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Environmental risk of pharmaceuticals in waters: Investigation on their occurrence and removal in conventional treatment plants.

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Glossary of Terms

AOP	Advanced Oxidation Process
AOX	Adsorbable Organic Halogen
°C	Degrees centigrade
C	Carbon
CAS	Conventional Activated sludge
CF	Conversion Factor
C_h	Concentration of Pharmaceuticals in Hospital Wastewater
CIS	Common Implementation Strategy
C_u	Concentration of Pharmaceuticals in Urban Wastewater
d	Day
DDD	Defined Daily Dose
Df_e	Dilution Factor, due to the discharge of WWTP into the receiving water body
Df_u	Dilution Factor, due to the discharge of hospital effluent in the sewer system
EC	European Comunity
EIAs	Environmental Impact Assessment
EMEA	European Agency for the Evaluation of Medicinal Products
EQSs	Environmental Quality Standards
ERA	Environmental Risk Assessment
ERQ	Environmental risk posed by pharmaceuticals originated from hospital wastewater
EU	European Union
F/M	Food to Microorganism ratio
FDA	United .States Food and Drug Administration
GWD	Ground Water Directive
GWRC	Global Water Research Coalition
h	Hour
HLB	Hydrophilic-lipophilic-balanced (reversed-phase sorbent)
HPLC	High-Performance Liquid Chromatography
HRQ	Risk Quotient posed by Pharmaceuticals in hospital wastewater
HRT	Hydraulic Retention Time
HWWs	Hospital WasteWaters
Ic	Wastewater treatment plant influent concentration of Pharmaceutical under investigation
Inh.	Inhabitants
IRQ	Wastewater treatment plant Influent Risk quotient posed by Pharmaceuticals under investigation
ISTAT	Istituto Nazionale di Statistica
K_{biol}	Reaction Rate Constant
K_d	solid-water distribution coefficient
Kg	Kilogram

LOD	Limit Of Detection
Log K_{ow}	Octanol Water Coefficient
LOQ	Limit Of Quantification
MBR	Membrane Bioreactor
MCDA	Multiple - Criteria Decision Analysis
MDD	Maximum Daily Dose
mg	Milligram
MS	Mass Spectrometry
MW	Molecular Weight
N	Nitrogen
ng	Nanogram
NI	Negative Ion
NSAIDs	NonSteroidal Anti-Inflammatory Drugs
OsMed	Osservatorio sull'impiego dei Medicinali
OSPAR	Oslo/Paris convention (for the Protection of the Marine Environment of the North-East Atlantic)
OTC	Over The Counter
P	Phosphorus
PBT	Persistence, Bioaccumulation and Toxic properties
PE	Person Equivalent
PEC	Predicted Environmental Concentration
pH	Power of Hydrogen
PhCs	Pharmaceutical Compounds
PILLS	Pharmaceutical Input and Elimination From Local Sources
Pk_a	Dissociation Constant
PNEC	Predicted No Effect Concentration
PPS	Pharmaceutical Products
QqLIT	Quadrupole-Linear Ion Trap
R	Percentage removal rate of pharmaceutical compound in WWTP
RQ	Risk Quotient
RSD	Relative Standard Deviation
SD	Standard Deviation
SRM	Selected Reaction Monitoring
SRT	Sludge retention Time
STP	Sewage Treatment Plant
STWs	Sewage Treatment Works
Sw	Surface Water
$t_{1/2}$	Half life time
µg	Microgram
µm	Micrometer

U.S. EPA	United states Environmental Protection Agency
UK	United kingdom
UWWs	Urban WasteWaters
V	Volume
VMPs	Veterinary Medicinal Products
WFD	Water Framework Directive
WHO	World Health Organization
WW	WasteWater
WWTPs	WasteWater Treatment Plants

Chapter 1: Introduction

1.1 The Scientific interest towards Pharmaceutical compounds as emerging contaminants

Pharmaceutical compounds (PhCs) have been characterized as "new" or "emerging" contaminants in the environment. However, PhCs have been around for several decades now. To that effect, a more accurate characterization is the fact that our attention to their presence in the environment is new or just emerging (Jjemba 2008).

Interest in their presence in the environment is directly or indirectly stimulated by the fact that they are produced in increasingly large quantities and the improvements in detection methods, in fact enhanced sensitivity of analytical chemistry methods has enabled the detection of low-levels of pharmaceuticals in the environment, resulting in questions about the safety of the ecosystem and surface waters used for drinking supplies. Furthermore, their use and diversity is also steadily increasing every year. In addition, the continually ageing population and improving quality of life worldwide mean that their consumption is set to increase in future years (Van der Aa et al., 2011), recent investigations document that PhCs production and administration may vary both between countries and over time (Goossens et al., 2007, Kümmerer, 2009a), fluctuating not only on an annual basis, but also from one year to the next (Alexy et al., 2006). In recent years, PhCs have provoked increasing concern, particularly as no legal requirements have been set for discharge into surface water bodies of these ubiquitous, persistent and biologically active substances (Furhacker, 2008; Salgot et al., 2006; Ternes et al., 2007). Hence, over the last ten to fifteen years, PhC concentrations in raw and treated urban wastewater (WW) have been extensively monitored. Nevertheless, this is still a largely unregulated area, and there is ongoing debate within the scientific community regarding which PhCs to include among the priority substances (Bottoni et al. 2010). According to the new European draft annex (EC 2012), the anti-inflammatory diclofenac and the hormones 17β-estradiol and 17α-ethinylestradiol are prime candidates to be added to the European Priority List, while according to the U.S. EPA, erythromycin, nitroglycerin, and 9 hormones (17α-ethinylestradiol, 17α-estradiol, 17β-estradiol, equilenin, equilin, estriol, estrone, mestranol and norethindrone), need to be considered a priority (Richardson and Ternes 2011).

Once administered, these compounds are only partially metabolized by the human body, and therefore enter the water cycle either as parent (unchanged) compounds, which

are excreted largely through urine (generally 55-80 % of the total, with few exceptions) and partially in the feces, or as a mixture of metabolites and/or conjugated compounds (Jjemba et al., 2006, Lienert et al., 2007).

Municipal wastewater treatment plants (WWTPs) were not designed to remove trace organic contaminants, and as a results PhCs found their way into the environment through the discharge of treated WWs (Bendz et al., 2005; Castiglioni et al., 2006; Glassmeyer et al., 2005; Gomez et al., 2007; Joss et al., 2005; Verlicchi et al., 2012b), and as a result, their occurrence in surface water has been documented by a number of authors (Ashton et al., 2004; Calamari et al., 2003; Fatta-Kassinos et al., 2011; Gros et al., 2006, Kolpin et al., 2002, 2004; Spongberg et al., 2011) from around the world.

While the presence of pharmaceuticals in the environment is established, sources of these compounds in the environment, the pathways by which they reach sensitive receptors and their effects on these receptors are less characterized, moreover the latter must be determined before the effectiveness of risk mitigation measures can be assessed. Hospital wastewater (HWWs) represent an important source of PhCs (Le Corre et al. 2012, Jean et al. 2012), but has only recently been investigated, and in a far fewer number of studies. Not only high analysis costs, but also the difficulties in organizing water-sampling campaigns inside health facilities have delayed these investigations. Nonetheless, according to the recent literature (Verlicchi et al. 2010a,b; Ort et al. 2010a) HWWs may be considered a hot spot in terms of PhC load generated, prompting the scientific community to question the acceptability of the general practice of discharging HWWs into public sewers (Verlicchi et al. 2010 b), where they are conveyed to municipal WWTPs and co-treated with UWWs (Verlicchi et al. 2010 a,b; Pauwels and Verstraete 2006; Kummerer and Helmers 2000).

Through the evaluation of a compound's risk quotient (RQ), that is the ratio between its measured or predicted concentration and its predicted no-effect concentration (PNEC), Escher et. al. (2011) found that the presence of PhCs in raw HWWs, UWWs pose a risk for the environment, and this risk remains high in the WWTP effluent. However, once the effluent is discharged into the receiving water body, its dilution with surface water can mitigate the effect of residual PhCs and the associated risk quotient may decrease (Gros et al. 2010).

1.2 Aim and Objectives

The general aims of this thesis were to characterized the sources and pathways of PhCs in the environment, to monitor the occurrence, to assess the removal and fate of selected PhCs in WWTPs and in the water environment, and to carry out environmental risk analysis based on their occurrence as a basis to prioritize the hazardous compounds and to manage the risk posed by their exposure. In particular, this work focused on HWWs in order to assess their potential as a point source of 73 PhCs and their role in spreading these compounds into the environment, and consequently the impact of WWTPs on the receiving water bodies. The last aim was to develop a tool to estimate the level of environmental risk posed by PhCs originated from HWWs at site specific catchment area to aid the authorities and decision makers in the management of HWWs and the reducing of PhCs discharged into the environment. To achieve these aims the following objectives were set:

Objective 1: To review of the current knowledge on the sources, pathways, fate, and behaviour of PhCs in WWTPs from the literature (Chapter 2).

In this Chapter, an in-depth literature review has been carried out, collating data pertaining to 264 WWTPs from various global locations, mostly in Europe. The data pertaining to a wide spectrum of PhCs, 118 compounds belonging to 17 different classes distinguished by their function or biological activity, were considered: 23 analgesics/anti-inflammatories, 36 antibiotics, 1 antidiabetic, 1 antifungal, 3 antihypertensives, 1 barbiturate, 12 beta-blockers, 2 diuretics, 9 lipid regulators, 10 psychiatric drugs, 6 receptor antagonists, 4 hormones, 4 beta-agonists, 3 antineoplastics, 1 topical product, 1 antiseptic and 1 contrast agent.

Objective 2: To assess the occurrence of selected PhCs in HWWs and to evaluate their potential as point sourc of PhCs to the total load in the Influent of WWTP (Chapter 3).

In this chapter, an experimental investigation has been conducted in the area of Ferrara, Italy. Sixteen water samples were withdrawal from the effluents of two different sized hospitals and the influent and effluent of the receiving municipal treatment plant of one of the examined hospitals. The aim was to investigate 73 selected pharmaceuticals, belonging

to twelve different classes, comparing their occurrence in the effluent directly exiting the hospital with that, mixed with the local urban effluent, at the points of its entry and exit from the treatment plant. PhCs were selected due to their high prescription rates or volumes, the availability of a reliable analysis methods, as well as due to their occurrence and ubiquity in the aquatic environment

Objective 3: To assess the removal and release of 27 selected PhCs in full-scale WWTPs and their impacts on the receiving water bodies (Chapter 4).

An investigation on the occurrence of 27 PhCs, belonging to different classes has been carried out. Twenty one water samples were withdrawal from the influent, effluent of two full-scale WWTPs and their receiving water bodies in the sensitive area of the Po Valley (northern Italy). The receiving water bodies were monitored upstream and downstream of the effluent discharge points in order to evaluate the effluent impact on the quality of surface waters, commonly used for irrigation.

Objective 4: To determine the relative accuracy of the prediction models, and the limitations of on-site monitoring campaigns, that regard the occurrence of PhCs in the influent, effluent of a large municipal WWTP and downstream of its discharge point in the receiving water body, and their effect on the estimation of the environmental risk. (Chapter 5).

Objective 5: To estimate the potential impact of HWWs on the environment, and to assess the relative importance of PhCs pathways (HWWS, UWWs) for the priority candidate diclofenac as a case study for individual WWTP. (Chapter 6).

Scientific researches that study the occurrence of pharmaceuticals in the environment are hindered by many challenges, chemical analysis and sampling protocol represent the most important and they are a key part in the process of gathering environmental data (Ort et al. 2010b). These two factors are time and monetary consuming,

indeed they required an adequate quality assurance and control protocols. For these reasons and in order to maximize the value of measured data for the experimental investigations, chemical analysis of investigated PhCs in the different withdrawal water samples (HWWs, raw UWWs, treated WWs and surface water) in this work are done thanks to the collaboration with the Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA), Spanish Council for Scientific Research (CSIC), Barcelona, Spain. Moreover, This work is conducted in parallel with the European project "PILLS" (www.pills-project.eu), that part of its aims were the aims investigated in this Ph.D thesis, and during the years of this work, our results and knowledge have been discussed.

The results obtained from this research have been presented at international conferences, workshops, as well as in book chapters (see Appendix D). Moreover , three scientific papers are published in international journals as following:

1. Verlicchi P, **Al Aukidy M**, Zambello E. Occurrence of Pharmaceutical Compounds in Urban Wastewater: Removal, Mass Load and Environmental Risk after a Secondary Treatment – A Review. Science of the Total Environment 429 (2012) 123– 155.
2. Verlicchi P., **Al Aukidy M.**, Galletti A., Petrovic M., Barceló D.. Hospital Effluent: Investigation of the Concentrations and Distribution of Pharmaceuticals and Environmental Risk Assessment. Science of the Total Environment 430 (2012) 109– 118.
3. **Al Aukidy** M., Verlicchi P., Jelic A., Petrovic M., Barcelo D. Monitoring release of pharmaceutical compounds: Occurrence and environmental risk assessment of two WWTP effluents and their receiving bodies in the Po Valley, Italy. Science of the Total Environment 438 (2012) 15 – 25.

Chapter 2: Background

2.1 Introduction

The problem of PhCs in the Environment, is not a new issue (there has much work in this area since the 1990s, and a lot of information is available in the scientific literature) but recently it has become a priority concern, particularly for politicians and the general public. PhCs have been found in various environmental compartments (waters, soils, sediments) and have been suspected of having an affect on the integrity of the aquatic ecosystems (Kummerer, 2008).

Pharmaceuticals are classified according to their purpose (e.g., antibiotics, analgesics, anti-neoplastics, anti-inflammatory substances, antihistamines, X-ray contrast media, etc.). PhCs are complex molecules with different physicochemical and biological properties and functionalities. They are developed and used because of their more or less specific biological activity and are most notably characterized by their ionic nature. The molecular weights of the chemical molecules range typically from 200 to 1000 Dalton. This chapter gives an introduction to the PhCs properties, consumption, toxicity, sources and pathways through a full litereature review that deals also with their occurrence, removal, fate and factors effecting their removal in WWTPs, and finaly the total discharged load of PhCs from WWTP and an evaluation of their risk posed to the envieonment is presented.

2.2 Physico-chemical properties of pharmaceutical compounds (PhCs)

Once a PhCs is discharged into wastewater, it will be distributed between the different environmental compartments (e.g. surface water, soil, sediment) according to its physico-chemical properties, including the solubility, volatility, acidity, lipophilicity and sorption potential. Moreover, its persistence will depend on its resistance to be degraded biologically or abiotically. Appendix A.1. shows the physico-chemical characteristics and biodegradability of selected PhCs.

2.2.1 Volatility

Volatility is the tendency of a compound to volatilize, that is to leave the liquid phase and enter into the gas phase. It is strictly correlated to Henry coefficient. Ternes and Joss (2006) observed that a significant amount of a compound will be stripped in a bioreactor with fine bubble aeration if Henry constant > 0.003. Therefore, since most of

PhCs has low henry constant (Appendix A.1.), it can be concluded that stripping process is in general not relevant for the removal of pharmaceuticals during WWTPs.

2.2.2 Acidity

Acidity (i.e. dissociation constant pK_a) describes the degree of ionization of the compound at a known pH. The pH of relevance in the environment ranges between 4 and 8, with activated sludge typically presenting a pH in the range of 7 – 8 (Christofi et al., 2003). The pK_a values that are less than 7 indicate that the compound is negatively charged under acidic conditions and vice versa. Most PhCs are acids or bases with pK_a values of 2 – 12. Weakly acidic pharmaceuticals such as the nonsteroidal anti-inflammatory drugs (NSAIDs) (i.e., naproxen, ibuprofen, and acetysalicylic acid) with pK_a values of 4.2, 5.2, and 3.5 as well as clofibric acid (pK_a = 2.95) have a low tendency to adsorb onto sludge.

2.2.3 Lipophilicity

Lipophilicity (Hydrophobicity) is related to the physical property of a compound to be repelled from a mass of water. Different coefficients were used to evaluate the tendency of a substance to stay in the water phase. The most common parameters are the octanol-water partition coefficient (K_{ow}) and the octanol-water distribution coefficient (D_{ow}). In the past, K_{ow} was generally used for evaluating and predicting pharmaceutical behavior in aquatic compartment by considering that high K_{ow} values are characteristics of hydrophobic substances, poor hydrosolubility and in some case of a high potential to sorb on organic material of sludge (Rogers, 1996).

As known, PhCs are complex molecules, multifunctional organic compounds in some cases ionized in the aquatic environment: the un-ionized species will be the predominant species to partition into octanol from water, the ionized species predominantly remaining in the aqueous compartment. The pH at which measurements are made for evaluating K_{ow} is a crucial parameter. For these reason, recently Cunnigham (2008) reported that K_{ow} does not properly describe environmental partitioning and dynamic in the environment of polar and ionizable compounds such as PhCs and for them the coefficient D_{ow} is more adequate as it is pK_a dependent at the pH of the environment.

2.2.4 Sorption Potential

The sorption potential of a given compound is indicated by the solid-water distribution coefficient (K_d), which combines two driving forces for sorption: acidity and lipophilicity. Ternes and Joss (2006) indicated that only compounds having K_d values higher than 500 L kg^{-1} will be sorbed significantly onto sludge during primary and secondary treatment. In the case of sludge treatment, Carballa et al. (2007) showed that the limit of relevance below which sorption can be neglected is around $K_d < l$ L kg^{-1}, since the sorbed amount is not only dependent on the distribution coefficient but also on the concentration of solids.

2.3 Pharmaceutical consumption

Large amounts of pharmaceuticals, representing a wide spectrum of therapeutic classes, are used and prescribed in human medicine world wide (Dìaz-Cruz and Barcelo 2004). In most cases, only a rough estimation of pharmaceutical consumption is available, because they are often sold as over-the-counter drugs (Dìaz-Cruz and Barcelo 2004; Stackelberg et al. 2004). A rough estimation of the global consumption of human PhCs showed that about 100 000 tons of PhCs are used each year, which corresponds to a worldwide average consumption of 15 g/pro capite year (Kummerer 2004). More detailed analyses about PhC consumption for area, country are available in terms of sales (WHO, 2004) of the specific therapeutic classes, but these data are not useful to evaluate the mass flow of PhCs consumed in a specific area and time. Usage data for active compounds sold in four different European countries are summarised in Table 2.1. These data indicate that, in general, the analgesic acetaminophen and the analgesic and anti-inflammatory drugs acetylsalicylic acid and ibuprofen are the pharmaceuticals sold in highest quantities, followed by the antibiotics, and the antiepileptic carbamazepine.

Table 2.1. Volume of pharmaceutically active compounds sold in different countries (kg/yr)

Therapeutic class	Compound	France (2004)[a]	UK (2004)[b]	Spain (2003)[c]	Italy (2010)[d]
Analgesics/anti-inflammatories	Acetaminophen	3303077	3534737	-	-
	Acetylsalicylic acid	396212	177623	-	-
	Diclofenac	9896	35361	32300	9602
	Ibuprofen	240024	330292	276100	-

Therapeutic class	Compound	France (2004)[a]	UK (2004)[b]	Spain (2003)[c]	Italy (2010)[d]
	Naproxen	37332	33580	42600	-
Antibiotics	Azithromycin	4073	756	-	13870
	Clarithromycin	15105	8807	-	64470
	Erythromycin	-	48654	8100	-
	Penicillin V	-	32472	-	-
	Amoxicillin	333223	149764	-	-
	Sulfamethoxazole	16730	3113	12700	-
	Sulfadiazine	-	362	-	-
	Ciprofloxacin	12186	16445	-	21672
	Tetracycline	-	2101	-	-
	Trimethoprim	3346	11184	3700	13896
Beta-blockers	Acebutolo	-	943	-	-
	Atenolol	18337	49547	-	18084
	Metoprolol	8786	3907	2300	-
	Propranolol	12487	9986	-	-
Hormones	Progesterone	-	751	-	-
	Testosterone	-	-	-	-
Lipid regulators	Gemfibrozil	-	1418	-	-
	Fenofibrate	85670	2815	-	-
	Atorvastatin	7924	-	-	7682
	Simvastatin	6943	14596	-	-
	Lovastatin	-	-	-	-
Psychiatric drugs	Fluoxetine	3740	4826	4200	-
	Paroxetine	5515	2663	-	-
	Citalopram	3487	4799	1600	-
	Carbamazepine	33514	52245	20000	31190
Contrast media	Iopromide	-	-	20000	-

Data from: [a] Besse et al. (2007), [b] Environment Agency (2008), [C] Carballa et al. (2008), [d] Al Aukidy et al. (2012)

2.4 Toxicity of PhCs

The most important issue of concern about the presence of pharmaceuticals in the aquatic environment and the main reason why they are of interest for inclusion in monitoring programs as environmental contaminants is the ecotoxicological effects that they may cause. Even today, little is known about this subject. Some studies, however, have reported that some compounds, such as diclofenac (anti-inflammatory), propranolol (β-blocker), and fluoxetine (antidepressant), show chronic lowest-observed-effect

concentrations for fish toxicity, zooplankton, and benthic organisms in the range of WWTP effluent concentrations (Fent et al.2006). This indicates that for some compounds, the margin of safety is narrow and that chronic effects at highly contaminated sites cannot be completely ruled out, particularly when combined effects of pharmaceutical mixtures are taken into account (Fent et al.2006). Nevertheless, dilution in receiving waters results in lower levels, enabling the reduction of environmental risks.

It is impossible to rule out acute effects entirely without further testing since certain species may be particularly susceptible to certain class of drugs. Pharmaceuticals are created with the intent of causing a biological effect, they often have similar types of physiochemical behavior that are characteristic of harmful xenobiotics (e.g. they are able to pass membranes, and they are relatively persistent in order to avoid being inactivated before having their therapeutic effect). In non-target aquatic life many pharmaceuticals act as baseline toxicants. However, some exhibit the therapeutic effect also in aquatic life as the unwanted estrogenic effects on fish (Kidd et al., 2007). Others act via a different specific mode of toxic action, as evidenced for fluoxetine effects on algae (Neuwoehner et al., 2009). It is generally accepted that mixtures with components exhibiting the same mode of action act according to the model of concentration addition. In wastewater pharmaceuticals are present as mixture with varying modes of toxic action, and their toxicity was found at concentrations at which the single compound showed no or only little effects. Thus it could be assumed that the toxicity of a very complex mixture is governed by the underlying baseline toxicity, not the specific mode of toxic action of single components (Escher et a.2011).

Pomati et al. (2006, 2008) investigated the effects and interactionsof a mixture of commonly used pharmaceuticals, including carbamazepine, ibuprofen and sulfamethoxazole at low concentrations, designed to mimic those found in the environment using in vitro tests on human and zebrafish cells. They concluded that a mixture of drugs at ng/L levels can inhibit cell proliferation by affecting their physiology and morphology and that waterborne pharmaceuticals may have an effect on aquatic life. Synergy remains an important topic with the complex mixtures of trace organic compounds being released to the environment (Stuart et al. 2012).

2.5 Legislation on PhCs in the aquatic environment

From a legislation point of view, it is quite important to note that the Directives concerning the protection of aquatic environments and related organisms are the Water Framework Directive 2000/60/EC (WFD), the daughter Directice 2006/118/EC (GWD) for the protection of groundwater and the daughter Directive 2008/105/EC (PSD) stating the List of Priority Substances (also known as Annex X to WFD) for surface waters and related Environmental Quality Standards (EQSs). Pharmaceuticals are not included among those compounds to be monitored, outwithstanding their occurrence have been documented since more than 20 years in many European countries. The revision of the list of compounds and the subsequent definition of pertinent new EQSs are based on significant risks to or via aquatic environment in compliance with Art. 16 of the WFD.

Bottoni et al. (2010) report that a simplified and pragmatic methodology was developed under the WFD Common Implementation Strategy (CIS), taking into consideration both monitoring data and modelling data. According to these Authors, possible priority pharmaceuticals could be antineoplastics (including tamoxifen and cyclophosphamide), synthetic estrogens and hormones. The inclusion of target PhCs in the EU List of Priority Substances implies the definition of their corresponding EQSs and the necessity to subject to monitoring ambient water, sediment and biota in the different EU countries. In addition further attempts to define prioritisation lists have been made by other Commissions. For instance that by Oslo and Paris Commission (OSPAR) including mainly antibiotics, psychiatric drugs, receptor antagonists, that by Global Water Research Coalition (GWRC 2008) that defined a high priority level for a group of substances belonging to different classes: carbamazepine, sulfamethoxazole, diclofenac, ibuprofen, naproxen, bezafibrate, atenolol, ciprofloxacin, erythromycin and gemfibrozil. National prioritisation procedures have also taken place and prioritised PhCs based on the potential risk that they are perceived to pose to aquatic environment. In the United Kingdom, 12 compounds were prioritised for targeted monitoring based upon their predicted environmental concentrations, predicted no effect concentration (PNECs), and persistence, bioaccumulation and toxic (PBT) properties: mainly analgesics, antidepressants, antibiotics,antineoplastics (Ashton et al., 2004). In the United States the contaminants candidate to be included into the priority lists are the antibiotic erythromycin and the estrogens ethinylestradiol, estradiol, equilenin, estriol, estrone, mestranol and norethindrone (Richardson and Ternes, 2011). All these attemps provide a good start in focusing efforts, but they should be considered with caution as they are based on acute,

principally lethal, ecotoxicological test data and may therefore not include those substances taht may be exerting effects following chronic exposure. Occurrence data have to be used not only to confirm the presence of a compound in the aquatic environment, but it is used in combination with relevant ecotoxicol test data to allow the refinement of risk assessments.

2.6 Environmental risk assessment of PhCs.

The European Union Directive 92/18/EEC introduced for the first time, the requirement for an environmental risk assessment, as a prerequisite to obtain marketing authorization for veterinary pharmaceuticals. For this purpose, the European Agency for the Evaluation of Medicinal Products (EMEA) published a "Note for Guidance" (EMEA 1998) where guidelines to assess the environmental risk of veterinary medicinal products are established. The European Commission extended its concerns to pharmaceuticals for human use by publishing Directive 2001/83/EC which was subsequently amended by Directive 2004/27/EC (EudraLex 2009). These directives established that marketing authorization for new medical products for human use should be accompanied by an environmental risk assessment, whose guidelines were set out by (EMEA,2006). Nevertheless, the environmental impact does not provide sufficient grounds for a refusal. Environmental risk assessment of both veterinary and human pharmaceuticals is assessed in a stepwise approach, divided into two phases. In Phase I, environmental exposure to the pharmaceutical or its metabolites is estimated. Phase II comprises its fate and effects in the environment. For this reason, Phase II is sub-divided into two parts: Tier A, in which possible fate and effects of the pharmaceutical and/or its major metabolites are evaluated; and Tier B, which focuses on the effects on fauna and flora within environmental compartments that are likely to be affected (EMEA 1998,2006). However, medicinal products for human use only require Phase II studies if the predicted environmental concentration in surface water is equal to or above 0.01 μgL^{-1} (EMEA 2006). In the US, issues concerning pharmaceuticals in the environment are regulated by the U.S. Food and Drug Administration (FDA). This institution requires environmental assessments to obtain marketing authorisations which are specified in the "Guidance for Industry-Environmental Assessment of Human Drug and Biologic Applications" (FDA 1998). However, an environmental assessment is required only if the estimated environmental concentration of the pharmaceutical at the point of the entry is above 1 μgL^{-1} (FDA 1998). As EMEA, the

FDA also requires environmental assessments for veterinary medicinal products, using a tiered approach. With a view to harmonising the guidelines that govern these environmental impact assessments, the EU, US and Japan elaborated two guidelines: "Environmental Impact Assessment (EIAs) for Veterinary Medicinal Products (VMPs)-Phase I" (EMEA 2000) and "Environmental Impact Assessment for Veterinary Medicinal Products-Phase II Guidance" (EMEA 2005) so that environmental fate and toxicity data obtained could be used to obtain marketing authorisation in all these regions.

In synthesis, the basic principle of Environmental Risk Assessment (ERA) is the comparison of a predicted or measured environmental concentration (PEC or MEC) of a substance with a predicted, no effect concentration (PNEC), the concentration at which no effects on environmental organisms are expected to occur. If the PEC or MEC of a substance is higher than or equal to the PNEC, i.e. the risk characterisation ratio is ≥ 1, and thus an unacceptable risk for the environment is indicated, either a refined ERA with improved data is conducted or a risk management with appropriate measures has to be realised. PECs are derived from model calculations, whereas MECs can be determined from monitoring studies. Preference should be given to adequately representative exposure data, the discrypancis between these two approches are discussed in Chapter 5.

2.7 Source and pathways of PhCs

The origins of aquatic pollution by PhCs are derived from diverse sources that can be divided into point sources which include municipal wastewater effluent from STWs, industrial effluent and leachates from waste disposal sites, and non point sources which may consist of agricultural run-off (Figure 2.1.). The principal sources of human pharmaceuticals that discharge into wastewater treatment facilities include hospitals, extended-care facilities, and private households, all of these sources also contribute via the disposal of unused medicines as trash. This occurs through the hospital sewage system for admitted patients and urban wastewaters. Pharmaceuticals applied in veterinary medicin, as growth promoters and for other purposes, are excreted by the animals, usually, it is assumed that emission from pharmaceutical manufacturing and production are low in Europe and north America (Kummerer 2010). Contaminants applied to the soil surface will migrate through the soil zone, the unsaturated zone and the saturated zone in the well established way. This may be the route for components of sewage sludge used as fertilizer.

The potential for organic contaminants present in sewage sludge to leach following application to agricultural land was highlighted by (Wilson et al. 1996, Montiero et al.2010).

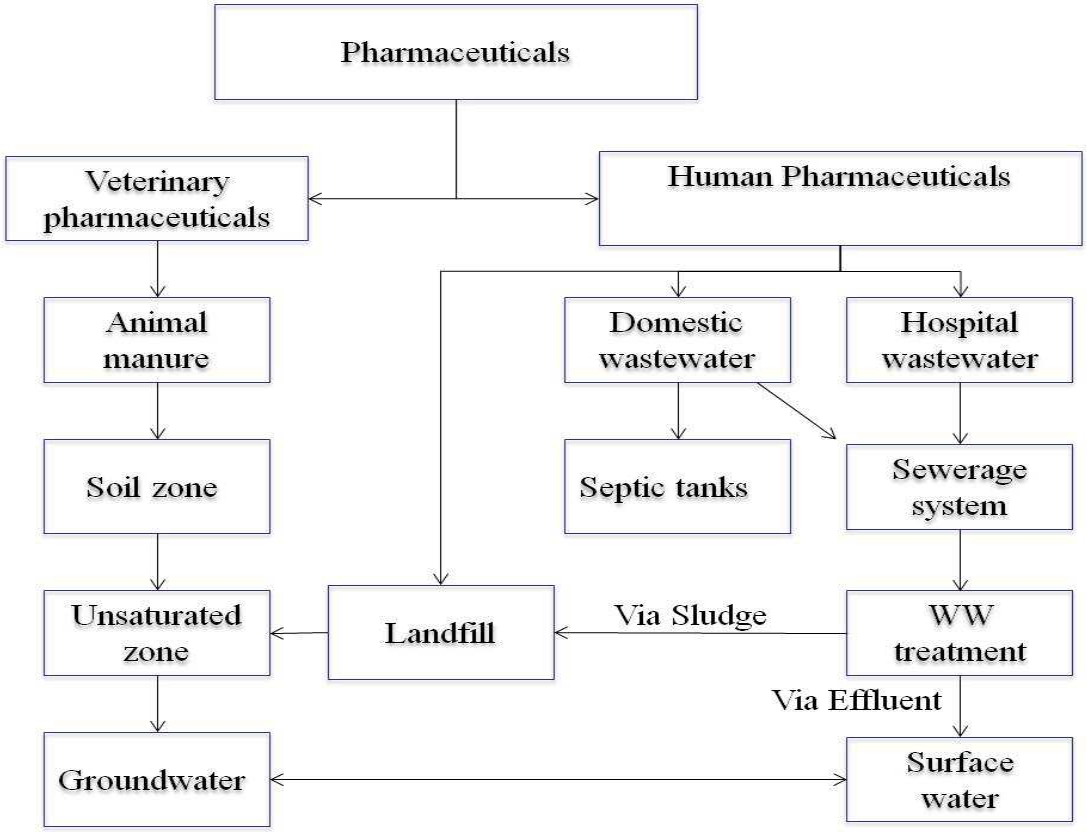


Figure 2.1. Sources and pathways of pharmaceutical compounds.

Another important pathway is groundwater–surface water interaction. In many instances treated effluent from industrial premises and sewage works is discharged to surface water. This may then infiltrate to groundwater from losing reaches of rivers (Stuart et al., 2012).

2.8 Occurrence and fate of PhCs in wastewaters

Once administered, PhCs are metabolised to varying degrees, and their excreted metabolites and unaltered parent compounds can also undergo further modification due to biological, chemical and physical processes in both sewage treatment facilities and receiving water bodies (Deblonde et al., 2011; Fatta-Kassinos et al., 2011; Miège et al., 2009; Monteiro et al., 2010; Onesios et al., 2009). Municipal wastewater treatment plants (WWTPs) are generally not equipped to deal with complex pharmaceuticals, as they were

built and upgraded with the principal aim of removing easily or moderately biodegradable carbon, nitrogen and phosphorus compounds and microbiological organisms, which regularly arrive at the WWTP in concentrations to the order of mg L^{-1} and at least 10^6 MPN/100 mL, respectively. PhCs in raw wastewaters are generally in the range of 10^{-3} – 10^{-6} mg L^{-1}, in addition, their chemical and physical properties, namely solubility, volatility, adsorbability, absorbability, biodegradability, polarity and stability, vary greatly (Le Minh et al., 2010; Ziylan and Ince, 2011), with obvious repercussions on their behaviour during the treatments and consequently their removal efficiencies.

Indeed, several PhCs have been found in river biota, some at high levels (Rimkus, 1999), thereby evidencing the risk that environmental concentrations of PhCs can be higher than their (PNECs) (Santos et al., 2007; Stuer-Lauridsen et al., 2000), especially in effluent-dominant rivers whose dilution capacity and self-purifying processes are insufficient to temper the risk to aquatic life (Kasprzyk-Horder et al., 2009).

Although much research has been conducted on this topic, studies have generally been limited to single treatment plants. Hence, in order to provide an overview of the findings, a full litereature review is set out, collating data pertaining to 264 WWTPs from various global locations, mostly in Europe. Reflecting the abundance of conventional activated sludge systems (CAS) among existing municipal WWTPs, 244 of them were considered in this study, the remaining 20 plants examined were membrane biological reactors (MBR), included for comparative purposes. Data pertaining to a wide spectrum of PhCs, 118 compounds belonging to 17 different classes distinguished by their function or biological activity, were considered: 23 analgesics/anti-inflammatories, 36 antibiotics, 1 antidiabetic, 1 antifungal, 3 antihypertensives, 1 barbiturate, 12 beta-blockers, 2 diuretics, 9 lipid regulators, 10 psychiatric drugs, 6 receptor antagonists, 4 hormones, 4 beta-agonists, 3 antineoplastics, 1 topical product, 1 antiseptic and 1 contrast agent.

Raw influent and secondary effluent concentrations for the 118 PhCs, and their removal efficiencies observed in CAS and MBRs were reported, the objective being to provide a snapshot of their occurrence and of the efficacy of suspended growth mass biological processes in their removal. Based on the collected data, the average daily mass load (mg/1000 inhabitants/ day) in the secondary effluent for the majority of the compounds under study has been evaluated, ranking them accordingly. The PhCs were then also ranked according to their environmental risk, using a quotient derived from the ratio between their measured concentrations in secondary effluents and their corresponding

PNEC. This strategy provides an overview of the situation, clearly identifying a group of compounds in need of more intensive monitoring further to safeguarding the environment.

Compounds are grouped according to their therapeutic class and presented in terms of their chemical formula and molecular weight; literature references are also provided for each (Table 2.2.). In addition, in the Appendix A.1., their main physical and chemical properties (protonation constant as pK_a, octanol-water partition coefficient as Log K_{ow}, solubility S_w, sludge-water distribution coefficient as Log K_d, reaction rate constant k_{biol}, molecular charge at pH 7) as well as their molecular structure are provided. The main features of the WWTPs investigated in each study and details of the corresponding experimental campaigns are compiled in Table 2.3. Through the last column of Table 2.2., it is possible to know the previous works investigating the substance under study and then, once known the cited work, through Table 2.3. to know the details of the experimental campaign and the characteristics of the WWTPs under consideration.

Based on the collected literature data, the variability ranges for the concentration of each examined compound in both raw urban influent has been defined (Figures 2.3.-2.8. and Appendix A.2.) and secondary effluent (Figures 2.9.-2.14. and Appendix A.3.), as well as for their corresponding removal efficiencies (Figures 2.16.-2.20. and Appendix A.4.). To complete the analysis of literature data, the percentage partitions, for some of the compounds under study, among biodegradation, sorption onto sludge and occurrence in the secondary effluent are provided (Table 2.4.) as well as removal efficiencies for the different selected PhCs with respect to the sludge retention time of the corresponding biological reactor (referring to CAS in Table 2.5. and MBR in Table 2.6.).

Subsequently, the average daily mass discharged from the secondary biological system was evaluated, where possible, for the examined compounds, and their corresponding risk quotients (average concentration/PNEC) in the secondary effluent (Figures 2.21. and 2.22.). As a whole, the results of these two analyses revealed the most critical compounds in terms of mass load and/or environmental risk.

2.8.1 Mostly Investigated PhCs

Table 2.2. reports the list of the investigated contaminants, grouped according to their therapeutic class, in addition to their molecular weight (MW) and chemical formula, together with the number and details of the references reviewed. The majority of the

compounds mentioned in the various studies are administrated orally, intramuscularly, endovenously or by inhalation, and in few cases on the skin.

An analysis of the data compiled in Appendix A.1., referring to selected PhCs evidences their very different molecular structures, also in terms of basic or acidic functional groups (charge at pH = 7). These, if found on the same molecule (e.g. ciprofloxacin), can cause it to be neutral, cationic, anionic or zwitterionic under different environmental conditions, (Kümmerer, 2009a; Ternes and Joss, 2006) resulting in (very) different behaviours during treatment processes as it will be discussed later.

2.8.2 Main features of the mostly investigated wastewater treatment plants (WWTPs)

Table 2.3. lists the main features of the WWTPs investigated in each study (second column), as well as the details of the experimental campaigns (sampling mode, number of samples, observation period, number of investigated PhCs). 244 CAS systems (242 full-scale and 2 pilot plants) and 20 MBRs (all pilot plants) situated in various world locations were included in this study: 68 % of the WWTPs are situated in European countries (Spain, Germany, Italy, Switzerland, Sweden, Austria, UK, Finland, France, Greece and Denmark), 14 % in the Americas (USA, Canada and Brazil), 14 % in Asia (China, Japan, South Korea and North Korea) and 4 % in Australia.

The raw wastewaters influent to these plants are generally subjected to preliminary treatments (bar screening and grit removal), then primary sedimentation followed by the secondary biomass growth treatment (CAS or MBR, the majority of the latter equipped with ultrafiltration or, in a few cases microfiltration, membranes). This final step usually included denitrification-nitrification and carbon removal processes, and in some cases simultaneous precipitation of phosphate by the addition of Fe salts. CAS operates at a HRT ranging from 2-24 h and at a SRT generally equal to 2-20 d with some exceptions, while MBR at a HRT of 7-15 h (with few exceptions) and at a SRT equal to 15-80 d (with a few exceptions). Figure 2.2. shows the historical development of the activated sludge process: from CAS for BOD removal to MBR and MBBR for enhancing the quality of the final effluent and upgrading the existing CAS maintaining the same footprint or reducing it.

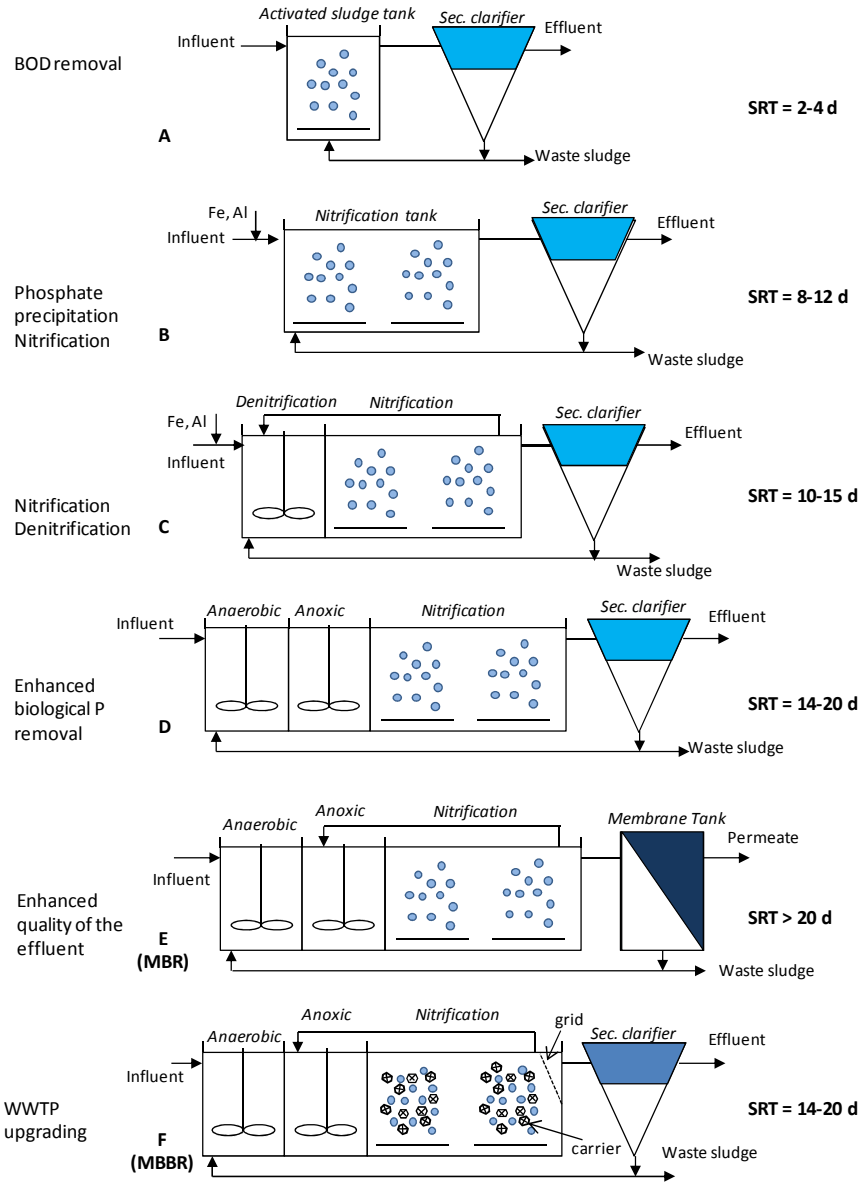


Figure 2.2. Historical development of the activated sludge process: from CAS for BOD removal to MBR and MBBR for enhancing the quality of the final effluent and upgrading the existing CAS maintaining the same footprint or reducing it.

In general, chemical analysis of PhCs was performed on 24-h composite water samples, quite often flow-proportional, thereby avoiding the risk of under- or over-estimating the average daily concentrations in the wastewater. Experimental investigations were mainly based on a number of samples ranging between 3 and 12. Few studies collected multiple data sets for each sampling point. Water samples were generally taken in dry days in order to avoid dilution of the influent in case of combine sewage and due to parasite streams and dilution of the effluent caused by washout of the biological tanks.

Table 2.2. Pharmaceutical compounds examined, grouped according to their therapeutical class. For each substance, chemical formula and molecular weight (MW) are provided as well as number of papers and references dealing with it.

Therapeutic class	Pharmaceutical compound	MW	Chemical formula	Number of papers	References
Analgesics/ Anti-inflammatories **A**	5-aminosalicylic acid	153	C7H7NO3	1	Kasprzyk-Hordern et al., 2009
	Acetaminophen	151	C8H9NO2	15	Choi et al., 2008; Coetsier et al., 2009; Foster, 2007; Gómez et al., 2007; Jones et al., 2007; Kasprzyk-Hordern et al., 2009; Khan and Ongerth, 2005; Kim et al., 2007; Radjenovic et al., 2007, 2009; Roberts and Thomas, 2006; Rosal et al., 2010; Snyder et al., 2006; Ternes, 1998; Yu et al., 2006
	Acetylsalicylic acid	180	C9H8O4	2	Kasprzyk-Hordern et al., 2009; Ternes, 1998
	Aminopyrine	231	C13H17N3O	2	Andreozzi et al., 2003; Ternes, 1998
	Codeine	299	C18H21NO3	5	Foster, 2007; Gómez et al., 2007; Kasprzyk-Hordern et al.,2009; Rosal et al., 2010; Wick et al., 2009
	Dextropropoxyphene	339	C22H29NO2	1	Roberts and Thomas, 2006
	Diclofenac	296	C14H11Cl2NO2	36	Andreozzi et al., 2003; Bendz et al., 2005; Bernhard et al., 2006; Clara et al., 2004, 2005a, 2005b; Coetsier et al., 2009; Gómez et al., 2007; Kasprzyk-Hordern et al., 2009; Kim et al., 2007; Kimura et al., 2005, 2007; Kreuzinger et al., 2004; Lindqvist et al., 2005; Lishman et al., 2006; Muñoz et al., 2009; Paxéus, 2004; Quintana et al., 2005; Radjenovic et al., 2007, 2009; Reif et al., 2008; Roberts and Thomas, 2006; Rosal et al., 2010; Santos et al., 2007, 2009; Snyder et al., 2006; Stumpf et al., 1999; Suárez et al., 2005; Tauxe-Wuersch et al., 2005; Ternes et al., 2003; Ternes, 1998; Thomas and Foster, 2005; Vieno et al., 2005; Weigel et al., 2004; Yu et al., 2006; Zorita et al., 2009
	Dipyrone	333	C13H16N3NaO4S	1	Gómez et al., 2007
	Fenoprofen	242	C15H14O3	6	Andreozzi et al., 2003; Bendz et al., 2005; Coetsier et al., 2009; Lishman et al., 2006; Nakada et al., 2006; Ternes, 1998
	Flurbiprofen	244	C15H13FO2	2	Andreozzi et al., 2003; Bendz et al., 2005
	Hydrocodone	299	C18H21NO3	1	Snyder et al., 2006
	Ibuprofen	206	C13H18O2	43	Andreozzi et al., 2003; Bendz et al., 2005; Bernhard et al., 2006; Carballa et al., 2004, 2005; Castiglioni et al., 2006; Clara et al., 2004, 2005a, 2005b; Coetsier et al., 2009; Gómez et al., 2007; Jones et al., 2007; Kasprzyk-Hordern et al., 2009; Khan and Ongerth, 2005; Kim et al., 2007; Kimura et al., 2005, 2007; Kreuzinger et al., 2004; Lindqvist et al., 2005; Lishman et al., 2006; Muñoz et al., 2009; Nakada et al., 2006; Paxéus, 2004; Quintana et al., 2005; Radjenovic et al., 2007, 2009; Reif et al., 2008; Roberts and Thomas, 2006; Rodriguez et al., 2003; Rosal et al., 2010; Santos

Therapeutic class	Pharmaceutical compound	MW	Chemical formula	Number of papers	References
					et al., 2007, 2009; Snyder et al., 2006; Stumpf et al., 1999; Suárez et al., 2005; Tauxe-Wuersch et al., 2005; Ternes et al., 2003; Ternes, 1998; Thomas and Foster, 2005; Vieno et al., 2005; Weigel et al., 2004; Yu et al., 2006; Zorita et al., 2009
	Indomethacin	358	C19H16ClNO4	8	Bendz et al., 2005; Lishman et al., 2006, Radjenovic et al., 2007, 2009; Rosal et al., 2010; Stumpf et al., 1999; Ternes et al., 2003; Ternes, 1998
	Ketoprofen	254	C16H14O3	21	Andreozzi et al., 2003; Bendz et al., 2005; Kasprzyk-Hordern et al., 2009; Khan and Ongerth, 2005; Kimura et al., 2005, 2007; Lindqvist et al., 2005; Lishman et al., 2006; Nakada et al., 2006; Quintana et al., 2005; Radjenovic et al., 2007, 2009; Rosal et al., 2010; Santos et al., 2007, 2009; Stumpf et al., 1999; Tauxe-Wuersch et al., 2005; Ternes, 1998; Thomas and Foster, 2005; Vieno et al., 2005; Yu et al., 2006
	Ketorolac	255	C15H13NO3	1	Rosal et al., 2010
	Meclofenamic acid	296	C14H11Cl2NO2	1	Ternes, 1998
	Mefenamic acid	241	C15H15NO2	9	Jones et al., 2007; Kasprzyk-Hordern et al., 2009; Kimura et al., 2005, 2007; Radjenovic et al., 2007, 2009; Roberts and Thomas, 2006; Rosal et al., 2010; Tauxe-Wuersch et al., 2005
	Naproxen	230	C14H14O3	30	Andreozzi et al., 2003; Bendz et al., 2005; Carballa et al., 2004, 2005; Kasprzyk-Hordern et al., 2009; Khan and Ongerth, 2005; Kim et al., 2007; Kimura et al., 2005, 2007; Lindqvist et al., 2005; Lishman et al., 2006; Nakada et al., 2006; Paxéus, 2004; Quintana et al., 2005; Radjenovic et al., 2007, 2009; Reif et al., 2008; Rodriguez et al., 2003; Rosal et al., 2010; Santos et al., 2007, 2009; Snyder et al., 2006; Stumpf et al., 1999; Suárez et al., 2005; Ternes, 1998; Ternes et al., 2003; Thomas and Foster, 2005; Vieno et al., 2005; Yu et al., 2006; Zorita et al., 2009
	Phenazone	188	C11H12N2O	3	Andreozzi et al., 2003; Rosal et al., 2010; Ternes, 1998
	Propyphenazone	230	C14H18N2O	3	Nakada et al., 2006; Radjenovic et al., 2007, 2009
	Salicylic acid	138	C7H6O3	4	Kasprzyk-Hordern et al., 2009; Khan and Ongerth, 2005; Lishman et al., 2006; Ternes, 1998
	Tolfenamic acid	262	C14H12ClO2	1	Ternes, 1998
	Tramadol	263	C16H25NO2	2	Kasprzyk-Hordern et al, 2009; Wick et al., 2009
Antibiotics **B**	Amoxicillin	365	C16H19N3O5S	1	Watkinson et al., 2007
	Azithromycin	749	C38H72N2O12	4	Ghosh et al., 2009; Göbel et al., 2005, 2007; Yasojima et al., 2006
	Cefaclor	368	C15H14ClN3O4S	1	Watkinson et al., 2007
	Cefalexin	347	C16H17N3O4S	4	Costanzo et al., 2005; Gulkowska et al., 2008; Li and Zhang, 2011; Watkinson et al., 2007

Therapeutic class	Pharmaceutical compound	MW	Chemical formula	Number of papers	References
	Cefotaxime	456	C16H17N5O7S2	2	Gulkowska et al., 2008; Li and Zhang, 2011
	Chloramphenicol	323	C11H12Cl2N2O5	3	Kasprzyk-Hordern et al., 2009; Li and Zhang, 2011; Peng et al., 2006
	Chlortetracycline	479	C22H23ClN2O8	2	Li and Zhang, 2011; Watkinson et al., 2007
	Ciprofloxacin	331	C17H18FN3O3	15	Andreozzi et al., 2003; Baumgarten et al., 2007; Castiglioni et al., 2006; Costanzo et al., 2005; Ghosh et al., 2009; Golet et al., 2003; Karthikeyan and Meyer, 2006; Li and Zhang, 2011; Lindberg et al., 2005, 2006; Muñoz et al., 2009; Rosal et al., 2010; Vieno et al., 2007; Watkinson et al., 2007; Zorita et al., 2009
	Clarithromycin	748	C38H69NO13	7	Castiglioni et al., 2006; Ghosh et al., 2009; Göbel et al., 2005, 2007; Sahar et al., 2011; Ternes et al., 2003; Yasojima et al., 2006
	Clindamycin	425	C18H33ClN2O5S	1	Watkinson et al., 2007
	Cloxacillin	436	C19H18ClN3O5S	1	Watkinson et al., 2007
	Doxycycline	463	C22H24N2O8	2	Lindberg et al., 2005; Watkinson et al., 2007
	Enoxacin	320	C15H17FN4O3	1	Andreozzi et al., 2003
	Enrofloxacin	359	C19H22FN3O3	3	Baumgarten et al., 2007; Ghosh et al., 2009; Watkinson et al., 2007
	Erythromycin	734	C37H67NO13	19	Castiglioni et al., 2006; Göbel et al., 2005, 2007; Gulkowska et al., 2008; Karthikeyan and Meyer, 2006; Kasprzyk-Hordern et al., 2009; Kim et al., 2007; Li and Zhang, 2011; Muñoz et al., 2009; Radjenovic et al., 2007, 2009; Reif et al., 2008; Roberts and Thomas, 2006; Rosal et al., 2010; Sahar et al., 2011; Snyder et al., 2006; Ternes et al., 2003; Watkinson et al., 2007; Xu et al., 2007
	Lincomycin	407	C18H34N2O6S	3	Castiglioni et al., 2006; Ghosh et al., 2009; Watkinson et al., 2007
	Lomefloxacin	351	C17H19F2N3O3	1	Andreozzi et al., 2003
	Metronidazole	171	C6H9N3O3	2	Kasprzyk-Hordern et al., 2009; Rosal et al., 2010
	Norfloxacin	319	C16H18FN3O3	12	Andreozzi et al., 2003; Coetsier et al., 2009; Costanzo et al., 2005; Ghosh et al., 2009; Golet et al., 2003; Gulkowska et al., 2008; Li and Zhang, 2011; Lindberg et al., 2005, 2006; Watkinson et al., 2007; Xu et al., 2007; Zorita et al., 2009
	Ofloxacin	361	C18H20FN3O4	12	Andreozzi et al., 2003; Brown et al., 2006; Castiglioni et al., 2006; Li and Zhang, 2011; Lindberg et al., 2005; Peng et al., 2006; Radjenovic et al., 2007, 2009; Rosal et al., 2010; Vieno et al., 2007; Xu et al., 2007; Zorita et al., 2009
	Oxytetracycline	460	C22H24N2O9	2	Li and Zhang, 2011; Watkinson et al., 2007
	Penicillin G	334	C16H18N2O4S	2	Gulkowska et al., 2008; Watkinson et al., 2007
	Penicillin V	350	C16H18N2O5S	1	Watkinson et al., 2007
	Roxithromycin	837	C41H76N2O15	12	Clara et al., 2005b; Ghosh et al., 2009; Göbel et al., 2005, 2007; Kreuzinger et al.,

Therapeutic class	Pharmaceutical compound	MW	Chemical formula	Number of papers	References
					2004; Li and Zhang, 2011; Reif et al., 2008; Ruel et al., 2010; Sahar et al., 2011; Ternes et al., 2003, Watkinson et al., 2007; Xu et al., 2007
	Spiramycin	843	C43H74N2O14	1	Castiglioni et al., 2006
	Sulfachloropyridazine	285	C10H9ClN4O2S	1	Choi et al., 2008
	Sulfadiazine	250	C10H10N4O2S	3	Li and Zhang, 2011; García-Galán et al., 2011; Peng et al., 2006
	Sulfadimethoxine	310	C12H14N4O4S	3	Choi et al., 2008; García-Galán et al., 2011; Ghosh et al., 2009
	Sulfamethazine	278	C12H14N4O2S	4	García-Galán et al., 2011, Karthikeyan and Meyer, 2006; Li and Zhang, 2011; Sahar et al., 2011
	Sulfamethoxazole	253	C10H11N3O3S	31	Andreozzi et al., 2003; Bendz et al., 2005; Brown et al., 2006; Carballa et al., 2004, 2005; Castiglioni et al., 2006; Choi et al., 2008; Clara et al., 2005b; Foster, 2007; García-Galán et al., 2011; Ghosh et al., 2009; Göbel et al., 2005, 2007; Karthikeyan and Meyer, 2006; Kasprzyk-Hordern et al., 2009; Kim et al., 2007; Kreuzinger et al., 2004; Li and Zhang, 2011; Lindberg et al., 2005; Muñoz et al., 2009; Peng et al., 2006; Radjenovic et al., 2007, 2009; Reif et al., 2008; Rosal et al., 2010; Ruel et al., 2010; Sahar et al., 2011; Snyder et al., 2006; Ternes et al., 2003; Watkinson et al., 2007; Xu et al., 2007
	Sulfapyridine	249	C11H11N3O2S	4	García-Galán et al., 2011; Göbel et al., 2005, 2007; Kasprzyk-Hordern et al., 2009
	Sulfasalazine	398	C18H14N4O5S	2	Kasprzyk-Hordern et al., 2009; Watkinson et al., 2007
	Sulfathiazole	255	C9H9N3O2S2	3	Choi et al., 2008; García-Galán et al., 2011; Watkinson et al., 2007
	Tetracycline	444	C22H24N2O8	5	Ghosh et al., 2009; Gulkowska et al., 2008; Li and Zhang, 2011; Karthikeyan and Meyer, 2006; Watkinson et al., 2007
	Trimethoprim	290	C14H18N4O3	25	Andreozzi et al., 2003; Batt 2006; Bendz et al., 2005; Brown et al., 2006; Foster, 2007; Ghosh et al., 2009; Göbel et al., 2005, 2007; Gulkowska et al., 2008; Karthikeyan and Meyer, 2006; Kasprzyk-Hordern et al., 2009; Choi et al., 2008; Kim et al., 2007; Li and Zhang, 2011; Lindberg et al., 2005, 2006; Paxéus, 2004; Radjenovic et al., 2009; Reif et al., 2008; Roberts and Thomas, 2006; Rosal et al., 2006; Sahar et al., 2011; Snyder et al., 2006; Ternes et al., 2003; Watkinson et al., 2007
	Tylosin	916	C46H77NO17	1	Watkinson et al., 2007
Antidiabetics **C**	Glibenclamide	494	C23H28ClN3O5S	1	Radjenovic et al., 2007, 2009
Antifungals **D**	Clotrimazole	345	C22H17ClN2	1	Roberts and Thomas, 2006
Antihypertensives	Diltiazem	415	C22H26N2O4S	3	Choi et al., 2008 Foster, 2007; Kasprzyk-Hordern et al., 2009;

Therapeutic class	Pharmaceutical compound	MW	Chemical formula	Number of papers	References
E	Enalapril	377	C20H28N2O5	1	Castiglioni et al., 2006
	Hydrochlorothiazide	298	C7H8ClN3O4S2	5	Castiglioni et al., 2006; Muñoz et al., 2009; Radjenovic et al., 2007, 2009; Rosal et al., 2010;
Barbiturates **F**	Phenobarbital	232	C12H12N2O3	1	Yu et al., 2006
Beta-blockers **G**	Acebutolol	336	C18H28N2O4	2	Andreozzi et al., 2003; Vieno et al., 2007
	Atenolol	266	C14H22N2O3	14	Alder et al., 2010; Bendz et al., 2005; Carucci et al., 2006; Castiglioni et al., 2006; Kasprzyk-Hordern et al., 2009; Maurer et al., 2007; Muñoz et al., 2009; Paxéus, 2004; Radjenovic et al., 2007, 2009; Rosal et al., 2010; Ternes et al., 2003; Vieno et al., 2007; Wick et al., 2009
	Betaxolol	307	C18H29NO3	3	Andreozzi et al., 2003; Ternes, 1998; Wick et al., 2009
	Bisoprolol	325	C18H31NO4	2	Ternes, 1998; Wick et al., 2009
	Carazolol	298	C18H22N2O2	1	Ternes, 1998
	Celiprolol	379	C20H33N3O4	2	Ternes et al., 2003; Wick et al., 2009
	Metoprolol	267	C15H25NO3	12	Alder et al., 2010; Andreozzi et al., 2003; Kasprzyk-Hordern et al., 2009; Maurer et al., 2007; Paxéus, 2004; Radjenovic et al., 2007, 2009; Rosal et al., 2010; Ternes, 1998; Ternes et al., 2003; Vieno et al., 2007; Wick et al., 2009
	Nadolol	309	C17H27NO4	1	Ternes, 1998
	Oxprenolol	265	C15H23NO3	1	Andreozzi et al., 2003
	Propranolol	259	C16H21NO2	12	Alder et al., 2010; Andreozzi et al., 2003; Bendz et al., 2005; Coetsier et al., 2009; Kasprzyk-Hordern et al., 2009; Maurer et al., 2007; Radjenovic et al., 2009; Roberts and Thomas, 2006; Rosal et al., 2010; Ternes, 1998; Ternes et al., 2003; Wick et al., 2009
	Sotalol	272	C12H20N2O3S	6	Alder et al., 2010; Maurer et al., 2007; Radjenovic et al., 2009; Ternes et al., 2003; Vieno et al., 2007; Wick et al., 2009
	Timolol	316	C13H24N4O3S	1	Ternes, 1998
Diuretics **H**	Bendroflumethiazide	421	C15H14F3N3O4S2	1	Kasprzyk-Hordern et al., 2009
	Furosemide	331	C12H11ClN2O5S	3	Castiglioni et al., 2006; Kasprzyk-Hordern et al., 2009; Rosal et al., 2010
Lipid regulators **I**	Bezafibrate	362	C19H20ClNO4	15	Andreozzi et al., 2003; Castiglioni et al., 2006; Clara et al., 2004, 2005a, 2005b; Kasprzyk-Hordern et al., 2009; Kreuzinger et al., 2004; Lindqvist et al., 2005; Quintana et al., 2005; Radjenovic et al., 2007, 2009; Rosal et al., 2010; Stumpf et al., 1999; Ternes, 1998; Vieno et al., 2005
	Clofibrate	243	C12H15ClO3	2	Andreozzi et al., 2003; Ternes, 1998

Therapeutic class	Pharmaceutical compound	MW	Chemical formula	Number of papers	References
	Clofibric acid	215	C10H11O3Cl	16	Andreozzi et al., 2003;Bendz et al., 2005; Bernhard et al., 2006; Kasprzyk-Hordern et al., 2009;Kimura et al., 2005, 2007; Lishman et al., 2006; Radjenovic et al., 2007;Roberts and Thomas, 2006; Rosal et al., 2010;Stumpf et al., 1999; Tauxe-Wuersch et al., 2005;Ternes, 1998; Ternes et al., 2003; Weigel et al., 2004; Zorita et al., 2009
	Etofibrate	364	C18H18ClNO5	1	Ternes, 1998
	Fenofibrate	361	C20H21ClO4	3	Andreozzi et al., 2003; Lishman et al., 2006;Ternes, 1998
	Fenofibric acid	319	C17H15ClO4	5	Muñoz et al., 2009; Rosal et al., 2010;Stumpf et al., 1999; Ternes, 1998; Ternes et al., 2003
	Gemfibrozil	250	C15H22O3	14	Andreozzi et al., 2003; Bendz et al., 2005; Khan and Ongerth, 2005;Kim et al., 2007; Lishman et al., 2006; Muñoz et al., 2009;Paxéus, 2004;Radjenovic et al., 2007, 2009;Rosal et al., 2010; Snyder et al., 2006; Stumpf et al., 1999; Ternes, 1998;Yu et al., 2006
	Pravastatin	425	C23H36O7	4	Coetsier et al., 2009; Kasprzyk-Hordern et al., 2009; Radjenovic et al., 2007, 2009
	Simvastatin	419	C25H38O5	1	Kasprzyk-Hordern et al., 2009
Psychiatric drugs **J**	Amitriptyline	277	C20H23N	1	Kasprzyk-Hordern et al., 2009
	Carbamazepine	236	C15H12N2O	31	Andreozzi et al., 2003;Bendz et al., 2005; Bernhard et al., 2006; Castiglioni et al., 2006; Clara et al., 2004, 2005a, 2005b; Conti et al., 2011; Coetsier et al 2009;Foster, 2007; Gómez et al., 2007;Kasprzyk-Hordern et al., 2009; Khan and Ongerth, 2005; Choi et al., 2008; Kim et al., 2007;Kreuzinger et al., 2004; Muñoz et al., 2009; Nakada et al., 2006; Paxéus, 2004;Radjenovic et al., 2007, 2009; Reif et al., 2008; Rosal et al., 2010;Santos et al., 2007, 2009;Snyder et al., 2006; Suárez et al., 2005; Ternes, 1998;Ternes et al., 2003; Vieno et al., 2007; Wick et al, 2009
	Diazepam	285	C16H13ClN2O	6	Clara et al., 2005b;Kreuzinger et al., 2004; Reif et al., 2008;Suárez et al., 2005;Ternes, 1998; Wick et al., 2009
	Fluoxetine	309	C17H18F3NO	8	Foster, 2007; Kim et al., 2007; Metcalfe et al., 2010; Muñoz et al., 2009; Radjenovic et al., 2009; Rosal et al., 2010; Snyder et al., 2006; Zorita et al., 2009
	Gabapentin	171	C9H17N1O2	2	Kasprzyk-Hordern et al., 2009; Yu et al., 2006
	Lorazepam	321	C15H10Cl2O2N2	1	Coetsier et al., 2009
	Norfluoxetine	295	C16H16F3NO	2	Metcalfe et al., 2010; Zorita et al., 2009
	Oxcarbazepine	252	C15H12N2O2	1	Conti et al., 2011
	Paroxetine	329	C19H20FNO3	2	Metcalfe et al., 2010; Radjenovic et al., 2007
	Valproic acid	144	C8H16O2	1	Yu et al., 2006
Receptor antagonists	Cimetidine	252	C10H16N6S	2	Choi et al., 2008; Kasprzyk-Hordern et al., 2009;
	Famotidine	337	C8H15N7O2S3	1	Radjenovic et al., 2009

Therapeutic class	Pharmaceutical compound	MW	Chemical formula	Number of papers	References
K	Loratadine	383	C22H23ClN2O2	1	Radjenovic et al., 2009
	Omeprazole		C17H19N3O3S	1	Rosal et al., 2010
	Ranitidine	314	C13H22N4O3S	6	Carucci et al., 2006; Castiglioni et al., 2006; Kasprzyk-Hordern et al., 2009; Radjenovic et al., 2007, 2009; Rosal et al., 2010
	Valsartan	436	C24H29N5O3	1	Kasprzyk-Hordern et al., 2009
Hormones **L**	Estradiol	272	C18H24O2	11	Andersen et al., 2003; Baronti et al., 2000; Carballa et al., 2004, 2005; Clara et al., 2005a; Foster, 2007; Joss et al., 2004; Kim et al., 2007; Lishman et al., 2006; Ternes et al., 1999a; Zorita et al., 2009
	Estriol	288	C18H24O3	4	Baronti et al., 2000; Clara et al., 2005a;Kim et al, 2007; Nakada et al., 2006
	Estrone	270	C18H22O2	12	Andersen et al., 2003; Baronti et al., 2000; Carballa et al., 2004, 2005; Clara et al., 2005a; Joss et al., 2004; Kim et al., 2007; Lishman et al., 2006; Nakada et al., 2006; Ternes et al., 1999a, 2003; Zorita et al., 2009
	Ethinylestradiol	296	C20H24O2	10	Andersen et al., 2003; Baronti et al., 2000; Clara et al., 2004, 2005a; Foster, 2007; Joss et al., 2004; Kim et al., 2007; Kreuzinger et al., 2004; Ternes et al., 1999; Zorita et al., 2009
Beta-agonists **M**	Clenbuterol	277	C12H18Cl2N2O	1	Ternes, 1998
	Fenoterol	303	C17H21NO4	1	Ternes, 1998
	Salbutamol	239	C13H21NO3	4	Castiglioni et al., 2006; Jones et al., 2007; Kasprzyk-Hordern et al., 2009; Ternes, 1998
	Terbutaline	226	C12H19NO3	1	Ternes, 1998
Antineoplastics **N**	Cyclophosphamide	261	C7H15Cl2N2O2P	1	Ternes, 1998
	Ifosfamide	261	C7H15Cl2N2O2P	3	Coetsier et al., 2009; Kümmerer et al., 1997; Ternes, 1998
	Tamoxifen	372	C26H29NO	2	Coetsier et al., 2009; Roberts and Thomas, 2006
Topical products **O**	Crotamitron	203	C13H17NO	1	Nakada et al., 2006
Antiseptics **P**	Triclosan	290	C12H7Cl3O2	13	Foster, 2007; Gómez et al., 2007; Kim et al., 2007; McAvoy et al., 2002; Muñoz et al., 2009; Nakada et al., 2006; Paxéus, 2004; Rosal et al., 2010; Ruel et al., 2010; Snyder et al., 2006; Thomas and Foster, 2005; Weigel et al., 2004; Yu et al., 2006
Contrast media **Q**	Iopromide	791	C18H24I3N3O8	5	Batt et al., 2006; Carballa et al., 2004; Clara et al., 2005b; Kim et al., 2007; Kreuzinger et al., 2004

Collected data report the pharmaceutical concentrations in raw urban wastewaters and in the corresponding treated biological effluent, as well as the global removal efficiencies achieved after the secondary treatment. The urban wastewater considered includes both the effluent produced by domestic users and that from (small) industrial activities, which, according to the local regulation, may be discharged into the public sewer network and conveyed to the municipal WWTP.

Experimental investigations were carried out at different times of the year, and the overall data therefore covers periods characterized by higher and lower PhC consumption, enabling this study to provide a balanced overview, bolstered by taking into account the different consumption habits in the different countries worldwide.

2.8.3 Quality assurance of literature data

As reported by the EC Technical Guidance Document on risk assessment (EC, 2003) and as remarked by many Authors (among them Liebig et al., 2006; Ternes and Joss, 2006), it is vital that the quality of literature data is assured. For this reason, to be included in the present study, references had to feature a description of the analytical methodology used for the assessment of measured concentrations and the quality assurance program adopted for sampling, analysis and elaboration. In particular, they provide the following information: list of analytes, solvents and chemicals used; details of sampling, transport and storage in addition to sample volume; analytical methods adopted, including pH adjustment, filtration and filter material, extraction and solvent evaporation techniques; derivatization and detection method; surrogate and/or instrumental standards used; methods and limits of quantification, recovery measurements, procedural and instrumental blanks used; sampling conditions, location, frequency and period and compartment characteristics.

Table 2.3 Main characteristics of the treatment plants and monitoring campaigns included in the litereature review.

	References	Details of treatment plants and experimental investigations
1	Alder et al., 2010	24-h flow-proportional composite samples were taken at the influent and effluent of a conventional WWTP of Niederglatt, Switzerland (33 000 inhabitants, 16800 m^3/d) and processed for four beta-blockers: atenolol, metoprolol, propanolol and sotalol. The plant includes nitrification-denitrification stages. Collected data refer to influent and effluent concentrations, average removal rates as well as average mass loads for each of the selected compounds.
2	Andersen et al., 2003	24-h flow-proportional composite samples were taken at the influent and effluent of a conventional WWTP in Wiesbaden, Germany (300 000 population equivalent, pe) and processed for three oestrogens (n = 2). The plant includes pretreatments (screening, aerated grit removal), primary clarification and activated sludge systems for biological and chemical phosphate removal, denitrification and nitrification. SRT is roughly 11-13 d.
3	Andreozzi et al., 2003	Grab samples and 24-h composite samples were taken between February and March 2001 at the inlet and outlet of the secondary treatment step of five CAS systems, treating domestic and industrial wastewaters, in different countries (Greece, Italy and Sweden). They serve populations ranging from 6000 to 900 000 inhabitants. All plants featured a primary settling phase and one a chemical phosphorus removal step. 26 PhCs were investigated.
4	Baronti et al., 2000	24-h composite samples of the influent and secondary effluent of six CAS systems in the area of Rome, Italy, were collected once a month over five months (n = 5) and processed for four oestrogens. The plants have flow rates ranging between 10 000 and 734 000 m^3/d and HRT in the range 12-14 h. They serve populations ranging between 40 000 and 1 200 000 inhabitants.
5	Batt et al., 2006	24-h flow-proportional composite samples were taken at the inlet and the outlet of the WWTP located in Amherst, NY. Samples were collected once a week for three consecutive weeks (n=3), in 2006, and processed for iopromide and trimethoprim. The plant includes a primary clarifier and a two-stage secondary biological process (slurry system). Stage 1 is a CAS for substrate removal with HRT 1 h and SRT 6 d . Stage 2 is a CAS designed for nitrogen removal with HRT 2 h and SRT 49 d.
6	Baumgarten et al., 2007	An investigation was carried out on an MBR pilot plant in order to evaluate the removal efficiencies of target pharmaceuticals during MBR treatment as well as to compare them with those obtained with simultaneously addition in the bioreactor of powdered activated carbon (PAC). Average elimination efficiencies are provided for some common antibiotics (in particular ciprofloxacin and enrofloxacin).
7	Bendz et al., 2005	24-h flow-proportional and composite samples were taken at the inlet and secondary effluent of the Kallby WWTP (Sweden) in October 2002 (n = 1) and processed for 14 PhCs.
8	Bernhard et al., 2006	The investigation carried out at the WWTP of Wiesbaden, Germany, receiving domestic (90 %) and industrial (10 %) wastewater, with a capacity equal to 282 000 pe. The plant consists of a grit removal tank, a clarification tank, a CAS for carbon and nitrogen removal (HRT = 22 h), a final clarification tank and microscreen. Moreover, a pilot submerged-MBR equipped with microfiltration membranes (pore size 0.4 μm) was installed and fed with preclarified water (HRT = 7-10 h). 24-h composite water samples (n = 10-11) were taken at the influent, the MBR permeate and the WWTP effluent between July 2004 and March 2005. Average removal rates for the two

	References	Details of treatment plants and experimental investigations
		investigated systems were provided for 4 PhCs (diclofenac, ibuprofen, clofibric acid and carbamazepine). In addition, the concentrations of diclofenac are also provided at the three sampling points .
9	Brown et al., 2006	48-h composite samples from the urban influent and the secondary effluent of the Albuquerque WWTP in New Mexico were taken and processed for 3 PhCs.
10	Carballa et al., 2004	24-h composite samples were taken at the inlet and outlet of Galicia municipal WWTP (Spain) in October 2001, and in January and April 2002, and analysed for 6 PhCs. The plant has a capacity of 100 000 p.e. and consists of preliminary treatments (coarse and fine screening and aerated chambers for grit and fat removal), primary sedimentation and CAS (HRT 24 h).
11	Carballa et al., 2005	24-h composite samples were taken at the influent and secondary effluent of the WWTP in Galicia (Spain, 100 000 inhabitants) in October 2001, January 2002, April 2002 and June 2002 and processed for five PhCs. The plant consists of preliminary treatment (fine screening, aerated chambre for grit and fat removal), primary sedimentation and CAS (mixed reactors followed by sedimentation tank). Average removal rates are provided for the selected compounds.
12	Carucci et al., 2006	The investigation refers to a 2-L lab-scale SBR, working through six 4-h cycles each day, SRT 8-14 d, using the activated sludge system coming from municipal WWTP as inoculum and municipal wastewater as feed. Average removal rates are provided for ranitidine and atenolol.
13	Castiglioni et al., 2006	Six Italian large WWTPs were monitored for 16 PhCs during Winter (January-March 2004) and Summer (June-September 2004). All investigated plants are equipped with pre-treatments, primary sedimentation and CAS. 24-h composite samples were collected at the inlet and the outlet of each plant, and their average removal rates are provided.
14	Choi et al., 2008	Grab samples (n = 3) were taken between April and August 2005 at the influent and secondary effluent of four large municipal WWTPs within Seoul city boundary (Korea) and analysed for 9 PhCs.
15	Clara et al., 2004	24-h composite samples were taken at the influent and the effluent of a CAS system in the South East of Austria (7000 pe, SRT 52-237 d) and in a pilot MBR (10-56 d, ultrafiltration membranes) during three monthly experimental campaigns in 2002. They were processed for 5 PhCs.
16	Clara et al., 2005a	24-h composite samples of influent and the secondary effluent of four full-scale CAS plants (SRTs: 2 d, 19 d, 48 d and 42 d) and a pilot MBR plant (SRT: 22-82) in Austria. Corresponding design capacities are 2.5 10^6 pe, 167 10^3 pe, 135 10^3 pe, 6 10^3 pe, and 50 pe. Mean average concentrations were provided for 8 PhCs.
17	Clara et al., 2005b	Three urban CAS WWTPs and one pilot MBR plant, equipped with ultrafiltration membranes, were monitored in the South East of Austria. 24-h composite samples were taken at the inlet and outlet of each plant and analysed for 8 PhCs. The corresponding SRTs are: 52-114 d (CAS 1), 2 d (CAS 2) and 46 (CAS 3) and 10-55 d (MBR).
18	Coetsier et al., 2009	24-h averaged flow-proportional samples were collected (n=8) between June 2007 and February 2008 at the effluent of the WWTP of Alès in France (90 000 pe). The plant consists of a CAS system with extended aeration and simultaneous phosphorus precipitation.
19	Conti et al., 2011	24-h flow-proportional samples were taken at the inlet of the large conventional WWTP in Pavia, Italy (160 000 inhabitants, HRT = 4 h) and processed for carbamazepine and oxcarbamazepine.
20	Costanzo et	Samples were taken (n = 2) at the influent and effluent of a CAS in Brisbane (Australia) and

	References	Details of treatment plants and experimental investigations
	al., 2005	processed for three antibiotics (ciprofloxacin, norfloxacin and cephalexin).
21	Foster 2007	Grab samples were taken at the raw influent and secondary effluent of the municipal WWTP of San Marco, Texas (USA) during periods of normal operation from October 2006 to March 2007. The plant includes preliminary treatments (screening, degritting), primary clarification and CAS. Average concentrations and variability ranges were provided for 10 PhCs.
22	García-Galán et al., 2011	Collected data refer to the removal efficiencies observed for selected sulphonamide antibiotics in three municipal wastewater treatment plants in Spain, along the Ebro river basin. The three WWTPs consist of primary treatments followed by a conventional activated sludge system. HRT and SRT were respectively 10 h and 4 d for the first plant, 10 h and 6 d for the second one, 24-46 h and 19 d for the third one.
23	Ghosh et al., 2009	Samples were collected at the influent and secondary effluent of four medium–large capacity CAS systems in Japan (flow rate: 576 000 m^3/d, 9500 m^3/d, 50 000 $m^{3/}$d, 57 000 m^3/d; SRT: 16-19 d, 13 d, 17 d, 14-18 d and HRT: 9.5-12 h, 14 h, 11 h, 2.8-5.5 h). Average influent concentrations and average removal rates are reported for 11 antibiotics.
24	Göbel et al., 2005	24-h composite samples were taken at the influent and effluent of two conventional municipal WWTPs in Switzerland (55 0000 pe and 80 000 pe) and processed for 7 antibiotics between March 2002 and November 2003. The plants consist of preliminary treatments (screening and aerated gritting), primary clarification and nitrification-denitrification steps.
25	Göbel et al., 2007	Two full-scale CAS systems (55 000 pe, HRT =15 h, SRT =10-12 d; and 80 000 pe, HRT = 31 h and SRT = 21-25 d, respectively) and one pilot MBR (100 pe, SRT = 16-80 d) were investigated in Switzerland in order to compare their capacity to remove 7 selected antibiotics. CASs include denitrification and nitrification tanks, and the MBR consists of a cascade of stirred anaerobic, anoxic, aerobic compartments. 24-h flow-proportional composite samples were taken three times at each sampling point in each of the three experimental campaigns (March 2002, February 2003 and November 2003, n= 9). Only percentage removal rates are provided.
26	Golet et al., 2003	24-h flow-proportional composite water samples were taken at the influent and secondary effluent of the largest urban WWTP in Zurich (600 000 pe), Switzerland and analysed for 2 antibiotics, ciprofloxacin and norfloxacin (n = 7), in October 2000. The plant consists of pretreatments (screening, gritting and primary clarification) and CAS steps (predenitrification-nitrification-secondary clarifier; HRT = 20 h and SRT = 11 d).
27	Gómez et al., 2007	The inlet and the outlet of the municipal CAS system in Almeria (Spain, 62 000 inhabitants) were monitored during July 2003 and April 2004. Ten 24-h composite water samples and 12 discrete samples (monthly) were analysed for 7 PhCs.
28	Gulkowska et al., 2008	Grab samples at the inlet and secondary effluent of two large CAS systems in Hong Kong operating at different HRTs (16 h and 21 h) but the same SRT (20 d) were processed for 7 antibiotics in December 2006.
29	Jones et al., 2007	Grab samples were taken every 6 hours at the inlet and outlet of a municipal CAS plant (150 000 pe) in southern England during the four dry investigation days in June 2004. The plant consists of preliminary treatments (screening, gritting), primary clarification and biological treatment (nitrification-denitrification), operating at a SRT of 13 d and HRT of 13.5 h. Average removal rates are provided for 4 selected PhCs (ibuprofen, acetaminophen, salbutamol and mefenamic acid).

	References	Details of treatment plants and experimental investigations
30	Joss et al., 2004	An experimental investigation was carried out in November 2002 at the conventional WWTP of Kloten (Switzerland) where a pilot-scale MBR was installed in parallel with the conventional WWTP of Altenrhein (Switzerland). The Kloten plant serves 55 000 pe and includes primary treatments (screening, aerated grit and primary clarifier), secondary treatments (denitrification, nitrification and simultaneous phosphorus removal with Fe^{+3}); its SRT is about 10-12 d. The MBR is a 100-pe pilot plant fed with primary effluent from the Kloten plant and equipped with stirred anaerobic and anoxic tanks followed by an aerobic filtration compartment, operating at SRT 30 d. Microfiltration and ultrafiltration membranes were tested. 24-h composite flow-proportional samples were taken at the influent and effluent of each plant and processed for 3 compounds (n =6).
31	Karthikeyan et al., 2006	24-h composite samples were collected from the inlet and the outlet of two WWTPs in the USA (serving 73 000 and 150 000 inhabitants) and processed for 6 PhCs (n = 2) in October 2001 and December 2002.
32	Kasprzyk-Hordern et al., 2009	24 h composite samples (n = 10) of urban influent and secondary effluent of the Coslech WWTP (UK) (flow rate range between 150–300 L/s) during the period April-August 2007. 35 compounds were investigated and their removal rates evaluated in the CAS plant deployed as an extended aeration/oxidation ditch for carbon and nitrogen removal.
33	Khan and Ongerth 2005	24-h composite samples were taken at the influent and the effluent of the municipal WWTP located in the outer western suburbs of Sidney, Australia (23 000 inhabitants). The plant consists of preliminary and primary treatments followed by a CAS system with additional phosphorus removal. Seven compounds were monitored over five week-days.
34	Kim et al., 2007	The influent and the secondary effluent of six South Korean urban CAS systems were sampled for 15 PhCs between 2004-2005 .
35	Kimura et al., 2005	Samples were taken at the inlet and the outlet of a full-scale CAS system and two pilot MBRs to compare the removal rates of 6 PhCs. The two pilot plants were equipped with hollow-fibre microfiltration membranes and fed by raw (the same feeding the full-scale plant) and pretreated (pre-coagulated/clarified) municipal wastewater, respectively. In both MBRs HRT was 9 h, in CAS, HRT was 13 h.
36	Kimura et al., 2007	Grab samples (n = 11) were taken at the influent and outlet of 1 full-scale CAS (Soseigawa, Japan, 125 000 m^3/d, HRT = 12 h and SRT =7 d) and two MBRs (equipped with hollow fiber microfiltration membranes, fed by the same influent as the conventional treatment plant and operating at the same flow rate = 0.624 m^3/d and HRT = 6.7 h but at different SRT: 15 d and 65 d) between August-November 2005. 6 compounds were monitored.
37	Kreuzinger et al., 2004	Samples were taken at the inlet and secondary effluent of two full-scale Austrian CAS systems and at a pilot MBR plant (equipped with ultrafiltration membranes) operating at different SRTs: 9.6 d and 96 d for the full-scale plants, 20 and 41 for the MBR over a period of 7-14 days. Average removal rates are given for 9 selected PhCs.
38	Kümmerer 1997	8-h composite samples were taken from the influent to a WWTP in Forchheim (Germany) between January and April 1995 (n = 7) and processed for ifosfamide.
39	Li and Zhang, 2011	Removal efficiencies for selected antibiotics were investigated in two conventional Chinese WWTPs: Shatin, 600 000 inhabitants served and Stanley 27 000 inhabitants served. The two systems include an anoxic-aerobic activated sludge process, the first is characterized by HRT of 10 h and SRT of 12 d, while the second by a RT of 17 h and SRT of 7 d.

	References	Details of treatment plants and experimental investigations
40	Lindberg et al., 2005	Fourteen 24-hour flow-proportional composite samples were taken at the inlet and outlet of four Swedish conventional WWTPs in August 2002 and February 2003 and analysed for 6 antibiotics. The plants receive municipal and industrial wastewaters and have a capacity ranging from 50 000 and 644 000 inhabitants. HRTs are: 8 h, 11 h, 16 h and 24 h and corresponding SRTs are: 20 d, 22 d, 11 d and 15 d. Each plant consists of preliminary treatments (screening, sand and fat removal, chemical phosphorus removal, primary clarification) followed by a CAS system. For three out of the four plants, nitrogen removal is also performed.
41	Lindberg et al., 2006	24-h composite samples of the influent and secondary effluent of the municipal WWTP of Umea, Sweden were investigated in the period November–December 2004. The influent is mechanically (3-mm split screen) and chemically (flocculation-precipitation) pretreated. Its HRT is 8 h and SRT 20 d; 3 antibiotics were monitored.
42	Lindqvist et al., 2005	24-h composite samples of the influent and secondary effluent of seven full-scale CAS systems in Finland were taken in September 2003 and processed for 5 PhCs. Four of the CAS systems used a denitrification-nitrification process for nitrogen removal and all of them feature a simultaneous biological treatment for removal of P.
43	Lishman et al., 2006	24-h composite samples were taken at the influent and secondary effluent of 7 CAS systems in Canada. The investigation lasted between October and December 2002 and monitored 12 PhCs.
44	Maurer t al., 2007	24-h composite samples were taken during a 3-day study period at the inlet and the outlet of two CAS systems (including nitrification-denitrification) near Zurich, Switzerland, and processed for 4 beta-blockers. The first plant has a capacity of 50 000 inhabitants, an HRT of 6.6 h and an SRT of 8-10 d. The second serves a population of about 36 000 inhabitants, operates at an HRT of 18 h and at an SRT of 14 d.
45	McAvoy et al., 2002	24-h flow-proportional composite samples (n = 2) were taken at the inlet and the outlet of one CAS plant in Loveland (27 000 p.e., 12 000 m^3/d, HRT = 6 h) in the USA. They were processed for triclosan in November 1997.
46	Metcalfe et al., 2010	24-h composite water samples were collected at the influent and secondary outlet of a WWTP, in Southern Ontario, serving a population of approximately 69 000 using conventional activated sludge system and tertiary treatment followed by UV disinfection. The WWTP consists of two parallel trains (HRT = 11.9 h in both lines and SRT of 8.1 d and 10.4 d).
47	Muñoz et al., 2009	Samples were taken at the outlet of two large WWTPs in Spain: El Ejido (64 0000 inhabitants) and Alcalá (375 000 inhabitants) and processed for 12 PhCs. The plants include coarse-solid and grease removal, primary settling and anoxic-aerobic biological treatment with activated sludge for C and N removal.
48	Nakada et al., 2006	24-h composite samples (*n* =16) of the influent and secondary effluent of five conventional activated sludge plants serving populations ranging from 464 000 to 2 020 000 inhabitants (HRT from 7.1 to 9.4 h and SRT from 3.8 to 8.4 d) in Tokyo, Japan, from December 2001 and February 2003. 10 PhCs from different classes were investigated.
49	Paxéus, 2004	24-h composite flow-proportional and grab samples were taken at the inlet and secondary effluent of 10 different full-scale CAS systems processing domestic and industrial wastewater in different European countries. All feature primary settling followed by CAS. Investigations were carried out between February 2001 and March 2003 on 9 PhCs (n = 2-10). Effluent average concentrations are provided for each compound for all plant and average removal rates where possible.

	References	Details of treatment plants and experimental investigations
50	Peng et al., 2006	Grab samples were taken at the influent and effluent of Guangzhou conventional WWTP (China, 195 000 pe) and processed for 4 antimicrobials (sulfadiazine, sulfamethoxazole, ofloxacin and chloramphenicol). Average influent and effluent concentrations were provided.
51	Quintana et al., 2005	24-h composite samples (n = 7) were taken at the inlet and outlet of a pilot MBR plant (HRT = 8.8-10 d; SRT = 37 d) equipped with Kubota plate membranes (0.4 μm) and fed by municipal wastewater in Germany. Average influent and effluent concentrations and average removal rates were provided for 5 PhCs (diclofenac, ketoprofen, bezafibrate, naproxen, ibuprofen) monitored between January and April 2004.
52	Radjenovic et al., 2007	24-h composite water samples were taken at the inlet and the outlet of the municipal CAS system in Rubi (Spain, 125 000 pe) and in a pilot MBR fed in parallel. Pretreatments consist of screening, gritting and primary sedimentation. Biological system includes denitrification-nitrification sedimentation and has SRT 3 d and HRT 12 h. MBR was equipped with Kubota flat sheet microfiltration membranes (0.4 μm) operating at HRT 14 h and "infinite" SRT (as no sludge was discharged from the reactor during the investigation period, May–June 2005). 22 selected PhCs were monitored, and their range of variability in the influent and the removal achieved by CAS and MBR were reported.
53	Radjenovic et al., 2009	24-h flow-proportional composite samples (n = 9) were taken at the influent and secondary effluent of the municipal conventional WWTP in Terrassa (Barcelona, Spain) and at the effluent of two pilot MBR plans fed in parallel after preliminary treatments and primary clarification. The full-scale plant serves 277 000 pe and has an average flow rate 42 000 m^3/d, SRT 10 d and HRT 11.5 h. It consists of preliminary treatment (grit and sand removal), primary clarification and aeration, followed by secondary clarification. The first pilot plant is equipped with hollow-fibre ultra-filtration membranes (nominal porosity 0.05 μm) and operates at HRT 7.2 h. The second features micro-filtration flat-sheet membranes (nominal porosity 0.4 μm) and operates at HRT 15 h. Variability ranges and average influent concentrations of 26 PhCs and their corresponding removal rate are given; data was collected between March and April 2007.
54	Reif et al., 2008	The investigation carried out on a pilot MBR plant equipped with submerged hollow-fibre membrane module (0.04 μm) fed by synthetic water simulating domestic sewage. Its HRT is 12-24 h and its SRT 44-72 d. Influent and permeate concentrations were sampled and processed for 9 PhCs.
55	Roberts and Thomas, 2006	24-h composite samples were taken at the influent and the effluent of Howdon WWTP (230 000 m^3/d) (UK) consisting of screening, primary clarification and CAS (SRT = 2.4 d, HRT = 12.5 h). 11 PhCs were investigated, and average concentrations at the two sampling points and average removal rates are provided.
56	Rodriguez et al., 2003	24-h composite samples were taken at the influent and effluent of a municipal WWTP in Spain (serving 100 000 inhabitants) and processed for ibuprofen and naproxen between October 2001 and February 2002. Their influent and effluent concentrations as well as their average removal rates are reported.
57	Rosal et al., 2010	The influent and secondary effluent of the 10 000 pe WWTP of Alcalà (Spain) was monitored every month over a year. The plant featured a traditional A2O multistage configuration with nitrification-denitrification and enhanced simultaneously phosphorus removal. 30 PhCs were monitored.

	References	Details of treatment plants and experimental investigations
58	Ruel et al., 2010	24-h composite water samples were taken at the influent and effluent of 6 different CAS plants (SRT range 13–26) in France and processed for 3 PhCs: the antibiotics roxythromycin and sulfamethoxazole and the antiseptic triclosan.
59	Sahar et al., 2011	Water samples were taken at the raw influent of one municipal WWTP in Tel Aviv (Israel) and at the inlet and outlet of a municipal WWTP in Berlin (Germany) and processed for 6 antibiotics. The Berlin plant consists of a conventional CAS (HRT = 24 h, SRT = 9-15 d) and an MBR (HRT = 15 h; SRT > 70 d, equipped with submerged non-woven flat sheet pillow membranes (10 μm). Variability ranges and average concentrations of the influents are provided, together with the average removal rates measured in the Berlin CAS and pilot MBR.
60	Santos et al., 2007	24-h flow-proportional composite samples (n = 21) were taken at the inlet and secondary effluent of four urban full-scale CAS systems in Spain. 5 PhCs were analysed for 21 days between July and September 2004. The plants have nominal capacity ranges between 20 000 and 950 000 pe, HRT between 12 and 17 h, and SRT between 1.5-5 d.
61	Santos et al., 2009	24-h flow-proportional composite samples (n = 63) were taken at the inlet and secondary effluent of two CAS systems in Spain between June 2004 and June 2005 and processed for 5 PhCs. Their design capacities are 350 000 pe and 950 000 pe, the corresponding operating conditions: HRT 12 h and 17 h and SRT 1.5 d and 2.7 d.
62	Snyder et al., 2006	The investigation refers to a pilot MBR equipped with ultrafiltration membranes (nominal pore size 0.08 μm) fed by primary effluent. 12 selected PhCs were monitored at the influent of the WWTP and at the MBR permeate.
63	Stumpf et al., 1999	24-h composite samples were taken at the inlet and the outlet of one CAS system in Rio de Janeiro (Brazil) during June 1997 (n = 6) and processed for 9 PhCs (anti-inflammatories and lipid regulators).
64	Suárez et al., 2005	Water samples were taken at the inlet and the outlet of a pilot CAS system and processed for 5 common PhCs of different therapeutic classes. The plant operated at SRT = 60 d and HRT = 1 d. It includes a denitrification-nitrification sequence.
65	Tauxe-Wuerch et al., 2005	24-h flow-proportional composite water samples were taken (n ranging between 4 and 7) at the inlet and outlet of three CAS systems in Berne (Switzerland, 23000 inhabitants, 9300 m^3/d), Morges (Switzerland, 29000 inhabitants, 8500 m^3/d) and Lausanne (Switzerland, 220 000 inhabitants, 100200 m^3/d). Each plant consists of a screen and sand trap, fat separator, primary clarifier and biological activated sludge reactor with simultaneous phosphorus chemical precipitation, and secondary clarifier. Variability ranges and average influent and effluent concentrations and average removal rates are provided for 5 PhCs.
66	Ternes et al., 1999	24-h flow-proportional composite samples (n=6) were taken at the influent and effluent of two CAS systems in Frankfurt Main (German) and Penha Rio de Janeiro (Brazil) in 1997 and processed for 3 oestrogens (estrone, 17β-estradiol, 17α-ethinylestradiol). In addition in the same periods, effluents of 16 municipal German WWTPs and 10 Canadian WWTPs were also investigated for the same PhCs.
67	Ternes et al., 2003	The effluent of a conventional municipal WWTP (380 000 pe) was monitored (n = 6) and analysed for 18 PhCs. The plant consists of mechanical pretreatment, followed by nitrification-denitrification, biological phosphate removal and secondary clarification.

	References	Details of treatment plants and experimental investigations
68	Ternes, 1998	24-h composite samples were taken at the inlet and outlet of a full scale conventional WWTP near Frankfurt (312 000 pe, preliminary clarification, followed by aerator tank and addition of Fe(II)chloride for phosphate removal and final clarification) over a period of six days covering 5 weeks in different periods between May 1996 and November 1997. Average removal rates are provided for 14 PhCs. 49 full-scale municipal treatment plants (all containing preliminary treatment, aeration tank and final clarification steps; 43 plants are equipped with phosphate removal, 25 plants with nitrification, and 13 denitrification steps) were also investigated between November 1995 and November 1997, and average effluent concentrations were provided for 35 PhCs. .
69	Thomas and Foster, 2005	24-h flow-and time integrated composite samples were collected at the influent and the secondary outlet of the urban WWTP in Arlington, VA, USA (194 000 served population) and processed for four analgesics/anti-inflammatories and one antiseptic. The same compounds were monitored in grab samples withdrawn at the influent and outlet of other two urban WWTPs (City of Alexandria Sanitation Authority and Noman M Cole Water Pollution Control Plant , serving a population of 375 000 and 500 000 respectively). Each WWTP consists of preliminary treatments (bar screens and grit removal), primary settling, conventional activated sludge/biological nutrient removal. In addition phosphorus precipitation, gravity filtration and disinfection are included.
70	Vieno et al., 2005	24-h composite samples were taken at the influent and effluent of Aura municipal WWTP (Finland) in four days between September 2003 and March 2004. The WWTP is a ditch oxidation tank, consisting of an activated sludge compartment (SRT 20 d and HRT 36 h) with simultaneous phosphorus precipitation by adding ferric salt. Average concentrations of 5 selected PhCs (bezafibrate, diclofenac, ketoprofen, naproxen and ibuprofen) were provided for the two sampling points.
71	Vieno et al., 2007	24-h composite samples were taken at the inlet and outlet of 9 full-scale conventional municipal plants (SRT range 2-15 d and HRT range 7-20 h) in Finland between 2004 and 2005 and processed for 7 common PhCs.
72	Watkinson et al., 2007	The urban influent and secondary effluent of a large CAS system (140 000 m^3/d) in Brisbane, Australia, were monitored for the 22 most commonly administered PhCs (n = 5). Bioreactor HRT was 11 h and SRT 12.5 d. Pretreatments consisted of screening, gritting and primary settling.
73	Weigel et al., 2006	Samples were taken at the influent and effluent of Hamburg WWTP (Germany) in November 2002, and processed for 4 PhCs (ibuprofen, diclofenac, clofibric acid, triclosan).
74	Wick et al, 2009	48-h and 72 h-composite samples were collected from the inlet and outlet of a German municipal WWTP in (1 350 000 pe) on 7 days in March 2007, May 2007 and July 2007 (n=9). The WWTP consists of a cascade of two CAS units operating under aerobic (HRT = 1 h and SRT = 0.5 d) and anoxic-aerobic conditions (HRT =5 h and SRT =18 d), respectively. Pretreatments include screen, aerated grit-removal tank and primary clarifier. The second biological step includes simultaneous phosphate precipitation. 11 PhCs (beta-blockers and psychiatric drugs) were monitored.
75	Xu et al., 2007	24-h composite water samples were taken at the inlet and effluent of the CAS system in New Territory (Hong Kong) and processed for 5 PhCs (n = 6). The plant serves 300 000 inhabitants and operates at HRT = 15-22 h and SRT = 5.6-8.2 d. It consists of preliminary treatments (screening, aerated gritting), primary clarifier and biological treatment, including denitrification-nitrification sequence. Sampling and analysis were performed in October 2005.

	References	Details of treatment plants and experimental investigations
76	Yasojima et al., 2006	24-hour flow-proportional composite samples were taken at the inlet and the outlet of six full-scale CAS systems in Japan and processed for two antimicrobials (clarythromycin and azythromycin). Their HRT range between 4 and12 h and their SRT 5-9 d.
77	Yu et al., 2006	24-h composite samples were taken at the inlet and the effluent of the Baltimore WWTP that receives about $8.5x10^5$ m^3/d of residential and urban wastewaters. The plant is a CAS system (SRT = 8-10 d) designed for biological nutrient removal. 10 between pharmaceuticals and antiseptics were monitored.
78	Zorita et al., 2009	24-h composite samples were collected from the inlet and outlet of the municipal WWTP in Kristianstad (Sweden, 150 000 inhabitants, HRT range 24 –40 h and SRT roughly 8 d) in June 2007 and April 2008 (n = 3) and processed for 12 PhCs. Pretreatments include screening, aerated grit removal and primary sedimentation, the biological section includes denitrification-nitrification.

2.9 Occurrence of PhCs in raw urban wastewaters (UWWs)

Literature data referring to the concentrations of PhCs, grouped in alphabetic order in their therapeutic classes, in the raw influent to a municipal WWTP are reported in Figures 2.3.-2.8. The average of the considered data is shown in brackets after the name of each compound on the X-axis. Influent data was not available for some compounds, for example the analgesic aminopyrine, but these are nevertheless included in the graphs as data referring to their secondary effluent concentrations and/or removal efficiencies were available.

Referring to Fig. 2.3., the variability of analgesics/anti-inflammatories was found to range between 0.0016 and 373 μg/L. The most commonly investigated compounds were ibuprofen, diclofenac, naproxen and ketoprofen. Ibuprofen was the compound with the highest registered absolute influent concentration (373 μg/L), followed by acetaminophen (246 μg/L), tramadol (86 μg/L) and naproxen (53 μg/L). Acetaminophen and ibuprofen also had the highest average influent concentrations (respectively 38 μg/L and 37 μg/L), followed by tramadol (32 μg/L).

As to Fig. 2.4., the range of variability of antibiotic concentrations was between 0.001 and 32 μg/L. The most commonly investigated compounds were trimethoprim, sulfamethoxazole, erythromycin and ciprofloxacin. The highest absolute concentrations were found for ofloxacin (32 μg/L), roxithromycin (17 μg/L) and ciprofloxacin (14 μg/L). Other antibiotics exhibiting measured concentrations greater than 10 μg/L are: sulfapyridine (12.4 μg/L), trimethoprim (10.5 μg/L) and erythromycin (10.2 μg/L). The highest average antibiotic concentrations were found for ofloxacin and sulfadiazine (5.1

µg/L), followed by sulfapyridine (3.3 µg/L) and cefalexim (3.2 µg/L). No data were provided for enoxacin, lomefloxacin and spiramycin concentrations in the raw urban wastewater.

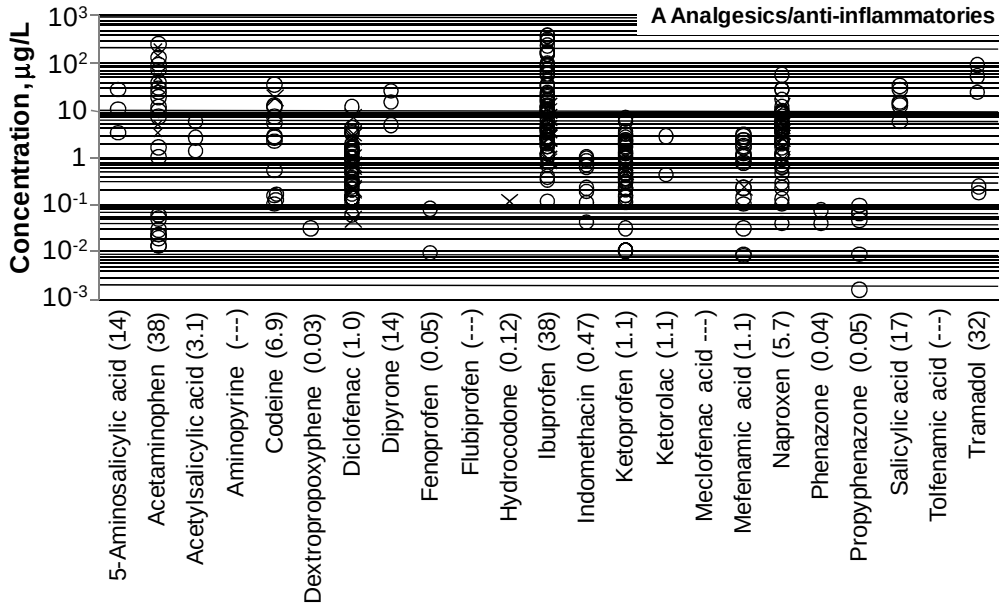


Figure 2.3. Concentration of selected analgesics/anti-inflammatories measured in the raw influent to municipal WWTP (o refers to CAS and x to MBR) and corresponding average values (in brackets).

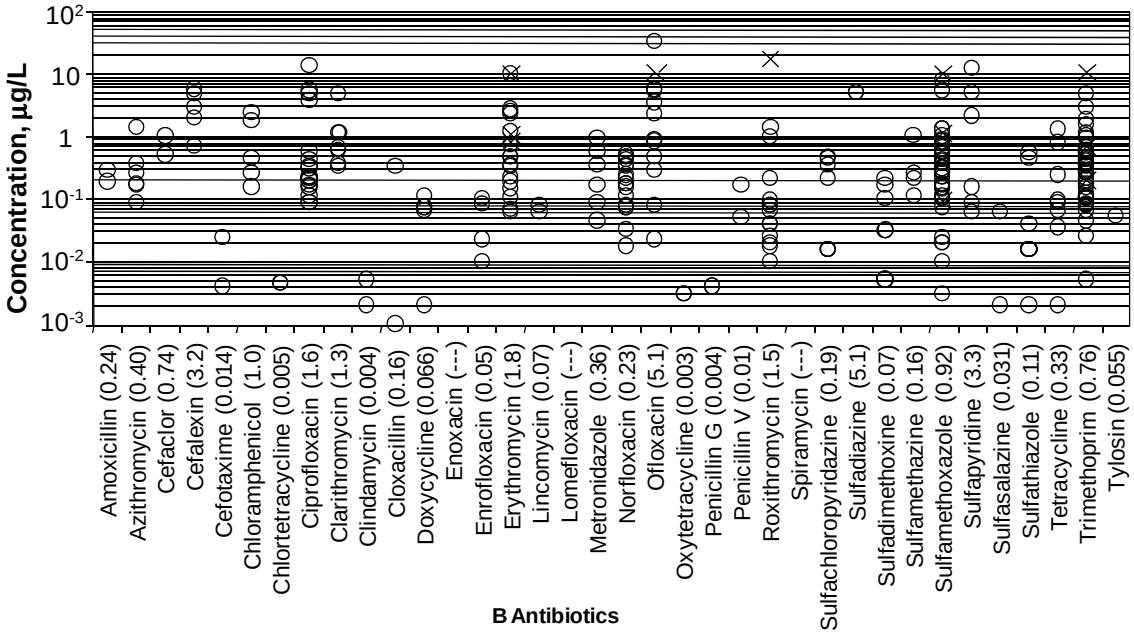


Figure 2.4. Concentrations of selected antibiotics measured in the raw influent to municipal WWTPs (o refers to CAS and x to MBR) and corresponding average values (in brackets).

Fig. 2.5. reports data for six, less investigated, classes, comprising 20 compounds; indeed, only one antifungal, barbiturate and antidiabetic were reported. The observed ranges of variability were: 0.12-16 μg/L for antidiabetics, 0.0025-10 μg/L for antihypertensives, 0.006-25 for beta-blockers, 0.004-6 for diuretics. The single values found for the antifungal and barbiturate were respectively: 0.029 μg/L (clotrimazole) and 0.07 μg/L (phenobarbital). The highest concentrations were found for the beta-blocker atenolol (25 μg/L), followed by the antidiabetic glibenclamide (16 μg/L) and the antihypertensive hydrochlorothiazide (10 μg/L). The highest average concentrations were found for glibenclamide (8.7 μg/L), followed by atenolol (4.5 μg/L), hydrochlorotiazide (3.9 μg/L) and furosemide (2.4 μg/L).

Raw urban wastewater concentration data were unavailable for five out of the 12 beta-blockers and the antihypertensive enalapril. The data spread within the observed variability range was the greatest for diltiazem, another antihypertensive.

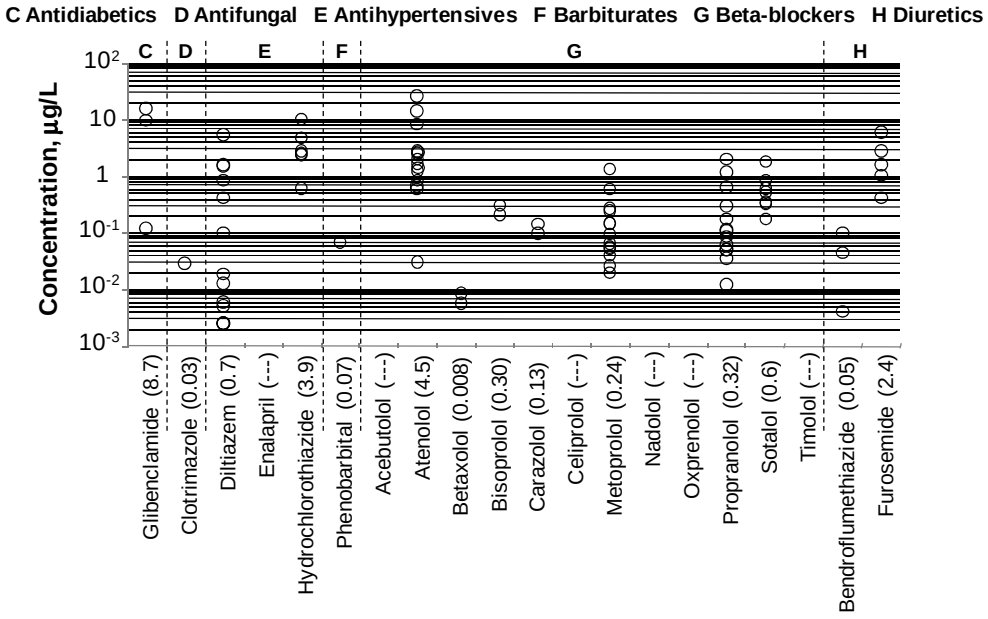


Figure 2.5. Concentrations of selected PhCs belonging to six therapeutic classes measured in the raw influent to municipal WWTPs (o refers to CAS and x to MBR) and corresponding average values (in brackets).

Referring to Fig.2.6., the variability for the selected lipid regulators was found to range between 0.001 and 30 μg/L, and for psychiatric drugs between 0.0025 and 25 μg/L. In the former class, the most commonly detected compounds were bezafibrate, gemfibrozil and clofibric acid, in the second one carbamazepine and fluoxetine. The highest absolute concentrations were found for bezafibrate (30 μg/L), gabapentin (25 μg/L), diazepam (23

µg/L), carbamazepine (22 µg/L) and gemfibrozil (17 µg/L), whereas the highest average concentrations were found for diazepam (22 µg/L), gabapentin (13 µg/L), bezafibrate (3.5 µg/L) and amitriptyline (3.1 µg/L). Only one datum are present for paroxetine (0.0016 µg/L) as well as for valproic acid (0.0014 µg/L). Data are not available for the lipid regulators clofibrate, etofibrate and fenofibrate, or for the psychiatric drug lorazepam.

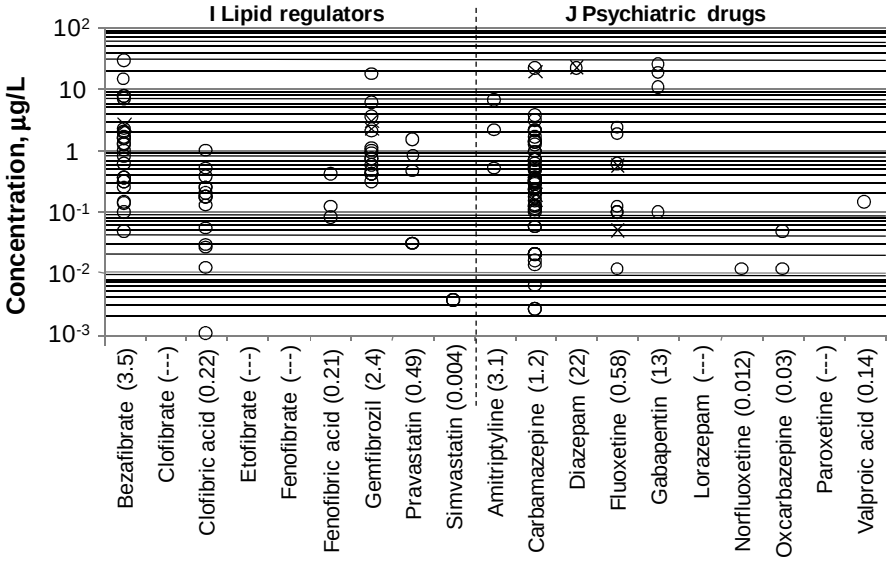


Figure 2.6. Concentrations of selected lipid regulators and psychiatric drugs measured in the raw influent to municipal WWTPs (o refers to CAS and x to MBR) and corresponding average values (in brackets).

As to Fig. 2.7., the variability range of selected receptor antagonists was between 0.014 and 11 µg/L, and that of hormones between 0.002-3 µg/L. The most frequently detected compounds were the four hormones (estrone, estradiol, ethinylestradiol and estriol) and cimetidine. The highest absolute concentrations were found for ranitidine (11 µg/L) and cimetidine (10 µg/L), while the highest average values were found for cimetidine (4.1 µg/L), ranitidine (2.7 µg/L) and valsartan (2.5 µg/L). Among the four hormones included in this study, the estradiol presented the highest absolute concentration (3 µg/L) as well as the highest average observed value (0.25 µg/L).

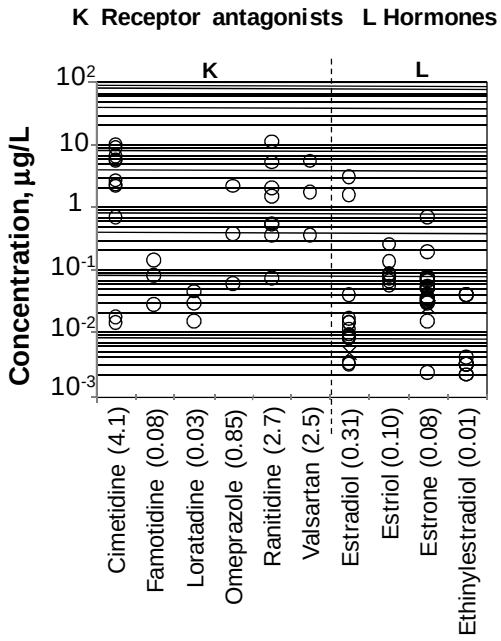


Figure 2.7. Concentrations of selected receptor antagonists and hormones measured in the raw influent to municipal WWTPs (o refers to CAS and x to MBR) and corresponding average values (in brackets).

Fig. 2.8. reports data pertaining to 5 classes, three of which (topical products, antiseptics and contrast media) feature only one investigated compound. Out of the four beta-agonists under review, only one (salbutamol) exhibits values of influent concentrations and, out of the three antineoplastics, only two compounds were found (ifosfamide and tamoxifen). The observed ranges of variability are: 0.05-0.15 µg/L for beta-agonists, 0.019-0.36 µg/L for antineoplastics, 0.38-3 µg/L for the topical product crotamiton, 0.22-7 µg/L for the antiseptic triclosan and 0.01-6.6 µg/L for the contrast agent iopromide. The highest absolute concentrations were found for triclosan (7 µg/L) and iopromide (6.6 µg/L). The highest average concentrations were found for iopromide (2.2 µg/L) and triclosan (1.9 µg/L).

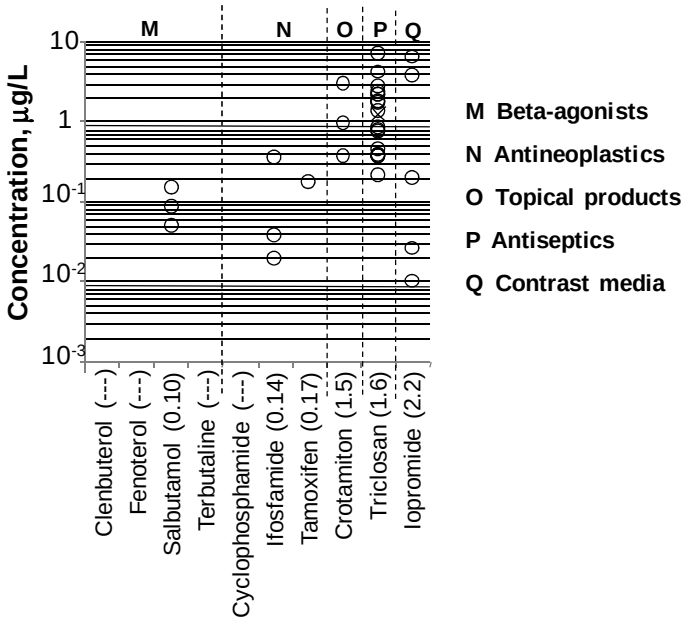


Figure 2.8. Concentrations of other classes of micropollutants measured in the raw influent to municipal WWTPs (o refers to CAS and x to MBR) and their corresponding average values (in brackets).

2.10 Occurrence of PhCs in secondary biological effluents

Figures 2.9.-2.14. refer to the concentrations of PhCs detected in the effluent of the WWTPs included in this study. As reported in Table 2.3., these generally consist of preliminary treatments (bar screening and grit removal), primary sedimentation and secondary biological suspended mass reactor, i.e. CAS (with different configurations, quite often including an anoxic-aerobic reactor and sometimes with a simultaneous precipitation of phosphate), followed by a secondary settler or an advanced MBR with anoxic-aerobic compartments. As reported above, in the X-axis of the figures 2.9.-2.14., average concentrations are reported alongside each compound in brackets.

Referring to Fig. 2.9., concentrations of analgesics/anti-inflammatories in the secondary effluent ranged between 0.001 and 57 µg/L. The most frequently detected compounds were ibuprofen, diclofenac, naproxen, ketoprofen and acetaminophen. The highest absolute concentrations were found for tramadol (57 µg/L), ibuprofen (48 µg/L) and diclofenac (11 µg/L), and the highest average values were found for tramadol (20 µg/L), dipyrone (4.9) and ibuprofen (3.6 µg/L).

Fig. 2.10. shows that, the range of variability for selected antibiotics in the secondary effluent was 0.001-6.7 µg/L. The most investigated compounds were trimethoprim, sulfamethoxazole, erythromycin, ciprofloxacin and norfloxacin. The highest

absolute concentrations were found for trimethoprim (6.7 μg/L), erythromycin (6.3 μg/L), ciprofloxacin (5.7 μg/L), sulfamethoxazole and roxithromycin (5 μg/L), while the highest average values were found for ciprofloxacin (0.86 μg/L), erythromycin (0.73 μg/L), roxithromycin (0.50 μg/L) and ofloxacin (0.45 μg/L).

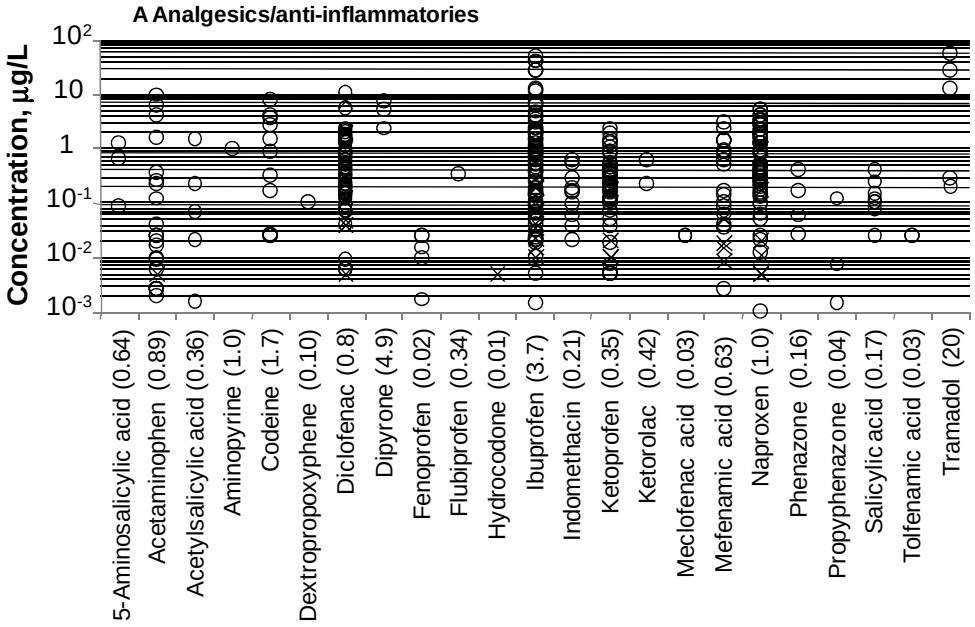


Figure 2.9. Concentration of selected analgesics/anti-inflammatories measured in the secondary effluent (o refers to CAS and x to MBR) and corresponding average values (in brackets).

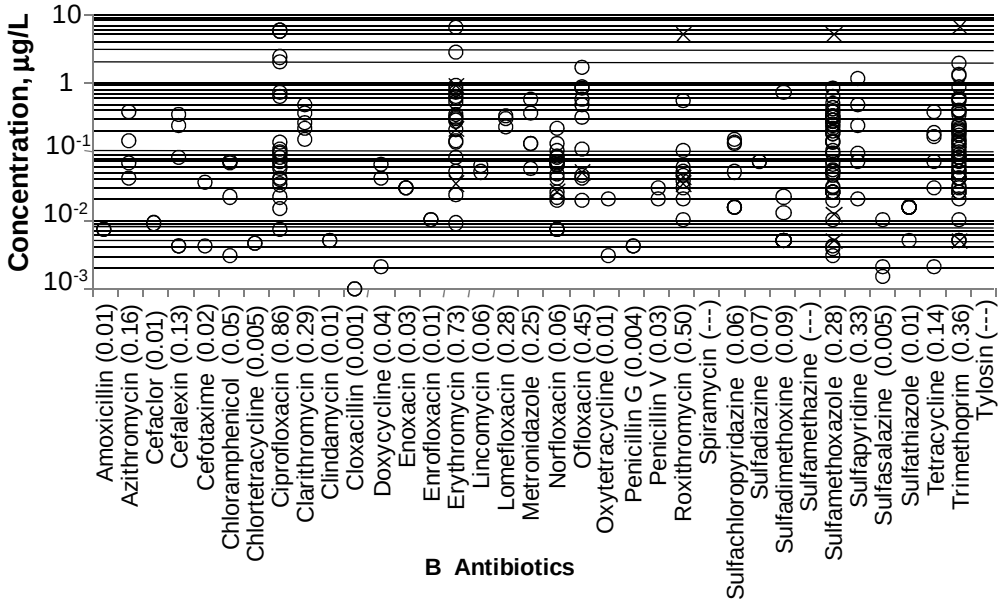


Figure 2.10. Concentration of selected antibiotics measured in secondary effluent (o refers to CAS and x to MBR) and corresponding average values (in brackets).

Referring to Fig. 2.11., two classes (antidiabetics and barbiturates), represented by only one compound, were never detected in any investigation. The range of variability for antihypertensives was 0.0025 to 11 μg/L, beta-blockers were detected between 0.005 and 73 μg/L, and diuretics between 0.004 and 1.8 μg/L. The most commonly detected compounds were the beta-blockers atenolol, metoprolol and propranolol and the antihypertensive diltiazem. The antifungal clotrimazole was found only once, while data are not available for the antihypertensive enalapril. The highest absolute concentrations in these classes were found for atenolol (73 μg/L), hydrochlorothiazide (11 μg/L) and furosemide (1.8 μg/L). The same compounds exhibited the highest average concentrations: atenolol 3.7 μg/L, hydrochlorothiazide 3.3 μg/L and furosemide 0.66 μg/L. It is worth remarking that the average concentration of all the other compounds remained less than 1 μg/L.

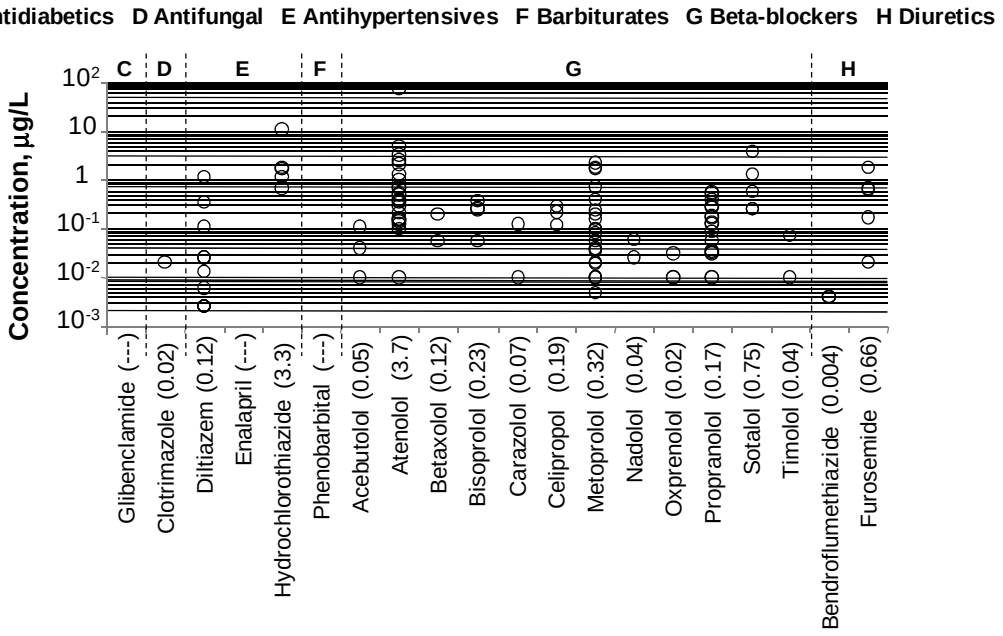


Figure2.11. Concentrations of selected PhCs from different classes measured in secondary biological effluent (o refers to CAS and x to MBR) and corresponding average values (in brackets).

As shown in Fig. 2.12., the range of variability observed in the secondary effluent was 0.0015-80 μg/L for lipid regulators and 0.001- 20 μg/L for psychiatric drugs. The most frequently investigated compounds were carbamazepine, gemfibrozil, bezafibrate and clofibric acid. Data were unavailable for oxcarbazepine and valproic acid. The highest absolute concentrations were found for fenofibric acid (80 μg/L), carbamazepine (20

µg/L), diazepam (19 µg/L) and gemfibrozil (5.2 µg/L), while the highest average concentrations were found for fenofibric acid (11 µg/L), diazepam (9.1 µg/L), gabapentin (2.6 µg/L) and carbamazepine (1.04 µg/L). All the other compounds had average values less than 1 µg/L. It is worth noting that the variability ranges are quite wide for most compounds: up to 5 orders of magnitude for carbamazepine.

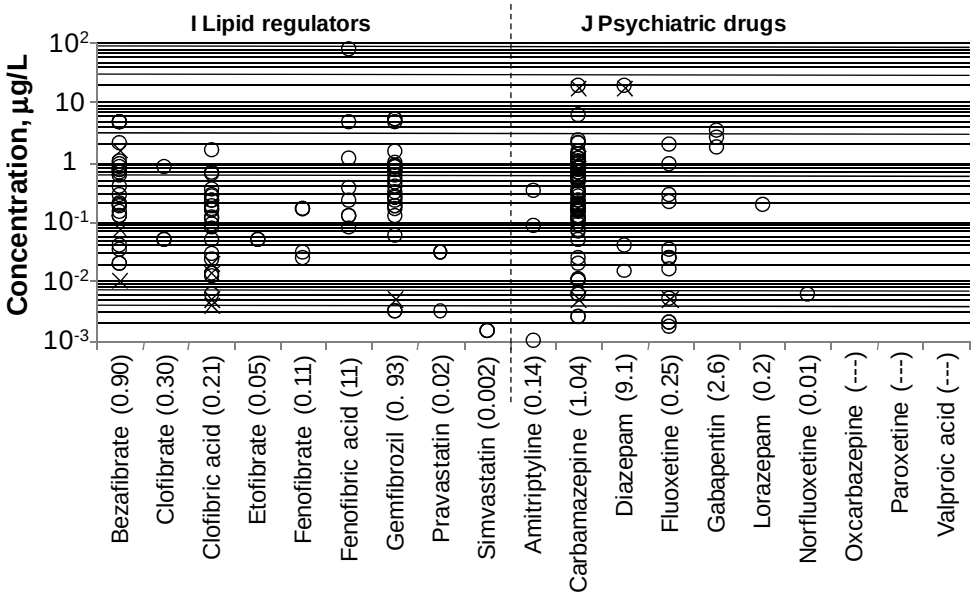


Figure2.12. Concentrations of selected lipid regulators and psychiatric drugs measured in secondary biological effluent (o refers to CAS and x to MBR) and corresponding average values (in brackets).

As to Fig. 2.13., the range of variability observed after the secondary treatment was 0.006-7.8 µg/L for receptor antagonists and 0.0002-0.11 µg/L for hormones. The most commonly investigated compounds were estrone, estradiol, ethinylestradiol and cimetidine. The highest absolute and average concentrations were found for cimetidine (7.8 µg/L and 3.5 µg/L, respectively), which was the only receptor antagonist found with an average concentration greater than 1 µg/L; famotidine and loratidine were never detected in the effluent. Hormones were found at consistently lower concentrations, always lower than 0.11 µg/L.

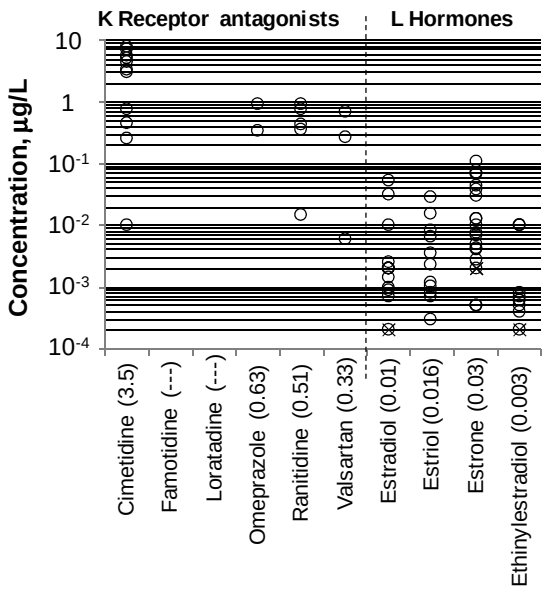


Figure 2.13. Concentrations of receptor antagonists and hormones measured in secondary biological effluent (o refers to CAS and x to MBR) and corresponding average values (in brackets).

Finally, the graph in Fig. 2.14. shows that the ranges of variability were 0.01-0.17 µg/L for beta-agonists, 0.002-2.9 µg/L for antineoplastics, 0.25-0.97 µg/L for topical products, 0.005-2.5 µg/L for antiseptics and 0.01-9.3 µg/L for contrast media. The most investigated compound was triclosan, while the others were monitored at a far lower frequency. Iopromide showed both the highest measured (9.3 µg/L) and the highest average concentration (2.5 µg/L).

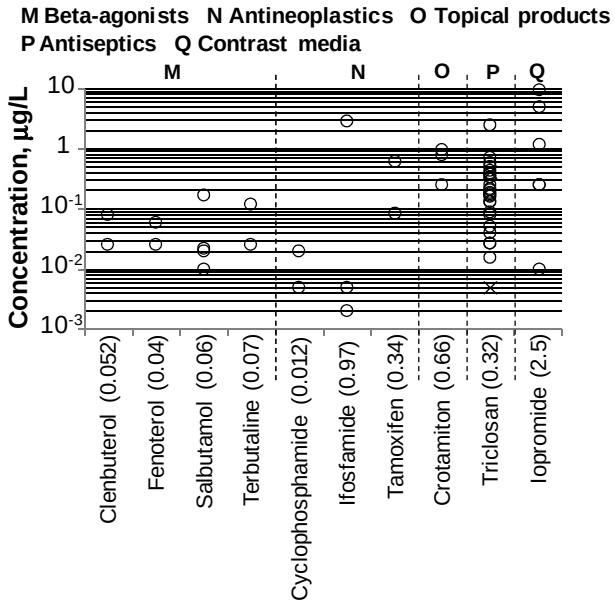


Figure 2.14. Concentrations of other classes of micropollutants measured in secondary biological effluent (o refers to CAS and x to MBR) and corresponding average values (in brackets).

Figure 2.15. summarizes the range of variabilities of the different classes based on collected data for the influent and effluent of all CAS (244 plants) and MBRs (20 plants). At the bottom of the figure, a table reports the number of collected data for each class in the influent and effluent of all the CAS (circle) and MBRs (cross) under study. It is important to remark that data pertaining to MBRs are quite limited and these systems were always pilot plants.

A rapid glance at these intervals shows that the different classes have different trends. In fact, the range of variability of measured concentrations in secondary effluents is narrower and lower than in the influent for analgesics/anti-inflammatories (A), antibiotics (B), antifungal (D), diuretics (H), psychiatric drugs (J), receptor antagonists (K), hormones (L), topical products (O) and antiseptics (P), being quite similar for antihypertensives (E) and beta-agonists (M), but higher for beta-blockers (G), lipid regulators (I), antineoplastics (N) and contrast media (Q). For antidiabetics (C), and barbiturates (F) the comparison is not possible as data are not available for the effluent. Moreover, ranges of variability referring to MBR permeates are narrower than those referring to CAS effluents for all of the investigated classes.

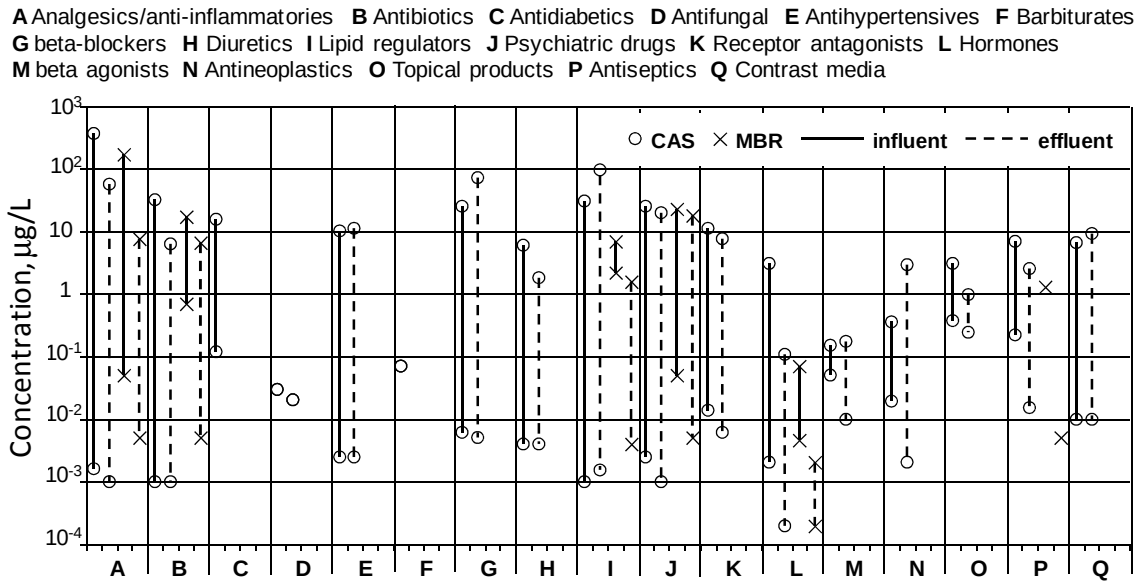


Figure 2.15. Comparison between the ranges of variability for the selected classes in the influent and effluent of all the CAS and MBRs under study.

2.11 Observed removal efficiencies in WWTPs

Figures 2.16.-2.20. report the observed removal efficiencies of PhCs from the aqueous phase achieved after secondary biological treatment in the WWTPs under study. These data are directly provided by listed references, in some cases, when it was possible, they were estimated by eq. 2.1, assuming a constant WWTP influent and effluent flow rate, equal to the average daily flow rate and as influent and effluent concentrations their corresponding average daily values (based on 24-h composite water samples). In the Appendix A-4 it is possible to distinguish between removal data provided by the Authors and those evaluated by means of eq. 2.1:

$$\eta = \frac{c_{inf} - c_{eff}}{c_{inf}} \text{ x } 100 \qquad \text{(eq. 2.1)}$$

η is the percentage removal efficiency and c is the average PhC concentration measured in the raw influent (subscript *inf*) or secondary effluent (subscript *eff*). As stated in Table 2.3., almost all the plants investigated include preliminary and primary treatments. As a consequence, η refers to the overall WWTP removal efficiency and takes into consideration removal by all the mechanisms occuring during preliminary, primary and secondary biological treatments: sorption onto coarse solids and sedimentation, in

preliminary and primary treatments, and a combination of biodegradation/biotransformation due to suspended biomass and sorption onto particles, flocs and then sludge in biological processes.

According to many Authors (Khan and Ongerth, 2005; Ternes and Joss, 2006; Yasojima et al., 2006; Watkinson et al., 2007; Zorita et al., 2009), the efficacy in removing PhCs by preliminary and primary treatments is in general quite poor, and in some cases compounds may even be released during the process, probably caused by the simultaneous presence of deconjugable substances, that is human metabolites, of these compounds in the raw influent (Carballa et al., 2004, Göbel et al., 2005). In particular, in the pre-treatment and sedimentation step no significant reduction was found for ibuprofen and naproxen (Carballa et al, 2004). This can be correlated to their acidic structures (negative charge of the molecule at pH 7, as shown in Appendix A.1., with very low solid-liquid partition coefficient K_d (according to Ternes et al., 2004, K_d < 500 L/kg or Log K_d < 2.7 implies very poor sorption onto sludge) which results in their presence mainly in the aqueous phase. For the hormone estrone, a higher concentration was observed at the end of the primary sedimentation with respect to the influent (Carballa et al., 2004), very likely due to the oxidation of the estradiol present, which explains the high negative removal efficiencies obtained for the estrone and the positive reduction of estradiol. (This is quite important to remember for the next sections as if the compound is found at a lower concentration in the secondary effluent than in the raw influent, the biological treatment is generally the greatest contributor).

As remarked above, biodegradation/biotransformation and sorption are the two main mechanisms occurring in the biological reactor, volatilization being quite scarce. The constant K_d and k_{biol} reported in Appendix A.1. may provide some first simple information on the potential behaviour of a compound during treatment, but, as it will be discussed in the following, it is quite complex to describe its real removal mechanisms.

Sorption on the sludge is a mechanism depending on many factors, including pH, redox potential, stereochemical structure and chemical nature of both the sorbent and the sorbed molecule (Kümmerer 2009b). It may occur by means of: (*i*) *absorption* due to hydrophobic interactions of the aliphatic and aromatic groups of a compound with the lipophilic cell membrane of the microorganisms or the lipid fractions of the suspended solids and (*ii*) *adsorption* due to electrostatic interactions of positively charged groups of chemicals with the negatively charhed surfaces of the microorganisms.

Biodegradation processes are strictly correlated to the characteristics of the biomass, the compounds (often quite persistent), the plant configuration and operation paramaters, in this case, in particular CAS and MBR. Apart from the final liquid/sludge separation stage, obtained by means of (ultrafiltration or microfiltration) membranes in MBR and sedimentation in CAS, these systems are mainly distinguished by their SRT, which is generally longer for MBR (15-80 d) with respect to CAS (7-20 d), as well as by their biomass concentration, generally higher in the MBR than in the CAS (8-10 kg/m^3 in MBRs and 3-5 kg/m^3 in CAS. Unfortunately these data were not always provided in the papers included in Table 2.3., hence the commonest operating values are reported). In order to better evidence the removal efficiencies achieved by both systems, at the bottom of each of Figures 2.16.-2.20. a table reports the average percentage removal achieved by CAS and MBR for each compound. It is important to remark again, that in any case, a comparison between these data has to consider that only 20 MBRs are included in the review (against 244 CAS), and they are always pilot plants, (against only 2 pilot CAS and 242 full scale plants) and finally a limited number of PhC concentration is available (and collected) for MBRs with respect to CAS.

Occasionally, negative removal efficiencies were found. These are not reported in the graphs of Figures 2.16.-2.20., but PhCs, with at least one negative percentage removal, are indicated with an asterisk and values are reported below the legend. While in some substances this phenomenon is clearly ascribable either to the presence of deconjugates interfering with biological transformation of the deconjugated compounds or to the release of PhC sorbed onto the particulate dissolving after the biological treatment, in others further investigation is required. Moreover, it is important to note that at the low level of concentrations found for some PhCs in the influent as well as in the secondary effluent, instrumental errors may lead to “apparent” releases of the investigated substance rather than a neglectable removal during the passage through the treatment plant. Sampling variation may also have contributed to this negative removal, as reported by Clara et al. (2005b), where the collection of effluent samples does not time-adjusted to account for long HRTs. Collecting composite samples over a period longer than plant HRT may improve the comparability between influent and effluent (Roberts and Thomas, 2006). Generally analysis were performed on influent and effluent water samples averaged over 24 h, a period higher than the corresponding WWTP HRT (Table 2.3.).

Fig. 2.16. reports the removal efficiencies for 18 out of 25 analgesics/anti-inflammatories in CAS and 9 out of 25 in MBR. The average percentage removals vary between 23 %

(tramadol) and 99 % (salicylic acid) in CAS, and between 43 % (indomethacin) and 99 % (acetaminophen) in MBR. For compounds investigated in both systems, MBR always exhibited a higher removal capacity than CAS. The graph shows that 12 compounds exhibited at least one value of their percentage removals in the range 90 – 100 % (5-aminosalycilic acid, acetaminophen, acetylsalycic acid, codeine, diclofenac, fenoprofen, hydrocodone, ibuprofen, ketoprofen, mefenamic acid, naproxen and salicylic acid).
Values lower than 10 % were found for five substances: diclofenac, ibuprofen, indomethacine, ketoprofen, mefenamic acid and tramadol. It is quite interesting to observe that some PhCs (diclofenac, ketoprofen, mefenamic acid) exhibited a wide range of variability in their removal by secondary treatments. According to Ziylan and Ince (2011), higher removal efficiencies of analgesics and anti-inflammatories are achieved at longer HRT and SRT, in reactors including nitrification and denitrification steps, at higher temperature. pH is another significant parameter especially for those compounds characterized by an increasing water-sludge partition coefficient and elevated acidity (acetaminophen, salicylic acid and ibuprofen).

In addition, negative removal efficiencies were observed for diclofenac and ibuprofen. Possible release of diclofenac can be explained by deconjugation of glucoronidated or sulphated diclofenac (Kimura et al., 2005) or its desorption from particles (Zorita et al., 2009). Ibuprofen is largely (90 %) transformed to its hydroxyl and carboxy derivatives that may later be hydrolyzed and converted to the parent compounds (Ziylan and Ince, 2011; Roberts and Thomas, 2006).

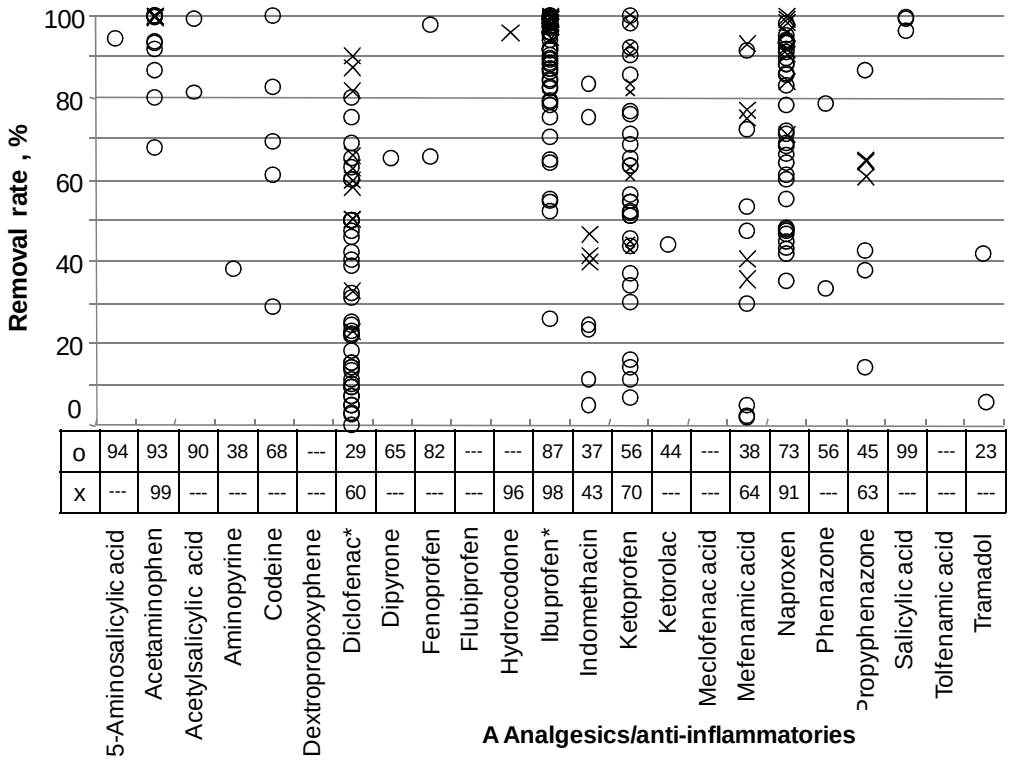


Figure 2.16. Percentage removal rates for analgesics/anti-inflammatories in WWTPs, and corresponding average values for CAS (o) and MBR (x).

Fig. 2.17. shows the removal efficiency variability for 29 antibiotics in CAS and 10 in MBR out of 37 reviewed substances. The most investigated compounds are sulfamethoxazole, trimethoprim, ciprofloxacin, roxithromycin, norfloxacin and erythromycin. Their ranges of variability are generally wide. The corresponding average values vary between 0 % (spiramycin) and 98 % (cefachlor) in CAS and between 15 % (azithromycin) and 94 % (ofloxacin) in MBRs. Only one (azithromycin) out of 10 compounds investigated in both systems featured higher average removal efficiencies in CAS than in MBR.

Antibiotic release was observed for nine compounds. For some of them the phenomenon has been investigated whereas for other it is not completely clear. Referring to clindamycin, very low concentrations (0.002-0.005 ng/L) were detected in the influent and effluent and possible instrumental errors may influence the evaluation of the negative removal efficiency (Watkinson et al., 2007). As to the two sulphonamides sulfamethoxazole and sulfasalazine, their main metabolites entering the sewage are biologically inactive N4-acetylated products and may retransform back to the intial parent compound (Göbel et al., 2007).

The presence of de-conjugable metabolites seems unlikely for the macrolides erythromycin and roxithromycin. Since they are mainly excreted with bile and feces, they are probably partly enclosed in feces particles and released during biological treatment. The load entering biological treatment is therefore underestimated, taking only in consideration the dissolved fraction and sorption to the suspended solids (Göbel et al., 2007). According to Lindberg et al. (2005), the increment in the effluent concentrations for trimethoprim can be explained by an underestimation of the actual amount entering the WWTP due to particulate matter with adsorbed antibiotics being filtered out during sample preparation. Higher concentration of ciprofloxacin, tetracycline and norfloxacin in the secondary effluent rather than the raw influent could be ascribed to a change in the adsorption behavior of the analytes to particles during treatment processes, influencing the ratio between influent and effluent (Gulkowska et al., 2008, Plósz et al., 2010).

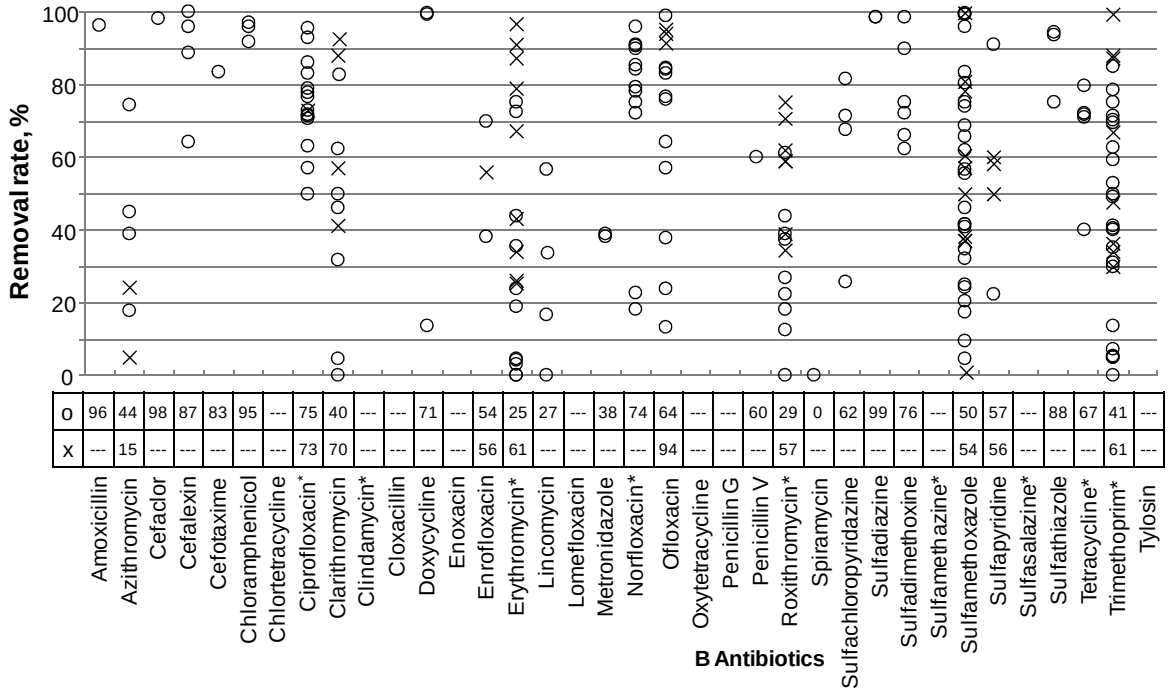


Figure 2.17. Percentage removal rates of antibiotics in WWTPs and corresponding average values for CAS (o) and MBR (x).

Fig. 2.18. refers to twenty PhCs from six classes, but data are available only for fourteen, all of which were investigated in CAS and six in MBRs. Five compounds were only reported in one study (clotrimazole, enalapril, phenobarbital, acetobutol and bisoprolol), while more data, spread over quite wide ranges, were available for the remaining compounds. For compounds investigated in both systems, the average removal

efficiencies are consistently higher in MBR than in CAS, except in the case of hydrochlorothiazide (45 % in CAS and 25 % in MBR).

Fig. 2.19. refers to the removal efficiencies obtained for selected lipid regulators and psychiatric drugs; the most investigated compounds were: bezafibrate, gemfibrozil, clofibric acid, pravastatin in the former group and carbamazepine and fluoxetine in the latter. Only one data set is available for fenofibrate, simvastatin, amitriptlyne, norfluoxetine and valproic acid in CAS and for paroxetine in CAS and MBR. No removal data were provided for clofibrate, etofibrate, lorazepam and oxcarbazepine, and few data sets were provided for the remaining compounds (fenofibric acid and gabapentin).For the most frequently investigated pharmaceuticals, the removal efficiencies variability ranges are generally quite wide, but, in general, higher removal efficiencies were achieved by MBRs except in the case of carbamazepine, which exhibited similar (low) average values in the two systems. This compound is not only one of the most persistent, but it can also be released in the WWTP, as shown in the data reported below the graph, presumably due to enzymatic cleavage of its glucuronic conjugate and release of the parent compound in the effluent (Radjenovic et al., 2007; Vieno et al., 2007).

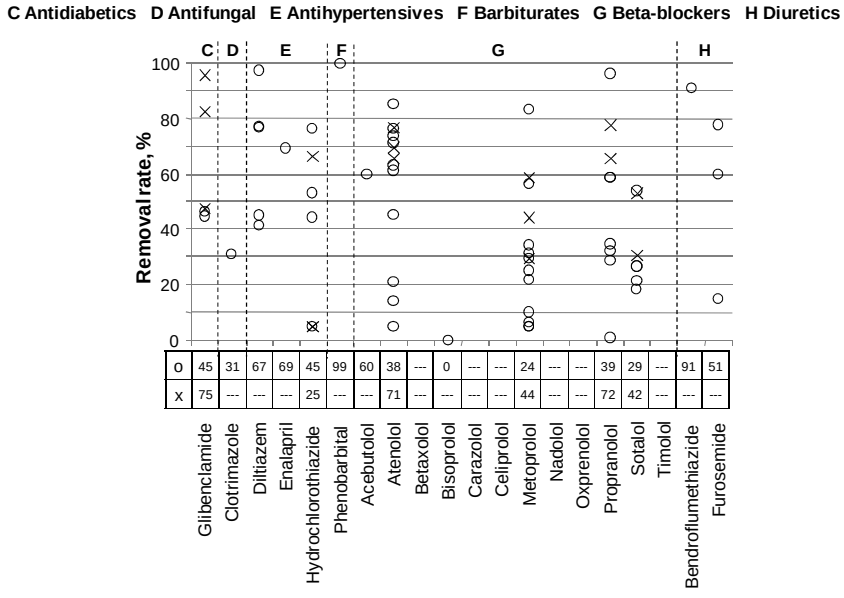


Figure 2.18. Percentage removal rates for some PhCs from different therapeutic classes in WWTPs, and corresponding average values for CAS (o) and MBR (x).

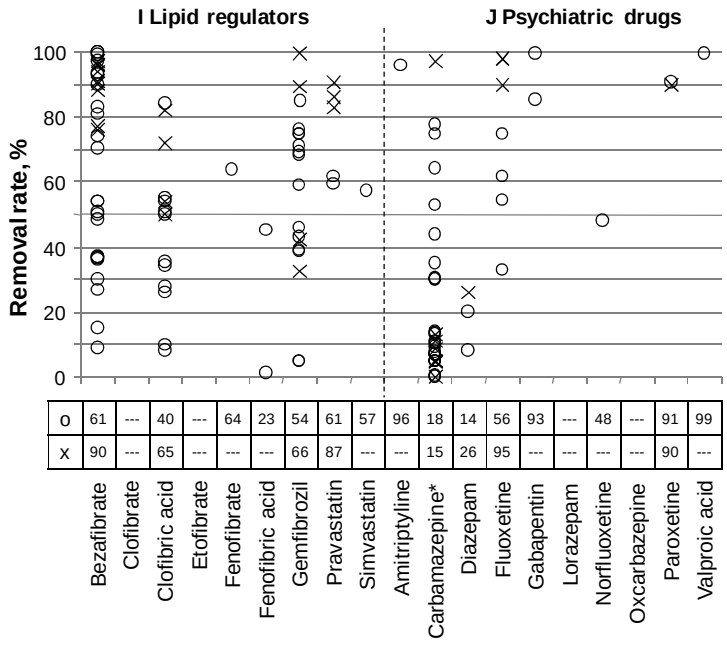


Figure 2.19. Percentage removal rates of selected lipid regulators and psychiatric drugs (o refers to CAS and x to MBR).

Fig. 2.20. refers to receptor antagonists and hormones; more data is available for the latter. Removal efficiencies for receptor antagonists were lower than 80 %, with the exception of ranitidine and valsartan, and average values were between 50-60 %, with a few exceptions: valsartan (84 % in CAS), loratadine (15 % in CAS and 19 % in MBR) and omeoprazol (9 % in CAS). In contrast, observed removal efficiencies for hormones were consistently higher, on average between 67 % and 80 % in CAS and 60 % and 99 % in MBRs. Estradiol is the compound most removed (on average 80 % in CAS and 99 % in MBR). However, negative removals of estrone were observed in CAS in several investigations, the assumption being that this is produced in the sewage treatment system by the oxidation of estradiol and by partial deconjugation of other estrogens present in the wastewater (D'Ascenzo et al., 2003).

Very few data are reported for the removal of the compounds belonging to the classes M-Q (Fig. 2.20.). A wide range was observed for the removal of salbutamol (0-98 %) and a slightly smaller one (21-65 %) for clotamitron. Triclosan is removed to a greater extent, even exceeding 98 % in both CAS and MBR, and its average removal efficiency is quite high (76 % in CAS and 99 % in MBR). Iopromide, on the other hand, was scarcely removed by biological processes, and in some investigations it was found to be released, as shown by the data reported below the graph. Its persistence is due to the fact that, as a

diagnostic agent, it is designed to be highly stable. No reasonable justification for the increasing of iopromide concentrations within the WWTP could be identified, according to Clara et al., (2005b). As to crotamiton, its releases can be explained by breakdown of conjugates of the pharmaceutical (Nakada et al., 2006).

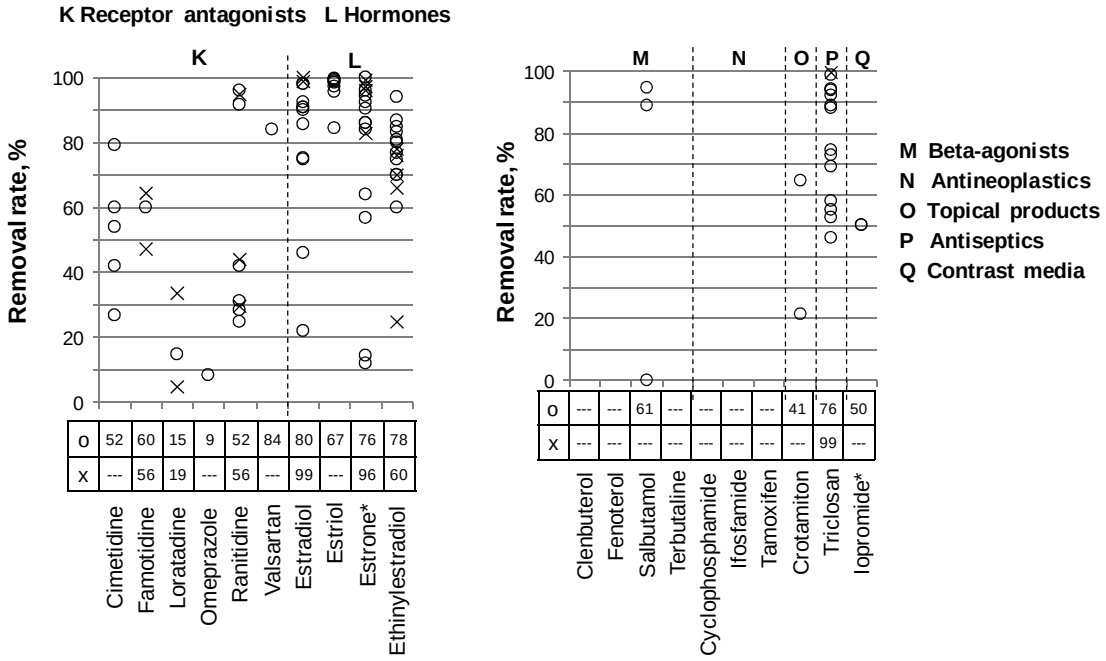


Figure 2.20. Percentage removal rates for selected receptor antagonists, hormones another PhCs, and corresponding average removal rates in CAS (o) and MBR(x).

All the data reported in the graphs above refer to PhC removal from aqueous phase, as defined by eq.2.1: in this way, attention is paid to the WWTP influent and effluent quality in order to evaluate how efficient is a specific treatment plant in *retaining* the selected compounds from the aqueous phase, without distinguishing between sorption onto sludge (hence transfer to another phase) and/or biological degradation/transformation processes. Sometimes it may be also called "apparent removal".

Another approach in evaluating PhC removal efficiencies considers the WWTP as a black box with one entrance (influent) and *two* outputs (liquid effluent and sludge). In this case, the removal efficiency, also called overall removal, η_{overall} is evaluated through equation 2.2:

$$\eta_{overall} = \frac{c_{inf}\ Q - \left(c_{eff}\ Q + c_{sludge}\ P_{sludge}\right)}{c_{inf}\ Q}\ x\,100 \qquad \text{(eq. 2.2)}$$

In eq. 2.2, c_{sludge} is the concentration of the selected PhC in the treated sludge (ng/g) and P_{sludge} is the daily sludge production for the plant under examination (g/d). Influent and effluent flow rates are assumed constant and equal to Q. The numerator represents the mass load of the selected PhC, subjected to biological reactions.

Few Authors investigated these two mechanisms in details, providing sorption and biodegradation contributions to the overall removal based on liquid and sludge concentration, influent and effluent flow rates and sludge production collected on full scale plants. Table 2.4. compiles these findings available only for some of the selected compounds with the corresponding references.

Table 2.4. Fractions with respect to the influent mass load of selected PhCs removed during secondary biological treatment, sorbed to sludge and discharged with secondary effluent. Data with a star as apex refer to MBR systems

Therapeutic class	Compound	Sludge age [d]	Biolog transform %	Sorption onto sludge %	Effluent %	References
Analgesic and anti-inflammatories **A**	Diclofenac	4-60 6 16 <20 >50	5-45 25 10 5 10-30	<5 <5 5 0 0	55-95 70-75 85 95 70-90	Joss et al.,2005 Jelic et al., 2011 Jelic et al., 2011 Suarez et al., 2010 Suarez et al., 2010
	Ibuprofen	4-60 2 10-55* <20 >50	90-100 <5 95-100 35-40 95	<5 <5 <5 0 0	0-10 95-100 0-5 60-65 5	Joss et al.,2005 Clara et al., 2005b Clara et al., 2005b Suarez et al., 2010 Suarez et al., 2010
	Indomethacin	6 16	27 40	0 <5	73 58-60	Jelic et al., 2011
	Ketoprofen	6 16	70 <95	0	30 5-10	Jelic et al., 2011
	Mefenamic acid	6 16	65 55-58	7 <30	28 <20	Jelic et al., 2011
	Naproxen	10-30 6 16 <20 >50	55-85 77 95-98 5 85-90	<5 0 0 0	15-45 23 <5 95 10-15	Joss et al.,2005 Jelic et al.2011 Jelic et al., 2011 Suarez et al., 2010 Suarez et al., 2010
Antibiotics **B**	Azithromycin	10-30	< 40	< 10	60-90	Gobel et al., 2007
	Chloramphenicol	6	0	0	100	Jelic et al., 2011
	Ciprofloxacin	10-12 20	< 10 < 10	70-80 77	≤30 <4	Golet et al., 2003 Lindberg et al., 2006
	Clarithromycin	< 20 >50 <20 6 16	< 10 90 <10 0 0	< 5 <5 ≤10 18 <45	75-90 10 >90 82 55-60	Gobel et al., 2007 Gobel et al., 2007 Gobel et al., 2007 Jelic et al., 2011 Jelic et al., 2011
	Enrofloxacin	20-25	19	65	17	Jia et al., 2012
	Erythromycin	<20	20		80	Suarez et al., 2010
	Lomefloxacin	20-25		60	40	Jia et al., 2010
	Metronidazole	6 16	15-18		100 82-85	Jelic et al., 2011

Therapeutic class	Compound	Sludge age [d]	Biolog transform %	Sorption onto sludge %	Effluent %	References
	Norfloxacin	10-12 20	< 10 < 10	80-90 72	≤ 20 < 4	Golet et al., 2003 Lindberg et al., 2006
	Ofloxacin	20-25		60	40	Jia et al., 2012
	Roxithromycin	4-30 <20	< 60 18	< 5 2	>35 80	Gobel et al., 2007 Suarez et al., 2010
	Sulfamethazine	6 16	<85 15-18	0 20	<20 60-65	Jelic et al., 2011
	Sulfamethoxazole	4-12 <20	50-90 20	< 5 0	10-50 80	Gobel et al., 2007 Suarez et al., 2010
	Sulfapyridine	10-30	≤ 70	< 10	≥30	Gobel et al., 2007
	Trimethoprim	<50 <20 6 16 <20	~90 <10 40 38-40 18	≤5 ≤5 < 5 5-10	~10 >90 < 60 50-55 72	Gobel et al., 2007 Gobel et al., 2007 Jelic et al., 2011 Jelic et al., 2011 Suarez et al., 2010
Antidiabetics **C**	Glibenclamide	6 16		<10 60	90-95 40	Jelic et al., 2011
Antihypertensives **E**	Enalapril	6 16	95-98 95-98		2-5 2-5	Jelic et al., 2011
	Hydrochlorothiazide	6 16		100 100		Jelic et al., 2011
Beta-blockers G	Atenolol	6	< 70	< 5	< 35	Jelic et al., 2011
	Metoprolol	6 16	~35 0	0 0	~65 100	Jelic et al., 2011
	Nadolol	6 16	35-40 70	<5 30	60	Jelic et al., 2011
	Sotalol	6 16	10 <50	< 5 <5	< 90 50	Jelic et al., 2011
	Timolol	6 16	< 40 40-45	<5 0	< 65 55-60	Jelic et al., 2011
Diuretics **H**	Furosemide	6 16	35-40 75-80	<5 2-5	60-65 20	Jelic et al., 2011
Lipid regulators **I**	Bezafibrate	6 16 2	12 <80 45-50	2 <5 <5	86 20-25 50	Jelic et al., 2011 Jelic et al., 2011 Clara et al., 2005b
	Fenofibrate	6 16	0 25-30	100 65-70	0	Jelic et al., 2011
	Gemfibrozil	6 16	0 90	3 <5	97 5-10	Jelic et al., 2011 Jelic et al., 2011

Therapeutic class	Compound	Sludge age [d]	Biolog transform %	Sorption onto sludge %	Effluent %	References
	Pravastatin	6 16	45 62	0 2	55 <40	Jelic et al., 2011
Psychiatric drugs **J**	Carbamazepine	4-60 6 16	<40 22 0	<5 3 5	>60 75 95	Joss et al., 2006 Jelic et al., 2011 Jelic et al., 2011
	Diazepam	6 16	0	42 65	58 35	Jelic et al., 2011
	Fluoxetine	<20 >50	80 90	0 0	20 10	Suarez et al., 2010 Suarez et al., 2010
	Lorazepam	6 16	30 30	<5 5-8	65-70 65	Jelic et al., 2011
Receptor antagonists **K**	Cimetidine	6 16	42 60	4 5-8	54 32-35	Jelic et al., 2011
	Famotidine	6 16	< 10 80	10 20	85 0	Jelic et al., 2011
	Ranitidine	6 16	< 20 75	< 5 <5	80 20-25	Jelic et al., 2011
Hormones **L**	Estradiol	10-30	85-99	<5	<15	Joss et al., 2004
	Estrone	10-30	35-97	≤5	5-60	Joss et al., 2004
	Ethinylestradiol	10-30 <20 >50	45-95 25 80-90	≤5 5 0	5-50 70 10-20	Joss et al., 2004 Suarez et al., 2010 Suarez et al., 2010
Beta-agonist **M**	Salbutamol	6 16	<60 40-42	<5 2	<45 55-60	Jelic et al., 2011
Contrast agent **Q**	Iopromide	10-30	20-95	<5	5-80	Joss et al.,2005

A rapid glance to the data compiled in Table 2.4. shows that sorption onto activated sludge is of minor importance for most of the selected PhCs: due to their hydrophilic characteristics (Log K_{ow} < 2.5 high hydrophilic compound, see Appendix A.1.), their sorption removal keeps quite low (< 20 %). According to a simple rule (Ternes and Joss, 2006), compounds with K_d > 500 L/kg (Log K_d > 2.7) potentially tend to adsorb onto sludge and particles. Appendix A.1. compiles Log K_d values for most of the selected substances and evidences that for most of them, they are less than 2.7 confirming their low tendency to adsorb. The value of the PhC molecular charge at pH 7 provides information about its potential to create electrostatic interactions with the (usually) negatively charged biomass surface.

Data of Table 2.4. show that, only for the antibiotics ciprofloxacin, norfloxacin, ofloxacin and lomefloxacin, the antihypertensive hydrochlorotiazide and the lipid regulator fenofibrate, the removal percentage due to sorption is in the range 60-100 %. The antibiotics appear not be readily biodegradable (Ternes and Joss, 2006; Jia et al., 2012) and their removal during activated sludge processes is assumed to be due to the formation of flocs by microbial activity, via electrostatic and hydrophobic interactions (Lindberg et al., 2006; Jia et al., 2012). The four antibiotics are characterized by high sorption constant Log K_d (> 4 as reported in Appendix A.1.), confirming a good tendency to sorption (Kümmerer, 2009a) and to create electrostatic interactions, as suggested by Vieno et al. (2007) and Göbel et al. (2007). Data of high removal by sorption referring to hydrochlorothiazide (Jelic et al., 2011) were not expected by the Authors during their investigation as this compound was never detected in the influent and effluent of the WWTP, but only it was detected in the sludge. Perhaps its presence in the sludge is correlated to previous processes of accumulation in the solid phase, inside the biological reactor. Further research is necessary to better investigate the fate of hydrochlorothiazide as well as fenofibrate (Jelic et al., 2011).Sorption of compounds is in generally pH dependent, however, in WWTPs it is not significantly affected by the narrow range of pH variability normally observed (Lindberg et al., 2006).

For compounds with a high sorption potential, the removal efficiency in an MBR may be slightly higher due to the absence of suspended solids in the effluent (Clara et al., 2004): Fig. 2.18. shows that ciprofloxacin and norfloxacin have higher removals in MBRs rather than CAS systems.

Attempts to correlate biodegradation removal of a compound to its molecular characteristics was made by Tunkel et al. (2000). On the basis of a large set of organic

chemicals, they found that compounds including esters, nitriles and aromatic alcohols have functional groups that may increase biodegradability, while aromatic amines, iodide, nitro and azo groups increase the persistence of the compound. Jones et al. (2005) reported that long and highly branched side chains (i.e. omeoprazole and ranitidine) render a compound more persistent as well as complicated aromatic ring structures (including norfluoxetine, diazepam) and halogen groups (i.e. iopromide, diazepam).

2.11.1 Considerations on the observed removal efficiencies of the selected PhCs.

As previously mentioned, compounds of the same class may have quite different chemical and physical properties (Ternes and Joss, 2006) resulting in different behaviours during treatment processes (tendency to remain in dissolved phase, to adhere to flocs or particles or to undergo biodegradation), which can explain why compounds belonging to the same therapeutic class do not exhibit similar removal efficiencies (Figures 2.16.-2.20.). However, as reported by Tadkaew et al. (2011), it is always difficult to correlate physical properties of pharmaceuticals to their corresponding removal efficiency achieved in an activated sludge system, as many other factors contribute to it, in particular operating parameters such as biomass concentration, SRT, HRT, pH, temperature, configuration and type of plant. A brief discussion is below reported.

2.11.1.1 Effect of biomass concentration and sludge retention time (SRT)

Many authors (among them Kreuzinger et al., 2004; Weiss and Reemtsma, 2008) have found that a long SRT promotes the adaptation of different kinds of microorganisms, as well as the presence of slower growing species that could have a greater capacity for removing xenobiotics while simultaneously greatly improving suspended solid separation: this is the case for ibuprofen and diclofenac as reported by Suarez et al. (2010) whose removal was only achieved after the growth of specific bacteria. Moreover, Kimura et al. (2007) found that a greater removal of diclofenac was achieved in an MBR operating at longer SRT (up to 65 d) with respect to a CAS (SRT on average 7 d) due to a different composition of the two sludges resulting in different sorption capacities with respect to the selected PhC.

Schröder (2002) suggested that MBR systems provide a competitive advantage for organisms able to degrade persistent compounds by eliminating bacterial washout. The

high biomass concentrations in an MBR not only lead to a decreased sludge production, but also a higher stability and persistence to shock loads (Lee et al., 2003).

The higher biomass concentration in MBRs results in a decrease of the food to microorganisms ratio (F/M). The relative shortage in biodegradable substance may induce microorganisms to metabolize also poorly degradable compounds. This can explain why removal efficiencies for some persistent PhCs (including ketoprofen and naproxen) are higher in MBRs than in CAS systems and why this can be obtained at lower HRT (Weiss and Reemtsma, 2008). High SRT combined with reduced F/M ratios may result in an increased biodiversity and may also favor elimination of compounds, like the antibiotics trimethoprim, erythromycin and other macrolides, by co-metabolism processes (Göbel et al., 2007).

High SRTs have also beneficial effects on the removal of PhCs that tend to accumulate in the sludge flocs, either due to intrisec hydrofobicity or via electrostatic interactions with the biomass (i.e. tetracycline, ciprofloxacin, ofloxacin, norfloxacin) (Kim et al., 2007). Moreover the biomass in an MBR has a more viable fraction compared to CAS system (Cicek et al. 1999) that can be attributed to an improved mass transfer due to the presence of smaller flocs (10-100 μm in MBR against 100-500 μm in CAS) and a large fraction of planktonic microorganisms. These factors favor the contact between microorganisms and pollutants and stimulate their biodegradation, as well as some enzymatic activities (Cirja et al., 2008). Radjenovic et al. (2009) found higher concentrations in MBR sludge rather than CAS sludge for hydrochlorothiazide, azythromycin, carbamazepine and ketoprofen.

Clara et al. (2005a) found that a SRT > 10 d is needed for some biodegradable PhCs (in particular hormones, bezafibrate and ibuprofen) to achieve low effluent concentrations, although other studies (Joss et al., 2005; Vieno et al., 2007) noticed no clear correlation between percentage elimination and SRT in particular for beta-blockers, carbamazepine and the antibiotics ciprofloxacin, ofloxacin and norfloxacin.

Table 2.5. and Table 2.6. report removal efficiencies for the selected compounds with the corresponding SRT and references distinguishing between CAS and MBR. The positive effect of increasing SRT appear for several compounds, in particular for hormones, ibuprofen, ketoprofen, naproxen, bezafibrate, gemfibrozil, fluoxetine, antibiotics mainly removed by biodegradation, as also confimed by (Strenn et al., 2004).

Increasing SRT beyond 30 days does not usually result in a consistent increment in the removal for most compounds, (Suarez et al., 2008). This could be explained with the

fact that biodegradation of micropollutants, including PhCs, is mostly due to cometabolic processes as the low concentrations do not likely sustain growth for specific microorganisms, because in this case the SRT necessary for an efficient biodegradation of the primary substrate is the relevant parameters (Sipma et al., 2010).

Clara et al. (2004) reported that they did not find significant differences in the removal efficiency of pharmaceuticals like diclofenac, ibuprofen, carbamazepine, bezafibrate and ethinylestradiol between CAS and MBR systems when operated at similar sludge retention times, which suggests that the reactor type is of less importance than the SRT. Although SRT has been reported as determinative for pharmaceutical biodegradation due to enrichment of certain microbial communities who excrete enzymes able to break down PhCs (Cirja et al., 2008), the effect of an increasing SRT does not become clear for other compounds, including naproxen and sulfamethoxazole (Lishman et al., 2006;Vieno et al., 2007): often very fluctuating removal efficiencies are encountered with increasing of its values, as reported in Tables 2.5. and 2.6.

Table 2.5. Average removal efficiencies obtained in CAS systems for the selected pharmaceuticals with respect to the operating SRT in the bioreactor and the corresponding references

Class	Pharmaceutical	SRT [d]	Removal efficiency CAS [%]	References
A	Acetaminophen	2.4/3 8/10/13	100/86.4 99.5/99.9/92	Roberts and Thomas, 2006/Radjenovic et al., 2007 Yu et al., 2006/Radjenovic et al., 2009/Jones et al., 2007
	Codeine	18.5	82	Wick et al., 2009
	Diclofenac	1.5/2/2.4/2.7/3 5/7/8/9.6/10 19/20 42/46/48/52/52/60	50/7.1/65.1/50/50 50/42/18/9/22 9.7/13 47/14/14/63/60/3	Santos et al.,2009/Clara et al., 2005a/Roberts and Thomas, 2006/Santos et al.2009/Radjenovic et al., 2007 Santos et al., 2007/Kimura et al., 2007/Yu et al., 2006/Kreuzinger et al., 2004/Radjenovic et al., 2009 Clara et al., 2005a/Vieno et al., 2005 Clara et al., 2005a /Clara et al., 2005b/Clara et al., 2005a /Clara et al., 2005b/Clara et al., 2004/Suarez et al., 2005
	Ibuprofen	1.5/1.5/2/2.4/2.7/3 5/7/8/8/9.6/10 13/19/20 42/46/48/52/60	89.5/87/-4.4/-13/84/82.5 88.4/98/87/99/92/99 86/92/99.8 99/98/98/97/82	Santos et al., 2007/Santos et al., 2009/Clara et al., 2005a/ Roberts and Thomas, 2006/ Santos et al., 2009/ Radjenovic et al., 2007 Santos et al., 2007/ Kimura et al., 2007/ Yu et al., 2006/Zorita et al., 2009/ Kreuzinger et al., 2004/ Radjenovic et al., 2009 Jones et al. 2007/Clara et al. 2005a/Vieno et al.2005 Clara et al., 2005a/ Clara et al., 2005b/ Clara et al., 2005a/Clara et al.,2004/Suarez et al.,2005
	Indomethacin	3/10	23/<10	Radjenovic et al., 2007;2009
	Ketoprofen	1.5/1.5/2.7/3 5/7/8/10/20	37/52/56/52 30/55/77/55/92	Santos et al., 2007/Santos et al.,2009/Santos et al.,2009/Radjenovic et al., 2007 Santos et al., 2007/Kimura et al.,2007/Yu et al., 2006/Radjenovic et al., 2009/Vieno et al., 2005
	Mefenamic acid	3/7/10/13	29/72/5/92	Radjenovic et al., 2007/Kimura et al., 2007/Radjenovic et al., 2009/Jones et al., 2007
	Naproxen	1.5/1.5/2.7/3 5/7/8/8/10 20/60	35/43/71/85 89/64/88/93/72 95/68	Santos et al., 2007/Santos et al.,2009/Santos et al., 2007/Radjenovic et al., 2007 Santos et al., 2007/Kimura et al.,2007/Yu et al., 2006/Zorita et al., 2009/Radjenovic et al., 2009 Vieno et al.. 2005/Suarez et al., 2005
	Propyphenazone	3/10	42/38	Radjenovic et al., 2007;2009
	Tramadol	18.5	4	Wick et al., 2009
B	Amoxicillin	12.5	96	Watkinson et al., 2007
	Azithromycin	5/18	74/39;45	Yasojima et al., 2006/Ghosh et al., 2009;
	Cefaclor	12.5	98	Watkinson et al., 2007
	Cefalexin	7 12/12.5/20	91 53/100/64;87	Li and Zhang, 2011 Li and Zhang, 2011/Watkinson et al., 2007/Gulkowska et al., 2008
	Cefotaxime	12/20	43/83	Li and Zhang, 2011/Gulkowska et al., 2008
	Chlortetracycline	7;12	82;85	Li and Zhang, 2011

Class	Pharmaceutical	SRT [d]	Removal efficiency CAS [%]	References
	Ciprofloxacin	7/8/11 11/12/12.5/15/18/20 22	55/71/78 93/18/83/96/50;73/79 72	Li and Zhang, 2011/Zorita et al., 2009/Golet et al., 2003/ Lindberg et al., 2005/ Li and Zhang, 2011/Watkinson et al., 2007/Lindberg et al., 2005/; Ghosh et al., 2009/Lindberg et al., 2006/ Lindberg et al., 2005
	Clarithromycin	5/9 11/18	46/62 4.5/50;83	Yasojima et al., 2006/Sahar et al., 2011 Göbel et al., 2007/Ghosh et al., 2009
	Doxycycline	11/15;20	14/100;99	Lindberg et al., 2005/Lindberg et al., 2005
	Enrofloxacin	18	70;38	Ghosh et al., 2009
	Erythromycin	3 5.6/7/9/10 11/12/20	24 4.4/26/19/35 3/15/19	Radjenovic et al., 2007 Xu et al., 2007/ Li and Zhang, 2011/Sahar et al., 2011/Radjenovic et al., 2009 Göbel et al., 2007/ Li and Zhang, 2011/Gulkowska et al., 2008
	Lincomycin	12.5/18	17/57;33	Watkinson et al., 2007/Ghosh et al., 2009
	Norfloxacin	5.6/7/8 11/11/12/12.5/15/18/20/20/20;22	18/45/-6 84/91/30/85/96/75;90/79/23;78/91;72	Xu et al., 2007/ Li and Zhang, 2011/Zorita et al., 2009 Golet et al., 2003/Lindberg et al., 2005/ Li and Zhang, 2011/Watkinson et al., 2007/Lindberg et al., 2005/Ghosh et al., 2009/Lindberg et al., 2006/Gulkowska et al., 2008/Lindberg et al., 2005
	Ofloxacin	3 5.6/7/8/10 11/12	24 38/59/13/76 84/26	Radjenovic et al.2007 Xu et al., 2007/ Li and Zhang, 2011/Zorita et al., 2009/Radjenovic et al.2009 Lindberg et al., 2005/ Li and Zhang, 2011
	Penicillin V	12.5	60	Watkinson et al., 2007
	Oxytetracycline	12	4	Li and Zhang, 2011
	Roxithromycin	2/5.6/7/9/9.6 11/12/18 46;52	27/12.5/40/22/-4 19/46/39;-32 -80;44	Clara et al., 2005b/Xu et al., 2007/ Li and Zhang, 2011/Sahar et al., 2011/Kreuzinger et al., 2004 Göbel et al., 2007/ Li and Zhang, 2011/Ghosh et al., 2009 Clara et al. 2005b
	Sulfadiazine	6/7/12	78-98/87/100	García-Galán et al., 2011/Li and Zhang, 2011
	Sulfadimethazine	6;19	100	García-Galán et al., 2011
	Sulfamethazine	4;6/7;12/19	100;16/100/100	García-Galán et al., 2011/Li and Zhang, 2011/ García-Galán et al., 2011
	Sulfamethoxazole	3/6/7/9/10 11/12/12.5/15/18/18 20/46	56/54;71/62/10/74 4.5/90/25/100/39/26 42/32	Radjenovic et al., 2007/García-Galán et al., 2011/Sahar et al., 2011/Radjenovic et al., 2009/ Li and Zhang, 2011 Göbel et al., 2007/ Li and Zhang, 2011/Watkinson et al., 2007/Lindberg et al., 2005/Ghosh et al., 2009/Ghosh et al., 2009 Lindberg et al., 2005/Clara et al., 2005b
	Sulfapyridine	4/6/19	20/77;89/6	García-Galán et al., 2011
	Sulfathiazole	4;6/12.5	100;65/75	García-Galán et al., 2011/Watkinson et al., 2007
	Tetracycline	7/12 18/20	36/24 40;72/-88;72	Li and Zhang, 2011 Ghosh et al., 2009/Gulkowska et al., 2008
	Trimethoprim	2.4 7/9/10	-56 42/0/40	Roberts and Thomas, 2006 Li and Zhang, 2011/Sahar et al., 2011/Radjenovic et al., 2009

Class	Pharmaceutical	SRT [d]	Removal efficiency CAS [%]	References
		11/11/12/12.5/15/18/20/20/22/55	-2/7/13/85/41/-88;35/14/-17;63/-34/53	Lindberg et al., 2005/Göbel et al., 2007/ Li and Zhang, 2011/Watkinson et al., 2007/Lindberg et al., 2005/Ghosh et al., 2009/Lindberg et al., 2006/Gulkowska et al., 2008/Lindberg et al., 2005/ Batt et al.,2006
C	Glibenclamide	3/10	44.5/46	Radjenovic et al., 2007;2009
D	Clotrimazole	2.4	31	Roberts and Thomas, 2006
E	Hydrochlorothiazide	3/10	76/<10	Radjenovic et al., 2007;2009
F	Phenobarbital	8	99.5	Yu et al., 2006
G	Atenolol	3/8/9/10 14.6/18.5	<10/71/76/61 73/44	Radjenovic et al., 2007/Carucci et al., 2006/Maurer et al., 2007/ Radjenovic et al., 2009 Maurer et al., 2007/Wick et al., 2009
	Bisoprolol	18.5	0	Wick et al., 2009
	Metoprolol	3/9/10 14.6/18.5	<10/31/25 29/21	Radjenovic et al., 2007/Maurer et al., 2007/Radjenovic et al., 2009 Maurer et al., 2007/Wick et al.,2009
	Propranolol	9/10 14.6/18.5	28/59 35/0	Maurer et al., 2007/Radjenovic et al., 2009 Maurer et al., 2007/Wick et al.,2009
	Sotalol	9/10 14.6/18.5	26/21 27/18	Maurer et al., 2007/Radjenovic et al., 2009 Maurer et al., 2007/Wick et al.,2009
I	Bezafibrate	2/3 9.6/10 19/20 42/46/48/52/52	36.8/48 36/81 37/94 90/53.9/53.8/99.9/97	Clara et al., 2005a/Radjenovic et al., 2007 Kreuzinger et al., 2004/Radjenovic et al., 2009 Clara et al., 2005a/Vieno et al., 2005 Clara et al., 2005a /Clara et al., 2005b/Clara et al., 2005a /Clara et al., 2005b/Clara et al., 2004
	Clofibric acid	2.4/3 7/8	84/28 50/55	Roberts and Thomas, 2006/Radjenovic et al., 2007 Kimura et al., 2007/Zorita et al., 2009
	Gemfibrozil	3/8/10	39/68/5	Radjenovic et al., 2007/Yu et al., 2006/Radjenovic et al., 2009
	Pravastatin	3/10	62/59	Radjenovic et al., 2007;2009
J	Carbamazepine	1.5/1.5/2/2.7/3 5/9.6/10 18.5/19 42/46/48/52/52/60	-4/11/-3/7/<10 -67/35/<10 -12/-47 -35/-43/-43/-11/0/<10	Santos et al., 2007/Santos et al. 2009/Clara et al. 2005a/Santos et al., 2009/Radjenovic et al. 2007 Santos et al., 2007/Kreuzinger et al., 2004/Radjenovic et al., 2009/ Wick et al, 2009/Clara et al., 2005a Clara et al., 2005a /Clara et al., 2005b/ Clara et al., 2005a/ Clara et al., 2005b/Clara et al., 2004/ Suarez et al., 2005
	Diazepam	60	8	Suárez et al., 2005
	Fluoxetine	8/10	54.5/33	Zorita et al., 2009/Radjenovic et al., 2009
	Gabapentin	8	99.5	Yu et al., 2006
	Norfluoxetine	8	48	Zorita et al., 2009
	Paroxetine	3	91	Radjenovic et al., 2007
	Valproic acid	8	>99	Yu et al., 2006
K	Famotidine	10	60	Radjenovic et al., 2009

Class	Pharmaceutical	SRT [d]	Removal efficiency CAS [%]	References
	Loratadine	10	15	Radjenovic et al., 2009
	Ranitidine	3/8/10	42/28.5/25	Radjenovic et al., 2007/Carucci et al., 2006/Radjenovic et al., 2009
L	Estradiol	8/10	22/98	Zorita et al., 2009/Joss et al., 2004
	Estrone	10/11/19 42/48	96/99/-35 94/99.9	Joss et al., 2004/Andersen et al., 2003/ Clara et al., 2005a Clara et al., 2005a /Clara et al., 2005a
	Ethinylestradiol	9.6/10 52	70/94 70	Kreuzinger et al., 2004/Joss et al., 2004 Clara et al., 2004
M	Salbutamol	13	95	Jones et al., 2007
P	Triclosan	8	69	Yu et al., 2006
Q	Iopromide	2/9.6 55	-32/50 50	Clara et al., 2005b/Kreuzinger et al., 2004 Batt et al., 2006

Table 2.6. Average removal efficiencies obtained in MBRs for the selected pharmaceuticals with respect to the operating SRT in the bioreactor and the corresponding references

Class	Pharmaceutical compound	SRT [d]	Removal efficiency MBR [%]	References
Analgesics/ Anti-inflammatories **A**	Diclofenac	10/15 22/27/37/65	60/51 33/51/23/82	Clara et al., 2004/Kimura et al., 2007 Clara et al., 2005a/Clara et al., 2005b/Quintana et al., 2005/Kimura et al., 2007
	Ibuprofen	10/11/15/20 22/27/37/65	97/99/95/97 97/99/97/98	Clara et al., 2004/Kreuzinger et al., 2004/Kimura et al., 2007/Kreuzinger et al., 2004; Clara et al., 2005a/Clara et al., 2005b/Quintana et al., 2005/Kimura et al., 2007
	Ketoprofen	15 37/65	83 62/99	Kimura et al., 2007 Quintana et al., 2005/Kimura et al., 2007
	Mefenamic acid	15/65	77/93	Kimura et al., 2007
	Naproxen	15 37/65	96 71/98	Kimura et al., 2007 Quintana et al., 2005/Kimura et al., 2007
Antibiotics **B**	Azithromycin	33/70	5/24	Göbel et al., 2007
	Clarithromycin	16 33/70/70	57 41/92/88	Göbel et al., 2007 Göbel et al., 2007/Sahar et al., 2011/Göbel et al., 2007/
	Erythromycin	16 33/70/70	34 26/79/87	Göbel et al., 2007 Göbel et al., 2007/Sahar et al., 2011/Göbel et al., 2007
	Roxithromycin	16/20 27/33/70/70	39/75 34/62/59/59	Göbel et al., 2007/Kreuzinger et al., 2004 Clara et al., 2005b/Göbel et al., 2007/ Sahar et al., 2011/Göbel et al., 2007
	Sulfamethoxazole	11/16 33/70/70	57/37 38/0/37	Kreuzinger et al., 2004/Göbel et al., 2007 Göbel et al., 2007/Sahar et al., 2011/Göbel et al., 2007
	Sulfapyridine	16	60	Göbel et al., 2007

Class	Pharmaceutical compound	SRT [d]	Removal efficiency MBR [%]	References
		33/70	50/58	Göbel et al., 2007/Göbel et al., 2007
	Trimethoprim	16 33/70/70	30 34/88/87	Göbel et al., 2007 Göbel et al., 2007/Sahar et al., 2011/Göbel et al., 2007
Lipid regulators **I**	Bezafibrate	10/11/20 22/27/37	97/94/76 77/96/91	Clara et al., 2004/Kreuzinger et al., 2004/Kreuzinger et al., 2004 Clara et al., 2005a/Clara et al., 2005b/Quintana et al., 2005
	Clofibric acid	15/65	50/82	Kimura et al., 2007
Psychiatric drugs **J**	Carbamazepine	10/11 22/27	0/11/ -13/4.4	Clara et al., 2004/Kreuzinger et al., 2004 Clara et al., 2005a/Clara et al., 2005b/
Hormones **L**	Estradiol	30	99	Joss et al., 2004
	Estrone	22/30	97/96	Clara et al., 2005a/Joss et al., 2004
	Ethinylestradiol	10/11;20 30	70/66;25 76	Clara et al., 2004/Kreuzinger et al., 2004 Joss et al., 2004

As MBRs generally operate at longer SRTs (at least 15 d, as stated in Table 2.3.) than CAS (generally at maximum 15 d), this could explain higher removal efficiencies achieved by the former with respect to the latter as reported in Clara et al. (2005b), Radjenovic et al. (2009), Weiss and Reemtsma (2008). Moreover, in MBRs, membranes detain particulate matter, including any adsorbed or absorbed PhCs, leading to an effluent free of suspended solids and relatively free of contaminants (for instance glibenclamide).

Weiss and Reemstma 2008 found that the major advantage of MBR lies in the range of compounds with moderate removal in CAS (including naproxen, diclofenac, phenazone, clofibric acid). For these MBR is capable of delivering lower and more stable effluent concentrations in comparison to CAS even with lower HRT.

2.11.1.2 Effect of Hydraulic retention time (HRT)

The influence of HRT on the removal efficiencies of selected PhCs was investigated by different Authors. Among them, Bernard et al. (2006) and Vieno et al. (2007) found no significant correlation between HRT and removal of respectively diclofenac and the beta-blockers atenolol, metoprolol, acebutolol and sotalol. Gros et al. (2010) and García-Galán et al. (2011) investigated in two full scale WWTPs in Spain operating at different HRT , respectively 7-10 h and 32 h, the removal of several compounds, covering different therapeutic classes: analgesics/anti-inflammatories, antibiotics, lipid regulators, diuretics, beta-blockers the former and sulphonamide antibiotics the latter. They correlated observed PhC removal efficiencies to the corresponding PhC half-lives $t_{1/2}$ evaluated on the assumption that a decrease of the concentration through time is proportional to the concentration remaining in the matrix (that is assuming a pseudo-first order kinetic for the degradation). Half-lives were estimated through eq. 2.3

$$t_{1/2} = \frac{ln\,2}{k} \qquad \text{(eq. 2.3)}$$

where k is the loss rate constant calculated according to eq. 2.4, where c is the PhC concentration in the influent (subscript *inf*) and effluent (subscript *eff*).

$$ln(c_{eff} / c_{inf}) = -k\,t \qquad \text{(eq.2.4)}$$

They found that those compounds with a half-live time $t_{1/2}$ less than WWTP HRT generally exhibited high removal efficiencies, concluding that $t_{1/2}$ gives an idea about the required permanence time of the compounds in the biological reactor to ensure an efficient removal of them.

In particular they found three different situations: (*a*) for compounds with high removal efficiency and high degradation rate (low $t_{1/2}$), like ibuprofen, naproxen, salicylic acid, acetaminophen and enalapril; (*b*) for compounds with poor or no elimination and low degradation (high $t_{1/2}$) like carbamazepine, HRT does not influence compound removal; (c) for compounds with medium removal and degradation rate, HRT seems to play a role, as their removal efficiencies were higher when increasing HRT (including famotidine, ranitidine and pravastatin). Gross et al. (2010) conclude that substances that are biodegradable (high k_{biol} or low $t_{1/2}$) and have low Log K_d (low sludge-water distribution coefficient, corresponding to low tendency to adsorb on sewage sludge) are more influenced by HRT, while compounds with high Log K_d and low k_{biol} are more influenced by SRT. However, there are other PhCs like ibuprofen with high k_{biol} and low Log K_d that are well removed independently of HRT and SRT. Based on experimental findings on Canadian WWTPs (SRT from 2 to 10 d), Metcalfe et al. (2003) proposed the following correlation for naproxen and ibuprofen, between HRT and PhC percentage removal η:

$$\eta = 1.735\ e^{0.886\ HRT} \qquad \text{(eq. 2.5)}$$

They conclude that due to high half-lives observed for most of the investigated compounds in WWTP effluents, higher HRT should be required in order to enhance compound degradation.

2.11.1.3 Effect of pH

pH values can also greatly affect the behaviour of PhCs, in particular antibiotics (ciprofloxacin, tetracycline and penicillin G), which possess different functional groups within the same molecule. In fact, under different pH conditions, the molecule can be neutral, cationic, anionic or zwitterionic and so its physical, chemical and biological

properties (sorption, photo-reactivity, antibiotic activity and toxicity) will change accordingly (Kümmerer, 2009b, Cirja et al., 2008). Tadkaew et al. (2010) investigated the effects of mixed liquor pH (pH between 5 and 9) on the removal of trace organics (sulfamethoxazole, carbamazepine, diclofenac, ibuprofen and ketoprofen) by a submerged MBR system. They found that removal efficiencies of ionisable compounds (sulfamethoxazole, diclofenac, ibuprofen and ketoprofen) were strong pH-dependent. At pH 5, the high removal of the ionisable compounds can be due to their speciation behaviour. At this pH, these compounds exist mainly in their hydrophobic form. As a consequence, they could readily adsorb onto the activated sludge, resulting in higher removal efficiencies in comparison to under less acidic conditions in the reactor. Removal efficiencies of the non-ionisable carbamazepine were relatively independent of the mixed liquor pH. These findings are consistent with those by Urase et al. (2005). Watkinson et al. (2007) found a strong pH sensitivity for resulting in the formation of a degraded erythromycin product (erythromycin-H2O) through the loss of a water molecule and the inability to detect the parent erythromycin at pH < 7.

2.11.1.4 Effect of temperature

Biological reactions are greatly affected by temperature, and lower efficiencies have been observed during winter seasons in colder climates (Vieno et al., 2005). Moreover, based on removal data collected on six different large WWTPs in Italy, Castiglioni et al. (2006) found that there are PhCs that present really higher removal efficiencies in summer than in winter: amoxicillin (with a median of 75 % in winter and 100 % in summer), atenolol (10 % and 55 %), bezafibrate (15% and 87 %), enalapril (18 % and 100 %), furosemide (8 % and 54 %), ibuprofen (38 % and 93 %), ranitidine (39 % and 84 %) and sulfamethoxazole (17 % and 71 %). Another group of compounds has similar removal in the two seasons: ciprofloxacin (60 %), hydrochlorotiazide (30 %) and ofloxacin (50 %). Finally a third group has removal efficiencies close to zero in winter and in summer: carbamazepine, clarithromycin, erythromycin and salbutamol.

Hai et al. (2011) investigated the effect of temperature on the removal of selected PhCs contained in a synthetic wastewater fed to a lab scale MBR. They reported that the removal of most hydrophobic compounds (including estrone, ethinyl-estradiol, estradiol and triclosan) was stable during operations under the temperature range of 10-35 °C. On the other hands, for the less hydrophobic compounds (salicylic acid, ketoprofen, naproxen,

metronidazole, ibuprofen, acetaminophen, diclofenac, gemfibrozil, carbamazepine and estriol) a comparatively more pronounced variation between removals in the lower temperature regimes (10-35 °C) was observed. With a few exception, operation at 45 °C clearly exerted detrimental effects on the removal efficiency of the investigated compounds.

However, it is still unclear whether temperature dependence, commonly observed for biological degradation of common pollutants (C, N and P compounds), also applies to the transformation of antibiotics or PhCs in general (Göbel et al., 2007; Tauxe-Wuersch et al., 2005; Ternes, 1998).

2.11.1.5 Effect of Treatment configuration

Nitrifying bacteria have been found capable of co-metabolizing a wide range of persistent compounds like iopromide and trimethoprim (Batt et al., 2006; Perez et al., 2005). Wastewater treatment processes performing a complete biological nutrient removal are characterized by separate zone with aerobic, anoxic and anaerobic conditions to optimize C and N removal that may affect PhCs removal as well (US EPA 2009Zwiener and Frimmel 2003). High removal efficiencies of PhCs have been suggested to occur in WWTPs with high levels of nitrogen removal (Batt et al., 2006; Clara et al., 2005a): Vieno et al. (2007) found that atenolol and sotalol were slightly more efficiently eliminated in the WWTPs where nitrogen removal was greater than 60 % compared with those that removed nitrogen only less than 30 %. Suarez et al. (2010) divided into three groups PhCs with respect to their potential to be removed in biological reactor: highly biodegradable compounds under aerobic and anoxic conditions, including ibuprofene, fluoxetine, natural estrogens; highly biodegradable compounds under aerobic conditions, but persistent in anoxic conditions, inbcluding diclofenac, naproxen, ethinylestradiol, roxythromycin and erythromycin and finally resistant compounds to biological transformations (sulfamethoxazole, trimethoprim, carbamazepine and diazepam).

It is important to remark that low removal efficiencies could also be due to the fact that contaminants are present at *very low* concentrations in the influent, and unavoidable instrumental errors may affect their “observed” removal values. At the other extreme, high removal efficiencies, greater than 99 %, corresponding to a reduction of two orders of magnitude of the influent concentrations, may not be enough to consistently reduce the

PhC concentrations to a low level of risk to aquatic life. For instance if ibuprofen presents an influent concentration at 350 μg/L and 99 % is removed, its final concentration would still amount to 3.5 μg/L, i.e. a consistent mass load discharged by the WWTP, as described below.

2.12 Average daily mass loads of PhCs in secondary effluents

Where possible, to complete this analysis, the average daily mass load, L_i, of each PhC, *i*, in the secondary biological effluent was estimated. L_i was evaluated as the average of mass load $L_{i,j}$ at WWTP *j*, provided by the cited literature or evaluated via eq. 2.6, on the basis of the average effluent concentration $c_{i,j}$ from the WWTP *j*, the average treated flow rate Q_j and the population served by the WWTP *j*. Each mass load is expressed in mg/1000 inhabitants/day.

$$L_{i,j} = \frac{c_{i,j}\ Q_j}{\text{served population}} \text{ x } 1000 \qquad i = \text{generic PhC};\ j = \text{generic WWTP} \qquad \text{(eq. 2.6)}$$

It was possible to evaluate the average mass load of 75 out of 118 compounds, as those WWTPs lacking one or more of the following variables were excluded: effluent concentration, treated flow rate and population served.

The graph in Figure 2.21 reports, in descending order, average mass loads L_i greater than 10 mg/1000 inh/day, and below is a list of the references used in the evaluation.

These findings may be affected by different sources of uncertainty as discussed in Ort and Gujer (2006), for this reason they have to be prudently considered.

The highest average mass loads (greater than 200 mg/1000 inh/d) were found for the antihypertensive hydrochlorothiazide (368 mg/1000 inh/day), the psychiatric drug carbamazepine (364 mg/1000 inh/day), the receptor antagonist cimetidine (332 mg/1000 inh/day) and the beta-blocker atenolol (316 mg/1000 inh/day), followed by the analgesics/anti-inflammatories: naproxen (295), ibuprofen (273), diclofenac (241), ketoprofen (217) and mefenamic acid (211). Antibiotics showed lower average daily mass loads: spyramycin (155), clarithromycin (140), trimethoprim (124), ofloxacin (123), erythromycin (100).

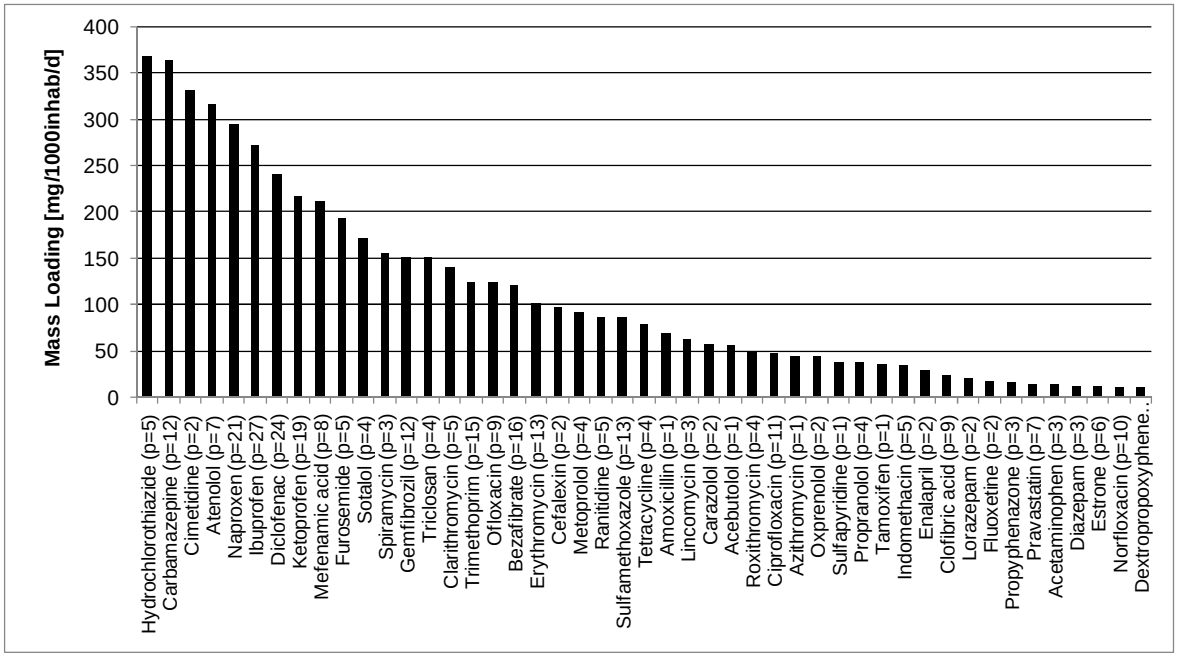


Figure 2.21. Average daily mass loads evaluated for most of the compounds under review. The number p of treatment plants considered in the analysis are shown in brackets after the name in the X-axis. Data from: Baronti et al., 2000; Bendz et al., 2005; Castiglioni et al., 2009; Conti et al., 2011; Gobel et al., 2005; Golet et al., 2003; Gulkulowska et al., 2008; Jones et al., 2007; Karthikejan and Meyer, 2006; Kimura et al., 2007; Lindberg et al., 2005; Lindqvist et al., 2005; Mc Avoy et al., 2002; Nakada et al., 2006; Paxéus et al., 2004; Radjenovic et al., 2007, 2009; Roberts and Tomas., 2006; Santos et al., 2009; Tauxe-Wuerch et al., 2005; Ternes 1998; Ternes et al., 1999, 2003; Vieno et al., 2005, 2007; Xu et al., 2007; Yu et al., 2006; Zorita et al., 2009.

Compounds with average mass loads of less than 10 mg/1000 inh/d (not reported in Fig. 2.21.) were: acetylsalicylic acid, doxycycline, cefotaxime, salbutamol, aminopyrine, glibenclamide, famotidine, loratadine, clotrimazole, phenazone, tylosil, cyclophosphamide, fenofibric acid, norfluoxetine, paroxetine, estradiol, estriol, ethinylestradiol, simvastatin, gabapentin, valproic acid, oxcarbazepine, fenoprofen, sulfamethazine and phenobarbital.

2.13 Environmental risk assessment of secondary biological effluent

The environmental risk posed by the presence of PhCs in water is still under discussion. Safety threshold values have been defined for a limited number of PhCs, but only in single compound-single organism toxicity studies, meaning that mixture effects have not yet been considered.

Moreover, many compounds themselves have not been extensively studied, and, when available, PhC toxicity data tends to refer only to acute rather than chronic effects.

Table 2.7. reports the PNEC values defined for 67 out of the 118 PhCs included in this study, the corresponding assayed species, the endopoint and the literature references.

Conforming to EC (EC, 2003), each of the reported PNECs is 1000 times lower than the toxicity concentration value found for the most sensitive species assayed, so as to take into account the effect on other, potentially more sensitive, aquatic species to those used in toxicity studies.

An evaluation of the environmental risk posed by PhCs in secondary effluent was carried out by means of the risk quotient (RQ), that is the ratio between the average PhC concentrations measured in the secondary effluent and its corresponding PNEC (EMEA, 2001). Average secondary effluent concentrations are reported in brackets after the name of the compounds in the *x*-axis of Figures 2.9.-2.14., and PNEC values are those reported in Table 2.7.

A commonly used ranking criterion was applied, according to De Sousa et al. (2009) and Hernando et al. (2006): RQ < 0.1 low risk to aquatic organisms, 0.1≤ RQ ≤ 1, medium risk; RQ ≥ 1, high risk. The RQ values were found within the range 6.8 x 10^{-6}-37 for the 67 compounds considered; compounds with RQ greater than 0.01 are reported in Fig. 2.22., in descending order. The dotted lines in the graph represent the thresholds defining the three environmental risk levels: high, medium and low.

Table 2.7**.** PNECvalues for the PhCs under investigation and corresponding assayed species.

Compounds	Species Assayed	Test (endpoint)	Toxicity (mg/l)	References	PNEC (µg/L)
Acetaminophen	*Daphnia*	EC50 (24h)	136	Stuer-Lauridsen et al., 2000	**1**
	Daphnia	EC50 (48h)	9.2	Stuer-Lauridsen et al., 2000	
	S. proboscideu	LC50(24h)	29.6	Stuer-Lauridsen et al., 2000	
	Fish	**EC50 ECOSAR**	**1**	**Sanderson et al., 2003**	
	Daphnia	EC50 ECOSAR	42	Sanderson et al., 2003	
	Algae	EC50 ECOSAR	2549	Sanderson et al., 2003	
	Invertebrates	EC50	300	Boillot, 2008	
	Algae	EC50	105	Boillot, 2008	
	Fish	EC50	900	Boillot, 2008	
	Daphnia	EC50 (48h-immobility)	9.2	Kühn et al., 1989	
Acetylsalicylic acid	*Fish*	EC50 ECOSAR	796	Sanderson et al., 2003	**61**
	Daphnia	EC50 ECOSAR	8858	Sanderson et al., 2003	
	Algae	**EC50 ECOSAR**	**61**	**Sanderson et al., 2003**	
	Daphnia	**EC50 ECOSAR**	61	US EPA, 1999	
Aminopyrine	*Fish*	EC50 ECOSAR	3.7	Sanderson et al., 2003	**1.3**
	Daphnia	EC50 ECOSAR	8.3	Sanderson et al., 2003	
	Algae	**EC50 ECOSAR**	1.3	**Sanderson et al., 2003**	
Codeine	*Fish*	EC50 ECOSAR	238	Sanderson et al., 2003	**16**
	Daphnia	**EC50 ECOSAR**	**16**	**Sanderson et al., 2003**	
	Algae	EC50 ECOSAR	23	Sanderson et al., 2003	
Dextropropoxyphene	*Fish*	EC50 ECOSAR	13	Sanderson et al., 2003	**1**
	Daphnia	EC50 ECOSAR	24	Sanderson et al., 2003	
	Algae	**EC50 ECOSAR**	**1**	**Sanderson et al., 2003**	
Diclofenac	*Fish*	EC50 ECOSAR	532	Sanderson et al., 2003	**9.7**
	Daphnia	EC50 ECOSAR	5057	Sanderson et al., 2003	
	Algae	EC50 ECOSAR	2911	Sanderson et al., 2003	
	Daphnia	EC50 (48h-mortality)	22.4	Ferrari et al., 2004	
	Algae	EC50 (96h-growth)	16.3	Ferrari et al., 2004	
	Bacteria	EC50 (30 min-luminescence)	11.4	Ferrari et al., 2004	
	Bacteria	**EC50 (15min-inhibition)**	**9.7**	**Ra et al., 2008**	
	Microtox	EC50 (30min)	11.45	Ferrari et al., 2003	
	Daphnia	EC50 (48h)	22.43	Ferrari et al., 2003	
	C. dubia	EC50 (48h)	22.7	Ferrari et al., 2003	

Compounds	Species Assayed	Test (endpoint)	Toxicity (mg/l)	References	PNEC (µg/L)
	Algae	EC50 (96h-growth)	14.5	Ferrari et al., 2004	
	Invertebrates	EC50	90	Boillot, 2008	
	Algae	EC50-inhibition	72	Cleuvers,2004	
	Daphnia	EC50-immobilization	68	Cleuvers, 2004	
Ibuprofen	*Fish*	EC50 ECOSAR	5	Sanderson et al., 2003	**1.65**
	Daphnia	EC50 ECOSAR	38	Sanderson et al., 2003	
	Algae	EC50 ECOSAR	26	Sanderson et al., 2003	
	Bacteria	EC50 (15min-inhibition)	37.5	Ra et al., 2008	
	Bacteria	EC50 (15min)	12.1	Farré et al., 2001	
	Daphnia	EC50 (48h)	9.06	Halling-Sørensen et al., 1998	
	Invertebrates	**EC50 (96h)**	**1.65**	**Quinn et al., 2008**	
	Invertebrates	EC50	100	Boillot, 2008	
	Algae	EC50	500	Boillot, 2008	
	Fish	EC50	110	Boillot, 2008	
	Algae	EC50-inhibition	342.2	Cleuvers, 2004	
	Daphnia	EC50-immobilization	101.2	Cleuvers, 2004	
Indomethacin	***Fish***	**EC50 ECOSAR**	**3.9**	**Sanderson et al., 2003**	**3.9**
	Daphnia	EC50 ECOSAR	26	Sanderson et al., 2003	
	Algae	EC50 ECOSAR	18	Sanderson et al., 2003	
Ketoprofen	*Fish*	EC50 ECOSAR	32	Sanderson et al., 2003	**15.6**
	Daphnia	EC50 ECOSAR	248	Sanderson et al., 2003	
	Algae	EC50 ECOSAR	164	Sanderson et al., 2003	
	Bacteria	**EC50 (15min)**	**15.6**	**Farré et al., 2001**	
Mefenamic acid		**EC50 ECOSAR**	**0.43**	**Jones et al. 2002**	**0.43**
Naproxen	*Fish*	EC50 ECOSAR	34	Sanderson et al., 2003	**2.62**
	Daphnia	EC50 ECOSAR	15	Sanderson et al., 2003	
	Algae	EC50 ECOSAR	22	Sanderson et al., 2003	
	Algae	EC50-inhibition	626	Cleuvers, 2004	
	Invertebrates	LC50(96h)	22.4	Quinn et al., 2008	
	Bacteria	EC50(15min)	21.2	Farré et al., 2001	
	Invertebrates	**EC50(96h)**	**2.62**	**Quinn et al., 2008**	
	Invertebrates	EC50	150	Boillot, 2008	
	Fish	EC50	600	Boillot, 2008	
	Daphnia	EC50-immobilization	166.3	Cleuvers, 2004	

Compounds	Species Assayed	Test (endpoint)	Toxicity (mg/l)	References	PNEC (µg/L)
Phenazone	*Fish*	EC50 ECOSAR	3	Sanderson et al., 2003	**1.1**
	Daphnia	EC50 ECOSAR	6.7	Sanderson et al., 2003	
	Algae	**EC50 ECOSAR**	**1.1**	**Sanderson et al., 2003**	
Propyphenazone	***Fish***	**EC50 ECOSAR**	**0.8**	**Sanderson et al., 2003**	**0.8**
	Daphnia	EC50 ECOSAR	3.5	Sanderson et al., 2003	
	Algae	EC50 ECOSAR	1	Sanderson et al., 2003	
Salicylic acid	***Fish***	**EC50 ECOSAR**	**1.28**	**Sanderson et al., 2003**	**1.28**
	Daphnia	EC50 ECOSAR	59	Sanderson et al., 2003	
	Algae	EC50 ECOSAR	48	Sanderson et al., 2003	
	Invertebrates	EC50 (48h)	1147	Marques et al., 2004	
	Invertebrates	LC50 (48h)	112	Han et al., 2006	
	Algae	EC50 (48h)	>100	Henschel et al., 1997	
	Bacteria	EC50 (15min)	43.1	Farré et al., 2001	
Tolfenamic acid	***Fish***	**EC50 ECOSAR**	**0.4**	**Sanderson et al., 2003**	**0.4**
	Daphnia	EC50 ECOSAR	1.7	Sanderson et al., 2003	
	Algae	EC50 ECOSAR	1.3	Sanderson et al., 2003	
Amoxicillin			0.1	Kümmerer et al., 2003	**0.0037**
	Algae	**EC 50**	**0.0037**	**Halling-Sørensen, 2000**	
Azithromycin			**0.15**	**Kümmerer et al., 2003**	**0.15**
Cefaclor	*Algae*	EC50 ECOSAR	734.05	Lee et al., 2008	**687.42**
	Daphnia	**EC50 ECOSAR**	**687.42**	**Lee et al., 2008**	
	Fish	EC50 ECOSAR	11524	Lee et al., 2008	
Cefalexin			**2.5**	**Kümmerer et al., 2003**	**2.5**
Cefotaxime			**0.04**	**Kümmerer et al., 2003**	**0.04**
Chloramphenicol			**1.6**	**Kümmerer et al., 2003**	**1.6**
Ciprofloxacin	*Fish*	EC50 ECOSAR	246000	Sanderson et al., 2003	**938**
	Daphnia	EC50 ECOSAR	991	Sanderson et al., 2003	
	Algae	**EC50 ECOSAR**	**938**	**Sanderson et al., 2003**	
Clarithromycin	*Invertebrates*	EC50	20	Boillot, 2008	**0.07**
	Algae	**EC50**	**0.07**	**Boillot, 2008**	
Clindamycin			**0.5**	**Kümmerer et al., 2003**	**0.5**
Doxycycline			**0.3**	**Kümmerer et al., 2003**	**0.3**
			316	Brain et al.,2004	
Enoxacin			**0.15**	**Kümmerer et al., 2003**	**0.15**

Compounds	Species Assayed	Test (endpoint)	Toxicity (mg/l)	References	PNEC (µg/L)
Erythromycin	*Fish*	EC50 ECOSAR	61	Sanderson et al., 2003	**0.02**
	Daphnia	EC50 ECOSAR	7.8	Sanderson et. al., 2003	
	Algae	EC50 ECOSAR	4.3	Sanderson et. al., 2003	
	Invertebrates	EC50	15	Boillot, 2008	
	Algae	**EC50**	**0.02**	**Boillot, 2008**	
	Fish	EC50	900	Boillot, 2008	
Lincomycin	*Fish*	EC50 ECOSAR	1391	Sanderson et al., 2003	**82**
	Daphnia	**EC50 ECOSAR**	**82**	**Sanderson et. al., 2003**	
	Algae	EC50 ECOSAR	86	Sanderson et. al., 2003	
Metronidazole			**2.5**	**Kümmerer et al., 2003**	**2.5**
	Algae	EC50	39.1	Halling-Sørensen, 2000	
	Algae	EC50	40.4	Halling-Sørensen, 2000	
Norfloxacin	***Algae***	**EC50**	**15**	**Boillot, 2008**	**15**
Ofloxacin	***Algae***	**EC50 (96h- growth)**	**0.016**	**Ferrari et al., 2004**	**0.016**
	Invertebrates	EC50	30	Boillot, 2008	
	Algae	EC50	1.5	Boillot, 2008	
	Fish	EC50	10	Boillot, 2008	
Oxytetracycline	***Algae***	**EC50**	**0.207**	**Halling-Sørensen, 2000**	**0.207**
	Fish	EC50 ECOSAR	166000	Sanderson et al., 2003	
	Daphnia	EC50 ECOSAR	2432	Sanderson et al., 2003	
	Algae	EC50 ECOSAR	2294	Sanderson et al., 2003	
	Invertebrates	EC50 (96h)	40.13	Quinn et al., 2008	
Penicillin G	***Algae***	**EC50**	**0.006**	**Halling-Sørensen, 2000**	**0.006**
Penicillin V	***Daphnia***	**EC50**	**177**	**Jones et al., 2002**	**177**
Roxythromycin	*Fish*	EC50 ECOSAR	50	Sanderson et al., 2003	**4**
	Daphnia	EC50 ECOSAR	6	Sanderson et al., 2003	
	Algae	**EC50 ECOSAR**	**4**	**Sanderson et al., 2003**	
Sulfachloropyridazine	*Bacteria*	**EC 50(15 min-florescence)**	**26.4**	**Kim et al., 2007**	**26.4**
Sulfadiazine			5	Kümmerer et al., 2003	**0.135**
	Algae	**EC50**	**0.135**	**Halling-Sørensen, 2000**	
Sulfadimethoxine	*Fish*	EC50 ECOSAR	226	Sanderson et al., 2003	**3.5**
	Daphnia	**EC50 ECOSAR**	**3.5**	**Sanderson et al., 2003**	
	Algae	EC50 ECOSAR	24	Sanderson et al., 2003	
Sulfamethoxazole	*Fish*	EC50 ECOSAR	890	Sanderson et al., 2003	**0.027**

Compounds	Species Assayed	Test (endpoint)	Toxicity (mg/l)	References	PNEC (µg/L)
	Daphnia	EC50 ECOSAR	4.5	Sanderson et al., 2003	
	Algae	EC50 ECOSAR	51	Sanderson et al., 2003	
	Fish	EC50 (96 h)	563	Kim et al., 2007	
	Daphnia	EC50 (48 h-mortality)	>100	Ferrari et al., 2004	
	Bacteria	EC50 (15 min)	78.1	Kim et al., 2007	
	Algae	EC50 (96 h-growth)	0.15	Ferrari et al., 2004	
	Algae	**EC50 (96 h- growth)**	**0.027**	**Ferrari et al., 2004**	
Sulfapyridine	***Invertebrates***	**EC50 (96h)**	**21.61**	**Quinn et al., 2008**	**21.61**
Sulfathiazole	*Daphnia*	**EC50 (96h-immobility)**	**85.4**	**Kim et al., 2007**	**85.4**
Tetracycline			0.3	Kümmerer et al., 2003	**0.09**
	Algae	**EC50**	**0.09**	**Halling-Sørensen, 2000**	
Trimethoprim	*Fish*	EC50 ECOSAR	795	Sanderson et al., 2003	**2.6**
	Daphnia	EC50 ECOSAR	4.8	Sanderson et al., 2003	
	Algae	**EC50 ECOSAR**	**2.6**	**Sanderson et al., 2003**	
	Bacteria	EC50 (15min)	177	Kim et al., 2007	
	Daphnia	EC50 (96h-immobility)	121	Kim et al., 2007	
	Invertebrates	LC50 (96h)	>100	Quinn et al., 2008	
	Fish	EC50 (48h)	>100	Kim et al., 2007	
	Invertebrates	EC50	110	Boillot, 2008	
	Algae	EC50	90	Boillot, 2008	
	Fish	EC50	100	Boillot, 2008	
Diltiazem	*Daphnia*	EC50 (96 h-immobility)	8.2	Kim et al., 2007	**1.9**
	Fish	EC50 ECOSAR	23	Sanderson et al., 2003	
	Daphnia	EC50 ECOSAR	2.9	Sanderson et al., 2003	
	Algae	**EC50 ECOSAR**	**1.9**	**Sanderson et al., 2003**	
Atenolol	***Invertebrates***	**EC50**	**30**	**Boillot, 2008**	**30**
Metoprolol	*Fish*	EC50 ECOSAR	116	Sanderson et al., 2003	**8**
	Daphnia	**EC50 ECOSAR**	**8**	**Sanderson et al., 2003**	
	Algae	EC50 ECOSAR	14	Sanderson et al., 2003	
	Invertebrates	LC50 (48h)	>100	Huggett et al., 2002	
	Invertebrates	LC50 (48h)	8.8	Huggett et al., 2002	
	Invertebrates	LC50 (48h)	63.9	Huggett et al., 2002	
	Fish	LC50 (48h)	>100	Huggett et al., 2002	
Nadolol	***Invertebrates***	**EC50**	**110**	**Boillot, 2008**	**110**

Compounds	Species Assayed	Test (endpoint)	Toxicity (mg/l)	References	PNEC (µg/L)
Propranolol	*Fish*	EC50 ECOSAR	29.5	Sanderson et al., 2003	**0.244**
	Daphnia	EC50 ECOSAR	2.3	Sanderson et al., 2003	
	Algae	EC50 ECOSAR	5.5	Sanderson et al., 2003	
	Bacteria	EC50 (30min-luminescence)	61	Ferrari et al., 2004	
	Algae	EC50 (48h)	0.7	Cleuvers, 2005	
	Diatoms	**EC50 (96 h- growth)**	**0.244**	**Ferrari et al., 2004**	
	Invertebrates	LC50 (48h)	29.8	Huggett et al., 2002	
	Invertebrates	LC50 (48h)	0.8	Huggett et al., 2002	
	Invertebrates	LC50 (48h)	1.6	Huggett et al., 2002	
	Fish	LC50 (48h)	24.3	Huggett et al., 2002	
	Invertebrates	EC50	11	Boillot, 2008	
	Algae	EC50	0.8	Boillot, 2008	
	Fish	EC50	20	Boillot, 2008	
Timolol	*Fish*	EC50 ECOSAR	126	Sanderson et al., 2003	**9**
	Daphnia	**EC50 ECOSAR**	**9**	**Sanderson et al., 2003**	
	Algae	EC50 ECOSAR	15.5	Sanderson et al., 2003	
Bezafibrate	***Fish***	**EC50 ECOSAR**	**5.3**	**Sanderson et al., 2003**	**5.3**
	Daphnia	EC50 ECOSAR	25	Sanderson et al., 2003	
	Algae	EC50 ECOSAR	18	Sanderson et al., 2003	
	Invertebrates	EC50	50	Boillot, 2008	
Clofibrate	*Fish*	EC50 ECOSAR	5	Sanderson et al., 2003	**0.5**
	Daphnia	EC50 ECOSAR	6.5	Sanderson et al., 2003	
	Algae	**EC50 ECOSAR**	**0.5**	Sanderson et al., 2003	
Clofibric acid	*Fish*	EC50 ECOSAR	53	Sanderson et al., 2003	**40.2**
	Daphnia	EC50 ECOSAR	293	Sanderson et al., 2003	
	Algae	EC50 ECOSAR	192	Sanderson et al., 2003	
	Algae	EC50 (96h-growth)	94	Ferrari et al., 2004	
	Bacteria	EC50 (30min)	91.8	Ferrari et al., 2003	
	Invertebrates	EC50 (48h)	83.5	Rosal et al., 2009	
	Invertebrates	EC50 (48h)	72	Cleuvers, 2003	
	Microtox	EC50 (30min)	91.8	Ferrari et al., 2003	
	Algae	**EC50 (96h- growth)**	**40.2**	**Ferrari et al., 2004**	
Fenofibrate	*Fish*	EC50 ECOSAR	0.8	Sanderson et al., 2003	**0.1**
	Daphnia	EC50 ECOSAR	0.35	Sanderson et al., 2003	

Compounds	Species Assayed	Test (endpoint)	Toxicity (mg/l)	References	PNEC (µg/L)
	Algae	**EC50 ECOSAR**	**0.1**	**Sanderson et al., 2003**	
Fenofibric acid	***Fish***	**EC50 ECOSAR**	**7.6**	**Sanderson et al., 2003**	**7.6**
	Daphnia	EC50 ECOSAR	38	Sanderson et al., 2003	
	Algae	EC50 ECOSAR	26	Sanderson et al., 2003	
Gemfibrozil	***Fish***	**EC50 ECOSAR**	**0.9**	**Sanderson et al., 2003**	**0.9**
	Daphnia	EC50 ECOSAR	6	Sanderson et al., 2003	
	Algae	EC50 ECOSAR	4	Sanderson et al., 2003	
	Bacteria	EC50 (15 min)	35.3	Rosal et al., 2009	
	Bacteria	EC50 (15 min)	18.8	Farré et al., 2001	
	Invertebrates	EC50 (48h)	10.4	Han et al., 2006	
	Invertebrates	EC50 (96h)	1.18	Quinn et al., 2008	
Pravastatin	***Fish***	**EC50**	**1.8**	**Ginebreda et al., 2010**	**1.8**
Carbamazepine	*Fish*	EC50 ECOSAR	101	Sanderson et al., 2003	**13.8**
	Daphnia	EC50 ECOSAR	111	Sanderson et al., 2003	
	Algae	EC50 ECOSAR	70	Sanderson et al., 2003	
	Algae	EC50 (3days)	74	Cleuvers, 2003	
	Bacteria	EC50 (15min)	52.2	Kim et al., 2007	
	Fish	EC50 (48h)	35.4	Kim et al., 2007	
	Daphnia	**EC50 (48h-mortality)**	**13.8**	**Ferrari et al., 2004**	
	Diatoms	EC50 (96h- growth)	31.6	Ferrari et al., 2004	
	C. dubia	EC50 (48h)	77.7	Ferrari et al., 2003	
Diazepam	*Fish*	EC50 ECOSAR	28	Sanderson et al., 2003	**2**
	Daphnia	**EC50 ECOSAR**	**2**	**Sanderson et al., 2003**	
	Algae	EC50 ECOSAR	5.5	Sanderson et al., 2003	
	Invertebrates	EC50	90	Boillot, 2008	
	Algae	EC50	12	Boillot, 2008	
	Fish	EC50	11	Boillot, 2008	
Fluoxetine	*Fish*	EC50 ECOSAR	1.7	Sanderson et al., 2003	**0.05**
	Daphnia	EC50 ECOSAR	0.17	Sanderson et al., 2003	
	Algae	EC50 ECOSAR	0.8	Sanderson et al., 2003	
	Invertebrates	EC50	0.9	Boillot, 2008	
	Algae	**EC50**	**0.05**	**Boillot, 2008**	
	Fish	EC50	2	Boillot, 2008	
Cimetidine	*Fish*	EC50 ECOSAR	571	Sanderson et al., 2003	**35**

Compounds	Species Assayed	Test (endpoint)	Toxicity (mg/l)	References	PNEC (µg/L)
	Daphnia	**EC50 ECOSAR**	**35**	**Sanderson et al., 2003**	
	Algae	EC50 ECOSAR	40	Sanderson et al., 2003	
	Daphnia	EC50 (96h-immobility)	271.3	Kim, 2007	
Ranitidine	*Fish*	EC50 ECOSAR	1076	Sanderson et al., 2003	
	Daphnia	**EC50 ECOSAR**	**63**	**Sanderson et al., 2003**	**63**
	Algae	EC50 ECOSAR	66	Sanderson et al., 2003	
Clenbuterol	*Fish*	EC50 ECOSAR	30	Sanderson et al., 2003	
	Daphnia	**EC50 ECOSAR**	**2**	**Sanderson et al., 2003**	**2**
	Algae	EC50 ECOSAR	10	Sanderson et al., 2003	
Fenoterol	*Fish*	EC50 ECOSAR	20	Sanderson et al., 2003	
	Daphnia	**EC50 ECOSAR**	**17.5**	**Sanderson et al., 2003**	**17.5**
	Algae	EC50 ECOSAR	25	Sanderson et al., 2003	
Terbutaline	***Fish***	**EC50 ECOSAR**	**1.05**	**Sanderson et al., 2003**	
	Daphnia	EC50 ECOSAR	27	Sanderson et al., 2003	**1.05**
	Algae	EC50 ECOSAR	32	Sanderson et al., 2003	
Cyclophosphamide	*Fish*	EC50 ECOSAR	70	Sanderson et al., 2003	
	Daphnia	EC50 ECOSAR	1795	Sanderson et al., 2003	**11**
	Algae	**EC50 ECOSAR**	**11**	**Sanderson et al., 2003**	
Ifosfamide	*Fish*	EC50 ECOSAR	140	Sanderson et al., 2003	
	Daphnia	EC50 ECOSAR	1795	Sanderson et al., 2003	**11**
	Algae	**EC50 ECOSAR**	**11**	**Sanderson et al., 2003**	
Iopromide	*Fish*	EC50 ECOSAR	865000	Sanderson et al., 2003	
	Daphnia	EC50 ECOSAR	766000	Sanderson et al., 2003	**370000**
	Algae	**EC50 ECOSAR**	**370000**	**Sanderson et al., 2003**	

As seen in Fig. 2.22., fourteen compounds pose a high risk: 7 antibiotics (erythromycin, ofloxacin, sulfamethoxazole, clarithromycin, amoxicillin, tetracycline and azithromycin), 2 psychiatric drugs (fluoxetine and diazepam), 2 analgesics-anti/inflammatories (ibuprofen and mefenamic acid) and 3 lipid regulators (fenofibric acid, fenofibrate and gemfibrozil). A medium risk is posed by twenty compounds: 7 analgesic-anti/inflammatories (acetaminophene, aminopyrine, naproxen, phenazone, salicylic acid, codeine and dextropropoxyphene), 8 antibiotics (penicillin G, sulfadiazine, cefotaxime, enoxacin, trimethoprim, doxycycline, roxithromycin and metronidazole), 2 beta-blockers (propranolol and atenolol), 2 lipid regulators (clofibrate and bezafibrate) and 1 receptor antagonist (cimetidine). For the remaining 17 compounds included in Fig. 2.22., the environmental risk is considered low, as is that of the 16 PhCs excluded from the graph due to an RQ of less than 10^{-2} (clindamycin, ranitidine, acetylsalicylic acid, clofibric acid, timolol, norfloxacin, sulfachloropyridazine, fenoterol, cyclophosphamide, ciprofloxacin, lincomycin, nadolol, sulfathiazole, penicillin V, cefaclor, iopromide).

Comparison of Figures 2.21. and 2.22. shows that the top compounds are not the same in the two rankings, with the exception of the two analgesics/anti-inflammatories ibuprofen and mefenamic acid. Compounds of different classes had the highest mass loads: the antihypertensive hydrochlorothiazide, the psychiatric drug carbamazepine, the receptor antagonist cimetidine, the beta-blocker atenolol and 5 analgesics/anti-inflammatories (naproxen, ibuprofen, diclofenac, ketoprofen and mefenamic acid), many of which are administered frequently and/or over long periods of time. In contrast, the highest risk is posed the 12 compounds cited just above belonging to the groups of antibiotics, lipid regulators and analgesics/anti-inflammatories. This fact onfirms the results obtained by other Authors (among them Escher et al., 2011) that high consumption does not mean high risk for the environment.

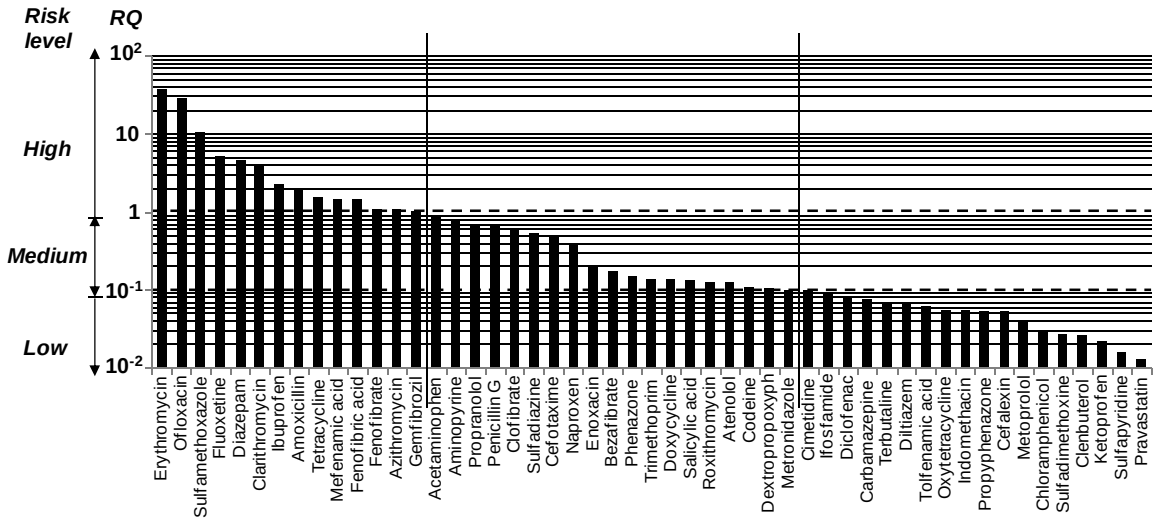


Figure 2.22. RQ of the investigated compounds

2.14 Conclusion

Most of the municipal WWTPs consist of preliminary, primary and secondary treatments (mainly activated sludge systems) with the final effluent being discharged into a surface water body and often indirectly reused for irrigation purposes or recreational activities. The present study shows that many PhCs are usually present in raw influent at concentrations in the range 10^{-3}-10^{2} μg/L and even more, and that common WWTPs are not able to efficiently remove all of them. Observed removal efficiencies vary in a wide range for the different compounds, as well as for the same substance, due to the different chemical and physical characteristics of PhCs and to operational conditions (mainly aerobic, anaerobic, anoxic reactors, SRT, pH and water temperature) as discussed above. MBRs seem (only 20 pilot plants were investigated and a limited number of PhCs were tested) to guarantee higher removal effciencies for most compounds and a better quality of the permeates with respect to CAS.

This study highlights the fact that the occurrence of some PhCs in the secondary effluent discharged into surface water bodies may pose a medium–high (acute) risk to aquatic life. Furthermore, many other compounds, even if their environmental risk was found to be low, are discharged at high daily mass loads, which could contribute to negative effects on aquatic organisms in the long term due to chronic and mixture toxicity.
For these reasons, it would be more prudent to begin monitoring the most frequently and most persistent administered PhCs, as well as those with the highest environmental risk, namely antibiotics (including erythromycin, ofloxacin, sulfamethoxazole, clarithromycin,

amoxicillin, tetracycline and azithromycin), psychiatric drugs (like fluoxetine, diazepam and carbamazepine), analgesics/anti-inflammatories (ibuprofen, mefenamic acid, naproxen, diclofenac and ketoprofen) and lipid regulators (fenofibric acid, fenofibrate and gemfibrozil).

Chapter 3: Hospital effluent: Investigation of the concentrations and distribution of PhCs and environmental risk assessment

3.1 Introduction

3.1.1 Hospital Wastewaters (HWWs)

During recent years, the issue of PhCs in WWs has become a major concern in terms of both human health and the environment. This has prompted the launch of several monitoring studies into the most commonly administered compounds in UWWs (Lishman et al., 2006; Santos et al., 2007; Terzic et al., 2009) and surface water (Kolpin et al., 2002). However, a considerably smaller number of studies have been devoted to characterizing PhCs sources, mainly hospital effluents (Boillot et al., 2008; Kosma et al., 2010; Kummerer 2001; Sim et al., 2011). In fact, in quite all countries worldwide, no distinction is usually made between these WWs and urban effluent, and they, along with their potentially hazardous loads, are generally discharged directly into the public sewage network and conveyed for co-treatment at the nearest municipal WWTP.

Nonetheless, considering the multiple research and laboratory activities carried out in these structures, as well as the treatments performed and pharmaceuticals administered and excreted within them, a wide range of concentrations of hazardous substances may be present in hospital effluent (Verlicchi et al., 2010b). HWWs are composed of the effluents of different services: kitchen, internal laundry, heating and cooling systems, laboratories, radiology departments, outpatients departments, transfusion centres and wards. Due to the nature and quantity of the micro-pollutants they harbour, such as active substances of medicines and their metabolites, chemicals, heavy metals, disinfectants, sterilizers, and radioactive markers, which are typically present at concentrations of $\mu g\ L^{-1}$, they should be earmarked for special consideration. Previous studies investigated the occurrence in hospital effluents of detergents, disinfectants, organic compounds (alcohols, acetone, formaldehyde, acetaldehyde, phenols) and several metals (Emmanuel et al., 2005; Boillot et al., 2008) and the proliferation of drug-resistant microorganisms (Hawkshead 2008). The issue of PhC occurrence in hospital effluents has already been investigated by different Authors, among them Thomas et al., 2007a; Gomez et al., 2006, Mahnik et al., 2007, Suarez et al., 2009, Kummerer, 2001.

It would therefore be of interest to discover the percentage contributions of PhCs from hospitals to those in the total municipal WWTP influent, in order to discover whether specific treatments for hospital effluent are necessary to reduce environmental

contamination by persistent and hazardous micropollutants. To date, however, very little data on this topic has been reported in the literature (Beier et al. 2011; Heberer and Feldman, 2005; Langford and Thomas, 2009; Ort et al., 2010a; Thomas et al., 2007a), and those studies have been conducted to a limited number of compounds.

In order to investigate the differences between hospital and urban wastewaters, an assessment of the (acute and chronic) risk posed to aquatic organisms by the two effluents would be advisable. In fact, although the ecotoxicological effect of PhCs in treated UWWs has been investigated (Ferrari et al., 2003; Kostich et al., 2008), once again, very little data is available regarding hospital effluent, and what is available generally relies on predicted, rather than measured, concentrations (Escher et al., 2011).

3.1.2 Ecotoxicity of HWWs

HWWs is often assumed to be the most toxic to aquatic life and there are indeed several studies in which genotoxic activity of HWWs has been confirmed. Guiliani et al. (1996). found that out of over 800 hospital effluent samples from a large cancer hospital 13% were genotoxic in the umuC assay. Genotoxic samples were detected throughout a 24-h period with the morning hours showing the highest activity. of the toxic wastewater samples 96% showed genotoxic potential without detectable cytotoxic effects. the authors considered that anti-neoplastic agents were the possible causative agents however they concluded that there was no obvious pollution hazard attributable to the waste because no genotoxic activity was detected in the influx of the sewage treatment plant(STP) receiving the wastewater of the hospital. Steger-Hartmann et al. (1997) have tried to identify the causal agents of genotoxicity activity in HWWs investigating the effects of cyclophosphamide in the umuC assay. They found that there were no genotoxic effects at a concentration of 1 g L^{-1}. this was in agreement with the SOS chromotest in which Hellmèr and Bolcsfoldi (1992). did not detect a genotoxic effect of cyclophosphamide at concentration of up to 4.6 g L^{-1}. Hartmann et al.(1998) has found evidence to suggest that one single class of antibiotic drug, the fluoroquinolone antibiotics (e.g. ciprofloxacin) were responsible for the genotoxic activity for a specific hospital under investigation. Recently the toxicological effects of PhCs in HWWs has been performed by PILLS (2012), and as a results, raw HWWs was found to be moderately cytotoxic, estrogenic and toxic to various test organisms compared to municipal WW.

3.1.3 Antibiotic resistant bacteria in HWWs

The widespread use of antibiotics in medicine and in intensive animal husbandry is indicative of the selection pressure exerted on bacteria (Klare et al. 1995). Although antibiotics have been used in large quantities for some decades, the existence of these substances in the environment has received little notice until recently. In the last years a more complex investigation of antibiotic has been undertaken in different countries in order to assess their environmental risks. It has been found that the concentrations of antibiotics are higher in hospital effluent than in municipal wastewater which are higher than in different surface waters, ground water and sea water (Kümmerer 2001).

Bacteria have developed different mechanisms to render ineffective the antibiotics used against them. The genes encoding these defence mechanisms are located on the bacterial chromosome or on extrachromosomal plasmids, and are transmitted to the next generation (vertical gene transfer). Genetic elements, such as plasmids, can also be exchanged among bacteria of different taxonomic affiliation (horizontal gene transfer) (Davison 1999). Horizontal gene transfer by conjugation is common in nature, or in technical systems, where the density of bacteria is high and so, accordingly, is the chance of two suitable bacterial cells coming close to each other (Muela et al. 1994).

Figure 3.1. shows the range of the measured concentrations of resistant integrons and the proportion of bacteria with resistant integrons in HWWs, domestic wastewater and in two rivers. As antibiotic resistant integrons are embedded on mobile genetic elements generally present in further copies, the relative abundance can be higher than 100%. Specialised medical centres with geriatric and psychiatric activities were not sources of bacteria harbouring resistant integrons. The elevated concentrations and relative abundance of hospital effluents (5 to 390%) when compared to the investigated rivers (0.6 to 1.9%) showed that the hospitals are a potential source of multidrug resistant bacteria. Furthermore, with regards to the relative abundance found in municipal waste water (13%) hospital effluents can be seen as a hotspot for antibiotic resistant bacteria (PILLS 2012).

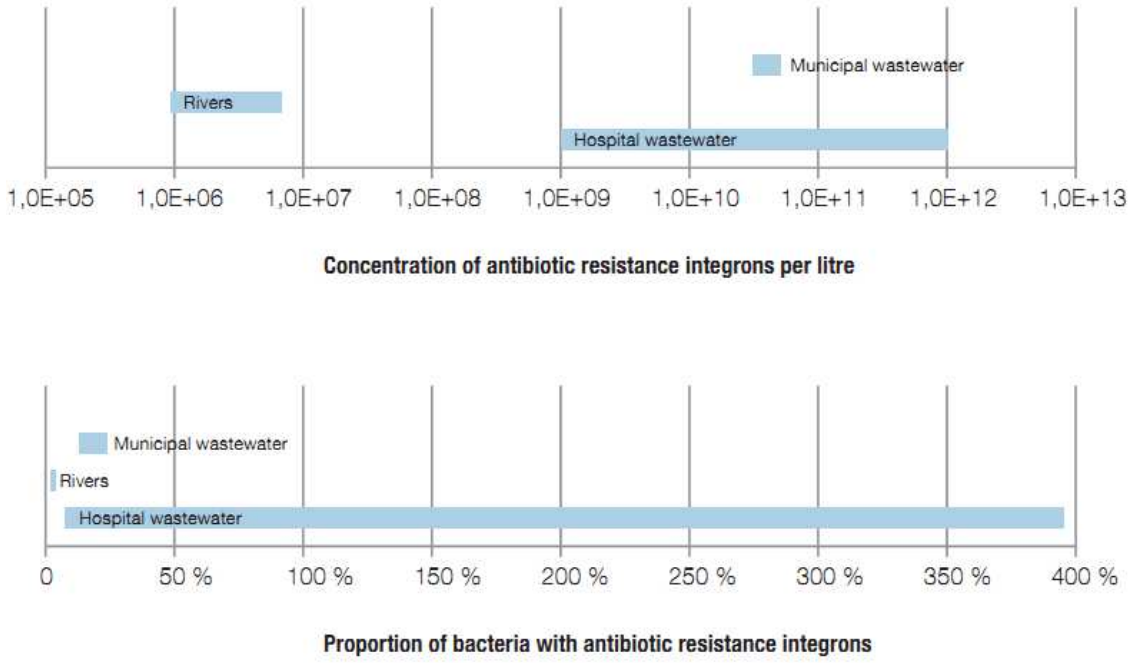


Figure 3.1. Concentration of antibiotic resistance integrons and proportion of bacteria with antibiotic resistance integrons in hospital waste water in comparison to municipal waste water and river water(adapted from PILLS 2012).

3.1.4 Hospitals as a point source of PhCs

The aims of this chapter were to investigate the occurrence of 73 common PhCs from 12 different therapeutic classes in the effluent of two hospitals (medium-sized and large) in the province of Ferrara, north Italy, and in the influent and effluent of the local municipal WWTP, which also receives and co-treats the wastewater from the larger hospital. In particular : (i) to compare the PhC concentrations discharged by the two hospitals over the same period, (ii) to evaluate the PhCs discharged by the large hospital over two different periods, (iii) to compare these concentrations with those found in the influent to the WWTP during the same period, (iv) to evaluate the contribution, in terms of the compounds detected, of the large hospital to the total influent to the WWTP, and finally (v) to assess and compare the potential environmental risk of hospital effluent and WWTP influent by mean of RQ. In this way, this study attempts to provide an initial assessment of these issues with a view to comparing the chemical and ecotoxicological characteristics of hospital effluent with those of the influent to the WWTP charged with co-treating hospital wastewater.

3.2 Experimental materials and methods

3.2.1 Hospitals and WWTP under Investigation

3.2.1.1 Lagosanto hospital (Hospital A)

It is a medium-sized hospital with 300 beds, 650 members of staff and twelve main wards. It is situated in the town of Lagosanto (5000 inhabitants), 30 km from Ferrara, in a coastal area that is densely populated in summertime due to tourist influx (in the peak months of July and August, the population is seven times higher than the resident one). Hospital flow rate is regularly monitored by the internal Water and Wastewater Network Managing Body. The resulting average flow rate is equal to 160 m^3 d^{-1}, corresponding to a specific water consumption of about 550 L bed^{-1} d^{-1}.

3.2.1.2 Ferrara hospital (Hospital B)

It is a large hospital with 900 beds, 2000 members of staff and a total of over 50 wards and departments. It is located in the centre of the city of Ferrara (135,000 inhabitants) and its effluent is directly discharged into the combined sewage network, conveyed to the Ferrara WWTP and co-treated with the urban WWs. Ferrara Hospital flow rate is regularly monitored by the internal Water and Wastewater Network Managing Body. The resulting average flow rate is equal to 603 m^3 d^{-1}, corresponding to a specific water consumption of about 670 L bed^{-1} d^{-1}, and its bed density, that is the number of beds per 1000 inhabitants, is roughly 6.5.

3.2.1.3 Ferrara WWTP

Designed for 120 000 population equivalent (pe), it performs preliminary treatments (screening and grit removal), a biological treatment and a final NaClO disinfection step. The biological treatment consists of a conventional activated sludge system including denitrification (V = 4000 m^3) and nitrification (V = 6100 m^3) steps, followed by secondary sedimentation (V = 6000 m^3) Figure 3.2. It operates at a low-to-medium load, at an average hydraulic retention time of 6 h, a sludge age of 8 d and a mixed liquor concentration of approximately 3.5 kg m^{-3}. The WWTP influent flow rate is on average 28 000 m^3 d^{-1}, and Hospital B contributes roughly 2 % of the influent hydraulic load.

Figure 3.2. WWTP of Ferrara

3.2.2 Target Compounds

The 73 PhCs under investigation are reported in Table 3.1., grouped according to their therapeutic class. These compounds were selected due to their high prescription rates or volumes, the availability of a reliable analysis methods (Gros et al., 2006), as well as due to their occurrence and ubiquity in the aquatic environment (Bell et al., 2011; Daughton and Ternes, 1999, Fatta-Kassinos et al., 2011, Pal et al., 2010). The selected compounds represent the most consumed within their corresponding therapeutical class. It is quite evident that analgesics and anti-inflammatories are the groups most investigated, followed by beta-blockers and lipid regulators.

Table 3.1. Investigated pharmaceutical compounds grouped according to therapeutic class.

	THERAPEUTIC CLASS	COMPOUNDS
A	Analgesics / Anti-inflammatories	Acetaminophen, Codeine, Diclofenac, Ibuprofen, Indomethacin, Ketoprofen, Mefenamic acid, Naproxen, Phenazone, Phenylbutazone, Propyphenazone, Salicylic acid
B	Antibiotics	Azithromycin, Chloramphenicol, Chlortetracycline, Ciprofloxacin, Clarithromycin, Danofloxacin, Doxycycline, Enoxacin, Enrofloxacin, Erythromycin, Josamycin, Metronidazole, Nifuroxazide, Norfloxacin, Ofloxacin, Oxytetracycline, Roxythromycin, Spiramycin, Sulfadiazine, Sulfamethazine, Sulfamethoxazole, Tetracycline, Tilmicosin, Trimethoprim, Tylosin A
C	Anti-diabetics	Glibenclamide
D	Anti-hypertensives	Enalapril, Hydrochlorothiazide, Lisinopril
E	Barbiturates	Butalbital, Pentobarbital, Phenobarbital

F	Beta-agonists	Clenbuterol, Salbutamol
G	Beta-blockers	Atenolol, Betaxolol, Carazolol, Metoprolol, Nadolol, Pindolol, Propranolol, Sotalol, Timolol
H	Diuretics	Furosemide
I	Lipid regulators	Atorvastatin, Bezafibrate, Clofibric acid, Fenofibrate, Gemfibrozil, Mevastatin, Pravastatin
J	Psychiatric drugs	Carbamazepine, Diazepam, Fluoxetine, Lorazepam, Paroxetine
K	Receptor antagonists	Cimetidine, Famotidine, Loratadine, Ranitidine
L	Antineoplastics	Tamoxifen

3.2.3 Sampling Sites and sample preparation

Four sampling points were monitored: the effluents from Hospitals A and B and the influent and the effluent of Ferrara WWTP. Two experimental campaigns were carried out in August 2009 (summer) and in March 2010 (winter). In the first period, water samples were taken from the raw effluent of Hospital A (n = 4) and Hospital B (n = 4), while in the second one, from the effluent of hospital B (n = 4) and the influent and the effluent of the Ferrara WWTP (n = 4). Manholes located on the property line of each hospital were selected as sampling points, based on their suitability for covering all of the sewage discharges from the facility. Portable auto samplers (Sigma 900) were used to collect samples from each sampling point.

24-hour composite water samples were collected over four days on each sampling point at a rate of one sample per hour (a total of 24 sub-samples, 125 mL each were collected over 24 hours). To insure representative sampling and consistency in the estimation of the mass loadings at the differing locations, identical sampling strategies (the same sampling frequencies) were used for both hospital B effluent and WWTP influent. Water samples were collected only in dry days in order to avoid dilution effects. Wastewater samples were collected in amber glass bottles, pre-rinsed with ultra-pure water, as 24-h composite samples. The samples were immediately transported to the near laboratory under cooled conditions (4 °C). Upon reception, samples were filtered through 0.45 μm Nylon filters (Whatman, Maidstone, UK) to eliminate suspended solid matter and then frozen until analysis (less than a week) at -20 °C. It is important to observe that the fraction of the selected pharmaceutical sorbed onto the suspended solids is removed during preparation phase and, as a consequence, the values of (measured) concentrations found correspond to the dissolved fraction of the investigated compounds.

3.2.4 Standards

All standard solutions used were of a high purity grade (>90%). Isotopically labelled compounds, used as internal standards, were: ^{13}C-phenacetin, fluoxetine-d_5 and flumequine from Sigma-Aldrich (Steinham, Germany), sulfathiazole-d_4 from Toronto Research Chemicals, diazepam-d_5 and phenobarbital-d_5 from Cerilliant (Texas, USA), atenolol-d_7, carbamazepine-d_{10}, ibuprofen-d_3 from CDN isotopes (Quebec, Canada) and mecoprop-d_3 from Dr. Ehrenstorfer (Augsburg, Germany). Both individual stock standard and isotopically labelled internal standard solutions were prepared on a weight basis in methanol, except fluoroquinolones, which were dissolved in a water:methanol mixture (1:1) containing 0.2% v/v hydrochloric acid (Golet et al., 2002). After preparation, standards were stored at −20°C. Due to their limited stability, fresh stock solutions of antibiotics were prepared monthly, while stock solutions for the other substances were renewed every three months. A mixture of all pharmaceuticals was prepared by appropriate dilution of individual stock solutions in methanol–water (25:75, v/v). Working standard solutions, also prepared in a methanol–water (25:75, v/v) mixture, were renewed before each analytical run. A separate mixture of isotopically labelled internal standards, used for internal standard calibration, was prepared in methanol, and further dilutions in methanol–water (25:75, v/v) mixture.

3.2.5 Analytical methods

The multiresidue analytical method developed by Gros et al. (2009) was used to measure the selected pharmaceuticals in wastewaters. Briefly, after filtration, an appropriate volume of aqueous solution of 5% Na2EDTA were added to 200 mL of WWTP effluent and 100 mL of influent (hospital and urban) wastewaters, respectively, to achieve a final Na2EDTA concentration of 0.1% in the samples. The measured volumes were afterwards preconcentrated onto a lipophilic–hidrophilic balanced Oasis HLB (60 mg and 3 mL) cartridge, using a Baker vacuum system (J.T. Baker, Deventer, The Netherlands) at a flow rate of 5 mL/min. After sample preconcentration, cartridges were rinsed with 5 mL of HPLC grade water and were dried under vacuum for 15–20 min, to remove excess of water. Elution of target compounds was performed with 2 × 4 mL pure methanol. Extracts were evaporated to dryness under a gentle nitrogen stream and reconstituted with 1 mL of methanol–water (25 :75, v/ v). Finally, 10 µL of a 1 ng μL^{-1}

standard mixture containing the internal standards were added in the extract for internal standard calibration. Instrumental analysis was performed by liquid chromatography, using an Agilent HP 1100 HPLC (Palo Alto, CA, USA) system, equipped with an auto sampler and connected in series with a 4000 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer operating with a Turbo Ion Spray source (Applied Biosystems-Sciex, Foster City, CA, USA). Chromatographic separation was achieved with a Purospher Star RP-18 endcapped column (125 mm × 2.0 mm, particle size 5 µm) preceded by a C18 guard column (4 × 4,5 µm), both supplied by Merck (Darmstadt, Germany). For the analysis in NI mode , eluent A was a mixture of acetonitrile–methanol (1:1, v /v) and eluent B was HPLC grade water at a flow rate of 0.2 mL/min, where as the analysis in PI mode was performed using acetonitrile as eluent A and HPLC grade water with 0.1% formic acid as eluent B. Appendix B.1. provides details of the optimized QqLIT-MS parameters (two SRMs, collision energies) for each investigated compound in negative and positive ionization modes. Limits of detection (LOD) for the investigated compounds were in the range 1-16 ng L^{-1} for the WWTP influent and the effluent form the two hospitals and in the range 1-18 ng L^{-1} for the WWTP effluent. Table 3.2. reports the values for each selected substance. Recoveries of the methods were determined by analysing fortified samples of each type of wastewater spiked in triplicate to 1 µg L^{-1}. They were in the range 22-145 %. The single values with relative standard deviation (RSD) are reported in Table 3.2.

3.2.6 Risk Quotients (RQ) and Ecotoxicological Risk Assessment

The potential risk of PhCs was assessed by means of their risk quotient values (RQ), calculated as the ratio between their MEC and PNEC. PNEC values were estimated on the basis of toxicity data reported for several aquatic organisms: bacteria, algae, invertebrates and fish (as reported in Table 2.7.). According to (EC 2003; Tauxe-Wuersch et al., 2005), PNEC values were estimated as 1000 times lower than the most sensitive species assayed (marked in bold in the Table 2.7.), so as to take into account the effect on other, potentially more sensitive, aquatic species to those used in toxicity studies. A commonly used risk ranking criterion was applied: RQ < 0.1, minimal risk to aquatic organisms, 0.1 ≤ RQ < 1, median risk; RQ ≥ 1, high risk (De Souza et al., 2009; Hernando et al., 2006; Zhao et al., 2010).

Table 3.2. Recovery and limits of detection (LOD) of the selected compounds.

Therapeutic Class	Compound	% Recovery (±RSD)					LOD (ng L^{-1})				
		Hospital A (Summer)	Hospital B (Summer)	Hospital B (Winter)	WWTP inf. (Winter)	WWTP eff. (Winter)	Hospital A (Summer)	Hospital B (Summer)	Hospital B (Winter)	WWTP inf. (Winter)	WWTP eff. (Winter)
Analgesic/anti-inflammatories **A**	Acetaminophen	92 (±3)	121 (±1)	96 (±4)	131 (±9)	80 (±15)	2	3	7	11	8
	Codeine	86 (±8)	78 (±5)	113 (±3)	75 (±6)	94 (±2)	3	3	2	6	7
	Diclofenac	127 (±12)	89 (±3)	78 (±11)	100 (±9)	102 (±5)	4	5	5	2	2
	Ibuprofen	83 (±13)	91 (±7)	105 (±5)	111 (±14)	133 (±8)	8	6	11	9	9
	Indomethacin	80 (±13)	94 (±6)	116 (±1)	103 (±3)	81 (±5)	2	3	3	6	7
	Ketoprofen	55 (±3)	112 (±6)	89 (±8)	62 (±4)	73 (±13)	3	4	7	7	8
	Mefenemic acid	128 (±1)	124 (±5)	95 (±2)	86 (±7)	63 (±15)	6	7	4	5	3
	Naproxen	98 (±4)	118 (±2)	116 (±1)	104 (±1)	95 (±3)	11	5	5	6	3
	Phenazone	100 (±3)	103 (±1)	96 (±15)	85 (±13)	78 (±11)	2	3	8	5	6
	Phenylbutazone	120 (±9)	111 (±4)	81 (±4)	67 (±3)	92 (±16)	3	5	4	6	3
	Propyphenazone	119 (±9)	130 (±3)	104 (±15)	123 (±12)	98 (±21)	2	6	3	2	5
	Salicylic acid	91 (±4)	88 (±8)	78 (±25)	56 (±6)	91 (±7)	12	9	8	11	6
Antibiotics **B**	Azithromycin	45 (±3)	58 (±1)	85 (±9)	78 (±7)	76 (±13)	3	4	2	2	4
	Chloramphenicol	87 (±13)	95 (±2)	96 (±25)	86 (±1)	78 (±6)	9	8	4	9	7
	Chlortetracycline	56 (±4)	90 (±7)	100 (±8)	56 (±1)	74 (±9)	12	11	8	14	9
	Ciprofloxacin	103 (±3)	62 (±5)	105 (±5)	107 (±7)	123 (±13)	3	4	3	3	2
	Clarithromycin	89 (±23)	95 (±2)	91 (±1)	78 (±6)	121 (±9)	4	3	6	6	2
	Danofloxacin	101 (±9)	109 (±6)	104 (±3)	103 (±4)	95 (±2)	7	8	5	9	3
	Doxycycline	94 (±7)	56 (±3)	67 (±10)	41 (±26)	103 (±3)	11	8	15	16	18
	Enoxacin	120 (±6)	98 (±7)	121 (±4)	133 (±9)	89 (±17)	3	6	5	7	2
	Enrofloxacin	89 (±1)	107 (±3)	88 (±1)	79 (±4)	93 (±3)	4	5	5	2	3
	Erithromycin	99 (±3)	96 (±9)	112 (±16)	103 (±3)	95 (±5)	7	5	8	7	8
	Josamycin	112 (±9)	91 (±4)	87 (±7)	46 (±4)	23 (±8)	3	2	3	2	1
	Metronidazole	37 (±5)	22 (±1)	47 (±9)	56 (±3)	45 (±7)	6	5	3	4	1
	Nifuroxazide	111 (±2)	56 (±4)	79 (±5)	96 (±1)	87 (±1)	11	14	12	9	7
	Norfloxacin	56 (±3)	43 (±9)	112 (±2)	118 (±7)	109 (±1)	8	5	6	6	3
	Ofloxacin	135 (±1)	94 (±7)	79 (±25)	98 (±23)	79 (±1)	1	2	1	1	1
	Oxytetracycline	100 (±23)	105 (±18)	95 (±12)	78 (±8)	45 (±9)	6	8	7	12	15
	Roxithromycin	120 (±1)	94 (±5)	56 (±3)	99 (±9)	78 (±8)	4	5	6	3	2
	Spiramycin	145 (±5)	80 (±4)	98 (±7)	93 (±6)	109 (±11)	2	3	2	3	2

Therapeutic Class	Compound	% Recovery (±RSD)					LOD (ng L^{-1})				
		Hospital A (Summer)	Hospital B (Summer)	Hospital B (Winter)	WWTP inf. (Winter)	WWTP eff. (Winter)	Hospital A (Summer)	Hospital B (Summer)	Hospital B (Winter)	WWTP inf. (Winter)	WWTP eff. (Winter)
	Sulfadiazine	131 (±6)	45 (±3)	105 (±3)	103 (±4)	56 (±24)	2	4	4	5	7
	Sulfamethazine	56 (±5)	97 (±8)	96 (±9)	124 (±3)	65 (±5)	2	4	5	2	6
	Sulfamethoxazole	73 (±1)	56 (±1)	98 (±3)	87 (±5)	120 (±3)	1	3	2	3	1
	Tetracycline	81 (±7)	85 (±28)	123 (±18)	99 (±5)	95 (±2)	7	9	12	14	13
	Tilmicosin	145 (±3)	103 (±3)	78 (±1)	94 (±1)	82 (±16)	1	2	1	3	6
	Trimethoprim	57 (±7)	51 (±7)	86 (±6)	119 (±11)	88 (±18)	1	1	2	1	1
	Tylosin A	103 (±3)	86 (±8)	107 (±19)	102 (±7)	145 (±1)	2	1	2	3	1
Anti-diabetic **C**	Glibenclamide	56 (±3)	76 (±1)	98 (±7)	112 (±21)	97 (±15)	3	5	2	4	2
Anti-hypertensives **D**	Enalapril	92 (±11)	106 (±17)	65 (±8)	93 (±3)	69 (±7)	2	1	3	2	4
	Hydrochlorothiazide	83 (±15)	86 (±19)	100 (±1)	103 (±3)	87 (±9)	6	9	8	12	13
	Lisinopril	91 (±4)	98 (±7)	134 (±8)	111 (±6)	95 (±3)	2	3	12	15	9
Barbiturates **E**	Butalbital	103 (±3)	56 (±12)	45 (±2)	47 (±1)	69 (±1)	2	1	5	6	3
	Pentobarbital	45 (±7)	51 (±23)	119 (±4)	99 (±19)	103 (±6)	5	2	3	4	3
	Phenobarbital	35 (±1)	48 (±4)	75 (±3)	44 (±8)	26 (±2)	1	1	2	3	2
Beta-agonists **F**	Clenbuterol	105 (±23)	91 (±7)	95 (±2)	115 (±8)	117 (±7)	2	2	1	1	1
	Salbutamol	80 (±20)	89 (±11)	135 (±1)	97 (±1)	78 (±9)	1	1	1	2	1
Beta-blockers **G**	Atenolol	34 (±5)	83 (±13)	145 (±8)	58 (±4)	118 (±6)	4	5	11	13	9
	Betaxolol	118 (±3)	56 (±3)	95 (±2)	101 (±1)	120 (±5)	2	3	2	2	1
	Cerazolol	99 (±1)	98 (±7)	92 (±16)	79 (±9)	91 (±6)	1	1	1	2	1
	Metoprolol	113 (±5)	107 (±3)	129 (±1)	136 (±6)	95 (±2)	1	2	1	3	1
	Nadolol	106 (±12)	96 (±9)	90 (±1)	87 (±8)	97 (±3)	2	2	1	1	2
	Pindolol	45 (±3)	75 (±23)	103 (±3)	108 (±9)	49 (±16)	3	1	3	2	4
	Propranolol	69 (±8)	61 (±5)	104 (±1)	70 (±8)	57 (±9)	2	2	1	1	1
	Sotalol	73 (±9)	117 (±19)	110 (±4)	56 (±7)	91 (±7)	3	5	9	8	10
	Timolol	45 (±12)	79 (±3)	62 (±15)	56 (±6)	101 (±14)	2	1	5	3	2
Diuretics **H**	Furosemide	78 (±19)	59 (±9)	100 (±1)	96 (±7)	92 (±3)	3	5	6	8	9
Lipid regulators **I**	Atorvastatin	89 (±19)	131 (±12)	111 (±1)	118 (±3)	85 (±3)	1	3	5	3	4
	Bezafibrate	134 (±1)	95 (±1)	97 (±5)	95 (±7)	56 (±3)	1	1	2	2	2
	Clofibric acid	135 (±1)	120 (±7)	108 (±15)	71 (±3)	98 (±7)	1	2	1	1	1
	Fenofibrate	110 (±9)	117 (±18)	92 (±1)	79 (±9)	107 (±3)	2	1	3	1	1
	Gemfibrozil	145 (±1)	64 (±23)	67 (±1)	87 (±5)	96 (±9)	2	3	2	1	1
	Mevastatin	126 (±9)	113 (±2)	110 (±7)	72 (±9)	87 (±9)	5	6	8	9	7

Therapeutic Class	Compound	% Recovery (±RSD)					LOD (ng L^{-1})				
		Hospital A (Summer)	Hospital B (Summer)	Hospital B (Winter)	WWTP inf. (Winter)	WWTP eff. (Winter)	Hospital A (Summer)	Hospital B (Summer)	Hospital B (Winter)	WWTP inf. (Winter)	WWTP eff. (Winter)
Psychiatric drugs **J**	Pravastatin	114 (±23)	69 (±3)	98 (±1)	90 (±7)	71 (±14)	12	11	9	13	15
	Carbamazepine	92 (±19)	68 (±9)	92 (±1)	145 (±8)	111 (±7)	3	4	2	4	5
	Diazepam	101 (±15)	45 (±26)	76 (±12)	103 (±3)	59 (±16)	1	1	2	1	2
	Fluoxetine	139 (±1)	96 (±5)	92 (±6)	109 (±9)	107 (±6)	3	2	2	1	2
	Lorazepam	100 (±3)	123 (±7)	103 (±3)	91 (±1)	98 (±12)	8	7	8	9	11
	Paroxetine	103 (±8)	135 (±15)	87 (±9)	45 (±18)	103 (±3)	2	3	2	2	3
Receptor antagonists **K**	Cimetidine	103 (±3)	56 (±25)	67 (±3)	78 (±1)	89 (±9)	1	3	3	5	2
	Famotidine	119 (±9)	109 (±13)	92 (±8)	95 (±2)	104 (±6)	2	3	2	4	3
	Loratadine	132 (±3)	79 (±1)	75 (±7)	103 (±3)	98 (±7)	3	1	2	3	2
	Ranitidine	138 (±4)	127 (±15)	94 (±9)	97 (±6)	135 (±1)	8	7	8	11	10
Cytostatic **L**	Tamoxifen	138 (±1)	65 (±3)	103 (±3)	145 (±2)	92 (±3)	1	1	2	1	1

3.3 Results and discussion

Table 3.3. shows the ranges of concentrations and the corresponding average values (in brackets) of the investigated compounds in the effluents from Hospital A (in summer), Hospital B (in summer and in winter) and in the influent and effluent of Ferrara WWTP (in winter). The final row reports the number of compounds detected during the investigation periods (occurrence). In descending order, the highest occurrence of PhCs was detected in the WWTP influent (63), in Hospital B effluent in winter (62), Hospital A effluent in summer (61) and in the WWTP effluent (58). The lowest number of detected substances was found in the Hospital B effluent in summer (49).

Among the analgesics/anti-inflammatories, also in descending order, the highest average concentrations were found for ketoprofen (5 μg L^{-1}), acetaminophen (4.5 μg L^{-1}μg/L) in Hospital A effluent, acetaminophen (4.1 μg L^{-1}) and indomethacin (2.2 μg L^{-1}) in Hospital B effluent in summer, naproxen (4.9 μg L^{-1}) and ibuprofen (2.6) in Hospital B effluent in winter, ibuprofen (1.0 μg L^{-1}) and naproxen (0.83 μg L^{-1}) in the WWTP influent, followed by mefenamic acid (0.66 μg L^{-1}) and diclofenac (0.28 μg L^{-1}) in the WWTP effluent.

Among the antibiotics, the most prevalent compounds were: ofloxacin (19 μg L^{-1}) and ciprofloxacin (12 μg L^{-1}) in Hospital A effluent, ofloxacin (3.7 μg L^{-1}) and sulfamethoxazole (1.8 μg L^{-1}) in Hospital B effluent in summer, ofloxacin (31 μg L^{-1}) and sulfamethoxazole (21 μg L^{-1}) in Hospital B effluent in winter, ciprofloxacin (2.2 μg L^{-1}) and ofloxacin (1.0 μg L^{-1}) in the WWTP influent, followed by ciprofloxacin (0.64 μg L^{-1}) and clarithromycin (0.28 μg L^{-1}) in the WWTP effluent.

Hydrochlorothiazide was the most present anti-hypertensive at the four sampling points, being detected at concentrations of 1.8 μg L^{-1} in Hospital A effluent, 0.68 μg L^{-1} in Hospital B effluent (summer), 2.2 μg L^{-1} in Hospital B effluent (winter), 2.7 μg L^{-1} in the WWTP influent, and 1.2 μg L^{-1} in the WWTP effluent. Among the barbiturates, pentobarbital had the highest concentrations in Hospital A effluent (0.035 μg L^{-1}), and butalbital the highest concentrations in Hospital B effluent in summer (0.032 μg L^{-1}) and winter (0.36 μg L^{-1}), while phenobarbital was most prevalent in the WWTP influent (0.21 μg L^{-1}) and effluent (0.14 μg L^{-1}). Salbutamol was the beta-agonist with the highest concentration in the effluent of Hospital A (0.062 μg L^{-1}) and Hospital B in summer (0.028

μg L^{-1}), whereas clenbuterol had the highest concentrations in Hospital B effluent in winter (0.18 μg L^{-1}). The most represented beta blockers were: atenolol at 5.1μg L^{-1} and sotalol at 4.8 μg L^{-1} in Hospital A effluent, and atenolol 2.4 μg L^{-1} in Hospital B effluent in summer; in winter atenolol was detected at 5.8 μg L^{-1} and sotalol at 5.1 μg L^{-1} in Hospital B effluent, while in the WWTP influent, atenolol was found at 2.1 μg L^{-1} and sotalol at 0.53 μg L^{-1}, in contrast with the 0.32 μg L^{-1} sotalol and 0.073 μg L^{-1} atenolol detected in the WWTP effluent. Among the lipid regulators, those with the highest concentrations were mevastatin in Hospital A effluent (1.1 μg L^{-1}) and in Hospital B effluent in summer (0.49 μg L^{-1}), atorvastatin in Hospital B effluent in winter (0.27 μg L^{-1}), and gemfibrozil in the WWTP influent (0.20 μg L^{-1}) and effluent (0.11 μg L^{-1}). The psychiatric drug carbamazepine and the receptor antagonist ranitidine displayed the highest concentrations of their type at all the sampling points.

There are limited data that allow for a comparison referring to PhC occurrence in hospital effluents, however Verlicchi et al. (2010b) reviewed the variability ranges for some compounds of different therapeutic classes in raw hospital wastewater. Based on these findings, measured concentrations for PhCs in hospital A and B effluents are in agreement with those reported in Verlicchi et al. (2010b), except for erythromycin (measured concentrations are 2 order of magnitude lower than those of the review), propranolol and gemfibrozil (1 order of magnitude lower). More literature data are available regarding the presence of PhCs in urban wastewaters. A comparison with the variability intervals found in different countries by Jelicic and Ahel, (2003), Kasprzyk-Hordern et al. (2009), Radjenovic et al. (2009), Roberts and Thomas (2006), Rosal et al. (2010) Sipma et al. (2010) Sui et al. (2010) and Verlicchi et al. (2010b) shows that measured concentrations in the influent of Ferrara WWTP is in good agreement with them except for codeine, erythromycin, propranolol and cimetidine that were at a concentrations of 1 order of magnitude lower than those reported by literature.

On the basis of the concentration data reported above, the following comparisons were made between: the two hospital effluents in summer, the effluent of Hospital B in summer and winter, and Hospital B effluent and Ferrara WWTP influent (which, in addition to urban wastewater, receives that of Hospital B) in winter.

3.3.1 Comparison of PhC Concentrations in the Effluent from Hospitals A and B in Summer

Data reported in Table 3.3. show that, for the majority of the compounds considered, concentrations were higher in the effluent of Hospital A than those in that of Hospital B. Only 12 out of the 73 investigated PhCs, codeine, phenylbutazone, azithromycin, chlortetracycline, josamycin, sulfadiazine, butalbital, phenobarbital, propranolol, atorvastatin, carbamazepine and fluoxetine, were detected in lower concentrations in Hospital A effluent than those found in Hospital B.

The relatively large dose/population ratios detected in Hospital A could be due to the fact that: (*i*) Hospital A is situated in a coastal area, densely populated by tourists in the summertime, the period in which the water samples were taken; thus, analyses may reflect that a higher consumption of PhCs than average occurred; and/or (*ii*) Hospital A has a lower daily water demand, resulting in lesser dilution of the micropollutants present.

3.3.2 Comparison between Summer and Winter Concentrations of PhCs in Hospital B Effluent

Data of Table 3.3. show that 49 compounds were detected in summer and 62 in winter. Five compounds were found only in summer and 18 only in winter. Only 6 compounds (phenazone, danofloxacin, enrofloxacin, tylosin A, fenofibrate and tamoxifen) were not detected at either sampling point at any time. Of the 44 compounds found at least in one sampling point, the winter concentrations were, on average, greater than those detected in the summer, with their ratio ranging between 1.1 (sulfamethoxazole) and 190 (clarithromycin), with an average value of 10.4, a standard deviation of 31.3, and a 95^{th}-percentile equal to 16.9. Only 2 anti-inflammatories (acetaminophen and indomethacin), 5 antibiotics (chlortetracycline, doxycycline, josamycin, oxytetracycline, tetracycline and trimethoprim), the anti-hypertensive lisinopril, the beta-blocker propanolol, the diuretic furosemide, the lipid regulator mevastatin and the psychiatric drug carbamazepine were found at 1.3-4 times higher summer concentrations than those detected in the winter (on average 2.3 times).

Table 3.3. Ranges and average concentration levels of pharmaceuticals in effluents from the two hospitals and in the influent and effluent of Ferrara WWTP.

Therapeutic Class	Compound, μg L^{-1}	Hospital A (Summer)	Hospital B (Summer)	Hospital B (Winter)	WWTP Influent (Winter)	WWTP Effluent (Winter)
Analgesics/anti-inflammatories **A**	Acetaminophen	3.3-5.9 (4.5)	3.5-4.7 (4.1)	1.4-3.4 (2.5)	0.50-1.2 (0.81)	0.012-0.058 (0.030)
	Codeine	0.26-0.43 (0.36)	0.42-0.64 (0.53)	0.41-3.2 (1.9)	0.09-0.15 (0.11)	0.052-0.082 (0.066))
	Diclofenac	0.17-0.46 (0.30)	0.18-0.27 (0.22)	0.48-0.53 (0.51)	0.36-0.48 (0.44)	0.22-0.33 (0.28)
	Ibuprofen	1.0-2.5 (1.7)	0.38-0.81 (0.60)	2.2-3.2 (2.6)	0.93-1.2 (1.0)	0.010-0.12 (0.081)
	Indomethacin	0.31-4.1 (2.5)	0.90-3.4 (2.2)	0.40-0.61 (0.53)	0.061-0.20 (0.16)	0.06-0.13 (0.10)
	Ketoprofen	2.2-9.8 (5.0)	0.83-1.4 (1.1)	1.1-1.8(1.4)	0.13-0.19 (0.17)	0.056-0.11 (0.085))
	Mefenamic acid	0.18-0.50 (0.33)	0.10-0.13 (0.12)	0.33-0.75 (0.55)	0.56-1.2 (0.90)	0.41-0.91 (0.66)
	Naproxen	1.2-3.2 (2.3)	0.34-0.48 (0.41)	1.1-11 (4.9)	0.78-0.91 (0.83)	0.10-0.21 (0.18)
	Phenazone	< LOD	< LOD	< LOD	< LOD	< LOD
	Phenylbutaz.	0.01-0.05 (0.04)	0.048-0.080 (0.063)	0.12-0.17 (0.14)	0.067-0.13 (0.11)	0.037-0.060 (0.052)
	Propyphen.	<LOD-0.020 (0.011)	< LOD	0.011-0.10 (0.038)	0.038-0.074 (0.053)	0.024-0.068 (0.042)
	Salicylic acid	0.90-1.9 (1.3)	0.99-1.1 (1.0)	1.9-2.4 (2.22)	0.21-1.1 (0.50)	0.11-0.13 (0.12)
Antibiotics **B**	Azithromycin	<LOD-0.11 (0.030)	0.045-0.050 (0.047)	0.58-1.04 (0.80)	0.01-0.33 (0.13)	0.07-0.18 (0.13)
	Chloramphenicol	<LOD-0.036 (0.012)	< LOD	< LOD-0.01 (0.078)	0.013-0.024 (0.019)	< LOD
	Chlortetracycline	0.02-0.06 (0.04)	0.063-0.094 (0.077)	< LOD	< LOD	< LOD
	Ciprofloxacin	10-15 (12)	1.4-1.9 (1.6)	15-26 (21)	1.1-3.7 (2.2)	0.29-1.1 (0.64)
	Clarithromycin	0.02-0.14 (0.06)	0.050-0.064 (0.058)	9.3-14 (11)	0.11-0.78 (0.31)	0.26-0.31 (0.28)
	Danofloxacin	< LOD	< LOD	< LOD	< LOD	< LOD
	Doxycycline	0.10-0.27 (0.17)	0.056-0.97 (0.078)	< LOD	< LOD	< LOD
	Enoxacin	0.33-0.48 (0.41)	0.058-0.10 (0.080)	0.18-0.45 (0.27)	0.081-0.13 (0.10)	0.03-0.10 (0.061)
	Enrofloxacin	< LOD	< LOD	< LOD	< LOD	< LOD
	Erythromycin	0.06-0.32 (0.16)	0.080-0.086 (0.082)	0.091-0.23 (0.16)	0.010-0.072 (0.045)	0.010-0.033 (0.016)
	Josamycin	<LOD-0.012 (0.003)	0.011-0.015 (0.012)	< LOD-0.01 (0.01)	< LOD -0.007 (0.0020)	< LOD
	Metronidazole	0.33-1.64 (0.72)	0.26-0.39 (0.033)	0.85-1.1 (0.96)	0.028-0.056 (0.042)	0.013-0.041 (0.028)
	Nifuroxazide	0.10-2.56 (1.4)	0.10-0.16 (0.14)	0.22-0.33 (0.29)	0.019-0.076 (0.052)	0.010-0.022 (0.013)
	Norfloxacin	0.04-0.10 (0.07)	0.023-0.044 (0.034)	0.22-0.51 (0.35)	0.15-0.31 (0.020)	0.14-0.17 (0.15)
	Ofloxacin	13-22 (19)	3.3-4.1 (3.7)	25-37 (31)	0.45-2.2 (1.0)	0.22-0.52 (0.39)
	Oxytetracycline	0.30-1.3 (0.78)	0.074-0.10 (0.089)	< LOD	< LOD	< LOD
	Roxithromycin	< LOD	< LOD	0.02-0.14 (0.079)	<LOD-0.14 (0.063)	0.013-0.053 (0.029))
	Spiramycin	<LOD-0.040 (0.010)	< LOD	0.034-0.11 (0.068)	< LOD-0.15 (0.061)	0.019-0.053 (0.029)

Therapeutic Class	Compound, μg L^{-1}	Hospital A (Summer)	Hospital B (Summer)	Hospital B (Winter)	WWTP Influent (Winter)	WWTP Effluent (Winter)
	Sulfadiazine	0.029-0.033 (0.032)	0.077-0.12 (0.10)	0.27-0.38 (0.33)	0.013-0.026 (0.022)	0.010-0.021 (0.017)
	Sulfamethazine	<LOD-0.014 (0.0070)	< LOD	0.013-0.03 (0.023)	0.010-0.033 (0.018)	0.010-0.015 (0.011)
	Sulfamethoxazole	3.0-6.5 (4.2)	0.90-2.7 (1.8)	0.94-3.4 (2.0)	0.28-0.74 (0.44)	0.17-0.24 (0.21)
	Tetracycline	<LOD-0.026 (0.014)	<LOD-0.033 (0.017)	< LOD	< LOD	< LOD
	Tilmicosin	0.05-0.07 (0.06)	0.014-0.020 (0.015)	0.12-0.35 (0.26)	0.021-0.46 (0.25)	<LOD-0.081 (0.036)
	Trimeth.	0.80-1.8 (1.2)	0.45-0.86 (0.65)	0.068-0.36 (0.18)	0.039-0.072 (0.058)	0.036-0.051 (0.040)
	Tylosin A	< LOD	< LOD	< LOD	< LOD	< LOD
Anti-diabetics **C**	Glibenclamide	0.05-0.10 (0.07)	0.066-0.071 (0.068)	0.072-0.11 (0.10)	0.081-0.96 (0.087)	0.01-0.08 (0.055)
	Enalapril	0.15-0.27 (0.20)	0.091-0.18 (0.13)	0.24-0.40 (0.31)	0.071-0.10 (0.082)	< LOD
Anti-hypertensives **D**	Hydrochlorotiazide	1.3-2.1 (1.8)	0.54-0.82 (0.68)	1.8-2.4 (2.2)	1.4-5.5 (2.7)	0.97-1.4 (1.2)
	Lisinopril	0.08-0.61 (0.25)	0.089-0.34 (0.21)	< LOD	< LOD	< LOD
	Butalbital	0.014-0.038 (0.022)	0.011-0.052 (0.032)	0.25-0.48 (0.36)	0.072-0.25 (0.13)	0.090-0.13 (0.10)
Barbiturates **E**	Pentobarbital	0.011-0.074 (0.035)	0.014-0.025 (0.019)	0.11-0.15 (0.13)	0.021-0.043 (0.021)	0.01-0.028 (0.018))
	Phenobarbital	<lod-0.029 (0.0014)	0.013-0.030 (0.021)	0.13-0.36 (0.25)	0.11-0.27 (0.21)	0.11-0.17 (0.14)
Beta-agonists **F**	Clenbuterol	< LOD	< LOD	0.86-1.19 (1.1)	0.22-0.29 (0.26)	0.13-0.21 (0.18)
	Salbutamol	0.04-0.10 (0.062)	0.026-0.030 (0.028)	0.10-0.14 (0.12)	0.011-0.020 (0.013)	0.010-0.017 (0.012)
	Atenolol	3.5-6.2 (5.1)	2.2-2.6 (2.4)	5.1-6.6 (5.8)	1.8-2.4 (2.1)	0.55-0.98 (0.073)
	Betaxolol	<LOD-0.020 (0.011)	< LOD	< LOD-0.01 (0.01)	< LOD-0.007 (0.002)	< LOD
	Cerazolol	< LOD	< LOD	< 0.0018-0.0023 (0.002)	< LOD-0.01	< LOD
	Metoprolol	0.58-0.99 (0.83)	0.51-0.97 (0.74)	0.86-1.2 (1.1)	0.22-0.29 (0.26)	0.13-0.21 (0.18)
Beta-blockers **G**	Nadolol	< LOD	< LOD	< LOD-0.0034 (0.0012)	< LOD-0.016 (0.011)	< LOD
	Pindolol	0.032-0.26 (0.12)	< LOD	0.034-0.048 (0.038)	<LOD-0.011 (0.0030)	< LOD
	Propranolol	<LOD-0.051 (0.023)	0.076-0.094 (0.085)	0.030-0.061 (0.043)	0.014-0.045 (0.026)	0.013-0.026 (0.018)
	Sotalol	3.8-5.9 (4.8)	0.35-0.61 (0.048)	3.3-6.7 (5.1)	0.37-0.64 (0.53)	0.21-0.47 (0.32)
	Timolol	< LOD	< LOD	0.022-0.039 (0.033)	0.010-0.016 (0.014)	< LOD-0.013 (0.010)
Diuretics **H**	Furosemide	11-18 (14)	6.4-7.7 (7.1)	5.3-6.3 (5.8)	0.39-0.47 (0.42)	0.08-0.35 (0.27)
	Atorvastatin	0.062-0.10 (0.083)	0.080-0.17 (0.13)	0.24-0.31 (0.27)	< lod -0.018 (0.011)	< LOD-0.010 (0.0060)
	Bezafibrate	0.057-2.9 (0.95)	< LOD	0.042-0.51 (0.20)	0.063-0.12 (0.090)	0.011-0.048 (0.036)
	Clofibric acid	<LOD-0.043 (0.017)	< LOD	0.010-0.014 (0.013)	< LOD-0.012 (0.010)	< LOD-0.0060 (0.0020)
Lipid regulators **I**	Fenofibrate	<LOD-0.026 (0.010)	< LOD	< LOD	< LOD-0.020 (0.0060)	< LOD-0.013 (0.0030)
	Gemfibrozil	0.018-0.020 (0.019)	< LOD	0.014-0.064 (0.033)	0.16-0.28 (0.20)	0.04-0.17 (0.11)
	Mevastatin	0.38-2.0 (1.1)	0.45-0.53 (0.49)	0.068-0.20 (0.015)	0.12-0.28 (0.17)	0.03-0.14 (0.083)
	Pravastatin	0.19-1.1 (0.62)	0.064-0.080 (0.077)	0.081-0.27 (0.17)	0.080-0.14 (0.11)	0.04-0.07 (0.54)

Therapeutic Class	Compound, μg L^{-1}	Hospital A (Summer)	Hospital B (Summer)	Hospital B (Winter)	WWTP Influent (Winter)	WWTP Effluent (Winter)
Psychiatric drugs **J**	Carbamazepine	0.64-0.87 (0.73)	0.76-1.2 (0.97)	0.75-1.1 (0.95)	0.30-1.17 (0.58)	0.28-0.44 (0.37)
	Diazepam	< LOD	< LOD	0.021-0.038 (0.031)	0.002-0.010 (0.076)	< LOD
	Fluoxetine	<LOD-0.018 (0.005)	0.024-0.033 (0.027)	0.035-0.069 (0.056)	0.055-0.19 (0.11)	0.010-0.063 (0.044)
	Lorazepam	0.62-0.79 (0.67)	0.17-0.20 (0.18)	0.46-0.70 (0.060)	0.17-0.25 (0.22)	0.08-0.14 (0.12)
	Paroxetine	< LOD	< LOD	0.056-0.076 (0.067)	0.020-0.080 (0.041)	0.010-0.018 (0.013)
Receptor antagonists **K**	Cimetidine	0.019-0.032 (0.026)	< LOD	0.033-0.26 (0.11)	0.029-0.061 (0.047)	0.012-0.049 (0.031)
	Famotidine	0.087-0.29 (0.16)	0.035-0.048 (0.042)	0.075-0.13 (0.10)	0.010-0.022 (0.014)	< LOD-0.0040 (0.0020)
	Loratadine	<LOD-0.014 (0.003)	< LOD	0.015-0.026 (0.020)	< LOD-0.020 (0.013)	< LOD-0.0050 (0.003)
	Ranitidine	0.24-2.2 (1.5)	1.1-1.5 (1.3)	1.4-4.1 (3.0)	0.093-0.13 (0.11)	0.04-0.10 0.078)
Cytostatic agents **L**	Tamoxifen	< LOD	< LOD	< LOD	< LOD	< LOD
	Occurrence, n°	**61**	**49**	**62**	**63**	**58**

3.3.3 Comparison between Winter Concentrations of PhCs in Hospital B effluent and WWTP Influent

The data reported in Table 3.3. show that average concentrations of PhCs in Hospital B effluent were higher than those found in the influent of Ferrara WWTP, with the exception of two analgesics/anti-inflammatories (mefenamic acid and propyphenazone), two antibiotics (chloramphenicol and roxythromycin), the anti-hypertensive hydrochlorothiazide, three beta-blockers (betaxolol, cerazolol and nadolol), two lipid regulators (gemfibrozil and mevastatin) and one psychiatric drug (fluoxetine).

As regards the other compounds, the ratio between Hospital B effluent and WWTP influent concentrations ranged between 1.03 and 35.5, with an average value of 7, standard deviation of 8.5 and 95th-percentile of 27.

3.3.4 Contribution of Hospital B Loads to WWTP influent

Table 3.4. reports the percentage average contribution of Hospital B to the load of the investigated compounds in WWTP influent. Compounds were classified according to the average percentage contributions (≤ 5 %, 5-15 %, >15 %). Hospital contributions were ≤ 5 % for 32 substances, between 5 and 15 % for 18 compounds and >15 for 12 PhCs (7 antibiotics, 2 receptor antagonists, 1 analgesic, 1 diuretic and 1 lipid regulator). The highest contributions were found for ofloxacin (67%), azithromycin (67 %), clarithromycin (53%), ranitidine (52%) and metronidazole (45%). This confirms that antibiotics represent a critical class of compound, as reported in Verlicchi et al. (2012c) due to their high consumptions inside the hospital and their stability once excreted.

Unfortunately, little data is available in the literature for comparison with our findings. Nevertheless, what little data is available is reported here below (Table 3.4.). For instance, (Thomas et al., 2007a; Langford and Thomas, 2009), evaluated the PhC contributions originating from the two main hospitals (in total 1800 beds) in the area of Oslo (440,000 inhabitants), Norway, with a bed density of 4 and (Ort et al., 2010a), evaluated the contributions for a 200-bed Australian hospital with a catchment area of 45,000 people (bed density = 4.4). In Germany, (Heberer and Feldman, 2005), analysed contributions from the Berlin hospitals (12,000 beds) and their catchment area (1 million people, bed density = 12) and (Beier et al., 2011), the contributions from Waldbrol hospital (342 beds) and its catchment area (10,200 inhabitants, bed density = 33.5). Their findings are reported in the last five columns of Table 3.4, which shows that percentage

hospital contributions for the detected compounds vary greatly, depending on bed density and the compound in question. Furthermore, differences are evident in the usage patterns of the various PhCs in the different countries, another influential factor. In fact, the highest levels of almost all compounds were found by (Beier et al., 2011); the hospital they studied had the highest bed density (33.5), of all those reported in the literature, thereby indicating the importance of this parameter.

Another interesting study was conducted by Escher et al., 2011 on a Swiss general hospital (338 beds, average flow rate 115690 m^3 $year^{-1}$) whose effluent is conveyed to the near WWTP with conventional biological treatment which serves 54 000 inhabitants. Based on consumption data of the top 40 pharmaceuticals sold in pharmacies, drug stores and doctor's practices, they found that the amount of pharmaceuticals discharged into the WWTP from households totals to 62 % of the total pharmaceutical load in the WWTP. Thus the remaining 38 % stems from the hospital.

Table 3.4. Hospital B average percentage contributions for the detected compounds with respect to the WWTP influent loads and comparison with other studies.

Classification	Compound	PhC Class	This study	Heberer and Feldman 2005	Thomas et al. 2007	Langford and Thomas 2009	Ort et al. 2010	Beier et al. 2011
Bed density			6.5	12	4	4	4.4	33.5
Contribution ≤ 5%	Betaxolol	G	0.99					
	Chloramphenicol	B	1.1					
	Gemfibrozil	I	1.2				4.1	
	Propyphenazone	A	1.4					
	Hydrochlorothiazide	D	1.7					
	Nadolol	G	1.7					
	Mefenamic acid	A	1.8					
	Roxythromycin	B	2.1				26	
	Diclofenac	A	2.1	10	1.6		1	7-9
	Fluoxetine	J	2.3					
	Sulfamethazine	B	2.3					
	Pravastatin	I	2.4					
	Glibenclamide	C	2.4					
	Cerazolol	G	2.4					
	Carbamazepine	J	2.5	15		1.7	0.4	3-8
	Mevastatin	I	2.5					
	Phenobarbital	E	2.6					
	Clofibric acid	I	2.6					
	Josamycin	B	3.0					
	Loratadine	K	3.2					
	Phenylbutazone	A	3.2					
	Trimethoprim	B	3.2		14		10	
	Naproxen	A	3.9				2.3	
	Ibuprofen	A	4.0		0.7		4.6	3-7
	Acetaminophen	A	4.2		12		5.1	
	Butalbital	E	4.3					
	Timolol	G	4.3					
	Enoxacin	B	4.3					

Classification	Compound	PhC Class	This study	Heberer and Feldman 2005	Thomas et al. 2007	Langford and Thomas 2009	Ort et al. 2010	Beier et al. 2011
Bed density			6.5	12	4	4	4.4	33.5
	Norfloxacin	B	4.6					
	Lorazepam	J	4.6					
	Propranolol	G	4.7			11.4		
	Atenolol	G	4.7			2.52	1.8	
	Cimetidine	K	5.6					
	Clenbuterol	F	5.7					
	Metoprolol	G	5.7		1.5		4.1	
	Paroxetine	J	5.9			0.5		
	Sulfamethoxazole	B	6.1		1.2		0.8	
	Indomethacin	A	6.2					
	Diazepam	J	6.8					
	Pentobarbital	E	6.8					
5% < Contribution ≤ 15%	Bezafibrate	I	7.0					27
	Enalapril	D	7.1					
	Erythromycin	B	7.7				2.6	
	Pindolol	G	8.2					
	Nifuroxazide	B	8.5					
	Tilmicosin	B	8.7					
	Salicylic acid	A	11				4.9	
	Sotalol	G	11					
	Ketoprofen	A	14			0.53		
	Salbutamol	F	14.7					
	Ciprofloxacin	B	15.5		311			19-36
	Famotidine	K	16					
	Sulfadiazine	B	19					
	Furosemide	H	21				5.8	
Contribution > 15%	Atorvastatin	I	25			2.3	3	
	Codeine	A	28				1.5	
	Spiramycin	B	28					
	Metronidazole	B	45					84
	Ranitidine	K	52				4.9	

Classification	Compound	PhC Class	This study	Heberer and Feldman 2005	Thomas et al. 2007	Langford and Thomas 2009	Ort et al. 2010	Beier et al. 2011
Bed density			6.5	12	4	4	4.4	33.5
	Clarithromycin	B	53					61-94
	Azithromycin	B	67					
	Ofloxacin	B	67					

3.3.5 Environmental Risk Analysis

A risk analysis was conducted on the effluent of Hospital B and the WWTP influent and effluent (all monitored in winter), using the quotient between the maximum MEC and the PNEC as a marker of risk. Each compound detected was subjected to evaluation, and values refer to acute toxicity. Neither chronic nor mixture toxicity was considered. Results for analgesic/anti-inflammatories, antibiotics and all the other classes are reported in Figure 3.3-3.5, respectively.

These analyses reveal that 9 substances in Hospital B effluent (the four analgesics/anti-inflammatories acetaminophen, ibuprofen, naproxen and salicylic acid, the four antibiotics clarithromycin, erythromycin, ofloxacin and sulfamethoxazole and the psychiatric drug fluoxetine) pose a potential ecotoxicological risk. A high risk was found only for 5 compounds (the same antibiotics and the psychiatric drug) in the influent and the effluent of Ferrara WWTP.

RQ classification proposed by (Hernando et al., 2006), showed that the levels of codeine, indomethacin, clenbuterol, atenolol, metoprolol and propranolol detected in the Hospital effluent pose a medium risk, as do the concentrations of acetaminophen, ibuprofen, naproxen, salicylic acid, clenbuterol, metoprolol, propranolol and gemfibrozil in the WWTP influent and, more importantly, salicylic acid, clenbuterol, propranolol, fenofibrate and gemfibrozil in the WWTP effluent.

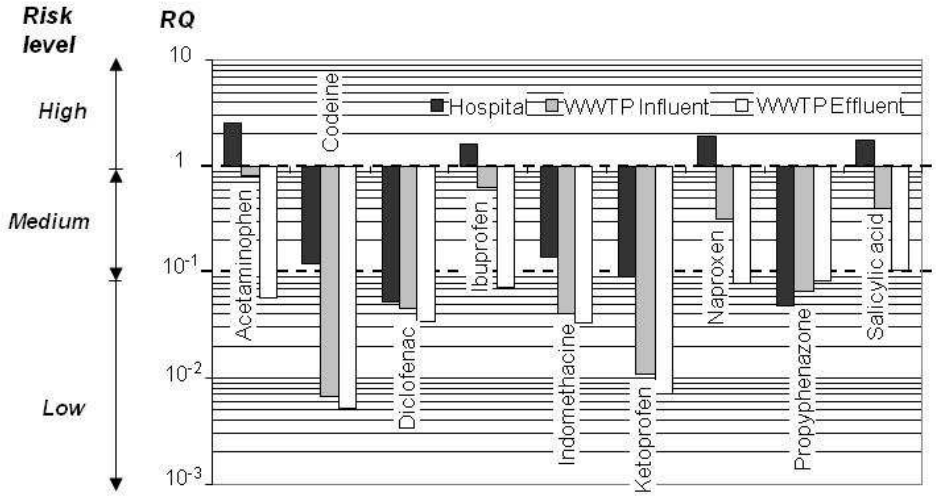


Figure 3.3. Risk quotients for analgesic/anti-inflammatories in Hospital B effluent and Ferrara WWTP influent and effluent

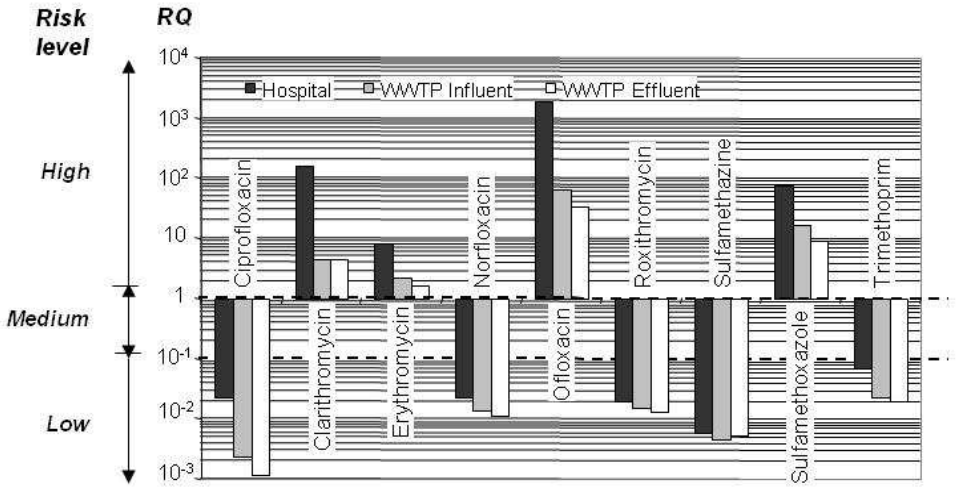


Figure 3.4. Risk quotients for antibiotics in Hospital B effluent and Ferrara WWTP influent and effluent.

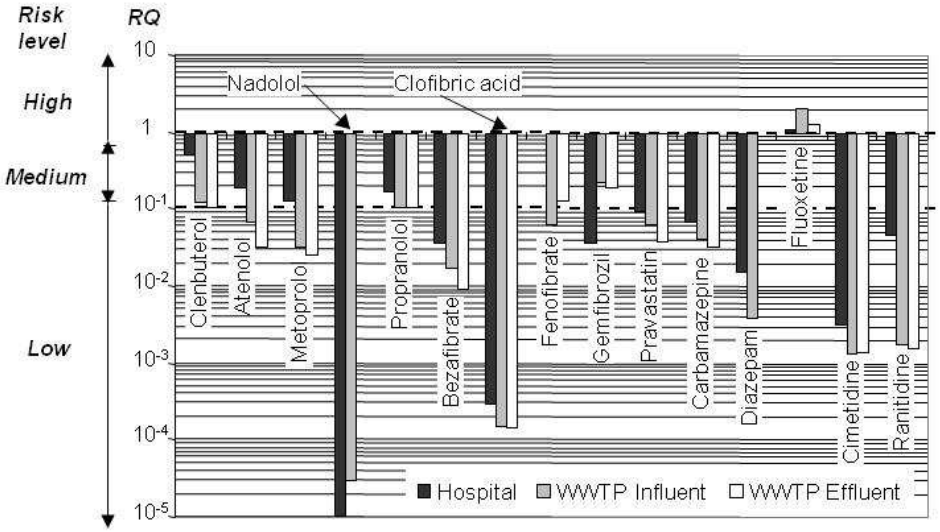


Figure 3.5. Risk quotients for other PhCs investigated in Hospital B effluent and Ferrara WWTP influent and effluent.

These findings are closely correlated to the fact that the hospital effluent contained higher concentrations for analgesics/anti-inflammatories and antibiotics than the influent to the WWTP. In addition, they confirm that antibiotics are one of the most critical therapeutic classes used in hospitals, being highly resistant to degradation and removal; indeed, the same 4 antibiotics whose concentrations were found to pose a high risk in hospital effluent were also those found at high levels of potential toxicity in the influent and the effluent of the WWTP.

This confirms that the conventional treatments exploited by this WWTP are unable to effectively remove these micropollutants, being constructed, and later upgraded, with

the aim of removing carbon, nitrogen and phosphorus compounds, pollutants which regularly arrive at the WWTP in concentrations to the order of mg L^{-1}.

This study evidences the fact that correct and specific management of hospital effluent on a *local scale* is necessary, and that further research is required to identify the best strategies for managing this type of effluent and evaluating the most suitable technologies for removing the most persistent contaminants, thereby reducing the risk posed to the environment and human health by these substances.

3.4 Conclusions

Hospital effluents are generally considered to possess the same pollutant nature as urban wastewaters and are therefore co-treated at the same WWTP, without any special consideration being given to the potentially harmful nature of the substances they may contain. This study, however, by means of an investigation into 73 PhCs from 12 different therapeutic classes, reveals that these compounds are found in consistently higher concentrations in hospital WW than in urban WW, particularly commonly used drugs such as analgesics and antibiotics.

The characteristics of the hospital effluent seem to be influenced by the size of the structure (the smaller hospital discharged higher mean concentrations than the larger one), and season (concentrations tended to be higher in winter than in summer). The ratio between PhC concentration in hospital effluent and WWTP influent was, on average, 7. The highest values were found for ofloxacin (31) and clarithromycin (36), ranitidine (27), atorvastatin (25), metronidazole (23). Antibiotics, analgesics/anti-inflammatories and lipid regulator were the pharmaceutical compounds found at the highest concentrations.

The percentage load contribution of the hospital varied among the investigated compounds; in particular 12 compounds yielded values between 16 and 67% (some antibiotics, receptor antagonists and lipid regulators).

Environmental risk analysis showed that 9 compounds posed a high risk at the concentrations detected in hospital effluent, while in the WWTP influent and effluent, only 5 of these PhCs were found to exhibit high ecotoxicity. As four out of these five PhCs were antibiotics, we can state that this class of compound should cause the most concern.

These results confirm that, due to their micropollutant content, HWWs require more specific management and treatment in order to protect and safeguard the environment, in

particular the surface water body which will receive the final (treated) effluent from the WWTP.

As co-treatment is common practice, and the usual (conventional) treatments are unable to efficiently remove PhCs, this issue needs urgent attention. Indeed, administrators and technicians will need to perform case-by-case analyses on a *local scale*, in particular during WWTP planning and design phases, in order to determine the best means of tackling the problem.

Chapter 4: Evaluation of the removal and release of PhCs from full-scale WWTPs and their impacts on the receiving water bodies

4.1 Introduction

At present, approximately 3000 different pharmaceutical ingredients are used in the European Union, including antibiotics, beta-blockers, lipid regulators, antidepressants and many more, for human consumptions (therapeutic or diagnostic purposes) (Ternes and Joss, 2006). One important emission source of pharmaceuticals in the water cycle is via human metabolism: in fact, once administered, these compounds are only partially metabolized by the human body, and therefore enter the water cycle either as parent (unchanged) compounds, which are excreted largely through urine (generally 55-80 % of the total, with few exceptions) and partially in the faeces, or as a mixture of metabolites and/or conjugated compounds (Jjemba et al., 2006, Lienert et al., 2007).

Unfortunately, municipal wastewater treatment plants (WWTPs) are generally unable to effectively remove either unaltered or metabolized forms of pharmaceutical compounds (PhCs) from wastewaters (Bendz et al., 2005; Castiglioni et al., 2006; Glassmeyer et al., 2004; Gomez et al., 2007; Joss et al., 2005; Verlicchi et al., 2012b). Their occurrence in surface water has been documented by a number of authors (Ashton et al., 2004; Calamari et al., 2003; Fatta-Kassinos et al., 2011; Gros et al., 2006, Kolpin et al., 2002, 2004; Spongberg et al., 2011) from around the world. Sometimes the load of the main compounds discharged with the treated effluent was also evaluated. Andreozzi et al. (2003), Castiglioni et al. (2005) and Zuccato et al. (2010) have monitored the occurrence of compounds such as antibiotics and some antiphlogistics, lipid regulators, beta-blockers, antiepileptics and anticancer drugs in a few Italian municipal WWTPs, although, generally speaking, little data is yet available regarding Italian secondary effluents. In contrast, many studies have been carried out in other countries in Europe, America, Asia and Australia (among them Gulkowska et al., 2008; Kasprzyk-Hordern et al., 2009; Lishman et al., 2006; McAvoy et al., 2002; Nakada et al., 2006; Watkinson et al., 2007), focusing principally on analgesics/anti-inflammatories and antibiotics. These studies have confirmed the occurrence of most of the abovementioned PhCs and, in some cases, their metabolites in the secondary effluent from municipal WWTPs.

As some of these substances have been detected in large concentrations (of the order of magnitude of hundred μg L^{-1}-mg L^{-1}) in the secondary effluent, there has been increasing interest in evaluating the environmental risk related to their discharge into

surface waters through treated effluent. PhCs are designed to exert specific biological effects within a given species; however, once excreted and released into the environment they may remain bioactive and could also pose a toxicological risk to non-target organisms, thereby altering the ecosystem dynamic (Boxall et al., 2002; Daughton and Ruhoy, 2009). As few of these substances are easily degradable, it is the characteristics of the receiving water body, in terms of (minimum and average) flow rate, biological, chemical and physical characteristics, auto-depurative capacity, water use and environmental quality standards, as well as environmental conditions (mainly solar radiation, temperature and precipitation), that will determine the extent to which it can tolerate the release of pharmaceuticals without perceptible adverse effects.

The aims of this chapter were therefore *(i)* to evaluate the removal efficiency of 27 PhCs, belonging to nine different therapeutic classes (six analgesics/anti-inflammatories, seven antibiotics, three lipid regulators, four beta-blockers, three psychiatric drugs, one antidiabetic, one antihypertensive, one diuretic and one beta-agonist) in a Full-scale WWTP,*(ii)* to evaluate the concentration of the same PhCs in the effluent from two municipal WWTPs, *(iii)* to evaluate the impact of the WWTPs (in terms of single compound concentrations and therapeutic class mass loads) on their respective receiving water bodies, which are characterized by different hydrodynamic characteristics. PhC concentrations were also monitored in the receiving surface waters, upstream and downstream of the effluent discharge point, and *(iv)* an environmental risk analysis assessment was performed for both the effluent and the receiving water body in the two case studies.

4.2 Materials and methods

4.2.1 Pharmaceutical compounds

Compounds analyzed in this study were selected on the basis of several criteria: high consumption by the resident population (Gruppo di lavoro OsMed, 2011), interest for environmental and public health (De Voogt et al. 2009) and availability of detection techniques. Table 4.1. reports the PhCs selected, grouped according to their therapeutic class and compiled in alphabetic order. For each compound, Table 4.1. also reports the amounts of active compound consumed in Italy in 2010 in terms of defined daily doses per 1000 inhabitants per day (DDD 1000 inh^{-1} d^{-1}) (Gruppo di lavoro OsMed, 2011), as well as

their annual consumption (kg). This figure was obtained by multiplying the DDD of each drug by the conversion factor (CF) (mg active compound/DDD) provided by WHO (2012). The Italian reference population was that documented in 2010 (58 640 000 inhabitants), except in the case of bezafibrate consumption, where data refer to 2001 (population 56 996 000 inhabitants). The same pattern of consumption of the selected PhCs found for the Italian population as a whole was assumed for the areas under study.

Table 4.1. also reports data from the literature on percentage excretion rate and removal efficiency achieved in secondary biological municipal WWTPs (including an activated sludge system as secondary treatment).

Table 4.1. Consumption of selected compounds in Italy in 2010, expressed as defined daily dose per 1000 inhabitants per day (DDD 1000 inh^{-1} d^{-1}) and the corresponding amount of compound consumed in that year (kg) evaluated using the conversion factor CF (mg active compound/DDD); percentage of excretion through human body after administration and percentage removal efficiency found in the literature.

Therapeutic class	Compounds	Amount used (DDD/1000 inh/d)	CF (mg/DDD)	Amount used (kg)	Excretion (%)	Removal efficiency (%)
Analgesics/Anti-inflammatories	Diclofenac	4.5	100	9602	39[b]	34[f]
	Indomethacin					
	Ketoprofen	4.3	150	13763		
	Mefenamic acid					36[f]
	Naproxen				1-10[d]	
	Propyphenazone					
Antibiotics	Azithromycin	1.3	500	13870		33[f]
	Ciprofloxacin	1.0	1000	21672	70[b]	83[f]
	Clarithromycin	3.0	1000	64470	25[d]	22[f]
	Metronidazole		1500	3.73	40[d]	24[f]
	Roxithromycin		300	577	1[d]	40[g]
	Sulfamethoxazole				39[b]	57[f]
	Trimethoprim		2000	13896	70[b]	32[f]
Antidiabetics	Glibenclamide	7.8	7	1165		
Antihypertensives	Enalapril	15.3	10	3265		
	Hydrochlorothiazide	11.8	25	6295		
Beta-agonists	Salbutamol	3.5	10	747	30[e]	95[f]
Beta-blockers	Atenolol	11.3	75	18084	90[b]	71[f]
	Metoprolol				10[e]	40[f]
	Sotalol					27[f]
	Timolol	4.5	20	1920		
Diuretics	Furosemide	21.8	40	18606	40[c]	42[f]
Lipid regulators	Atorvastatin	18	20	7682	5[c]	0[f]
	Bezafibrate		600	7600[a]	69[b]	
Psychiatric drugs	Carbamazepine		1000	31190	5[b]	0[f]
	Diazepam	1.5	10	320	1[c]	0[f]
	Lorazepam	13.3	2.5	709		

[a] Zuccato et al. (2005) (data referring to 2001). [b] Pal et al. (2010) . [c] Jjemba (2006) . [d] Verlicchi et al. (2010b) . [e] Kasprzyk-Horde rn et al. (2009) . [f] Escher et al. (2011) .[g] Karthikeyan and Meyer (2006) .

4.2.2 sampling area and sample collection

4.2.2.1 Area under study

Two conventional activated sludge plants WWTP A and WWTP B situated in the Po Valley, northern Italy, and their corresponding receiving water bodies were monitored in this study(Figure 4.1.). Table 4.2. summarizes the characteristics of the WWTPs investigated (population served, expressed as number of inhabitants *N*; capacity, as population equivalent PE; average influent flow rate; type of wastewater; hydraulic retention time, HRT and sludge retention time, SRT) and of the two receiving bodies (in terms of average flow rate during the observation period).

4.2.2.2 Case study A

WWTP A discharges its final effluent into canal A, that is part of the local surface water body network commonly used for irrigation during summertime. This canal crosses an urban area, and many buildings and commercial premises have long been situated along its banks. At the time of the monitoring program, its flow rate was on average 50 $m^3 s^{-1}$, as reported in Table 4.2. Based on historical data sets, this may range between 7 and 55 $m^3 s^{-1}$, with an average value of 15 $m^3 s^{-1}$ on a monthly basis. Assuming the WWTP flow rate reported in Table 4.2. (47 520 $m^3 d^{-1}$ = 0.549 $m^3 s^{-1}$), its dilution factor, i.e. the ratio between canal flow rate and WWTP flow rate, equals 91 during the monitoring period. It may vary between 13 and 100 depending on the season with its lowest values occurring during summertime.

4.2.2.3 Case study B

WWTP B discharges its treated effluent into the small and shallow canal B, that is part of the local surface water network that flows through a rural area. The surrounding land is predominantly agricultural, there are also herds of cattles and chickens and there are no buildings or commercial activities along its banks. Water from the canal is generally used for irrigation from May to October. The canal receives the runoff from agricultural land during rainy periods, and in dry periods, the WWTP effluent contributes about 50 % of the total average canal flow rate. As a consequence the corresponding dilution factor

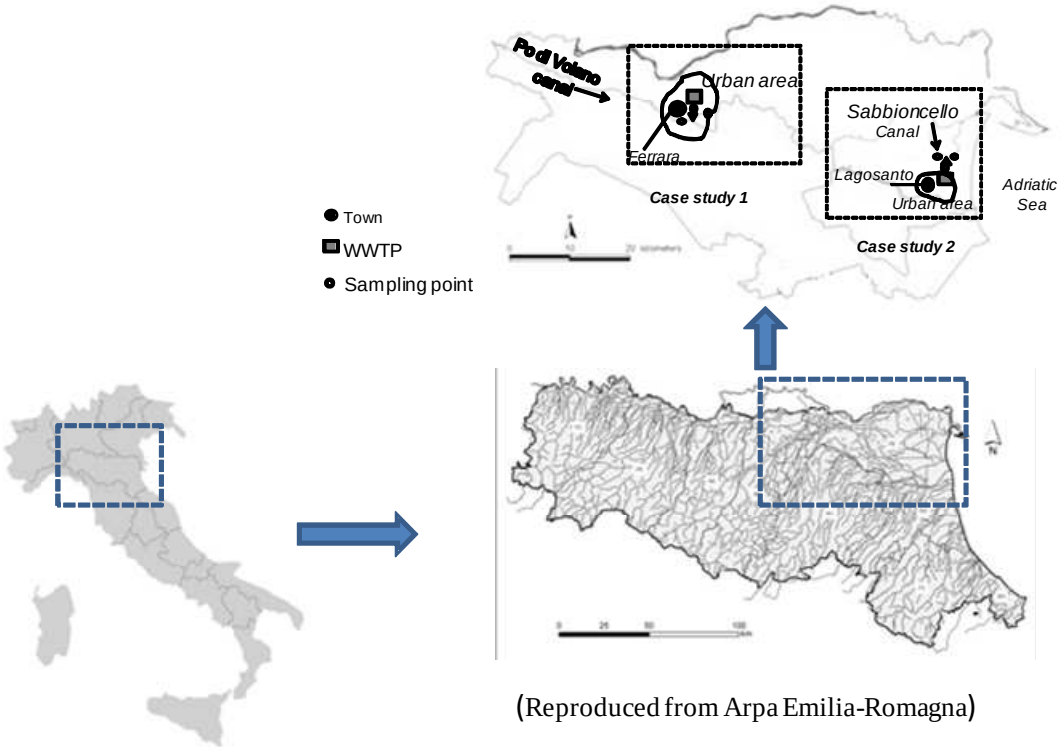
amounts to 1. The flow rate of canal B is quite almost constant along the year as it is regularly controlled by the local irrigation Agencies.

These two case studies were chosen because they belong to an area that was first declared at *risk of environmental crises*, as recurrent prolonged drought periods could drastically reduce the availability of fresh water for the different needs and then declared as a *sensitive area* due to eutrophication phenomena. Conventional macropollutants, including carbon, nitrogen and phosphorus compounds, suspended solids, *Escherichia coli*, have long been and still are regularly monitored according to the Italian regulation (Decreto Legislativo 152/2006) In the last years, some research groups (Zuccato et al.2010, Verlicchi et al., 2010a, 2012a, b) have been carried on experimental investigations in this area aiming to monitor unregulated parameters such as pharmaceuticals, in order to provide useful information for local and regional environmental protection agencies (their occurrence and load) to evaluate the potentially ecological risks associated with the discharge of pharmaceuticals. Moreover these two case studies can be considered representative of two fairly common situations in the Po Valley: the presence of a large WWTP receiving the wastewater from an urban as well as industrial catchment area and discharging its final effluent in a medium size canal (case study A) mainly used for agricultural purposes, and the presence of a small WWTP treating the wastewater from an urban catchment area, discharging its final effluent into a small canal whose flow rate is regulated for irrigation needs and kept quite constant (case study B).

4.2.2.4 Sampling

24-h composite flow proportional water samples of the influent, effluents of WWTP B and the effluent of WWTP A were taken over a period of three dry consecutive days in May 2011. In both cases, sampling sites were chosen on the basis of available access to the canal banks Fig 4.1. Unfortunately they were not at the same distance from the discharge point: roughly 1000 m upstream and downstream of the point of treated effluent discharge in canal A and 500 m in canal B. 4-h composite samples upstream and downstream of the WWTP discharge points. Surface water was collected from the central part of the canals, using 2 L clean plastic bottles for this purpose. All water samples were transferred to amber polyethylene terephthalate (PET) bottles and immediately transported

to the laboratory under cooled conditions (4 °C). Upon reception, samples were filtered through 0.45 μm nylon filters (Whatman, Maidstone, UK) to eliminate suspended solid matter, and then frozen (-20 °C) until analysis (less than a week later).



(Reproduced from Arpa Emilia-Romagna)

Figure 4.1 Location of the WWTPs and the receiving surface water canals sampled in the case studies.

Table 4.2. Characteristics of the two wastewater treatment plants under study

WWTP (inhabitants)	Capacity (PE)	Average flow rate $m^3\ d^{-1}$	Receiving water body, average flow rate m^3s^{-1}	Type of wastewater	HRT (h)/ SRT(d)	Secondary treatment
A (138 000)	240 000	47 520	canal A, 50	Urban (60 %) and industrial (40 %)	6/8	Activated sludge
B (5000)	5500	1360	canal B, 0.016	Urban	6/6	Activated sludge

4.2.3 Standards

All the pharmaceuticals and the corresponding isotopically labelled internal standards were of high purity grade (>90%). Detailed information on the providers of the analytical standards, as well as about the preparation of the mixture solutions can be found elsewhere (Jelic et al., 2009). The solvents, HPLC grade methanol, acetonitrile, water (Lichrosolv) and formic acid (98%) were provided by Merck (Darmstadt, Germany).

4.2.4 Analytical methods

Influent, Effluent wastewater and surface water samples were vacuum filtered through 1µm glass fibre filters and 0.45µm nylon membrane filters, after which an appropriate volume of aqueous solution of 5% Na_2EDTA was added to 100mL of WWTP influent, 200 mL of WWTP effluent and 500 mL of surface water to achieve a final Na_2EDTA concentration of 0.1% in the samples. The measured volumes were subsequently preconcentrated onto Oasis® HLB cartridges (60 mg and 3 mL) (Waters, Milford, MA, USA), at a flow rate of 5 mL/min, using a Baker vacuum system (J.T. Baker, Deventer, The Netherlands). After sample preconcentration, cartridges were rinsed with 5 mL of HPLC grade water and vacuum dried for 15–20 min to remove excess water. Elution of target compounds was performed with 2 × 4 mL pure methanol. Extracts were evaporated to dryness under a gentle nitrogen stream, and reconstituted with 1 mL of methanol–water (25:75, v/v). Prior to analysis, all the samples were spiked with a standard mixture of isotope-labelled standards at concentration of 20 ng ml^{-1}. Instrumental analysis was performed by high performance liquid chromatography coupled to a hybrid triple quadrupole – linear ion trap mass spectrometer (HPLC-QLIT-MS/MS) according to the previously developed multi-residual methodology for analysis of pharmaceuticals in wastewater (Gros et al., 2009).

The internal standard calibration approach was used for quantification. To determine the recoveries, three samples of each matrix were spiked with a standard mixture of target analytes. For wastewater, the recoveries ranged from 43 to 121 % (RSD<13%) for effluent wastewater, and from 45 to 135 (RSD<15 %) for surface water samples. Matrix effect was 20 to 60% for most of the compounds. The instrumental intra-day precision ranged from 2 to 11%, for six injections/day of a 50 ng ml^{-1} standard mixture. Limit of detection (LOD) and limit of quantification (LOQ) were calculated as the minimum detectable amount of analyte with a signal-to-noise ratio of 3 and 10, respectively. LOD , LOQ and RSD (%) values are reported in Table 4.3.

4.2.5 Statistical analysis

Statistical analysis was performed by means of Student's test in order to determine the significance of the differences found: between the average concentrations in the effluents of the two WWTPs, between average concentrations upstream and downstream in the two case studies, at a general confidence level of 99% (p-value = 0.01).

Table 4.3.Limits of detection (LOD), limits of quantification (LOQ) and precision of the method, expressed as a relative standard deviation (n=3).

Classe	compounds	WWTP influent			WWTP effluent			Surfacewater		
		LOD ng L^{-1}	LOQ ng L^{-1}	RSD%	LOD ng L^{-1}	LOQ ng L^{-1}	RSD%	LOD ng L^{-1}	LOQ ng L^{-1}	RSD%
Analgesics/anti-inflammatories	Diclofenac	5	17	11	4	13	5	1	3	14
	Indomethacine	4	13	8	3	10	13	1	3	9
	Ketoprofen	8	27	14	5	17	12	4	13	9
	Mefenamic acid	13	43	18	4	13	9	1	3	12
	Naproxen	12	40	13	4	13	12	1	3	16
	Propyphenazone	4	13	5	2	7	4	1	3	8
Antibiotics	Azithromycin	7	23	5	5	17	10	1	3	17
	Ciprofloxacin	5	17	9	2	7	13	1	3	17
	Clarithromycin	5	17	3	4	13	10	0.5	2	5
	Metronidazole	6	20	9	2	7	19	0.5	1	3
	Roxithromycin	3	10	5	2	7	9	0.5	2	3
	Sulfamethoxazole	4	13	7	2	7	18	1	3	6
	Trimethoprim	3	10	2	2	7	12	0.5	2	16
Antidiabetic	Glibenclamide	5	17	7	4	13	15	1	3	8
Antihypertensive	Enalapril	3	10	5	1	3	12	0.5	2	11
	Hydrochlorothiazide	11	37	6	6	20	5	5	17	12
Beta-agonists	Salbutamol	2	7	13	2	7	7	0.5	2	3
Beta-blockers	Atenolol	10	33	13	8	27	16	0.5	2	9
	Metoprolol	4	13	6	3	10	2	1	3	11
	Sotalol	5	17	16	3	10	11	1	3	14
	Timolol	1	3	3	1	3	7	0.5	2	9
Diuretics	Furosemide	3	10	12	2	7	13	0.5	2	16
Lipid regulators	Atorvastatin	4	13	16	3	10	9	0.2	1	12
	Bezafibrate	4	13	12	1	3	7	0.5	2	14
Psychiatric drugs	Carbamazepine	5	17	3	3	10	1	0.5	2	7
	Diazepam	4	13	12	2	7	5	1	3	6
	Lorazepam	7	23	11	5	17	8	1.5	5	9

4.2.6 Environmental risk assessment

The potential risk posed by each PhC was assessed by calculating its risk quotient (RQ) as the ratio between its maximum measured environmental concentration (MEC) and its predicted no-effect concentration (PNEC), as discussed in section 3.2.6.

PNEC values assumed for this risk analysis correspond to the lowest ecotoxicological PNEC values found in literature. For 19 out of the 27 investigated compounds, ecotoxicological data are available. Table 4.5. compiles the assumed values. Conforming to EC (EC, 2003), the values of PNECs to use in the risk analysis (those of Table 4.5.) are 1000 times lower than the ecotoxicity concentration values found for the most sensitive species assayed (among bacteria, algae, invertebrates and fish), so as to take into account the effect on other, potentially more sensitive, aquatic species to those used in toxicity studies. A commonly used risk ranking criterion was applied, after Hernando et al. (2006), De Souza et al. (2009) and Zhao et al. (2010): RQ < 0.1, minimal risk to aquatic organisms, $0.1 \leq RQ < 1$, median risk; $RQ \geq 1$, high risk.

4.3 Results and discussion

Experimental data are reported in Table 4.4., in terms of measured average values, standard deviations SD, corresponding variability ranges and percentage detection frequencies *f* of each selected compound in the two sampled effluents. PhCs are grouped according to their therapeutic class and listed in alphabetical order. The last two columns of Table 4.4. show measured concentration intervals reported in secondary effluents from other WWTPs in Italy and other world countries.

Analytical variations due to analysis for WWTP effluents are in general % RSD <18 , RSD < 19, and RSD <17 (n=3) in influent , effluent and surface water respectively.

4.3.1 Removal of selected pharmaceuticals in the WWTP B.

Influent and effluent concentrations of selected PhCs in the WWTP B are depicted together in Figure 4.2. in order to evaluate percentage removal efficiency of this WWTP. Data are ranked with decreasing percentage removal efficiency that shown between the parenthesis after the name of the selected compounds in Figure 4.2. The observed effluent concentration of selected PhCs in Table 4.4. is clearly correlate to the influent concentration and the percentage elimination in the WWTP, all the detected

pharmaceuticals in the influent of the WWTP B, are detected in the effluent of the same with the exception of Enalapril, Atorvastatin and Glibenclamide due the complete Removal in the WWTP. The average percentage removal efficiency vary between 0% (Carbamazepine) and 97% (Bezafibrate and Naproxen), negative removal efficieny were observed for Diazepam, Furosemide, Azithromycin and hydrochlorothiazide.

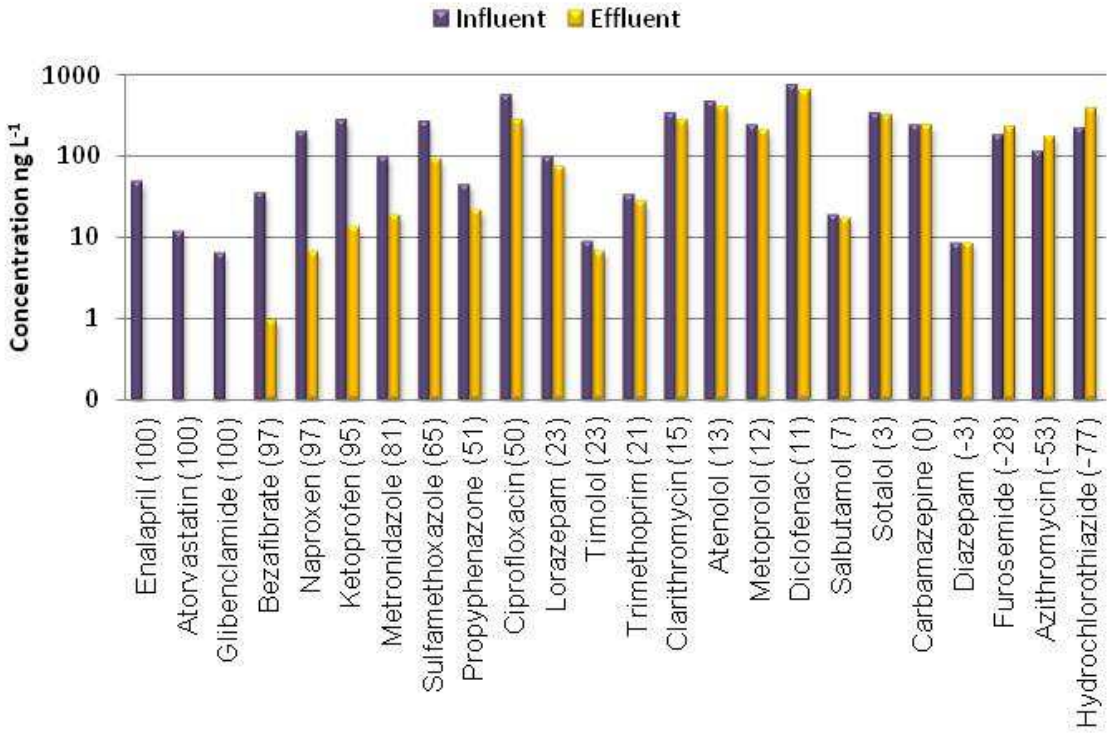


Figure 4.2. Influent and effluent concentration of selected PhCs in WWTP B.

4.3.2 Occurrence of selected pharmaceuticals in the investigated WWTP effluents

Overall, 24 out of the 27 selected pharmaceuticals were detected at least once in each sample of the two monitored WWTP effluents, 10 compounds were always detected (*f* = 100 %). The compounds indomethacine, atorvastatin and enalapril were those never detected in the two effluents investigated during this study. The average number of detected compounds in each water sample was 17 (SD = 2). No compound has never exceeded 1 μg L^{-1}: the maximum concentrations were found for diclofenac (800, 605, 589, 533 ng L^{-1}) and hydrochlorothiazide (520 ng L^{-1}). The highest overall average values were found for diclofenac (502 ng L^{-1}), hydrochlorothiazide (265 ng L^{-1}), atenolol (264 ng L^{-1}) and sotalol (262ng L^{-1}).

In 47 cases out of 162 (= 27 compounds x 2 sampling sites x 3 samplings), measured concentration was > 100 ng L^{-1}. It happened 6 times for diclofenac, sotalol and metoprolol, 5 times for clarithromycin, atenolol, hydrochlorothiazide and carbamazepine, 3 times for azithromycin and furosemide, twice for ciprofloxacin and once for sulfamethoxazole. The value of 100 ng L^{-1} was chosen as it was previously used by Beier et al. (2011) as a target value for pharmaceuticals discharged with treated effluents. Overall, rather broad ranges of variability were observed for diclofenac (578 ng L^{-1}), ciprofloxacin (470 ng L^{-1}), hydrochlorothiazide (436 ng L^{-1}) and atenolol (409 ng L^{-1}), while much smaller intervals were observed for timolol (9 ng L^{-1}), diazepam (10 ng L^{-1}), roxithromycin (13 ng L^{-1}) and metronidazole (18 ng L^{-1}).

Average concentrations found in this investigation are consistent with those reported by Andreozzi et al. (2003), Castiglioni et al. (2005) and Zuccato et al. (2010) referring to the occurrence of selected PhCs in different Italian WWTP effluents (Table 4.4), except in the case of the analgesic naproxen and the antibiotic clarithromycin. With respect to variability ranges found in this investigation, occurrence of naproxen has been reported at an order of magnitude higher (literature range equal to 290–5220 ng L^{-1} against our measured range < LOD–21 ng L^{-1}), and clarithromycin at an order of magnitude lower (literature data included in the range 8–37 ng L^{-1} against our measured range 189–374 ng L^{-1}). Other studies carried out in different countries in Europe, America, Asia and Australia found ranges of PhC variability (Table 4.4., last column) even two (diclofenac, ketoprofen, naproxen, trimethoprim, hydrochlorothiazide, atenolol, sotalol, carbamazepine) or three (diazepam) orders of magnitude higher than those found in this investigation.

4.3.2.1 WWTP A Effluent

As shown in Table 4.4. and in Fig. 4.3., out of the 27 selected substances, 19 PhCs were detected in WWTP A effluent and among these 12 pharmaceuticals were always detected (f = 100 %): the analgesic diclofenac, the antibiotics azithromycin, ciprofloxacin, clarithromycin, metronidazole and roxithromycin, the antidiabetic glibenclamide, the antihypertensive hydrochlorothiazide, the beta-blockers atenolol, metoprolol and sotalol and the psychiatric drug carbamazepine. In addition, the 4 compounds: ketoprofen, mefenamic acid, furosemide and lorazepam were detected with f = 67 % and finally the three compounds sulfamethoxazole, salbutamol and timolol were detected at a frequency

of 33 %. The number of detected compounds was on average 16 in the three samples (SD = 2).

The highest average values were found for the analgesic diclofenac (339 ng L^{-1}), the beta-blockers sotalol (197 ng L^{-1}) and metoprolol (184 ng L^{-1}). The other compounds exhibiting an average concentration > 100 ng L^{-1} were: the antihypertensive hydrochlorothiazide (145 ng L^{-1}), the psychiatric drug carbamazepine (125 ng L^{-1}), the beta-blocker atenolol (111 ng L^{-1}) and the antibiotic clarithromycin (102 ng L^{-1}).
On the basis of the all 81 data collected for this effluent (=27 compounds x 3 samples), 17 times measured concentrations were > 100 ng L^{-1}. The highest ranges of variability were observed for diclofenac (310 ng L^{-1}), followed by hydrochlorothiazide (134 ng L^{-1}) and sotalol (106 ng L^{-1}), while the smallest one was found for roxithromycin (3 ng L^{-1}).

4.3.2.2 WWTP B Effluent

As shown in Table 4.4. and in Fig. 4.4., out of the 27 selected substances, 21 PhCs were detected in WWTP B effluent and among these 17 PhCs were always detected (f = 100 %): the analgesic diclofenac, the antibiotics azithromycin, ciprofloxacin, clarithromycin, metronidazole, sulfamethoxazole and trimethoprim, the antihypertensive hydrochlorothiazide, the beta-agonist salbutamol, the beta-blockers atenolol, metoprolol, sotalol and timolol, the diuretic furosemide and all the psychiatric drugs carbamazepine, diazepam and lorazepam. In addition, the 2 compounds: ketoprofen and propyphenazone were detected with f = 67 % and finally the two compounds naproxen and bezafibrate were detected at a frequency of 33 %. The average number of detected compounds in this sampling point was 19 (SD = 1).

The highest average values were found for diclofenac (665 ng L^{-1}), atenolol (417 ng L^{-1}), hydrochlorothiazide (385 ng L^{-1}), sotalol (327 ng L^{-1}), ciprofloxacin (284 ng L^{-1}) and clarithromycin (283 ng L^{-1}). Other compounds detected at concentrations > 100 ng L^{-1} were: carbamazepine (240 ng L^{-1}), furosemide (235 ng L^{-1}), metropol (210 ng L^{-1}) and azithromycin (175 ng L^{-1}). On the basis of all the 81 data collected for this effluent, 30 times measured concentrations were > 100 ng L^{-1}. The highest ranges of variability were observed for hydrochlorothiazide (474 ng L^{-1}), followed by ciprofloxacin (453 ng L^{-1}) and carbamazepine (230 ng L^{-1}), while the smallest one was found for timolol (2 ng L^{-1}).

4.3.2.3 Comparison between the two effluents

Out of the 27 investigated compounds, 16 were found in both effluents. An analysis of data reported in Table 4.4. shows that for 13 out of the common 16 detected pharmaceuticals average concentrations were higher for WWTP B effluent than WWTP A effluent. The only exceptions were for ketoprofen, sulfamethoxazole and timolol exhibiting quite similar values.

This could be explained by different reasons: (*i*) the fact that WWTP A treats both urban and industrial wastewaters, the latter of which, being from petrochemical activities that do not release PhCs, have a dilution effect on their inlet concentrations; (*ii*) possible different pharmaceutical consumption patterns between the two areas under investigation, resulting in different inlet concentration and (*iii*) possible different removal efficiencies achieved in the two activated sludge systems whose values depend on many design and operational factors, mainly reactor configuration, types and way of feeding in the biological tank, SRT, HRT, temperature as discussed in Verlicchi et al. (2012b).

As reported above, average concentrations > 100 ng L^{-1} were found for 7 compounds in effluent A and 10 in effluent B (the same pharmaceuticals of effluent A and 3 in addition). These elevated figures could be due to their high consumption, which range from 6–64 tons on a national basis, and to the inefficiency of conventional treatments in removing most of them, the percentage removal rates being less than 40 % (Table 4.1.). These results are also illustrated in Figures 4.3. and 4.4. which report, in descending order, the average concentrations of the monitored compounds measured in the two effluents.

The seven PhCs found at the highest concentrations in both effluents (diclofenac, clarithromycin, hydrochlorothiazide, atenolol, metoprolol, sotalol and carbamazepine) are generally consumed for long periods, especially among the elderly (beta-blockers, antihypertensives, anti-inflammatories and diuretics).

To complete the comparison, a statistical analysis has been conducted for the two effluents as well as for the classes of analgesics/anti-inflammatories, antibiotics, beta-blockers and psychiatric drugs. The aim was to verify if the average concentrations of compounds are different in the two effluents from a statistical point of view and how significant are these differences. Student's test applied to the whole effluents and to the single reported classes showed that there is enough evidence (*p*-value lower than 0.01) for the whole effluents and for the class of antibiotics to state that the corresponding. average concentrations are

different (level of significance greater than 99%). For the other classes the differences should seem not statically significant but, in our opinion, further research and experimental data should be necessary to confirm this result.

Table 4.4. Occurrence of pharmaceuticals in the effluents of WWTP A and WWTP B: Concentration ranges (ng L^{-1}) with their mean values (n=3), their standard deviation SD and the frequency of detection *f*. Comparison with literature values referring to Italy and worldwide

Class	Compounds	WWTP A			WWTP B			Range in Italy[a]	Worldwide[b]
		Mean±SD (ng L^{-1})	Range (ng L^{-1})	*f* (%)	Mean±SD (ng L^{-1})	Range (ng L^{-1})	*f* (%)	(ng L^{-1})	(ng L^{-1})
Analgesics/anti-Inflammatories	Diclofenac	339±138	223-533	100	665±96	589-800	100	470-5450	6-10000
	Indomethacine	n.d.*	n.d.	n.d.	n.d.	n.d.	n.d		20-600
	Ketoprofen	23±15	n.d.-23	67	21±10	n.d. -21	67		n.d. -2270
	Mefenamic acid	26±12	n.d.-27	67	n.d.	n.d.	n.d		2-3000
	Naproxen	n.d.	n.d.	n.d	21±10	n.d. -21	33	290-5220	1-5090
	Propyphenazone	n.d.	n.d.	n.d	33±20	n.d -48	67		1-120
Antibiotics	Azithromycin	44±16	22-55	100	175±47	109-209	100		40-380
	Ciprofloxacin	25±11	10-33	100	284±186	46-499	100	27-514	7-5700
	Clarithromycin	102±10	89-112	100	283±75	189-374	100	8-37	150-460
	Metronidazole	16±5	9-21	100	19±6	14-27	100		55-561
	Roxithromycin	12±1	10-13	100	n.d	n.d.	n.d		10-540
	Sulfamethoxazole	97±46	n.d. -97	33	91±67	35-185	100	10-317	3-840
	Trimethoprim	n.d.	n.d.	n.d	27±8	21-39	100	30-130	5-1880
Antidiabetics	Glibenclamide	36±7	27-43	100	n.d	< n.d	n.d		
Antihypertensives	Enalapril	n.d.	n.d.	n.d	n.d	n.d	n.d		
	Hydrochlorothiazide	145±56	85-219	100	385±98	294-520	100		679-11000
Beta-agonists	Salbutamol	9±4	n.d. -9	33	18±6	13-26	100	1.1-18	10-170
Beta-blockers	Atenolol	111±41	65-164	100	417±48	356-474	100	27-1168	10-73000
	Metoprolol	184±17	161-199	100	210±9	198-219	100	10-100	5-2200
	Sotalol	197±45	152-258	100	327±33	285-366	100		249-1320
	Timolol	9±4	n.d.-9	33	7±1	6-8	100		10-70
Diuretics	Furosemide	14±7	n.d.-18	67	235±68	184-331	100	0.2-2102	20-1823
Lipid regulators	Atorvastatin	n.d.	n.d.	n.d	n.d	n.d	n.d		
	Bezafibrate	n.d.	n.d.	n.d	3±1	n.d -3	33	0.3-910	4800
Psychiatric drugs	Carbamazepine	125±24	94-152	100	240±2	216-265	100		2-19800
	Diazepam	n.d	n.d	n.d	9±1	7-10	100		15-19300
	Lorazepam	46±22	n.d -46	67	76±5	71-82	100		196
N of detected compounds		19			21			11	24

n.d. = not detected, [a] Data from: Castiglioni et al., 2005; Andreozzi et al., 2003; Zuccato et al., 2010, [b] Data from: Verlicchi et al., 2012b.

4.3.3 Occurrence of selected pharmaceuticals in the two receiving surface water bodies

Overall, 22 out of the selected 27 substances were detected in surface water; no compound was always detected in each sample (see Appendix C.1.); the most detected PhCs were: carbamazepine (10 out of 12 times), naproxen, atenolol and sotalol (9 times), azithromycin, ciprofloxacin, clarithromycin and bezafibrate (7 times). Atorvastatin, enalapril, mefenamic acid and roxithromycin and ketoprofen were never detected. The number of compounds detected in surface water samples ranged between 5 and 22, with an average value equal to 11 (SD = 7).

The highest concentrations were found for sotalol (504, 502 and 373 ng L^{-1}), hydrochlorotiazide (128 and 116 ng L^{-1}), clarithromycin (128 and 103 ng L^{-1}), ciprofloxacin (124 ng L^{-1}) and furosemide (114 ng L^{-1}). Overall, concentrations were > 100 ng L^{-1} for 9 times and for the just reported 5 compounds. Overall, rather broad ranges of variability were observed for sotalol (504 ng L^{-1}), atenolol (231 ng L^{-1}), clarithromycin and hydrochlotothiazide (128 ng L^{-1}), while much smaller intervals were observed for naproxen, bezafibrate, metronidazole, propyphenazone (16 ng L^{-1}), timolol, (8 ng L^{-1}).

Out of the 27 selected compounds investigated in this work, only 13 compounds have previously been monitored in major Italian surface water bodies, including the Rivers Po, Arno and Lambro (Calamari et al. 2003; Ferrari et al. 2011; Perrett et al. 2006; Zuccato et al. 2000, 2006, 2008, 2010). The ranges of variability measured in these studies are consistent with our findings.

A further comparison between our data and ranges of variability of the same PhCs found in water surface bodies observed in other countries (Gros et al., 2010; Fatta Kassinos et al., 2011; Spongberg et al., 2011; Tamtam et al., 2008; Wang et al. 2010) reveals that reported surface concentrations can be much higher than those found in our study (for instance two orders of magnitude higher for the antibiotics ciprofloxacin and clarithromycin), presumably reflecting different patterns of PhC consumption in different countries.

Figures 4.3. and 4.4. report the average concentrations of each selected compound measured in the receiving water bodies of the two WWTP effluents (canals A and B), upstream and downstream of the corresponding discharge point (WWTP A and WWTP B),

together with the corresponding average concentrations found in the secondary effluents. In each graph, the X-axis reports the selected compounds, in order of decreasing concentrations found in the secondary effluent. The two numbers in brackets after each name correspond to the upstream and downstream detection frequencies, respectively. PhC concentration profiles detected in the two case studies are analyzed below. For easier viewing, the error due to analysis (RSD %) are not shown in the figures, and they ranged from 3-17% (n=3) depending on compound (see table 4.3.).

4.3.3.1 Case study A (WWTP A- canal A)

As reported in Figure 4.3., out of the 19 PhCs detected in the WWTP A effluent, only five (clarithromycin, atenolol, sotalol, carbamazepine and salbutamol) were detected upstream of the WWTP discharge (for three compounds f = 67 % and for the remaining two f = 100 %), while eleven (diclofenac, clarithromycin, sulfamethoxazole, azithromycin, ciprofloxacin, atenolol, sotalol, metoprolol, carbamazepine, lorazepam and furosemide) were found downstream at detection frequencies of 33 % (four compounds), 67 % (five compounds) and 100 % (six compounds). Although the two compounds naproxen and bezafibrate were never detected in WWTP A effluent, they were always detected (f = 100 %) both upstream and downstream in the canal A. Although trimethoprim and propyphenazone were never detected in the effluent, they were found downstream (f = 67 % each) of the discharge point and not upstream.

This could be explained by the presence of illegal raw discharge from buildings and commercial activities present along the banks of the canal, or, in some cases, of buildings not yet connected to the sewage network. Further reasons could be the release or resuspension of settled materials. Contaminations during extraction analysis have to be excluded.

Average measured concentrations of the selected compounds were generally below 10 ng L^{-1} in canal A, with a few exceptions: sotalol (30 ng L^{-1}), ciprofloxacin (25 ng L^{-1}) and atenolol (11 ng L^{-1}) (Fig. 4.3.). Student's test applied to the two series of upstream and downstream concentrations showed that there is enough evidence (p-value lower than 0.01) to state that the measured average concentrations are different with a level of significance greater than 99%. Referring to all the collected data in this case study, 29 out of 81 times pharmaceutical concentration was detected in the effluent A and not in canal A

downstream the discharge point, 10 out of 81 times pharmaceutical concentration was found only downstream the discharge point but not in the effluent and 22 out of 81 times selected pharmaceuticals were not detected in both the sampling points. In the remaining 20 cases, surface water concentration was found on average 20 times lower than the corresponding effluent concentration (with the ratio effluent concentration/surface water concentration varying between 6 and 64, SD = 19).

These considerations lead to think that the high dilution factor in canal A (roughly 91 during the observation period, estimated as above) could explain why surface water concentrations of the investigated PhCs remain quite low after effluent discharge. Sorption onto solids and sediments and photodegradation could also contribute to the decrease of pharmaceutical concentrations between the discharge point and the sampling site during the campaign. According to data reported in Verlicchi et al. (2012b) among the selected compounds, only azithromycin, ciprofloxacin, hydrochlorothiazide and diazepam tend to adsorb. Referring to Fig. 4.3. this mechanism could be of interest for the first three compounds, being the last one always not detected. Further attenuation of PhCs could be due to different biological, chemical and photochemical degradation processes occurring within the surface water (Jones et al., 2005): for many of organic compounds, photochemistry may be expected to play a much larger role than biodegradation, especially in sunlit waters. In particular, PhCs containing aromatic rings , heteroatoms (all the 27 compounds), and other functional groups or structural moieties such as phenol, nitro, and napthoxyl groups (most of them), thought to undergo consistent reduction in their concentration along the course of the receiving water body (Boreen et al., 2003, Andreozzi et al., 2003, Jones et al., 2005). Half-life time is the factor to consider to evaluate if the photodegradation can be of interest between the discharge and sampling points. According to Buser et al (1998) for those compounds with half-life time for direct photolysis < 1 h, such as diclofenac, photodegradation significantly contributes to reduce concentration in surface water together with dilution factor. For most of the investigated compounds, half-life time is much higher (Buser et al., 1998). Carbamazapine is the worst compound among those investigated: as it does not biodegrade, it does not adsorb onto solids and it requires more than 100 days to photodegrade (Andreozzi et al., 2003), it is only subjected to dilution effect.

4.3.3.2 Case study B (WWTP B- Canal B)

As reported in Figure 4.4., out of the 21 PhCs detected in the WWTP B effluent, only four (ciprofloxacin, azithromycin, trimethoprim and carbamazepine) were detected upstream of the discharge point, all with a detection frequency of 100 %, except for trimethoprim (*f* = 33 %), while 20 (hydrochlorothiazide, diclofenac, indomethacin, naproxen, propyphenazone, azithromycin, ciprofloxacin, clarithromycin, metronidazole, sulfamethoxazole, trimethoprim, glibenclamide, atenolol, sotalol, timolol, metoprolol, furosemide, lorazepam, salbutamol, carbamazepine, diazepam and bezafibrate) were found downstream. At this sampling point, their detection frequencies were always 100 %, except for bezafibrate and glibenclamide, which occurred in only 33 % of samples. The antidiabetic glibenclamide was never found in the effluent, and its presence downstream of the discharge point cannot rationally be explained by the very scarce literature data available about its concentration and behaviour upon release into the environment. Sorption onto solids and sediments and photodegradation could contribute to the decrease of surface water concentration, downstream only for the four compounds discussed above: hydrochlorothiazide, ciprofloxacin, azithromycin and diazepam.

Student's test applied to the two series of upstream and downstream average concentrations showed that there is enough evidence (*p*-value lower than 0.01) to state that the measured average concentrations are different at a level of significance greater than 99%.

Referring to all the collected measures, only 2 out of 81 times (=27 compounds x 3 samples) pharmaceutical concentration was detected in the effluent B and not in canal B downstream the discharge point, 7 out of 81 times pharmaceutical concentration was found only downstream the discharge point but not in the effluent and 17 out of 81 times selected pharmaceuticals were not detected in both the sampling points. In the remaining 55 cases, surface water concentration was found on average 3 times lower than the corresponding effluent concentration (with the ratio effluent concentration/surface water concentration varying between 0.35 and 14 and SD = 2.6). Moreover, based on average concentrations (Fig. 4.4.), canal B clearly shows a PhC concentration profile similar to that found in the WWTP B effluent: the dilution effect of the receiving surface water almost always results in a reduction of the final surface water concentration by one order of magnitude: the ratio between average effluent concentration and average downstream concentration was in the

range 0.7–12, with an average value of 2.9. In canal B, average measured concentrations were generally much higher than 10 ng L^{-1}, as shown in Figure 4.4. (The value of 10 ng L^{-1} was chosen as it is one order of magnitude lower than the target value adopted by Beier et al. (2011) as discussed above). The highest values were found for sotalol (460 ng L^{-1}) and atenolol (160 ng L^{-1}), followed by hydrochlorothiazide, ciprofloxacin, clarithromycin, carbamazepine, furosemide and azithromycin, which occurred at an average concentration ranging between 80 and 100 ng L^{-1}.

This analysis and the concentration profiles in Figures 4.3. and 4.4. confirm that, due to an (expected) incomplete removal of PhCs in conventional WWTPs, the discharge of these treatment plants seems to represent an important source of PhCs in surface waters. The reason for the higher concentrations in canal B with respect to canal A is mainly the modest flow rate of the former and the resulting poor dilution after mixing with the discharge. Even if for most compounds, effluent B exhibited higher average concentrations than effluent A, WWTP B flow rate is much lower than WWTP A flow rate (Table 4.2.), resulting in lower overall mass loads (referred to the whole catchment area, as discussed later) discharged in the receiving water body B than in canal A. This fact confirms a greater dilution capacity of canal A than canal B. As reported above, water sampling was possible at a different downstream distances from the discharge points: 1000 m in canal A and 500 m in canal B. A contribution in the reduction of measured concentration of pharmaceuticals in canal A could be also due to degradation processes occurring in the river before sampling. But as the selected compounds are scarcely subjected to photodegradation reactions, as discussed above, this contribution keeps quite modest. According to Gros et al. (2010) the dilution capacity of the receiving water bodies can be considered the first and the primary measure in mitigating the potential toxicological effects of PhCs released into the environment.

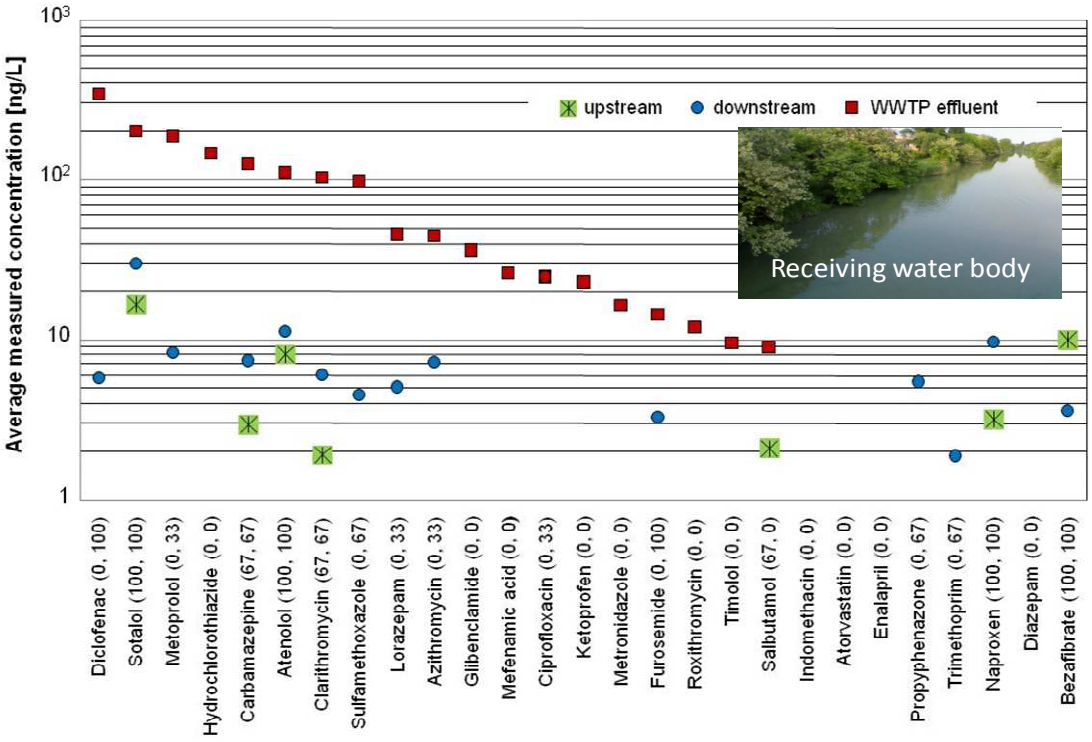


Figure 4.3 Average concentrations of pharmaceuticals detected in WWTP A effluent and its receiving water body, the canal A (case study A).

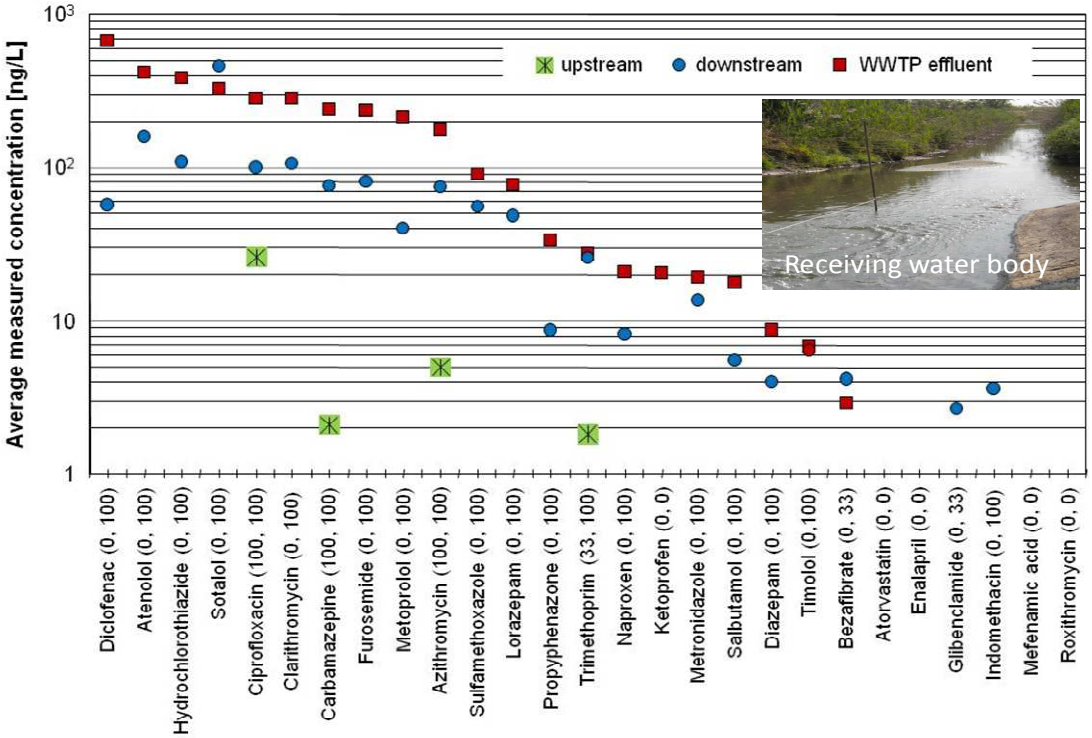


Figure 4.4 Average concentrations of pharmaceuticals detected in WWTP B effluent and its receiving water body, the canal B (case study B).

4.3.4 Pharmaceutical load discharged into the environment

In order to evaluate environmental input of PhCs due to WWTP effluent, an initial rough estimate of the average mass load *L* of each therapeutic class *j* (*j* = 1, 2, ..., 9) associated with the WWTP effluent *h* (*h* = 1, 2) and the 27 selected compounds was calculated by multiplying the average WWTP effluent flow rate Q_h by the sum of the average concentrations measured for each compound *i* belonging to the same class *j* (as reported in Table 4.4.), and dividing the result by the population served *N* (*N* values reported in Table 4.2.). The mass load usually refers to 1000 inhabitants, as in eq. 4.1:

$$L_j = \frac{Q_h \sum_i c_{i,j}}{N} x\ 1000 \qquad \text{(eq.4.1)}$$

The average mass loads for the nine classes and the two WWTP effluents are shown in Figure 4.5., in descending order of values found for the WWTP B effluent. They ranged from 3 to 173 mg/d/1000 inhabitants in WWTP A effluent and from 0 to 262 mg/d/1000 inhabitants in WWTP B effluent, the WWTP B mass loads being consistently greater than those in the WWTP A effluent, except for that of the sole antidiabetic selected, which was not found to be present in the WWTP B effluent ($L_{\text{antidiabetic}} = 0$). In both cases, the highest mass loads were found for beta-blockers, although the descending order distribution is different for the two WWTP effluents, i.e. WWTP A effluent: beta-blockers > analgesics/anti-inflammatories > antibiotics > psychiatric drugs > antihypertensives > antidiabetics > diuretics > beta-agonists (lipid regulators were never detected); and WWTP B effluent: beta-blockers > antibiotics > analgesics/anti-inflammatories > antihypertensives > psychiatric drugs > diuretics > beta-agonists > lipid regulators (antidiabetics were not detected).

As regards the single selected PhCs, the highest average mass loads were found for diclofenac in both effluents: 181 mg 1000 inhabitants^{-1} d^{-1} for the case study B and 117 for the case study A. Among the selected antibiotics, the highest values were found for clarithromycin and ciprofloxacin (77 mg 1000 inhabitants^{-1} d^{-1}) and azithromycin (48 mg 1000 inhabitants^{-1} d^{-1}) in the effluent of WWTP B, and for clarithromycin (35 mg 1000 inhabitants^{-1} d^{-1}) in the effluent of WWTP A.

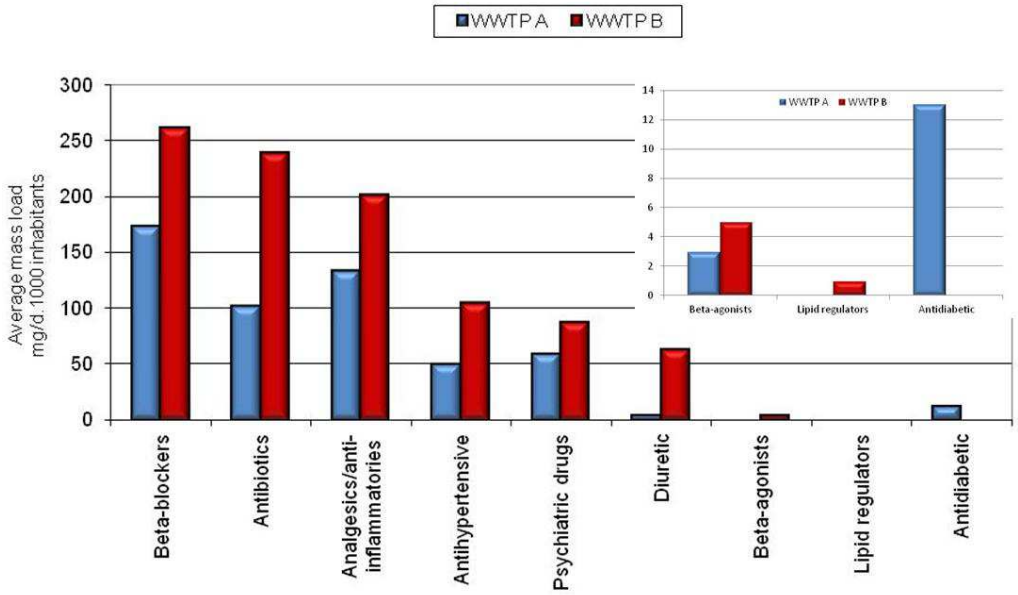


Figure 4.5. Average mass loads for the nine therapeutic classes based on occurrence of the selected pharmaceuticals.

Considerable mass loads were found in WWTP B effluent for atenolol (114 mg 1000 inhabitants^{-1} d^{-1}), hydrochlorothiazide (105 mg 1000 inhabitants^{-1} d^{-1}), sotalol (89 mg 1000 inhabitants^{-1} d^{-1}), carbamazepine (65 mg 1000 inhabitants^{-1} d^{-1}) and metoprolol (57 mg 1000 inhabitants^{-1} d^{-1}). In WWTP A effluent, the highest mass loads were consistently lower: sotalol (68 mg 1000 inhabitants^{-1} d^{-1}), hydrochlorothiazide (50 mg 1000 inhabitants^{-1} d^{-1}) and carbamazepine (43 mg 1000 inhabitants^{-1} d^{-1}), except for metoprolol, whose mass load was slightly higher (63 mg 1000 inhabitants^{-1} d^{-1}).

The total (referred to the selected compounds) average mass loads discharged by the two effluents were estimated at 539 mg 1000 inhabitants^{-1} d^{-1} for WWTP A and 965 mg 1000 inhabitants^{-1} d^{-1} for WWTP B. It is important to observe that the daily *overall* pharmaceutical mass load discharged with the whole effluent into the receiving water body depends on the whole resident population (hence on the WWTP flow rate). Assuming the values reported in Table 4.2. for the two WWTPs, the overall mass loads discharged with effluent A amounts to 74 g d^{-1} and it is much higher than that discharged with effluent B (5 g d^{-1}).

4.3.5 Environmental impact of the selected PhCs

Based on EMEA guidelines (EMEA, 2006), the risk quotient RQ between MEC and PNEC was calculated for the different compounds to estimate their potential adverse effects on aquatic organisms in both WWTP effluents and surface water. Unfortunately PNECs are known only for 19 out of the 27 selected compounds investigated here. In this study, in order to simulate the worst-case scenario referring to this observation period, *maximum* measured concentrations were used to calculate RQ for both the WWTP effluents and receiving water bodies. Table 4.5. reports the calculated RQ values. Values greater than 1 (high risk) or between 0.1 and 1 (medium risk) are reported in bold.

Sulfamethoxazole, clarithromycin and azithromycin were found to be the most critical compounds, due to their high RQ values. In fact, according to the risk ranking system proposed by Hernando et al. (2006), these compounds posed a high environmental risk (RQ > 1): sulfamethoxazole and clarithromycin in the two WWTP effluents and in canal B and azithromycin in effluent B and canal B. Moreover a medium risk (RQ in the range 0.1-1) was found to be posed by sulfamethoxazole and clarithromycin in canal A, azithromycin in effluent A.

For all the other PhCs, calculated RQs were consistently < 0.1, corresponding to a minimal risk. It is important to underline that the two receiving surface water bodies were investigated at different distances from the discharge points, defined by the characteristics of the banks not always accessible, as presented above. Comparison of the results must take into account this fact. Experimental investigations evidence that the discharge of a small WWTP in a small receiving water body may result in high RQs (even >1), as in the case study B.

Previously, other studies have reported high RQs in surface water due to the presence of pharmaceuticals in high concentrations, such as analgesics, psychiatric drugs and antibiotics: ibuprofen, naproxen, ketoprofen and carbamazepine (Hernando et al., 2006), diclofenac (Hernando et al., 2006, Zhao et al., 2010), mefenamic acid (Jones et al., 2002; Tauxe-Wuersch et al., 2005), sulfamethoxazole (Garcia-Galan et al., 2011), paracetamol, amoxicillin and oxytetracycline (Jones et al., 2002). These data all indicate

that WWTP discharges may pose a high risk for the aquatic environment, and that the hydraulic characteristics of the receiving water bodies should therefore be taken into consideration in their management, as supported by Gros et al. (2010), in order to mitigate their potential toxicological effects.

Table 4.5. Predicted no-effect concentrations (PNECs, ng L^{-1}) and corresponding risk quotients (RQs) for the selected compounds in the both two WWTP effluents and receiving water bodies.

			Case study A - *RQ*		Case study B- *RQ*	
Compounds	PNEC	Reference	WWTP effluent	Surface water	WWTP effluent	Surfacewater
Diclofenac	9700	Ra et al., 2008	0.05	0.001	0.08	0.006
Indomethacin	3900	Sanderson e al., 2003	-	-	-	0.001
Ketoprofen	15600	Farré et al., 2001	0.001	-	0.001	-
Mefenamic acid	428	Jones et al., 2002	0.06	-	-	-
Naproxen	2620	Quinn et al., 2008	-	0.006	0.008	0.003
Propyphenazone	800	Sanderson et al., 2003	-	0.009	0.06	0.02
Azithromycin	150	Kummerer and Henninger, 2003	**0.366**	0.046	**1.3933**	**0.59**
Ciprofloxacin	938000	Sanderson et al., 2003	0.00004	0.00003	0.001	0.0001
Clarithromycin	70	Boillot, 2008	**1.6**	**0.100**	**5.3**	**1.8**
Metronidazole	2500	Kummerer and Henninger, 2003	0.0084	-	0.01	0.0064
Roxithromycin	4000	Sanderson et al., 2003	0.003	-	-	-
Sulfamethoxazole	27	Ferrari et al., 2004	**3.6**	**0.19**	**6.9**	**3.4**
Trimethoprim	2600	Sanderson et al., 2003	-	0.001	0.015	0.01
Atenolol	30000	Boillot, 2008	0.01	0.0005	0.02	0.008
Metoprolol	8000	Sanderson et al., 2003.,	0.02	0.001	0.03	0.006
Timolol	9000	Sanderson et al., 2003	0.001	-	0.0008	0.0008
Bezafibrate	5300	Sanderson et al., 2003	-	0.003	0.001	0.0008
Carbamazepine	13800	Ferrari et al., 2004	0.01	0.001	0.02	0.006
Diazepam	2000	Sanderson et al., 2003	-	-	0.005	0.003

- = not detected

Moreover, it should be mentioned that the environmental risk analysis conducted in this work was based on the acute toxicity of single compounds, not taking into account the synergistic effects of a mixture of pharmaceuticals, which, according to previous study (Gros et al., 2010), are likely to be even more harmful at lower single concentrations.

4.4 Conclusions

These results show and confirm that PhC concentrations may exceed their PNECs in the effluents from conventional municipal WWTPs. In the area under investigation, the most critical compounds are the antibiotics sulfamethoxazole, clarithromycin and azithromycin. Other substances, including some analgesics/anti-inflammatories, other antibiotics and the antiepileptic carbamazepine, could also be considered as PhCs to add to the list of potential critical compounds from an environmental risk point of view. From a legislative point of view, up to now, limits for the concentrations of PhCs in WWTP effluent have not yet been set. The discussion is open, as reported in Verlicchi et al., 2012b. In this context, the study by Perazzolo et al. (2010) discussed a method to determine a list of pharmaceuticals to survey in surface water. Inclusion of substances on the list was based on a screening procedure, the analytical feasibility, and previous knowledge of pharmaceuticals detected in water. A recent review made by European Community Commission about the new priority substances to begin monitoring in aquatic environment include hormones (ethinylestradiol, estradiol) and diclofenac. (EC, 2012).

Hydrodynamic characteristics of the receiving water body, principally its average flow rate, contribute to mitigating the risks to the environment associated with the presence of toxic substances. The dilution capacity of the receiving water bodies can therefore be considered of prime importance in reducing and controlling the potential toxicological effects of PhCs released into the environment. Nonetheless, even after the discharge of the treated effluent into a receiving body characterized by a high flow rate, PhC concentrations do not appear to be reduced to level of minimal environmental risk. Furthermore, if environmental risk analysis is extended to a mixture of compounds, even more harmful effects are likely to be seen due to synergistic effects. Hence, further measures are needed to reduce the environmental risk posed by PhCs, including source control of the most critical compounds and enhancement of PhC removal by appropriately upgrading existing WWTPs.

Chapter 5: Predicted and measured concentration of selected PhCs: Towards an accurate environmental risk assessment

5.1 Introduction

Antibiotic drugs have been identified as a category of trace chemical contaminants that warrant close scrutiny (Al Aukidy et al., 2012; Verlicchi et al., 2012b). Much of the concern regarding the presence of antibiotics in wastewater and their persistence after treatment processes is related to suspicions that they may generate antibiotic resistance in bacterial species in wastewaters and surface waters down the discharge of the wastewater treatment plants (WWTPs) (Baquero et al., 2008), which may have consequences on the ecosystem as a whole.

Once this issue was brought to the fore, researchers began to investigate the environmental occurrence of PhCs, first via chemical analysis (Daughton and Ternes 1999), and later risk assessment studies (Carlsson et al., 2006;Verlicchi et al., 2012a, b). Environmental risk assessments are generally conducted by either monitoring programs, which provide *measured environmental concentrations* MECs) (as presented in Chapter 3 and 4) or prediction models, based mainly on consumption data, which furnish *predicted environmental concentrations* PECs) (Cunningham, 2008; Escher et al., 2011).

Hence the aim of this chapter is to determine the relative accuracy of the prediction models, and the limitations of on-site monitoring campaigns in order to investigate their effect on the estimation of the environmental risk, measured and predicted environmental concentrations of 12 selected prescription drugs (11 antibiotics and one antiepileptic) at three sampling points: the influent and the effluent of a large municipal WWTP and downstream of its discharge point in the receiving water body have been compared.

5.2 Materials and methods

5.2.1 WWTP and receiving water body

The investigated WWTP is the WWTP of Ferrara that investigated in Chapter 3 and its water receiving body that investigated in Chapter 4 under the name of “case study A”. This case study is representative of many other catchment areas in the Po Valley that feature similar environmental conditions (meteorological conditions, water body characteristics and destination of receiving water body use, catchment size, legal standards for the discharge into the receiving water body, etc.).

5.2.2 Selected compounds

Selected compounds were all common prescription drugs, 11 antibiotics and one antiepileptic, chosen on the basis of their high prescription and sale figures. The annual consumption of each could be documented through Territorial Office records and databases. Their consumptions in the investigated area are reported in the fourth column of Table 5.1. in terms of total amount (kg year^{-1}). The analytical technology required to detect each selected pharmaceutical in water is well known and documented.

5.2.3 Measured environmental concentration (MEC)

Measured environmental concentration of the selected PhCs under investigation are reported in Chapter 3 for the influent and effluent from the WWTP, while chapter 4 reports the measured environmental concentration in surface water, in this case maximum observed concentrations have been considered.

5.2.4 Predicted environmental concentrations (PEC) for the selected PhCs

5.2.4.1 WWTP Influent and effluent

The quantity of each selected pharmaceutical consumed in one year in the investigated catchment area $A_{area,\ j}$ (j = 1, 2, ...12) was evaluated by means of eq. 5.1. According to this model, already adopted in other studies (Le Corre et al., 2012; Ort et al., 2010a) $A_{area,\ j}$ corresponds to a fraction of the national consumption $A_{Italy,\ j}$, and depends on the Italian resident population P_{Italy}, which, is equal to 58.6 million people, and the local population P_{area}, equal to 138 thousand (ISTAT, 2010).

$$A_{area,j} = \frac{A_{Italy,j}}{P_{Italy}} \times P_{area} \qquad \text{(eq. 5.1)}$$

Eq. 5.1 assumes that in the area under investigation the pattern of consumption for the selected compounds is the same as that determined for the Italian population as a whole. The amounts of pharmaceuticals consumed in Italy in 2010 are provided (by the local Territorial Pharmaceutical Office and the OsMed Work Group, 2011) in terms of Defined Daily Doses (DDD). In order to obtain their annual consumption $A_{Italy,j}$ expressed

in kg year^{-1}, DDD values were multiplied by the corresponding conversion factor (CF_j) (mg active compound DDD^{-1}, defined by WHO, 2012).

Table 5.1. reports the selected compounds: first the 11 antibiotics, in alphabetical order, and then the anti-epileptic carbamazepine. The amount of active compound consumed in Italy in 2010 in terms of DDD, as well as its annual consumption $A_{Italy,j}$ (kg) and local consumption $A_{area,j}$ (kg) is reported for each compound. Table 5.1. also reports literature data on the percentage excretion rate E_j, as well as the removal efficiencies R_j observed in the WWTP monitored by Galletti, (2011) (except for a few compounds for which literature data were used).

Table 5.1. Amounts of the selected compounds used in Italy in 2010, in terms of DDD and kg/year, together with the conversion factor, excretion rate (from the literature) and removal efficiency (observed value) of each compound

Compound	Amount used (DDD)	Conversion factor CF (mg DDD^{-1})	Amount used in Italy (kg year^{-1})	Amount used in the area (kg year^{-1})	E_j [%]	R_j [%]
Azithromycin	27 739 328	500	13 870	33	14[c]	11
Ciprofloxacin	21 672 142	1000	21 672	51	55[a]	71
Clarithromycin	64 469 749	1000	64 470	152	25[a]	8
Doxycycline	3 961 205	100	396	0.93	41[c]	14[d]
Erythromycin	60	2000	0.12	0.00028	5[b]	73
Metronidazole	2492	2000	4.98	0.011	80[c]	34
Norfloxacin	3 548 335	800	2839	6.68	30[a]	25
Ofloxacin	198 300	400	79.3	0.18	80[c]	61
Roxithromycin	1 924 410	300	577	1.35	85[a]	65
Tetracycline	2 037 101	1000	2037	4.8	58[c]	40[e]
Trimethoprim	6 948 177	400	2780	6.54	80[b]	31
Carbamazepine	31 189 639	1000	31 190	73	30[a]	36

[a] Ternes and Joss 2006; [b] Verlicchi et al. 2010b; [c] rxlist (http://www.rxlist.com/); [d] Lindberg et al. 2005; [e] Ghosh et al. 2009

Predicted environmental concentrations for each compound *j*, $PEC_{j,k}$ in the influent (k = *inf*) and the effluent (k = *eff*) of the WWTP were calculated according to eq. 5.2, suggested by Tauxe-Wuersch et al. 2005:

$$PEC_{j,k} = \frac{A_{area,j} \times 10^9 \times E_j \times (1-R_j)}{WW_{inh} \times P_{area} \times 365} \qquad \text{(eq. 5.2)}$$

where A_{area} is the amount of pharmaceutical consumed per year in the catchment area under investigation (Table 5.1.), R_j is the fraction of the compound j removed during sewage treatment, (by adsorption to sludge particles, hydrolysis and biodegradation) (Table 5.1.); E_j is the fraction assumed to be excreted by a human body (Table 5.1.) of the compound *j*, P_{area} is the number of residents in the catchment area under investigation, and WW_{inh} is the volume of wastewater produced per capita per day in the catchment area, assumed to be equal to 200 L inh $^{-1}$ d^{-1}.

For each compound, $PEC_{j,\ inf}$ was evaluated assuming that no removal occurred, that is R_j= 0 in eq. 5.2, while in evaluating $PEC_{j,eff}$, R_j values were taken as those listed in Table 5.1.

5.2.4.2 Surface water

Predicted concentrations of the selected compounds in the receiving water body $PEC_{j,sw}$I of the WWTP final discharge were estimated by applying the procedure of Environmental risk assessment according to EMEA guidelines (EMEA, 2006). These suggest first evaluating a crude measure based on their maximum daily dose *MDD* (*Phase I*) by means of eq. 5.3:

$$PEC_{j,sw}\text{I}=\frac{MDD_j \times F_{pen,j}}{WW_{inh} \times D} \times 1000 \qquad \text{(eq. 5.3)}$$

where MDD_j is expressed in mg inh^{-1} d^{-1}, $F_{pen,j}$ is the market penetration, that is the fraction of the local population being treated daily with a specific drug substance (default value is equal to 0.01, corresponding to 1% of the population), WW_{inh} is the volume of wastewater produced daily by each inhabitant (default value equal to 200 L inh $^{-1}$ d^{-1}) and *D* is the factor for dilution of the wastewater by surface water flow rate (default value equal to 10). The guidelines recommend that any drug exceeding the concentration of 0.01 µg L^{-1} in surface water, considered as a threshold for environmental risk, should progress to *Phase II*.

Phase II consists of two steps in series: *Phase IIA* and *Phase IIB. Phase IIA* involves the refinement of the $PEC_{j,sw}$ values. By means of eq. 5.4, a revision of $F_{pen,j}$ is made, taking into consideration specific commercial information about the geographical distribution of the drug. Its calculation is based on total prescription quantities in the catchment area $A_{area,j}$, in addition to DDD data. In general, the figures do not include over-the-counter (OTC) sales, which is why the compounds selected were prescription drugs.

$$F_{pen,j}=\frac{A_{area,\,j}\times 100}{\mathrm{DDD}_j\times P_{area}\times 365} \qquad \text{(eq. 5.4)}$$

The surface concentration is then recalculated using this new $F_{pen,j}$ figure, providing $PEC_{j,sw}$ IIA values. These are then compared with the corresponding predicted no-effect concentration (PNEC) reported in Table 5.2.
Each PNEC value was assumed to be 1000 times lower than the toxicity concentration value found for the most sensitive species assayed, so as to take into account the effect on other, potentially more sensitive aquatic species to those used in toxicity studies (Verlicchi et al., 2012b).

From these figures the ratio $PEC_{j,sw}$ IIA/PNEC was then calculated. For compounds whose ratio is higher than 1, a new refinement of the surface concentration must be performed (*Phase IIB*), according to eq. 5.5, which takes into account excretion rate (E_j) and removal processes (R_j) during the passage through the WWTP.

$$PEC_{j,sw}\mathrm{IIB}=\frac{\mathrm{MDD}_j\times F_{pen,j}}{WW_{\mathrm{inh}}\times \mathrm{D}}\times E_j\times\left(1\text{-}R_j\right)\times 1000 \qquad \text{(eq. 5.5)}$$

5.3 Results and discussion

5.3.1 Predicted environmental concentrations

Table 5.2. reports the values of predicted concentrations for the selected compounds (j = 1,.., 12) in the WWTP influent ($PEC_{j,inf}$) and effluent ($PEC_{j,eff}$), estimated by means of eq. 5.2, and in the receiving water body ($PEC_{j,sw}$ I), on the basis of eq. 5.3. As all values exceeded the threshold of 0.01 μg/L, a refinement of $PEC_{j,sw}$ was conducted in all cases,

involving first an estimation of $F_{pen\ j}$ (through eq. 5.4) and then the recalculation of $PEC_{j,sw}$ IIA, once again applying eq. 5.3 with the re-evaluated $F_{pen\ j}$ (Table 5.2.). In this step, a re-evaluation of the surface water concentration is required for compounds with $PEC_{j,sw}$ IIA > $PNEC_j$, and was performed by means of eq. 5.5, which yields $PEC_{j,sw}$ IIB. Eq. 5.5 relies on MDD values, which are duly listed in Table 5.2. This procedure was only necessary for azithromycin, clarithromycin and tetracycline, and their corresponding $PEC_{j,sw}$ IIB values are also reported in Table 5.2.

For the remaining compounds, $PEC_{j,sw}$ IIA are considered. The predicted surface concentrations for the selected compounds are those listed in the last column of Table 5.2.

5.3.2 Measured environmental concentrations

The results of the monitoring campaign at the three sampling points (influent, effluent and receiving water body) that reported and discussed well in Chapter 3 for the influent and effluent of the WWTP and in Chapter 4 for surface water body are re-summarised in Table 5.3. as average values of the measured concentrations (MEC), alongside the corresponding standard deviation SD.

Again, in the influent and effluent samples, 10 out of the 12 selected compounds were detected in every sample (the antibiotics doxycycline and tetracycline were never found), whereas in surface water samples azithromycin, ciprofloxacin, clarithromycin, trimethoprim and carbamazepine were found in only one sample, and the remaining compounds, metronidazole, roxithromycin were never detected in any sample. doxycycline, erythromycin, norfloxacin, ofloxacin and tetracycline were not monitored. For this reason, SDs for surface water are not reported in Table 5.3.

Table 5.2. Maximum daily dose, predicted environmental concentrations and predicted no effect concentrations for the selected compounds in WWTP influent, effluent and receiving water body

Compounds	MDD mg/inh d	$PEC_{j,inf}$ µg/L	$PEC_{j,eff}$ µg/L	$PEC_{j,sw}$ I µg/L	$F_{pen,j}$ %	$PEC_{j,sw}$ IIA µg/L	PNEC$_j^a$ µg/L	$PEC_{j,sw}$ IIB µg/L	$PEC_{j,sw}$ µg/L
Azithromycin	800	0.454	0.40	4.0	0.13	0.52	0.15	0.064	0.064
Ciprofloxacin	1200	2.78	0.81	6.0	0.10	0.60	938		0.60
Clarithromycin	1000	3.76	3.46	5.0	0.30	1.5	0.07	0.35	0.346
Doxycycline	200	0.0380	0.033	1.0	0.019	0.018	0.3		0.018
Erythromycin	4000	$1.4\ 10^{-6}$	$4\ 10^{-7}$	20	$2.8\ 10^{-7}$	$5.6\ 10^{-6}$	0.02		$5.6\ 10^{-6}$
Metronidazole	2000	0.00093	$6\ 10^{-4}$	10	$1.2\ 10^{-5}$	$1.1\ 10^{-4}$	2.5		$1.1\ 10^{-4}$
Norfloxacin	800	0.20	0.15	4.0	0.016	0.066	15		0.066
Ofloxacin	800	0.015	0.0058	4.0	$9.2\ 10^{-4}$	0.0037	0.016		0.0037
Roxithromycin	600	0.12	0.040	3.0	0.0089	0.026	4		0.026
Tetracycline	2000	0.28	0.17	10	0.0095	0.095	0.09	0.033	0.033
Trimethoprim	640	0.52	0.36	3.2	0.032	0.10	2.6		0.10
Carbamazepine	1600	2.2	1.4	8.0	0.15	1.2	13.8		1.16

[a] PNEC are from Verlicchi et al., 2012b

Table 5.3. Average environmental concentrations and standard deviations (µg L^{-1}) for the selected pharmaceuticals in the WWTP influent, effluent and receiving surface water body

Compound	MEC ± SD influent	MEC± SD effluent	MEC± SD surface water
Azithromycin	0.11 ±0.15	0.13±0.046	0.007
Ciprofloxacin	2.2±1.8	0.63±0.349	0.025
Clarithromycin	0.30±0.32	0.28±0.024	0.006
Doxycycline	< LOD	< LOD	n.m
Erythromycin	0.058±0.016	0.023±0.014	n.m
Metronidazole	0.042±0.013	0.028±0.012	<LOD
Norfloxacin	0.20±0.07	0.15±0.013	n.m
Ofloxacin	1.0±0.82	0.39±0.138	n.m
Roxithromycin	0.084±0.049	0.029±0.018	<LOD
Tetracycline	< LOD	< LOD	n.m
Trimethoprim	0.058±0.014	0.040±0.007	0.002
Carbamazepine	0.58±0.39	0.37±0.069	0.007

n.m: not measured

Selected pharmaceuticals were found in the range 0.042–2.2 µg L^{-1} in the influent, 0.023–0.64 µg L^{-1} in the effluent and 0.002–0.07 µg L^{-1} in the receiving body. On the

whole, these results are in accordance with those found at other Italian municipal WWTPs and their corresponding receiving water bodies (Al Aukidy et al., 2012; Andreozzi et al., 2003; Castiglione et al., 2005; Ferrari et al., 2011; Zuccato et al., 2010).

5.3.3 Comparison of PEC and MEC

A comparison of the predicted and measured concentrations for the investigated compounds at the three sampling points was first performed by means of the ratio PEC/MEC, to establish whether the model underestimates or overestimates measured values, and then by the ranking criteria proposed by the Knappe Project and used in the study by Coetsier et al. (2009) to assess the acceptability of the results of the adopted model.

Predicted values used for evaluating this ratio are those reported in Table 5.2., in particular $PEC_{j,\ inf}$ (third column of Table 5.3.) for the influent, $PEC_{j,eff}$ (four column) for the effluent, and $PEC_{j,sw}$ (last column) for the surface water. The 3D diagrams in Fig. 5.1. clearly show that PECs are greater than the corresponding MECs in 6 out of the 10 detected compounds (as mentioned above, two antibiotics were not detected at any of the three sampling points). In particular, for azithromycin, ciprofloxacin, clarithromycin, trimethoprim and carbamazepine, PEC/MEC was greater than 1 at all the three sampling points, whereas for roxithromycin, the ratio is greater than 1 in the WWTP influent and effluent. In contrast, the influent and effluent concentrations of erythromycin, ofloxacin and metronidazole were underestimated by the prediction formula (PEC/MEC < 1). Only for norfloxacin were the predicted and measured concentrations of the same order of magnitude (PEC/MEC=1). For erythromycin, the PEC was roughly zero at influent and effluent sampling point.

These findings evidence that predicted values are often greater than measured values, but not in all cases, as noted by other studies carried out in France (Coetsier et al., 2009), the UK (Bound and Voulvolis 2006) and Spain (Carballa et al., 2008).

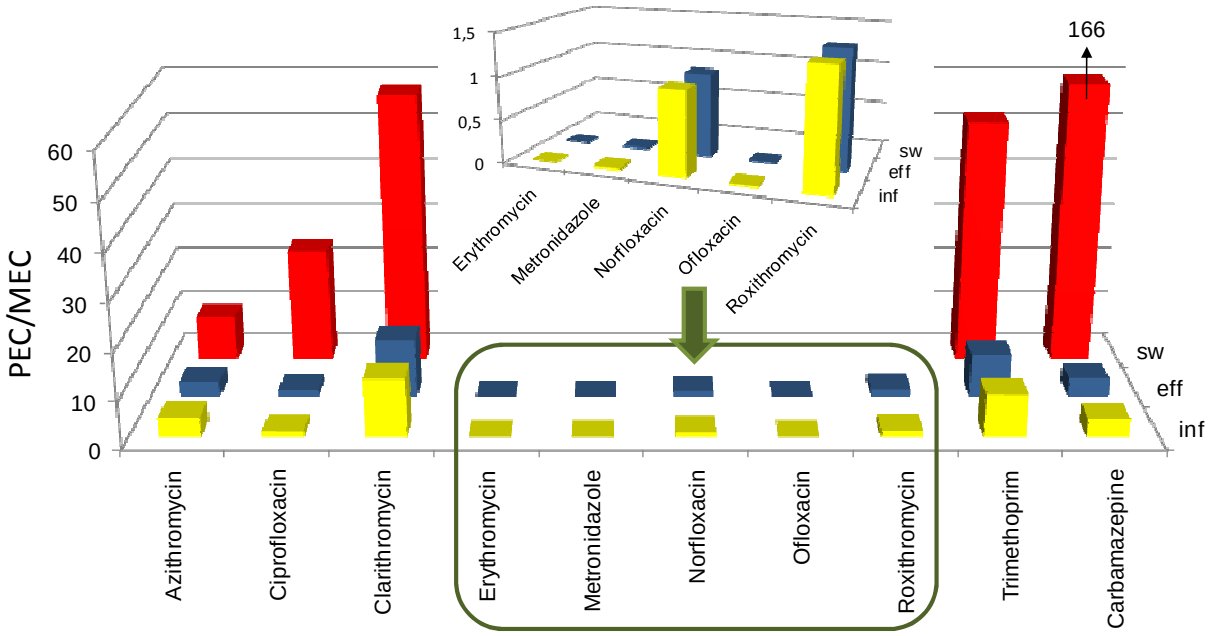


Figure 5.1. Comparison of predicted and measured concentrations of the selected compounds at the three sampling points by means of the ratio PEC/MEC. In the third axis of the graph: inf = WWTP influent, eff = WWTP effluent, sw= receiving surface water.

In order to evaluate whether predicted values may be accepted or rejected, it can be useful to adopt the following ranking criteria (Coetsier et al., 2009):

0.2 < PEC/MEC <1, PEC acceptable, slightly underestimated;
1 < PEC/MEC < 4, PEC acceptable, slightly overestimated;
4 < PEC/MEC < 8, PEC significantly overestimated;
PEC/MEC > 8, PEC strongly overestimated.

Table 5.4. shows that in this case study the adopted model yields acceptable predicted values for almost the same compounds in the influent and effluent (norfloxacin, ciprofloxacin, carbamazepine and, only in the effluent, azithromycin), while for surface water, the equations adopted always furnished a large overestimation. The worst predictions were found for the antibiotics clarithromycin and trimethoprim (PEC/MEC always > 8) and for azithromycin (PEC/MEC> 4 in the influent and >8 in surface water).

Table 5.4. Evaluation of the PEC/MEC ratio for the study area

Sampling point	PEC/MEC ≤ 0.2	0.2 <PEC/MEC ≤ 1	1 <PEC/MEC ≤ 4	4 <PEC/MEC ≤ 8	PEC/MEC > 8
Influent	Erythromycin, Metronidazole, Ofloxacin	Norfloxacin	Ciprofloxacin, Roxithromycin, Carbamazepine	Azithromycin	Clarithromycin, Trimethoprim
Effluent	Erythromycin, Metronidazole, Ofloxacin	Norfloxacin	Azithromycin, Ciprofloxacin, Roxithromycin Carbamazepine		Clarithromycin, Trimethoprim
Surface water					Azithromycin, Ciprofloxacin, Clarithromycin, Trimethoprim, Carbamazepine

5.3.4 Explanation of discrepancies

Discrepancies found between MEC and PEC at the three sampling points can be ascribed to various different causes. The most important of these causes are reported in Table 5.5. Depending on the compound and on the sampling point, some of these factors can be considered as principle or secondary. For instance, inaccuracy of the sales data pertaining to the twelve investigated compounds in this area could be due to a local consumption pattern different from the national one, as the substances are not OTC products and other sources of PhCs such as veterinary use on farms are not present in the catchment area.

Excretion rate is a critical parameter in these calculations, as it is strictly correlated to individual human characteristics (gender, age, health status, consumption of other pharmaceuticals) as well as to those of a particular pharmaceutical. Regarding the latter, the latest generations of compounds have been designed with a view to consistently

incrementing their adsorption rate during metabolism. Furthermore, it is important to note that for many substances a wide range of (observed) values of *E* are reported in the literature, and the choice of the most appropriate value to adopt in PEC estimation may therefore be difficult. However, as a precautionary measure it is wise to use the highest values in the model.

In addition, improper disposal of unused medicines, i.e., by flushing them down the toilet or throwing them out with the household waste rather than returning them to a pharmacist, will also affect the prediction accuracy. In this case the medicine would bypass the metabolic processes within the body that would modify it to different extents, and only the residual fraction would be excreted. Some compounds are almost completely metabolized to inactive metabolites prior to excretion with little or no parent compound appearing in urine or feces, so the appearance of significant concentrations of the parent form of these pharmaceuticals in wastewater might suggest that they were not introduced by excretion (Mankes and Silver 2013, Jelic et al., 2012).

It is rare that the removal efficiencies used in PhC prediction are the fruit of direct measurement at the WWTP under investigation; more often they refer to “similar” WWTPs whose data are available in literature. But, as Verlicchi et al. (2012b) have previously pointed out, removal efficiencies are strictly correlated to the *specific* WWTP configuration (C, N and P removal, biological reactor shape), operating conditions (SRT, HRT, pH, T, redox conditions, etc.) and feeding mode, and PEC calculations are therefore at high risk of inaccuracy. To compensate, Le Coetsier et al. (2009) suggest adopting the mean of a wide range of literature data when available, or a removal efficiency equal to 0, should data be lacking, in order to simulate the worst case scenario. Estimating the dilution in the receiving water body is another calculation highly susceptible to error, which is therefore passed on to the PEC. If the receiving body has a fairly constant flow rate (for instance in a mechanically regulated artificial canal) the error plaguing the estimation of dilution is quite small, but in other cases it depends on the flow rate of the receiving body and on the flow rate of the discharged effluent during the monitoring period.

On a related note, it is also important to point out that the investigation was limited to unchanged molecules, and did not encompass their corresponding metabolites.

Another aspect that EMEA guidelines fail to consider in their model is an estimation of the pharmaceutical removal processes occurring once the effluent is discharged into the surface water body. Indeed, processes such as partitioning, interactions with environmental media, photoreactions, photodegradation, settlements, biodegradation, etc., can all result in an overestimation of the PEC.

Last but not least, sampling protocols, as remarked by Ort et al. (2010b) and Johnson et al. (2008), as well as instrument and human errors, may cause further discrepancies between MEC and PEC, especially for those compounds detected at very low concentrations (several ng L^{-1}).

Table 5.5. Factors behind discrepancies between measured and predicted environmental concentrations of pharmaceuticals in raw wastewater, treated effluent and surface waters.

	Factor	WWTP Influent	WWTP effluent	Surface water
1	Erroneous estimation of pharmaceutical consumption (sales data and consumption pattern: over-the-counter OTC products are not included in the consumption data; presence of further sources such as farms)	✓	✓	✓
2	Inaccurate excretion rate assumed in PEC evaluation	✓	✓	✓
3	Improper disposal of unused medicines (in household waste or via the toilet)	✓	✓	✓
4	Inaccurate expected removal efficiency for the compound under investigation, after its passage through the WWTP		✓	✓
5	Inaccurately evaluated dilution effects due to possible variability in the flow rate in the receiving water body			✓
6	Failure to consider further removal mechanisms occurring in the surface water body after the discharge of the treated effluent, also due to photoreactions and photodegradation, etc.			✓
7	Sampling protocols	✓	✓	✓
8	Instrument error, especially for those compounds detected at very low concentrations (ng/L).	✓	✓	✓

5.4 Conclusions

Although in our case the observed differences between PEC and MEC varied among the selected compounds and the sampling points investigated (influent, effluent and surface water), both predicted and measured concentrations are plagued by uncertainty.

In fact, unless MEC values can be extrapolated to a longer period characterized by identical PhC consumption patterns and environmental conditions (including flow rate, meteorological conditions), they can only be considered valid for a particular sampling period. Indeed, to obtain annual data, monitoring campaigns would be even more complex and expensive. On the other hand, irrespective of the model used, PEC values need to be considered as *theoretical* values, extrapolated from annual data (the year the values assumed for each variable in the EMEA models refer to).

As exposure assessment is the first (screening) step in environmental risk assessment, it is vital that PECs should not underestimate actual environmental concentrations to avoid putting the environment under considerable strain. These considerations have prompted several Authors (among them Bound and Voulvoulis 2006; Castiglioni et al., 2004; Coetsier et al., 2009; Liebig et al., 2006) to question whether predicted concentrations should be used at all. According to Carballa et al. (2008), PECs should not be used in place of direct measurements, and instead should merely be considered a useful tool for defining target compound classes for monitoring or for identifying the forms of the compound (conjugated or free forms) and compartments (liquid or solid fraction) to be investigated.

Indeed, the differences between MECs and PECs documented by our findings, as well as in other studies, indicate that calculation models still need considerable refinement to increase model reliability and discriminative power. At present, however, great discrepancies between measured and predicted values are discouraging, as risk assessments should always err on the side of caution and produce false positives that lead to further investigation rather than false negatives, which could leave a potential risk unexplored.

Chapter 6: Environmental risk assessment of PhCs as a tool for the management of hospital effluents

6.1 Introduction

The consumption of PhCs is on the increase in both hospitals and households (van der Aa et al. 2011). As the human body only metabolizes a fraction of the administered PhC, it enters the water cycle as the parent compound and/or its metabolites via excretion, mainly in urine and to a lesser extent the faeces. (Jjemba et al. 2006). As shown in Chapter 2 and 3, conventional municipal WWTPs are unable to efficiently remove all the different compounds found in sewage, and treated effluent is therefore one of the main sources of PhC release into the environment. Hence, over the last ten to fifteen years, PhC concentrations in raw and treated urban WW have been extensively monitored. Nevertheless, this is still a largely unregulated area, and there is ongoing debate within the scientific community regarding which PhCs to include among the priority substances (Bottoni et al. 2010). Indeed, according to the European Draft (EC 2012), the anti-inflammatory diclofenac and the hormones 17β-estradiol and 17α-ethinylestradiol are prime candidates to be added to the European Priority List, while according to the U.S. EPA, erythromycin, nitroglycerin, and 9 hormones (17α-ethinylestradiol, 17α-estradiol, 17β-estradiol, equilenin, equilin, estriol, estrone, mestranol and norethindrone), need to be considered a priority (Richardson and Ternes 2011).

HWWs represents a particular concern, but has only recently been investigated, and in a far fewer number of studies. Not only high analysis costs, but also the difficulties in organizing water-sampling campaigns inside health facilities have delayed these investigations. Nonetheless, according to the recent literature(Verlicchi et al. 2012c, Verlicchi et al. 2010a,b; Ort et al. 2010a) HWWs may be considered a hot spot in terms of PhC load generated, prompting the scientific community to question the acceptability of the general practice of discharging HWWs into public sewers (Verlicchi et al. 2010 b), where they are conveyed to municipal WWTPs and co-treated with urban WWs (Verlicchi et al. 2010 a,b; Pauwels and Verstraete 2006; Kummerer and Helmers 2000). Initially the discussion centered on the concentrations of regulated (e.g. organic substances, N and P compounds, and microorganisms) and unregulated (residual of PhCs) pollutants in both hospital and urban WWs (Pauwels and Verstraete 2006). Then the focus shifted to evaluation of the load of selected (the most critical) PhCs produced by a hospital and its catchment area (Verlicchi et al. 2012a; Ort et al. 2010a). This made it possible to estimate the relative contributions of each investigated compound made by the hospital and its

catchment area, revealing that in some cases the hospital is indeed the main source of certain PhCs in WW, for example the antibiotics ciprofloxacin, spiramycin, clarithromycin, azithromycin and oflaxacin (Verlicchi et al. 2012a, Le Corre et al. 2012) and the lipid regulator atorvastatin (Verlicchi et al. 2012a, Ort et al. 2010a).

At the same time, several research groups set out to quantify the environmental risk generated by selected PhCs in raw hospital and urban WWs as well as in municipal WWTP effluents (Eascher et al. 2011, Verlicchi et al. 2012a). Through evaluation of a compound's risk quotient (RQ), that is the ratio between its measured or predicted concentration and its predicted no-effect concentration (PNEC), these studies have shown that for some compounds the risk is high (RQ > 1) in raw WWs and remains high in the WWTP effluent. However, once the effluent is discharged into the receiving water body, its dilution with surface water can mitigate the effect of residual PhCs and the associated risk quotient may decrease (Gros et al. 2010) sometimes even to moderate or low levels.

All cited studies were conducted with the aid of local PhC consumption data and/or field monitoring campaigns. Unfortunately, however, in the real world these types of investigations are unfeasible due to time and monetary constraints. Therefore, in the case of the construction of a new hospital, for example, a simple and rapid tool able to provide a rough estimation of the potential impact on the local environment of the PhCs in its effluent would be invaluable for the authorities and decision-makers responsible for hospital management and environmental protection. To this end, the aim of this chapter is to provide The authorities responsible for hospital management and environmental health a tool to evaluate the potential impact of hospital effluents taking in consideration the site specific information such as the contribution of human population and hospital sizes, their location in the catchment area, WWTP capacity, and available dilutions which can differ between catchment area.

This chapter also aims to assess the relative importance of PhCs pathways (HWWs, UWWs) for the priority candidate diclofenac as a case study for individual WWTP. Such information will then be discussed to demonstrate its potential to assist with options for reducing PhCs risk in discharges, and to highlight the need to adopt management options

6.2 Methodology

The PhCs discussed in this study focused on the minimum number of compound that should be considered in any study on PhC in water management and defined as high priority or priority substances by different research groups worldwide (GWRC 2008, Sui et al.,2012, Perazzolo et al., 2010, Roos et al., 2012, Ruel et al. 2012, Besse et al., 2008, Ginebreda et al.,2012, NRMMC[1] 2008, Richardson and Ternes 2011, Verlicchi et al., 2012b), six analgesics and anti-inflammatories, eleven antibiotics, one antihypertensive, three beta-blockers, one contrast media, three hormones, one Lipid regulators, one Psychiatric Receptor and one antagonists drugs (Table 6.1.). These pollutants are quite often unregulated as yet but may be included in the ongoing and future reviews of the Priority Substances List under WFD (Bottoni et al.2010). Some of these compounds are candidate to be within the list of priority substances (Diclofenac, Erythromycin, 17α-ethinylestradiol, 17α-estradiol, 17β-estradiol, equilenin, equilin, estriol, estrone, mestranol and norethindrone) in Europe and United state (EC, 2012, Richardson and Ternes 2011).

To understand the significance level of PhC in HWWs, removal rate in WWTP and PNEC values a systematic review of literature and experimental investigations were carried out as reported in Chapters 2,3 and 4.

Table 6.1. PhCs proposed to be a priority compounds by different research groups.

Therapeutic Class	Compounds	GWRC 2008	Sui et al. 2012	Perazzolo et al. 2010	Roos et al. 2012	Ruel et al. 2012	Besse et al. 2008	Ginebreda et al. 2012	NRMMC[1] 2008	Richardson et al. 2011	Verlicchi et al.2012	N. of studies suggesting the substance as priority
Analgesic/Anti-inflammatory	Acetaminophen	✓		✓	✓		✓		✓			5
Analgesic/Anti-inflammatory	Codeine	✓							✓			2
Analgesic/Anti-inflammatory	Diclofenac	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	10
Analgesic/Anti-inflammatory	Ibuprofen	✓	✓	✓			✓			✓	✓	6
Analgesic/Anti-inflammatory	Ketoprofen										✓	1
Analgesic/Anti-inflammatory	Naproxen	✓	✓	✓			✓	✓			✓	6
Antibiotics	Chlortetracycline								✓			1
Antibiotics	Ciprofloxacin	✓	✓	✓			✓					4
Antibiotics	Clarythromycin										✓	1
Antibiotics	Doxycycline	✓					✓					2
Antibiotics	Erythromycin	✓	✓	✓					✓	✓	✓	6
Antibiotics	Lincomycin	✓	✓						✓			3
Antibiotics	Metronidazol			✓			✓	✓				3
Antibiotics	Norfloxacin			✓								1
Antibiotics	Ofloxacin	✓		✓			✓				✓	4
Antibiotics	Sulfamethoxazole	✓	✓	✓		✓	✓		✓		✓	7
Antibiotics	Tetracyclin										✓	1

Therapeutic Class	Compounds	GWRC 2008	Sui et al. 2012	Perazzolo et al. 2010	Roos et al. 2012	Ruel et al. 2012	Besse et al. 2008	Ginebreda et al. 2012	NRMMC[1] 2008	Richardson et al. 2011	Verlicchi et al.2012	N. of studies suggesting the substance as priority
Antibiotics	Trimethoprim	✓	✓	✓			✓	✓	✓			6
Antihypertensive	Diltiazem	✓										1
B-blockers	Atenolol	✓		✓	✓		✓	✓				5
B-blockers	Metoprolol	✓		✓					✓			3
B-blockers	Propranolol			✓			✓		✓			3
Contrast media	Iopromide	✓		✓								2
Hormones	Estradiol			✓						✓		2
Hormones	Estriol			✓						✓		2
Hormones	Estrone			✓						✓		2
Lipid regulators	Bezafibrate	✓	✓	✓			✓	✓				5
Lipid regulators	Gemfibrozil	✓	✓	✓				✓			✓	5
Psychiatric drugs	Carbamazepine	✓	✓	✓	✓	✓	✓	✓			✓	8
Receptor antagonists	Ranitidine	✓					✓					2

[1] NRMMC 2008: Australian guidelines for water recycling: Managing health and envieonmental risks (Phase 2).

6.2.1 Evaluation of the environmental Risk posed by PhCs in HWWs.

The expected range of risk associated with the presence of PhCs in HWWs is calculated by the mean of minimum and maximum risk quotient (RQ), RQ is calculated by dividing the minimum and maximum pharmaceutical concentration in HWWs by the PNEC values for each compound (equation 6.1). Data are re-summarized in Table.6.2. regard the occurrence of PhCs in HWWs investigated in Chapter 3 and those reported by literature (Verlicchi et al. 2010b, Nagarnaik et al. 2010,2011).

$$\left[\boldsymbol{HRQ_{min,}HRQ_{max}}\right] = \left[\frac{\boldsymbol{Ch_{min}}}{\boldsymbol{PNEC}}, \frac{\boldsymbol{Ch_{max}}}{\boldsymbol{PNEC}}\right] \qquad \text{(eq. 6.1)}$$

Where:

HRQ$_{min}$: minimum risk associated with the presence of PhCs in HWWs.

HRQ$_{max}$: maximum risk associated with the presence of PhCs in HWWs.

C*h*$_{min}$: minimum HWWs pharmaceutical concentration in μg L^{-1} (Literature data)

C*h*$_{max}$: maximum HWWs pharmaceutical concentration in μg L^{-1} (Literature data)

PNEC: predicted no effect concentration in μg L^{-1} (Literature data)

6.2.2 Evaluation of the environmental risk in surface water posed by PhCs originated from HWWs

In order to quantify the range of the risk posed by HWWs due to the presence of PhCs in the environment (surface water), the following reference scenario has been considered: The hospital discharges its effluent in the sewers system where the pharmaceutical concentration was reduced by the dilution factor that depends on hospital and catchment area size, subsequently this effluent is treated by the local WWTP undergoing the various removal mechanisms and finally the discharge into receiving water body where the reduction in the concentration is due to the dilution factor of surface water. In this case the dilution in sewers system, removal in WWTP and the dilution in the surface water, should be taken into account and case by case should be evaluated and RQ in the environment (surface water) is calculated by Eq.(6.2)

$$[ERQ_{min,}ERQ_{max}] = [\frac{Ch_{min}\times Df_u\times(1-R)\times Df_e}{PNEC} , \frac{Ch_{max}\times Df_u\times(1-R)\times Df_e}{PNEC}] \quad \text{(eq. 6.2)}$$

Where:

ERQ_{min}: minimum risk in surface water posed by PhCs originated from HWWs.

ERQ_{max}: maximum risk in surface water posed by PhCs originated from HWWs.

R: percentage removal rate of PhCs in WWTP (literature data). For conservative reason, minimum values reported in literature has been adopted.

Df_u: dilution factor, due to the discharge of HWWs in the sewer system (from local conditions data). The dilution factor is the ratio by which a HWWs will be diluted in a sewers system, and is dependent on two variables: the first being the size of the hospital and the second being the size of the catchment area-Eq. (6.3).

Df_e: dilution factor, due to the discharge of WWTP into the receiving water body (from local conditions data). The dilution factor is the ratio by which a STW effluent will be diluted in a receiving water body, and is dependent on two variables, the first being the size of the STWs and the second being the size of the receiving water body (Metcalf & Eddy, 2004). It is clearly that flow of receiving water body varies with the season, and even within a season a great variation can occur, so The lowest flow of receiving water body should be accounted for when calculating the dilution factor to avoid the worst case. When the flow of the receiving water body is not available a 10 value was used Ashton et al.2004.-Eq.(6.4).

$$Df_u = \frac{Bed\times WW_{bed}}{(Bed\times WW_{bed}+Inhabitants\times WW_{inhabitant})} \quad \text{(eq. 6.3)}$$

$$Df_e = \frac{Inhabitants\times WW_{inhabitant}}{volume\ of\ recieving\ water\ body} \quad \text{(eq. 6.4)}$$

Where:

Bed: number of hospital beds under investigation (local conditions data)

WW_{bed}: the volume of WWs per bed and day (local conditions data)

Inhabitants: number of inhabitants in the catchment area under investigation (local conditions data)

$\mathbf{WW_{inhabitants}}$: the volume of WWs per capita and day (200 L) Ashton et al.2004.

This tool is applied for three case studies with different characteristics (different Bed density) as discussed below in order to estimate the environmental risk in different catchments area. The values of RQ were classified into three risk levels: low (values < 0.1), medium (between 0.1 and 1) and high (values >1) (Hernando et al., 2006).

Table 6.2. Minimum and maximum concentration of selected PhCs in HWWS, Percentage removal rate in WWTPs and PNEC values.

Class	Compound	Concentration in HWWs (μg L^{-1})		Removal in WWTP %	PNEC (μg L^{-1})
		min	max		
Analgesic/Anti-inflammatory	Acetaminophen	5.4	330	80	1
Analgesic/Anti-inflammatory	Codeine	0.2	50	29	16
Analgesic/Anti-inflammatory	Diclofenac	0.2	15	5	9.7
Analgesic/Anti-inflammatory	Ibuprofen	0.069	22	26	1.65
Analgesic/Anti-inflammatory	Ketoprofen	1.7	17.4	7	15.6
Analgesic/Anti-inflammatory	Naproxen	0.698	13	35	2.62
Antibiotics	Chlortetracycline	0.011	0.011	-	-
Antibiotics	Ciprofloxacin	0.038	125	50	938
Antibiotics	Clarithromycin	0.058	11	4.5	0.07
Antibiotics	Doxycycline	0.0005	7	14	0.3
Antibiotics	Erythromycin	0.019	83	4.3	0.02
Antibiotics	Lincomycin	0.3	4.82	-	-
Antibiotics	Metronidazole	0.2	6	38.7	2.5
Antibiotics	Norfloxacin	0.029	44	-	-
Antibiotics	Ofloxacin	0.2	35.5	-	-
Antibiotics	Sulfamethoxazole	0.004	83	10	0.027
Antibiotics	Tetracyclin	0.0015	2	24	0.09
Antibiotics	Trimethoprim	0.05	15	5.1	2.6
Antihypertensive	Diltiazem	0.71	1.6	-	-

Class	Compound	Concentration in HWWs (μg L^{-1})		Removal in WWTP %	PNEC (μg L^{-1})
		min	max		
B-blockers	Atenolol	1.6	3166	14	30
B-blockers	Metoprolol	0.4	25	7	8
B-blockers	Propranolol	0.054	22	1	0.244
Contrast media	Iopromide	0.2	2500	-	-
Hormones	Estradiol	0.017	0.04	-	-
Hormones	Estriol	0.353	1	-	-
Hormones	Estrone	0.017	0.13	-	-
Lipid regulators	Bezafibrate	0.2	7	9.1	5.3
Lipid regulators	Gemfibrozil	0.4	1.2	-	-
Psychiatric drugs	Carbamazepine	0.037	1	5	13.8
Receptor antagonists	Ranitidine	0.98	3	24.5	63

6.2.3 Evaluation of the relative importance of hospitals and catchments area on the risk of PhCs in the influent of WWTPs

The expected range of risk has been also investigated in the influent of each WWTP by means of Equations 6.5 and 6.6. Taking in consideration the pharmaceutical loads originated from both HWWS and its catchment area, in order to estimate the importance of the risk posed by these latter for a single compound. As a case study we examined the case of the analgesics diclofenac which is candidate to be among the priority substances that European states should control and monitor (EC 2012).

$$IRQ = \frac{I_c}{PNEC} \qquad \text{(eq. 6.5)}$$

$$I_c = \frac{(C_h \times Bed \times WW_{bed}) + (C_U \times Inhabitants \times WW_{inhabitants})}{(Bed \times WW_{bed} + Inhabitants \times WW_{inhabitants})} \qquad \text{(eq. 6.6)}$$

Where:

IRQ: WWTP Influent Risk quotient of PhC under investigation (diclofenac)

Ic: WWTP influent concentration of PhC under investigation (diclofenac) μgL^{-1}

$\mathbf{C_h}$: Concentration of PhC under investigation (diclofenac) in HWWs μgL^{-1} (literature data)

$\mathbf{C_U}$: Concentration of PhC under investigation (diclofenac) in UWWs μgL^{-1} (literature data)

The results obtained from Equation 6.5, represent the WWTP influent risk generated from the occurrence of diclofenac in HWWs and UWWs. We simulated all the possible scenarios at each WWTP under investigation by assuming that the concentration of the diclofenac in both WWs is occurred within the variable observed range in Chapters 2 and 3, and the results of their combination represent the influent concentration in a site-specific WWTP. the results is a 3D surface chart.

6.2.4 Case studies

case study 1:

A large size hospital with 900 beds, 2000 people of the medical staff and more than 50 between wards and departments. It is placed in the centre of the town of Ferrara (138000 inhabitants) and its effluent is directly discharged into the combined sewage network and conveyed to the Ferrara WWTP (design capacity 120 000 p.e.) and co-treated with the UWWs. The hospital bed density for the whole STP catchment is 6.5 beds per 1000 inhabitants. Average flow rate from the hospital is about 603 m^3 d^{-1}, corresponding to a specific water consumption of about 670 L bed^{-1} d^{-1}. The average urban influent flow rate to the WWTP is about 28 000 m^3 d^{-1}, hence the hospital contributes for the 2 % to the influent hydraulic load.

Case study 2:

A medium size hospital with 300 beds, 650 people of the medical staff working in twelve main wards. It is placed 30 km far from Ferrara, in the town of Lagosanto (5000 inhabitants), in a coastal and tourist area, densely populated in summertime. Its effluent is directly discharged into the public combined sewage and conveyed to a small WWTP in Lagosanto (design capacity 5500 p.e.) where it is co-treated with the local UWWs. The hospital bed density for the whole STP catchment is 60 beds per 1000 inhabitants. Hospital effluent has an average flow rate of about 160 m^3 d^{-1}, resulting in a specific water consumption of about 550 L bed^{-1} d^{-1}. The average WWTP total influent flow rate is about 1360 m^3 d^{-1} and the hospital flow rate corresponds to the 12 % of the total influent.

Case study 3:

A large size hospital with 900 beds and a staff of 2400, including medical, administrative and technical services, in addition to 250 university students and elderly people staying in the on-site accommodations. It is situated six kilometres from the town of Ferrara in the first outskirts, in the small urban centre of Cona. Due to the building growth connected with the hospital construction, the nearby urban centres (Cona and Gualdo) are under expansion and their estimated residential population is expected to climb to 1700 persons over the next years. In addition, there are local businesses and industries, corresponding to 500 p.e. Currently in this area, combined urban and industrial

wastewaters (respectively UWWs and IWWs) are conveyed to a small WWTP at Gualdo designed for 1000 p.e. Treatment includes screening, primary sedimentation, conventional activated sludge treatment and disinfection. This WWTP is not adequate to treat all the wastewaters coming from the new hospital and the new urban development. The hospital bed density for the whole WWTP catchment is 529 beds per 1000 inhabitants. The Expected average flow rate from the hospital is about 603 m^3 d^{-1}, corresponding to a specific water consumption of about 670 L bed^{-1} d^{-1}

6.3 Results

6.3.1 Environmental risk posed by PhCs in HWWs

The results of the expected range of Risk posed by PhCs in HWWs are presented in Figure 6.1. The variability of the range for each compound is determinant by the fact that some compounds have been well investigated in HWWs, so a wide range of data regards their occurrence in HWWs are available with respect to another compounds (Table 6.2.). The data are ranked with decreasing RQ_{max}. For ten compounds values of PNEC were not available, so the results were depicted for only twenty compounds. For some compounds (erythromycin, acetaminophen, clarithromycin) the range of the risk vary within the intervals of high level (RQ>1), which mean that based on the investigated occurrence of these compounds in HWWs, they always pose a high risk in the HWWs. sulfamethoxazole, propanolol, naproxen and ketoprofen have a risk ranged within the intervals of medium and high level (RQ> 0.1), atenolol, doxycycline, tetracycline, ibuprofen, trimethoprim, codeine, metoprolol, metronidazole, diclofenac and bezafibrate have a Risk range between low and high level . Ciprofloxacin and carbamazepine and ranitidine have a risk range within the low interval level (RQ<0.1).

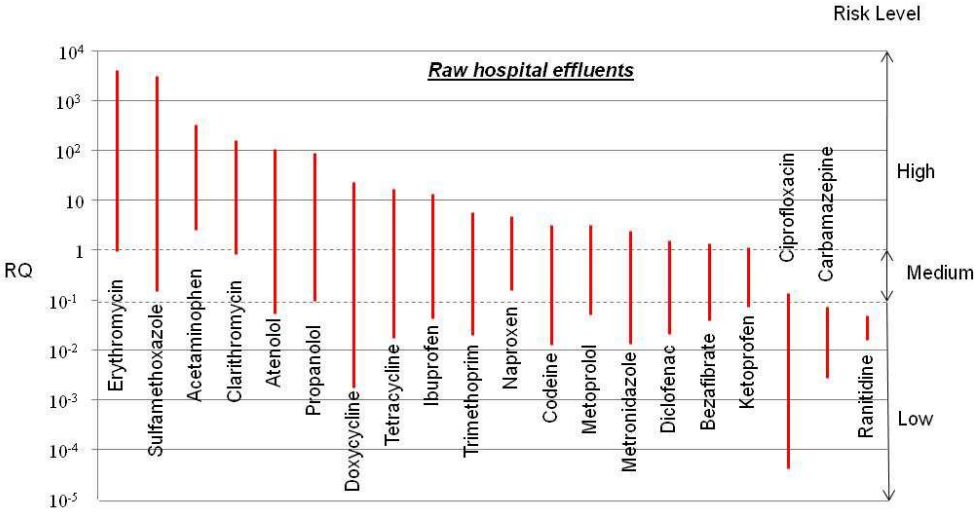


Figure 6.1. Expected range of Risk posed by PhCs in HWWs

6.3.2 Environmental Risk in surface water posed by PhCs originated from HWWs

Figure 6.2. shows the expected range of risk downstream the WWTP in each catchment area. Due to the assumptions of the proposed tools, the expected risk's range of each compounds is keep constant in each catchment area with the variation of solely risk level. As expected, the number of compounds that posed high risk is increased with the increased of the bed density. Among twenty compounds , the compounds that observed to have an expected range that fall within the high Risk level(RQ>1) were: two compounds (erythromycin and sulfamethoxazole) in case study 1, nine compounds (erythromycin, sulfamethoxazole, acetaminophen, clarithromycin, atenolol, propanolol, doxycycline, tetracycline, ibuprofen) in case study 2 and thirteen compounds (erythromycin, sulfamethoxazole, acetaminophen, clarithromycin, atenolol, propanolol, doxycycline, tetracycline, ibuprofen, trimethoprim, naproxen, codeine, metoprolol) in case study 3 .

The Antibiotics erythromycin and sulfamethoxazole were found to pose the highest risk in HWWS and their risk is still high downstream the WWTP in all the case studies notwithstanding the dilution in sewer systems , removal in WWTPs, and dilution in surface water have been occurred. This is determinate by their high toxicity (low PNEC values) and high exposure. The high RQ associated with the beta-blocker atenolol is found to be determinant by their high exposure which found to be 3166 μg/L in HWWS. Carbamazepine and ranitidine were found to have a low risk level (RQ<1) due to their low

toxicity and exposure, while the antibiotic ciprofloxacin, has a low RQ due to its low toxicity nevertheless it high exposure in HWWs that could arrive to 125 µg/L.

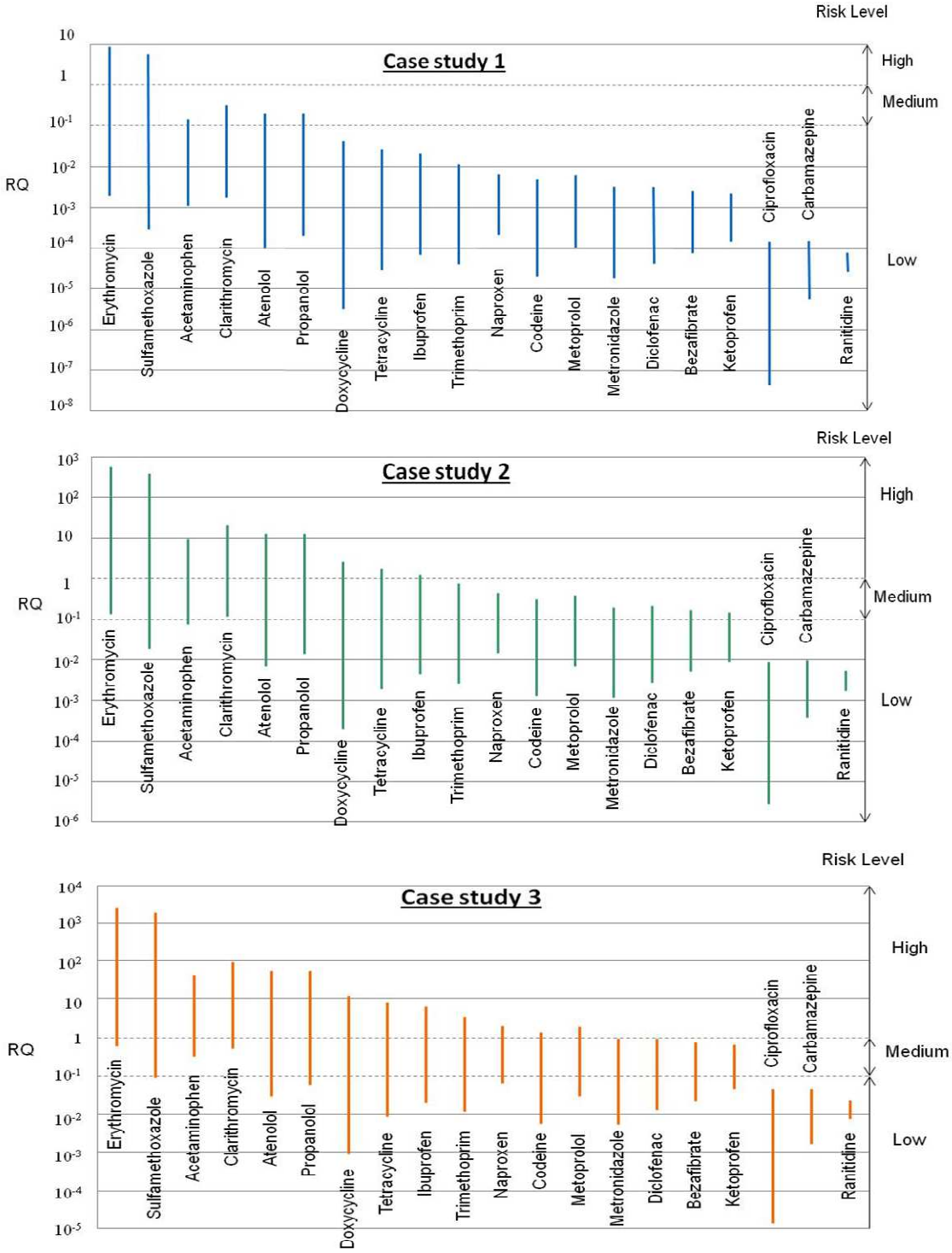


Figure 6.2. Expected environmental range of risk in surface water posed PhCs originated from HWWs in different catchments area.

Since the removal in WWTP was assumed to be equal in all the case studies, it is evident that dilution in sewer systems and surface water had a larger effect on the decrease of RQ and this is also evidenced by Escher et al. 2011. In general , simulating the current situation of the management of HWWS (co-treatment with UWWS in the municipal WWTP), the risk posed by HWWS due to the presence of PhCs could be reduced with various degree, and it is relevant to the characteristics of each catchment area where the hospital is situated. The range of the risk posed by HWWS (Fig. 6.1.) is reduced three order of magnitude in case study 1, one order of magnitude in case study 2 and still in same order of magnitude in case study 3. The Analgesics Acetaminophen exhibit a little more reduction with respect to the another compounds and this is effected by its high removal rate in WWTP (80%).

6.3.3 The relative importance of hospitals and catchments area on the risk of PhCs in the influent of WWTPs

The estimated risk posed by PhCs originated from HWWs and its catchment area (UWWs) in the influent of site-specific WWTP is depicted in Figure 6.3. X-Y axes are the two input parameter which represent the literature concentration range of PhC under investigation (diclofenac) in HWWs and UWWs respectively, while the vertical Z axes represents the associated RQ value for each of the X and Y point.

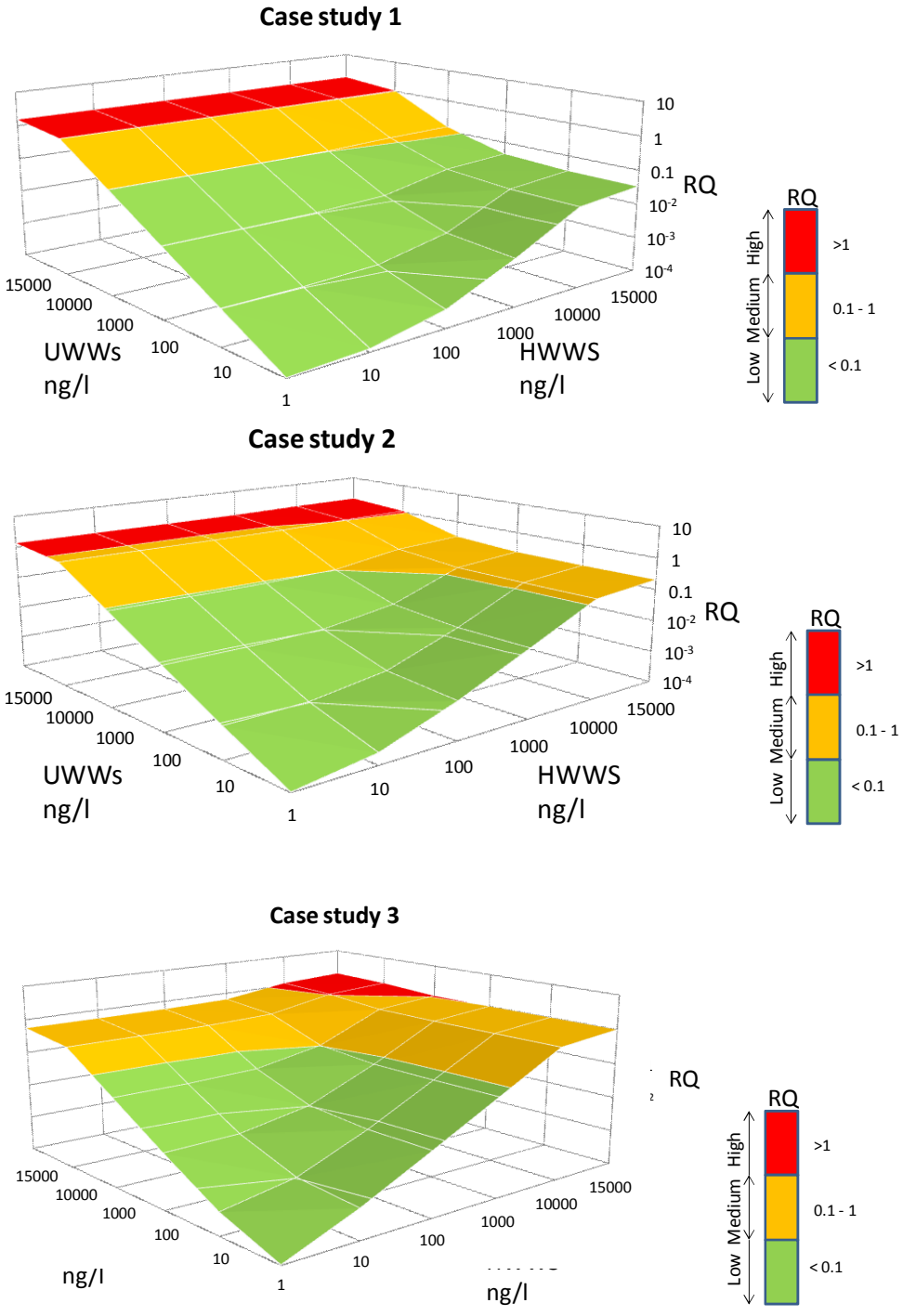


Figure 6.3. Risk patterns posed by diclofenac in the influent of WWTP. case study 1 (Low bed density), case study 2 (medium bed density), case study 3 (high bed density).

In all the case studies the results showed that the risk posed by diclofenac in the influent of WWTP varies from low to high risk with a maximum value of RQ= 1.5. In case study 1, medium and high risk could be present when the occurrence of diclofenac in

UWWs is in high concentration (> 1 μg L^{-1}) independently of the level of concentration in HWWS. This pattern is different in case study 2,3 where the effect of HWWs to the Risk began to be present, in these cases the medium risk could be present also when the diclofenac occurred in high concentration in HWWS independently of the concentration in UWWs. The observed high Risk in case study 1 and 2 with respect to study case 3, is due to the high load discharged in sewer systems from their catchments area.

6.4 Discusion

The results generated from the proposed tool, suggest that due to the presence of PhCs, HWWs could pose a risk for the receiving environment and their risk is relevant to many factors. Erythromycin and sulfamethoxazole are potentially compounds of concern in the HWWs and they required a management, whilst other compounds may not required any management due to their low risk. In some cases, HWWs contribute significantly to the risk in the influent of a site-specific WWTP, and their contribution is correlated to the bed density. In fact the measured contribution of the hospital effluent to the total load of diclofenac in the influent of WWTP of case study 1 was observed to be 2% as shown in Chapter 3 while the contribution in another catchment area with different hospital bed density from another countries was observed to be 10, 1.6, 1 and 7-9% in Germany (bed density= 12), Norway (bed density= 4), Australia (bed density= 4.4) and Germany (bed density= 33.5) respectively (Chapter 3).

Based on the results obtained from the proposed tool, the implementation of decentralized WWTP for the HWWs as a strategy to reduce pharmaceutical impacts seems not efficient in case study 1 and 2, where the RQ could by >1 even when the concentration in HWWs is at low levels, while for case study 3 seems efficient since RQ>1 is caused mainly by HWWs. Using a Multiple-Criteria Decision Analysis (MCDA) , Lienert et al. (2011) evaluated different alternatives that decrease pharmaceuticals in the hospitals' wastewater, based on two case studies(general hospital and psychiatric hospital). The technical alternatives included were reverse osmosis, ozonation, and activated carbon ; while organizational alternatives included urine separation. For the general hospital that contributed 38% to the total pharmaceuticals load at the wastewater treatment plant, alternatives removing all pharmaceuticals (especially reverse osmosis, or vacuum-toilets

and incineration), performed systematically better than releasing wastewater to municipal wastewater treatment plant or urine separation , despite higher costs. For the psychiatry with a lower pharmaceutical load(5%), costs were more critical. Stakeholder feedback concerning MCDA was very positive, especially because the results were robust across different stakeholder-types.

As a result, Proper management of HWWs should take into consideration the characteristics of the catchment area in which the hospital is situated, i.e. (*i*) size of area, number of residents and non residents (p.e.), average and maximum urban flow rate, (*ii*) industrial activities present in the area (type, WW flow rate, adopted pre-treatments within the battery limits, final disposal of the effluent, cotreatment with other kind of WWs), (*iii*) characteristics of existing WWTPs (nominal capacity, residual capacity, treatment sequence, authorized limits for the final discharge), (*iv*) characteristics of the receiving water body (hydraulic regime, auto-depurative capacity, irrigation, recreational and industrial uses), (*v*) legal and regulatory constraints.

In case of cotreatment of HWW and UWW, it is important to evaluate the percentage of hospital flow rate with respect to the total WWTP influent flow rate (Verlicchi et al. 2010a). This value depends on hospital size (small size with < 300 beds, medium size with 300-700 beds and large size with > 700 beds), and on the size of the resident population in the urban centre.

6.5 Method limitations

Evaluating the potential risk of HWWs due the occurrence of PhCs requires the availability of data regarding the concentration of PhCs in HWWs and removal rate in WWTP, PNEC values. Finding previous studies on actual pharmaceutical levels yielded a paucity of information and the final data that were employed by the calculation were limited to twenty compounds. The assumptions made by employing these data suggest that pharmaceutical concentration will be the same for each hospital, and the variation between hospitals will only be a result in variation flow (resulting from variation in beds number). This is obviously not the case as within each hospital there will be variations in pharmaceuticals concentration levels due to the differences in consumption profile, services, department and research activity.

6.6 Application of the tool and management options

Despite the limitation of the proposed tool, estimating the risk posed by PhCs originated from HWWs to the receiving environment could provide a viable information on the magnitude of the risk posed by HWWs and subsequently on the type of management options that should be adopted. As there is no specific treatment able to remove, to a high percentage, the many kinds of PhCs typically found in HWws, due to their differing behaviour during treatments, and as many PhCs are resistant to conventional treatments, innovative solutions to this problem are required. Different operational configurations should be developed and calibrated, in order to provide information for potential practitioners about the financial aspects and overall risks associated with putative treatments of HWWs (Pauwels and Verstraete 2006).

As soon as the risk identified through this tool, the different options to reduce this risk could be examined by applying the parameters input that regard each scenario to the tool. The options available to reduce the risk of HWWs could be dedicated treatment, upgrading of the existing municipal WWTP and Source management. A dedicated treatment for HWWs is always desirable, especially for large hospitals in rural areas, where its treated effluent may be indirectly reused for irrigation after its discharge into a surface water body. In fact, although co-treatment with UWWs at a municipal WWTP is common practice, it has several fundamental drawbacks. In the first place, dilution of HWWs with UWWs is not the correct procedure, as some substances in the hospital effluents may cause inhibition of the treatment plant biomass and thereby reduce the removal efficiency.

Furthermore, as many micro-pollutants tend to adsorb/absorb to the biomass flocks, efficient solid/liquid separation can greatly improve their removal from wastewater and, at the same time, guarantee a consistently good effluent quality. MBRs have been suggested for this purpose by many authors (Daigger et al. 2005; Pauwels et al. 2006; Radjenovic et al. 2009), some of whom found that ultrafiltration (UF) membranes are more efficient than MF membranes (Beier et al. 2010, Verlicchi et al., 2010b). MBR processes have also been suggested as better alternatives for the removal of pathogenic microorganisms, including some viruses (Ottoson et al. 2006; Zhang and Farahbakhash 2007).

Ozonation and advanced oxidation processes (AOPs) are also promising candidates for efficient degradation of pharmaceuticals in water and wastewaters (Zwiner and Frimmel 2003; Chiang et al. 2003; Huber et al. 2003; Balcioglu and Otker 2003; Ternes et al. 2003;

Machado et al. 2007; Zimmermann et al. 2008). In fact, treatment with ozone can reduce the concentration of many pharmaceuticals: 15 mg L^{-1} of ozone at 18 min contact time could be an adequate dose (Gagnon et al. 2008). However, as AOPs are not affordable at many municipal WWTPs, Kim et al. 2008 proposed that prolonging the SRT in biological WWTP may be the best practicable solution to reducing levels of pharmaceuticals in treated WWs.

An alternative to end-of-pipe upgrading of treatment plants, and an effective precautionary measure, could be source control . As reported above, administered PhCs are excreted from the human body via faeces and urine at a percentage that depends on the compounds in question. Although it will never be the perfect solution, separate collection of urine can contribute to keeping these substances away from wastewaters. Furthermore, source separation of urine (Nomix technology) can be conveniently adopted for other reasons, for instance, limitation of nutrient pollution of water. In this case, facilitated removal of pharmaceuticals could be a very welcome side effect (Lienert et al. 2007). In fact, Larsen et al. 2004 found that source separation of urine, which contains many of the pharmaceuticals and their transformation products from human metabolism, may offer the most effective solution to the problem of pharmaceuticals contaminating the environment. Due to the higher concentrations of micropollutants, biological as well as physical processes are expected to be more efficient for urine than for diluted wastewater. However, economic and practical feasibility must be carefully evaluated.

6.7 Conclusion

This chapter developed a tool to provide the authorities responsible for hospital management and environmental health a useful information on the magnitude of environmental risk posed by PhCs originated from HWWs, taking in consideration the site specific information such as the contribution of human population and hospital sizes, their location in the catchment area, WWTP capacity, and available dilutions in the receiving water body.

The results suggest that due to the presence of PhCs, HWWs could pose a risk for the receiving environment and their risk is relevant to many factors. Erythromycin and sulfamethoxazole are potentially compounds of concern in the HWWs and they required a management, whilst other compounds may not required any management due to their low

risk. The risk posed by HWWS due to the presence of PhCs could be reduced with various degree, and it is relevant to the characteristics of each catchment area where the hospital is situated. In some cases, the pathway of HWWs contribute significantly to the risk in the influent of a site-specific WWTP, and their contribution is correlated to the bed density. Nevertheless the limitation that the proposed tool experienced, it is provide a useful information about the management options that should be adopted to reduce the risk of HWWs.

Chapter 7: Conclusions

The general aims of this thesis were to characterized the sources and pathways of PhCs in the environment, to assess the occurrence, removal and fate of selected PhCs in WWTPs and in the water environment, and carry out environmental risk analysis based on their occurrence as a basis to priorities the hazardous compounds and to manage the risk posed by their exposure. In particular, this work focused on HWWs in order to assess their potential as a point source of selected 73 PhCs and their role in spreading these compounds into the environment, and consequently the impact of WWTPs on the receiving water bodies in terms of 27 PhCs concentration. The last aim was to develop a tool to estimate the level of environmental risk posed by PhCs originated from HWWs at site specific catchment area to aid the authorities and decision makers in the management of HWWs and the reduction of PhCs discharged into the environment.

7.1 Main Findings

- The literature review highlighted that:
  - PhCs are usually present in raw influent at concentrations in the range 10^{-3}-10^{2} μg L^{-1} and even more. Common WWTPs are not able to efficiently remove all of PhCs and observed removal efficiencies vary in a wide range for the different compounds, as well as for the same substance, due to the different chemical and physical characteristics of PhCs and to operational conditions (mainly aerobic, anaerobic, anoxic reactors, SRT, pH and water temperature). MBRs seem to guarantee higher removal effciencies for most compounds and a better quality of the permeates with respect to CAS.
  - The occurrence of some PhCs in the secondary effluent discharged into surface water bodies may pose a medium–high (acute) risk to aquatic life. Furthermore, many other compounds, even if their environmental risk was found to be low, are discharged at high daily mass loads, which could contribute to negative effects on aquatic organisms in the long term due to chronic and mixture toxicity.
  - For these reasons, it would be more prudent to begin monitoring the most frequently and most persistent administered PhCs, as well as those with the highest environmental risk, namely antibiotics (including erythromycin, ofloxacin,

sulfamethoxazole, clarithromycin, amoxicillin, tetracycline and azithromycin), psychiatric drugs (like fluoxetine, diazepam and carbamazepine), analgesics/anti-inflammatories (ibuprofen, mefenamic acid, naproxen, diclofenac and ketoprofen) and lipid regulators (fenofibric acid, fenofibrate and gemfibrozil).

- Raw HWWs has a higher ecotoxicity potential compared to municipal waste water.
- Raw HWWs is a hot spot for antibiotic resistant bacteria.

- The experimental investigation conducted in the area of Ferrara, Italy, on the effluent of two different sized hospitals and the influent and effluent of the receiving municipal WWTP of one of the examined hospitals and on in the effluent from two wastewater treatment plants (WWTPs) and their receiving water bodies highlighted these results:
  - The investigated PhCs are found in consistently higher concentrations in HWWs than in UWWs, particularly commonly used drugs such as analgesics and antibiotics.
  - The characteristics of the HWWs seem to be influenced by the size of the structure (the smaller hospital discharged higher mean concentrations than the larger one), and season (concentrations tended to be higher in winter than in summer).
  - The ratio between PhC concentration in HWWs and WWTP influent was, on average, 7. The highest values were found for ofloxacin (31) and clarithromycin (36), ranitidine (27), atorvastatin (25), metronidazole (23). Antibiotics, analgesics/anti-inflammatories and lipid regulator were the pharmaceutical compounds found at the highest concentrations.
  - The percentage load contribution of the hospital varied among the investigated compounds; in particular 12 compounds yielded values between 16 and 67% (some antibiotics, receptor antagonists and lipid regulators), and as a result hospital could be a hot spot for pharmaceutical emission.
  - Environmental risk analysis showed that 9 compounds posed a high risk at the concentrations detected in hospital effluent, while in the WWTP influent and effluent, only 5 of these PhCs were found to exhibit high ecotoxicity. As four out of these five PhCs were antibiotics, we can state that this class of compound should cause the most concern.

- Due to their micropollutant content, HWWs require more specific management and treatment in order to protect and safeguard the environment, in particular the surface water body which will receive the final (treated) effluent from the WWTP.

- As co-treatment is common practice, and the usual (conventional) treatments are unable to efficiently remove PhCs, this issue needs urgent attention. Indeed, administrators and technicians will need to perform case-by-case analyses on a *local scale*, in particular during WWTP planning and design phases, in order to determine the best means of tackling the problem.
- PhC concentrations may exceed their PNECs in the effluents from conventional municipal WWTPs. In the area under investigation, and the most critical compounds are the antibiotics sulfamethoxazole, clarithromycin and azithromycin. Other substances, including some analgesics/anti-inflammatories, other antibiotics and the antiepileptic carbamazepine, could also be considered as PhCs to add to the list of potential critical compounds from an environmental risk point of view.
- Hydrodynamic characteristics of the receiving water body, principally its average flow rate, contribute to mitigating the risks to the environment associated with the presence of toxic substances. The dilution capacity of the receiving water bodies can therefore be considered of prime importance in reducing and controlling the potential toxicological effects of PhCs released into the environment.
- Nonetheless, even after the discharge of the treated effluent into a receiving body characterized by a high flow rate, PhC concentrations do not appear to be reduced to level of minimal environmental risk.
- If environmental risk analysis is extended to a mixture of compounds, more harmful effects are likely to be seen due to synergistic effects. Hence, further measures are needed to reduce the environmental risk posed by PhCs, including source control of the most critical compounds and enhancement of PhC removal by appropriately upgrading existing WWTPs.
- The environmental risk analysis conducted in this work was on the basis of selected PhCs concentration in the water, and did not consider the quantity of the compound under investigation adsorbed onto sludge and sediments that could be released again to the water.

- Assessing the relative accuracy of the prediction models, and the limitations of on-site monitoring campaigns, that environmental risk assessment depends on their accuracy, indicate that:
    - differences between PEC and MEC varied among the selected compounds and the sampling points investigated (influent, effluent and surface water), both predicted and measured concentrations are plagued by uncertainty.
    - differences between MECs and PECs documented by our findings, as well as in other studies, indicate that calculation models still need considerable refinement to increase model reliability and discriminative power. At present, however, great discrepancies between measured and predicted values are discouraging, as risk assessments should always err on the side of caution and produce false positives that lead to further investigation rather than false negatives, which could leave a potential risk unexplored.

- A low cost tool to provide the authorities responsible for hospital management and environmental health a useful information on the potential impact of PhCs originated from hospital effluents, taking in consideration the site specific information such as the contribution of human population and hospital sizes, their location in the catchment area, WWTP capacity, and available dilutions in the receiving water body, and to assess the relative importance of PhCs pathways (HWWs, UWWs) at site specific WWTP has been developed. The results indicate that:
    - The environmental risk posed by PhCs originated from HWWs, varies from low to high level.
    - Due to the presence of PhCs, HWWs could pose a risk for the receiving environment and their risk is relevant to many factors.
    - Erythromycin and sulfamethoxazole are potentially compounds of concern in the HWWs and they required a management, whilst other compounds may not required any management due to their low risk.
    - In some circumstances, HWWs may represent an important point source of the risk posed by priority candidate compound “diclofenac” in the influent of WWTP.

- A dedicated treatment for HWWs is always a good solution, especially in the case of a large hospital in a scarcely populated area. The treatment sequence that seems to be the most appropriate one is a multi barrier system with a combination of biological, physical and chemical mechanisms (ultrafiltration MBR followed by advanced oxidation processes by means of O3/UV). In this way a combination of different mechanisms can occur in the different treatment phase, giving the possibility to compounds with great differences in their chemical-physical characteristics to find operational conditions where they can be effectively removed.

7.2 Suggestions for future research.

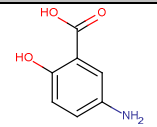
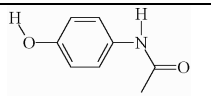
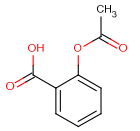
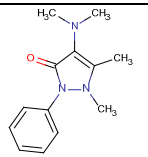
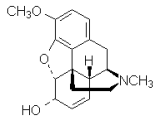
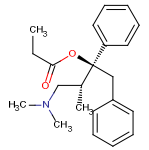
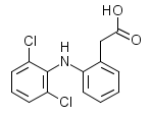
Due to the gap of data in the literature and in order to understand the issue of PhCs in the environment in a comprehensively way , the following recommendations are suggested:

1. Carrying out a monitoring program to analyse the occurrence of scarcely investigated PhCs in the influent and effluent of municipal WWTPs.
2. Define PNECs values for a wider spectrum of compounds.
3. Further researches are needed to evaluate the environmental impact of mixtures of different PhCs.
4. Evaluate the chronic effect of authentic PhC mixtures on the aquatic life.
5. Evaluate the best end-of-pipe measures for the existing WWTPs to guarantee better removal of the most persistent compounds.
6. Suggest source control options to reduce the quantity and variety of PhCs in the water cycle.

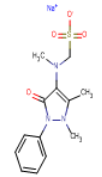
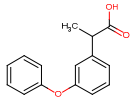
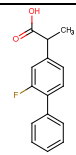
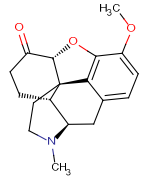
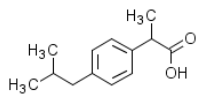
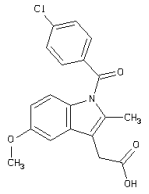
Appendix A

Physico-chemical properties of the selected PhCs, and their ranges of concentration in the influent and effluent of WWTPs and removal efficiencies reported in literature.

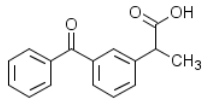
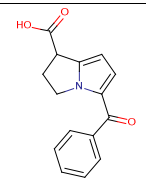
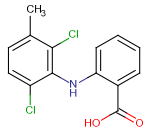
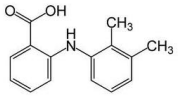
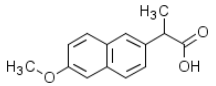
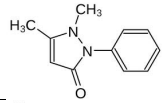
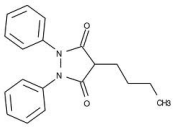
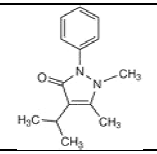
Table A.1. Physico-chemical properties of the selected pharmaceuticals. Data with a star as apex refer to MBR systems.

	Pharmaceutical	*Henry's Law constant (atm-m^3/mole)*	*pKa*	Log K_{ow}	S_w 25°C (mg l^{-1})	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	Charge at pH 7	Molecular structure
Analgesics/Anti-inflammatories	5-aminosalicylic acid CAS # 89-57-6	5.02E-012						Negative	
	Acetaminophen CAS # 103-90-2	6.42E-013	9.38	0.46	3.035 10^4	3.06[t]	58-80 106*-240*	Neutral	
	Acetylsalicylic acid CAS # 50-78-2	1.3E-009	3.5[h]	1.13	5295			Negative	
	Aminopyrine CAS # 58-15-1	1.38E-011		0.6	4191			Neutral	
	Codeine CAS # 76-57-3	7.58E-014	8.21	1.19	1.21 10^4	1.15[j]	4.7-4.8 [j]	Positive	
Analgesics/Anti-inflammatories	Dextropropoxyphene CAS # 469-62-5	2.34E-009						Positive	
	Diclofenac CAS # 15307-86-5	4.73E-012	4.15[a]	4.51/0.7	4.52	1.2[l]	<0.04-1.2 [o] ≤0.1 ≤0.1* <0.002*-<0.1* [s]	Negative	

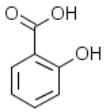
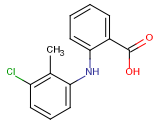
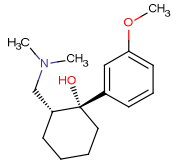
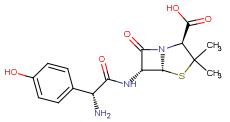
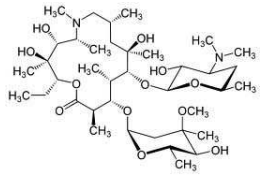
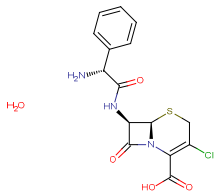
Appendix A

	Pharmaceutical	*Henry's Law constant (atm-m^3/mole)*	*pKa*	Log K_{ow}	S_w 25°C (mg l^{-1})	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	Charge at pH 7	Molecular structure
	Dipyrone CAS # 68-89-3	1.1E-015		-4.76	1 10^6				
	Fenoprofen CAS # 31879-05-7	1.28E-009	7.3	3.9	30.13		10-14 3.3*-5.9*	Negative	
	Flurbiprofen CAS # 5104-49-4	5.26E-009		3.81	17.7.13			Negative	
Analgesics/Anti-inflammatories	Hydrocodone CAS # 125-29-1	6.37E-012	8.48	2.16	1788	1.23[j]		Positive	
	Ibuprofen CAS # 15687-27-1	1.5E-007	4.51[e]	3.97/0.45	41.05	0.9[l]	1.5-20 [o] 21-35 9*-22* 1.33*->3* [s]	Negative	
	Indomethacin CAS # 53-86-1	3.13E-014	4.5	4.27	3.114		≤0.3 ≤0.21*	Negative	

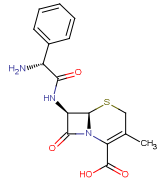
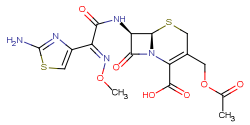
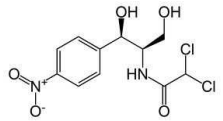
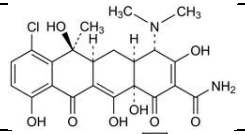
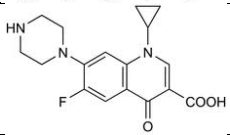
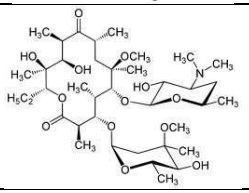
Appendix A

	Pharmaceutical	*Henry's Law constant (atm-m^3/mole)*	*pKa*	Log K_{ow}	S_w 25°C (mg l^{-1})	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	Charge at pH 7	Molecular structure
	Ketoprofen CAS # 22071-15-4	2.12E-011	4.45[f]	3.12/-0.44	120.4	1.2[t]		Negative	
	Ketorolac CAS # 74103-06-3	3.35E-013		2.32	572.3			Negative	
	Meclofenamic acid CAS # 644-62-2	1.28E-011		6.02	0.0934			Negative	
	Mefenamic acid CAS # 61-68-7	2.57E-011	4.2	5.12	1.121	2.6[t]		Negative	
	Naproxen CAS # 22204-53-1	3.39E-010	4.2[b]	3.18/-0.34	144.9	1.1[o]	<0.2-9 [o] 1.0-1.9 0.4*-0.8* 0.08*-0.4* [s]	Negative	
	Phenazone CAS # 60-80-0	6.65E-010	1.4	0.38	2.376 10^4			Neutral	
	Phenylbutazone CAS # 50-33-9	6.56E-009	4.5	3.16	21.95			Negative	
Anti-inflammato	Propyphenazone CAS # 479-92-5	1.84E-009	---	1.96	668.2			Neutral	

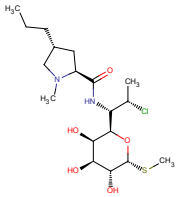
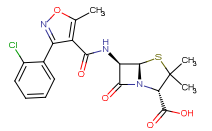
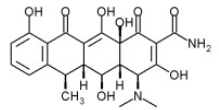
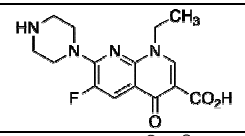
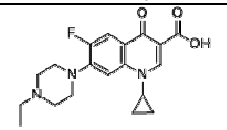
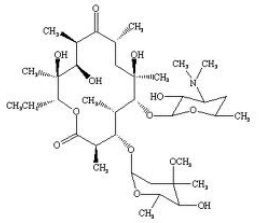
Appendix A

	Pharmaceutical	*Henry's Law constant (atm-m^3/mole)*	*pKa*	Log K_{ow}	S_w 25°C (mg l^{-1})	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	Charge at pH 7	Molecular structure
	Salicylic acid CAS # 69-72-7	7.34E-009	3.5 [b]	2.26/-2.42	3808			Negative	
	Tolfenamic acid CAS # 13710-19-5	1.73E-011		5.38	0.782			Negative	
	Tramadol CAS # 27203-92-5	1.54E-011		3.01	1151	1.11 [j]	≤0.11-≤0.13 [j]	Positive	
Antibiotics	Amoxicillin CAS # 26787-78-0	2.49E-021	2.4 [d]	0.87 [b]	3433			Neut./Neg.	
Antibiotics	Azithromycin CAS # 83905-01-5	5.3E-029	pK_1 =8.7 pK_2= 9.5	4.02	0.06204	2.5-2.7 [k]	≤0.1 ≤1.2* 0.17* [s]	positive	
Antibiotics	Cefaclor CAS # 53994-73-3	1.27E-017		0.35	119				

Appendix A

	Pharmaceutical	*Henry's Law constant (atm-m^3/mole)*	*pKa*	Log K_{ow}	S_w 25°C (mg l^{-1})	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	Charge at pH 7	Molecular structure
	Cefalexin CAS # 15686-71-2	2.77E-017						Neut./Neg.	
	Cefotaxime CAS # 63527-52-6	3.09E-024		0.64	394.5			Negative	
Antibiotics	Chloramphenicol CAS # 56-75-7	2.29E-018	5.5	1.14	388.5			Neut./Neg.	
	Chlortetracycline CAS # 57-62-5	3.45E-024	pK_1= 3.3 pK_2= 7.4 pK_3= 9.3	-0.62	615.7			Negative	
	Ciprofloxacin CAS # 85721-33-1	5.09E-019	6.38[g]	0.4[j]	1.148 10^4	4.3[k]		Pos./Neut.	
	Clarithromycin CAS # 81103-11-9	1.73E-029	8.99	3.16	0.342	2.5-2.6[k]	≤0.4 ≤1.7* 0.034*-0.2* [s]	Positive	

Appendix A

	Pharmaceutical	*Henry's Law constant (atm-m^3/mole)*	*pKa*	Log K_{ow}	S_w 25°C (mg l^{-1})	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	Charge at pH 7	Molecular structure
	Clindamycin CAS # 18323-44-9	2.89E-022		2.01	30.61			Pos./Neut.	
Antibiotics	Cloxacillin CAS # 61-72-3	1.89E-017		3.22	13.94			Negative	
	Doxycycline CAS # 564-25-0	4.66E-024	pK_1= 3.5 pK_2= 7.7 pK_3= 9.5	-0.02	312.9				
	Enoxacin CAS # 74011-58-8	1.14E-021	pK_1= 6.3 pK_2= 8.7	-0.2	3.43 10^4			Neutral	
	Enrofloxacin CAS # 93106-60-6	1.5E-018	6.27[g]	1.1[h]	3397	4.5[u]		Neut./Neg.	
	Erythromycin CAS # 114-07-8	5.42E-029	8.8-8.9 [b]	3.06	0.5168	2.2[l]	0.15-6 [o]	Positive	

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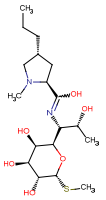
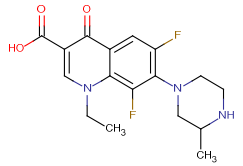
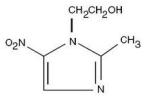
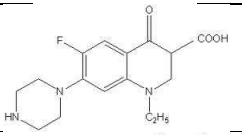
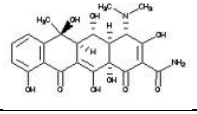
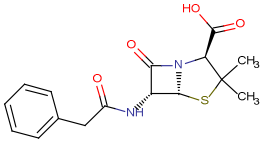
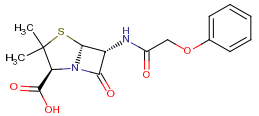
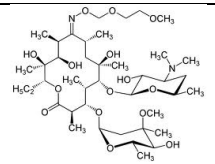
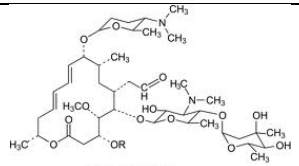
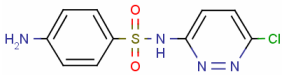
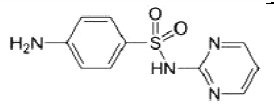
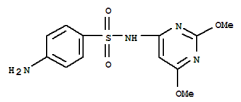
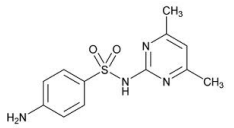
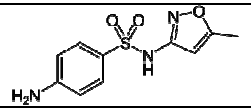
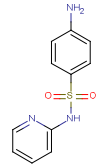
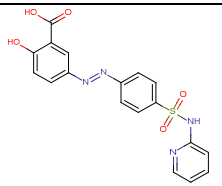
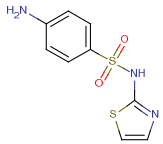
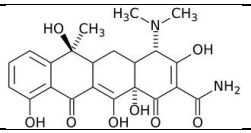
	Pharmaceutical	*Henry's Law constant (atm-m^3/mole)*	*pKa*	Log K_{ow}	S_w 25°C (mg l^{-1})	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	Charge at pH 7	Molecular structure
Antibiotics	Lincomycin CAS # 154-21-2	3E-023		0.29	92.19			Pos./Neut.	
	Lomefloxacin CAS # 98079-51-7	1.35E-018		0.31	2.72 10^4	4.16[u]		Neutral	
	Metronidazole CAS # 443-48-1	1.69E-011	2.5	-0.1; -0.02	2.573 10^4			Neutral	
	Norfloxacin CAS # 70458-96-7	8.7E-019	pK_1= 6.3, pK_2= 8.4	-1.03	1.779 10^5	4.2[k]		Positive	
	Ofloxacin CAS # 82419-36-1	4.98E-020	5.97	0.35	2.826 10^4	4.2[u]		Neut./Neg.	
Antibiotics	Oxytetracycline CAS # 79-57-2	1.7E-025	pK_1= 3.27 pK_2= 7.3 pK_3= 9.1	-0.90; -1.6 (pH 7.5) 1.22	1399			Negative	

Appendix A

	Pharmaceutical	*Henry's Law constant (atm-m^3/mole)*	*pKa*	Log K_{ow}	S_w 25°C (mg l^{-1})	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	Charge at pH 7	Molecular structure
	Penicillin G CAS # 61-33-6	1.16E-014	2.74					Negative	
	Penicillin V CAS # 87-08-1	4.42E-015	2.79	1.87	101.1			Negative	
	Roxithromycin CAS # 80214-83-1	4.97E-031	8.8[c]	2.75	0.01887	2.2-2.7[k] 2.3-2.6[l]	0.2-9 [o] ≤0.2 ≤0.3* 0.022*-0.023* [s]	Positive	
	Spiramycin CAS # 8025-81-8		8.0					Positive	
Antibiotics	Sulfachloropyridazine CAS # 80-32-0	2.05E-012		0.31	8235			Neut./Neg.	
	Sulfadiazine CAS # 68-35-9	1.58E-010	pK_1= 6.36 pK_2= 2.1	-0.09	2.814 10^4			Neut./Neg.	
	Sulfadimethoxine CAS # 122-11-2	1.3E-014		1.17	433.1			Neut./Neg.	

Appendix A

	Pharmaceutical	*Henry's Law constant (atm-m^3/mole)*	*pKa*	Log K_{ow}	S_w 25°C (mg l^{-1})	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	Charge at pH 7	Molecular structure
	Sulfamethazine CAS # 57-68-1	3.05E-013	2.65[q]	0.89[h]	1.124 10^4			Neut./Neg.	
Antibiotics	Sulfamethoxazole CAS # 723-46-6	6.42E-013	5.7[c]	0.89[i]	3942	2.1-2.7[k] 2.3-2.6[l]	0.3 [o]	Neut./Neg.	
	Sulfapyridine CAS # 144-83-2	1.08E-013	Pk1= 8043 Pk2=2.3	0.35	1.199 10^4	2.3-2.6[k]		Neut./Neg.	
	Sulfasalazine CAS # 599-79-1	2.19E-018		3.81	2.44			Negative	
	Sulfathiazole CAS # 72-14-0	5.85E-014		0.72	2.003 10^4			Negative	
Antibiotics	Tetracycline CAS # 60-54-8	4.66E-024	pK_1= 3.3 pK_2= 7.7 pK_3= 9.7	-1.30	3877	3.9[k]		Negative	

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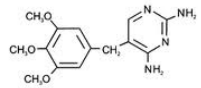
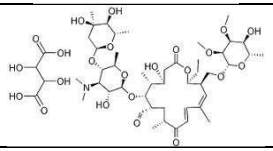
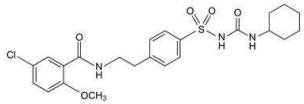
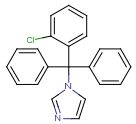
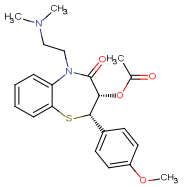
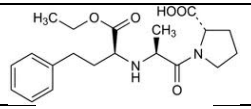
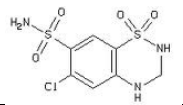
	Pharmaceutical	*Henry's Law constant (atm-m^3/mole)*	*pKa*	Log K_{ow}	S_w 25°C (mg l^{-1})	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	Charge at pH 7	Molecular structure
	Trimethoprim CAS # 738-70-5	2.39E-014	7.2	0.91	2334	2.2-2.6[k] 2.3[l]	0.15 [o]	Pos./Neut.	
	Tylosin CAS # 1401-69-0	5.77E-038	7.1[p]	1.63	0.5065			Pos./Neut.	
Antidiabetics	Glibenclamide CAS # 10238-21-8	7.56E-019	5.3	4.8	0.0635	2.4[t]		Negative	
Antifungals	Clotrimazole CAS # 23593-75-1	3.12E-008		6.26	0.0299			Pos./Neut.	
Antihypertensives	Diltiazem CAS # 42399-41-7	8.61E-017		2.79	12.3			Positive	
Antihypertensives	Enalapril CAS # 75847-73-3	3.34E-016	---	2.45	34.88			Negative	
	Hydrochlorothiazide CAS # 58-93-5	4.39E-012	7.9	-0.07	1292	1.8[t]		Negative	

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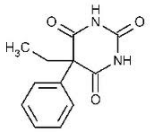
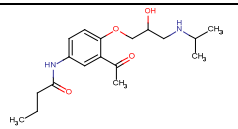
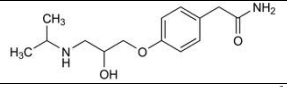
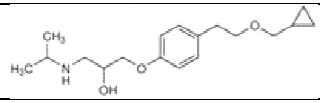
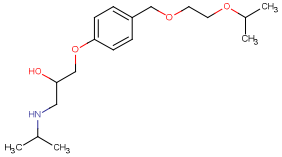
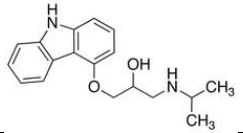
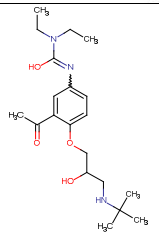
	Pharmaceutical	*Henry's Law constant (atm-m^3/mole)*	*pKa*	Log K_{ow}	S_w 25°C (mg l^{-1})	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	Charge at pH 7	Molecular structure
Barbiturates	Phenobarbital CAS # 50-06-6	3.8E-016	7.3	1.47	1644			Negative	
Beta-blokers	Acebutolol CAS # 37517-30-9	3.01E-020		1.71[i]	259				
	Atenolol CAS # 29133-68-7		9.6	0.16	685.2	-0.68[i]	1.1-1.9 [j]	positive	
	Betaxolol CAS # 63659-18-7	1.45E-013	---	2.81	450.7		6.0 [j]	Positive	
	Bisoprolol CAS # 66722-44-9	2.89E-015		1.84	2240		0.64-0.77 [j]	Positive	
Beta-blokers	Carazolol CAS #57775-29-8	5.56E-016	---	3.59	8.254			Positive	
	Celiprolol CAS # 56980-93-9	6.27E-021		1.93	93.92		0.18-0.24 [j]	Positive	

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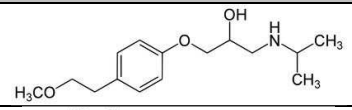
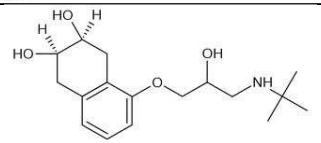
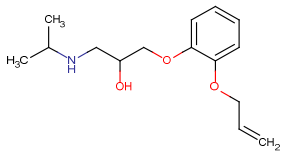
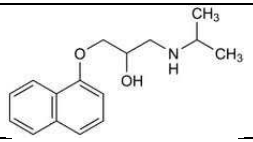
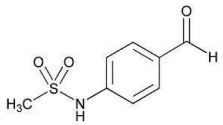
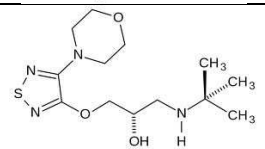
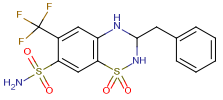
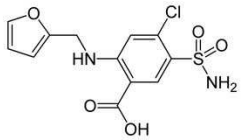
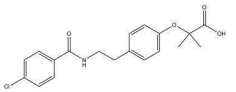
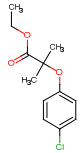
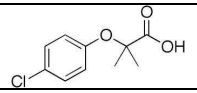
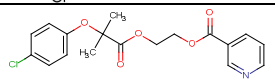
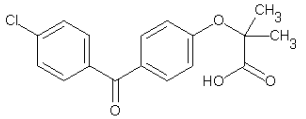
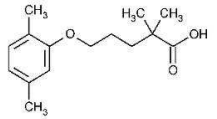
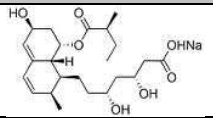
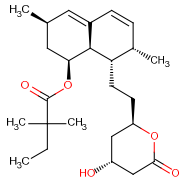
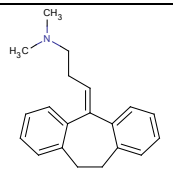
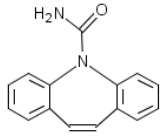
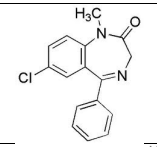
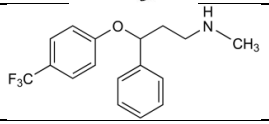
	Pharmaceutical	*Henry's Law constant (atm-m^3/mole)*	*pKa*	Log K_{ow}	S_w 25°C (mg l^{-1})	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	Charge at pH 7	Molecular structure
	Metoprolol CAS # 37350-58-6	1.4E-013	9.6	1.88	4777		0.35-0.40 [j]	Positive	
	Nadolol CAS # 42200-33-9	1.37E-014	9.67	0.81	2.24 10^4			Positive	
	Oxprenolol CAS # 6452-71-7	6.35E-013		1.83	3182			Positive	
Beta-blokers	Propranolol CAS # 525-66-6	7.98E-013	9.42	3.48	228	2.6[t]	0.36-0.46 [j]	Positive	
	Sotalol CAS # 3930-20-9	2.49E-014	pK_1=8.2 pK_2=9.8	0.24	5513		0.40-0.43 [j]	positive	
	Timolol CAS # 26839-75-8	4.35E-017	9.21	1.83	2741			Positive	
Diuretics	Bendroflumethiazide CAS # 73-48-3	5.51E-012		1.82	4.87			Neut./Neg.	

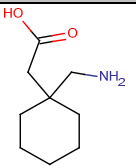
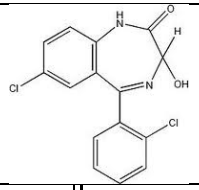
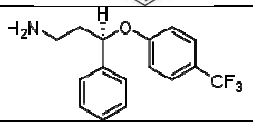
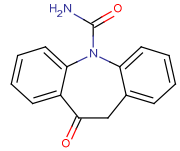
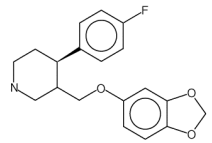
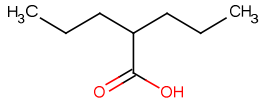
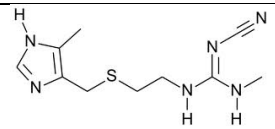
Appendix A

	Pharmaceutical	*Henry's Law constant (atm-m^3/mole)*	*pKa*	Log K_{ow}	S_w 25°C (mg l^{-1})	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	Charge at pH 7	Molecular structure
	Furosemide CAS # 54-31-9	3.94E-016	3.9	2.03	149.3			Negative	
Lipid regulators	Bezafibrate CAS # 41859-67-0	2.12E-015	3.6[c]	4.25	1.224		2.1-3.0 3.4*-4.5* 0.77*->2.9* [s]	Negative	
Lipid regulators	Clofibrate CAS # 637-07-0	9.31E-006		3.62	20.97			Neutral	
	Clofibric acid CAS # 882-09-7	2.19E-008	-3.18[m]	2.57	582.5		0.3-0.8 0.1*-0.23* 0.09*-0.1* [s]	Negative	
	Etofibrate CAS # 31637-97-5	5.74E-012		3.43	6.033			Neutral	
	Fenofibrate CAS # 49562-28-9	4.46E-009	---	5.19	0.1957			Neutral	
	Fenofibric acid CAS # 42017-89-0	7.9E-012		2.9			7.2-10.8 0.4*-1.7*;	Negative	
	Gemfibrozil CAS # 25812-30-0	1.19E-008	4.8	4.77	4.964	1.28[t]	6.4-9.6 0.5*-1.8*	Negative	

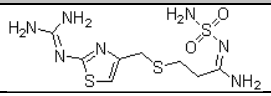
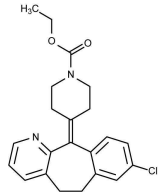
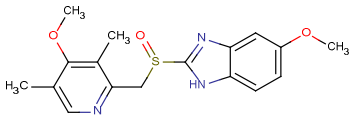
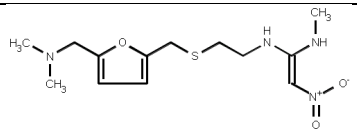
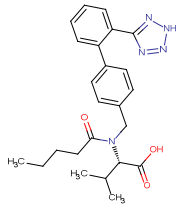
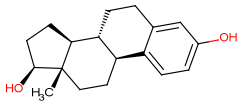
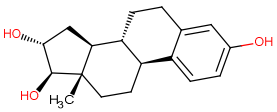
Appendix A

	Pharmaceutical	*Henry's Law constant (atm-m^3/mole)*	*pKa*	Log K_{ow}	S_w 25°C (mg l^{-1})	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	Charge at pH 7	Molecular structure
	Pravastatin CAS # 81093-37-0		---	-0.23	2464			Negative	
Lipid regulators	Simvastatin CAS # 79902-63-9	2.81E-010		5.19	0.765			Neutral	
Psychiatric drugs	Amitriptyline CAS # 50-48-6	6.85E-008		4.95	0.823			Positive	
	Carbamazepine CAS # 298-46-4	1.08E-010	13.9 [b]	2.45	17.66	0.1[l]	≤0.1 [j] <0.03-<0.06 [o] <0.005*- <0.008* [s]	Neutral	
	Diazepam CAS # 439-14-5	3.64E-009	3.4	2.82	58.78	1.3[l]	≤0.16 [j] <0.25-<0.4 [o]	Neutral	
	Fluoxetine CAS # 54910-89-3	8.9E-008	9.5	4.05	38.35	0.7[n]	5-9 [o]	positive	

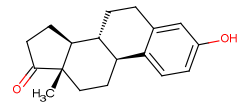
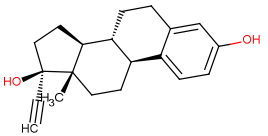
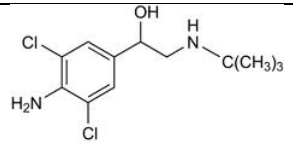
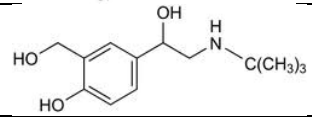
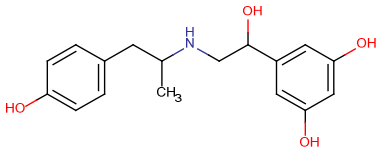
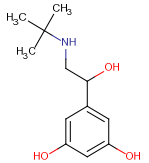
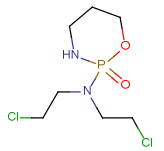
Appendix A

	Pharmaceutical	*Henry's Law constant (atm-m^3/mole)*	*pKa*	Log K_{ow}	S_w 25°C (mg l^{-1})	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	Charge at pH 7	Molecular structure
	Gabapentin CAS # 60142-96-3	1.81E-010			4491			Neutral	
Psychiatric drugs	Lorazepam CAS # 846-49-1	4.1E-010	pK_1=1.3 pK_2=11.5	2.39	83.87			Neutral	
	Norfluoxetine CAS # 126924-38-7		9.05[d]	4.07[d]					
	Oxcarbazepine CAS # 28721-07-5	6.92E-013		1.11	202.8			Neutral	
	Paroxetine CAS # 61869-08-7	1.78E-012	9.0	3.95	35.27			Positive	
	Valproic acid CAS # 99-66-1	3E-006		2.96	894.6			Negative	
Receptor antagonist	Cimetidine CAS # 51481-61-9	9.5E-016	6.8	0.40	1.046 10^4			Pos./Neut.	

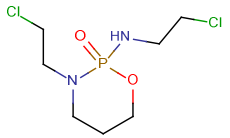
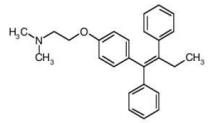
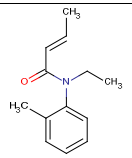
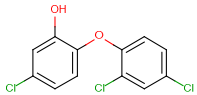
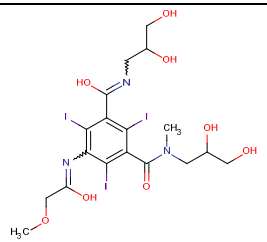
Appendix A

	Pharmaceutical	*Henry's Law constant (atm-m^3/mole)*	*pKa*	Log K_{ow}	S_w 25°C (mg l^{-1})	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	Charge at pH 7	Molecular structure
	Famotidine CAS # 76824-35-6	5.44E-024	---	-0.64	1271			Positive	
Receptor antagonists	Loratadine CAS # 79794-75-5	3.19E-013	---	5.20	0.01099	3.5[t]		Neutral	
	Omeprazole CAS # 73590-58-6	3.04E-019		3.4	82.28			Neutral	
	Ranitidine CAS # 66357-35-5	3.42E-015	2.4	0.27	2.466 10^4			Positive	
	Valsartan CAS # 137862-53-4							Negative	
Hormones	Estradiol CAS # 50-28-2	3.64E-011	10.27[m]	3.94	81.97	2.4-2.8[l]	175-460 [r] 280*-950* [r]	Neutral	
	Estriol CAS # 50-27-1	1.33E-012		2.81	440.8			Neutral	

Appendix A

	Pharmaceutical	*Henry's Law constant (atm-m^3/mole)*	*pKa*	Log K_{ow}	S_w 25°C (mg l^{-1})	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	Charge at pH 7	Molecular structure
	Estrone CAS # 53-16-7	3.8E-010	10.25[m]	3.43	146.8	2.4-2.9[l]	10-162 [r] 28*-430* [r] >20 [s]	Neutral	
	Ethinylestradiol CAS # 57-63-6	7.94E-012	10.24[m]	4.12	116.4	2.5-2.8[l]	0.4-20 [o] 1.2-8 [r] 1.5*-6* [r] >0.5->0.7 [s]	Neutral	
Beta-agonists	Clenbuterol CAS # 037148-27-9	2.96E-014	---	2.00	3320			Positive	
	Salbutamol CAS # 35763-26-9		pK_1= 9.3, pK_2=10.3	0.6, 0.01	--			Positive	
	Fenoterol CAS # 13392-18-2	1.04E-023		1.22	4.13 10^4			Positive	
	Terbutaline CAS # 23031-25-6	1.65E-018		0.67	2.128 10^5			Positive	
Antineoplastic	Cyclophosphamide CAS # 50-18-0	1.4E-011		0.97	5943			Neutral	

Appendix A

	Pharmaceutical	*Henry's Law constant (atm-m^3/mole)*	*pKa*	Log K_{ow}	S_w 25°C (mg l^{-1})	Log K_d	k_{biol} (L gSS^{-1} d^{-1})	Charge at pH 7	Molecular structure
	Ifosfamide CAS # 3778-73-2	1.36E-011		0.97	3781			Neutral	
	Tamoxifen CAS # 10540-29-1	4.49E-010		6.30	0.1936			Positive	
Topical Products	Crotamiton CAS # 483-63-6	1.53E-007		2.73	195.3			Neutral	
Antiseptics	Triclosan CAS # 3380-34-5	4.99E-009	8.1[n]	5.34	4.621			Neut./Neg.	
Contrast media	Iopromide CAS # 73334-07-3	1E-028		-2.49	23.75	1[l]	1.6-2.5 1.0*-2.0* 0.12*-0.026* [s]	Pos./Neut.	

Data were from Ternes and Joss, 2006;http:// esc.syrres.com/interkow/physdemo.htm (Henry's Law constant), Petrovic and Barcelò 2007 (pKa), EPISuite v4.00 (S_w, logK_{ow}, logK_{oc}); Chemamox (charge at pH=7). For LogK_d, references are specified.

References

[a]Avdeef et al. 2002; [b] Jones et al. 2002; [c] Huber et al. 2003; [d] Khan and Ongerth 2002; [e] Wan et al. 2002; [f] Tixier et al. 2003; [g] Nowara et al. 1997; [h] Meylan 1993; [i] Vieno et al.,2007; [j] Wick et al.,2009; [k] Le-Minh et al., 2010; [l] Suárez et al., 2008; [m] Zorita et al.2009; [n] Munoz et al.2009; [o] Suárez et al.,2010; [p] Wollenberger 2000; [q] Papastephanou and Frantz 1997; [r] Joss et al., 2004 [s] Abegglen et al., 2009; [t] Radjenovic et al., 2009; u Jia et al., 2012

Appendix A

Simple criteria

k_{biol} < 0.1 L/(gSS d)	poor degradability
0.1< k_{biol} < 10 L/(gSS d)	quite good biodegradability
k_{biol} > 10 L/(gSS d)	very good degradability
Log K_{ow} < 2.5	high hydrophilic compound
2.5 < Log K_{ow} < 4	moderate hydrophilic compound
Log K_{ow} > 4	high lipophilic compound
Log K_d < 2.7	low adsorption potential
Log K_d > 2.7	high adsorption potential

Table A.2. Ranges of concentration in the influent for the selected pharmaceuticals together with their corresponding references. Data with an asterix as apex (*) refer to MBRs; (loq= limit of quantification)

Therapeutic class	Pharmaceutical compound	Municipal WWTP influent	References
Analgesics/ Anti-inflammatories **A**	5-aminosalicylic acid	3.16-27.9	Kasprzyk-Hordern et al., 2009
	Acetaminophen	0.013-0.057 18-71 29-246 104 7.1-11.4 4.16 1.57-37.5 172* 0.96	Choi et al., 2008; Foster, 2007; Gómez et al., 2007; Khan and Ongerth, 2005; Radjenovic et al., 2007, 2009; Roberts and Thomas, 2006; Rosal et al., 2010; Snyder et al., 2006; Yu et al., 2006
	Acetylsalicylic acid	1.32-5.44	Kasprzyk-Hordern et al., 2009
	Codeine	0.1-35 2.8-11 2.49-12.6 0.15-2.09 0.12	Foster, 2007; Gómez et al., 2007; Kasprzyk-Hordern et al.,2009; Rosal et al., 2010; Wick et al., 2009
	Dextropropoxyphene	0.03	Roberts and Thomas, 2006
	Diclofenac	0.16 3.19*/0.9-3.19 0.9-4.1 0.2-3.6 0.06-1.16 0.25-1 0.3-0.6 0.2 0.2-0.7 2.8* 1-1.6 7 0.98* 0.23 <loq <loq 0.05* 0.78 11 0.3-2.09 0.33-0.49 0.46 1.23 0.11 0.23	Bendz et al., 2005; Clara et al 2005a, Clara et al 2005b; Gómez et al., 2007; Kasprzyk-Hordern et al., 2009; Kimura et al 2007; Lindqvist et al., 2005; Lishman et al., 2006; Paxéus, 2004; Quintana et al., 2005; Radjenovic et al., 2009; Reif et al., 2008; Roberts and Thomas, 2006; Rosal et al., 2010; Santos et al., 2007, Santos et al., 2009; Snyder et al., 2006; Stumpf et al., 1999; Suárez et al., 2005; Tauxe-Wuersch et al., 2005; Thomas and Foster, 2005; Vieno et al., 2005; Weigel et al., 2004; Yu et al., 2006; Zorita et al., 2009
	Dipyrone	4.7-24	Gómez et al., 2007
	Fenoprofen	<loq <loq 0.009-0.08	Bendz et al., 2005; Lishman et al., 2006; Nakada et al., 2006;
	Flurbiprofen	<loq	Bendz et al., 2005
	Hydrocodone	0.11*	Snyder et al., 2006
	Ibuprofen	3.59 2.6-5.7 2.44*/1.2-3.6 1.2-2.6 34-168 0.98-6.32 2.7 1.9	Bendz et al., 2005; Carballa et al., 2004, Clara et al., 2005a, Clara et al., 2005b; Gómez et al., 2007; Kasprzyk-Hordern et al., 2009; Khan and Ongerth, 2005; Kimura et al., 2007;

Appendix A

Therapeutic class	Pharmaceutical compound	Municipal WWTP influent	References
		9.8-19.8 8.45 0.38-1.13 0.8-11 5.7* 14.6-31.3 9.8* 2.6 2.8-5.8 <loq-4.11 12.1-373 <loq-353 12* 0.32 10 1.1-4.6 9.5-14.7 23.4 1.66 1.9 6.9	Lindqvist et al., 2005; Lishman et al., 2006; Nakada et al., 2006; Paxéus, 2004; Quintana et al., 2005; Radjenovic et al., 2009; Reif et al., 2008; Roberts and Thomas, 2006; Rodriguez et al., 2003; Rosal et al., 2010; Santos et al., 2007, Santos et al., 2009; Snyder et al., 2006; Stumpf et al., 1999; Suárez et al., 2005; Tauxe-Wuersch et al., 2005; Thomas and Foster, 2005; Vieno et al., 2005; Weigel et al., 2004; Yu et al., 2006; Zorita et al., 2009
	Indomethacin	<loq 0.23 0.66-1 <loq-0.11 0.95	Bendz et al., 2005; Lishman et al., 2006, Radjenovic et al., 2009; Rosal et al., 2010; Stumpf et al., 1999;
	Ketoprofen	0.94 0.031-0.34 0.9 0.97 1.3-3 0.15 0.1-0.37 0.5* 0.7-1.2 <loq-0.8 <loq-3.59 <loq-6.47 0.52 0.15-0.41 0.41-0.52 2.9 1.2	Bendz et al., 2005; Kasprzyk-Hordern et al., 2009; Khan and Ongerth, 2005; Kimura et al., 2007; Lindqvist et al., 2005; Lishman et al., 2006; Nakada et al., 2006; Quintana et al., 2005; Radjenovic et al., 2007, Rosal et al., 2010; Santos et al., 2007, Santos et al., 2009; Stumpf et al., 1999; Tauxe-Wuersch et al., 2005; Thomas and Foster, 2005; Vieno et al., 2005; Yu et al., 2006
	Ketorolac	<loq-2.8	Rosal et al., 2010
	Mefenamic acid	<0.017-0.03 0.22 0.8-1.2 0.23 0.1-0.22 0.75-2.9	Kasprzyk-Hordern et al., 2009; Kimura et al., 2007; Radjenovic et al.,2009; Roberts and Thomas, 2006; Rosal et al., 2010; Tauxe-Wuersch et al., 2005
	Naproxen	3.65 1.79-4.6 0.62-3.5 6.5 0.27 3.6-8.2 5.58 0.04-0.23 1.8-3.6 1* 0.13-0.67	Bendz et al., 2005; Carballa et al., 2004, Kasprzyk-Hordern et al., 2009; Khan and Ongerth, 2005; Kimura et al., 2007; Lindqvist et al., 2005; Lishman et al., 2006; Nakada et al., 2006; Paxéus, 2004; Quintana et al., 2005; Radjenovic et al.,2009;

Appendix A

Therapeutic class	Pharmaceutical compound	Municipal WWTP influent	References
		6.2* 3.5-4.5 1.19-5.23 1.1-27.4 2.02-52.1 12.5* 0.6 10 10.3-12.8 8.6 3.2 4.9	Reif et al., 2008; Rodriguez et al., 2003; Rosal et al., 2010; Santos et al., 2007, 2009; Snyder et al., 2006; Stumpf et al., 1999; Suárez et al., 2005; Thomas and Foster, 2005; Vieno et al., 2005; Yu et al., 2006; Zorita et al., 2009
	Phenazone	<loq-0.07	Rosal et al., 2010;
	Propyphenazone	0.0016-0.07 0.04-0.09	Nakada et al., 2006; Radjenovic et al., 2009
	Salicylic acid	5.6-32.08 13 13.7	Kasprzyk-Hordern et al., 2009; Khan and Ongerth, 2005; Lishman et al., 2006;
	Tramadol	23.03-85.8 0.23-0.47	Kasprzyk-Hordern et al, 2009; Wick et al., 2009
Antibiotics **B**	Amoxicillin	0.19-0.28	Watkinson et al., 2007
	Azithromycin	0.16-1.34 0.09-0.38 0.26	Ghosh et al., 2009; Göbel et al., 2005, Yasojima et al., 2006
	Cefaclor	0.5-0.98	Watkinson et al., 2007
	Cefalexin	2 0.67-2.9 4.6	Costanzo et al., 2005; Gulkowska et al., 2008; Watkinson et al., 2007
	Cefotaxime	0.004-0.024	Gulkowska et al., 2008
	Chloramphenicol	0.15-0.45 1.73-2.43	Kasprzyk-Hordern et al., 2009; Peng et al., 2006
	Chlortetracycline	<loq	Watkinson et al., 2007
	Ciprofloxacin	0.09 0.231-0.195 0.315-0.57 0.21 0.09-0.194 0.21-0.228 0.16-13.6 3.8 0.32	Costanzo et al., 2005; Ghosh et al., 2009; Golet et al., 2003; Karthikeyan and Meyer, 2006; Lindberg et al., 2005, Lindberg et al., 2006; Rosal et al., 2010; Watkinson et al., 2007; Zorita et al., 2009
	Clarithromycin	1.129-4.82 0.33-0.6 0.647	Ghosh et al., 2009; Göbel et al., 2005, Yasojima et al., 2006
	Clindamycin	0.002-0.005	Watkinson et al., 2007
	Cloxacillin	<loq	Watkinson et al., 2007
	Doxycycline	<loq-0.11 -	Lindberg et al., 2005; Watkinson et al., 2007
	Enrofloxacin	0.023-0.085 0.01	Ghosh et al., 2009; Watkinson et al., 2007
	Erythromycin	0.06-0.19 0.47-0.74 0.48-1.2 0.14-10.02 0.32-2.7 10* 0.11 0.34 1.05* 0.22	Göbel et al., 2005, Gulkowska et al., 2008; Karthikeyan and Meyer, 2006; Kasprzyk-Hordern et al., 2009; Radjenovic et al., 2007, Reif et al., 2008; Roberts and Thomas, 2006; Rosal et al., 2010; Snyder et al., 2006; Xu et al., 2007

Appendix A

Therapeutic class	Pharmaceutical compound	Municipal WWTP influent	References
	Lincomycin	0.06-0.08	Watkinson et al., 2007
	Metronidazole	0.34-0.962 0.044-0.17	Kasprzyk-Hordern et al., 2009; Rosal et al., 2010
	Norfloxacin	0.155-0.468 0.343-0.52 0.46 0.075-0.174 0.246-0.319 0.17 0.033 0.018	Ghosh et al., 2009; Golet et al., 2003; Gulkowska et al., 2008; Lindberg et al., 2005, 2006; Watkinson et al., 2007; Xu et al., 2007; Zorita et al., 2009
	Ofloxacin	0.47 0.287 0.52-5.56 0.89-31.7 0.84-5.29 0.077 0.022	Brown et al., 2006; Lindberg et al., 2005; Peng et al., 2006; Radjenovic et al., 2009; Rosal et al., 2010; Xu et al., 2007; Zorita et al., 2009
	Oxytetracycline	<loq	Watkinson et al., 2007
	Penicillin G	<loq <loq	Gulkowska et al., 2008; Watkinson et al., 2007
	Penicillin V	0.05-0.16	Watkinson et al., 2007
	Roxithromycin	0.025-0.078 0.096-0.209 0.01-0.04 17* 0.08 0.018 0.04	Clara et al., 2005b; Ghosh et al., 2009; Göbel et al., 2005, Reif et al., 2008; Ruel et al., 2010; Watkinson et al., 2007; Xu et al., 2007
	Sulfachloropyridazine	<loq-0.47	Choi et al., 2008
	Sulfadiazine	5.1-5.15	Peng et al., 2006
	Sulfadimethoxine	<loq-0.21	Choi et al., 2008;
	Sulfamethazine	0.11-0.21 <loq	Karthikeyan and Meyer., 2006; Sahar et al., 2011
	Sulfamethoxazole	0.02 0.39 <loq-0.58 0.15-0.98 0.02-0.075 <0.2 0.23-0.57 0.17-1.25 0.02-0.27 0.14-0.23 5.45-7.91 0.25-1.3 10* 0.16-0.53 0.53 1.11* 0.36 0.01	Bendz et al., 2005; Brown et al., 2006; Carballa et al., 2004, Choi et al., 2008; Clara et al., 2005b; Foster, 2007; Göbel et al., 2005, Karthikeyan and Meyer, 2006; Kasprzyk-Hordern et al., 2009; Lindberg et al., 2005; Peng et al., 2006; Radjenovic et al., 2009; Reif et al., 2008; Rosal et al., 2010; Ruel et al., 2010; Snyder et al., 2006; Watkinson et al., 2007; Xu et al., 2007
	Sulfapyridine	0.06-0.15 2.16-12.39	Göbel et al., 2005, Kasprzyk-Hordern et al., 2009
	Sulfasalazine	<loq-0.06	Watkinson et al., 2007
	Sulfathiazole	<0.03-0.53 0.002	Choi et al., 2008; Watkinson et al., 2007
	Tetracycline	0.065-0.089 0.096-1.3 0.24-0.79	Ghosh et al., 2009; Gulkowska et al., 2008; Karthikeyan and Meyer, 2006;

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Therapeutic class	Pharmaceutical compound	Municipal WWTP influent	References
		<loq	Watkinson et al., 2007
	Trimethoprim	0.53 0.08 0.59 0.1 0.011-0.026 0.21-0.44 0.12-0.32 0.58-1.1 1.51-4.67 <loq-0.49 0.17-0.65 1.03-1.86 0.1-0.3 0.15-0.43 10.5* 0.25 0.07-0.2 0.69* 0.34	Batt 2006; Bendz et al., 2005; Brown et al., 2006; Foster, 2007; Ghosh et al., 2009; Göbel et al., 2005, Gulkowska et al., 2008; Karthikeyan and Meyer, 2006; Kasprzyk-Hordern et al., 2009; Choi et al., 2008; Lindberg et al., 2005, 2006; Paxéus, 2004; Radjenovic et al., 2009; Reif et al., 2008; Roberts and Thomas, 2006; Rosal et al., 2006; Snyder et al., 2006; Watkinson et al., 2007
	Tylosin	<loq-0.055	Watkinson et al., 2007
Antidiabetics **C**	Glibenclamide	0.12-15.9	Radjenovic et al., 2009
Antifungals **D**	Clotrimazole	0.029	Roberts and Thomas, 2006
Antihypertensives **E**	Diltiazem	<0.005-0.019 <0.2-1.6 0.405-5.258	Choi et al., 2008 Foster, 2007; Kasprzyk-Hordern et al., 2009;
	Hydrochlorothiazide	2.3-4.8 0.61-10	Radjenovic et al., 2009; Rosal et al., 2010;
Barbiturates **F**	Phenobarbital	0.07	Yu et al., 2006
Beta-blockers **G**	Atenolol	2.29 0.03 8.1-25.14 1.69-2.54 0.84-2.8 0.66-2.43 0.72	Alder et al. 2010 Bendz et al., 2005; Kasprzyk-Hordern et al., 2009; Maurer et al., 2007; Radjenovic et al., 2009; Rosal et al., 2010; Wick et al., 2009
	Betaxolol	0.006-0.009	Wick et al., 2009
	Bisoprolol	0.21-0.38	Wick et al., 2009
	Celiprolol	0.1-0.16	Wick et al., 2009
	Metoprolol	0.24 0.056-0.14 0.14-0.23 0.3 0.026-0.063 0.02 1.2	Alder et al., 2010; Kasprzyk-Hordern et al., 2009; Maurer et al., 2007; Paxéus, 2004; Radjenovic et al., 2009; Rosal et al., 2010; Wick et al., 2009
	Propranolol	0.05 0.05 0.11-1.9 0.05-0.17 0.1-1.13 0.08 0.012-0.06 0.073	Alder et al., 2010; Bendz et al., 2005; Kasprzyk-Hordern et al., 2009; Maurer et al., 2007; Radjenovic et al., 2009; Roberts and Thomas, 2006; Rosal et al., 2010; Wick et al., 2009
	Sotalol	0.29 0.3 0.17-0.85 1.1	Alder et al., 2010; Maurer et al., 2007; Radjenovic et al., 2009; Wick et al., 2009
Diuretics **H**	Bendroflumethiazide	<0.008-0.1	Kasprzyk-Hordern et al., 2009
	Furosemide	1.58-6.02	Kasprzyk-Hordern et al., 2009;

Appendix A

Therapeutic class	Pharmaceutical compound	Municipal WWTP influent	References
		0.41	Rosal et al., 2010
Lipid regulators **I**	Bezafibrate	6.84*/1.55-7.6 1.55-7.6 0.6 0.1-1 2.6* 1.9-29.8 0.048-0.36 1.18 2.2	Clara et al., 2005a, Clara et al., 2005b; Kasprzyk-Hordern et al., 2009; Lindqvist et al., 2005; Quintana et al., 2005; Radjenovic et al., 2009; Rosal et al., 2010; Stumpf et al., 1999; Vieno et al., 2005
	Clofibric acid	0.028 0.49 0.03 1 0.17-0.37 0.17 0.05	Kimura et al., 2007; Roberts and Thomas, 2006; Rosal et al., 2010; Stumpf et al., 1999; Tauxe-Wuersch et al., 2005; Weigel et al., 2004; Zorita et al., 2009
	Fenofibric acid	<loq-0.12/0.079 0.42	Rosal et al., 2010; Stumpf et al., 1999;
	Gemfibrozil	0.71 1.5 0.45 0.6-1.1 2-5.9 0.41-17.1 2.21* 0.3 0.41	Bendz et al., 2005; Khan and Ongerth, 2005; Lishman et al., 2006; Paxéus, 2004; Radjenovic et al., 2009; Rosal et al., 2010; Snyder et al., 2006; Stumpf et al., 1999; Yu et al., 2006
	Pravastatin	<0.06 0.46-1.5	Kasprzyk-Hordern et al., 2009; Radjenovic et al., 2009
	Simvastatin	<0.007	Kasprzyk-Hordern et al., 2009
Psychiatric drugs **J**	Amitriptyline	0.504-6.7	Kasprzyk-Hordern et al., 2009
	Carbamazepine	1.68 0.7*/0.32-0.7 0.32-1.2 0.3 <0.2-0.59 0.12-0.31 0.1-3.11 0.5 <0.005-0.45 0.015-0.27 1.3-2 0.054-0.22 19.5* 0.1-0.17 <loq/2.15 <loq-3.78 0.2* 21.5 1	Bendz et al., 2005; Clara et al., 2005a, Clara et al., 2005b; Conti et al., 2011; Foster, 2007; Gómez et al., 2007; Kasprzyk-Hordern et al., 2009; Khan and Ongerth, 2005; Choi et al., 2008; Nakada et al., 2006; Paxéus, 2004; Radjenovic et al., 2009; Reif et al., 2008; Rosal et al., 2010; Santos et al., 2007, Santos et al., 2009; Snyder et al., 2006; Suárez et al., 2005; Wick et al, 2009
	Diazepam	23* 21	Reif et al., 2008; Suárez et al., 2005;
	Fluoxetine	0.1 0.191 0.12-2.3 0.58 <0.1* 0.011	Foster, 2007; Metcalfe et al. 2010; Radjenovic et al., 2009; Rosal et al., 2010; Snyder et al., 2006; Zorita et al., 2009
	Gabapentin	10.67-25	Kasprzyk-Hordern et al., 2009;

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Therapeutic class	Pharmaceutical compound	Municipal WWTP influent	References
		0.1	Yu et al., 2006
	Norfluoxetine	0.011 0.011	Metcalfe et al. 2010; Zorita et al., 2009
	Oxcarbazepine	0.011-0.046	Conti et al., 2011
	Paroxetine	0.016	Metcalfe et al. 2010;
	Valproic acid	0.14	Yu et al., 2006
Receptor antagonists **K**	Cimetidine	0.014-10 0.68-6.5	Choi et al., 2008 Kasprzyk-Hordern et al., 2009;
	Famotidine	0.027-0.14	Radjenovic et al., 2009
	Loratadine	0.015-0.043	Radjenovic et al., 2009
	Omeprazole	0.057-2.13	Rosal et al., 2010
	Ranitidine	2-11.15 0.072-0.54 0.52	Kasprzyk-Hordern et al., 2009; Radjenovic et al., 2009, Rosal et al., 2010
	Valsartan	0.35-5.3	Kasprzyk-Hordern et al., 2009
Hormones **L**	Estradiol	0.012-0.02 0.008-0.016 0.035-0.067/0.067* <0.08-3 0.04*/0.003 0.01 0.003	Andersen et al., 2003; Baronti et al., 2000; Clara et al., 2005a Foster, 2007; Joss et al., 2004; Lishman et al., 2006; Zorita et al., 2009
	Estriol	0.05-0.12 0.023-0.336/0.326* 0.08-0.25	Baronti et al., 2000; Clara et al., 2005a Nakada et al., 2006
	Estrone	0.05-0.07 0.03-0.07 0.002 0.071*/0.034-0.67 0.025*/0.032 0.03 0.02-0.19 0.014	Andersen et al., 2003; Baronti et al., 2000; Carballa et al., 2004, Clara et al., 2005a; Joss et al., 2004; Lishman et al., 2006; Nakada et al., 2006; Zorita et al., 2009
	Ethinylestradiol	0.002-0.004 0.004-0.07/0.02* 0.04 0.002	Baronti et al., 2000; Clara et al., 2005a Foster, 2007; Joss et al., 2004;
Beta-agonists **M**	Salbutamol	0.05-0.15	Kasprzyk-Hordern et al., 2009;
Antineoplastics **N**	Ifosfamide	0.038-0.36	Kummerer et al., 1997
	Tamoxifen	0.17	Roberts and Thomas, 2006
Topical product **O**	Crotamitron	0.38-3.03	Nakada et al., 2006
Antiseptic **P**	Triclosan	1.7-2.7 0.39-4.2 7 0.21-1.8 0.4-2.2 0.86 0.45 1.28* 3-3.6 0.38 0.8	Foster, 2007; Gómez et al., 2007; McAvoy et al., 2002; Nakada et al., 2006;, Paxéus, 2004; Rosal et al., 2010; Ruel et al., 2010; Snyder et al., 2006; Thomas and Foster, 2005; Weigel et al., 2004; Yu et al., 2006
Contrast agent **Q**	Iopromide	0.2 6.6 0.03-3.84	Batt et al., 2006; Carballa et al., 2004; Clara et al., 2005b

Table A.3. Ranges of concentration for the selected pharmaceuticals in the effluent of CAS and MBR together with their corresponding references. Data with an asterix as apex (*) refer to MBRs. (loq= limit of quantification)

Therapeutic class	Pharmaceutical compound	Secondary effluent	References
Analgesics/ Anti-inflammatories **A**	5-aminosalicylic acid	0.17-1.21	Kasprzyk-Hordern et al., 2009
	Acetaminophen	<0.005-0.009 0.11 0.025 <loq-4.3 0.08-1.57 0.23 0.0018-0.019 <20 <0.01 6	Choi et al., 2008; Coetsier et al., 2009; Foster, 2007; Gómez et al., 2007; Kasprzyk-Hordern et al., 2009; Khan and Ongerth, 2005; Kim et al., 2007; Roberts and Thomas, 2006; Snyder et al., 2006; Ternes, 1998
	Acetylsalicylic acid	<0.003-0.065 1.5-0.22	Kasprzyk-Hordern et al., 2009; Ternes, 1998
	Aminopyrine	<loq 1	Andreozzi et al., 2003; Ternes, 1998
	Codeine	0.025 0.9-8.1 1.45-4.17 0.16 0.025	Foster, 2007; Gómez et al., 2007; Kasprzyk-Hordern et al.,2009; Rosal et al., 2010; Wick et al., 2009
	Dextropropoxyphene	0.1	Roberts and Thomas, 2006
	Diclofenac	0.47-5.45 0.12 2.14*/0.78-1.68 2.03*/0.78-1.5 0.4 0.14-2.2 0.006-0.5 0.008-0.12 0.04*/0.07 0.046*-0.12*/0.145 0.15-0.33 0.19 0.006-1.3 0.14-1.48 1.9* 7.5* 0.34 0.006-0.43 <loq 0.07 <0.01* 0.19 10.67 0.6-2.4 1.3 0.81 0.068-0.083 0.4 0.09 0.48	Andreozzi et al., 2003; Bendz et al., 2005; Clara et al 2005a, Clara et al 2005b; Coetsier et al., 2009; Gómez et al., 2007; Kasprzyk-Hordern et al., 2009; Kim et al., 2007; Kimura et al., 2005, Kimura et al 2007; Lindqvist et al., 2005; Lishman et al., 2006; Muñoz et al., 2009; Paxéus, 2004; Quintana et al., 2005; Reif et al., 2008; Roberts and Thomas, 2006; Rosal et al., 2010; Santos et al., 2007, Santos et al., 2009; Snyder et al., 2006; Stumpf et al., 1999; Suárez et al., 2005; Tauxe-Wuersch et al., 2005; Ternes et al., 2003; Ternes, 1998; Thomas and Foster, 2005; Vieno et al., 2005; Yu et al., 2006; Zorita et al., 2009
	Dipyrone	2.4-7.5	Gómez et al., 2007
	Fenoprofen	<loq <loq 0.015 -	Andreozzi et al., 2003; Bendz et al., 2005; Coetsier et al., 2009; Lishman et al., 2006;

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Therapeutic class	Pharmaceutical compound	Secondary effluent	References
		0.0015-0.009 <loq	Nakada et al., 2006; Ternes, 1998
	Flurbiprofen	0.34 <loq	Andreozzi et al., 2003; Bendz et al., 2005
	Hydrocodone	<0.01*	Snyder et al., 2006
	Ibuprofen	0.02-0.18 0.15 2.1 0.02-2.4/0.069* <loq-2.4/0.02* 0.067 0.24-7.1 0.065-0.49 0.22 0.01-0.13 0.01*-0.2* 0.04/0.03*-0.1* 0.05-3.9 0.77 0.54 0.0014-1.18 0.02-1.9 0.18* 2.9 0.91-1.87 <loq-0.65 0.78-48.24 <loq-40.2 0.04* 0.08 1.8 0.1-2.1 1.13 3.4 0.015-0.023 0.005 0.08	Andreozzi et al., 2003; Bendz et al., 2005; Carballa et al., 2004, Clara et al., 2005a, Clara et al., 2005b; Coetsier et al., 2009; Gómez et al., 2007; Kasprzyk-Hordern et al., 2009; Khan and Ongerth, 2005; Kim et al., 2007; Kimura et al., 2005, Kimura et al., 2007; Lindqvist et al., 2005; Lishman et al., 2006; Muñoz et al., 2009; Nakada et al., 2006; Paxéus, 2004; Quintana et al., 2005; Roberts and Thomas, 2006; Rodriguez et al., 2003; Rosal et al., 2010; Santos et al., 2007, Santos et al., 2009; Snyder et al., 2006; Stumpf et al., 1999; Suárez et al., 2005; Tauxe-Wuersch et al., 2005; Ternes et al., 2003; Ternes, 1998; Thomas and Foster, 2005; Vieno et al., 2005; Zorita et al., 2009
	Indomethacin	<loq 0.19 0.02-0.05 0.16 0.1 0.27	Bendz et al., 2005; Lishman et al., 2006, Rosal et al., 2010; Stumpf et al., 1999; Ternes et al., 2003; Ternes, 1998
	Ketoprofen	<loq 0.33 0.007-0.37 0.59 0.01*-0.02*/0.28 <0.02*-0.17*/0.44 0.05-0.9 0.12 0.06-0.21 0.18* 0.27-0.53 <loq-1.5 <loq-2.27 0.19 0.1-0.37 0.2 0.015-0.041 0.23	Andreozzi et al., 2003; Bendz et al., 2005; Kasprzyk-Hordern et al., 2009; Khan and Ongerth, 2005; Kimura et al., 2005, 2007; Lindqvist et al., 2005; Lishman et al., 2006; Nakada et al., 2006; Quintana et al., 2005; Rosal et al., 2010; Santos et al., 2007, Santos et al., 2009; Stumpf et al., 1999; Tauxe-Wuersch et al., 2005; Ternes, 1998; Thomas and Foster, 2005; Vieno et al., 2005;

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Therapeutic class	Pharmaceutical compound	Secondary effluent	References
		0.28	Yu et al., 2006
	Ketorolac	<loq-0.607	Rosal et al., 2010
	Meclofenamic acid	0.025	Ternes, 1998
	Mefenamic acid	<0.005-0.1 0.008*-0.018*/0.035 0.015*-0.05*/0.062 0.96 0.087-0.16 0.5-3	Kasprzyk-Hordern et al., 2009; Kimura et al., 2005, 2007; Roberts and Thomas, 2006; Rosal et al., 2010; Tauxe-Wuersch et al., 2005
	Naproxen	0.29-5.2 0.25 0.8-2.6 <0.002-0.2 0.35 0.02-0.4 0.005*-0.02*/0.05 <0.01*-0.01*/0.099 0.15-1.93 0.45 0.012-0.14 0.2-1.51 0.17* 1* 1.87-2.1 0.35-2.2 0.22-4.28 0.22-5.09 <0.01* 1.32 3.2 0.3 0.1 0.012-0.038 0.42 0.38 0.34	Andreozzi et al., 2003; Bendz et al., 2005; Carballa et al., 2004, Kasprzyk-Hordern et al., 2009; Khan and Ongerth, 2005; Kim et al., 2007; Kimura et al., 2005, 2007; Lindqvist et al., 2005; Lishman et al., 2006; Nakada et al., 2006; Paxéus, 2004; Quintana et al., 2005; Reif et al., 2008; Rodriguez et al., 2003; Rosal et al., 2010; Santos et al., 2007, 2009; Snyder et al., 2006; Stumpf et al., 1999; Suárez et al., 2005; Ternes, 1998; Ternes et al., 2003; Thomas and Foster, 2005; Vieno et al., 2005; Yu et al., 2006; Zorita et al., 2009
	Phenazone	<loq <loq-0.058 0.16-0.41	Andreozzi et al., 2003; Rosal et al., 2010; Ternes, 1998
	Propyphenazone	0.0014-0.12/0.007	Nakada et al., 2006
	Salicylic acid	<0.001-0.39 0.38 0.1 0.14	Kasprzyk-Hordern et al., 2009; Khan and Ongerth, 2005; Lishman et al., 2006; Ternes, 1998
	Tolfenamic acid	0.025	Ternes, 1998
	Tramadol	12.77-56.81 0.23-0.37	Kasprzyk-Hordern et al, 2009; Wick et al., 2009
Antibiotics **B**	Amoxicillin	0.007	Watkinson et al., 2007
	Azithromycin	0.04-0.38 0.06	Göbel et al., 2005, Yasojima et al., 2006
	Cefaclor	0.009	Watkinson et al., 2007
	Cefalexin	0.08 0.24-0.33 <loq	Costanzo et al., 2005; Gulkowska et al., 2008; Watkinson et al., 2007
	Cefotaxime	<loq-0.034	Gulkowska et al., 2008
	Chloramphenicol	<0.006-0.069 <loq	Kasprzyk-Hordern et al., 2009; Peng et al., 2006
	Chlortetracycline	<loq	Watkinson et al., 2007
	Ciprofloxacin	0.04-0.07 0.13	Andreozzi et al., 2003; Costanzo et al., 2005;

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Therapeutic class	Pharmaceutical compound	Secondary effluent	References
		0.079-0.1 0.06 0.007-0.032 0.03-0.05 2 2.37 0.64 0.094	Golet et al., 2003; Karthikeyan and Meyer, 2006; Lindberg et al., 2005, Lindberg et al., 2006; Muñoz et al., 2009; Rosal et al., 2010; Watkinson et al., 2007; Zorita et al., 2009
	Clarithromycin	0.15-0.46 0.21 0.35	Göbel et al., 2005, Ternes et al., 2003; Yasojima et al., 2006
	Clindamycin	0.005	Watkinson et al., 2007
	Cloxacillin	0.001	Watkinson et al., 2007
	Doxycycline	0.064 <loq-0.04	Lindberg et al., 2005; Watkinson et al., 2007
	Enoxacin	0.03	Andreozzi et al., 2003
	Enrofloxacin	0.01	Watkinson et al., 2007
	Erythromycin	0.05-0.14 0.52-0.6 0.27-0.3 0.023-2.77 0.0089-0.29 0.89 0.9* 0.2 0.33 0.03* 0.62 <loq 0.21	Göbel et al., 2005, Gulkowska et al., 2008; Karthikeyan and Meyer, 2006; Kasprzyk-Hordern et al., 2009; Kim et al., 2007; Muñoz et al., 2009; Reif et al., 2008; Roberts and Thomas, 2006; Rosal et al., 2010; Snyder et al., 2006; Ternes et al., 2003; Watkinson et al., 2007; Xu et al., 2007
	Lincomycin	0.05-0.06	Watkinson et al., 2007
	Lomefloxacin	0.22-0.32	Andreozzi et al., 2003
	Metronidazole	0.13-0.56 0.055	Kasprzyk-Hordern et al., 2009; Rosal et al., 2010
	Norfloxacin	0.06-0.07 0.12 0.21 0.06-0.07 0.08-0.1 0.007-0.021 0.046-0.07 0.025 0.027 0.019	Andreozzi et al., 2003; Coetsier et al., 2009; Costanzo et al., 2005; Golet et al., 2003; Gulkowska et al., 2008; Lindberg et al., 2005, 2006; Watkinson et al., 2007; Xu et al., 2007; Zorita et al., 2009
	Ofloxacin	0.31-0.58 0.11 0.045 0.04-0.86 0.81 0.048 0.019	Andreozzi et al., 2003; Brown et al., 2006; Lindberg et al., 2005; Peng et al., 2006; Rosal et al., 2010; Xu et al., 2007; Zorita et al., 2009
	Oxytetracycline	<loq-0.02	Watkinson et al., 2007
	Penicillin G	<loq 0.004	Gulkowska et al., 2008; Watkinson et al., 2007
	Penicillin V	0.02-0.03	Watkinson et al., 2007
	Roxithromycin	0.042*/0.045/0.057/0.036 0.01-0.03 5* 0.05 0.54	Clara et al., 2005b; Göbel et al., 2005, Reif et al., 2008; Ruel et al., 2010; Ternes et al., 2003,

Therapeutic class	Pharmaceutical compound	Secondary effluent	References
		0.1 0.035	Watkinson et al., 2007; Xu et al., 2007
	Sulfachloropyridazine	<0.03-0.14	Choi et al., 2008
	Sulfadiazine	0.07	Peng et al., 2006
	Sulfadimethoxine	<0.01-0.7	Choi et al., 2008
	Sulfamethoxazole	0.01-0.09 0.07 0.31 0.25 0.025-0.5 0.05-0.09/<loq* 0.025 0.13-0.84 0.05-0.21 0.004-0.044 0.003-0.4 0.13 0.18 <loq 5* 0.1-0.3 0.3 <0.01* 0.62 0.27	Andreozzi et al., 2003; Bendz et al., 2005; Brown et al., 2006; Carballa et al., 2004, Choi et al., 2008; Clara et al., 2005b; Foster, 2007; Göbel et al., 2005, Karthikeyan and Meyer, 2006; Kasprzyk-Hordern et al., 2009; Kim et al., 2007; Lindberg et al., 2005; Muñoz et al., 2009; Peng et al., 2006; Reif et al., 2008; Rosal et al., 2010; Ruel et al., 2010; Snyder et al., 2006; Ternes et al., 2003; Watkinson et al., 2007
	Sulfapyridine	0.02-0.23 0.46-1.11	Göbel et al., 2005, Kasprzyk-Hordern et al., 2009
	Sulfasalazine	0.0015 <loq-0.01	Kasprzyk-Hordern et al., 2009; Watkinson et al., 2007
	Sulfathiazole	<0.03 0.005	Choi et al., 2008; Watkinson et al., 2007
	Tetracycline	0.18-0.37 0.07-0.16 0.03	Gulkowska et al., 2008; Karthikeyan and Meyer, 2006; Watkinson et al., 2007
	Trimethoprim	0.04-0.13 0.25 0.04 0.18 0.025 0.08-0.4 0.12-0.14 0.55 0.38-1.2 <0.01-0.87 0.01-0.18 0.21-1.34 0.61-1.88 0.02-0.24 6.7* 0.4 0.099 <0.01 0.34 0.05	Andreozzi et al., 2003; Batt 2006; Bendz et al., 2005; Brown et al., 2006; Foster, 2007; Göbel et al., 2005, Gulkowska et al., 2008; Karthikeyan and Meyer, 2006; Kasprzyk-Hordern et al., 2009; Choi et al., 2008; Kim et al., 2007; Lindberg et al., 2005, Lindberg et al., 2006; Paxéus, 2004; Reif et al., 2008; Roberts and Thomas, 2006; Rosal et al., 2006; Snyder et al., 2006; Ternes et al., 2003; Watkinson et al., 2007
	Tylosin	<loq	Watkinson et al., 2007
Antifungals **D**	Clotrimazole	0.02	Roberts and Thomas, 2006
Antihypertensives **E**	Diltiazem	<0.005-0.013 0.025 0.1-1.15	Choi et al., 2008 Foster, 2007; Kasprzyk-Hordern et al., 2009
	Hydrochlorothiazide	1.8-11 0.67-1.7	Muñoz et al., 2009; Rosal et al., 2010

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Therapeutic class	Pharmaceutical compound	Secondary effluent	References
Barbiturates **F**	Phenobarbital	< loq	Yu et al., 2006
Beta-blockers **G**	Acebutolol	0.01-0.11	Andreozzi et al., 2003;
	Atenolol	1.33 0.16 1.3-3.16 0.4-0.6 0.14-73 0.01-0.73 0.51-2.4 0.36 0.37	Alder et al., 2010; Bendz et al., 2005; Kasprzyk-Hordern et al., 2009; Maurer et al., 2007; Muñoz et al., 2009; Paxéus, 2004; Rosal et al., 2010; Ternes et al., 2003; Wick et al., 2009
	Betaxolol	<loq 0.057-0.19 <loq	Andreozzi et al., 2003; Ternes, 1998; Wick et al., 2009
	Bisoprolol	0.37 0.21-0.27	Ternes, 1998; Wick et al., 2009
	Carazolol	<loq-0.12	Ternes, 1998
	Celiprolol	0.28 0.12-0.16	Ternes et al., 2003; Wick et al., 2009
	Metoprolol	0.16 0.01-0.1 0.034-0.057 0.103-0.161 <0.01-0.39 <loq-0.038 2.2 1.7 1.1	Alder et al., 2010; Andreozzi et al., 2003; Kasprzyk-Hordern et al., 2009; Maurer et al., 2007; Paxéus, 2004; Rosal et al., 2010; Ternes, 1998; Ternes et al., 2003; Wick et al., 2009
	Nadolol	0.025-0.06	Ternes, 1998
	Oxprenolol	0.01-0.03	Andreozzi et al., 2003
	Propranolol	0.03 0.01-0.09 0.03 0.56 0.13-0.523 0.032-0.123 0.39 <loq-0.057 0.17-0.29 0.18 0.058	Alder et al., 2010; Andreozzi et al., 2003; Bendz et al., 2005; Coetsier et al., 2009; Kasprzyk-Hordern et al., 2009; Maurer et al., 2007; Roberts and Thomas, 2006; Rosal et al., 2010; Ternes, 1998; Ternes et al., 2003; Wick et al., 2009
	Sotalol	0.21 0.249-0.251 1.32 1.2	Alder et al., 2010; Maurer et al., 2007; Ternes et al., 2003; Wick et al., 2009
	Timolol	<loq-0.07	Ternes, 1998
Diuretics **H**	Bendroflumethiazide	<0.008	Kasprzyk-Hordern et al., 2009
	Furosemide	<0043-1.823 <loq-0.666	Kasprzyk-Hordern et al., 2009; Rosal et al., 2010
Lipid regulators **I**	Bezafibrate	<loq-0.91 0.692-4.8/1.55* <loq-4.8/0.073* 0.094-0.393 <loq-0.83 0.01* 0.033-0.28 0.59 2.2-4.6 0.14	Andreozzi et al., 2003; Clara et al., 2005a, Clara et al., 2005b; Kasprzyk-Hordern et al., 2009; Lindqvist et al., 2005; Quintana et al., 2005; Rosal et al., 2010; Stumpf et al., 1999; Ternes, 1998; Vieno et al., 2005
	Clofibrate	<loq-0.8	Andreozzi et al., 2003;

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Therapeutic class	Pharmaceutical compound	Secondary effluent	References
		<loq	Ternes, 1998
	Clofibric acid	<loq-0.68 <loq <0.001-0.048 0.028/0.004*-0.02* 0.014/0.005*-0.014* - 0.078 <loq-0.091 0.66 0.15-0.27 0.36-1.6 0.12 0.11 0.024	Andreozzi et al., 2003; Bendz et al., 2005; Kasprzyk-Hordern et al., 2009; Kimura et al., 2005, 2007; Lishman et al., 2006; Roberts and Thomas, 2006; Rosal et al., 2010; Stumpf et al., 1999; Tauxe-Wuersch et al., 2005; Ternes, 1998; Ternes et al., 2003; Weigel et al., 2004; Zorita et al., 2009
	Etofibrate	0.05	Ternes, 1998
	Fenofibrate	0.16 <loq <loq-0.03	Andreozzi et al., 2003; Lishman et al., 2006; Ternes, 1998
	Fenofibric acid	4.7-80 <loq-0.129 0.231 0.38-1.2 0.13	Muñoz et al., 2009; Rosal et al., 2010; Stumpf et al., 1999; Ternes, 1998; Ternes et al., 2003
	Gemfibrozil	0.71-4.76 0.18 0.2 0.004-0.017 0.246-0.436 0.003-5.2 0.06-0.84 0.003-5.233 <0.01* 0.162 0.4-1.5	Andreozzi et al., 2003; Bendz et al., 2005; Khan and Ongerth, 2005; Kim et al., 2007; Lishman et al., 2006; Muñoz et al., 2009; Paxéus, 2004; Rosal et al., 2010; Snyder et al., 2006; Stumpf et al., 1999; Ternes, 1998
	Pravastatin	<0.007 <0.06	Coetsier et al., 2009; Kasprzyk-Hordern et al., 2009
	Simvastatin	<0.003	Kasprzyk-Hordern et al., 2009
Psychiatric drugs	Amitriptyline	<0.002-0.335	Kasprzyk-Hordern et al., 2009

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Therapeutic class	Pharmaceutical compound	Secondary effluent	References
J	Carbamazepine	0.3-1 1.18 0.794*/0.465-0.952 1.147*/0.465-1.337 1.519 <0.05-0.15 0.11-0.23 0.15-2.32 0.5 <0.005-0.195 0.073-0.729 0.14-0.26 0.011-0.16 0.1-1.2 17.8* 0.069-0.173 <loq-1.29 <loq-1.29 <0.01* 19.8 2.1-6.3 2.1 0.74-0.92	Andreozzi et al., 2003; Bendz et al., 2005; Clara et al., 2005a, Clara et al., 2005b; Coetsier et al 2009; Foster, 2007; Gómez et al., 2007; Kasprzyk-Hordern et al., 2009; Khan and Ongerth, 2005; Choi et al., 2008; Kim et al., 2007; Muñoz et al., 2009; Nakada et al., 2006; Paxéus, 2004; Reif et al., 2008; Rosal et al., 2010; Santos et al., 2007, Santos et al., 2009; Snyder et al., 2006; Suárez et al., 2005; Ternes, 1998; Ternes et al., 2003; Wick et al, 2009
	Diazepam	<loq*/<loq 17* 19.3 <loq-0.04 -	Clara et al., 2005b; Reif et al., 2008; Suárez et al., 2005; Ternes, 1998; Wick et al., 2009
	Fluoxetine	<0.05-0.025 0.0017 0.127-0.154 0.016-2 0.034-0.929 <0.01* <loq	Foster, 2007; Kim et al., 2007; Metcalfe et al. 2010; Muñoz et al., 2009; Rosal et al., 2010; Snyder et al., 2006; Zorita et al., 2009
	Gabapentin	1.786-3.514 <loq	Kasprzyk-Hordern et al., 2009; Yu et al., 2006
	Lorazepam	0.196	Coetsier et al., 2009
	Norfluoxetine	<loq 0.006	Metcalfe et al. 2010; Zorita et al., 2009
	Paroxetine	0.007	Metcalfe et al. 2010;
	Valproic acid	<loq	Yu et al., 2006
Receptor antagonists **K**	Cimetidine	0.02-7.763 0.253-0.781	Choi et al., 2008 Kasprzyk-Hordern et al., 2009
	Omeprazole	<loq-0.922	Rosal et al., 2010
	Ranitidine	0.015-0.783 <loq-0.942	Kasprzyk-Hordern et al., 2009; Rosal et al., 2010
	Valsartan	0.006-0.711	Kasprzyk-Hordern et al., 2009
Hormones **L**	Estradiol	<0.001 0.0007-0.002 <loq <loq-0.03/<loq* <0.02-0.054 0.0002 <0.001 <loq 0.0025	Andersen et al., 2003; Baronti et al., 2000; Carballa et al., 2004, Clara et al., 2005a; Foster, 2007; Joss et al., 2004; Kim et al., 2007; Lishman et al., 2006; Zorita et al., 2009
	Estriol	0.00072-0.0036 <loq-0.275/<loq* 0.0089-0.025 0.0003-0.0008	Baronti et al., 2000; Clara et al., 2005a; Kim et al, 2007; Nakada et al., 2006

Therapeutic class	Pharmaceutical compound	Secondary effluent	References
	Estrone	0.0005 0.005-0.044 <loq-0.0044 <loq-0.072/0.002* 0.002/0.002* 0.002-0.036 0.0076-0.038 0.0028-0.11 0.07	Andersen et al., 2003; Baronti et al., 2000; Carballa et al., 2004, Clara et al., 2005a; Joss et al., 2004; Kim et al., 2007; Lishman et al., 2006; Nakada et al., 2006; Zorita et al., 2009
	Ethinylestradiol	0.0004-0.0008 <loq-0.005/0.004* <0.02-0.01 0.0002/0.0002* 0.0013 <loq	Baronti et al., 2000; Clara et al., 2005a; Foster, 2007; Joss et al., 2004; Kim et al., 2007; Zorita et al., 2009
Beta-agonists **M**	Clenbuterol	<loq-0.08	Ternes, 1998
	Fenoterol	<loq-0.06	Ternes, 1998
	Salbutamol	<0.001-0.022 <loq-0.17	Kasprzyk-Hordern et al., 2009; Ternes, 1998
	Terbutaline	<loq-0.12	Ternes, 1998
Antineoplastics **N**	Cyclophosphamide	<loq-0.02	Ternes, 1998
	Ifosfamide	<0.0038 <loq-2.9	Coetsier et al., 2009; Ternes, 1998
	Tamoxifen	0.083 0.6	Coetsier et al., 2009; Roberts and Thomas, 2006
Topical product **O**	Crotamitron	0.245-0.968	Nakada et al., 2006
Antiseptic **P**	Triclosan	0.015-0.039 0.08-0.4 0.0013-0.032 0.41 0.052-2.5 0.0266-0.33 0.09-0.58 <loq-0.512 <loq <0.01* 0.054-0.082 0.18 0.25	Foster, 2007; Gómez et al., 2007; Kim et al., 2007; McAvoy et al., 2002; Muñoz et al., 2009; Nakada et al., 2006;, Paxéus, 2004; Rosal et al., 2010; Ruel et al., 2010; Snyder et al., 2006; Thomas and Foster, 2005; Weigel et al., 2004; Yu et al., 2006
Contrast agent **Q**	Iopromide	0.1 9.3 <loq-5.06/<loq* 1.17-4.03	Batt et al., 2006; Carballa et al., 2004; Clara et al., 2005b; Kim et al., 2007

Table A.4. Ranges of removal efficiency for the selected pharmaceuticals with their corresponding references. Data with an asterix as apex (*) refer to MBRs; data in italics and underlined are obtained by applying eq. 2.1 of the manuscript to the provided data in the reported reference.

Therapeutic class	Pharmaceutical compound	**Removal Efficiencies for CAS and MBR**	References
Analgesics/ Anti-inflammatories **A**	5-aminosalicylic acid	*94*	*Kasprzyk-Hordern et al., 2009*
	Acetaminophen	*80-93* *99.94* *99.8* 91.93 *100*	*Choi et al., 2008;* *Foster, 2007;* *Gómez et al., 2007;* Jones et al., 2007; *Khan and Ongerth, 2005;*

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Therapeutic class	Pharmaceutical compound	**Removal Efficiencies for CAS and MBR**	References
		86.4/99.6* 99.9/99.8*-99.9* *100* 100 *100** 99.5	Radjenovic et al., 2007, Radjenovic et al., 2009; *Roberts and Thomas, 2006;* Rosal et al., 2010; *Snyder et al., 2006;* Yu et al., 2006
	Acetylsalicylic acid	*99.2* 81	*Kasprzyk-Hordern et al., 2009;* Ternes, 1998
	Aminopyrine	38	Ternes, 1998
	Codeine	*99.86* *29* *60.9* 69.3 *81.66*	*Foster, 2007;* *Gómez et al., 2007;* *Kasprzyk-Hordern et al.,2009;* Rosal et al., 2010; *Wick et al., 2009*
	Diclofenac	22 24/58* 60/60* *7.14-47.34/32.92** *7.1-62.7* 50.6* *40* *31.2* *42/51*-82** 9-46 9-60 *0* 5-80 23* 50.1/87.4* 21.8/62.6*-65.8* *-7** *65.1* 5 *50* *50* *90** 75 3 *-11-3* 69 *13* *-36.6* 18 *-111*	Bendz et al., 2005; Bernhard et al., 2006; Clara et al., 2004, *Clara et al 2005a,* *Clara et al 2005b;* Coetsier et al., 2009; *Gómez et al., 2007;* *Kasprzyk-Hordern et al., 2009;* *Kimura et al 2007;* Kreuzinger et al., 2004; Lindqvist et al., 2005; *Lishman et al., 2006;* Paxéus, 2004; Quintana et al., 2005; Radjenovic et al., 2007, Radjenovic et al., 2009; *Reif et al., 2008;* *Roberts and Thomas, 2006;* Rosal et al., 2010; *Santos et al., 2007,* *Santos et al., 2009;* *Snyder et al., 2006;* Stumpf et al., 1999; Suárez et al., 2005; *Tauxe-Wuersch et al., 2005;* Ternes, 1998; *Vieno et al., 2005;* *Weigel et al., 2004;* Yu et al., 2006; *Zorita et al., 2009*
	Dipyrone	*65*	*Gómez et al., 2007*
	Fenoprofen	65.6-97.5	Nakada et al., 2006
	Hydrocodone	*95.76**	*Snyder et al., 2006*
	Ibuprofen	96 97/99* *64* 70 55 97/97* *-4.35-99.18/97.18** *-4.3-98/99.2** *92* 86 *93.8* *92* *98/95*-98**	Bendz et al., 2005; Bernhard et al., 2006; *Carballa et al., 2004,* *Carballa et al.*, 2005; Castiglioni et al., 2006; Clara et al., 2004, *Clara et al., 2005a,* *Clara et al., 2005b;* *Gómez et al., 2007;* Jones et al., 2007; *Kasprzyk-Hordern et al., 2009;* *Khan and Ongerth, 2005;* Kimura et al., *2007;*

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Therapeutic class	Pharmaceutical compound	**Removal Efficiencies for CAS and MBR**	References
		92-99/97*-99* 78/100 *96* 84.3/99.7/ 52/99 97* 82.5/99.8* 99.1/99.2*-99.5* *98** *-12.8* *65* 95 *88.4-89.5* *84-87* *100** 75 82 *26-79* 90 *99.8* *98.2* 87 *99*	Kreuzinger et al., 2004; Lindqvist et al., 2005; *Lishman et al., 2006;* Nakada et al., 2006; Paxéus, 2004; Quintana et al., 2005; Radjenovic et al., 2007, Radjenovic et al., 2009; *Reif et al., 2008;* *Roberts and Thomas, 2006;* *Rodriguez et al., 2003;* Rosal et al., 2010; *Santos et al., 2007,* *Santos et al., 2009;* *Snyder et al., 2006;* Stumpf et al., 1999; Suárez et al., 2005; *Tauxe-Wuersch et al., 2005;* Ternes, 1998; *Vieno et al., 2005;* *Weigel et al., 2004;* Yu et al., 2006; *Zorita et al., 2009*
	Indomethacin	*24* 23.4/46.6* 5/39.7*-41.4* 11.1 83 75	*Lishman et al., 2006,* Radjenovic et al., 2007, Radjenovic et al., 2009; Rosal et al., 2010; Stumpf et al., 1999; Ternes, 1998
	Ketoprofen	65 *85.4* *34* *55/83*-99** 51/100 *16* 14-68.4 62* 51.5/91.9* 54.6/43.9*-44* 11.2 *30-37* *52-56* 63 *7-51* *92.1* 77	Bendz et al., 2005; *Kasprzyk-Hordern et al., 2009;* *Khan and Ongerth, 2005;* *Kimura et al., 2007;* Lindqvist et al., 2005; *Lishman et al., 2006;* Nakada et al., 2006; Quintana et al., 2005; Radjenovic et al., 2007, Radjenovic et al., 2009; Rosal et al., 2010; *Santos et al., 2007,* *Santos et al., 2009;* Stumpf et al., 1999; *Tauxe-Wuersch et al., 2005;* *Vieno et al., 2005;* Yu et al., 2006
	Ketorolac	43.9	Rosal et al., 2010
	Mefenamic acid	91.54 *72/77*-93** 29.4/74.8* 5/35.5*-40.5* 1.8 *2-53*	Jones et al., 2007; Kimura et al., *2007;* Radjenovic et al., 2007, 2009; Rosal et al., 2010; *Tauxe-Wuersch et al., 2005*
	Naproxen	93 *47* 47 *85.5* *95* *64/96*-98** 55-98 *93*	Bendz et al., 2005; *Carballa et al., 2004,* *Carballa et al.,* 2005; *Kasprzyk-Hordern et al., 2009;* *Khan and Ongerth, 2005;* *Kimura et al., 2007;* Lindqvist et al., 2005; *Lishman et al., 2006;*

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Therapeutic class	Pharmaceutical compound	**Removal Efficiencies for CAS and MBR**	References
		-1.89-82.9 42-93 71* 85.1/99.3* 71.8 *84** *44.62* 60.9 *35.1-89.4* 43-71/90.7*-91.6* *100** 78 68 66 *95.1* 88 *93*	Nakada et al., 2006; Paxéus, 2004; Quintana et al., 2005; Radjenovic et al., 2007, Radjenovic et al., 2009; *Reif et al., 2008;* *Rodriquez et al., 2003;* Rosal et al., 2010; *Santos et al., 2007,* *Santos et al.*, 2009; *Snyder et al., 2006;* Stumpf et al., 1999; Suárez et al., 2005; Ternes, 1998; *Vieno et al., 2005;* Yu et al., 2006; *Zorita et al., 2009*
	Phenazone	87.2 33	Rosal et al., 2010; Ternes, 1998
	Propyphenazone	14-86.4 42.7/64.6* 37.6/60.7*-64.5*	Nakada et al., 2006; Radjenovic et al., 2007, Radjenovic et al., 2009
	Salicylic acid	*99.4* *97* *99*	*Kasprzyk-Hordern et al., 2009;* *Khan and Ongerth, 2005;* *Lishman et al., 2006;*
	Tramadol	*42* *4.16*	*Kasprzyk-Hordern et al, 2009;* *Wick et al., 2009*
Antibiotics **B**	Amoxicillin	*96*	*Watkinson et al., 2007*
	Azithromycin	45/39 *18* 5*-24* *74.3*	Ghosh et al., 2009; *Göbel et al., 2005,* Göbel et al., 2007; *Yasojima et al., 2006*
	Cefaclor	*98*	*Watkinson et al., 2007*
	Cefalexin	*96* *64.2-88.6* 53-91 *100*	*Costanzo et al., 2005;* *Gulkowska et al., 2008;* Li and Zhang, 2011 *Watkinson et al., 2007*
	Cefotaxime	*83.3* 43	*Gulkowska et al., 2008* Li and Zhang, 2011
	Chloramphenicol	*92* Not evaluated *96-97*	*Kasprzyk-Hordern et al., 2009;* Li and Zhang, 2011 *Peng et al., 2006*
	Chlortetracycline	82-85	Li and Zhang, 2011
	Ciprofloxacin	73* 63 *-44* 50-73 *78* *71.43* 18/55 *72-96* *79* 57 86 *83* *71*	Baumgarten et al., 2007 Castiglioni et al., 2006; *Costanzo et al., 2005;* Ghosh et al., 2009; *Golet et al., 2003;* *Karthikeyan and Meyer, 2006;* Li and Zhang, 2011; *Lindberg et al., 2005,* *Lindberg et al., 2006;* Rosal et al., 2010; Vieno et al., 2007; *Watkinson et al., 2007;* *Zorita et al., 2009*
	Clarithromycin	0 50-83 *32* 4.5/41*-88* 62/92*	Castiglioni et al., 2006; Ghosh et al., 2009; *Göbel et al., 2005,* Göbel et al., 2007; Sahar et al., 2011;

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Therapeutic class	Pharmaceutical compound	**Removal Efficiencies for CAS and MBR**	References
		45.9	*Yasojima et al., 2006*
	Clindamycin	*-150*	*Watkinson et al., 2007*
	Doxycycline	*14-100*	*Lindberg et al., 2005*
	Enrofloxacin	56* 38-70	Baumgarten et al., 2007; Ghosh et al., 2009
	Erythromycin	0 *-14* 26*-87* *-11-18.9* *43.75-75* *72* 15-26 23.8/67.3* 35.4/25.2*-43* *91** *-84* 4.3 19/79* *97** *4.42*	Castiglioni et al., 2006; *Göbel et al., 2005*, Göbel et al., 2007; *Gulkowska et al., 2008;* *Karthikeyan and Meyer, 2006;* *Kasprzyk-Hordern et al., 2009;* Li and Zhang, 2011 Radjenovic et al., 2007, Radjenovic et al., 2009; *Reif et al., 2008;* *Roberts and Thomas, 2006;* Rosal et al., 2010; Sahar et al., 2011; *Snyder et al., 2006;* *Xu et al., 2007*
	Lincomycin	0 33/57	Castiglioni et al., 2006; Ghosh et al., 2009;
	Metronidazole	*38* 38.7	*Kasprzyk-Hordern et al., 2009;* Rosal et al., 2010
	Norfloxacin	75-90 *84* *22.7-78.3* 30/45 *72-96* 79 *85* *18.18* *-6*	Ghosh et al., 2009; *Golet et al., 2003;* *Gulkowska et al., 2008;* Li and Zhang, 2011 *Lindberg et al., 2005*, Lindberg et al., 2006; *Watkinson et al., 2007;* *Xu et al., 2007;* *Zorita et al., 2009*
	Ofloxacin	*77* 57 26-59 *84* *85-99* 23.8/94* 75.8/91.3*-95.2* 64 83 *37.66* *13*	*Brown et al., 2006;* Castiglioni et al., 2006; Li and Zhang, 2011 *Lindberg et al., 2005;* *Peng et al., 2006;* Radjenovic et al., 2007, Radjenovic et al., 2009; Rosal et al., 2010; Vieno et al., 2007; *Xu et al., 2007;* *Zorita et al., 2009*
	Oxytetracycline	44	Li and Zhang, 2011
	Penicillin V	*60*	*Watkinson et al., 2007*
	Roxithromycin	*-80-43.8/34.4** -32-39 *0* 19/39*-62* -4-61/75* 40-46 *71** *37.5* 22/59* *12.5*	*Clara et al., 2005b;* Ghosh et al., 2009; *Göbel et al., 2005,* *Göbel et al.*, 2007; Kreuzinger et al., 2004; Li and Zhang, 2011 *Reif et al., 2008;* *Ruel et al., 2010;* Sahar et al., 2011; *Xu et al., 2007*
	Spiramycin	0	Castiglioni et al., 2006
	Sulfachloropyridazine	*26-82*	*Choi et al., 2008*

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Therapeutic class	Pharmaceutical compound	**Removal Efficiencies for CAS and MBR**	References
	Sulfadiazine	78-98 87-100 *99*	García-Galán et al., 2011 Li and Zhang, 2011 *Peng et al., 2006*
	Sulfadimethoxine	*66-90* 100	*Choi et al., 2008* García-Galán et al., 2011
	Sulfamethazine	16-100 100	García-Galán et al., 2011 Li and Zhang, 2011
	Sulfamethoxazole	*21* *57* 46 24 *41-80* *32* *75* 54-71 26-39 *35* 4.5/37*-38* *-24-96* *83* 62/57* 62-90 *42-100* *99* 55.6/60.5* 73.8/78.3*-80.8* *50** 17.3 *41.5* 10/0* *100** *25* *-20*	*Brown et al., 2006;* *Carballa et al., 2004,* Carballa et al., 2005; Castiglioni et al., 2006; *Choi et al., 2008;* *Clara et al., 2005b;* *Foster, 2007;* García-Galán et al., 2011 Ghosh et al., 2009; *Göbel et al., 2005,* Göbel et al., 2007; *Karthikeyan and Meyer, 2006;* *Kasprzyk-Hordern et al., 2009;* Kreuzinger et al., 2004; Li and Zhang, 2011 *Lindberg et al., 2005;* *Peng et al., 2006;* Radjenovic et al., 2007, Radjenovic et al., 2009; *Reif et al., 2008;* Rosal et al., 2010; *Ruel et al., 2010;* Sahar et al., 2011; *Snyder et al., 2006;* *Watkinson et al., 2007;* *Xu et al., 2007*
	Sulfapyridine	6-89 *22* 50*-60* *91*	García-Galán et al., 2011 *Göbel et al., 2005,* Göbel et al., 2007; *Kasprzyk-Hordern et al., 2009*
	Sulfasalazine	*-50*	*Kasprzyk-Hordern et al., 2009*
	Sulfathiazole	*94-95* 65-100 *75*	*Choi et al., 2008;* García-Galán et al., 2011 *Watkinson et al., 2007*
	Tetracycline	40-72 *-87-71.5* *70.8-79.7* 24-36	Ghosh et al., 2009; *Gulkowska et al., 2008;* *Karthikeyan and Meyer, 2006* Li and Zhang, 2011
	Trimethoprim	*52.8* 49 *69* *75* -88-35 *31* 7/30*-87* *-17-62.5* *50* *70* *-11-79* 13-42 *-106-41* 14 30-40 40.4/47.5*-66.7*	*Batt 2006;* Bendz et al., 2005; *Brown et al., 2006;* *Foster, 2007;* Ghosh et al., 2009; *Göbel et al., 2005,* Göbel et al., 2007; *Gulkowska et al., 2008;* *Karthikeyan and Meyer, 2006;* *Kasprzyk-Hordern et al., 2009;* *Choi et al., 2008;* Li and Zhang, 2011 *Lindberg et al., 2005,* Lindberg et al., 2006; Paxéus, 2004; Radjenovic et al., 2009;

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Therapeutic class	Pharmaceutical compound	**Removal Efficiencies for CAS and MBR**	References
		36 *-56* 5.1 0/88* *99** *85*	*Reif et al., 2008;* *Roberts and Thomas, 2006;* Rosal et al., 2010; Sahar et al., 2011; *Snyder et al., 2006;* *Watkinson et al., 2007*
Antidiabetics **C**	Glibenclamide	44.5/47.3* 46.1/82.2*-95.6*	Radjenovic et al., 2007, Radjenovic et al., 2009
Antifungals **D**	Clotrimazole	*31*	*Roberts and Thomas, 2006*
Antihypertensives **E**	Diltiazem	*41-77* *97* *77*	*Choi et al., 2008* *Foster, 2007;* *Kasprzyk-Hordern et al., 2009*
	Enalapril	69	Castiglioni et al., 2006
	Hydrochlorothiazide	44 76.3/66.3* 5* 53.2	Castiglioni et al., 2006; Radjenovic et al., 2007, Radjenovic et al., 2009 Rosal et al., 2010
Barbiturates **F**	Phenobarbital	99.5	Yu et al., 2006
Beta-blockers **G**	Acebutolol	60	Vieno et al., 2007
	Atenolol	41 70.9 21 *85* *73.35-76.11* 65.5* 61.2/69.5*-76.7* 14 63 *44.44*	Alder et al., 2010; Carucci et al., 2006; Castiglioni et al., 2006; *Kasprzyk-Hordern et al., 2009;* *Maurer et al., 2007;* Radjenovic et al., 2007, Radjenovic et al., 2009; Rosal et al., 2010; Vieno et al., 2007; *Wick et al., 2009*
	Bisoprolol	*0*	*Wick et al., 2009*
	Metoprolol	31 *56* *29.45-31.48* 10 58.7* 24.7/29.5*-44.2* 7 83 34 *20.98*	Alder et al., 2010; *Kasprzyk-Hordern et al., 2009;* *Maurer et al., 2007;* Paxéus, 2004; Radjenovic et al., 2007, Radjenovic et al., 2009; Rosal et al., 2010; Ternes, 1998; Vieno et al., 2007; *Wick et al., 2009*
	Propranolol	*33* *59* *28.48-34.69* 58.8/65.5*-77.6* 1 96 *0*	Alder et al., 2010; *Kasprzyk-Hordern et al., 2009;* *Maurer et al., 2007;* Radjenovic et al., 2009; Rosal et al., 2010; Ternes, 1998; *Wick et al., 2009*
	Sotalol	*27* *26.3-26.6* 21.4/30.4*-53.1* 54 *18.3*	Alder et al., 2010 *Maurer et al., 2007;* Radjenovic et al., 2009; Vieno et al., 2007; *Wick et al., 2009*
Diuretics **H**	Bendroflumethiazide	*91*	*Kasprzyk-Hordern et al., 2009*
	Furosemide	15 *77* 59.8	Castiglioni et al., 2006; *Kasprzyk-Hordern et al., 2009;* Rosal et al., 2010
Lipid regulators **I**	Bezafibrate	30 97/97* *36.6-89.8/77.34** *36.8-99.98/96**	Castiglioni et al., 2006; Clara et al., 2004, *Clara et al., 2005a,* *Clara et al., 2005b;*

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Therapeutic class	Pharmaceutical compound	**Removal Efficiencies for CAS and MBR**	References
		71 36-99/76*-94* -11/100 91* 48.4/95.8* 80.8/88.2*-90.3* 9.1 50 83 *93.6*	*Kasprzyk-Hordern et al., 2009;* Kreuzinger et al., 2004; Lindqvist et al., 2005; Quintana et al., 2005; Radjenovic et al., 2007, Radjenovic et al.,2009; Rosal et al., 2010; Stumpf et al., 1999; Ternes, 1998; *Vieno et al., 2005*
	Clofibric acid	26/54* *50/50*-82** 27.7/71.8* *84.2* 54.2 34/51 *8-10* *35.3* *55*	Bernhard et al., 2006; *Kimura et al., 2007;* Radjenovic et al., 2007; *Roberts and Thomas, 2006;* Rosal et al., 2010; Stumpf et al., 1999; *Tauxe-Wuersch et al., 2005;* *Weigel et al., 2004;* *Zorita et al., 2009*
	Fenofibrate	64	Ternes, 1998
	Fenofibric acid	1.3 45	Rosal et al., 2010; Stumpf et al., 1999
	Gemfibrozil	75 *87* *39* 43-75 38.8/89.6* 32.5*-42.2* 76 *100** 46 69 68	Bendz et al., 2005; *Khan and Ongerth, 2005;* *Lishman et al., 2006;* Paxéus, 2004; Radjenovic et al., 2007, Radjenovic et al., 2009; Rosal et al., 2010; *Snyder et al., 2006;* Stumpf et al., 1999; Ternes, 1998; Yu et al., 2006
	Pravastatin	61.8/90.8* 59.4/83.1*-86.1*	Radjenovic et al., 2007, Radjenovic et al., 2009
	Simvastatin	*57*	*Kasprzyk-Hordern et al., 2009*
Psychiatric drugs	Amitriptyline	*96*	*Kasprzyk-Hordern et al., 2009*

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Therapeutic class	Pharmaceutical compound	**Removal Efficiencies for CAS and MBR**	References
J	Carbamazepine	30 7/13* 0 0/0* *-47-(-3)/-13** *-43-(-3)/4.4** *75* *13* *13* *0* *30-64* 14/35/11* -122-77.6 10-53 5 5 *9** 9.5 *-67-(-4)* 7-11 *97** 7.9 7 -44 *-12*	Bendz et al., 2005; Bernhard et al., 2006; Castiglioni et al., 2006; Clara et al., 2004, Clara et al., *2005a*, Clara et al., *2005b;* *Foster, 2007;* *Gómez et al., 2007;* *Kasprzyk-Hordern et al., 2009;* *Khan and Ongerth, 2005;* *Choi et al., 2008;* Kreuzinger et al., 2004; Nakada et al., 2006; Paxéus, 2004; Radjenovic et al., 2007, Radjenovic et al., 2009; *Reif et al., 2008;* Rosal et al., 2010; *Santos et al., 2007,* Santos et al., 2009; *Snyder et al., 2006;* Suárez et al., 2005; Ternes, 1998; Vieno et al., 2007; *Wick et al, 2009*
	Diazepam	20 *26** 8.1	Kreuzinger et al., 2004; *Reif et al., 2008;* Suárez et al., 2005
	Fluoxetine	*75* 33.1/98* 61.9 *90** *54.5*	*Foster, 2007;* Radjenovic et al., 2009; Rosal et al., 2010; *Snyder et al., 2006;* *Zorita et al., 2009*
	Gabapentin	*86* 99.5	*Kasprzyk-Hordern et al., 2009;* Yu et al., 2006
	Norfluoxetine	*47.8*	*Zorita et al., 2009*
	Paroxetine	90.6/89.7*	Radjenovic et al., 2007
	Valproic acid	99.5	Yu et al., 2006
Receptor antagonists **K**	Cimetidine	*27-60* *79*	*Choi et al., 2008* *Kasprzyk-Hordern et al., 2009*
	Famotidine	60.1/47.4*-64.6*	Radjenovic et al., 2009
	Loratadine	15/<10*-33.5*	Radjenovic et al., 2009
	Omeprazole	8.5	Rosal et al., 2010
	Ranitidine	28.5 96 *92* 42.2/95* 24.7/29.5*-44.2* 31.2	Carucci et al., 2006; Castiglioni et al., 2006; *Kasprzyk-Hordern et al., 2009;* Radjenovic et al., 2007, Radjenovic et al., 2009; Rosal et al., 2010
	Valsartan	*84.1*	*Kasprzyk-Hordern et al., 2009*
Hormones **L**	Estradiol	*75-92.22* 46 *98* 98/99* *75* 99.9 *21.8*	*Baronti et al., 2000;* *Carballa et al.*, 2005; *Foster, 2007;* Joss et al., 2004; *Lishman et al., 2006;* Ternes et al., 1999a; *Zorita et al., 2009*
	Estriol	*84.55-99.19* 99.7-99.8	*Baronti et al., 2000;* Nakada et al., 2006

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Therapeutic class	Pharmaceutical compound	**Removal Efficiencies for CAS and MBR**	References
	Estrone	*99.24* *12-92.53* *-83* -40 *112-99.93/97.18** 96/96* *57* 83.9-90.3 83	*Andersen et al., 2003;* *Baronti et al., 2000;* *Carballa et al., 2004*, *Carballa et al.*, 2005; *Clara et al., 2005a;* Joss et al., 2004; *Lishman et al., 2006;* Nakada et al., 2006; Ternes et al., 1999
	Ethinylestradiol	*60-86.66* 70/70* *75* 94/76* 70-81/25*-66* 78	*Baronti et al., 2000;* Clara et al., 2004, *Foster, 2007;* Joss et al., 2004; Kreuzinger et al., 2004; Ternes et al., 1999
Beta-agonists **M**	Salbutamol	0 94.6 *89*	Castiglioni et al., 2006; Jones et al., 2007; *Kasprzyk-Hordern et al., 2009*
Topical product **O**	Crotamitron	-32.5-64.5	Nakada et al., 2006
Antiseptics **P**	Triclosan	*98.8* *88.89* *94.14* 46.2-92.3 55-94 74.5 *99.61** *52.6* 69	Foster, 2007; Gómez et al., 2007; McAvoy et al., 2002; Nakada et al., 2006;, Paxéus, 2004; Rosal et al., 2010; *Snyder et al., 2006;* *Weigel et al., 2004;* Yu et al., 2006
Contrast agent **Q**	Iopromide	*50* *-41* *-32* 50	*Batt et al., 2006;* *Carballa et al., 2004;* *Clara et al., 2005b*; Kreuzinger et al., 2004

Appendix B

Details of the optimized QqLIT-MS parameters (two SRMs, collision energies) for each investigated compound in negative and positive ionization modes.

Table B.1. Investigated compounds and their optimized QqLIT-MS/MS parameters in SRM-negative and -positive ionization modes.

Compounds	Precursor ion (m/z)	SRM 1	Collision Energy 1	SRM 2	Collision Energy 2	R_t (min)	IS used for quantification
Compounds Analysed in Negative Mode							
Acetaminophen	150	107	22			3.6	mecoprop-d3
Bezafibrate	360	274	26	154	38	16.7	ibuprofen-d3
Butalbital	223	180	16	85	18	16.6	ibuprofen-d3
Chloramphenicol	323	152	22	194	18	15.1	ibuprofen-d3
Clofibric acid	213	127	26	85	14	12.9	mecoprop-d3
Diclofenac	294	250	16	214	30	19.9	ibuprofen-d3
Furosemide	329	205	22	285	32	13.3	ibuprofen-d3
Gemfibrozil	249	121	20	127	14	24.3	ibuprofen-d3
Hydrochlorothiazide	296	78	28			6.1	mecoprop-d3
Ibuprofen	205	161	10			19.2	ibuprofen-d3
Ibuprofen-d3 (IS)	208	164	10			19.1	
Indomethacin	356	312	12	297	24	20.6	ibuprofen-d3
Ketoprofen	253	209	12	197	6	14.9	mecoprop-d3
Mecoprop-d3 (IS)	218	146	24			14.8	
Mefenamic acid	240	196	20	180	38	21.1	ibuprofen-d3
Naproxen	229	185	10	169	38	14.3	mecoprop-d3
Pentobarbital	225	182	18	85	18	18.6	ibuprofen-d3
Phenobarbital	231	188	14			14.2	phenobarbital-d5
Phenobarbital-d5 (IS)	236	193	16			14.2	
Salicylic acid	137	93	20	66	38	4.1	mecoprop-d3
Compounds Analysed in Positive Mode							
13C-Phenacetin (IS)	181	139	23			12.7	
Atenolol	267	145	35	190	35	6.2	atenolol-d7
Atenolol-d7 (IS)	274	145	37			6.2	
Atorvastatin	559	440	27	250	63	19.8	carbamazepine-d10
Azithromycin	749	591	43	573	50	10.9	carbamazepine-d10
Betaxolol	308	116	40	121	40	12.9	atenolol-d7
Carazolol	299	116	35	222	35	11.8	atenolol-d7
Carbamazepine	237	194	29			14.7	carbamazepine-d10
Carbamazepine-d10 (IS)	247	204	31			14.5	
Chlortetracycline	479	462	29	444	29	11.4	13C-Phenacetin
Cimetidine	253	95	30	159	23	6.3	atenolol-d7
Ciprofloxacin	332	288	25	231	51	9.4	flumequine
Clarithromycin	748	591	35	158	40	14.6	carbamazepine-d10
Clenbuterol	277	203	23	132	33	10.3	atenolol-d7
Codeine	300	152	85	115	105	7.4	
Danofloxacin	358	340	35	314	27	9.7	flumequine
Diazepam	285	193	45	154	50	17.6	diazepam-d5
Diazepam-d5 (IS)	290	198	43			17.6	
Doxycycline	445	410	29	154	41	9.7	13C-Phenacetin
Enalapril	377	234	29	303	35	12.5	diazepam-d5
Enoxacin	321	303	30	234	33	8.9	flumequine
Enrofloxacin	360	316	29	245	39	9.9	flumequine
Erythromycin	734	158	41	576	35	13.4	carbamazepine-d10
Famotidine	338	189	27	259	20	6.3	atenolol-d7

Appendix B

Compounds	Precursor ion (m/z)	SRM 1	Collision Energy 1	SRM 2	Collision Energy 2	R_t (min)	IS used for quantification
Fenofibrate	361	139	43			25.2	diazepam-d5
Flumequine (IS)	262	202	47			15.4	
Fluoxetine	310	44	93	148	13	15.1	fluoxetine-d5
Fluoxetine-d5 (IS)	315	153	13			15.3	
Glibenclamide	494	369	23	169	55	20.7	carbamazepine-d10
Josamycin	828	174	45	600	37	15.6	carbamazepine-d10
Lisinopril	406	84	75			8.1	atenolol-d7
Loratadine	383	337	33	267	47	17.5	carbamazepine-d10
Lorazepam	323	174	45	229	45	15.7	diazepam-d5
Metoprolol	268	121	35	133	35	10.2	atenolol-d7
Metronidazole	172	172	21	82	37	5.8	13C-Phenacetin
Mevastatin	391	185	19	159	39	21.5	carbamazepine-d10
Nadolol	310	254	30	201	35	8.5	atenolol-d7
Nifuroxazide	276	121	25	65	73	12.8	13C-Phenacetin
Norfloxacin	320	302	35			9.3	flumequine
Ofloxacin	362	261	39			9.2	flumequine
Oxytetracycline	461	426	25	201	51	9.2	13C-Phenacetin
Paroxetine	330	192	31	123	45	14.4	fluoxetine-d5
Phenazone	189	56	40	147	33	9.8	carbamazepine-d10
Phenylbutazone	309	77	77	160	29	20.7	carbamazepine-d10
Pindolol	249	116	30	98	30	8.8	atenolol-d7
Pravastatin	447	327	29			14.2	carbamazepine-d10
Propranolol	260	116	35	183	30	12.5	atenolol-d7
Propyphenazone	231	56	57	189	35	15.3	carbamazepine-d10
Ranitidine	315	176	25	130	39	6.5	atenolol-d7
Roxythromycin	838	158	49	679	31	15.1	carbamazepine-d10
Salbutamol	240	148	25	166	20	5.7	atenolol-d7
Sotalol	273	213	25	255	25	6.1	atenolol-d7
Spiramycin	843	174	53	540	43	10.7	carbamazepine-d10
Sulfadiazine	253	156	25	92	43	7.3	sulfathiazol-d4
Sulfamethazine	279	186	25	124	33	9.5	sulfathiazol-d4
Sulfamethoxazole	254	156	25	92	41	12.5	sulfathiazol-d4
Sulfathiazol-d4 (IS)	260	160	23			8.2	
Tamoxifen	372	72	43	327	35	19.4	carbamazepine-d10
Tetracycline	445	428	20			11.8	13C-Phenacetin
Tilmicosin	869	696	61	174	55	11.8	carbamazepine-d10
Timolol	317	261	30	244	30	9.8	atenolol-d7
Trimethoprim	291	230	33	261	31	8.8	
Tylosin A	916	174	63	773	41	14.1	carbamazepine-d10

Appendix C

Concentrations of selected PhCs in surface waters and summary of the experimental investigations.

Table C. 1.Concentration of Selected PhCs (ng L^{-1}) upstream and downstream the discharge point of WWTPs A and B.

Class	Compounds	Ferrara (case study A)						Lagosanto (case study B)					
		Up stream			Down stream			Up stream			Down stream		
		1 st sample	2 nd samples	3 rd samples	1 st sample	2 nd samples	3 rd samples	1 st sample	2 nd samples	3 rd samples	1 st sample	2 nd samples	3 rd samples
analgesics/anti-inflammatories	Diclofenac	<LOQ	<LOQ	<LOQ	8	4	4	<LOQ	<LOQ	<LOQ	44	62	63
analgesics/anti-inflammatories	Indomethacine	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4	4	3
analgesics/anti-inflammatories	Ketoprofen	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
analgesics/anti-inflammatories	Mefenamic acid	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
analgesics/anti-inflammatories	Naproxen	3	4	3	8	16	6	<LOQ	<LOQ	<LOQ	7	8	9
analgesics/anti-inflammatories	Propyphenazone	<LOQ	<LOQ	<LOQ	7	<LOQ	4	<LOQ	<LOQ	<LOQ	5	6	16
antibiotics	Azithromycin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	7	3	3	9	56	77	89
antibiotics	Ciprofloxacin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	25	30	9	40	91	124	88
antibiotics	Clarithromycin	2	<LOQ	2	5	<LOQ	7	<LOQ	<LOQ	<LOQ	86	103	128
antibiotics	Metronidazole	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	14	11	16
antibiotics	Roxithromycin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
antibiotics	Sulfamethoxazole	<LOQ	<LOQ	<LOQ	4	<LOQ	5	<LOQ	<LOQ	<LOQ	30	45	92
antibiotics	Trimethoprim	<LOQ	<LOQ	<LOQ	2	<LOQ	2	<LOQ	2	<LOQ	18	28	30
lipid regulators	Atorvastatin	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Class	Compounds	Ferrara (case study A)						Lagosanto (case study B)					
		Up stream			Down stream			Up stream			Down stream		
		1 st sample	2 nd samples	3 rd samples	1 st sample	2 nd samples	3 rd samples	1 st sample	2 nd samples	3 rd samples	1 st sample	2 nd samples	3 rd samples
lipid regulators	Bezafibrate	3	11	16	3	4	4	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	4
lipid regulators	Hydrochlorothiazide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	83	116	128
diuretics	Furosemide	<LOQ	<LOQ	<LOQ	5	2	2	<LOQ	<LOQ	<LOQ	49	76	114
beta-agonists	Salbutamol	<LOQ	2	2	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	6	5	6
beta-blockers	Atenolol	4	14	7	14	12	7	<LOQ	<LOQ	<LOQ	117	132	231
beta-blockers	Metoprolol	<LOQ	<LOQ	<LOQ	8	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	36	33	49
beta-blockers	Sotalol	12	24	14	35	30	25	<LOQ	<LOQ	<LOQ	373	502	504
beta-blockers	Timolol	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	5	6	8
antihypertensive	Enalapril	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
psychiatric drugs	Carbamazepine	3	<LOQ	3	9	<LOQ	6	2	2	2	76	77	74
psychiatric drugs	Diazepam	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3	4	5
psychiatric drugs	Lorazepam	<LOQ	<LOQ	<LOQ	5	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	37	47	59
antidiabetic	Glibenclamide	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	3

Table C. 2. Summary of experimental investigations carried out in this thesis.

Site	Type of analyzed waters	Period	Searched	Detected	Samples	Kind of samples	Section	Table	Page
Ferrara	HWW	08/2009	73	49	4	24 hours	3.3	3.3	108
Ferrara	HWW	03/2010	73	62	4	24 hours	3.3	3.3	108
Lagosanto	HWW	08/2009	73	61	4	24 hours	3.3	3.3	108
Ferrara	UWW	03/2010	73	63	4	24 hours	3.3	3.3	108

Appendix C

Site	Type of analyzed waters	Period	Searched	Detected	Samples	Kind of samples	Section	Table	Page
Ferrara	Treated UWW	03/2010	73	58	4	24 hours	3.3	3.3	108
Ferrara	Treated UWW	05/2011	27	19	3	24 hours	4.3.2	4.4	138
Ferrara	Surface water(up stream)	05/2011	27	7	3	4 hours	4.3.3	C.1.	253
Ferrara	Surface water (down stream)	05/2011	27	14	3	4 hours	4.3.3	C.1.	253
Lagosanto	UWW	05/2011	27	24	3	24 hours	4.3.1	Fig 4.2.	133
Lagosanto	Treated UWW	05/2011	27	21	3	24 hours	4.3.2	4.4	138
Lagosanto	Surface water (up stream)	05/2011	27	4	3	4 hours	4.3.3	C.1.	253
Lagosanto	Surface water (down stream)	05/2011	27	21	3	4 hours	4.3.3	C.1.	253

Appendix D

This Appendix reports the scientific pubblications and partecipation in conferences made during the years of this Ph.D work. Above each paper the number of journal impact factor and number of citations are reported.

List of Publications

Juornal's Impact factor is 3.28, Number of Citations of this paper in scopus till February 2013 is 13.

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Review

Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment—A review

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ABSTRACT

This review focuses on 118 pharmaceuticals, belonging to seventeen different therapeutic classes, detected in raw urban wastewater and effluent from an activated sludge system, a usual treatment adopted for urban wastewaters worldwide prior to final discharge into surface water bodies. Data pertaining to 244 conventional activated sludge systems and 20 membrane biological reactors are analysed and the observed ranges of variability of each selected compound in their influent and effluent reported, with particular reference to the substances detected most frequently and in higher concentrations. A snapshot of the ability of these systems to remove such compounds is provided by comparing their global removal efficiencies for each substance. Where possible, the study then evaluates the average daily mass load of the majority of detected pharmaceuticals exiting the secondary treatment step. The final part of the review provides an assessment of the environmental risk posed by their presence in the secondary effluent by means of the risk quotient that is the ratio between the average pharmaceutical concentration measured in the secondary effluent and the predicted no-effect concentration.

Finally, mass load rankings of the compounds under review are compared with those based on their risk level. This analysis shows that the highest amounts discharged through secondary effluent pertain to one antihypertensive, and several beta-blockers and analgesics/anti-inflammatories, while the highest risk is posed by antibiotics and several psychiatric drugs and analgesics/anti-inflammatories. These results are reported with a view to aiding scientists and administrators in planning measures aiming to reduce the impact of treated urban wastewater discharge into surface water bodies.

Contents

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Hospital effluent: Investigation of the concentrations and distribution of pharmaceuticals and environmental risk assessment

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ABSTRACT

A study was conducted in an area in north, Italy, on the effluent of two different sized hospitals and the influent and effluent of the receiving municipal treatment plant of one of the examined hospitals. The aim was to investigate 73 selected pharmaceuticals, belonging to twelve different classes, comparing their occurrence in the effluent directly exiting the hospital with that, mixed with the local urban effluent, at the point of its entry and exit from the treatment plant.

Consistent differences were found in the concentrations of some antibiotics, analgesics and lipid regulators in the two wastewaters, confirming that hospital effluents should not be considered as possessing the same pollutant nature as urban wastewater. Furthermore, analysis of percentage contributions of the hospital to the treatment plant influent evidences that hospitals represent one of the main sources of pollutants, in particular antibiotics, receptor antagonists and lipid regulators.

Hence, an environmental risk assessment, performed on the effluent from the hospital and the influent and effluent from the treatment plant, revealed a high risk for 9 pharmaceuticals in hospital effluent and for 4 of the 9 substances in the treatment plant influent and effluent, with antibiotics being the most critical compounds in terms of contribution and potential environmental risk for the hospital.

1. Introduction

During recent years, the issue of pharmaceutical compounds (PhCs) in wastewater has become a major concern in terms of both human health and the environment. This has prompted the launch of several monitoring studies into the most commonly administered compounds in urban wastewater (Lishman et al., 2006; Santos et al., 2007; Terzic et al., 2009) and surface water (Kolpin et al., 2002).

However, a considerably smaller number of studies have been devoted to characterizing PhCs sources, mainly hospital effluents (Boillot et al., 2008; Kosma et al., 2010; Kummerer, 2001; Sim et al., 2011). In fact, in quite all countries worldwide, no distinction is usually made between these wastewaters and urban effluent, and they, along with their potentially hazardous loads, are generally discharged directly into the public sewage network and conveyed for co-treatment at the nearest municipal wastewater treatment plant (WWTP).

Nonetheless, considering the multiple research and laboratory activities carried out in these structures, as well as the treatments performed and pharmaceuticals administered and excreted within them, a wide range of concentrations of hazardous substances may be present in hospital effluent (Verlicchi et al., 2010). Hospital wastewaters are composed of the effluents of different services: kitchen, internal laundry, heating and cooling systems, laboratories, radiology departments, outpatients departments, transfusion centres and wards. Due to the nature and quantity of the micro-pollutants they harbor, such as active substances of medicines and their metabolites, chemicals, heavy metals, disinfectants, sterilizers, and radioactive markers, which are typically present at concentrations of μg/L, they should be earmarked for special consideration. Previous studies investigated the occurrence in hospital effluents of detergents, disinfectants, organic compounds (alcohols, acetone, formaldehyde, acetaldehyde, phenols) and several metals (Emmanuel et al., 2005; Boillot et al., 2008) and the proliferation of drug-resistant microorganisms (Hawkshead, 2008). The issue of PhC occurrence in hospital effluents has already been investigated by different Authors, among them Thomas et al., 2007; Gomez et al., 2006; Mahnik et al., 2007; Suarez et al., 2009; Kummerer, 2001.

It would therefore be of interest to discover the percentage contributions of PhCs from hospitals to those in the total municipal WWTP influent, in order to discover whether specific treatments for hospital effluent are necessary to reduce environmental contamination by persistent and hazardous micropollutants. To date, however, very

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Monitoring release of pharmaceutical compounds: Occurrence and environmental risk assessment of two WWTP effluents and their receiving bodies in the Po Valley, Italy

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HIGHLIGHTS

- 27 pharmaceuticals of different classes were monitored.
- Two WWTP effluents and the corresponding receiving water bodies were investigated.
- Hydrodynamic characteristics and pharmaceutical impact on water bodies were studied.
- Based on the risk quotient, antibiotics are the most critical compounds.
- The flow rate of the surface water body contributes to mitigate the risk.

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ABSTRACT

This study describes an investigation on the occurrence of 27 pharmaceutical compounds, belonging to different classes, in the effluent from two wastewater treatment plants (WWTPs) and their receiving water bodies in the sensitive area of the Po Valley (northern Italy). These canals were monitored upstream and downstream of the effluent discharge points in order to evaluate the effluent impact on the quality of surface waters, commonly used for irrigation. An environmental risk assessment was also conducted by calculating the risk quotient, i.e. the ratio between measured concentration and predicted no effect concentration. Collected data show that, although average values of the selected compounds were in general higher in the effluent than in the surface waters, some compounds not detected in the WWTP effluent were detected in the receiving water (upstream as well as downstream), indicating that sources other than treated effluents are present as contaminations during extraction and analysis have to be excluded. The most critical compounds for the environment were found to be the antibiotics sulfamethoxazole, clarithromycin and azithromycin. The study shows that the potential toxicological effects of persistent micropollutants can be mitigated to some extent by a high dilution capacity, i.e. a high average flow rate in the receiving water body with respect to the effluent.

1. Introduction

At present, approximately 3000 different pharmaceutical ingredients are used in the European Union, including antibiotics, beta-blockers, lipid regulators, antidepressants and many more, for human consumptions (therapeutic or diagnostic purposes) (Ternes and Joss, 2006). One important immission source of pharmaceuticals in the water cycle is via human metabolism: in fact, once administered, these compounds are only partially metabolized by the human body, and therefore enter the water cycle either as parent (unchanged) compounds, which are excreted largely through urine (generally 55–80% of the total, with few exceptions) and partially in the feces, or as a mixture of metabolites and/or conjugated compounds (Jjemba, 2006; Lienert et al., 2007).

Unfortunately, municipal wastewater treatment plants (WWTPs) are generally unable to effectively remove either unaltered or metabolized forms of pharmaceutical compounds (PhCs) from wastewaters (Bendz et al., 2005; Castiglioni et al., 2006; Glassmeyer et al., 2005; Gomez et al., 2007; Joss et al., 2005; Verlicchi et al., 2012b). Their occurrence in surface water has been documented by a number of authors (Ashton et al., 2004; Calamari et al., 2003; Fatta-Kassinos et al., 2011; Gros et al., 2006; Kolpin et al., 2002, 2004; Spongberg

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A project of reuse of reclaimed wastewater in the Po Valley, Italy: Polishing sequence and cost benefit analysis

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SUMMARY

The paper presents a study carried out in the environmentally sensitive area of the Po Valley in northern Italy, with the aim of evaluating, from technical and economic perspectives, a project to reuse part of the final effluent from the Ferrara wastewater treatment plant for irrigation and to develop the site for recreational purposes.

Although this area features plentiful supplies of surface water, the Ministry of the Environment has declared it to be at risk of environmental crises due to eutrophication and the drought recurring over the last decade. Thus the availability of fresh water, particularly for agricultural purposes, is threatened, and prompt water saving and protection measures are required. Hence, the possibility of reusing reclaimed wastewater from this plant was investigated, with the aim of exploiting the space around the WWTP, situated within a large urban park, to install natural polishing treatment systems and create green spaces for recreational use.

Based on experimental investigation on a pilot plant (featuring both natural and conventional treatments), the study outlines the rationale behind the treatment train selected for the project, details the initial and ongoing costs involved, evaluates the benefits deriving from the project, and assesses public acceptance of the project by the contingent valuation method. A cost–benefit analysis completes the study, and various economic indicators (net present value, benefit–cost ratio, pay-back period, and internal rate of return) revealed that the proposed project was financially feasible.

1. Introduction

In recent decades, increasingly unpredictable precipitation patterns and the deterioration of surface water quality, even in countries with a plentiful supply, have challenged the idea that water be considered as a *reliable* and *inexhaustible* resource (Bixio et al., 2006). Institutions charged with managing water resources are faced with the urgent problem of how to promote surface water saving and improvement.

In these environmentally sensitive times, reuse of reclaimed wastewater is a particularly appealing solution (Asano, 1998; Lazarova et al., 2001; Mujeriego et al., 2008), and various wastewater polishing sequences have been investigated with the aim of finding ways to comply with the, often strict, legal requirements for direct reuse of reclaimed wastewater in agriculture, industry or urban applications (Asano, 1998; Nurizzo et al., 2001; Lazarova et al., 2001; Meneses et al., 2010).

Thus, several reclaimed wastewater reuse schemes, relying on supplementary treatments such as rapid filtration and disinfection, have been designed as add-ons to conventional treatment processes. Efficient and reliable disinfection systems are governed by strict legal microbiological limits, and tried-and-tested chlorination and UV are therefore the most common; nonetheless, the so-called *natural* solutions, including lagoons, horizontal or vertical subsurface flow beds, and their combination in *hybrid systems*, are becoming more popular (Cirelli et al., 2007; Herrera Melian et al., 2010).

Although natural polishing strategies generally consume less energy with respect to conventional treatments (0–1 kW h per person equivalent per year, kW h/(p.e. year) as compared to at least 2–3 kW h/(p.e. year)) (Masotti and Verlicchi, 2005), they require far larger surface areas, i.e. 1–4 m^2/p.e. with respect to 0.001–0.002 m^2/p.e. Thus, land acquisition needs to be taken into account when projecting investment costs. Generally speaking, evaluation of the feasibility of a reuse project is focused on the internal costs, i.e. initial investment, operation and maintenance (Nurizzo et al., 2001; Fine et al., 2006; Mujeriego et al., 2008),

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Submitted Papers

- Verlicchi P., Galletti A., **Al Aukidy M.**, Zambello E., Petrovic M., Barcelo D. Removal of Selected Pharmaceuticals from Domestic Wastewater in an Activated Sludge System followed by a Horizontal Subsurface Flow Bed – Analysis of their respective contributions. Submitted to the journal Science of the Total Environment.

Book Chapters

- Verlicchi P., Galletti A., **Al Aukidy M**., Masotti L., Perspectives in Water disinfection, In. WATER DISINFECTION, Nova Publisher, Kelly M. Buchanan, 2011, p. ISBN 978-1-61122-021-6.

- Verlicchi P, Galletti A, **Al Aukidy M**. Hospital Wastewaters: Quali-quantitative Characterization and Strategies for Their Treatment and Disposal, Springer. In: SK Sharma and R Sanghi (Eds), Water Treatment And Pollution Prevention: Advances in Research, Springer, UK, 2012.

Attendance at conferences - Platform presentations:

- Galletti A**., AL Aukidy M**., Verlicchi P., Petrovic M., Barcelò D.;Pharmaceuticals removal in a H-SSF treating a secondary domestic wastewater – an experimental investigation, Proc. IWA Conference Wetland Systems for Water Pollution Control, Venice October 2010, 382-384, ISBN 978-88-6060-300-5.

- Verlicchi P., Galletti A., **Al Aukidy M.**, Pasti L., Marchetti N., Evaluation of sulphametoxazole and ciprofloxacin removal processes in a horizontal subsurface flow system: an experimental investigation. Proc. IWA Conference Wetland Systems for Water Pollution Control, Venice October 2010, 1045-1047, ISBN 978-88-6060-300-5.

- Verlicchi P., **Al Aukidy M.**, Galletti A., Chiloiro E., Ranieri E., Experimental investigations on Heavy Metals Removal in H-SSF Systems in Northern and Southern Italy, Proc. IWA Conference Wetland Systems for Water Pollution Control, Venice October 2010, 1048-1050, ISBN 978-88-6060-300-5.

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- Verlicchi P., **Al Aukidy M.**, Jelic A., Petrovic M. Exposure assessment of pharmaceuticals in wastewaters and surface water - the case study of the catchment area of Ferrara. oral presentation at 12th on Tecnologie Sostenibili per la Tutela dell'Ambiente, Milano, 26 – 29 Giugno 2012.

- **Al Aukidy M.**, Verlicchi P., Galetti A., Masotti L. Gli Effluenti Ospedalieri: Caratterizzazione, Trattabilità E Sequenze Di Trattamento Più Adeguate. Il monitoraggio, il controllo e la rimozione dei composti xenobiotici ed emergenti nelle acque reflue civili industriali e di falda: il contributo della ricerca nei programmi di Ricerca di Interesse Nazionale (PRIN), Acquaria Verona, 24 ottobre 2012.

Attendance at conferences - Poster presentations:

- **M. Al Aukidy** , P. Verlicchi, A. Jelic , M. Petrovic. Monitoring and Risk Assessment of Pharmaceutical Compounds in Two WWTP Effluents and Their Receiving Rivers in the Po Valley, Italy. oral presentation at the Ecotechnologies for Wastewater Treatment 2012 International Conference, 25-27 June 2012 Santiago de Compostela, Spain.
- **Al Aukidy M**, Verlicchi P, Zambello E. Environmental Risk Assessment of Pharmaceutical Compounds as a Tool for the Management of Hospital Effluents. Accepted for poster presentation at the Micropol & Ecohazard Conference 2013 (Zurich, Switzerland 16-20 June 2013).

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