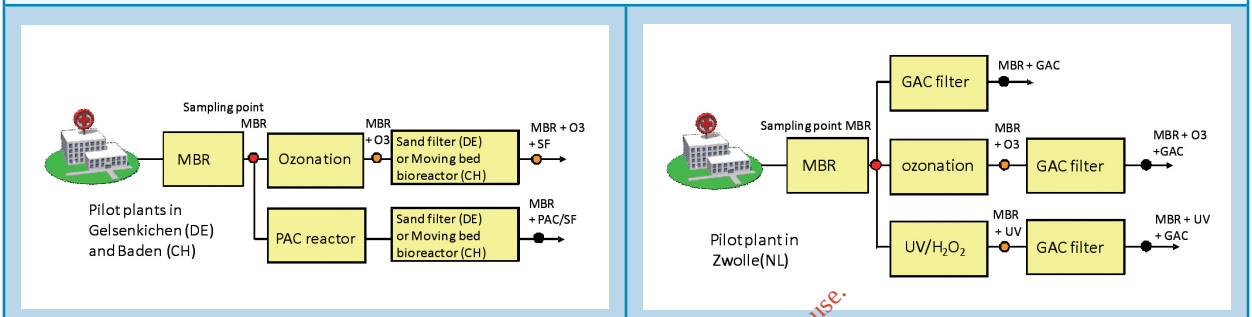

Table 4-5: Samples investigated for the evaluation of wastewater treatment performance

Bioassays	Effluent of MBR	MBR + O3	MBR + O3 + SF	MBR + PAC/ SF	MBR + GAC	MBR + O3 +GAK	MBR + UV	MBR + UV + GAC
Tests with samples (24h-comp. samples) from the pilot plant in Gelsenkirchen, Germany								
MTT test (with T47D cells) Er-Calux (with T47D cells) A-YES test (AQUA 1.0)	n=9	0.45 gO3/g DOC, n=9	0.45 gO3/g DOC, n=6	20 mgPAC/L, n=3				
Ames test *(Salmonella thyphimurium, strain YG7108)*	n=6	Ozone dose 0.45 gO3/g DOC, n=6		20mg PAC/L, n=6				
Algae growth test (72h), growth rate	n=3	0.45 gO3/g DOC, n=3		20 mgPAC/L, n=3				
Daphnia Population test	21d, semi-static, samples renewal three times per week	21d, semi-static, samples renewal three times per week, 0.45 gO3/gDOC						
Fish embryo test (48h, DIN)		0.45 gO3/g DOC, n=3		20 mgPAC/L, n=3				
Potamopyrgus antipodarum test *Lumbriculus variegatus* test	flow-through. 28d	flow-through, 28d, 0.45 gO3/gDOC						

Tests with samples (24h-comp. samples) from the pilot plant in Baden, Switzerland							
Bacteria test *(Vibrio fischeri)*	n = 3	6 and 10 mgO3/L, n=2, 1-week composite sample or grab sample (at constant inflow conc.)	40 mg/L PAC, n=1, grab sample (at constant inflow conc.)				
Test with *Gammarus fossarum*	n = 3	6 mgO3/L, n=2, 1-week composite sam-ple or grab sam-ple (at constant inflow conc.)	40 mg/L PAC, n=1, grab sample (at constant inflow conc.)				
Tests with samples (grab samples) from the pilot plant in Zwolle, The Netherlands							
Waterscan (antibiotics test)	n=8	n=3		n=5	n=2	n=5	n=5
Bacteria test *(Vibrio fischeri)*	n=8	n=2		n=6	n=2	n=5	n=5
Algal photosynthesis test	n=4	n=1		n=2	n=1	n=4	n=4

4.4.1 Performance assessment regarding specific toxicity effects

The performance assessment of the wastewater treatment processes regarding specific toxicity effects are shown in Table 4-6. Information about the investigated samples is listed in Table 4-5.

Table 4-6: Evaluation of the performance of treatment processes measured in the specific toxicity tests

Bioassays	Endpoints	Effluent of MBR	MBR + O3	MBR + O3 + SF	MBR + PAC/SF	MBR + O3 + GAC	MBR	MBR + UV	MBR + UV
MTT test (with T47D cells) [1]	Cytotoxicity (viability)	↓	↗	↗					
Er-Calux (with T47D cells) [1]	Estrogenicity (EE2 equivalent)	Estrogenicity (EE2 equivalent)	0.1 ng/L	< LOD	< LOD				
A-YES test (AQUA 1.0) [1]	Estrogenicity (EE2 equivalent)	0.235 ng/L	0.261 ng/L	0.176 ng/L	0.079 ng/L				
Ames test *(Salmonella thyphimurium, strain YG7108)*[1]	Mutagenicity (No. of histidine revertants		↑	↘					
Waterscan (antibiotics test) 2	Sulfanomides (concentration factor EC50)								
Waterscan (antibiotics test) [2]	Macrolides/ß-lactam (concentration factor EC50)								

Origin of samples: [1] pilot plant in Gelsenkirchen, Germany; [2] pilot plant in Zwolle, the Netherlands
Evaluation is performed on average values. Change of toxicity value after treatment process: ↑ and ↓ indicate increasing toxicity or decreasing toxicity of > 20%, ↗ and ↘ indicate slightly increasing toxicity or slightly decreasing toxicity of < 20%. Color codes:

	Cell viability in the cytotoxicity test (according DIN EN ISO 10993-5)	EC50 values based on expert judgment	Other values (change of effect compared to negative control)
no negative effects	81–100%	EC50 > 100	< 5%
weak or moderate effects	61–81%	20 < EC50 < 100	5 - 20%
strong effects	0–60%	EC50 < 20	> 20%

Cytotoxicity

The above mentioned moderate cytotoxic effects of hospital wastewater decreased during the biological treatment in the MBR in DE in a way that no toxic effects could be measured in the MBR effluent. After ozonation and the following sand filtration the cytotoxicity slightly increased, but the respective effluents were both on average not cytotoxic to cells.

Estrogenic activity

The estrogenic effects of hospital wastewater (measured by A-YES test) decreased by >99% after biological treatment in the MBR in DE. The subsequent advanced treatment steps led to a further decrease of the estrogenic effects of the MBR effluent. PAC addition (20 mg/L) had a better performance than ozonation (5 mg/L) and subsequent sand filtration. The results derived by the ER-CALUX test indicated remaining estrogenic effects in the MBR, but these effects could be reduced by the follow-up ozonation to a 17ß-estradiol-equivalent value below the LOD.

Mutagenicity

While raw hospital wastewater had moderate mutagenic effects, no mutagenic effects could be observed in native and enriched MBR samples at the pilot plant in DE. In contrast, mutagenic effects significantly increased after ozonation. After the subsequent sand filtration (continuous moving bed filtration), mutagenicity was partly removed but still significantly increased compared to the MBR effluent. After PAC followed by sand filtration no mutagenic effects were detected.

Antibiotic effects

At the pilot plant in NL moderate antibiotic effects (based on sulfonamides and macrolides/ß-lactam antibiotic groups) were detected after biological treatment in the MBR. After the post-treatment of the MBR effluent by UV treatment, ozonation or GAC filtration no antibiotic effects were detected in the respective effluents.

4.4.2 Performance assessment regarding acute toxicity effects

Table 4-7 gives an overview of the performance assessment of the different wastewater treatment processes regarding acute toxicity effects.

Bacteria luminescence

V. fischeri assays were performed in CH and NL to evaluate the performance of the respective pilot plants. The results showed decreasing toxicity effects of hospital wastewater on bacteria over different treatment steps. Already after MBR treatment there was a high decrease of toxicity in CH. But, at both pilot plants MBR effluent still showed moderate toxic effects to bacteria. The following advanced treatment with ozone and post-treatment in a biofilter (moving bed bioreactor) or PAC in CH or in GAC in NL led to a further decrease of toxicity resulting in a non toxic effluent, respectively. In NL the UV treated effluent of the MBR was still moderately toxic to *V. fischeri*, but after post-treatment by GAC no toxic effects were detectable.

Algal photosynthesis

The effluents of MBR treatment at the pilot plant in NL and the subsequent GAC filtration were strongly toxic to algae with respect to their photosynthetic activity. UV treatment led to a decrease of the toxic effects in the MBR effluent, but this effluent was still moderately toxic for algal photosynthesis. In contrast, after GAC filtration of the UV treated wastewater no negative effects were detected.

Algae growth

In the algae growth inhibition test all raw wastewater samples investigated at the pilot plant in DE had strong toxic effects on *D. subspicatus* in the two dilutions of 50% and 80% wastewater. Biological wastewater treatment in the MBR, as well as subsequent ozonation and PAC addition resulted in a reduced algal toxicity in comparison to raw wastewater. However, after ozonation the inhibition of algae growth increased slightly compared to the MBR effluent and showed the same trend in all samples. After sand filtration of the ozonation effluent in a moving bed sand filter variable effects were measured.

Table 4-7: Evaluation of the performance of treatment processes measured in the acute toxicity tests

Bioassays	Endpoints	Effluent of MBR	MBR + O3	MBR + O3 + SF	MBR + PAC/SF	MBR + O3 + GAC	MBR + GAC	MBR + UV	MBR + UV + GAC
Bacteria test *(Vibrio fischeri)* [2]	Inhibition of luminescence (concentration factor EC50)					↓	↓		↓
Bacteria test *(Vibrio fischeri)* [3]	Inhibition of luminescence (concentration factor EC50)	↓	↓	↘	↓				
Algal photosynthesis test (4.5 and 24hr) [2]	Inhibition of photosynthetic efficiency (concentration factor EC50)							↓	↓
Algae growth test (72hr) [1]	growth rate inhibition, average for dilutions 80% and 50% wastewater	↓	↗						
est with *Gammarus fossarum* [3]	Mortality after 48hr	variable effects	variable effects	variable effects	variable effects				
Fish embryo test (48hr, DIN) [1]	lethal mortality								

Origin of samples: [1] pilot plant in Gelsenkirchen, Germany; [2] pilot plant in Zwolle, The Netherlands; [3] pilot plant in Baden, Switzerland Evaluation is performed on average values. Change of toxicity value after treatment process: ↑ and ↓ indicate increasing toxicity or decreasing toxicity of > 20%, ↗ and ↘ indicate slightly increasing toxicity or slightly decreasing toxicity of < 20%. Variable effects = varying results between different measurement campaigns. Color codes:

	EC50 values based on expert judgment	Other values (change of effect compared to negative control)
no negative effects	EC50 > 100	< 5%
weak or moderate effects	20 < EC50 < 100	5 - 20%
strong effects	EC50 < 20	> 20%

Gammarus fossarum

G. fossarum bioassays were performed in the pilot study in CH. Here effects with high variability in all treatment processes were measured. When exposed to samples of one sampling campaign, no significant mortality in comparison to the control occurred, neither in the MBR permeate nor after treatment with ozone or with PAC. However, the variability in mortality among the replicates was high. When exposed to samples of another sampling campaign, a high mortality was measured in the MBR permeate and after treatment of the permeate with ozone. One of the reasons for these variable effects might be differences in the wastewater composition (McArdell et all, 2011).

Fish embryo

Fish embryo tests were performed to evaluate the treatment processes of the pilot plant in DE. No treatment related effects were observed in the various treatment steps. Thus, no indications of acute effects on the embryo development of *Danio rerio* were found.

4.4.3 Performance assessment regarding long-term toxicity effects

In-vivo long-term bioassays were conducted in DE with Daphnia as semi-static test and worms and snails in flow-through systems to evaluate the performance of the treatment processes at the pilot plant and to assess possible effects of oxidation by-products after wastewater ozonation. The results are shown in Table 4-8.

Table 4-8: Evaluation of the effects measured in the long-term toxicity tests

Bioassays	Endpoints	Effluent of MBR	MBR + O3	MBR + O3 + SF	MBR + PAC/SF
Daphnia population test (21d, semistatic)	Mean abundance		↗	↘	
	Mean of sectional growth		↗	↗	
	Sum of neonates				
	Sum of dead animals				
	Sum of aborted eggs				
	Sum of ephippial eggs				
	Body length on day 21				
	Portion of adult daphnids		↗	↘	
Potamopyrgus antipodarum test (28d, OECD)	Mortality	↗	↗	↓	
	Shell size		↑	↓	
	Number of embryos/female	↑	↘	↑	
Lumbriculus variegatus test (28d, flow though)	Reproduction		↗	↘	
	Biomass		↑	↘	
	Biomass per worm		↑	↘	

All tests performed on samples from the pilot plant in Gelsenkirchen, Germany, Evaluation is performed on average values. Change of toxicity value after treatment process: ↑ and ↓ indicate increasing toxicity or decreasing toxicity of > 20%, ↗ and ↘ indicate slightly increasing toxicity or slightly decreasing toxicity of < 20%. Variable effects = varying results between different measurement campaigns. Color codes:

	Other values (change of effect compared to negative control)
no negative effects	< 5%
weak or moderate effects	5 - 20%
strong effects	> 20%

Daphnia population

In the *Daphnia* population experiment possible acute and sub-lethal effects on these invertebrates can be investigated. The test results indicated negative effects of the ozonation treatment on the population abundance and the population structure. After ozonation the population size was increased, but the portion of larger daphnids was decreased. These effects remained present after the subsequent post-treatment in the sand filter. In contrast, the MBR permeate did not result in significant negative effects on the population dynamic.

Worm toxicity

In the ozonated effluent *L. variegatus* revealed a significant inhibition of reproduction compared to the membrane bioreactor permeate and therefore a significant reduction of the worm number compared to MBR effluent was observed. After sand filtration of the ozonated influent this effect

was slightly reduced but worm number was still significantly reduced compared to MBR effluent. The biomass per worm comparison showed also a similar pattern with a significant decrease after ozonation and the following moving bed sand filtration compared to the MBR treatment. These findings are consistent with the results of the above mentioned algae growth tests.

Snail reproduction

The survival of the snails was significantly reduced during exposure to MBR permeate and ozonation effluent. Adverse effects on the shell size of the snails exposed to ozonation effluent were also detected compared to the MBR permeate. Furthermore, snails exposed to a positive control (100 ng/L 17α-Ethinylestradiole EE2) exhibited a reduced shell height. The organisms in the positive control produced significantly more embryos than snails in reference water and hence indicating the snails' sensitivity to EE2. The snails exposed to MBR permeate showed higher reproductive output than the snails in the negative control, whereas the snails exposed to the ozonated wastewater showed a slightly decreased embryo production compared to MBR permeate. After sand filtration of the ozonated effluent snails exhibited a significantly higher embryo production compared to the previous treatment steps.

The performance of GAC filtration and UV treatment were not measured by *in-vivo* bioassays. But, the results of the applied *in-vitro* may allow their evaluation regarding possible chronic effects when the acute-to-chronic ratios of the effects causing substances are known.

4.5 Discussion

The tests battery performed in PILLS are adequate for the research objectives of the project.

The tests performed with sample enrichment are in principle suitable for the assessment of the treatment processes in cases where standard tests are not sensitive enough for tests with original samples. But, *in- vitro* tests might underestimate potential hazards of ozonation by-products as these substances are supposed to be readily degradable and consequently storage- and transportation time (Petala et al. 2006) as well as insufficient extraction procedures (Daughton 2003) might lead to a significant loss of toxic by-products. Therefore, whole organism tests with original samples or conducted on site in flow-through systems are important to evaluate the alteration of toxicity of wastewater after oxidation by ozone or UV as well as the detoxifying potential of post treatments because loss of non extractable or unstable compounds is minimized (Magdeburg et al. 2012; Stalter et al. 2010a, b).

The used *in-vivo* tests are in line with other studies. For example, in a pilot study at the wastewater treatment plant Waldbröl long-term tests were performed with *Lemna minor* and *Daphnia magna* (Pinnekamp et al. 2009), both standard organisms in risk assessment. In order to show possible toxic effects resulting from ozonation of secondary treated wastewater, *in-vivo* long-term tests have been applied in the NEPTUNE project (Stalter et al. 2010). Tests here were conducted with *Lemna minor, Potamopyrgus antipodarum* (snail), *Lumbriculus variegatus* (worm), *Chironomus riparius* (midge) and also with the vertebrate (secondary consumer) *Oncorhynchus mykiss* (rainbow trout) in an early life stage test. The two last organisms proved to be sensitive to the ozonated wastewater and showed some negative effects post ozonation, which could be reduced/eliminated with subsequent sand filtration (Magdeburg et al., 2009). Similar results were observed in Switzerland in two pilot studies at the WWTPs Regensdorf and Lausanne (Abegglen et al. 2009; Margot et al. 2011), where ecotoxicological effects of the advanced wastewater treatment methods ozonation followed by sand filtration (in both pilot studies) and powdered activated carbon treatment with subsequent filtration (in one pilot study) were assessed.

Though, some *in vitro* bioassays can measure the effects of all substances in an environmental sample with the same mode of action, e.g. estrogenic substances in the YES or ER-CALUX assay. But, the detected toxicity effects cannot be related to individual chemical compounds. The toxicity tests therefore don't allow conclusions neither on the toxicity of specific single substances nor on the toxicity of the mixture of pharmaceuticals or other compounds.

Some bioassay results showed a high variability which may be related to the highly variable nature of the composition of hospital wastewater and the different source from other wastewater treatment plants. The variable composition of hospital wastewater and the dilution effects in municipal wastewater may also be the reason for the higher toxicity of hospital wastewater when compared to municipal wastewater. However, it should be considered that wastewater samples from different locations and only one or a few number of samples were tested. Thus, the results of the different tests may not be directly comparable or statically relevant. But, they may provide initial information on the ecotoxicity potential of hospital wastewater in comparison with municipal wastewater.

In all bioassays with sample enrichment the biological treatment in a MBR reduced the toxic effects in the raw wastewater. However, the resulting MBR permeate was strongly toxic to algal photosynthesis, moderate toxic (estrogenicity, antibiotic effects and inhibition of bacteria luminescence) or not toxic (cytotoxicity and mutagenicity). The MBR permeate (without sample enrichment) was not toxic in the *in-vivo* tests with worms and daphnids. But, MBR permeate was toxic to algae growth and snails and furthermore affected the reproduction of snails.

In the pilot study in Wadbröl MBR treatment reduces toxicity considerably. The MBR effluent was toxic to bacteria and regarding mutagenic effects, but was not toxic to the algae growth (Beier et al. 2008). However, in this study another test design and the standard test medium were used according to the standard DIN 38412-L33. Recently the two different test media for the algal growth inhibition test – the „Altenburger test medium" (Altenburger et al. 2010) and the standard medium according to DIN 38412-L33 – were both used in parallel to investigate the eco-toxicological potential of wastewater from the municipal treatment plant in Bad Sassendorf. In this study algal toxicity was regularly observed in the MBR effluent when the Altenburger test medium was used and a clear dose-response relationship was found, while the standard procedure according to DIN was mostly not able to detect these effects due to masking effects by macronutrients in the wastewater (Schmidt et al. 2011).

The advanced treatment of MBR permeate by PAC addition or GAC filtration had a decreasing effect on the toxicity when compared to that of raw wastewater. However, both bioassays (photosynthesis inhibition with pre-concentrated samples and growth inhibition with diluted original samples) with the primary producers revealed that even after activated carbon treatment the effluent contained algal toxic compounds. In contrast, in the pilot studies in Waldbröl (Pinnekamp et al. 2009) and at the effluent Vidy in Lausanne (Margot et al. 2011) no toxic effects to algae were detected after GAC filtration of the MBR effluent or PAC addition to the wwtp effluent.

In the PILLS project ozonation of MBR permeate was efficient in reducing antibiotic and estrogenic effects. However, as well in different acute toxicity tests with and without sample enrichment as in long-term *in-vivo* bioassays an increasing toxicity was observed after the oxidation of MBR permeate by ozonation or UV treatment. Adverse effects of the oxidation process were detected for bacteria, algae, daphnids, snails and worms. The consistency of the outcome of the test systems indicates the formation of transformation products during wastewater oxidation with an enhanced toxicity for these organisms.

With regard to the results of wastewater ozonation and the formation of by-products indicating toxic effects after ozonation the above mentioned results are in contrast with some studies, where no such effects could be observed (e.g. Mišík et al., 2011; Petala et al., 2006; Reungoat et al., 2010; Takanashi et al., 2002). But, the occurrence of adverse effects after wastewater ozonation is in line with other experiments at other treatment plants equipped with an ozonation step [WWTP Neuss, Germany (Stalter et al., 2010b); WWTP Wüeri in Regensdorf (Magdeburg et al. 2012) and WWTP Vidy in Lausanne (Margot et al. 2011), both Switzerland and treatment plants in North Rhine-Westphalia, Germany (Schmidt et al., 2011)]. Stalter et al. (2010a) observed an increased mortality of rainbow trout after long-term exposure (64 days) to ozonated wastewater of the municipal WWTP Wüeri. However, in these experiment sand filtration reduced toxic effects, indicating that these ozonation by-products are readily degradable or adsorbable (Stalter et al. 2010b).

A post-treatment of the oxidated effluent by biological treatment in a biofilter at the pilot plant in CH or GAC filtration at the pilot plant in NL removed these adverse effects. But, the adverse effects were only partly removed with sand filtration (moving bed sand filter) as post-treatment of the ozonation process at the pilot plant in DE. This is in contrast to the previous experiments, where adverse effects were significantly removed with sand filtration. Hacker et al. (1994) demonstrated that ozonation by-products are mainly removed via biological degradation. Accordingly, the extent of biological activity in the sand filter and/or its hydraulic retention time might be parameters with a potential to improve the elimination of oxidation products.

4.6 Conclusions

- The used battery of bioassays was adequate to characterise hospital wastewater and to evaluate the performance of the investigated treatment processes from the ecotoxicological point of view.
- Raw hospital wastewater was moderately cytotoxic, estrogenic and toxic to various test organisms. In general hospital wastewater was more toxic compared to municipal wastewater.
- The biological treatment in a MBR decreased toxic effects considerably in raw hospital wastewater. But, MBR permeate may still contain toxic effects to some organisms like bacteria, algae and snails.

- Advanced treatment by PAC addition or GAC filtration had decreasing effects on the toxicity of raw wastewater, but the effluent of this treatment process may still contain algal toxic compounds.
- Ozonation reduced considerably antibiotic and estrogenic effects of hospital wastewater. But, increasing toxicity is detected after the oxidation processes by ozonation or UV treatment, presumably due to the formation of by-products.
- A post-treatment of the ozonated effluent in a biofilter (e.g. sand filter with biological activity) reduced the adverse effects of the advanced treatment by oxidation significantly but did not remove it totally (e.g. toxic effects on algae).
- A post-treatment of the UV treatment effluent by GAC filtration removed the adverse effects of the oxidation process.
- In the present study, the sand filter was not as efficient as observed at previously evaluated treatment plants to reduce oxidation product induced toxicity. Further studies on oxidation product removal after the ozonation process are desirable.

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