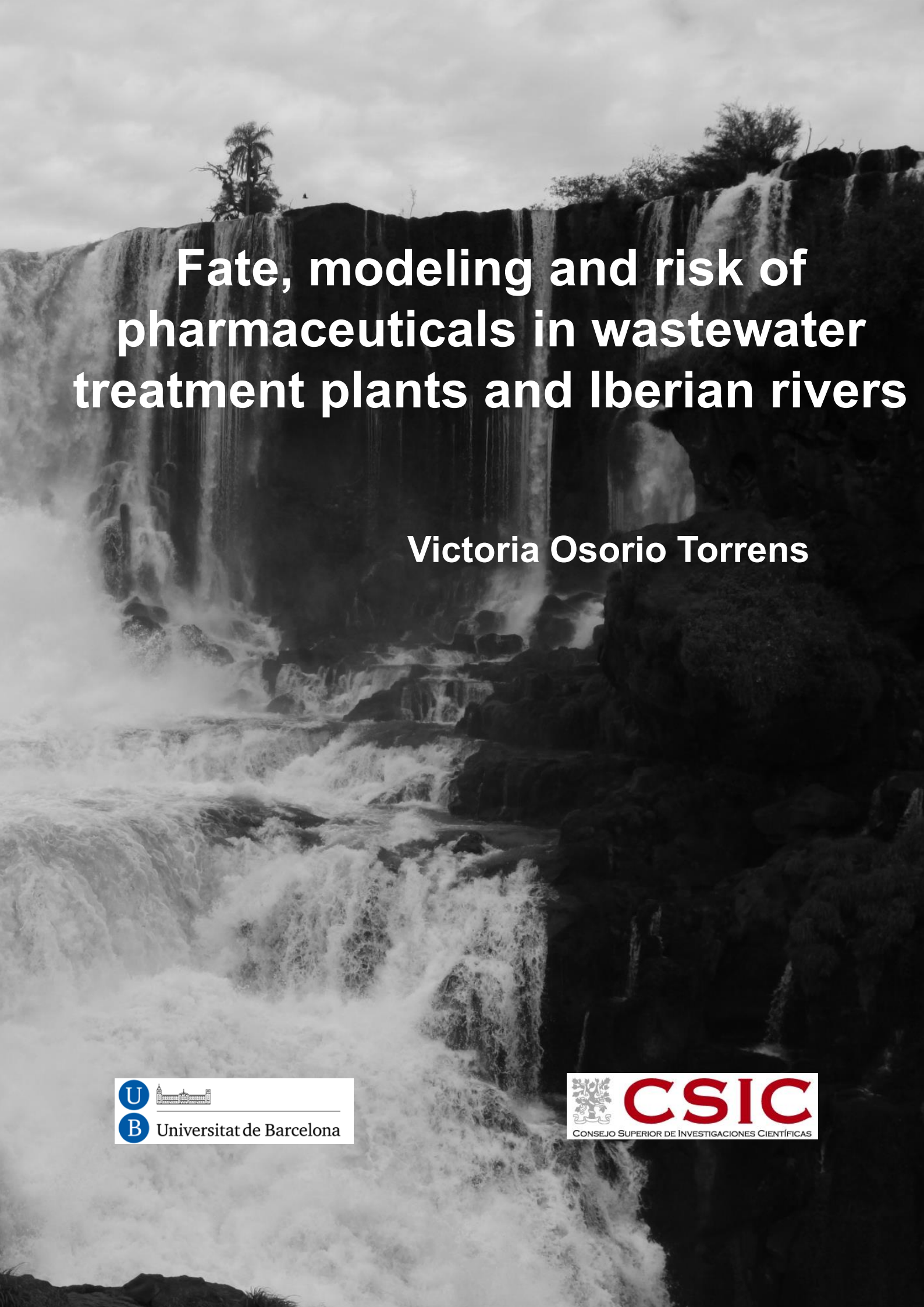
UNIVERSITAT DE BARCELONA

Fate, modeling, and risk of pharmaceuticals in wastewater treatment plants and Iberian rivers

Victoria Osorio Torrens



Fate, modeling and risk of pharmaceuticals in wastewater treatment plants and Iberian rivers

Victoria Osorio Torrens

Universitat de Barcelona

CSIC
Consejo Superior de Investigaciones Científicas

Programa de Doctorat
"Química Analítica del Medi Ambient i de la Pol·lució"

Fate, modeling, and risk of pharmaceuticals in wastewater treatment plants and Iberian rivers

Memòria presentada per optar al títol de
Doctora per la Universitat de Barcelona per
Victoria Osorio Torrens

Tutora

Dra. Mercè Granados Juan
Prof. Titular de Química Analítica
Facultat de Química
Universitat de Barcelona

Codirector

Dr. Damià Barceló i Cullerés
Professor d´investigació
Dep. de Química Ambiental
IDAEA-CSIC

Codirectora

Dra. Sandra Pérez Solsona
Científica contractada del CSIC
Dep. de Química Ambiental
IDAEA-CSIC

A Papá y a Bubu.

“Do or do not, there is no try”

Master Yoda

Acknowledgements to projects:

This work has been supported by the finantial support of the The Spanish Ministry “Ministerio de Economía y Competitividad” through the projects, VIECO (2008 009/RN08/011), CEMAGUA (CGL2007-64551/HID) and SCARCE (Consolider-Ingenio 2010 CSD2009-00065); by the European Community 7th Framework Programme Funding under grant agreement no. 603629-ENV-2013-6.2.1-Globaqua; and by the Generalitat de Catalunya (Consolidated Research Groups “2014 SGR 418 – Water and Soil Quality Unit”).

Acknowledgements

En primer lloc vull agrair al Prof. Damià Barceló i a la Dra. Sandra Pérez per acceptar la direcció d' aquesta tesi i per la seva revisió continuada i les seves importants aportacions. També agraeixo a la Dra. Mercè Granados per acceptar la tutoria d' aquesta tesi, la seva revisió continuada i per el seu suport en el decurs de la mateixa.

Then I would like to acknowledge all those researchers with whom I had the opportunity to conduct such valuable scientific collaborations that enhanced the quality and interest of my investigations: Lorenzo Proia, Marta Ricart, Sergi Sabater, Marinel·la Farré, Antoni Ginebreda, Rafael Marcè, Jose Luís Abad, Josep-Àngel Sanchís, Aitor Larrañaga, Marta Imbert-Bouchard, Jaume Aceña, Bozo Zonja, Carlos Gonçalves, Manuela Barbieri, Despo Fatta-Kassinos, Irene Michael and Joana Aldekoa.

Això acaba tal i com va començar: nit, música, laboratori. Una gran diferència hi ha però, manqueu tots vosaltres. Han estat tants els companys amb el qui he tingut la sort i el plaer de fer aquest camí junts que potser me'n oblidi d' algú; però no per això voldrà dir que heu estat menys importants:

Gràcies a les germanes grans, Meri, Lina, Cris, Paula, Alícia, Marianne, Chus, Asun, Àngels, Elena i Laia per estar sempre disponibles per aclarir-me tots el dubtes. També gràcies a l' equip de jefas Marinel·la, Miren, Ethel, Silvia i Mira i al jefe Antoni. Muito obrigado à comunidade brasileira, Bianca, Renata, Karla y Mariana y a otros tantos visitantes como Lei, Carlos, Pablo y Piero por tan buenos momentos en el despachito de masas. Gracias a Juan y Christian, por compartirlo siempre todo. Als àngels de masses, Maria, Dori i Roser i les ICRA girls, Marta i Sara, moltes gràcies pel suport tècnic i el ànims! A Romain y mis niñas Noemí, Marine, Charlotte i a la Marta Imbert, por aprender conmigo. Grazie mille a le sorelle rubias Aleks e Maria Luisa per il vostro sostegno e le vostre lezioni importanti non solo i professionisti, ma anche della vita. Gracias al Doctor Gago (ahora sí) por compartir tantas laboratorio-partys en las noches de verano y a mi alcahueta Rebeca, por confiar en mi habilidad al volante en los muestreos. Grazie Niko per le terapie comune di progetti y Paqui por tus cantos de sirena. Gracias a mi vecino de la derecha Daniel San y las conversaciones tan profundas por el face. Gracias a Mar por nuestras charlas sobre cómo hacer (o deshacer) el mundo. Als nous vinguts, Zoran, Cris, Àlex, Dani2, Dani3, Jenni, Núria, Òscar (algú mes?) per rebre'm sempre com una més de la casa. Thanks to blondie Maja, for the olives on the roof. A mis freestylers Kyo y la rubia Noemia, gracias por el curso avanzado de cómo aparcar una barca. A mis compañeras de ICRA, Belinda y Laura siempre disponibles para la terapia de fármacos y demás cosas... Gràcies als meus germanets en Jaume i en Bozo pels nombrosos favors i deixar-me sempre el primer torn. Gràcies a la Marina, per les nostres llargues xerrades de confessions, no callàvem fins que fotien cops a la paret! Gracias a mi rubia Giselle, porque sin tí esto no hubiera sido posible. A la meva sister Marta, gràcies pel teu constant suport per poder fer "el salt del salmó" davant cada dificultat. Gràcies al simpòsium hispano-holandès, per les lliçons de surrealisme i frikisme, a en Josep i en Kike, per haver-me recolzat en qualsevol moment i fer-me sentir com si mai hagués marxat. I encara més gràcies a tots dos per la guia 24 hores de com preparar i dipositar una tesi.

No sols els de dins, sinó també els he de fora he contribuït d' alguna manera a aconseguir aquest repte:

Gracias Mari, Cris y Montse; preparad vuestras agendas! Gracias a mi padawan Coral, que aunque me demostró que el alumno supera al maestro, siguió creyendo en la fuerza de mis midiclorianos. Moltes gràcies a Zamira, Ester, Xesca, Maria, Lorena; Núria, Laia, Belén i totes les meves seitonetes avinagrades pels vostres ànims i la vostra comprensió; de nuevo gracias Belén por esa pedazo de figura con tantos colorines!

Gràcies als Intrèpids Seitons de Sant Adrià pel recolzament i la força que m' heu transmès e pure grazie mille a tutti gli Altitudi di Roma per trasmettere tutta la forza e il desiderio di porre fine. Especialmente a Bubu, a la fine o usato il google translate ma per escrivere a te o deciso meglio di parlare Vichiese, grazie per aiutarmi a finire questa tesi come la nostra ultima partita, come una grande victoria!

I would also like to ackowledge my colleagues from KWR, to Annemieke and Merijn for their support, to Dennis and Pascal for their willingness to reply everything everyday, to my no- microwave lunch-mates Rosa, Steff, Yuki, Bea, Roberto and Ana; thanks to all of you. Roberto attento che ci vediamo presto in Sardenya. Anakin, gracias por compartir cada día de tu tesis y de tu vida conmigo y dejarme compartir los míos contigo.

Anche a la mia famiglia romana, gracie a "Testa...", a bella Anna e a gli megli padre e madre Roberto e Enrica, grazie mille per gli vostri parolle di incoraggiamento.

Molt especialment voldria agraïrli a la meva familía que m' hagi recolzat tantíssim tots aquests anys i per fer-me sentir orgullosa de la meva feina, tot i que em sento encara més orgullosa de la meravellosa família que tinc: els meus germans Jesús i Quique, les meves cunyades Susana i Remei, els meus nebots Patrícia, Carlos, Cristina, Quique IV i Alba, la meva tieta Susi i els meus pares Enrique i Amèlia. Imagino Papá que tus palabras serían: "lo ves Meliquita? Ya te lo decía yo que la niña tenía que estudiar Químicas...", muchas gracias Papá, como siempre acertaste, todo esto te lo debo a tí. Y també a tu Amelita, perquè m' has recordat cada dia el que el Papa creia i el que tu també creus amb fermesa; que puc amb això i amb tot el que em proposi a la vida. Moltes gràcies Mama.

Finalment, agraïrte a tu, TOT. Gràcies per l' aigua que em vas portar tot xop el primer dia, gràcies per l' abraçada que em vas fer tot i fer-te esperar una hora al punt de mostreig, gràcies per les nits d' espera a engegar la seqüència, gràcies per tot el que m' has ensenyat a la tesi i a la vida, gràcies per haver-me pres la mà i guiar-me i per no deixar-me-la mai, ni en els moments més complicats ni després de nits sense dormir arreglant formats. Gràcies Lorenzo, saps que sense tu avui no podria acabar aquesta frase, gràcies.

Index

Fate, modeling, and risk of pharmaceuticals in wastewater treatment plants and Iberian rivers.

List of Tables

List of figures

Abbreviations and acronym list

•OH = hydroxyl radicals

4'-OH-DCF = 4'-hydroxydiclofenac

5-OH-DCF = 5-hydroxydiclofenac

4',5-diOH-DCF = 4',5-dihydroxydiclofenac

5-OHD-DCF = Lactam of 5-OH-DCF

ABR = Abrera

AMO = Ammonia MonoOxygenase

AOA = Ammonia Oxidizing Archaea

AOB = Ammonia Oxidizing Bacteria

AOPs = Advanced Oxidation Processes

APCs = Active Pharmaceutical Compounds

APCI = Atmospheric Pressure Chemical Ionization

ATT = Advanced Tertiary Treatment

CAS = Conventional Activated Sludge

CO_2 = carbon dioxide

COD = Chemical Oxygen Demand

CPAB = 2-[(2-chlorophenyl) amino] benzaldehyde

CYP = CYtochrome P

Des-SMX = Desamino-sulfamethoxazole

DCF = Diclofenac

DCF-Gluc = 1-O-acyl glucuronide

DDD = Defined Daily Dose

DOC = Dissolved Organic Carbon

DT_{50} = 50 % dissipation half-life time

DW = Drinking Water

DWTP = Drinking Water Treatment Plant

E2 = Estradiol

EC_{50} = 50% Effective Concentrations

ECDPC = European Centre for Disease Prevention and Control

EDC = Endocrine Disruption Compounds

EE2 = Ethynyl Estradiol

EFPIA = European Federation of Pharmaceutical Industries and Associations

EMA = European Medicines Agency

EPI = Enhanced Product Ion

EQSs = Environmental Quality Standards

ERA = Environmental Risk Assessment

ESI = Electrospray Sources of Ionization

EU = European Union

Fe^{2+}/H_2O_2 = cationic iron/hydrogen peroxide, photo-Fenton treatment

FDA = US Food and Drug Administration

GST = Glutathione S-Transferases

GW = Ground Water

GWRC = Global Water Research Coalition

HOA = Hydroxylamine Oxidoreductase

HPLC = High Performance Liquid Chromatography

HRMS = High Resolution Mass Spectrometry/Spectrometer

HRT = Hydraulic Retention Time

ICM = Iodinated Contrast Media

IDA = Information-Dependent Acquisition

IMS Health = IMS Institute for health care and informatics

IT = Ion Trap

K_d = solid-liquid partition coefficient

K_{oc} = soil organic carbon partition coefficient

K_{ow} = octanol-water partition coefficient

LC_{50} = 50% Lethal Effective concentration

LDTD = Laser Diode Thermal Desorption

LE = Liquid extraction

LOEC = Lowest Observed Effect Concentration

MRM = Multiple Reaction Monitoring

MS = Mass Spectrometry/Spectrometer

MSPD = Matrix Solid Phase Dispersion

MS/MS = Tandem Mass Spectrometry

MS^E = Elevated-Energy Mass Spectrometry

MS^n = n fragmentation Mass Spectra/Spectrometry

MSPD = Matrix Solid Phase Dispersion

NAS = Nitrifying Activated Sludge

NH_3 = ammonia

NH_4^+–N = ammonium nitrogen species

NH_2OH = hydroxylamine

NO_2^-N = nitrite nitrogen species

NO_3^-N = nitrate nitrogen species

NOEC = Non Observed Effect Concentrations

NOR = Nitrite OxidoReductase

O_3 = Ozone

OC = Oseltamivir Carboxylate

OE = Oseltamivir Ester

OECD = European Organisation for Economic Cooperation and Development

PhACs = Pharmaceutically Active Compounds

Photo-TPs = PhotoTransformation Products

PHSs = Priority Hazardous Substances

pka = acid dissociation constant

PLE = Pressurized Liquid Extraction

PAC = Powdered Activated Carbon

PSs = Priority Substances

Q = single Quadrupole

QqLIT = Quadrupole Linear Ion Trap

QqQ = triple Quadrupole

QTOF = Quadrupole Time Of Flight

SIM = Selected Ion Monitoring

SJD = Sant Joan Despí

SMX = Sulfamethoxazole

SNS = Spanish National health System

SPE = Solid Phase Extraction

SPM = Suspended Particulate Matter

SRM = Selected Reaction Monitoring

SRT = Sludge Retention Time

SULT = SULfoTransferases

SW = Surface Waters

TiO_2 = titanium dioxide

TPs = Transformation Products

UGT = UDP-GlucuronosylTransferases

UHPLC = Ultra High Performance Liquid Chromatography

US = UltraSound

USE = Ultrasonic Solvent Extraction

UV = ultraviolet

WFD = Water Framework Directive

WW = WasteWater

WWe = WasteWater effluent

WWi = WasteWater influent

WWTPs = WasteWater Treatment Plants

Chapter 1

General introduction about pharmaceuticals and their transformation products

Chapter 1

General introduction about pharmaceuticals and their transformation products

1.1. Description of pharmaceuticals studied

Pharmaceutically active compounds (PhACs) are synthetic or natural chemicals used for diagnosis, treatment or disease prevention and health condition. They are designed to have pharmacological effects and confer significant benefits to society. These substances cover a broad range of chemical structures and physicochemical properties.

Figure 1.1 lists the human and veterinary PhACs grouped according to their therapeutic activity assessed in this thesis and being frequently studied as well. CAS number, molecular formula, molecular weight, estimated and experimental physicochemical properties (acid dissociation constant , pka; water solubility; octanol-water partition coefficient, (K_{ow}); solid-liquid partition coefficient, (K_d) and soil organic carbon partition coefficient (K_{oc}) are provided in table A.1 (see Annex). Although PhACs are frequently classified according to their pharmacological mode of action, the physicochemical properties of compounds within a given group can vary widely. For instance, acetaminophen and diclofenac (DCF) are usually grouped together as analgesics and antiinflammatories according to medical uses and prescriptions. Acetaminophen is a weak acid that tends strongly to be solved in the aqueous phase, such as surface waters (SW) (water solubility of 14 g L^{-1} and log K_{ow} = 0.46). Conversely, DCF is acidic compound (pka 4.00) that tends to distribute between the aqueous (e.g. SW) and solid phase (e.g. fluvial sediments or suspended solids) due to its moderate solubility and hydrophobicity (2.37 mg L^{-1} and log K_{ow} = 4.51).

1.2 Human consumption and veterinary use of PhACs

PhACs are consumed not only by humans, but also in animal husbandry and aquaculture. These compounds are common in prescription medicines or as over-the-counter therapeutic drugs.

Since the beginning of commercialization of PhACs in the late 19th century, their total number has reached up to 4,000 for human and veterinary use (Daughton, 2013). Such a trend is likely to continue, particularly in developing countries with aging human population (European Environmental Agency, 2010). The annual worldwide consumption of active pharmaceutical compounds (APCs) is estimated to reach the 100,000 tons (Sadezky et al., 2008) and the amount of PhACs approved for use over 25,000 (Daughton, 2013). Of these, 9,524 and 9,700 APCs have been approved for human use and human/veterinary use, respectively. However, little information about their use or consumption is usually available.

The total use of PhACs in human medicine is reported in monetary value or number of prescriptions. In other cases consumption data is also expressed as pharmaceutical expenditure at current prices and pharmacy purchasing prices, pharmaceutical expenditure per capita or as the total amount of sales. These data are currently provided by diverse agencies such as the Organisation for Economic Cooperation and Development (OECD); the IMS Institute for health care and informatics (IMS Health); the European Federation of Pharmaceutical Industries and Associations (EFPIA); the Spanish National Health System (SNS) or the National Trade Association of the Spanish based pharmaceutical industry (farmaindustria).

Psychiatric drugs

- Fluoxetine
- Paroxetine
- Diazepam
- Lorazepam
- Carbamazepine
- Sertraline
- Citalopram
- Venlafaxine
- Olanzapine
- Trazodone
- Alprazolam

Antibiotics

- Erythromycin
- Metronidazole
- Azithromycin
- Roxithromycin
- Clarithromycin
- Tylosin A
- Josamycin
- Spiramycin
- Tilmicosin
- Ofloxacin
- Ciprofloxacin
- Enoxacin
- Danofloxacin
- Enrofloxacin
- Tetracycline
- Doxycycline
- Sulfamethoxazole
- Sulfadiazine
- Trimethoprim
- Chloramphenicol
- Nifuroxazide
- Flumequine
- Dimetridazole
- Ronidazole
- Cefalexin

Analgesics and antiinflamatories

- Ketoprofen
- Naproxen
- Ibuprofen
- Indomethacine
- Diclofenac
- Mefenamic acid
- Acetaminophen
- Propyphenazone
- Phenazone
- Phenylbutazone
- Codeine
- Piroxicam
- Meloxicam
- Tenoxicam

Figure 1.1. List of studied PhACs in this thesis classified by their therapeutic activity.

The world pharmaceutical market was worth an estimated € 655,222 million ($ 870,200 million) in 2013. Figure 1.2 shows the yearly pharmaceutical expenditure at current prices and pharmacy purchasing prices (in US dollars) covering fourteen countries during the period [2004-2012] extracted from the OECD health statistics database (OECD, 2014). Among the major markets, the North American market (USA & Canada) has remained the world's largest market well ahead of Europe and Japan with corresponding sales share at of 41.0; 27.4; and 9.7% in 2013 (EFPIA, 2014). Regarding Spain, it has remained on the fourth position in Europe's top five PhACs market (after Germany, France and Italy and before UK) (figure 1.2). Moreover, Spain was the eighth largest world market in 2010 (IMS Health, 2011). According to IMS Health (2011), the total world market on PhACs grew 36% from 2003 to 2010 to a total of $875 billion. As for the Spanish market, it has grown substantially, showing the highest market development among the other countries (see figure 1.2) with sales in 2009 that almost doubled those of 2001 (IMS Health, 2011) but dropped again after

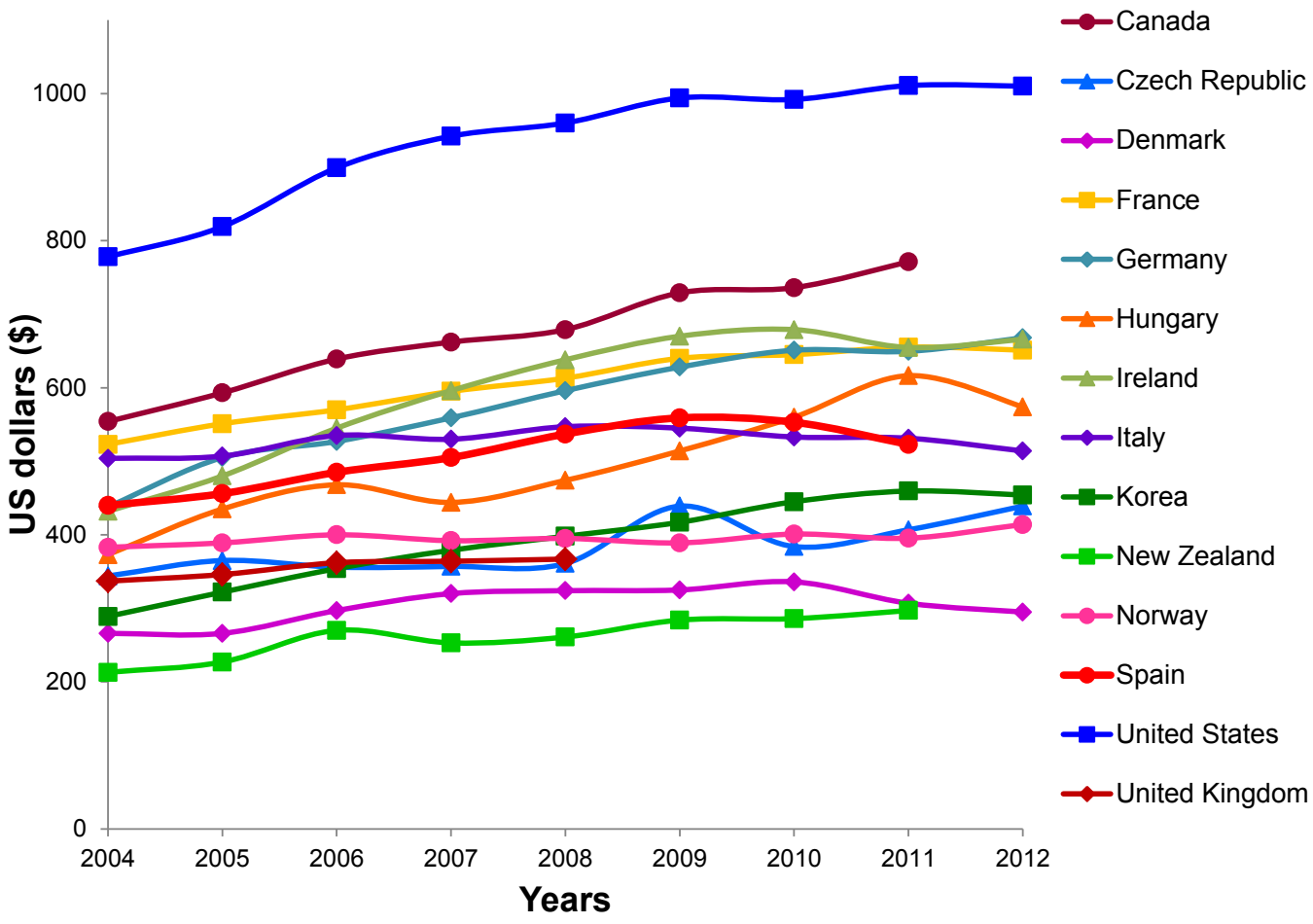


Figure 1.2. Worldwide pharmaceutical expenditure per capita (at current prices in US$ and pharmacy purchasing prices) of thirteen selected countries. Source: OECD Health Data: Health expenditure and financing: OECD Health Statistics (database) (2014)

significant spending cuts in the national health system.

Considering more specific data at a local level, figure 1.3 shows defined daily dose (DDD) in 2010 of the most consumed therapeutic drug classes in Spain, as well as the individual APCs (SNS, 2012). The DDD is the assumed average maintenance dose per day for a given drug in adults. The most consumed therapeutic groups are the antiulcerants and among these omeprazole is the most used (1,519.85 millions of DDD) (SNS, 2012). These are followed by analgesics and belonging to these, paracetamol and metamizole. Antiinflammatories, such as acetylsalicylic acid and ibuprofen are also highly consumed as well as lipid regulators and cholesterol lowering statin drugs like simvastatin and atorvastatin. Also of relevant use are antidiabetics like metformin and psychiatric treatment drugs, such as lorazepam. The consumption of antihypertensives such as enalapril is also substantial (SNS, 2012).

The consumption of most of these PhACs increased in recent years. Spanish statistics reported that consumption of analgesics and antiinflammatories, from 1999 to 2003, increased by 93.6% (García del Pozo and De Abajo, 2005). The use of β-blockers grew by 200.8% from 2000 to 2008 (García del Pozo, 2009). The total consumption of antidepressants increased by 107% between 1997 and 2002 (Martín, 2005). Between 1997 and 2006, the use of anxiolytics and hypnotics increased by 113.6% (García del Pozo et al. 2006). As

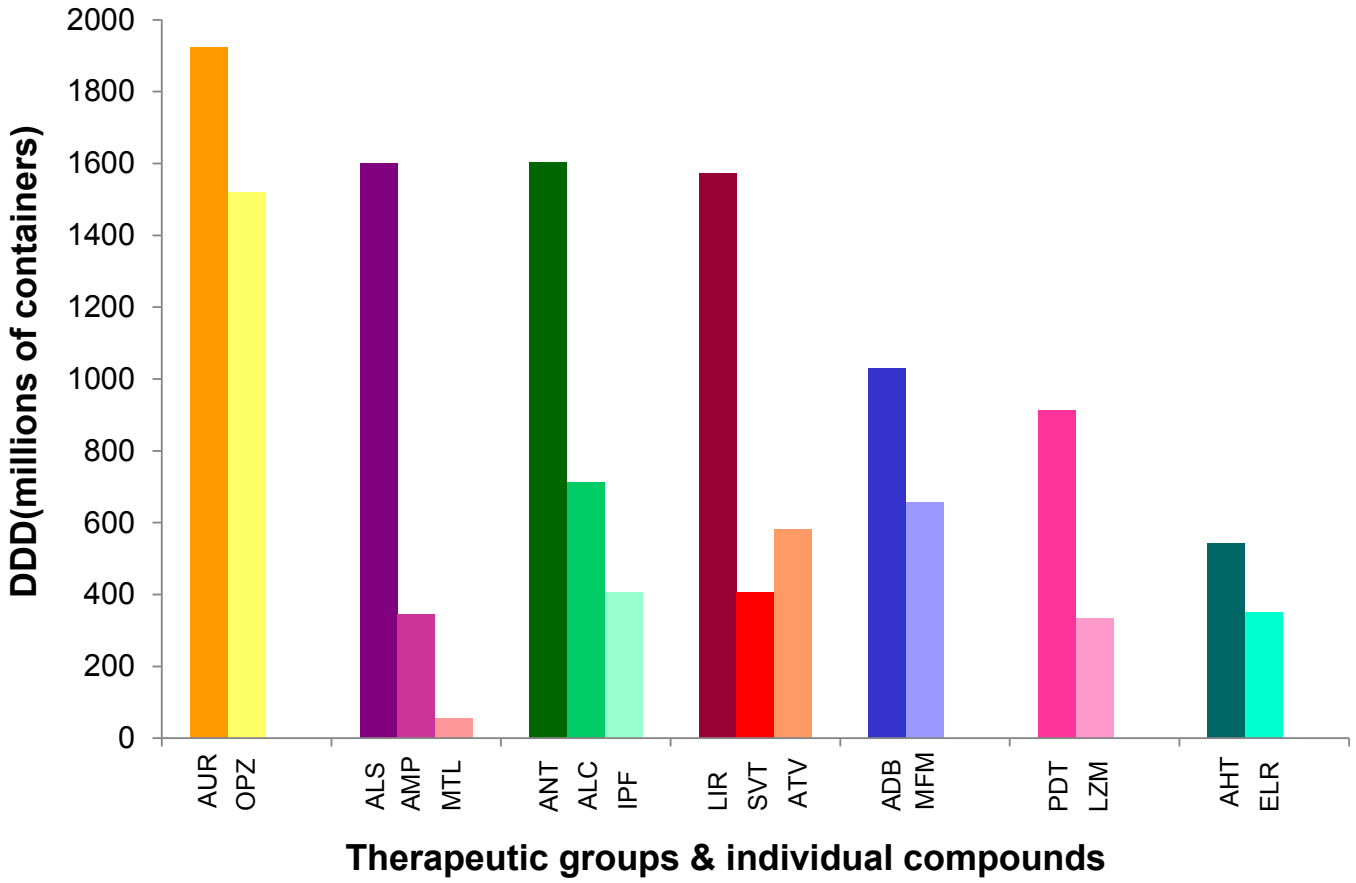


Figure 1.3. Number of DDD expressed in millions of containers of the ten therapeutic groups and individual drugs mostly consumed in the year 2010 in Spain. AUR = Antiulcerants and OPZ = Omeprazole; ALS = Analgesics, AMP = Acetaminophen and MTL = Metamizole; ANT = Antiinflammatories, ALC = Acetylsalicylic acid and IPF = Ibuprofen; LIR = Lipid regulators cholesterol lowering stating drugs, SVT = Simvastatin and ATV = Atorvastatin; ADB = Antidiabetics and MFM = Metformin; PDT = Psyquiatric treatment drugs and LZM = Lorazepam; AHT = Antihypertensives and ELR= Enalapril (SNS, 2012).

for antibiotics, Spain has been characterized by a high use per capita, as is the case for other countries in southern Europe (Lázaro and Montero, 2010).

As reported by the IMS Health (2014), the global spending on medicines is forecasted to reach nearly $1.3 trillion by 2018, an increase about 30% over the 2013 level. This growth is driven by population increase, an aging population, and improved access of the BRICS countries (i.e. Brazil, Russia, India, China and South America).The developed markets, led by the United States, the major five European markets and Japan, are foreseen as the primary drivers of this increased growth. Moreover, the so-called 21 “pharmerging countries” are also expected to increase their contribution to growth over the next five years accounting for nearly 50% of absolute growth in 2018 (IMS Health, 2014). On the contrary, the spending growth trend in other developed countries such as France and Spain is estimated to slow down. This is due to implementation of economic policies to constrain growth in healthcare spending, and especially PhACs (IMS Health, 2014).

PhACs of veterinary use encompass a broad range of products, including anti-parasitic drugs, anti-inflammatory medications, anesthetics, pain medications, antibiotics and specialized products used to manage reproductive, cardiovascular or metabolic conditions. An estimated 6,051 tons of APCs went into the production of veterinary medicines for the treatment of food animals in the European Union (EU) in 2004, including 5,393 tons of antibiotics and 194 tons of antiparasitics (Kools et al., 2008a). With global meat production projected to increase (Alexandratos and Bruisma, 2012) and the growing market for “pets” PhACs (National Office of Animal Health, UK), the use of veterinary drugs will continue to increase. However, there are approximately 2,000 PhACs of veterinary use in the EU, most of which a full test of their environmental risk has not been conducted (Kools et al., 2008b).

In addition, the continuous ageing population and improving quality of life worldwide means that human PhACs consumption is also set to increase in future years (Van der Aa et al., 2011). In fact, a recent study assessing the levels of PhACs in vertical cores of sediments of the Jamaica Bay (USA) has revealed an increase of concentration of PhACs during the last 60 years directly related to their use and, therefore, disposal into the environment. Lara-Martín et al. (2015) calculated the sediment–pore water partition coefficients of some detected PhACs and they reported that the sorption of PhACs onto these sediments has doubled within the last 9.2 years (Lara-Martín et al., 2015).

1.3 Metabolism of PhACs

Before entering the environment PhACs metabolize in humans and animals. The metabolism of PhACs in the human body, involves enzyme-catalyzed chemical modification of the drug. This biotransformation process generally alters the physiochemical properties of the drug, such as the lipophilicity and turns the molecule into a more hydrophilic form. This metabolic strategy usually creates pharmacologically inactive metabolites successively more polar than the parent compound, thereby enhancing excretion in urine and faeces (Holčapek et al., 2008). The biotransformation processes that PhACs can undergo during human metabolism are shown in figure 1.4. In mammals and in vertebrate aquatic species as well, these metabolic processes are governed by two different reaction steps:

(i) Phase I involves in-vivo biochemical reactions where metabolites are the result of oxidations, reductions, and hydrolysis, by the use of monooxygenases (e.g., CYP), reductases, and hydrolases

(for esters and epoxides) (Holčapek et al., 2008). These enzymes convert lipophilic organic molecules to more water-soluble compounds by introducing or unmasking functional groups such as –OH, or –COOH (Celiz et al., 2009; Parkinson et al., 2011).

(ii) Phase II uses covalent conjugation to make the molecule hydrophilic and more excretable. Phase II metabolites are the result of biochemical subsequent reactions of the parent drug or the Phase I metabolites, with other molecules present in the body to form O- and N-glucuronides, sulfates, and glutathione adducts (Celiz et al., 2009), as well as conjugation with amino acids (such as glycine, taurine, and glutamic acid) (Holčapek et al., 2008). These reactions are catalyzed by UDP-glucuronosyltransferases (UGT) and sulfotransferases (SULT) (for hydroxyaromatics), glutathione S-transferases (GST) (for electrophilic functional groups such as halogens, nitro groups, or unsaturated/conjugated sites), acetyltransferases (for primary amines or hydrazines), and aminoacyltransferases (for forming peptides from carboxy groups using free amino acids). Phase II metabolites (often termed conjugated metabolites) are transported out of the liver for elimination in bile or urine, respectively (Parkinson et al., 2011).

A common pathway of drug metabolism involves hydroxylation by cytochrome P450 (CYP450) (Phase I) followed by glucuronidation by UGT in Phase II (figure 1.4). However, some PhACs are directly conjugated

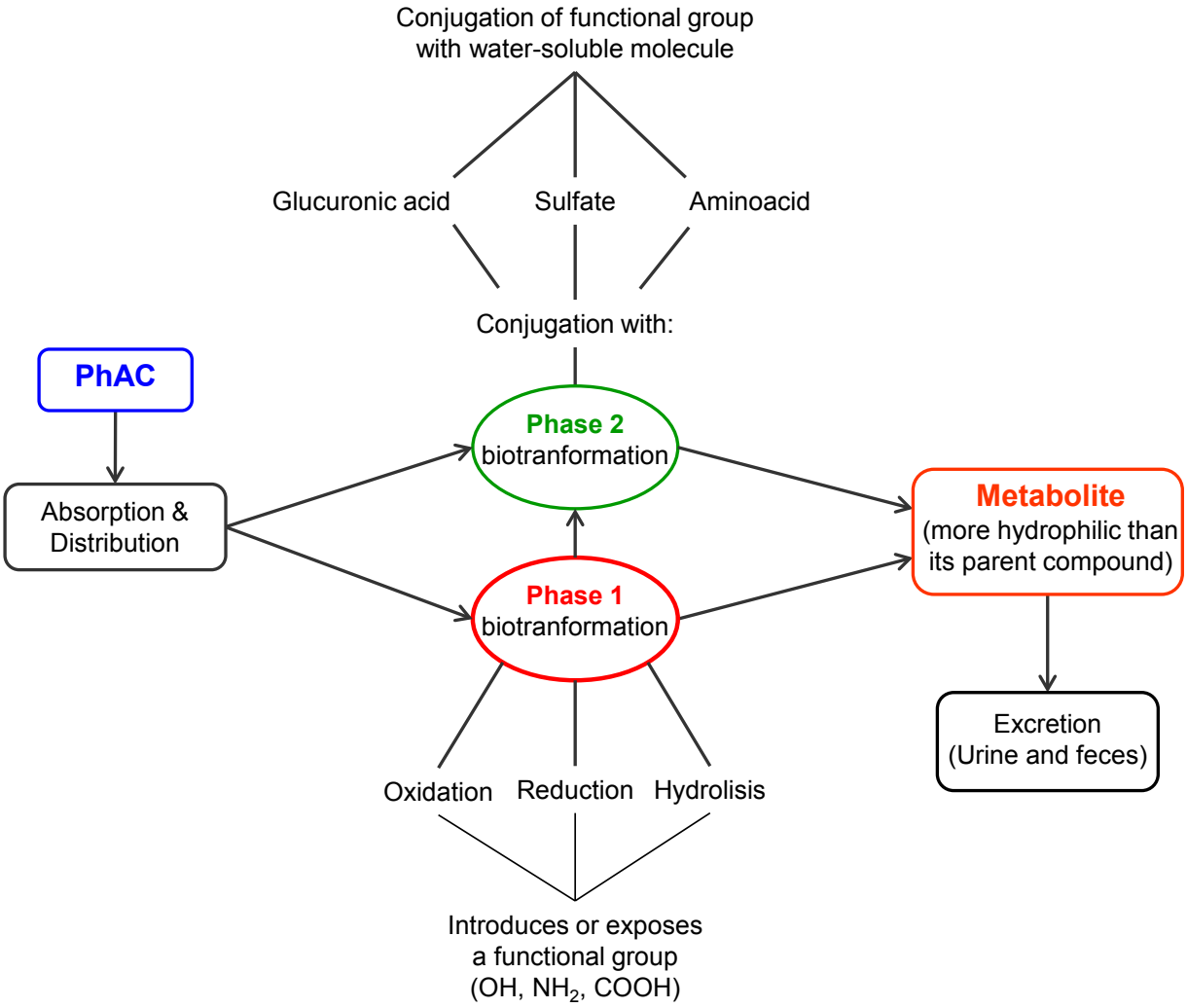


Figure 1.4. Biotransformation processes of PhACs during human metabolism.

without being biotransformed to Phase I metabolites. I.e. acetaminophen, which undergoes direct metabolism by both Phase I and Phase II enzymes. In a few cases, the two steps occur in reverse order, drug conjugates (Phase II) are metabolized by CYP as the glucuronide conjugate of DCF (Parkinson et al., 2011; Kumar et al., 2002).

As an example, figure 1.5 shows the biotransformation pathways of DCF after oral intake and the diverse subsequent metabolites generated. Approximately 65% of the oral dosage of DCF is excreted in urine, 15% as the parent compound (Forrez et al., 2011) and the rest as metabolites at different percentages (figure

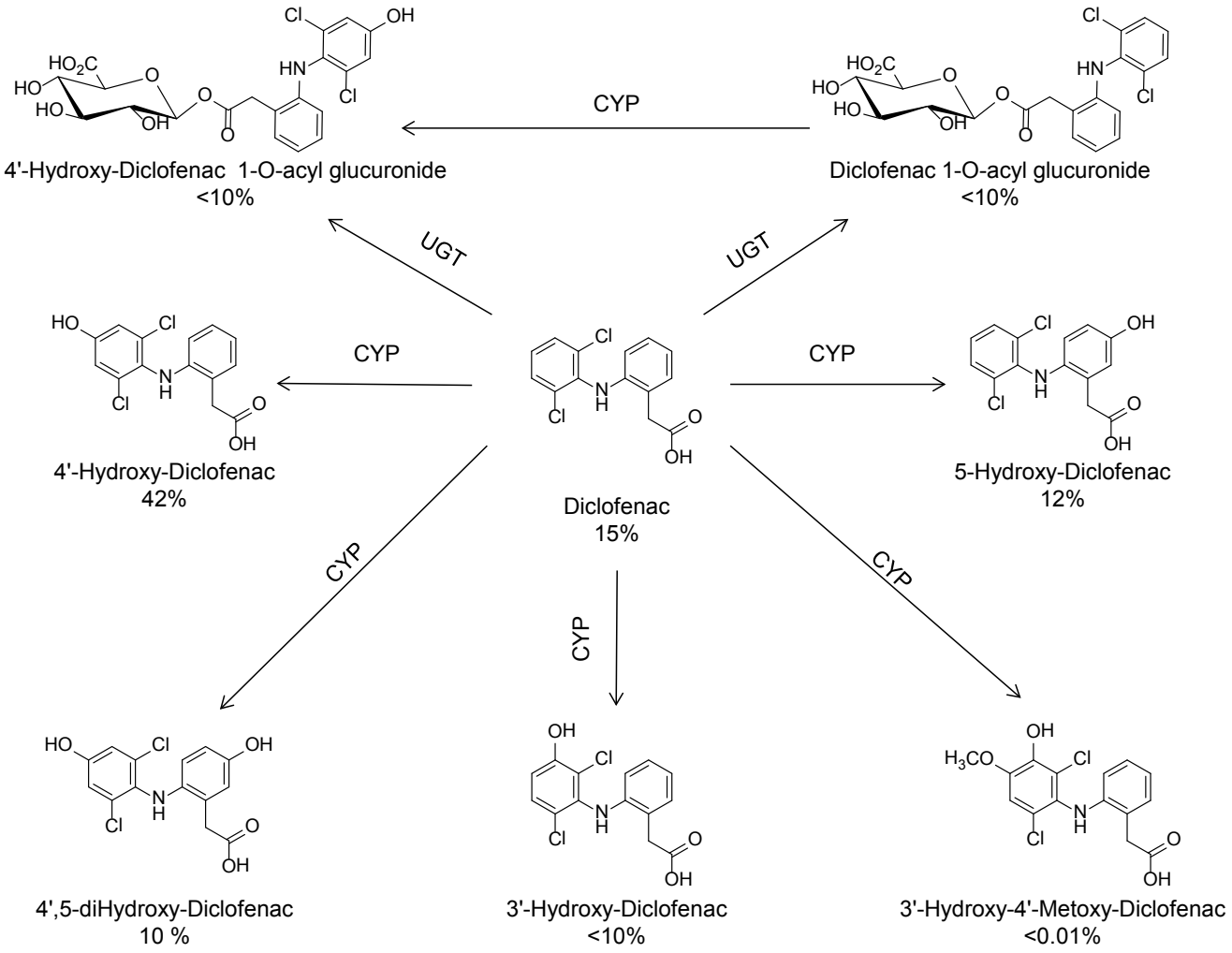


Figure 1.5. Human biotransformation routes of DCF. Percentages of excretion are indicated as well as enzymes involved.

1.5). During hepatic metabolism in the human body, DCF undergoes hydroxylation to yield predominantly 4'-hydroxydiclofenac (4'-OH-DCF) and 5-hydroxydiclofenac (5-OH-DCF) while glucuronidation of the carboxylic acid produces 1-O-acyl glucuronide (DCF-Gluc) (Stierlin et al., 1979a; Stierlin and Faigle, 1979b) (figure 1.5). However, DCF-Gluc can undergo hydroxylation to the 4'-hydroxydiclofenac acyl glucuronide (Kumar et al., 2002). The formed metabolites are excreted via biliary (faeces) and urine routes.

Metabolism of PhACs is usually incomplete and a percentage of the non-altered parent compound is excreted in urine and faeces. This percentage can vary depending on the physicochemical and biological

properties of the drug. For example up to a 90, 70, >70, >70, <40, <40, <5, <5 and <5% of atenolol, bezafibrate, trimethoprim, ciprofloxacine; sulfamethoxazol, salycilic acid, ibuprofen, acetaminophen and carbamazepine, respectively, are excreted unmetabolized (Pal et al., 2010). Owing to that, a combination of the non-altered drugs and their metabolites, considered hereafter as Transformation Products (TPs), will be excreted in urine and faeces ending up in the waste water treatment plants (WWTPs) (Kümmerer et al., 2008: Kunkel and Radke, 2012).

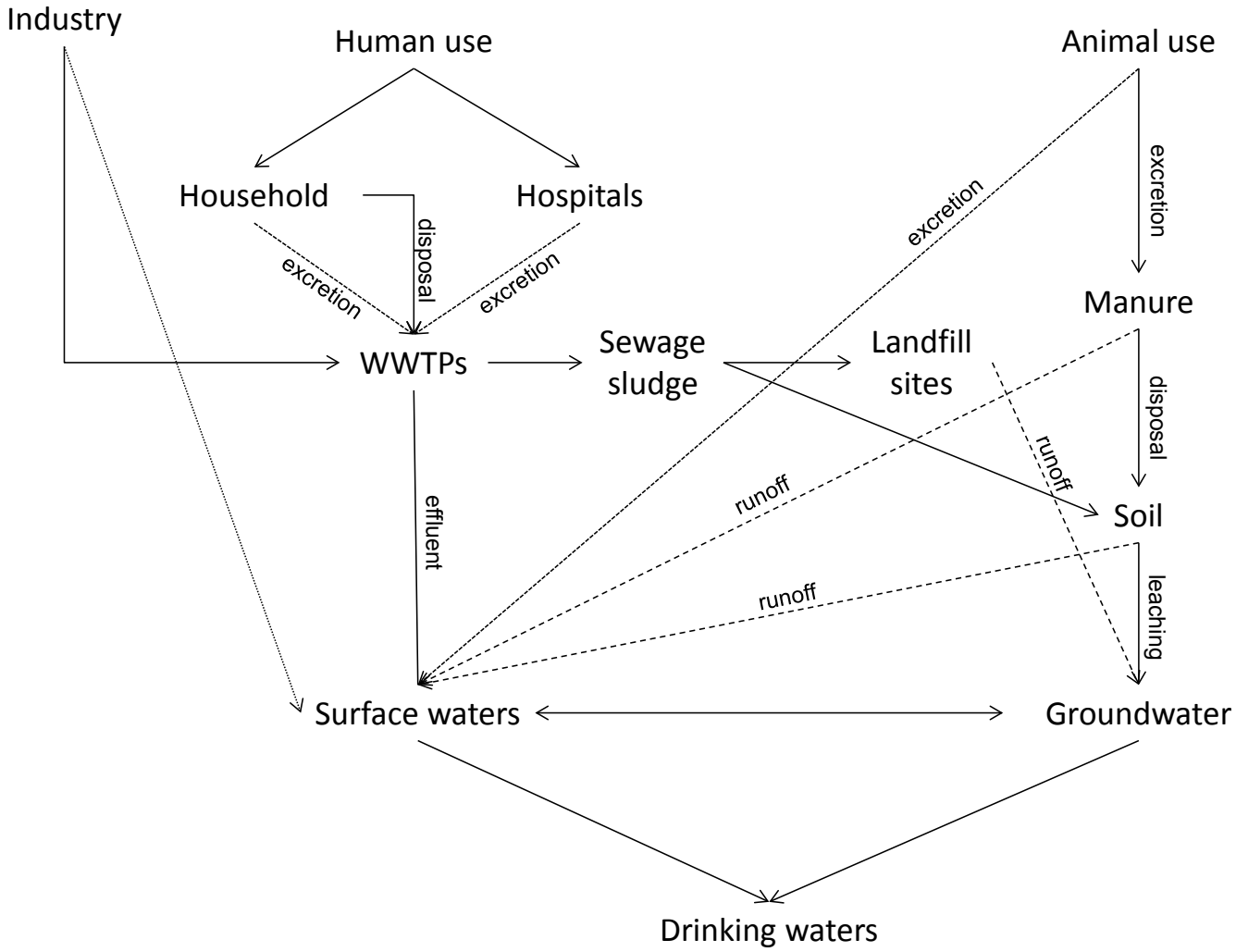


Figure 1.6. Environmental fate of PhACs along the water cycle.

1.4 Sources of emission and routes of entry of PhACs and their TPs into the aquatic environment.

PhACs can be introduced into aquatic systems by different emission sources (figure 1.6). These include industrial waste, disposal in household waste via the sink/drain animal husbandry practices, hospital or urban wastewater (WW) (Daughton 2013). After PhACs uptake and excretion by an organism, they enter the aquatic environment together with their TPs.

Despite “good management practices” (EPA, 1997) applied in manufacture of pharmaceutical products and the treatment of their industrial WW, the subsequent treated waters are also regarded as a potential source of PhACs to receiving waters (Larsson et al., 2015).

In addition, disposal of unused PhACs is another relevant source of these substances into the sewage. According to the EU legislation (EC, 2004), all unused or expired pharmaceutical products should be appropriately collected and destroyed. This waste is usually incinerated, however, if it is land-filled, disposal site effluents can be a source for the contamination of SW and ground water (GW) (Metzger, 2004). Furthermore, the EU legislation is usually violated in households by discarding remaining and expired drug products into the toilet (Seehusen and Edwards, 2006). Moreover, non-metabolized PhACs may also end-up in the aquatic environment through the urban WW system and WWTPs by direct flush of unused drugs or washing off of those topically applied (Daughton and Ruhoy, 2013).

Nevertheless, the main point source of PhACs into the aquatic environment is the excretion of PhACs in the present or metabolized form by humans and animals (figure 1.6). The following routes of exposure to SW of PhACs consumed by humans differ considerably from those used in animal treatment. Veterinary PhACs used in pasture animals and aquaculture, can be directly released into the SW (or sea waters in the particular case of marine aquaculture), or indirectly with manure into SW or soils (Kools et al., 2008a). PhACs entering the terrestrial environment can reach SW by leaching from the soil into GW or by run-off from agricultural fields, treated with livestock slurries (Kümmerer et al., 2010). In contrast, PhACs and their human metabolites excreted by humans, end up in WWTPs by direct disposal through domestic and hospital WW (Ternes and Joss, 2007; Schuster et al., 2008). Hospital patients are administered relatively high quantities of drugs and therefore hospital WW can consistently contribute to the total load of PhACs in sewage wastewater influents (WWi) (Al Aukidy et al., 2014). During WW treatment, PhACs mainly undergo biodegradation or sorption into the activated sludge (Pérez et al., 2006 Pérez and Barceló, 2008; Jelić et al., 2011). At this stage, PhACs and their TPs that are not completely removed, end up in receiving freshwater systems through wastewater effluents (WWe). Adsorbed compounds can reach the terrestrial environment when sludge is used as an agricultural fertilizer and, following the same pathway of those in animal treatment, end up in GW (Daughton, 2013).

Importantly, human and veterinary PhACs, and their TPs present in GW and SW can finally reach drinking waters (DW), if treatment at the drinking water treatment plant (DWTP) is not sufficient (Benotti et al., 2008; de Jongh et al., 2012; Petrović et al., 2014).

1.5 Analyisis of PhACs and their TPs

The fact that ultratrace amounts of PhACs can reach the environment implies the need for developing selective and sensitive analytical methods. Afterwards the fate and degradation of PhACs in the WWTPs and receiving waters can be measured. The different steps followed in any analytical method are: sampling and preservation of the sample, sample preparation, detection and identification, quantification and data processing.

Several analytical procedures have been developed and applied to a wide diversity of studies focused on PhACs. For instance, the analysis of their occurrence and distribution along different environmental compartments, aquatic systems and biota; or the structural elucidation and identification in real samples of novel TPs of PhACs formed by anthropogenic or natural transformation processes. Table 1.1 provides representative examples of diverse analytical procedures applied in different studies conducted recently (Carmona et al., 2014; Darwano et al., 2014; Machado et al., 2015; Huntscha et al., 2012; Kostich et al., 2014; Li et al., 2014; Jank et al., 2015; Haddad and Kümmerer 2014; de Almeida et al., 2015; Boleda et al.,

2015; Boix et al., 2014; Chitescu et al., 2015; Kosma et al., 2015; Wang and Gardinali, 2014; Radović et al., 2015; Patrolecco et al., 2015; Li et al., 2012; Kosjek et al., 2012). The following details are provided to describe the cited studies: number of target compounds, sample type, sampling strategy, sample treatment, technique used, chromatographic conditions, and Mass Spectrometry (MS) analysis applied. As it can be noticed in table 1.1, the occurrence, fate and behavior of PhACs is assessed in all kind of matrices (e.g. WW; SW; GW; DW; sediments; Suspended Particulate Matter, SPM).

The different steps followed and to be optimized in any analytical method depending on the aims of the study are: sampling and preservation of the sample, sample preparation, detection and identification. The sampling procedure (i.e. grab or composite sample collection) is selected according to the questions addressed in a given study. Similarly, the type of matrix and physicochemical properties of the target compounds determine the strategy followed during the preparation of the sample for further analysis. This step requires optimization by means of recovery tests in order to evaluate and decide the most appropriate procedure (e.g. solid phase extraction (SPE) for aqueous samples and liquid extraction (LE) matrix solid phase dispersion (MSPD), ultrasonic sound extraction (USE) or pressurized liquid extraction (PLE) for solid samples). Due to its versatility, specificity and selectivity; high performance liquid chromatography (HPLC) or ultra high performance liquid chromatography (UHPLC) coupled to mass spectrometry (MS), high resolution mass spectrometry (HRMS) or tandem mass spectrometry (MS/MS) are the preferred analytical tools applied for the detection and identification of PhACs and their TPs in environmental samples (Agüera et al., 2013; Ferrer and Thurman, 2013; Richardson and Ternes, 2014) (and see table 1.1). In addition, the LC-MS interfaces most commonly used are the atmospheric pressure sources electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI) (Richardson and Ternes, 2014). However, other techniques can also be appropriate such as the laser diode thermal desorption (LDTD) sample introduction interface directly coupled to an MS/MS instrument, can lead to rapid identification, confirmation, and quantification of target analytes (see table 1.1, Darwano et al., 2014). A wide panel of different mass spectrometers and combinations of them can be used for the analysis of PhACs and TPs, from the single quadrupoles (Q) and Ion Traps (IT) to the hybrid triple quadrupoles (QqQ), quadrupole linear ion traps (QqLIT), and the high resolution quadrupole time of flight (QTOF) or hybrid Orbitrap analyzers (table 1.1). These instruments provide a broad possibility of MS experiments making them suitable for the quantification, identification and structural elucidation of PhACs and TPs (e.g. selected ion monitoring (SIM), selected reaction monitoring (SRM), multiple reaction monitoring (MRM), n fragmentation mass spectrometry (MS^n), elevated mass spectrometry (MS^E), independent full scan & data-dependent scanning, information dependent acquisition (IDA), enhanced product ion (EPI) (table 1.1).

1.6 Presence of PhACs and their TPs in WWTPs and rivers

The environmental occurrence of a drug can vary depending on the quantity manufactured, the amount and frequency of consumption, the persistence, the compound metabolism, and the effectiveness of WWTPs in removing these micro-contaminants from WWi. Since the 1980s, different studies conducted worldwide reported the occurrence in the environment of PhACs and their TPs (Richardson and Bowron, 1985). The first substance of medicinal origin detected was clofibric acid, a metabolite of the lipid regulators clofibrate and etofibrate. This compound was reported in US treated WWe at concentrations ranging from 0.8 to 2 μg L^{-1} (Garrison et al., 1976). Since then, clofibric acid has become the most widely detected PhAC. However, scientific attention was primarily drawn to the antibiotics group, as a consequence of the observed alterations on the structure of microbial communities and the development of resistance in potential human pathogens

Table 1.1 Summary of the diverse methodologies that have been applied for the analysis of PhACs and their TPs.

Target compounds	Sample Type	Sampling description	Sample treatment	Technique	Chromatographic conditions	MS analysis	Study and Reference
10 PhACs	WWi, WWe	Grab samples	Off-line SPE Cartridge: Strata-X 33U Eluent: methanol	UHPLC-(-)ESI MS/MS	Column: Waters Sunfire C18 (2.1 x 50 mm, 3.5 µm) Mobile phase: A)1 mM ammonium fluoride in H_2O B) 1 mM ammonium fluoride in methanol	6410 Triple Quad (QqQ MS) SRM mode	Analytical method development and validation for occurrence & distribution assessment Carmona et al., 2014
	SW						
	DW						
	Sediments		MSPD Solvent: acetonitrile Extraction material: $MgSO_4$, NaCl, trisodium citrate dihydrate, sodium sesquihydrate citrate, PSA, C18.				
10 PhACs	Sediments	Grab samples	USE Eluent: methanol & acetone (3:1, v/v)	LDTD-(+)-(APCI)-MS/MS	Thermal desorption by infrared laser diode (980 nm 20 W,continuous) carrier gas : medical-grade purified air	TSQ Quantum Ultra AM (QqQ MS) SRM mode	Analytical method development and validation for occurrence & distribution assessment Darwano et al., 2014
	SW SPM	Integrated samples using an SPM trap					
	WW SPM	Grab samples by decantation & filtration of WW	Off-line SPE Cartridge: Strata-C18 Eluent: acetonitrile: H_2O (7:3, v/v)				
	GW						
	SW						
	WWe						

Rosuvastatin (cholesterol lowering statin drug) & TPs	WWe after AOP treatment (photocatalisis)	Sequenced collection of samples from the reactor at fixed times	Centrifugation	nano-UHPLC–(+)ESI-HRMS/MS	Column: Acquity UPLC BEH 130 C18 (18 µm x 100 mm, 1.7 µm) Mobile phase: A) 0.1 % formic acid in water B) 0.1 % formic acid in acetonitrile	Q-TOF Micro™ Full scan & product ion MS spectra mode	Identification of Photo-TPs Machado et al., 2015
88 polar organic micropollutants including 33 PhACs & TPs	GW	Grab samples	On-line SPE Cartridge: mixed-mode multibed Oasis HLB (15 µm) &1:1:1.5 mixture of Strata X-AW, Strata X-CW, Isolute ENV+ Elution: 0.1% formic acid in methanol	HPLC-(±)ESI-MS/MS	Column: Atlantis T3 (3.0 x150 mm, 3 µm) Mobile phase: A) 5 mM ammonium acetate in H_2O B) Acidic methanol with 0.1% formic acid	TSQ Quantum MS (QqQ MS) SRM mode	Analytical method development and validation for occurrence & distribution assessment Huntscha et al., 2012
	SW						
	WWe	Composite (24h) samples					
56 PhACs	WWe	Composite (24h) samples	Method 1 Off-line SPE Cartridge: Oasis MCX Eluent: - acetonitrile for acidic and neutral analytes -5% ammonium hydroxide in acetonitrile for basic analytes	UHPLC-(±)ESI-MS/MS	Column: BEH C18 (1.0 x 100mm, 1.7 µm) Mobile phase: A) 0.3% formic acid in H_2O B) methanol:acetonitrile (2:1)	Micromass Quattro Micro (QqQ MS) MRM mode	Quantitative analysis for occurrence assessment Kostich et al., 2014

56 PhACs	WWe	Composite (24h) samples	Method 2 Off-line SPE (for antibiotics) Cartridge: Oasis HLB Eluent: 1% ammonium hydroxide in acetonitrile	HPLC-(+)ESI-MS/MS	Column: BEH Phenyl (1.0 x 100 mm, 1.7 µm) Mobile phase: A) acetonitrile B) methanol C) 0.3% formic acid in H_2O	LCQ Advantage (IT-MS) Independent full scan & data-dependent scanning modes	Quantitative analysis for occurrence assessment Kostich et al., 2014
Unknowns	Spiked artificial river water	Sequenced collection of samples from the water/sediment compartment at fixed times	Direct injection	UHPLC-(±)ESI-HRMS/MS	Column: HSS T3 (100 mm × 2.1 mm, 1.8 µm) Mobile phase: A) 10 mM formic acid 5% acetonitrile in H_2O B) 10 mM formic acid 5% water in acetonitrile	QToF Premier MS Full scan and MS^E modes	Screening of PhACs and their TPs formed in river sediment Li et al., 2014
8 antibiotics	WWi, WWe	Grab samples	Off-line SPE Cartridge: Oasis HLB Eluent: -50mM formic acid in methanol -50mM formic acid in acetone	UHPLC-(±)ESI-HRMS/MS	Column: Zorbax Eclipse XDB-C18 (150 x 4.6 mm, 5 µm) Mobile phase: A) 0.1 % formic acid in water B) 0.1 % formic acid in acetonitrile	API 5000 (QqQ MS) MRM mode	Quantitative analysis for occurrence assessment Jank et al., 2015
	SW						

Ciprofloxacin & TPs	SW after artificial solar irradiation	Sequenced collection of irradiated aliquots of the sample at fixed times	Not described	HPLC-(+)-ESI-MS/MS	Column: Nucleodur C18 EC (125 mm x4 mm, 5 µm) Mobile phase: A) 0.1 % formic acid in H_2O B) acetonitrile	Esquire 6000 Plus (IT MS) Full scan & product ion MS^3 spectra mode	Quantification & structural elucidation of natural Photo-TPs Haddad et al., 2014
				UHPLC-(+)-ESI-HRMS/MS		LTQ-Orbitrap XL MS Full scan mode	
Carbamazepine, diazepam &their TPs	Hospital WWe	Sequenced collection of samples at fixed times	Off-line SPE Cartridge: C18 Premium Eluent: methanol:0.1 % acetic acid in water(80:20, v/v)	HPLC-(+)-ESI-MS/MS	Column: Zorbax SB C18 (4.6×150 mm, 5 µm) Mobile phase: A) 1 % formic acid in water B) 1 % formic acid in methanol	API 400 QTRAP (QqLIT MS) MRM, IDA and EPI modes	Determination & identification of PhAC TPs Almeida et al., 2015
54 PhACs	DW	Grab samples	Off-line SPE Cartridge: Oasis HLB Eluent: methanol	UHPLC-(±)ESI-MS/MS	Column Acquity BEH C18 column (100 × 2.1 mm., 1.7 µm) Mobile phase: (+)-ESI mode: A) 6.5 mM ammonium acetate in H_2O B) acetonitrile:methanol (2:1) Mobile phase: (-)-ESI mode: A) 6.5 mM ammonium acetate/ acetic acid in H_2O B) methanol	Micromass Quattro Micro (QqQ MS) SRM mode	Quantitative analysis for occurrence assessment & confirmation of positive detections Boleda et al., 2015
				UHPLC-(±)ESI-HRMS/MS		Q-Exactive (Q-Orbitrap MS) SIM & t-MS^2 modes	

Omeprazole (treatment of gastric diseases) & its TPs	Human urine	Sequenced collection of samples after intake at fixed times	Centrifugation & Hydrolization (β-D-glucuronidase)	UHPLC-(±)ESI-HRMS/MS	Column Acquity BEH C18 column (100 × 2.1 mm., 1.7 µm) Mobile phase: A) 0.01 % formic acid in water B) 0.01 % formic acid in methanol	QToF Premier MS Full scan and MS^E modes	Structural elucidation of PhACs' metabolites & Identification in real samples Boix et al., 2014
	WWe	Grab samples	Not described	UHPLC-(±)ESI-MS/MS		TQD (QqQ MS)	
	SW						
67 PhACs	SW	Grab samples	Off-line SPE Cartridge: Strata-X Eluent: methanol	UHPLC-(±)ESI-HRMS/MS	Column HSS T3 (100 × 2.1 mm, 1.8 µm) Mobile phase: A) 2mM ammonium formiate in H_2O (pH 3.5) B) 2mM ammonium formiate in methanol (pH 3.5)	Q-Exactive (Q-Orbitrap MS) Full scan mode	Multiresidue quantitative analysis for occurrence assessment Chitescu et al., 2015
Metformin and its TP Guanylurea	WWi, WWe	Composite (24h) samples	Off-line SPE Cartridge: Oasis HLB Eluent: methanol	HPLC-UV/Vis-(+)-ESI/MS	Column C18 (150 x4.6 mm, 5 µm) Mobile phase: A) 0.1% formic acid in water B) 0.1% formic acid in acetonitrile	SPD 20A UVevis coupled to 2010 EV MSD (Q MS) SIM mode	Quantitative analysis for occurrence assessment & confirmation of positives Kosma et al., 2015
				HPLC-(+)ESI-HRMS	Column hypersil gold (150 x 2.1 mm, 5 µm) Mobile phase: A) 0.1% formic acid in water B) 0.1% formic acid in acetonitrile	Orbitrap MS Full scan mode	

Sulfamethoxazole & its TPs	Reclaimed water	Grab samples	Off-line SPE Cartridge: Oasis HLB Eluent: methanol	HPLC-(±)-ESI-HRMS/MS	Column: Hypersil GOLD (50 x 2.1 mm, 3 µm) Mobile phase: A)0.1% formic acid in water B) acetonitrile	Q-Exactive (Q-Orbitrap MS) Full scan & data dependent mode	Identification & quantitative analysis for occurrence assessment Wang et al., 2014
13 PhACs and TPs	SW GW Sediments	Grab samples	Off-line SPE Cartridge: Oasis HLB Eluent: methanol USE Eluent: methanol:dichloromethane (1:1)	HPLC-(+)ESI MS/MS	Column: Zorbax Eclipse® XDB–C18 (75 mm×4.6 mm, 3.5 µm) Mobile phase: A) Water B) methanol C) 10% acetic acid in water	LCQ Advantage (QqLIT MS) SRM mode	Analytical method development and validation for occurrence & distribution assessment Radovic et al., 2015
12 PhACs	WWi, WWe SW	Composite (24h) samples Grab samples	Off-line SPE Cartridge: Strata-X Eluent: acetone	HPLC-(±)ESI MS/MS	Column: Hypersil Gold (100×2.1 mm, 5 µm) Mobile phase: A) 0.1 % formic acid in water B) acetonitrile	LCQ DECA XP MAX MS (IT-MS) SRM mode	Quantitative analysis for occurrence assessment Patrolecco et al., 2015

22 antibiotics	Plant Animal Tissue Sediment	Grab samples	PLE (ASE 350) Solvent: methanol temperature: 70ºC static time: 10 min cycles: 2. & Off-line automatized SPE (AutoTrace SPE 280) Cartridge: Oasis HLB Eluent: 5% ammonium hydroxide in methanol	HPLC-(+)ESI MS/MS	XTerra MS C_{18} column (100 x 2 mm, 3 µm) Mobile phases: A)Methanol–acetonitrile (1:1, V/V) B) 0.3% formic acid/water containing 0.1% ammonium formate, V/V, pH = 2.9	API 3200 MS (QqQ MS) MRM mode	Quantitative analysis for occurrence & distribution assessment Li et al., 2012
	SW		Off-line automatized SPE (AutoTrace SPE 280) Cartridge: Oasis HLB Eluent: 5% ammonium hydroxide in methanol				
Diazepam, oxazepam & bromazepam	WWi, WWe SW	Grab samples	SPE Cartridge: Oasis HLB Eluent: - acetone acetone:ethylacetate(3:7) - ethylacetate	UPLC-(+)ESI-HRMS/MS	Acquity UPLC™ BEH C_{18} (3cm x 2.1mm, 1.7 µm) Mobile phases: A) 0.1% formic acid B) Acetonitrile	QTOF-MS Full scan & product ion MS spectra mode	Study of behavior in water treatment Kosjek et al., 2012

(Halling-Sørensen et al., 1998). Later, PhACs were recognized as compounds of emerging concern (Daughton and Ternes, 1999). Nowadays, PhACs are well known widespread micro-contaminants in the environment, being present in numerous compartments including WW streams, rivers, estuaries, seawaters, GW, soils, sediments and biota (Daughton and Ruhoy, 2009). Moreover, PhACs have been detected even in supposedly pristine areas as the Antarctic environment (Emnet et al., 2015). Several extensive reviews provided detailed information about the occurrence of PhACs, and their TPs in the aquatic environment (e.g., Halling-Sørensen et al., 1998; Daughton and Ternes, 1999; GWRC, 2004; Fent et al., 2006; Sadezky et al., 2008; Mompelat et al., 2009; Kumerer et al., 2010; Monteiro and Boxall, 2010; Pal et al., 2010; Hughes et al., 2012; Ying et al., 2013).

Of the 3,193 PhACs commercially available worldwide, only 275 drugs have been analyzed in the environment (Howard and Muir, 2011). In general, PhACs and their TPs occur in the range of μg L^{-1} in WWi and WWe while in SW and GW they are detected in the ng L^{-1} range (Fent et al., 2006; Celiz et al., 2009; Fatta-Kassinos et al., 2011a; Kunkel and Radke, 2012; Ying et al., 2013, Michael et al., 2014a).

An overview of the presence of PhACs in WWTPs was reported by Verlicchi et al. (2012). The authors reviewed the levels of 118 PhACs, belonging to different therapeutic classes, determined in WWi and WWe of 264 WWTPs across Europe. The diverse PhAC families showed variable concentrations ranges and were higher in WWi compared to the trends reviewed for WWe (see figure 1.7). Analgesics and antiinflammatories were reviewed as the most concentrated therapeutic group in WWi; while their levels in WWe were below those observed for lipid regulators and β-blockers. The most investigated and frequently detected individual compounds were ibuprofen, DCF, naproxen, ketoprofen and tramadol. Of these, ibuprofen showed the highest absolute concentrations in WWi, followed by tramadol. On the contrary, atenolol was reported as the most concentrated PhAC among the WWe reviewed. Interestingly, levels of psychiatric drugs as carbamazepine and diazepam did not differed too much between WWi and WWe, which pointed out the recalcitrant behavior of these PhACs during WW treatment. As for antibiotics, trimethoprim, sulfamethoxazole (SMX), erythromycin and ciprofloxacin were found the most ubiquitous and among these, ciprofloxacin was detected at the highest concentrations. As it is noticed on figure 1.7, levels of lipid regulators and β-blockers were higher in WWe relative to WWi. Among lipid regulators, bezafibrate and gemfibrozil were the most concentrated and frequently detected; while atenolol was for the β-blockers therapeutic group.

At the regional scale level, the occurrence of 15 of the more studied and frequently detected PhACs in WWi and WWe was reviewed among different countries of the globe (i.e. China, Greece, Korea, Spain, UK, Western Balkan WB, Sweeden, Switzerland and USA) (Luo et al., 2014). The PhACs reviewed belonged to the considered more relevant therapeutic groups: analgesics and antiinflamatories (i.e. acetaminophen, DCF, ibuprofen, ketoprofen, naproxen and salicylic acid); lipid regulators (i.e. bezafibrate and gemfibrozil); antibiotics (i.e. SMX, trimethoprim and erithromycin); psychiatric treatment drugs (i.e. carbamazepine) and β-blockers (i.e. atenolol, metoprolol and propanolol). According to the average values reported in the different studies reviewed by Luo et al. (2014), the family of analgesics and antiinflamatories was detected at the highest concentrations in WWi of Spain reaching individual levels of 44,800; 1,450; 86,367; 1,645; 5,467 and 35,100 ng L^{-1} for acetaminophen, DCF, ibuprofen, ketoprofen, naproxen and salicylic acid, respectively (Santos et al., 2008; Gracia-Lor et al., 2012). As for the levels determined in WWe, Spain presented the highest concentration of analgesics an antiinflamatories (6,993; 840; 2,057; and 969 ng L^{-1} for ibuprofen, ketoprofen, naproxen, and salicylic acid) (Santos et al., 2008; Gracia-Lor et al., 2012), with the exception of

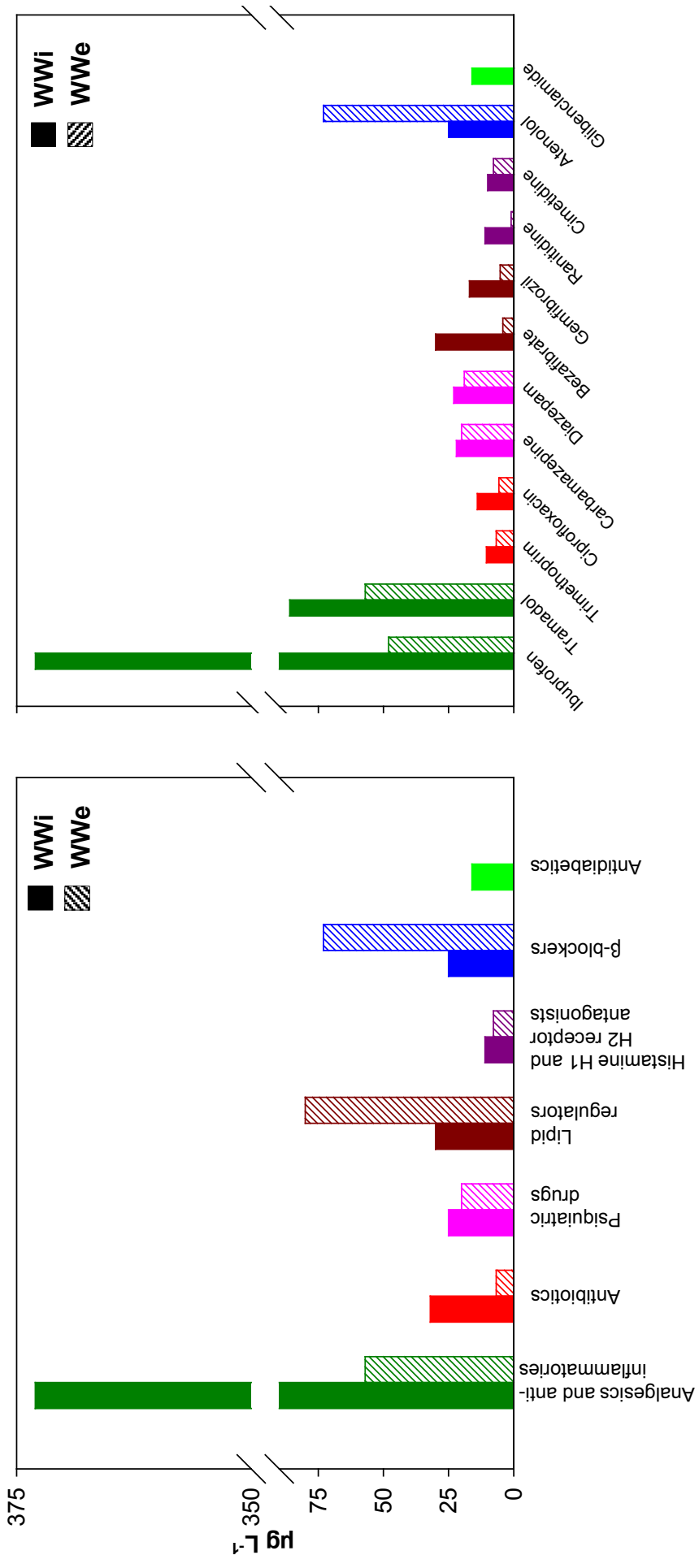


Figure 1.7. Maximum levels of therapeutic groups and individual PhACs detected in WWi and WWi reported in Verlicchi et al. (2012).

acetaminophen and DCF which were detected at highest levels (191 and 690 ng L^{-1}, respectively) in Greece (Stamatis and Konstatinou, 2013) and Switzerland (Singer et al., 2010), respectively. As for lipid regulators, bezafibrate was detected at the highest concentrations in both WWi and WWe of UK with average levels of 800 and 376 ng L^{-1} (Kasprzyk-Hordern et al., 2009); while for gemfibrofil maximum levels were observed in both WWi and WWe of Spain (1110 and 540 ng L^{-1}, respectively) (Gracia-Lor et al., 2012). Levels of carbamazepine were detected at maximum values in both WWi (1,820 ng L^{-1}) and WWe (2,620 ng L^{-1}) of UK (Kasprzyk-Hordern et al., 2009). Regarding antibiotics, SMX showed highest levels in WWi of the WB (1,180 ng L^{-1}) (Terzić et al., 2008); while for WWe these were highest in Spain (310 ng L^{-1}) (Ruel et al., 2010). As for the remaining compounds, trimethoprim and erythromycin top concentrations were observed in both WWi and WWe of UK with respective levels of 3,630 and 3499 ng L^{-1} in WWi; and 1,839 and 1,567 ng L^{-1} in WWe (Kasprzyk-Hordern et al., 2009). Finally, β-blockers atenolol and propanolol were detected in both WWi and WWe of UK at highest levels of 18,098 and 1,044 ng L^{-1} in WWi and 4,431 and 263 ng L^{-1} in WWe (Kasprzyk-Hordern et al., 2009); while for metoprolol maximum levels of 953 ng L^{-1} were observed in WWi of WB (Terzić et al., 2008) that were closely followed by those determined in China (917 ng L^{-1}), were also levels in WWe were maximum (241 ng L^{-1}) (Zhou et al., 2010).

Even more interesting is the work of Hughes et al. (2012), who analyzed at a global scale the reported data on the presence of 203 PhACs in WWe and SW across 41 countries (Hughes et al., 2012). The levels map on figure 1.8 shows the occurrence of PhACs in WWe impacted SW at a global scale. The database (Hughes et al., 2012) corresponds to median concentrations of a wide diversity of PhACs determined in

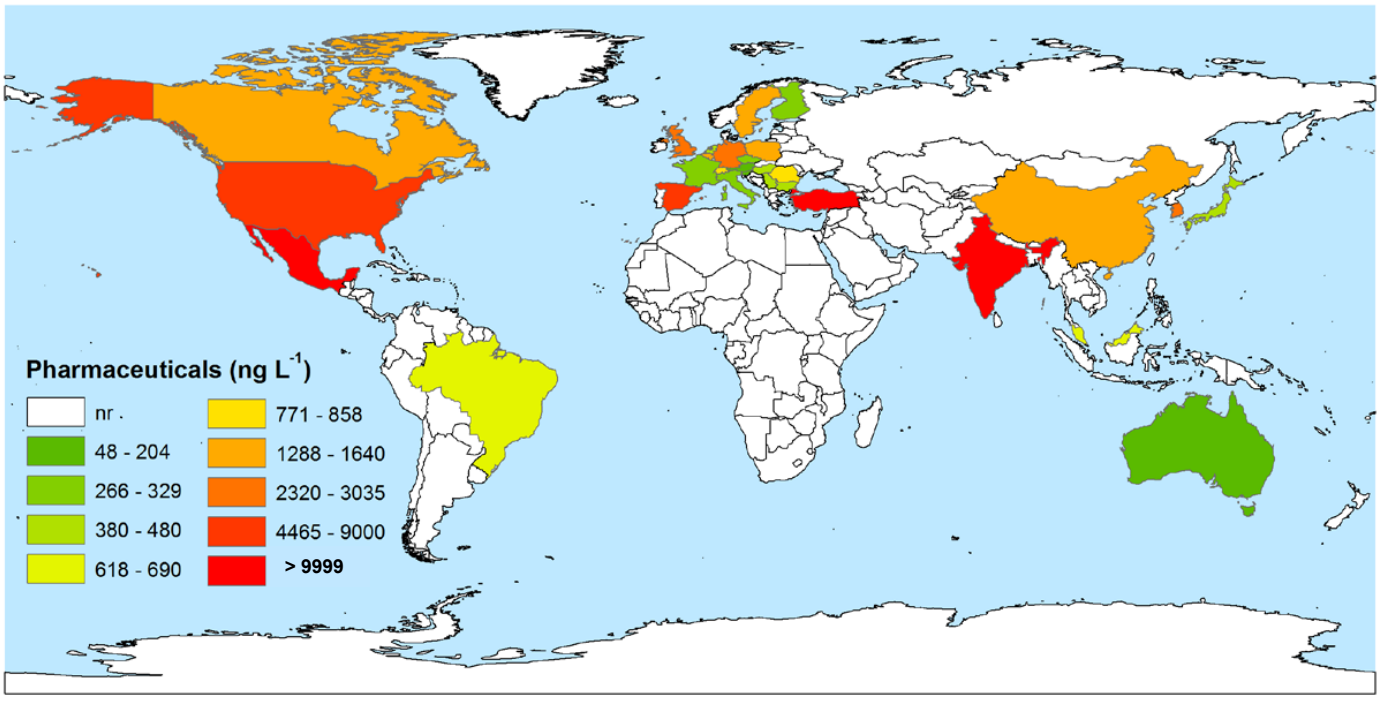


Figure 1.8. Occurrence of PhACs in SW receiving WWe. Data provided by Dr. Stephen Hughes (JBA consulting, NY, UK) (Hugues et al., 2010).

236 studies conducted worldwide during the period 1998-2010. These substances were globally present at concentrations ranging from 50 to 1,800,000 ng L^{-1}. As it can be clearly seen, Spain and the US were among the countries with higher levels of medicinal drugs present in its SW, with median concentrations ranging from 4,400 to 9,000 ng L^{-1}. However, the nations were the highest levels of PhACs were detected, among those reviewed in the study (Hughes et al., 2012) were India, Mexico and Turkey, with median concentrations reaching levels that exceeded the 9,999 ng L^{-1}.

Of the total number of drugs studied, 61 substances were reported as frequently detected both in WWe and SW (Hughes et al., 2012). Of these 61 compounds, 39, 21, 20 and 3 % were antibiotics, analgesics, cardiovascular drugs (like β-blocking agents, diuretics and calcium channel blockers) or blood lipid regulators, and antidepressants (psychiatric drugs), respectively. The frequency of detection of PhACs in SW, classified by their therapeutic activity was compared among WWe-receiving streams from over the world. Analgesics were the most ubiquitous in Europe accounting for the 34% of the reported studies, while antibiotics were the most frequently detected in North America and Asia (38% and 42%, respectively). Nevertheless, at a global scale, analgesics were the most ubiquitous therapeutic class with 31% of all data reported and a median concentration of 230 ng L^{-1} followed by antibiotics (21 %, 8,128 ng L^{-1}). Importantly, in a comparison among the top 10 most studied countries, levels of PhACs in Spain were substantially above the global mean concentration (171− 441%) for the principal therapeutic families namely: analgesics, cardiovascular drugs, blood lipid regulators and antidepressants.

Regarding individual compounds, carbamazepine, bezafibrate, clofibric acid, ibuprofen, and DCF were found the most relevant in WWe and SW, among the 61 PhACs most frequently detected according to Hughes et al. (2012). More recently, Ying et al. (2013) reported the levels of 61 PhACs belonging to different therapeutic classes detected in SW collected from rivers of 14 countries over the world. Among the reported antibiotics, SMX, ciprofloxacin, norfloxacin, ofloxacin, and clarithromycin were the more ubiquitous ones at levels of up to several μg L^{-1}. At a global scale, the reported concentrations for the most frequently detected analgesics and antiinflammatories (i.e. ibuprofen, DCF, mefenamic acid, naproxen, ketoprofen, salicylic acid, acetylsalicylic acid, meclofenamic acid, tolfenamic acid, and indomethacin) spanned from several ng L^{-1} to several μg L^{-1}. Several lipid regulators, such as clofibric acid, bezafibrate and gemfibrozil, were observed to be also ubiquitous in SW worldwide. As for psychiatric drugs, carbamazepine was the most frequently detected compound at concentrations varying from not detected to several μg L^{-1}. The last group reviewed were β-blockers, of which metoprolol, propranolol, and atenolol were the most widespread ones at levels ranging from not detected to several thousand ng L^{-1}.

Contrarily to the extensive reported data on PhACs occurrence in environmental waters, the number of studies assessing their presence in sediments is substantially lower. A good example is the previously mentioned review conducted by Ying et al. (2013). Apart from SW, it also includes sediments for the assessment of the occurrence of PhACs in rivers at a global scale. However, the presence of PhACs in sediments was only reported in five out of the fourteen countries reviewed. For instance, the antibiotics norfloxacin, ofloxacin, and ciprofloxacin were frequently detected in three Chinese rivers at concentrations of 5,770, 1,290 and 653 ng g^{-1}, respectively (Zhou et al., 2011). Conversely, analgesics and antiinflamatories such as ibuprofen, DCF, and clofibric acid were rarely found in sediments from the Spanish Mediterranean region and detected at levels below the limits of quantification of the analytical method (Vázquez-Roig et al., 2012). In this study, the lipid regulators fenofibrate, clofibric acid; the psychiatric drugs carbamazepine

and diazepam and the β-blockers metoprolol and propranolol were among the compounds more frequently detected and at high concentrations as well. Other studies reported concentrations of psychiatric drugs at the ng g^{-1} range in sediments from US streams (Schultz et al., 2010). Among them, venlafaxine and fluoxetine were the predominant drugs observed, determined at levels of 26 and 19 ng g^{-1}, respectively. In the line of the work of Vázquez-Roig et al. (2011), the distribution of a larger list of PhACs was studied along the water column of a Spanish river (da Silva et al., 2011). Out of the 34 compounds studied, the higher concentrations were measured for acetaminophen (222 ng g^{-1}), mevastatin (99 ng g^{-1}) and tylosin A (71 ng g^{-1}). Other PhACs, such as erythromycin, ibuprofen and ranitidine were detected at maximum concentrations of 33, 19 and 25 ng g^{-1}, respectively, while cimetidine and clofibric acid were detected at levels below 20 ng g^{-1}. The remaining compounds were found at concentrations below 10 ng g^{-1}.

Although the occurrence of PhACs in the aquatic environment was well documented, the lack of literature on the presence of PhACs' TPs was evidenced by Celiz et al. (2009) and Mompelat et al. (2009). However, diverse studies have reported the identification and levels of TPs in environmental waters. For instance, norfluoxetine, the main human metabolite of fluoxetine, was detected at concentrations between 4 and 25 ng L^{-1} in WWe (Vanderford et al., 2006); 0.9 and 14 ng L^{-1} in SW; and 0.02 and 3 ng g^{-1} in sediments (Schultz et al., 2010). In the last study cited, the levels of norsertraline, the main human metabolite of sertraline, were determined in SW ranging from 1.13 to 26.7 ng L^{-1} and in sediment spanning from 0.02 to 10.7 ng g^{-1}. Similarly, five human metabolites of carbamazepine were detected at levels between 8.5 and 1,571 ng L^{-1} in WWi and between 9.3 and 1,325 ng L^{-1}in WWe (Miao et al., 2003). Only 10,11-dihydro-10,11 dihydroxycarbamazepine was found in SW but at ~3 times higher concentrations than that of its parent compound. Importantly, the studies assessing the occurrence, fate and behavior of PhAC's TPs in the aquatic environment have substantially increased in recent years (Fatta-Kassinos et al., 2011a; Michael et al., 2014a; Evgenidou et al., 2015). For example, 13 metabolites of PhACs belonging to different therapeutic classes, such as 4'-OH-DCF, oxazepam glucuronide or N-acetylsulfamethoxazole, were detected in SW from a Spanish river at levels that spanned from 0.96 to 1,670 ng L^{-1} (López-Serna et al., 2012). More recently, the presence of six iodinated contrast media (ICM) and their phototransformation products (photo-TPs) has been determined in SW (Zonja et al., 2015). The median concentration of the parent compounds was 110 ng L^{-1} reaching up to 6 ng L^{-1} for iomeprol, while TPs were found at median concentration of 8 ng L^{-1}, reaching up to 0.4 ng L^{-1} for iomeprol 's TP651-B.

However, although conjugated metabolites have been identified in both human and animal excreta, they are rarely detected in SW. This is mainly because the appropriate analytical methods capable to detect these TPs were not used. Nevertheless, the work of Ferrer and Thurman (2010), among the few examples found in the literature, presented a specific analytical method developed for the detection of the psychiatric drug lamotrigine and its human metabolite 2-*N*-glucuronide in environmental waters. They measured levels of the metabolite in WWe and SW of to 209 and 195 ng L^{-1}, respectively. However, the detections were sparse (frequencies of detection of 21 and 13 % in respective WWe and SW) and the locations assessed were highly impacted by WWTPs pressure. In addition, these compounds may undergo de-conjugation to transform back to their parent drug (commented previously in section 1.4). This possibility could also explain the reported increase of PhACs levels in WWe of Spanish WWTPs compared to those determined in WWi (Gros et al 2007, Jelić et al. 2011).

In other interesting study, the presence of ibuprofen and its main microbial TPs: ibuprofen carboxylic

acid, 2-hydroxy-ibuprofen and 1-hydroxy-ibuprofen were monitored quantitatively for the first time in WWi, WWe and the receiving SW (Ferrando-Climent et al., 2012). These TPs were found in WW samples at higher concentration than ibuprofen. The maximum concentrations in WWi samples were 13.7, 5.8, 38.4, 94.0 μg L^{-1} for ibuprofen, 1-hydroxylated ibuprofen, ibuprofen carboxylic acid and 2-hydroxylated ibuprofen respectively; whereas maximum levels in WWi samples were 1.9, 1.4, 10.7, 5.9 μg L^{-1} for ibuprofen, 1-hydroxylated ibuprofen, ibuprofen carboxylic acid and 2-hydroxylated ibuprofen, respectively. High levels were also found in river samples, in particular for ibuprofen carboxylic acid, which was detected up to 3.9 μg L^{-1}.

On the basis of the numerous evidences for the presence of PhACs and their TPs in the aquatic environment worldwide (Halling-Sørensen et al., 1998; Daughton and Ternes, 1999; GWRC, 2004; Fent et al., 2006; Sadezky et al., 2008; Mompelat et al., 2009; Pal et al., 2010; Hughes and Brown, 2013; Ying et al., 2013; Michael et al., 2014a; Evgenidou et al., 2015), research efforts have been directed towards the knowledge of the distribution and fate of these trace pollutants in the aquatic ecosystems.

1.7 Behavior of PhACs and their TPs

1.7.1 Fate and transformation in WWTPs

WWTPs principally operate applying primary and secondary treatments, where the second one is based on ***Conventional Activated Sludge (CAS)*** (figure 1.9). Conventional systems rely on the activated sludge process, a complex biological WW treatment system designed to remove carbon and/or nitrogen constituents WW. CAS treatment is widely employed as WW treatment mostly because it produces WWe of acceptable quality at reasonable operating and maintenance costs (Jelić et al., 2011). In more sophisticated WWTPs advanced tertiary treatments (**ATTs**) are included mainly after the secondary treatment (figure 1.9).

The fate of PhACs in WWTPs is governed by the physicochemical and biological properties of the substance (table A.1) and the treatment processes in use at the WWTP. The mechanisms that determine PhACs pathways along the WW treatment and their possible degradation/distribution along the WWTP are the ***adsorption*** to particles and the ***biodegradation*** in the sludge (Fent et al., 2006). Very few PhACs are volatile, thus ***evaporation*** from the plants is not significant. The fate of PhACs along the WWTPs is shown in figure 1.9.

Adsorption is dependent on both hydrophobic and electrostatic interactions of PhACs with particulates and microorganisms. Some, but not many, PhACs bind strongly to the sludge and are handled at the sludge treatment step. In general, sorption of acidic drugs to sludge is suggested to be not very important and thus levels of PhACs in digested sludge are expected to be relatively low (Ternes et al, 2004; Urase and Kikuta, 2005). However, basic compounds and zwitterions can be adsorbed to sludge to a significant extent, as observed for fluoroquinolone and tetracycline antibiotics (Golet et al., 2002) or the macrolide antibiotics azithromycin and clarithromycin, with a K_d >0.2 L g^{-1} (Joss et al., 2006). Nevertheless, the majority of PhACs show no significant sorption to solids (K_d< 0.1 L g^{-1}), thus occurring mainly in the dissolved phase (Joss et al., 2006). Interestingly, a more recent study evidenced that PhACs can occur both in the aqueous and the particulate phase of the activated sludge as is the case of DCF, mefenamic acid, bezafibrate, fenobibrate, gemfibrozil, atorvastatin, diazepam, lorazepam, carbamazepine, clarithromycin, cimetidine, fanitidine, famotidine, sulfamethazine, trimethoprim, atenolol, sotalol, nadolol, glibenclamide and furosemide (Jelić et al., 2011). Moreover, no simple pattern for the distribution of these substances between the dissolved and the

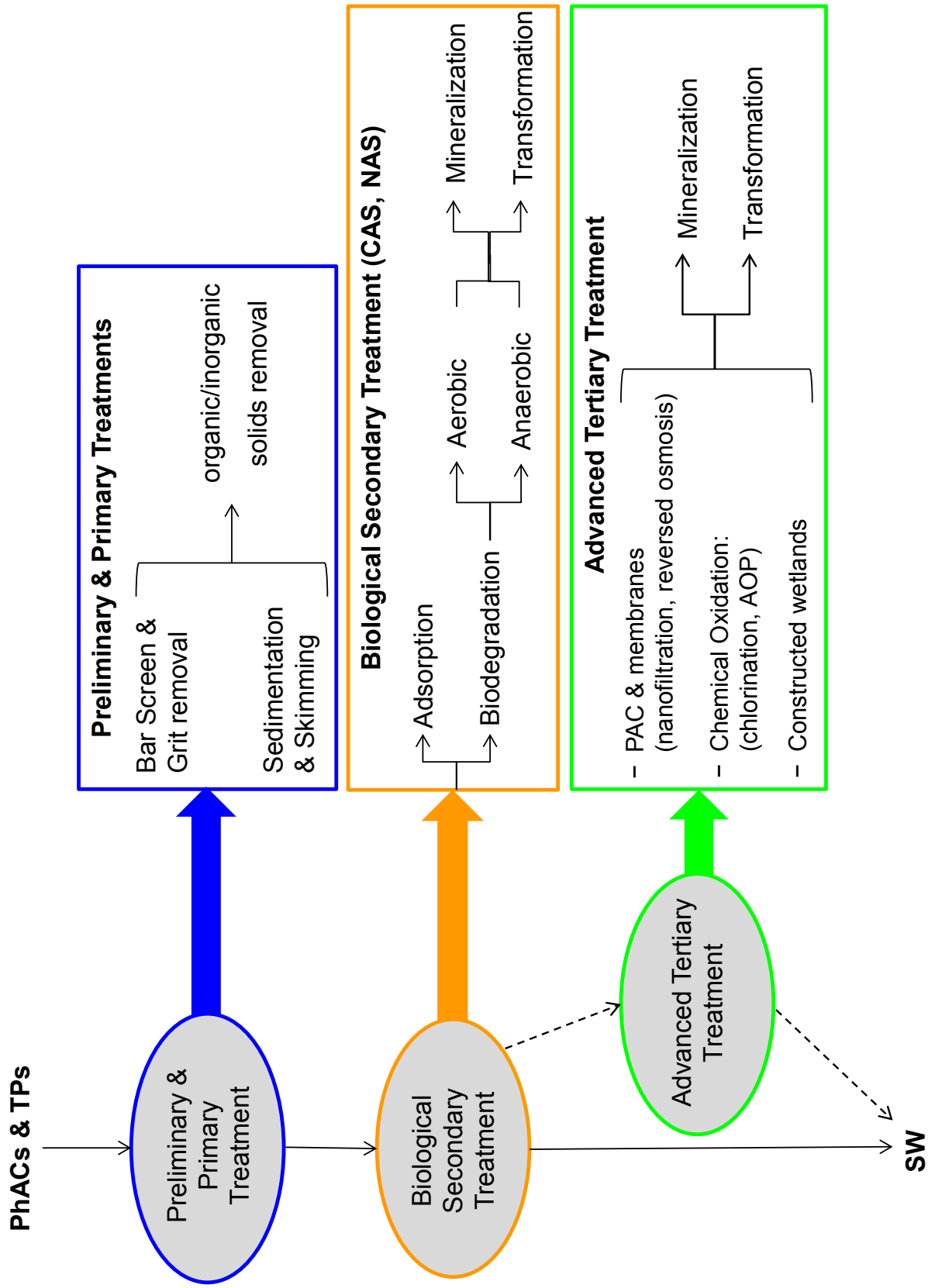


Figure1.9. Scheme of the treatment steps applied in a WWTP. Advanced processes are mainly included after the secondary biological treatment as a tertiary treatment.

solid phase of the activated sludge was observed. Still and all, since the majority of PhACs are characterized by high water solubility (table A.1) their release into receiving fresh waters through WWe is favored.

Biodegradation is suggested to be the most important PhACs removal mechanism during the biological CAS treatment (Joss et al., 2006; Johnson et al., 2008). Biodegradation is known as the process of partial or total elimination of organic matter by microorganisms (such as bacteria and/or fungi) that use compounds as an energy source. Such process can occur either in the aerobic zone of the activated sludge tank or anaerobically during the sewage sludge digestion (Fent et al., 2006). Anaerobic digestion is carried out to reduce the volume of sludge to be handled, for biogas production and for reduction of pathogens. However, few PhACs are degraded under anaerobic conditions, and thus biodegradation under aerobic conditions is the main PhACs removal process (Fent et al., 2006). Nitrogen removal from WW has become a common side stream process in WWTPs performing anaerobic sludge digestion (Rodriguez-Caballero et al., 2013). The treatment of this WW with high ammonium nitrogen species content (~1 g L^{-1} NH_4^+—N) and low chemical oxygen demand (COD) is normally achieved via nitrification-denitrification over nitrite nitrogen species (NO_2^--N) or nitrification combined with Anammox. Ammonia (NH_3) is converted to nitrate nitrogen species (NO_3^--N) during aerobic nitrification and subsequent anoxic denitrification removes the nitrate by producing N_2 gas. The first step in nitrification converts NH_3 into NO_2^- by two sequential oxidation steps (Bock et al., 2006). First, NH_3 is oxidized to hydroxylamine (NH_2OH) by the enzyme ammonia monooxygenase (AMO), and conversion of NH_2OH to NO_2^- is catalyzed by the enzyme hydroxylamine oxidoreductase (HOA). Ammonia oxidizing bacteria (AOB) are normally the main group of bacteria in these systems in which oxidation of NH_3 to NO_2^- is enhanced. To be precise, the bacteria responsible for the nitritation belong to the genera *Nitrosomonas*, *Nitrosospira* (*β-Proteobacteria*), and *Nitrosococcus* (*γ-Proteobacteria*) (Koops et al. 2001). The detection of a unique archaeal amoA gene and the presence of archaeal amoA in various ecosystems showed the additional involvement of ammonia oxidizing archaea (AOA) in nitritation. The next step in the nitrification from NO_2^- to NO_3^- is catalyzed by the enzyme nitrite oxidoreductase (NOR) delivered by the genera *Nitrobacter (α-Proteobacteria)*, *Nitrospina* (*δ-Proteobacteria*), and *Nitrospira* (phylum *Nitrospirae*) (Koops et al. 2001). *Nitrosomonas* and *Nitrospira* are the dominant nitrifiers in WWTPs, and archaea were found as the predominant causal organisms in low nutrient, low pH, and sulfide environments (Erguder et al. 2009). Biodegradation of PhACs is mainly attributed to co-metabolic activities of both heterotrophic and autotrophic microorganisms. AOB and AOA co-metabolize a variety of PhACs via non-specific enzymes such as AMO. For instance, Vader et al. (2000) demonstrated the degradation of ethynyl estradiol (EE2) by nitrifying sludge with a high ammonia-oxidizing activity and batch tests with nitrifying mixed cultures suggested that the enzyme AMO could mediate in EE2/NH_3 co-metabolism. Metabolism of drugs can only be observed by heterotrophic microbes. For example, a pure culture of *Sphingomonas Ibu-2* and *Delftia tsuruhatensis* and *P.aeruginosa* were reported to be capable of degrading ibuprofen and acetaminophen, respectively, as the sole carbon source (Murdoch and Hay 2005; de Gusseme et al. 2011).

As a consequence of the poor elimination of some PhACs under CAS treatment, ATTs have been suggested to be applied in WWTPs in order to improve the chemical quality of the WWe (Klavarioti et al., 2009; Ziylan and Ince, 2011) (figure 1.9). Various ATTs have been evaluated in recent years with the purpose to increase PhACs removal rates. These include the use of powdered activated carbon (PAC) and membranes (e.g. nanofiltration, reverse osmosis) (Mailler et al., 2015; García et al., 2013), chemical oxidation such as chlorination or advanced oxidation processes (AOPs) (WHO, 2011; Oller et al., 2011; Hey et al., 2012; Lester

et al., 2013; Prieto-Rodríguez et al., 2013; Fatta-Kassinos et al., 2011a; Malato et al., 2014); and constructed wetlands (Matamoros and Bayona, 2013, Verlicchi and Zambello, 2014; Luo et al., 2014) (figure 1.9). The advantages and disadvantages of the different technologies have been widely discussed in many reviews (Oulton et al. 2010; Oller et al., 2011; Malato et al., 2014; Verlicchi and Zambello, 2014).The fate of PhACs during WWTP under conventional and advanced treatment methodologies was reviewed by Oulton et al. (2010). Among the literature surveyed, it was found that PhACs were removed at maximum 90% of efficiency after a primary and secondary treatment. These removal rates were slightly enhanced for a few compounds at facilities operating with solids removal and CAS while the majority showed removal rates far below the 90%. On the contrary, the use of ATTs and particularly ozonation and/or membranes, improved the elimination of all classes of PhACs achieving removal rates beyond the 90% and levels in the WWe were frequently below the analytical limits of detection.

Among all these advanced/tertiary treatments, ***chemical oxidation*** and particularly ***AOPs***, being able to oxidize and degrade a wide variety of organic pollutants in water and WW (Ikehata et al., 2006) are the most effective ones in WW treatment and mostly studied at lab scale (Oller et al., 2011). These treatments are based on oxidation methods in aqueous phase that generate powerful reagents, such as hydroxyl radicals (•OH), that oxidize recalcitrant and non-biodegradable compounds to various TPs and eventually convert them into carbon dioxide (CO_2), water vapor and inorganic salts (Ikehata et al., 2006; Klavarioti et al., 2009; Antoniadis et al., 2010; Klamerth et al., 2010; Oller et al., 2011). Examples of AOPs that can be applied in WW treatment are ultraviolet (UV) oxidation, ozonation, photo-Fenton (cationic iron/hydrogen peroxide, Fe^{2+}/H_2O_2), ultrasound (US), the combinations ozone (O_3)/H_2O_2, O_3/UV, O_3/H_2O_2/UV, H_2O_2/UV, photo-Fenton US/H_2O_2, US/O_3 and UV/US electrochemical oxidation, supercritical water oxidation, photocatalysis using titanium dioxide (TiO_2/hv), ionizing radiation and sono-photocatalysis. Some of these processes are commercially available, like UV photolysis, which has more than 3,000 applications in Europe and a large number in the US (Parsons et al. 2004). Similarly, ozonation is one of the AOPs most employed as a pre-oxidation step in a combined treatment line (Oller et al., 2011). As shown in figure 1.9, ozonation and AOPs applied to water treatment operations, as a part of disinfection steps, may considerably decrease the concentration of PhAC residues in WWe and reduce toxicity (Ikehata et al., 2006; Comninellis et al., 2008; Ziylan and Ince, 2011). As an example, several analgesics and antiinflamatories (namely ketoprofen, naproxen, DCF, mefenamic acid and paracetamol) were efficiently removed under UV/H_2O_2 treatment at lab scale Kim et al. (2009). Nevertheless, these methodologies are not widely applied mainly because they involve high expenses on materials and equipment; relatively large amounts of oxidants and/or catalysts used (e.g., O_3, H_2O_2, and iron-based AOPs); as well as energy requirements and high electricity demand (e.g., ozonation and UV-based AOPs) (Ikehata et al., 2006; Antoniadis et al., 2010; Oller et al., 2011).

Most WWTPs are generally designed for the efficient removal of organic matter and inorganic nutrients (e.g. biodegradable carbon, nitrogen and phosphorus), that are present in WWi at the mg L^{-1} to g L^{-1} range. These are generally conventional WWTPs, which are not particularly equipped to remove emerging organic micro-pollutants such as medicinal drugs (Verlicchi et al., 2012). For that reason, conventional biological treatment systems have often been demonstrated not to be fully efficient and have presented varying degrees of removal rates of PhACs, ranging from less than 20% to greater than 90% (Chiron et al., 2010; Forrez et al., 2011). Table 1.2 provides the removals of selected PhACs and TPs after WW treatment reported in several studies. For some compounds, such as DCF, clofibric acid, propranolol or SMX, the elimination rates are

Table 1.2 Percentage of removal efficiencies of PhACs and their metabolites after WW treatment reported in several studies.

Pharmaceutical	Removal (%)	Sources
Analgesics and antiinflammatories		
Ketoprofen	8; 30; 56; 62; 77; 83; 92; 98	[1]; [2]; [3]; [4]; [5]; [6];[7]; [8]
Naproxen	35; 43; 71; 96; 55-98; 100	[9]; [3]; [4]; [6]; [10]; [8]
Ibuprofen	12-86; 52-99; 53-79; 60-70; >90; 96; 99	[11]; [12]; [1]; [13];[14]; [15]; [16]
Diclofenac	0; 7; 18; 23; 42; 65; 75	[1]; [17]; [5]; [4];[6];[18]; [19]
Acetaminophen (paracetamol)	86; 92; 99.5; 100	[20];[21]; [5]; [18]
Tramadol	20-56; 55->97	[50]
o-Desmethyltramadol	17-27; 28->88	[50]
Acetylsalicylic acid	81, 99-100	[22]; [53]
Salicylic acid	99; 77-98	[54]; [55]
Lipid regulators and cholesterol lowering stating drugs		
Clofibric acid	0; 28; 51; 84; 91	[1]; [20]; [22]; [18]; [23]
Gemfibrozil	5; 39; 68; 75	[2]; [20]; [5]; [24]
Bezafibrate	15; 27; 48; 81; 91	[25]; [19]; [20]; [2]; [4]
Psychiatric drugs		
Fluoxetine	33; 54.5; >70%	[2]; [26];[27]
Paroxetine	91; 94	[20]; [27]
Diazepam	0-25; 8; 93	[16]; [28]; [29]
Carbamazepine	0; 4; 8; 14; 30	[25]; [30]; [31];[16]; [24]
Sertraline	11	[27]
Citalopram	29	[27]
Venlafaxine	37-56; 56->95;	[50]
o-Desmethylvenlafaxine	29-41; 44->98;	[50]
Histamine H_1 and H_2 receptor antagonists		
Ranitidine	25; 28.5; 42; 39-84	[2]; [32]; [20]; [25]
β-Blocking agents		
Atenolol	0-10; 10-55; 58; 71; 84	[12]; [25]; [33]; [32]; [34]
Metoprolol	0-10; 17; 31; 65; 83	[12]; [33]; [35]; [34]; [22]
Propanolol	0; 32; 59; 65; 96;	[23]; [24]; [2]; [34]; [22]
Barbiturates		
Phenobarbital	99,5	[5]
Diuretics		
Hydrochlorothiazide	<10; 24-44; 76	[2]; [25]; [20]
Furosemide	8-54	[25]
Antihypertensives		
Enalapril	18-100	[25]
X-ray contrast agents		
Iopromide	50; 50	[16]; [36]
Cancer treatment drugs		
Tamoxifen	0	[23]
Antibiotics		
Erythromycin	0; 24; 25; 26	[25]; [20]; [34]; [37]
Azithromycin	22-55; 39-45; 74	[38]; [39]; [40]
Roxithromycin	12.5; 22; 40; 58-61	[37]; [41]; [42]; [16]
Clarithromycin	0; 4.5; 46; 54; 62; 50-83	[25]; [38]; [40]; [34]; [41]; [39]
Spiramycin	0	[25]

Ofloxacin	13; 24; 43-57; 84; 75-88	[26]; [20]; [25];[43]; [33]
Ciprofloxacin	18; 50-73; 60-63; 84; 93	[42]; [39]; [25]; [33];[43]
Tetracycline	24; 36; 40-72; 0-89	[39]; [42]; [44]; [51]
4-Epitetracycline	0-100	[51]
4-Epioxytetracycline	0-100	[51]
Isochlortetracycline	22-100	[51]
Sulfamethoxazole	4.5; 10; 25; 56; 74; 100; 0-85; 65	[38]; [2]; [45]; [20]; [42]; [43]; [51],[52]
n-Acetyl sulfamethoxazole	0-34; 84	[51]; [52]
Sulfadiazine	50; 78-98; >97; 100; 11-59; 93	[37]; [46]; [47]; [42]; [51]; [52]
n-Acetyl sulfadiazine	9-100; 87	[51]; [52]
Sulfamethazine	0-100; 82	[51]; [52]
n-Acetyl sulfamethazine	0-71; 100	[51]; [52]
Trimethoprim	0; 7; 14; 49; 69; 85	[41]; [38]; [44]; [24]; [34];[43]
Fluoroquinolone	80	[48]; [49]
Ashtma treatment drugs		
Salbutamol	0; 95	[25]; [21]

[1] Tauxe-Wuersch et al., 2005; [2] Radjenovic et al., 2009; [3] Santos et al., 2009; [4] Quintana et al., 2005; [5] Yu et al.,2006; [6] Kimura et al., 2007; [7] Vieno et al., 2005; [8] Thomas and Foster, 2004; [9] Santos et al.,2007; [10] Lindqvist et al., 2005; [11] Strenn et al., 2004; [12] Andreozzi et al., 2003; [13] Carballa et al., 2004; [14] Gros et al., 2007; [15] Buser et al., 1999; [16] Kreuzinger et al., 2004; [17] Clara et al., 2005; [18] Roberts and Thomas, 2006; [19] Stumpf et al., 1999; [20] Radjenovic et al., 2007; [21] Jones et al., 2007; [22] Ternes, 1998; [23] Roberts and Thomas, 2005; [24] Bendz et al., 2005; [25] Castiglioni et al., 2006; [26] Zorita et al., 2009; [27] Vasskog et al., 2006; [28] Suárez et al., 2005; [29] Van Der Hoeven, 2004; [30] Clara et al., 2004; [31] Herber, 2002; [32] Carucci et al., 2006; [33] Vieno et al., 2007; [34] Ternes et al., 2007; [35] Maurer et al., 2007; [36] Batt et al., 2006; [37] Xu et al., 2007; [38] Göbel et al., 2007; [39] Ghosh et al., 2009; [40] Yasojima et al., 2006; [41] Sahar et al., 2011; [42] Li and Zhang, 2011; [43] Lindberg et al., 2005; [44] Gulkowska et al., 2008; [45] Watkinson et al.,2007; [46] García-Galán et al., 2011; [47] Peng et al., 2006; [48] Giger et al., 2003; [49] Golet et al., 2002 [50] Rúa-Gómez et al., 2012; [51] Zhang et al., 2015; [52] García-Galán et al., 2012; [53] Nakada et al., 2006; [54] Metcalfe et al., 2003; [55] Matamoros and Bayona, 2006

widely varying (from no removal up to total removal). Others show steady trends like carbamazepine, which is generally recalcitrant to the WW treatment; while acetaminophen, is efficiently eliminated from WW in all cases.

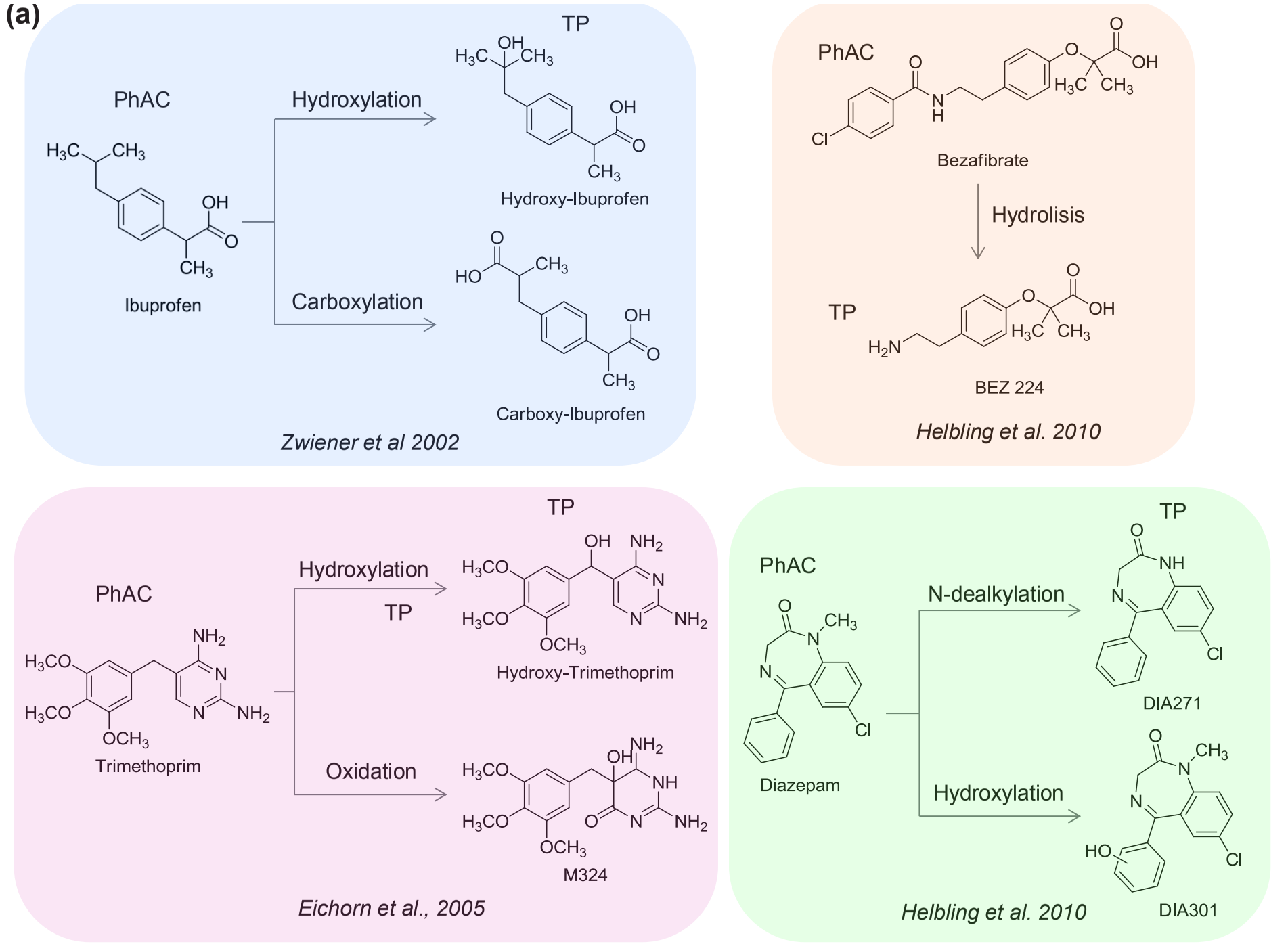
The variability in elimination rates of PhACs in WWTPs may be due to differences in treatment technologies and operational parameters as well as environmental parameters (Verlicchi et al., 2012) (see table 1.2). Among PhACs, relevant differences in removal rates are frequently explained by their diverse physicochemical and biological properties (Verlicchi et al., 2012) (table A.1). In addition, climate and meteorological conditions can affect the efficiency of PhACs elimination in WWTPs through changes in water temperature and dilution by rain water, eventually influencing the biological state of the microbial community (Fent et al., 2006; Castiglioni et al., 2006; Vieno et al., 2005; 2007; Zhang et al., 2015). For instance, Vieno et al. (2005) reported lower removal efficiencies during winter seasons in cold climates and the changes in biological reactions, which were strongly affected by temperature, were pointed out as the main cause. On the other hand, different seasonal effects on PhACs removal were observed in six different large WWTPs in Italy, (Castiglioni et al. 2006). A substantial number of compounds showed markedly 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 SMX (17% and 71%). Another group of compounds presented similar removal rates regardless the season: ciprofloxacin (60%), hydrochlorotiazide (30%) and ofloxacin (50%).

Finally, other PhACs, carbamazepine, clarithromycin, erythromycin and salbutamol, were not removed at all, neither in winter nor in summer.

The WWTP design and operational factors such as sludge retention time (SRT), hydraulic retention time (HRT), temperature in the biological reactor, and properties of the activated sludge may influence removal (Suárez et al., 2008; 2012; Alvarino et al., 2014). For instance, those equipments and treatment steps, operating for nitrogen removal have demonstrated to increase removal of organic micro-pollutants (Batt et al., 2006). Since the degree of biodegradation of PhACs depends on the number and type of microorganisms present (Alvarino et al., 2014), it is also important to keep a critical SRT. Keeping SRT promotes the growth of a more diverse biological community that is probably able to degrade compounds, such as PhACs, more efficiently (Kreuzinger et al., 2004; Clara et al., 2005; Oppenheimer, 2007). For example, DCF is only significantly biodegraded at an SRT > 8 days (Kreuzinger et al., 2004), while carbamazepine removal is normally less than 10 %, independent from SRT (Metcalfe et al., 2003). The properties of the activated sludge community such as biomass activity (Majewsky et al., 2010) and nitrification potential may also influence on the removal (Koh et al., 2009; McAdam et al., 2010). HRT and SRT govern both reaction time and loading (McAdam et al., 2010), thus affecting biomass activity and concentration. Several studies showed that bacteria of the ***Nitrifying Activated Sludge (NAS)*** are capable to degrade emerging micro-pollutants through co-metabolism (Batt et al., 2006; Yi et al., 2007; Forrez et al., 2009; Zhou and Oleszkiewicz, 2010; Martínez-Hernández et al., 2011). Moreover, since the nitrification process improves the elimination of selected PhACs, removal efficiency can also be enhanced by the enrichment of nitrifiers in the activated sludge (Tran et al., 2009). For instance, enhanced removal rates in NAS compared to CAS were observed for the X-ray contrast agent iopromide and the antibiotic trimethoprim in laboratory-scale experiments (Pérez et al. 2005; Batt et al., 2006).

Biodegradation in the secondary tank can transform PhACs into TPs (Richardson and Ternes, 2014). Figure 1.10 shows several TPs that have been identified during degradation studies. In all instances, batch experiments at lab scale simulating CAS (figure 1.10a) or NAS (figure 1.10b) WW treatments were carried out in order to elucidate their chemical structures and eventually describe the transformation mechanisms. Several enzyme-catalyzed reactions are commonly involved in the biotransformation of PhACs during CAS or NAS WW treatments such as hydroxylation, oxidation, hydrolysis, N-de-alkylation, carboxylation, de-carboxylation, nitration and nitrosation. By contrast, when AOPs are applied in WW treatments, the main reactions occurring during the photodegradation process are hydroxylation, de-hydroxylation and oxidation.

Importantly, the excreted human metabolites of PhACs can undergo transformation along the WW treatment. For instance, acetylsulfamethoxazole, a metabolite of the sulfonamide drug SMX, can be transformed back to the parent form in WWTPs (Göbel et al., 2005). Therefore, not only the study of transformation processes of PhACs to their structurally related derivatives but also conversion back to the parent compound, are important questions to be addressed. Consequently, current research is attempting to understand the cleavage mechanisms of metabolites and conversion back to their active parent forms after biodegradation and how this process is accomplished by bacteria during the CAS treatment (Celiz et al., 2009; Helbling et al., 2012; Ferrando-Climent et al, 2012; Tran et al., 2013). The work of Ferrando-Climent et al. (2012) tackled the issue of whether the TPs detected in the aquatic environment are generated from human metabolism and/or from the activity of different microorganisms present in natural waters, soils and sediments, as well as in sludge of WWTPs, which increases the probability to find them in the environment. In this work the elimination and fate of ibuprofen and generation of its TPs in biodegradation batch experiments


(a)
PhAC
Hydroxylation
TP
Hydroxy-Ibuprofen
Ibuprofen
Carboxylation
Carboxy-Ibuprofen
Zwiener et al 2002
PhAC
Bezafibrate
Hydrolisis
TP
BEZ 224
Helbling et al. 2010
PhAC
Hydroxylation
TP
Hydroxy-Trimethoprim
Trimethoprim
Oxidation
M324
Eichorn et al., 2005
PhAC
N-dealkylation
TP
DIA271
Diazepam
Hydroxylation
DIA301
Helbling et al. 2010

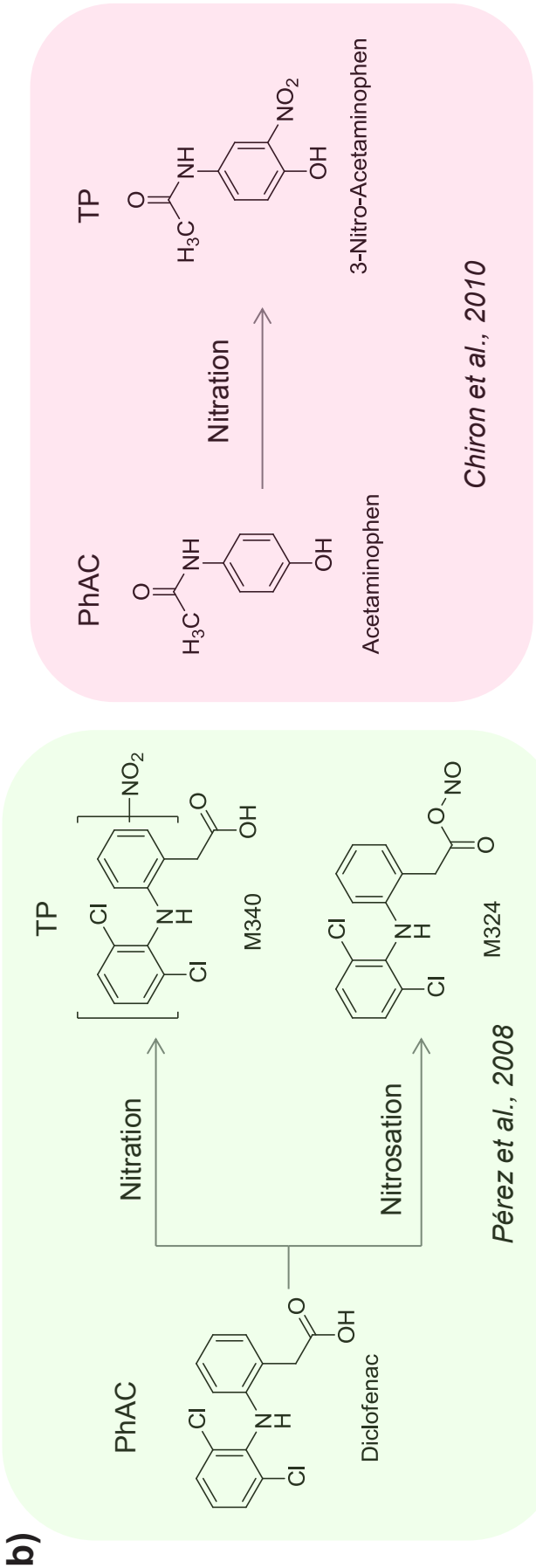


Figure 1.10. TPs of PhACs and their metabolites identified and characterized in batch experiments simulating different WW treatments in the (a) activated sludge and (b) nitrifying activated sludge.

with activated sludge was assessed. Against the excretion rates, 2-hydroxyl-ibuprofen was found at higher levels than carboxy ibuprofen in WWi samples, which points out the contribution of biological degradation and concomitant formation of ibuprofen metabolites before the entrance to WWTPs. These findings provided further insight into which metabolites generated in the process of ibuprofen biodegradation through activated sludge.

The structural elucidation and identification of new TPs of PhACs formed in engineered systems has increased substantially, especially in the field of the ATTs (a et al., 2014). These studies are mainly conducted aimed to evaluate the performance of ATTs and the possibility of implementing them in WWTPs. For instance, Machado et al. (2015) investigated on the photocatalic degradation of rosuvastatin, which is a cholesterol lowering statin drug, employing ZnO as a catalyst and under UV irradiation. The authors carried out nano-UPLC–MS/MS analyses to detect and identify the byproducts generated during the application of the AOP treatment in WWe (see Table 1.1. in section 1.5), which allowed to propose a degradation pathway for the the ZnO-assisted photocatalysis of rosuvastatin. These byproducts were characterized as resulting from the subsequent oxidation of the parent compound, which leaded to the formation of a carboxylic acid derivative of rosuvastatin.

In a follow-up study of the same group, ten derivatives of rosuvastatin were temptatively identified during the application of the same photocatalitic treatment to demineralised water (Segalin et al., 2015). These TPs, which included hydroxilated and dihydroxilated derivatives of rosuvastatin among other structures, were identified following the same analytical method described for Machado et al. (2015) (see table 1.1). Interestingly, the use of computational analysis facilitated the structural elucidation of some of the most abundant or persistent TPs. In addition, this analysis allowed the calculations for different isomers and showed the most stable structures and, consequently, the most likely to be found. The application of computational approaches was demonstrated to be a helpful tool for the elucidation of structures of unknown molecules. These advances on the analytical procedures applied for the elucidation of new TPs evidence the increase of the scientific community interest on the assessment of transformation of organic pollutants such as PhACs in the environment.

Overall, depending on the removal rates and the transformation of PhACs and their TPs during their fate along the WWTP, a cocktail of unchanged PhACs, excreted human metabolites and also TPs is expected to be discharged through WWe into receiving water bodies.

1.7.2 Natural attenuation along urban WW systems and rivers

Apart from the anthropogenic attenuation processes that PhACs and TPs undergo during the WWTP described in previous section, these substances may also experience natural attenuation processes during their way to the WWTP and after their discharge into receiving SW. Once PhACs and their TPs have reached the urban WW system or the rivers, their fate is subjected to numerous factors, including their physicochemical properties (table A.1), environmental factors and climate conditions (e.g. water temperature and pH, and solar radiation) and, most importantly the presence and activity of microorganisms capable to biodegrade them (Jelic et al., 2015; Caracciolo et al., 2015).

As shown in figure 1.11, PhACs and their TPs can be naturally attenuated by: (i) ***dilution*** in SW; (ii) ***sorption*** onto SPM, colloids and partitioning into sediments; (iii) biotic ***biodegradation***; (iv) abiotic

processes such as direct and indirect ***photodegradation***; and (v) ***bioaccumulation*** in biota and food chain biomagnification (Mompelat et al., 2009).

When entering the rivers via WWe, levels of PhACs are mainly attenuated by ***dilution*** in receiving SW. Generally, the concentration of these micro-pollutants in WWe decrease at least by one order of magnitude in the river (from high ng L^{-1}-µg L^{-1} range to low ng L^{-1} range) (Gros et al., 2010). The dilution capacity of the river is strongly influenced by its hydraulic conditions i.e. the mixing ratio between surface water and the WWe and the upstream background concentrations. For instance, Gros and coworkers estimated dilution factors of the Ebro River basin (NE, Spain) along different sections of the catchment (Gros et al., 2007; 2010). The Ebro river basin, with an average river flow of 600 $m^3\,sec^{-1}$ and receiving several urban and industrial WW discharges, averaged a dilution factor of 30-40. For example, at a given location where 1.9 $m^3\,sec^{-1}$ of WWe were mixed with 150 $m^3\,sec^{-1}$ of river flow, the estimated dilution factor was of 70. Conversely, PhACs were estimated to be diluted only 5 times at a section of the basin characterized by low river flow.

The ***sorption*** of PhACs to solids in the aquatic environment is dependent on their physicochemical

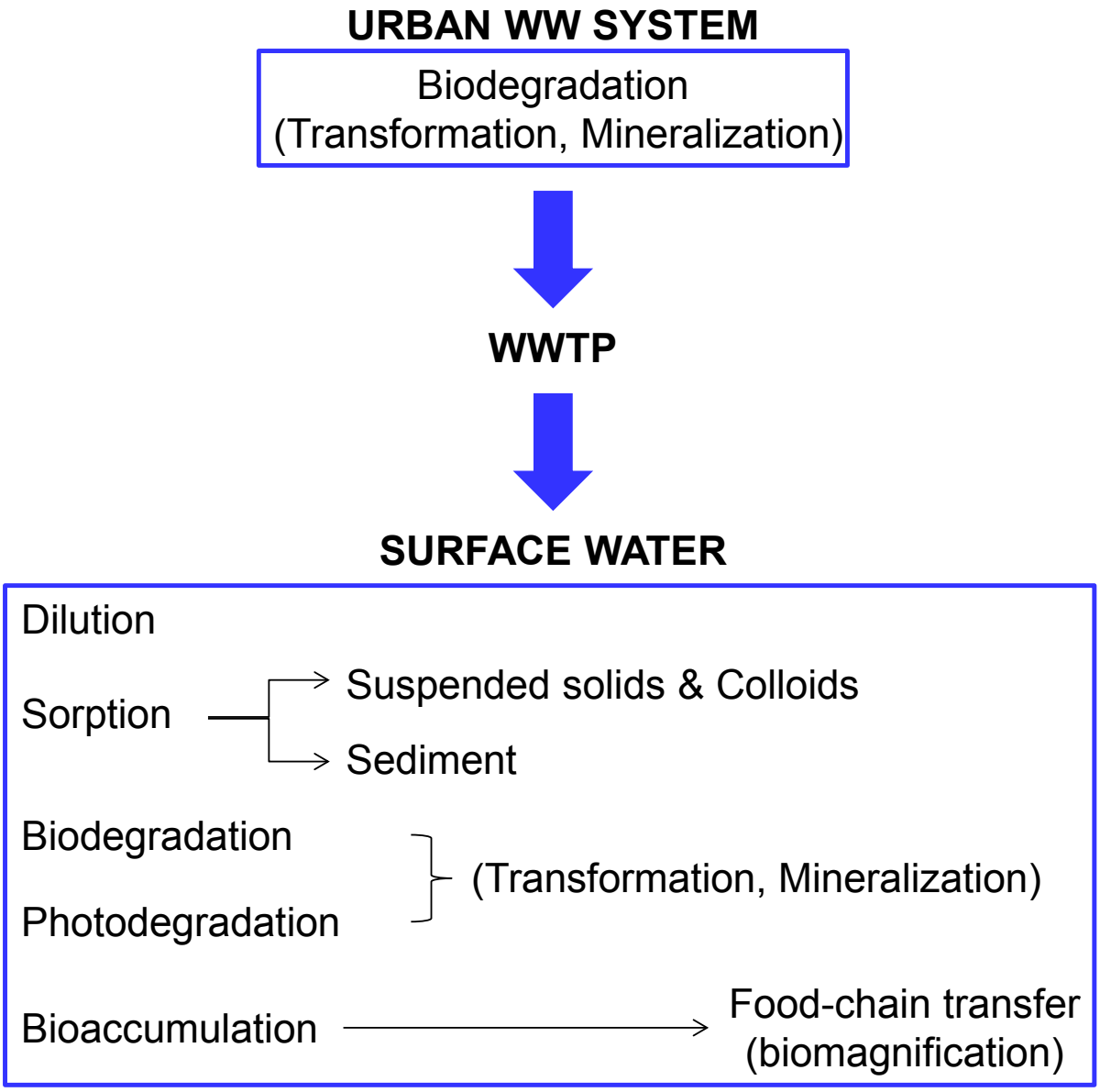


Figure 1.11. Natural attenuation processes of PhACs and their TPs along the urban WW system and rivers.

properties, such as pK_a, molecular weight, log k_{ow}, log K_{oc}; and many environmental parameters, like ion exchange capacity, organic carbon content, quality of solids, pH and presence and type of ionic and colloidal materials (Delle Site, 2001). Due to the polar and often ionic nature of PhACs, their sorption to solids is governed by several processes such as hydrophobic partitioning, ion exchange, surface related adsorption, complexation and hydrogen bonding (Tolls, 2001; Schwarzenbach et al., 2003). Depending on the compound type and heterogeneity of the river, PhACs are expected to bind to sediment (da Silva et al., 2011; Zhou et al., 2011), SPM (Maskaoui and Zhou, 2010; da Silva et al., 2011) and/or colloids (Yang et al., 2011). For instance, 34 PhACs belonging to different therapeutic families were detected in at least 3 out of the 22 sediment samples collected along the Ebro River Basin (da Silva et al., 2011). Acetaminophen, mevastatin and tylosin A were found at the highest concentration (222, 99.4 and 71 ng g^{-1}, respectively). These were followed by erythromycin, ibuprofen and ranitidine at respective maximum levels of 33.5, 19.2 and 25.1 ng g^{-1}. The remaining PhACs were detected at levels below 10 ng g^{-1}. Importantly, this study included the analysis of these substances in SPM. A total of 31 PhACs were detected in at least one out of the 20 SPM samples analyzed, at concentrations generally higher than those measured in sediments. These levels ranged from 0.44 ng g^{-1} for trimethoprim to 657 ng g^{-1} for acetaminophen (da Silva et al., 2011). Similarly, Yang and coworkers (2011) determined the levels of five selected PhACs in the soluble, SPM and colloidal phase and calculated their partition coefficients between the different phases. The partition coefficients of PhACs between colloids and the soluble phase, which were substantially greater than intrinsic partition coefficients, indicated that aquatic colloids are more powerful sorbents for accumulating PhACs than sediments and SPM. Furthermore, average mass balance calculations of PhACs concentrations demonstrated that 45% of propanolol, 40% of SMX, 22% of carbamazepine, 39% of indomethacine, and 37% of DCF were associated with colloidal particles, evidencing that sorption to colloids provides an important sink for the PhACs in the aquatic environment (Yang et al., 2011).

Biodegradation in the aquatic environment is governed by microorganisms attached to biofilms at the water/sediment interface or in bed sediments (Radke and Maier 2014). PhACs are naturally attenuated via biodegradation to an extent that depends on the number and type of microorganisms present as well as on the physicochemical properties of the drug (Fent et al., 2006; Alvarino et al., 2014). Several studies have evaluated the efficiency of this natural process to eliminate some PhACs from SW (Radke and Maier 2014). For example, Löffler et al. (2005) investigated the fate of ten PhACs and metabolites in water/sediment lab scale systems. Ibuprofen, 2-hydroxyibuprofen and paracetamol showed the lowest 50 % dissipation values (DT_{50}), while diazepam, carbamazepine, 10, 11-dihydrocarbamazepine and clofibric acid exhibited the high persistence. Similarly, a field study carried out in a WWe collecting river reported the efficient dissipation of gemfibrozil, ibuprofen, metoprolol and naproxen; with concentrations that decreased by between 60% and 90% as the water moved downstream (Fono et al., 2006). By contrast, ibuprofen and clofibric acid were the only compounds eliminated along a small stream in central Sweeden (Kunkel and Radke, 2012). The variability of reported field data about microbial degradation rates of PhACs in the aquatic environment is attributed to the heterogeneity of the river in terms of: (i) the specific microbial communities with substantially different degradation capacities or (ii) the interactions between SW and the sediment compartment, the hyporheic zone, that control the extent to which PhACs are biodegraded (Fent et al., 2006; Radke and Maier 2014). For instance, gemfibrozil which was initially reported to be not biodegradable (Stumpf et al., 1999), was found to be degraded in a liquid culture by the fungus *Cunninghamella elegans* (Kang et al., 2009). Another study, which reported gemfibrozil to be a quite persistent compound in river water (with a half-life of about

70 days), suggested the role of the *Gamma-Proteobacteria* group of microorganisms in its biodegradation (Grenni et al., 2013). On the other hand, the differences in attenuation of PhACs observed in different rivers by Radke and Maier (2014) were explained by the variance of the hydraulic exchange between the water column and sediment. Recently, it has been demonstrated that PhACs and their TPs can experiment natural attenuation and further transformation by in-sewer anaerobic biodegradation processes occurring along their fate through the urban WW system (Jelić et al., 2015). In the cited work, the concentrations of diltiazem, citalopram, clarithromycin, bezafibrate and amlodipine were substantially decreased (25-60%) during their pass through a pressurized pipe. Moreover, the phenomenon of reconversion of conjugated metabolites back to their parent compound was also conjectured for sulfamethoxazole and irbesartan, since negative removals of these compounds were calculated (-66±15 and -58±25 %, respectively).

Direct or indirect ***photodegradation*** is the principal abiotic mechanism of attenuation of PhACs in the aquatic environment, since the majority of these substances are designed for oral intake and thus being resistant to hydrolysis. While direct photolysis of substances is caused by direct absorption of solar light, the indirect photolysis involves strong oxidant species (e.g. hydroxyl radicals and singlet oxygen) generated by naturally occurring photosensitizers like nitrate and humic acids (Andreozzi et al., 2003). Several works reported the evaluation of the photodegradability of PhACs at lab scale (Challis et al., 2014). For instance, ranitidine, SMX, DCF, ofloxacin, atorvastatin and propanolol have been reported to be direct or indirectly photo-degraded relatively quickly (Buser et al., 1998; Andreozzi et al., 2003; Latch et al., 2003; Jasper et al., 2013). Importantly, recent studies of the photolysis of PhACs in SW are including the detection of photo-TPs that might be formed by the effect of the natural light on PhACs (Fatta-Kassinos et al., 2011a). As recent example, Gonçalves and co-authors (2011) evaluated the photodegradation of the antivirals Oseltamivir Ester (OE) and its human metabolite Oseltamivir Carboxylate (OC) in SW from rivers. Firstly, they assessed the photolysis process of OE and OC by simulated UV radiation experiments at lab scale, then they identified and characterized the TPs formed from OE and OC, and finally they monitored the TPs in a river. In the monitoring survey of the antivirals, OE and its photo-TPs, formed under natural solar irradiation, TP330 and 312 were detected confirming that photolysis is one of the processes involved in their disappearance in rivers. Other PhACs, such as carbamazepine, levofloxacin, cimetidine, and clofibric acid, have been observed to largely resist photodegradation (Andreozzi et al., 2003; Latch et al., 2003; Jasper et al., 2013). Photodegradation capacity in the river is affected by its turbidity and water depth (Fono et al., 2006; Robinson et al., 2007). Measurements of PhACs in the Trinity River (Dallas, U.S.), which were carried out in a period when WWe accounted for nearly the entire flow of the river, suggested that biotransformation was a more important attenuation mechanism than photolysis (Fono et al., 2006). Besides, the latter is restricted to the uppermost layer of SW (Bartels and von Tümpling, 2007). Other important factors affecting photodegradation are water constituents involved in indirect photolysis processes (Fatta-Kassinos et al., 2011a). For instance, the rate of photodegradation can be reduced due the increase of humic acids levels that may act as solar radiation filters (Andreozzi et al., 2003).

The ***bioaccumulation*** of PhACs in biota has been suggested to be determined by active transport through biological membranes (Daughton and Brooks, 2011). In general, these substances are moderately lipophilic and hence, their potential to bio-accumulate is low. However, in aquatic biota some compounds have been detected, such as psychiatric drugs. Brooks and colleagues (2005) reported for the first time residues of fluoxetine and sertraline, as well as their respective metabolites norfluoxetine and desmethylsertraline, in all

tissues of fish residing within municipal effluent-dominated systems at levels greater than 0.1 ng g^{-1}. A more recent study analyzed PhACs in fish tissues of eleven fish species from four heavily impacted Mediterranean rivers. DCF, citalopram, carbamazepine, venlafaxine, clopidogrel, carazolol, propanolol, sotalol and salbutamol were measured at concentrations higher than the method detection limits. Highest levels were found in trout liver with a maximum concentration of 18 ng g^{-1} for carbamazepine whereas the most ubiquitous compound was DCF (Huerta et al. 2013).

The effectiveness of natural attenuation processes is highly influenced by climate and meteorological conditions such as, solar radiation intensity and temperature or river hydraulic regime (Vieno et al., 2005). For example, Boreal winter climate conditions with low temperatures and low daylight hours may lead to decreased bio- and photodegradation of PhACs compared with summer (Bartels and von Tümpling, 2007). Increasing river flow and turbulence due to rainfall events can also affect natural attenuation of PhACs in the aqueous phase by dilution (decrease in concentration) or sediment re-suspension and suspended solid and colloids re-dissolution (increase in concentration). For example, an increase in the concentrations of pesticides was observed when flooding events occurred in the Ebro River (Gómez-Gutiérrez et al., 2006). The higher levels of these micro-pollutants were associated with the influence of floods on soil leaching, runoff and sediment re-mobilization processes.

1.8 . Effects of PhACs and their TPs on aquatic ecosystems.

As described in previous section the occurrence of PhACs in the aquatic environment is due to their large usage, incomplete human metabolism, relative persistence in WWTPs, and their slow degradation in the aquatic environment. The concentrations of PhACs are in the range of ng L^{-1}, at levels below those inducing toxic effects to humans (Christensen, 1998). However, aquatic organisms may experience continuous exposure via WW residues over their whole life and consequently these substances may cause adverse effects in non-target organisms (Oaks et al., 2004), especially to highly vulnerable aquatic life (Di Giulio and Hinton, 2008). Moreover, owing to their structural diversity, the complex mixtures of drugs can exhibit effects different than those from a single compound (Pomati et al., 2008). As a consequence of the presence of myriads of PhACs in the aquatic environment, significant chronic effects on aquatic organisms including additivity, antagonism, and synergism are likely to occur (Daughton and Ternes, 1999; Forrez et al., 2011). However, the direct attribution of ecosystem effects to PhACs is a difficult point to prove and must be regarded with caution due to the simultaneous concurrence of many other chemicals, as well as, other environmental stressors (e.g. nutrients or hydrological conditions).

Concerned about the aforementioned ecotoxicological effects, studies to evaluate the potential effects on aquatic organisms have increased considerably (Halling-Sørensen et al., 1998; Daughton and Ternes, 1999; Boxall et al., 2003; Jones et al., 2004; Fent et al., 2006; Kümmerer et al., 2009; Escher and Fenner, 2011; Michael et al., 2014a). In the literature, various ecotoxicological tests using freshwater invertebrates (such as daphnids), fish, algae, mussels, bacteria (such as *Vibrio fischeri*), biofilms, bacterial strains and cells have been assessed. Of the test species used, daphnids have been found to be most susceptible to environmental pharmaceutical contaminants, followed by fish and algae (Sanderson et al., 2004). Table 1.3 provides several examples of the ecotoxicological effects of PhACs on aquatic organisms, it compiles the experimental conditions, the organisms used, the endpoints and the measured effects in every ecotoxicity test.

Regarding acute toxicity data of PhACs over 90 % of the studies reviewed by the Global Water Research Coalition (GWRC, 2004), presented an effect concentration of >1 mg L^{-1} in the most commonly studied aquatic species (e.g., daphnids; algae; fish; bacteria; and macrophytes). Most of the acute lowest observed effect concentrations (LOECs) for PhACs are generally between μg L^{-1} and mg L^{-1}, typically at least one order of magnitude higher than concentrations normally found in SW (ng L^{-1}) (Forrez et al., 2011; Corcoran et al., 2010). Consequently, the risk of acute toxicity to aquatic organisms is thought to be negligible (Enick et al., 2007). For example, propranolol showed effective concentrations, (EC_{50}) in *Daphnia magna* and *Desmodesmus subspicatus* (see table 3) that were approximately 1,000 times higher than levels detected in the WWe (Ferrari et al., 2004; Huggett et al., 2002).However, for some substances used for treating human nervous diseases, such as the psychiatric drug fluoxetine, acute ecotoxicological effects have been found at WWe and SW levels of PhACs (EC_{50} at 48 h of 0.024 mg L^{-1} in algae) (Brooks et al., 2003; GWRC, 2004).

Despite the he low likelihood of acute ecotoxicity for the majority of PhACs their “pseudo-persistence” in the aquatic environment leads to address the question of effects by a long-term exposure. For instance, the acute cytotoxic effects of DCF, which were assessed by in vitro tests on rainbow-trout hepatocyte cell lines, determined EC_{50} (24 h) values at concentrations as high as 6 mg L^{-1} (Laville et al., 2004). Differently, the in vivo chronic effects observed in rainbow trout (LOEC = 1 μg L^{-1}) were in the range of WWe discharge levels (< 4 μg L^{-1}) (Triebskorn et al., 2004). Similarly, though no relevant acute toxic effects were observed at environmental concentrations, the LOEC of the β-blocker propranolol (30 μg L^{-1}) for zooplankton and benthic organisms was also near the maximal measured WWe levels (Fent et al., 2006).These contrasted findings suggest that the investigation of chronic effects might provide more realistic and suitable data for the risk assessment in aquatic ecosystems. The ecotoxicological effects from acute toxicity tests have been extensively reported (Brausch et al., 2012). The initial steps on environmental risk assessment were focused on the potential short-term effects on non-target organisms (Kümmerer, 2009). However, the knowledge of long-term exposure of non-target aquatic organisms to PhACs and the effects on ecosystem functioning has gained relevance (Oaks et al., 2004; Brooks et al., 2005; Fent et al., 2006; Kim et al., 2007; Sumpter and Johnson, 2008; Corcoran et al., 2010; Schultz et al., 2010; Richards et al., 2011; Lazarus et al., 2015).

Natural and synthetic estrogen hormones and other mimic substances (e.g., nonylphenol or bisphenol) have been classified as *endocrine disruptors* (Sumpter and Johnson, 2008). The presence of these compounds in WWe, even at extremely low concentrations (low and below ng L^{-1}) can cause feminization of male aquatic organisms, alterations of other sexual characteristics, and decrease of egg fertilization in fish.

Antibiotics are a class of PhACs with relevant ecological concern because of their potential active role against environmental bacteria and in the spread of resistance among natural communities. A relatively small amount of the antibiotics consumed by humans and animals is actually absorbed, with some about 30–90% of antibiotics excreted unchanged and released into WWTPs or directly into the environment (Chow et al., 2015). Previous studies have shown the detrimental effect of antibiotics to the freshwater environment because of their effects on autochthonous bacteria and the impairment on key roles they intervene, such as many biogeochemical processes or the degradation of organic pollutants (Buesing and Gessner, 2006; Garcia-Armisen et al., 2011; Roose-Amsaleg et al., 2015). Moreover, antibiotic residues released in the environment are suspected to induce the development/selection of antibiotic resistance genes (ARGs) and antibiotic resistant bacteria (ARB) (Tello et al., 2012). Recently, many more evidences suggest that aquatic habitats, especially rivers and streams, are ideal vectors for the antibiotic resistance dissemination (Lupo

Table 1.3. Examples of eco-toxicological effects of selected PhACs and their TPs to aquatic organisms reported in different studies.

Compound/s	Exp-scale	Time-scale	Exposure levels	Target	Classification	Endpoint	Effect	Reference
Diclofenac (& TPs)	Lab	Acute (28 days)	1 to 500 µg L^{-1}	Oncorhynchus mykiss	Fish	Histopathological	Kidney and gills alterations Bioaccumulation	Schwaiger et al.,2004
	Lab	Acute (28 days)	1 to 500 µg L^{-1}	*Oncorhynchus mykiss*	Fish	Cytopathological	Cytological alterations in liver, kidney and gills	Triebskorn et al.,2004
	Field	-	-	*Lutra lutra*	Mammal	Bioaccumulation in hair	Detected in 53.6% of samples	Richards et al., 2011
	Lab	Acute (144 h)	50 mg L^{-1}	*Scenedesmus vacuolatus*	Algae	Cell reproduction	Phototransformation products enhanced toxicity	Schmitt-Jansen et al., 2007
	Lab	Acute (24 h)	0.49 to 250 mg L^{-1}	*Scenedesmus vacuolatus*	Algae	Cell reproduction	EC_{50} DCF = 48.1 mg L^{-1} EC_{50} CPAB = 4.8 mg L^{-1} (Photo-TPs)	Schulze et al., 2012
	Lab	Chronic (8 weeks)	5 µg L^{-1}	Fluvial biofilms	-	EPS Bacteria biomass and community structure C utilization Protozoan	↓ bacterial biomass ↓ C utilization ↓ protozoan population Significant changes in EPS and bacterial community composition	Lawrence et al., 2012
	Field/Lab	Chronic (6 weeks) Acute (48 h)	$1.6x10^{-3}$ to 3.1 mM	Fluvial biofilm	-	β-Glucosidase activity (PICT test)	↑ Tolerance in communities grown at higher concentrations	Corcoll et al., 2014
	Lab	Chronic (6 generations)	0.36 µg L^{-1}	*Daphnia magna*	Crustacean	Age at1st reproduction Body length Offsprings	↑ age at 1st reproduction ↑ body length of neonates	Dietrich et al., 2010
	Lab *(in vitro)*	Acute (24 h)	0 to 500 µM	*Onchorynchus mykiss* Hepatocytes	Fish	Citotoxicity	EC_{50} = 420 µM	Laville et al., 2004
Propanolol (& metabolites)	Lab	Acute (6-24 h)	0.9 to 900000 µg L^{-1}	Fluvial biofilm	-	Phothosynthetic efficiencyt	Inhibition (85 %)	Bonnineau et al., 2010
	Lab	Acute (24h)	0-10 mg L^{-1}	*Spirostomum ambiguum*	Protozoan	Deformations (study of metabolites)	EC_{50} = 1.8 mg L^{-1} Metabolites show similar activity than parent compound	Naleçz-Jawecki et al., 2008

Propanolol (& metabolites)	Lab	Acute (24h)	0-10 mg L^{-1}	*Thamnocephalus platyurus*	Crustacean	Deformations (study of metabolites)	EC_{50} = 3.9 mg L^{-1} Metabolites show similar activity than parent compound	Naleçz-Jawecki et al., 2008
	Lab	Acute (48 h)	n.r.	*Daphnia magna*	Crustacean	Mortality	EC_{50} = 2.7 mg L^{-1}	Ferrari et al., 2004
	Lab	Acute (48 h)	n.r.	*Ceriodaphnia dubia*	Crustacean	Mortality	EC_{50} = 1.5 mg L^{-1}	
	Lab	Acute (48 h)	0.5 to 500 µg L^{-1}	*Daphnia magna*	Crustacean	Mortality	LC_{50} = 1.6 ± 0.3mg L^{-1}	Huggett et al., 2002
	Lab	Acute (48 h)	0.5 to 500 µg L^{-1}	*Ceriodaphnia dubia*	Crustacean	Mortality	LC_{50} = 0.8 ± 0.02 mg L^{-1}	
Ibuprofen	Field/Lab	Chronic (6 weeks) Acute (48 h)	2.2x10^{-3} to 4.4x 10^{1} mM	Fluvial biofilm	-	β-Glucosidase activity (PICT test)	↑ Tolerance in communities grown at higher concentrations	Corcoll et al., 2014
	Lab	Chronic (8 weeks)	10 µg L^{-1}	Fluvial biofilm	-	Biomass EPS Bacterial community structure	↓ Cyanobacteria biomass. Altered EPS composition Shift of bacteria community	Lawrence et al., 2005
Diltiazem	Lab	Acute (5, 15min)	n.r.	*Vibrio fisheri*	Bacteria	Luminescence	EC_{50} = 263.7 mg L^{-1}	Kim et al., 2007
	Lab	Acute (96h h)	n.r.	*Daphnia magna*	Crustacean	Immobilisation	EC_{50} = 8.2 mg L^{-1}	
	Lab	Acute (96h h)	n.r.	*Oryzias latipes*	Fish	Mortality	LC_{50} = 15.0 mg L^{-1}	
	Field	Chronic (3 years)	-	*Pandion haliaetus*	Bird	Bioaccumulation	100% detection (540-8630 ngL^{-1})	Lazarus et al., 2015
Fluoxetine (&metabolites)	Lab	Acute	n.r.	*Pseudokirchneriella subcapitata*	Algae	Growth	EC_{50} = 0.024 mg L^{-1}	Brooks et al., 2003
	Lab	Acute	n.r.	*Pseudokirchneriella subcapitata*	Algae	Photosynthesis Growth	EC_{50} = 0.09 -91 mg L^{-1}	Neuwoehner et al., 2009

Fluoxetine (&metabolites)	Lab	Acute/ chronic (96h/36 days)	n.r.	*Oryzias latipes*	Fish	Mortality Bioaccumulation	LC_{50} = 0.20-5.5 mg L^{-1} Bioconcentrated in body and liver	Nakamura et al., 2008
	Lab	Chronic (28 days)	0.64 µg L^{-1}	*Oryzias latipes*	Fish	Bioaccumulation and depuration	Bioaccumulated (BCF = 74-80)	Paterson and Metcalfe, 2008
Metoprolol	Lab	Acute (6-24 h)	0.9 to 9000 µg L^{-1}	Fluvial biofilm	-	Bacteria mortality	Increase (50 %)	Bonnineau et al., 2010
	Lab	Chronic (6 generations)	1.20 µg L^{-1}	*Daphnia magna*	Crustacean	Age at1st reproduction Body length Offsprings	↑ age at 1st reproduction ↓ body length	Dietrich et al., 2010
Carbamazepine	Lab	Chronic (8 weeks)	10 µg L^{-1}	Fluvial biofilm	-	Several	↓ Bacteria biomass ↓ Cyanobacteria biomass ↓ EPS	Lawrence et al., 2005
	Lab	Acute (5 and 15min)	n.r.	*Vibrio fisheri*	Bacteria	Luminescence	EC_{50} = 52.2 mg L^{-1}	Kim et al., 2007
	Lab	Acute (96h h)	n.r.	*Daphnia magna*	Crustacean	Immobilisation	EC_{50} = 76.3 mg L^{-1}	
	Lab	Acute (96h h)	n.r.	*Oryzias latipes*	Fish	Mortality	LC_{50} = 35.4 mg L^{-1}	
	Lab	Chronic (6 generations)	0.50 µg L^{-1}	*Daphnia magna*	Crustacean	Age at1st reproduction Body length Offsprings	↑ age at 1st reproduction	Dietrich et al., 2010
	Lab *(in vitro)*	Acute (24 h)	0 to 500 µM	*Onchorynchus mykiss* Hepatocytes	Fish	Citotoxicity	EC_{50} = 318 µM	Laville et al., 2004
Furosemide	Lab	Chronic (8 weeks)	10 µg L^{-1}	Fluvial biofilm	-	Several	↑ bacterial and algal biomass ↓ Cyanobacteria biomass Shift of bacteria community	Lawrence et al., 2005

Acetaminophen	Lab	Chronic (8 weeks)	5 µg L^{-1}	Fluvial biofilms	-	EPS Bacteria biomass and community structure C utilization Protozoan	↓ bacterial biomass Significant changes in bacterial community composition	Lawrence et al., 2012
	Lab	Acute (5, 15min)	n.r.	*Vibrio fisheri*	Bacteria	Luminescence	EC_{50} = 567.5 mg L^{-1}	Kim et al., 2007
	Lab	Acute (96h h)	n.r.	*Daphnia magna*	Crustacean	Immobilisation	EC_{50} = 26.6 mg L^{-1}	
	Lab	Acute (96h h)	n.r.	*Oryzias latipes*	Fish	Mortality	LC_{50} >160 mg L^{-1}	
57 PhACs mixture	Field/Lab	Chronic (34 days)	-	Fluvial biofilm	-	Photosynthesis, Extracellular enzymatic activities, Biomass	Ibuprofen and Acetaminophen affected photosynthesis. Diclofenac enhance phosphatase activity	Proia et al., 2013b
29 PhACs mixture	Field	Chronic	-	Invertebrate community	-	Biomass, abundances and community structure	Anti-inflammatories and β-blockers related with invertebrate community changes	Muñoz et al., 2009

et al., 2012). Consequently, the spread of resistant bacteria in freshwater systems can reach DW supplies and thus enter the human food chain causing a serious threat for public health (Walsh et al., 2011). Recent data from the European Centre for Disease Prevention and Control (ECDPC) and the European Medicines Agency (EMA) evidenced that every year approximately 25,000 European citizens die from infections caused by bacteria that have developed antibiotics resistance (Borg, 2012). Moreover, it is estimated that more than 70% of bacteria causing these infections are resistant to at least one of the antibiotics commonly used to treat them (Muto, 2005). Importantly, Spain, as many other countries in southern Europe, has been characterized by a high rate of antibiotic resistance (Lázaro and Montero, 2010).

A compound resistant to degradation or pseudo-persistent and relatively well-absorbed by biota (log K_{ow} > 3) may still *bioaccumulate* to biologically relevant concentrations even when it is present at low environmental concentrations. This effect is particularly relevant in WWe dominated streams where the PhACs uptake has been observed in aquatic organisms (Brooks et al., 2005; Schultz et al., 2010). For example, the relatively hydrophobic DCF (see table A.1), can accumulate in liver (up to 2–3 μg g^{-1}, wet weight), kidneys, and gills (~1 μg g^{-1}, wet weight) of rainbow trout after 28-day exposure to 1 μg L^{-1} (Schwaiger et al., 2004). Detrimental effects may occur if compounds are transferred within the food chain. For instance, diltiazem was detected in water, fish and ospreys from WWe impacted waterways in Chesapeake Bay (USA) at environmentally relevant concentrations. Even though concentrations of diltiazem were detected at one order of magnitude below the acute toxicity reported for Japanese medaka fish (lethal effective concentration, LC_{50} of 15 mg L^{-1}) (Kim et al., 2007); it was predicted to bioaccumulate in fish-eating birds, which may be more sensitive (Oaks and Watson, 2011; Shore et al., 2015), up to 4 times the levels found in fish plasma (Lazarus et al., 2015). Similar studies on the Eurasian otter (Lutra lutra) from the UK, detected DCF and ibuprofen residues in hair samples of these freshwater mammals (Richards et al., 2011) and determined renal lesions observed during carcass necropsies (Simpson et al., 2011). These findings prompted recommendations for further studies on the exposure of otters to neprotoxic PhACs such as DCF and ibuprofen (Shore et al., 2015).

TPs have also been reported to be present in fish tissues (Nakamura et al., 2008; Paterson et al., 2008; Metcalfe et al., 2010). For instance, the de-methylated TPs of three psychiatric drugs (i.e. citalopram; sertraline and fluoxetine) were detected in tissues of fathead minnows. However, the source of these compounds, whether they were directly up-taken from the aquatic environment or the resulting in vivo metabolism of the parent compound, was not clear (Metcalfe et al., 2010).

It is naturally assumed that metabolism and transformation of PhACs leads to decreased toxicity. For instance, the hydroxylated TPs of DCF showed pharmacological activities lower than those of DCF itself, or no activity at all (Menassé et al., 1978). However, several drugs (including acetaminophen, carbamazepine and DCF) have been reported to produce bioactive TPs which have been associated with adverse drug reactions (e.g. hepatotoxicity) (Walgren et al., 2005). Although the toxicity of drugs is mostly known, knowledge of the ecotoxicity of their derivatives in the aquatic environment is still scarce (Michael et al., 2014a). Nevertheless, published data has demonstrated that even metabolites can exert detrimental effects on aquatic organisms (Celiz et al., 2009; Escher et al., 2011). The pharmacologically active human phase I metabolites can exhibit ecotoxicological effects similar to those of the parent compound, such as propranolol and its metabolites to protozoa (Nałęcz-Jawecki et al., 2008); fluoxetine and its metabolites to algae, (Neuwoehner et al., 2009); or oseltamivir and its metabolites to *Vibrio fischeri* (Escher et al., 2010). Furthermore, TPs can show even higher toxicity than their parent compounds. For instance, norfluoxetine was found to be 50% more toxic in

24h lethality tests to protozoa than fluoxetine (Nałęcz-Jawecki et al., 2007). Apart from human metabolites, a number of other TPs are expected to exhibit comparable or more toxicity to their chemical precursors (van Zelm et al., 2010). For example, natural photo-TPs showed increased toxicity compared to the parent compound. For instance, photo-TPs of DCF showed phytotoxicity to the algae *Scenedesmus vacuolatus* after exposure to DCF under natural midsummer sunlight (Schmitt-Jansen et al., 2007). While the test solution containing the parent compound at mg L^{-1} levels did not exert any toxicity, the formation of photo-TPs, clearly correlated with inhibition of algal growth. Further research on these compounds identified 2-[(2-chlorophenyl) amino] benzaldehyde (CPAB) as a photo-TP of DCF, with an enhanced acute toxicity to *S. vacuolatus* (Schulze et al., 2010). The phytotoxicity of CPAB, was explained by its likely higher lipophilicity (Schmitt-Jansen et al. 2007, Schulze et al., 2010).

Similarly, TPs generated by microbial transformation taking place in environmental compartments (e.g., sediments and soils) and engineered systems (e.g activated sludge) may pose an ecotoxicological risk to aquatic species (Escher and Fenner, 2011). For instance, higher levels of norfluoxetine and norsertraline compared to their parent compounds (i.e., fluoxetine and sertraline) were determined in tissues from fish living in WWe-dominated streams. Biodegradation taking place in the river was pointed out as the source of these metabolites detected in fish (Schulz et al., 2010).

Likewise, the disappearance of parent PhACs during the WW treatment does not necessarily indicate a reduction of the toxicity associated to WWe since the TPs formed may still retain biologically activity. For instance, after the photo-catalytic AOP treatment of WWe, low TiO_2 and radiation doses resulted highly effective for the detoxification and mineralization of small concentrations of drugs, such as DCF. However, when a high amount of TiO_2 was applied to the treatment of high levels of DCF, the toxicity of the treated samples increased. This higher toxicity was conjectured to be due to the formation of TPs (e.g. hydroxyl- and bi-hydroxyl derivatives) (Calza et al. 2006). Similarly, in the work of Machado et al. (2015) , acute toxicity tests on *D. magna* were performed to evaluate the toxicity of the untreated rosuvastatin solution and the reactor effluent. Products generated by the ZnO-assisted photocatalytic oxidation of rosuvastatin proved to be more toxic than their parent drug. Owing to that, the authors concluded that, though rosuvastatin undergoes photocatalytic degradation, the safety, efficiency and feasibility of the treatment process may be compromised by the production of toxic byproducts and by presence of dissolved ZnO (Machado et al., 2015).

Due to the fact that TPs of PhACs can exhibit the same mode of action as their parent compounds and may occur in complex mixtures in the aquatic environment, additive and synergistic effects are expected to enhance the overall toxicity of the mixture (Escher and Fenner, 2011).

Stream biofilms are complex biological communities composed mainly of algae, cyanobacteria, bacteria, fungi and microfauna that live on submerged substrata (Lock, 1993). The microbial communities attached to biofilms in freshwater ecosystems can play a key role in the trophic web and in the biogeochemical cycles within aquatic ecosystems. The short life cycle of biofilm microorganisms and the trophic interactions among microbiota (algae, bacteria, fungi, protozoa) allow for the detection of both short and long-term, and direct and indirect effects on the biofilm consortia (Proia et al., 2012). Besides, in rivers and streams, biofilms are the first to interact with dissolved substances and can integrate the effects of changing conditions over extended periods of time. This behavior of biofilms turns them a useful descriptor of the effects of pollutants of environmental concern, such as PhACs, on the ecosystem and thus as proper bioindicators of the ecological

status of rivers (Sabater et al., 2007). Several studies tested the acute and chronic effects of PhACs on fluvial biofilm communities both in the field and laboratory (Lawrence et al., 2005; Bonnineau et al., 2010; Lawrence et al., 2012; Rosi-Marshall et al., 2013; Proia et al., 2013a; 2013b; Corcoll et al., 2014) (table 1.3.). For instance, Bonnineau et al. (2010) assessed the acute effects of propranolol and metoprolol on fluvial biofilms at lab scale. They observed that a 24 h exposure of biofilm algae to 531 μg L^{-1} of propranolol inhibited their photosynthetic process by up to 85%, while a metoprolol concentration of 503 μg L^{-1} caused 50% mortality of biofilm bacteria. However, estimated no observed effect concentrations (NOEC) were in the range of environmental concentrations of these compounds. Diversely, Lawrence et al. (2005) investigated the effects of chronic exposure of fluvial biofilms to ibuprofen, carbamazepine and furosemide at lab scale. PhACs levels of 10 μg L^{-1} exhibited both nutrient-like and toxic effects on biofilm communities by marked changes in their architecture and composition. For instance, furosemide increased the bacterial biomass, while carbamazepine and ibuprofen reduced it. Other populations significantly altered by all compounds were the gamma-proteobacterial beta-proteobacteria. Furthermore, furosemide, carbamazepine and ibuprofen influenced the ratio of live-to-dead cells of the biofilms, with corresponding increasing values of 1.9, 3.2 and 3.5.

1. 9. Regulation and risk assessment of the aquatic environment

The concerns about PhACs have increased in the last years, particularly as no legal requirements have been set for the discharge of these ubiquitous, persistent and biologically active substances into surface water bodies (Furhacker, 2008; Salgot et al.,2006; Ternes et al., 2007).

The main tool of the European water policy to reduce chemical pollution of SW bodies is the Water Framework Directive (WFD, 2000/60/EC). The WFD aims to provide a common action framework to the different member states of the European Union (EU) and establishes the basis to regulate the water bodies in Europe with the aim of conserving, protecting and improving their quality and its sustainable use, its ultimate objective being the achievement of a good ecological and chemical status of all European SW bodies by 2015. Whereas chemical status is essentially defined by compliance with established environmental quality standards (EQSs) of a list of selected key compounds, the so-called ‘‘Priority Substances’’ (PSs) and Priority Hazardous Substances (PHSs), which have been fixed by Directive 2008/105/EC and updated by Directive 2013/39/EU, the ecological status is defined in terms of biological, hydromorphological and physicochemical (thermal, acidity, salinity, oxygenation and nutrients) parameters. According to the WFD, PhACs are not included in the list of priority or dangerous priority substances (Directives 2008/105/EC and 2013/39/EU) and thus no environmental quality standards are stipulated. Nevertheless, the same directive establishes clearly that substances discharged into the basin, which is the case of PhACs, should be controlled. Furthermore, Directive 2013/39/EU recognizes the relevance of PhACs for the EU water environment (Art. 8c “Specific provisions for pharmaceutical substances) and commits the Commission to develop a strategic approach by 2015 and propose specific measures by 2017. In fact, the Commission has established a watch list of substances for which Union-wide monitoring data are to be gathered for the purpose of supporting future prioritization exercises (Directive 2013/39/EU and Decision 2015/495/EU). Importantly, in the aforementioned list, 6 PhACs are included, namely: the NSAID DCF, the two hormones ethynyl estradiol (EE2) and estradiol (E2); and the antibiotics erythromycin, clarithromycin and azithromycin.

The EU Directive 2004/27/EC on human medicine and Directive 2004/28/EC on veterinary medicine set out an Environmental Risk Assessment (ERA) in the frame of the approval process for new medicinal

products. According to Directive 2004/27/EC on human PhACs, for all new authorizations of PhACs, the environmental effects must be examined and this assessment must accompany the approval application. However, the granting of a marketing authorization of human medicine cannot be refused using only the environmental impact as criterion. On the contrary, if veterinary drugs pose an unacceptable risk for the environment, the granting of their marketing authorization can be denied. In this case, the environmental safety criterion is as relevant as consumer safety in the concluding risk-benefit assessment, deciding about the authorisation or non-authorisation of a new veterinary drug. As well as in the EU, Directives set by the US Food and Drug Administration (FDA) and the FDA Centre for Veterinary Medicine stipulate that an ERA should be part of the approval procedure of new medical substances.

Pseudo-persistent TPs of PhACs, are required to be considered in ERA because the effects resulting from exposure of aquatic organisms to a mixture of parent drugs and its TPs may be quite different from what could be observed based on toxicity tests using only a single compound (Filby et al., 2007; Wilson et al., 2003). Despite this, only the European Medicines Agency (EMA) set guidelines for reporting total concentrations of drugs (sum of the parent compound and its metabolites) excreted in aquatic or terrestrial environments (EMA, 2006; 2008). In addition, the EMA set out that any metabolite formed at a concentration greater than 10% of the parent compound should be further investigated (phase II tier B) for potential ecotoxicological effects (EMA, 2006; 2008). According to the European threshold safety value of 0.01 μg L^{-1} (EMA, 2006), only compounds exceeding this concentration in the environment are subjected to an ERA. Procedures for conducting ERA on PhACs have been developed on the basis of ecotoxicological data reported, mostly through the estimation of toxic units (TUs) or hazard quotients (HQs). TUs or HQs are associated to the ecotoxicological risk of a given compound, or a mixture of compounds, to exert short-term or long-term effects on non-target organisms (Gros et al. 2010, Ginebreda et al., 2010; Ginebreda et al., 2014). These TU and HQ values are defined as the ratio of the measured environmental concentration (MEC) of a given compound to its EC_{50} or LC_{50} acute toxicity (Sprague, 1970) or its associated chronic toxicity, usually expressed as NOEC (non-observed effect concentrations) (Castiglioni et al., 2004; Cooper et al., 2008). EC_{50}, LC_{50} and NOEC values are commonly determined using standard aquatic ecotoxicity tests to algae, daphnids or fish. These TU and HQ values can also be predicted if NOEC or MEC values are not available. If chronic toxicity has not been tested, which occurs frequently for PhACs, predicted non-observed effect concentrations (PNEC) can be extrapolated by dividing the EC_{50} or LC_{50} acute ecotoxicity by an assessment factor (AF; usually 1,000). Similarly, when MEC values are not determined, predicted environmental concentrations (PEC) can be applied. The PEC value is commonly estimated based on the percentage of market penetration, maximum daily dose, metabolism excretion rates, amount of WW per inhabitant, removal rates in WWTPs and a dilution factor (EMA, 2006; Riva et al., 2015).

These HQ or TU values are determined for every compound present on the environment using individual concentrations or for mixtures of compounds, using aggregations by simply concentration addition (Ginebreda et al., 2014). Concentration Addition (CA) is usually accepted as a first tier approach estimation of the toxic risk of mixtures (Backhaus and Faust, 2012). According to the safety guidance documents (EMEA, 2006) on ERA, if HQ values estimated for a given compound or a mixture are below the unit, no ecotoxicological risk is expected. However if the estimated HQ value equals or exceeds the unit, a potential environmental risk situation is anticipated.

Many PhACs prioritization exercises have been conducted in US and Europe (Oldekamp et al.,

2013). For instance, 40 parent compounds and 14 metabolites were prioritized in France following theoretical approaches (i.e. PEC, PNEC) and environmental measurements (Besse and Garric, 2008). Similarly, 14 PhACs were prioritized in Italy (Riva et al., 2015); while 12 PhACs were selected as the more concerning in UK (Donnacchie et al., 2015).

For the proper risk assessment of PhACs on the aquatic environment, time-consuming and costly intensive monitoring to measure concentrations in the field are required. As a consequence, the fate of PhACs along the water courses is frequently predicted. In this context, fate and transport models emerged as an appropriate alternative or complement (Johnson et al. 2008) to be applied in the assessment of the potential environmental risk of a given PhAC or a mixture of them.

1.10. Water quality modeling to assess the fate of PhACs in the aquatic environment

The use of models as predictive tools to interpret the complex reality in the presence of limited experimental information, as is the case of chemical fate assessment, has grown substantially during the last decades of modeling science (e.g. Beven, 2006). Incorporation of Geographic Information Systems (GIS) to modeling has greatly enhanced its possibilities (Pistocchi 2014). Complementary to monitored concentrations, water quality models have been developed to generate PECs from estimated point or diffuse emissions of chemicals to the environment. Hence, modeling studies have shown that concentrations of PhACs in WWe and in SW can be predicted with reasonable accuracy when realistic data on chemical emissions and water discharge are available (Pistocchi et al., 2010). Conversely, estimation of emissions from measured environmental concentrations is possible by inverse modeling (Pistocchi et al. 2012; Boxall et al. 2014; Banjac et al. 2015)

Water-quality modeling has evolved a great deal, and specially with the evolution of computational tools, since the first dissolved oxygen model was developed in the early 20th century (Streeter et al., 1958). Today, the concentration of a chemical can be basically predicted by two categories of modeling: generic computer models, such as EUSES (Vermeire et al., 1997) and in-stream water quality models, such as GREAT-ER.

Generic models use a multimedia ‘unit world’ approach to estimate regional PECs (e.g. EUSES (1996) or HAZCHEM (1994)), aiming to determine the global risk among environmental compartments, but these do not account for spatial and temporal variability in landscape characteristics, river flows and/or chemical emissions. Hence, the results offer no realistic predicted fate data, and are merely applicable on a generic screening level (Feijtel et al., 1998).

To identify hotspots in a river catchment, different in-stream water quality models for down the- drain chemicals, such as PhACs, are proposed (Ort et al., 2009). The use of simple flow approaches in the field of chemical fate modeling, has allowed the assessment of concentration patterns of compounds arising from a given source. Among these models, the “plug-flow” (PF) model is often the elective tool for simulation of river quality (e.g. Chapra, 1997). This approach describes the concentration of a chemical substance along the stream network downstream of an emission source. In addition, these simplified models, can be implemented directly using Geographical Information System (GIS) analytical capabilities, providing reasonably realistic predicted spatial distributions of chemical concentrations, through extremely simple mathematical calculations. This has been shown with reference to the continental distribution of many chemicals of environmental

concern, though their application on PhACs is very limited (Pistocchi et al., 2010). Nevertheless, water quality models relying on geo-referenced computer programs have become more popular. Examples for such models are GREAT-ER (Geography-referenced Regional Exposure Assessment Tool for European Rivers) (Feijtel et al., 1998) or its U.S. equivalent PhATE (Pharmaceutical Assessment and Transport Evaluation) (Anderson et al., 2004), LF2000-WQX (Keller et al. 2004; Johnson et al. 2007) among others (Pistocchi et al. 2010). Such models show the predicted environmental concentrations of chemicals within an entire river catchment as a concentration profile on a regional scale and also hot-spots, thus enabling to locate point sources. These approaches, which integrate the processes that influence the fate of PhACs in the aquatic environment (e.g. human metabolism, removal in WWTPs, dilution in receiving waters and other natural attenuation processes), can predict spatially resolved concentrations for compounds being released into SW via WWe discharge from WWTPs as their emission source (Alder et al., 2010). The advantages of such simulation programs over generic models are the increased realism of the chemical exposure assessment by incorporating spatial and temporal characteristics of the receiving environment. Moreover, these methodologies are easy to use and highly cost-effective (Alder et al., 2010). One of the most relevant geo-referenced computer simulation programs is GREAT-ER, a GIS-based computer program developed and validated by ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals), as an accurate aquatic chemical exposure prediction tool for use within the EU environmental risk assessment schemes. The software couples specific substance market data with relevant environmental substance information in order to calculate the distribution of real-world PECs of consumer chemicals in SW, for individual river stretches as well as for entire catchments, in a geo-referenced output map (Schowanek and Webb, 2000). GREAT-ER has already been successfully applied and validated for a number of consumer-product ingredients in European river catchments (Wind et al., 2004).

For instance, Alder et al. (2010) compared MECs of β-blockers (atenolol, sotalol, metoprolol, and propranolol) in WW and SW with PECs using an implementation of the geo-referenced model GREAT-ER for the Glatt Valley Watershed (Switzerland). They demonstrated the ability of the model to predict spatially resolved river concentrations based on average consumption and excretion data, removal in WWTPs and dissipation and degradation processes in surface water within an accuracy factor of 2.

Similarly, Anderson et al. (2004) developed and validated the PhATE model to estimate concentrations of PhACs in U.S. SW resulting from human consumption. Overall, they demonstrated the capability of the PhATE model to predict screening-level concentrations of PhACs and related compounds in the environment as well as to evaluate the suitability of existing fate information for a given drug (Anderson et al., 2004).

However, the accuracy of PECs, depends critically on the assumptions considered in the model and the variability of many factors (e.g. human consumption rates and patterns, chemical removal efficiency in distinct WWTPs, physicochemical properties of the compounds, hydrological conditions in rivers) (Pistocchi et al., 2010). Consequently, model predictions require to be compared with measured real data in order to evaluate their associated uncertainty. In the specific case of spatially fate models of chemicals, only spatial observational data for large regions or the globe should be used (Pistocchi et al., 2010). The current limitations of the application of science modeling to fate and exposure assessment of chemicals of environmental concern, such as PhACs, reveal that further refinement of the models implementing realistic approximations are needed. In consequence, the extensive assessment of fate of PhACs in SW systems at large scale is still demanded.

Chapter 2

Objectives

Chapter 2

Objectives

In view of the aforementioned concerns about the presence of PhACs and their TPs in the aquatic environment, the goal of this thesis is to study the fate of these substances in WWTPs and Iberian River basins and the ecotoxicological risk that PhACs and their TPs may pose to aquatic organisms.

The specific objectives are:

1. To develop an LC-MS/MS-based analytical methodology for trace quantification of DCF and SMX, their human metabolites and their nitrifying/denitrifying TPs, which were previously described to be formed in batch-reactors (Pérez et al., 2008; Nödler et al., 2012), in order to assess if they are also present in real WWTP samples.
2. To investigate if close structural analogs of DCF (2-anilinophenylacetic acid, mefenamic acid, tolfenamic acid, meclofenamic acid and flufenamic acid) are also able to form nitrosation/nitration TPs under experimental conditions identical to those used in the degradation studies on DCF (Pérez et al., 2008) employing HRMS.
3. To conduct large-scale monitoring studies of up to 96 PhACs on water and sediment samples collected across four Iberian river basins, characterized by high anthropogenic pressure, identifying the key factors affecting their occurrence.
4. To use chemometrics for the evaluation of the temporal and spatial distribution of a selected list of 76 PhACs in the SW and sediment samples measured in point 3, and to apply a “plug-flow” model (Pistocchi etal., 2010) to predict the natural attenuation of another selected list of 14 PhACs at the Llobregat river catchment.
5. To assess the ecotoxicological risk that PhACs may pose to aquatic ecosystems by (i) measuring the acute toxicity of PhACs and their TPs towards *Daphnia magna* and *Vibrio fischeri*; (ii) identifying the main contributors among the PhACs to the overall ecotoxicological risk of a given SW sample and (ii) examining the impact of changing PhAC levels and water flow conditions on the structure and function of river biofilms.

Chapter 3

Analysis and identification of diclofenac, related compounds and their transformation products

Chapter 3

Analysis and identification of diclofenac, related compounds and their transformation products.

3.1. Introduction

This chapter describes the study of the fate and behaviour of PhACs under the NAS treatment in WWTPs and after their release into receiving SW via WWe discharge. The chapter is divided in two sub-sections. First sub-section includes the publication reporting the development and validation of a sensitive analytical protocol for the simultaneous determination of DCF, its main human metabolites and TPs in WW (Osorio et al., 2014b). The method was further optimized and validated for the additional analysis of SMX and its TPs in WW and SW. Although the corresponding publication *(submitted to Journal of Hazardous Materials)* is not included in this chapter, the subsequent results are discussed together in the general discussion of chapter 6. The second sub-section presents the un-published findings of the investigation on the microbial mediated biotransformation of diclofenac and other related pharmaceutical structures into nitro and nitroso derivates that may occur in the NAS *(submitted to Journal of Hazardous Materials)*.

3.2. Article: "Simultaneous determination of diclofenac, its human metabolites and microbial nitration/nitrosation transformation products in wastewaters by liquid chromatography/quadrupole-linear ion trap mass spectrometry"

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Simultaneous determination of diclofenac, its human metabolites and microbial nitration/nitrosation transformation products in wastewaters by liquid chromatography/quadrupole-linear ion trap mass spectrometry

Victoria Osorio[a], Marta Imbert-Bouchard[a], Bozo Zonja[a], José-Luis Abad[b], Sandra Pérez[a,*], Damià Barceló[a,c]

[a] Water and Soil Quality Research Group, IDAEA-CSIC, c/ Jordi Girona 18-26, 08034 Barcelona, Spain
[b] RUBAM-IQAC-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain
[c] Catalan Institute for Water Research (ICRA), Scientific and Technological Park of the University of Girona, Emili Grahit 101, 17003 Girona, Spain

ARTICLE INFO

Article history:
Received 15 December 2013
Received in revised form 26 March 2014
Accepted 17 April 2014
Available online 24 April 2014

Keywords:
Diclofenac
Transformation products
Nitration
Nitrosation
High resolution mass spectrometry
LC–MS

ABSTRACT

An analytical method was developed and validated for the first determination of five major human metabolites of the non-steroidal anti-inflammatory drug diclofenac as well as two microbial transformation products in wastewater. The method was based on the extraction of diclofenac and the chemically synthetized compounds by solid-phase extraction (SPE), using a hydrophilic–lipophilic balanced polymer followed by liquid chromatography (LC) coupled to hybrid quadrupole-linear ion trap mass spectrometry (QqLIT-MS). Quantitation was performed by the internal standard approach, to correct for matrix effects. The accuracy of the method was generally higher than 40% for raw and treated wastewater with a precision below 12%. In wastewater influent and effluent samples the detection limits for the majority of target compounds were 0.3–2.5 ng L^{-1} and 0.1–3.1 ng L^{-1}, respectively. The method was applied to the analysis of influent and effluent wastewater samples from urban wastewater treatment plants. Moreover, to obtain an extra tool for confirmation and identification of the studied diclofenac-derived compounds, Information-Dependent Acquisition (IDA) experiments were performed, with selected reaction monitoring (SRM) as the survey scan and an enhanced product ion (EPI) scan as the dependent scan. Diclofenac and its major human metabolite, 4′-hydroxydiclofenac were detected in all samples at concentrations of 331–1150 ng L^{-1} and 585–6000 ng L^{-1}, respectively. Neither microbial transformation product of diclofenac was detected in any of the influent samples analyzed, but in effluents, their concentrations ranged from 4 to 105 ng L^{-1}.

1. Introduction

Diclofenac (DCF) is a non-steroidal anti-inflammatory drug (NSAID) widely used for the treatment of inflammatory disorders and painful conditions. In humans the extensive first-pass metabolism reduces the oral bioavailability to about 50% [1,2]. During hepatic metabolism, DCF undergoes hydroxylation to yield predominantly 4′-hydroxydiclofenac (4′-OH-DCF) and to minor extent 5-hydroxydiclofenac (5-OH-DCF), as well as glucuronidation of the carboxylic acid to produce the 1-O-acyl glucuronide (DCF-gluc) (see Table S-1 for structures) [3]. Thus DCF together with its human metabolites enter wastewater treatment plants (WWTPs) through sewers. DCF has been frequently detected in effluents samples collected at European WWTPs at concentrations ranging from 0.1 to over 5 μg L^{-1} [4]. Incomplete removal efficiencies of DCF during conventional activated sludge treatment (7–80%; [5]) translate into its frequent appearance in surface waters. Widespread use of DCF as over-the-counter drug in conjunction with relatively high doses at short dosing intervals and low removals in WWTPs lead to its continuous discharge into the aquatic environment, making it a pseudo-persistent pollutant therein. By definition, removal rates in WWTPs only reflect the disappearance of the compound itself without addressing the processes leading to its removal. If biochemical processes are involved in the removal of organic contaminants, biodegradation proceeds

* Corresponding author at: IDAEA-CSIC, Department of Environmental Chemistry, Jordi Girona 18-26, Barcelona 08034, Spain. Tel.: +34 934006100.
E-mail addresses: spsqam@idaea.csic.es, spsqam@cid.csic.es (S. Pérez).

http://dx.doi.org/10.1016/j.chroma.2014.04.058
0021-9673/

via the formation of a series of intermediates before the compound is – in the ideal case – ultimately mineralized. The treated effluents may thus contain not only remaining parent drug and excreted human metabolites but also microbial transformation products, all of which being discharged into receiving water bodies.

Although the major human metabolites were identified more than three decades ago [6], their presence in wastewater samples has been described only recently: the two hydroxylated metabolites 4′-OH-DCF and 5-OH-DCF were reported to occur in raw wastewater at concentrations ranging from 0.06 to 3.0 $\mu g\,L^{-1}$ and from 0.06 to 0.7 $\mu g\,L^{-1}$, respectively [7–9]. The major metabolite of DCF, 4′-OH-DCF, together with 5-OH-DCF and the lactam of 4′-OH-DCF (4′-OHD-DCF), were detected at levels of 0.71 $\mu g\,L^{-1}$, 0.45 $\mu g\,L^{-1}$, and 0.42 $\mu g\,L^{-1}$, respectively, while DCF concentrations ranged from 1.3 to 3.3 $\mu g\,L^{-1}$ in wastewater samples [10]. However, the human metabolites have been never analyzed in environmental samples. Moreover, to date no quantitative information is available on the occurrence of 4′,5-dihydroxydiclofenac (4′,5-diOH-DCF) and DCF-gluc in WWTPs, but it has been speculated that the conjugate presents hydrolytic instability of its ester bond which may lead to the release of DCF during the biological wastewater treatment and thereby explain the occasional observation of effluent DCF concentrations exceeding those measured in the corresponding influents [11].

Besides the presence of human metabolites of DCF in the WWTPs, formation of microbial transformation products (TPs) is a second aspect to be considered in assessing the overall fate of pharmaceuticals. There is growing interest in the study of the whereabouts of pharmaceuticals in WWTPs and the aquatic environment which is largely facilitated by technological advances in instrumentation suitable for analyzing polar organic compounds in complex matrices. Fate studies were the objective of the work presented by Pérez and Barceló [7] who investigated the transformation of DCF in lab-scale bioreactors loaded with mixed liquor from a municipal WWTP. Through the application of several mass spectrometric approaches, two hitherto unknown TPs of DCF, namely a nitroso derivative (TP323) and nitro derivative (TP339) were described for the first time. However, no detection of the two TPs in WWTP samples was attempted due to the lack of availability of pure standards. One of the main hurdles for measuring TPs in environmental samples is the need for available standards for method development and samples quantification; these are rarely commercially available. An alternative to obtain reference compounds is via “in-house” classical organic synthesis or biochemical synthesis. In order to generate human metabolites not commercially available at the time for further analysis in wastewater samples, Pérez and Barceló [7] biosynthesized 4′-OH-DCF by means of recombinant human cytochrome P450.

Importantly, DCF (along with the pharmaceuticals 17-β-estradiol and 17-α-ethinylestradiol) has been proposed to be included in the EU Commission first watch list of substances in order to gather monitoring data for the purpose of facilitating the determination of appropriate measures to address the risk posed by those substances [12]. In view of the environmental concern about DCF-related metabolites and TPs which are expected to be discharged with wastewater effluent into surface waters, the goal of the present work was to develop and validate a sensitive analytical protocol for the simultaneous determination of DCF, five human metabolites as well as the two chemically synthesized compounds, TP323 and TP339 in order to better understand the overall fate of DCF. The methodology, which relied on solid-phase extraction (SPE) and liquid chromatography/tandem mass spectrometry (LC–MS/MS), was applied to monitor influent and effluent water samples from Spanish WWTPs. To achieve an additional level of confidence in the detection of the target analytes, routinely analyzed in selected reaction monitoring (SRM) mode, the so-called instrument-dependent analysis (IDA) mode was activated on the hybrid triple quadrupole/linear ion trap (QqLIT) mass spectrometer to generate high-sensitivity product ion spectra.

2. Experimental

2.1. Chemicals

While diclofenac was obtained from Sigma–Aldrich (Steinheim, Germany), 4′-OH-DCF, 5-OH-DCF and DCF-gluc were purchased from Toronto Research Chemicals (Toronto, Canada). The human metabolites, 4′,5-diOH-DCF and the lactam form of 5-OH-DCF (5-OHD-DCF), as well as the microbial nitration/nitrosation transformation products TP339 and TP323 were chemically synthesized, purified and characterized according to the information provided in Sections 2.2 and 2.3 (see also supplementary content: Table S-2, Figs. S-1 and S-2). Isotopically labeled compounds, used as internal standards, were mefenamic acid-d_3 (MFA-d_3) purchased from Toronto Research Chemicals (Toronto, Canada) and niflumic acid-d_5 (NFA-d_5) purchased from Santa Cruz Biotechnologies (Santa Cruz, Canada). Sulfadimethoxine-d_6 (SDM-d_6) and lumiracoxib (LMX), used as surrogates, were provided by Sigma–Aldrich and Toronto Research Chemicals, respectively. Individual stock solutions of the analytes and the isotopically labeled internal standards were prepared on a weight basis in methanol (1000 $\mu g\,L^{-1}$) and stored at −20 °C. A mixture of all target analytes were prepared by appropriate dilution of individual stock solutions in methanol/water (5:95, v/v). Working standard solutions were prepared freshly in the same solvent mixture before each analytical run. A separate mixture of isotopically labeled internal standards, used for internal standard calibration, was prepared in methanol (1000 $\mu g\,L^{-1}$) and further dilutions were prepared in methanol/water (5:95, v/v). They were generated using linear regression analysis and afforded good fits over the established concentration range of 0.1–100 $ng\,mL^{-1}$ ($r^2 > 0.999$). For quantification purposes, the internal standard calibration approach was used, performing eight-point calibration standards daily, and the possible fluctuation in signal intensity was checked by injecting a standard solution at two concentration levels after each 8–10 injections.

The cartridges used for SPE were Oasis HLB (200 mg, 6 mL) and Oasis MAX (150 mg, 6 mL) (Waters, Milford, MA, USA). Glass fiber filters Whatman (Maidstone, Kent, UK) (0.7 μm) and nylon membrane filters (0.45 μm) were purchased from Teknokroma (Barcelona, Spain). HPLC-grade methanol, acetonitrile, water (Lichrosolv), hydrochloric acid (37%) and formic acid (98%) were supplied by Merck (Darmstadt, Germany). Ascorbic acid, ammonium hydroxide and ammonium acetate (99%) were from Sigma–Aldrich.

2.2. UPLC/ESI-high resolution MS analysis of synthetized standards

Accurate mass measurements of the chemically synthetized metabolites and TPs were carried out in full-scan and product ion scan mode using an LTQ Orbitrap Velos interfaced with an Accela 1250 UPLC system (Thermo Scientific, San Jose, CA, USA). Samples were separated on a Waters Acquity BEH C_{18} column (100 mm × 2.1 mm, 1.7 μm particle size) equipped with a precolumn (50 mm × 2.1 mm) of the same packing material. The LC–MS analysis was carried out using an ESI interface working in positive and negative ion modes. For the positive ion mode the mobile phases were (A) formic acid (0.1%) in water, and (B) acetonitrile. After 1 min of isocratic conditions at 85% A, the portion of A was linearly decreased to 3% within 8 min. This condition was held for 2 min and then the initial mobile phase composition was

Table 1
Optimized QqLIT-MS/MS parameters by SRM negative and positive ionization precursor ion in the (−)-ESI corresponds to the deprotonated molecule, while in (+)-ESI corresponds to the protonated molecule. In case of TP323, precursor ion corresponds to $[M-NO+H]^+$ (surr: surrogate SPE control standard; (IS 1): internal standard 1; (IS 2): internal standard 2 and numbers in superscript indicate which internal standard was used for ion suppression correction).

Compound	R_t (min)	Precursor ion	DP	Product ion 1	CE–CXP	Product ion 2	CE–CXP	SRM ratio (SRM1/SRM2)
Analyzed by (−)-ESI mode								
DCF[2]	5.27	294	30	214	30–11	250	16–13	17
4′-OH-DCF[2]	4.72	310	45	266	22–11	230	12–13	6.8
5-OH-DCF[2]	4.40	310	45	266	22–11	230	12–13	–
4′,5-diOHDCF[1]	3.90	326	30	282	18–19	246	26–13	3.2
5-OHD-DCF[1]	5.31	292	55	146	28–13	228	28–13	1.2
DCF-gluc[1]	4.37	470	40	193	12–7	113	34–7	1.5
TP339[2]	5.55	339	25	259	32–20	295	19–15	30
LMX (surr) [2]	5.29	292	35	212	32–15	248	16–11	8.7
MFA-d_3 (IS 1)	4.20	243	45	199	20–5	–	–	–
NFA-d_5 (IS 2)	6.01	286	45	242	28–13	–	–	–
Analyzed by (+)-ESI mode								
TP323[2]	6.28	295	71	242	27–14	214	39–16	1.3
NFA-d_5 (IS 2)	6.97	288	61	270	35–16	–	–	–
SDM-d_6 (surr) [2]	5.17	317	61	162	33–8	92	47–14	1.02

restored within 0.3 min and maintained for column regeneration for another 1.7 min. The flow rate was 300 μL min^{-1}. The injection volume was 10 μL. The MS analysis in the positive ion mode was performed applying a spray voltage of +3500 V. The source heater temperature and the capillary temperature in the ion transfer tube were 300 and 350 °C, respectively. The sheath gas pressure was set to 35 psi and the auxiliary gas flow to 5 psi. For the analysis in negative ion mode the mobile phases used were (A) ammonium acetate (10 mM) in water and (B) acetonitrile. Gradient and flow rate were the same as for the positive ion mode. The injection volume was 10 μL. The MS analysis in the negative ion mode was performed applying a spray voltage of −2500 V. The sheath gas pressure was set to 35 psi and the auxiliary gas flow to 10 psi. The Orbitrap analyzer was operated at a resolution of 60,000. FT mass calibration was carried out by infusion of the LTQ Velos Ion Calibration Solution (Thermo Scientific Pierce). All MS data acquisition and processing were done using the software package XCalibur V2.2.

2.3. NMR analysis of synthetized standards

Compounds were characterized by nuclear magnetic resonance (NMR) using a Shielded Varian Innova 500 and a VNMRS Varian 400 spectrometer (Illinois, USA). ^{1}H NMR and ^{13}C NMR spectra were acquired working in basic frequencies described in the experimental section. Chemical shifts (δ) are given in parts per million (ppm). Trimethylsilane signal in $CDCl_3$ was used as internal reference, while $CDCl_3$ triplet signal was used in the case of ^{13}C NMR. In ^{1}H NMR spectra, each signal is given in parenthesis with designation, integration, multiplicity values of coupling constant (J) in Hz.

2.4. LC–ESI-QqLIT-MS analysis of wastewater samples

Instrumental analysis was performed by LC using a Symbiosis™ Pico (Spark, Emmen, Netherlands), equipped with an autosampler and connected in series with a 4000 QTRAP QqLIT-MS equipped with a Turbo Ion Spray source (Applied Biosystems-Sciex, Foster City, CA, USA). Chromatographic separation was achieved with a Hypersil Gold PFP endcapped column C_{18} (50 mm × 2.1 mm, particle size 3 μm) preceded by a Hypersil Gold PFP drop in guard cartridge (10 mm × 2.1 mm, particle size 3 μm), both from Thermo Scientific. The analysis in the positive ion mode was performed using acetonitrile as eluent A and HPLC grade water with 0.1% formic acid as eluent B. The elution gradient started with 5% eluent A, increasing to 95% in 8 min and then, back to initial conditions within 4.5 min. The column was re-equilibrated for 1.5 min before the next injection with a total time for chromatographic analysis of 14 min. For the analysis in the negative ion mode, eluent A was acetonitrile and eluent B was 10 mM ammonium acetate (pH 6.8). The elution gradient started with 5% eluent A, increasing to 95% in 7 min, and then initial conditions were reached in 4.5 min and re-equilibration time was 1.5 min. Chromatographic analysis lasted 13 min. In both chromatographic methods the flow rate was 300 μL min^{-1} and the sample injection volume was set at 10 μL. The optimization of compound-dependent SRM parameters (declustering potential (DP), entrance potential (EP), collision energy (CE) and cell exit potential (CXP)) for each transition was performed by infusing standards of each compound at 1000 μg L^{-1} into the mass spectrometer. The optimized parameters are summarized in Table 1. All transitions were recorded in a single retention time window, setting the values for the dwell time for each transition to 50 ms and the inter-channel delay to 5 ms.

In addition, for compounds with low intensity qualifier ions, an IDA experiment was performed with SRM as the survey scan and an EPI scan, at different collision energies, as dependent scan (see more details in Section 3). Only the main SRM transition of each compound could trigger an EPI scan. The parameters of the IDA experiment were: minimum signal intensity of 5000 cps, dynamic fill time 50 ms, LIT scan rate of 4000 Da s^{-1} and collision energies between 20 and 50 eV. Dynamic exclusion, which defines the time for which a transition is excluded after acquiring an EPI scan, was set to 20 s. The resulting EPI spectra obtained from standards were then used as a reference to confirm the presence of the target analytes in real samples. The settings for source-dependent parameters were determined by flow injection analysis (FIA) and were as follows: curtain gas (CUR), 30 V; nitrogen collision gas (CAD) high; source temperature (TEM) was 600 °C, ion source gases GS1 and GS2 were set at 55 V and 60 V. Ion spray voltages in (−)-ESI and (+)-ESI modes were set at −4500 and 5500 V, respectively. To achieve higher sensitivity, resolution at the first quadrupole (Q1) was fixed at low while the resolution at the third quadrupole (Q3) was set to unit.

2.5. Sample pretreatment, preconcentration and clean-up of wastewater samples

The method was developed and validated for wastewater influent and effluent using samples from nine Catalan (NE Spain) WWTPs: Vilafranca, Sant Feliu de Llobregat, Besòs, Riu Sec, Montcada, Granollers, Rubí and Teià located in the province of Barcelona; and the ones located in the capital province of Girona and Tarragona receiving urban, domestic and industrial wastewaters

Table 2
Conditions of the SPE optimization.

SPE	Method A	Method B	Method C
Cartridge	Oasis HLB	Oasis HLB	Oasis MAX
Sample pH	8.0	2.0	8.0
Conditioning	5 mL MeOH 5 mL H_2O	5 mL MeOH 5 mL H_2O (pH = 2)	5 mL MeOH 5 mL H_2O
Sample volume	100 mL WWI 200 mL WWE	100 mL WWI 200 mL WWE	100 mL WWI 200 mL WWE
Wash	2 mL H_2O	2 mL 5% MeOH	2 mL 2% NH_4OH
Elution	2 × 4 mL MeOH	2 × 4 mL MeOH	2 × 4 mL MeOH + 5% HCOOH
Reconstitution	5% MeOH (1 mL)	5% MeOH (1 mL)	5% MeOH (1 mL)

(see Fig. S-3). Amber glass bottles pre-rinsed with ultrapure water were used for sample collection. Bottles were placed in a cooler (at 4 °C) and delivered to the laboratory within 2 h and then they were preserved with ascorbic acid (1%). Samples were immediately pre-treated (filtered through 0.7 μm glass fiber filters followed by 0.45 μm nylon membrane filters) and stored in a freezer (−20 °C) until analysis within two days. To optimize the extraction method, the extraction efficiencies for the lipophilic–hydrophilic balanced Oasis HLB (200 mg, 6 mL) and the mixed reversed phase/anionic exchange sorbent Oasis MAX (150 mg, 6 mL) were compared (Table 2). To this end, wastewater samples were spiked prior to the extraction with standard mixtures containing the target analytes at to levels (0.2 and 0.6 μg L^{-1}). For the preconcentration of the water samples, a vacuum system (J.T. Baker, Deventer, The Netherlands) was used. In all cases, samples were loaded onto the cartridges at a flow rate of approximately 5 mL min^{-1}, the cartridges were dried and then eluted. The obtained extracts were evaporated to dryness under a gentle nitrogen stream and reconstituted with 1000 μL of 5% methanol. Finally, 25 μL of a 1 μg L^{-1} standard mixture containing the internal standards MFA-d_3 and NFA-d_5 for (−)-ESI mode and NFA-d_5, for (+)-ESI mode, were added to the final extracts (Table 1). Since the internal standards were added to the final extracts prior to injection into the LC, the final concentrations were calculated by multiplying the recovery (WWI and WWE samples spiked with target analytes (n = 3)) with the concentration obtained by internal calibration. Additionally, the two standards LMX (NI mode) and SDM-d_6 (PI mode) were added to all the samples before the extraction at a concentration level of 50 μg L^{-1} and used as controls of the entire method.

3. Results and discussion

3.1. Purification and characterization of the chemically synthetized standards

The human metabolites of DCF (4′,5-diOH-DCF and the lactam form of 5-OH-DCF) chemically synthetized were purified up to 90% using chromatographic separation in columns packed with silica gel or XAD-4 (see supplementary content) and used as standards for method development and quantitative analysis. All these compounds were characterized by NMR and high resolution/MS spectroscopy (Table S-2).

NMR spectra (^{1}H, ^{13}C, DEPT) of 4′-OH-DCF, 5-OH-DCF and the lactam form of 5-OH-DCF were in agreement with those previously reported by Kenny et al. [3]. Ullmann coupling of 2,6-dichloro-4-methoxyaniline and (6-iodo-3-methoxy)phenyl-*N*,*N*-dimethylacetamide guaranteed the presence of the two-fold hydroxylation of DCF at 4′ and 5 positions in the synthetic pathway of 4′,5-diOH-DCF (Fig. S-1). NMR spectra (including both phenyl couplings and quaternary carbons) of the final compound supported this structure.

The (−)-ESI MS/MS spectrum of 4′,5-diOH-DCF (Fig. S-1) shows a deprotonated molecule at m/z 325.9920 confirming the molecular composition of $C_{14}H_{12}NO_4Cl_2$. The loss of 44 Da (CO_2) from the deprotonated molecule resulted in the formation of a major fragment ion at m/z 282.00946 and less intense fragment ions at m/z 246.03275 and m/z 210.05687 corresponding to successive losses of two HCl molecules from m/z 282.00946. The (+)-ESI product ion profile of 5-OH-DCF gave a protonated molecule at m/z 294.00831 confirming the molecular composition of $C_{14}H_{10}Cl_2NO_2$. Two fragment ions appeared at m/z 231.04490 and m/z 148.03931. The former ion was formed by concurrent loss of CO and one chlorine radical while the latter originated from homolytic cleavage of the N—C bond and loss of the dichlorophenyl ring (Table S-2).

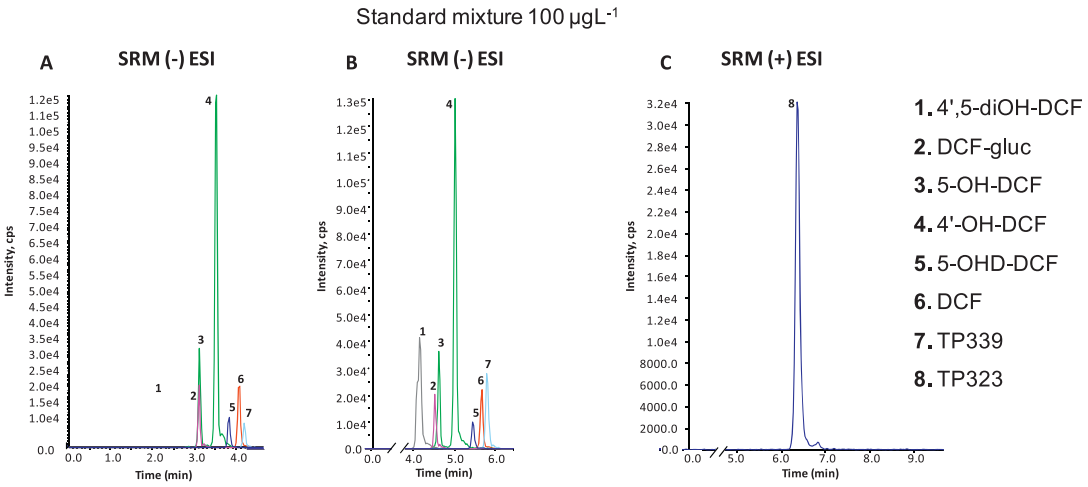
In the publication of Pérez and Barceló [7] on the biodegradation of DCF in mixed liquor from a WWTP, compounds referred to as TP339 and TP323 were tentatively identified and attributed to originate from nitration of one of the phenyl rings (TP339) and from nitrosation of nitrogen in the DCF molecule (TP323). Therefore, standards for these two compounds were also chemically synthetized and purified. Given that the MS fragmentation pattern observed for TP339 did not allow for definitive assignment of the position of the nitro group. TP339 was prepared, purified and characterized as 5-NO_2-DCF (2-(2,6-dichloroanilino)-5-nitrophenylacetic acid) which is a major compound of chemical nitration of DCF according the procedure described by Fleming et al. [13]. In this case, NMR analyses (^{1}H, ^{13}C, DEPT, COSY and HSQC) confirmed, through the unambiguosly identification of the ^{1}H NMR chemical shifts, the positions of the different phenyl hydrogens and their corresponding couplings and ultimately the position of the nitro group.

Likewise, a N-nitroso-diclofenac (TP323) standard was synthesized using the methodology reported by Zolfingol et al. [14] and purified by crystallization using as solvent $CDCl_3$. In this case, the TP323 structure (N-NO-DCF) coexists as a rotamer mixture (85:15) due to the stereochemical nature of the N—NO moiety [15]. Therefore, two contributing resonance structures were observed by NMR in $CDCl_3$ (two very clear CH_2-singlets at 3.94 and 3.50 ppm and an aromatic shift range with a complicated deconvolution for the small chemical shifts). In order to confirm its structure the (−)-ESI-MS/MS mode analysis was performed for this TP.

3.2. Method optimization

3.2.1. Optimization of QqLIT MS/MS conditions

Selection of the ionization mode was performed by comparing the intensities of the molecular ions in full-scan mode at different DP values. Of the ten compounds investigated, DCF, 4′-OH-DCF 5-OH-DCF, 4′,5-diOH-DCF, 5-OHD-DCF, DCF-gluc, TP339, MFA-d_3, and LMX showed higher response in the (−)-ESI mode; while SDM-d_6 and TP323 showed higher response in the (+)-ESI mode. The internal standard NFA-d_5 was eventually used in both ESI modes. With the exception of TP323 for which the pseudomolecular ion



A

Time (min)	Flow (mLmin-1)	Ammonium acetate (%)	Acetonitrile (%)
0:01	0.4	85	15
1:00	0.4	85	15
5:30	0.4	15	85
6:00	0.4	5	95
8:00	0.4	5	95
8:30	0.4	85	15
10:00	0.4	85	15

B

Time (min)	Flow (mLmin-1)	Ammonium acetate (%)	Acetonitrile (%)
0:01	0.3	95	5
1:00	0.3	95	5
8:00	0.3	5	95
10:00	0.3	5	95
10:30	0.3	95	5
12:00	0.3	95	5

C

Time (min)	Flow (mLmin-1)	Formic Acid 0.1% (%)	Acetonitrile (%)
0:01	0.3	95	5
1:00	0.3	95	5
9:00	0.3	5	95
11:00	0.3	5	95
10:30	0.3	95	5
13:00	0.3	95	5

Fig. 1. Chromatographic separations of target compounds for three different elution gradients applying (−)-ESI ((A) and (B)) or (+)-ESI (C).

[M+H−NO] was selected, protonated and deprotonated molecules were selected as percursor ions for (+)-ESI and (−)-ESI mode, respectively. Identification of the two most abundant fragment ions and selection of the optimum collision energies (CEs) and collision cell exit potentials (CXP) for each compound was carried out in the product ion scan mode. Dwell time of 50 ms was set to monitor three transitions and ten transitions for (+)-ESI and (−)-ESI mode, respectively. By this, each chromatographic peak was defined by 13–20 points. Table 2 shows the SRM transitions with the optimum DP, CE, and CXP values for each analyte and transition.

Quantitative and qualitative analysis. Two SRM transitions were monitored for each analyte, one for quantitation purposes, the second for confirmation. Thus, the identification criteria needed to confirm the detection of target analytes according to the EU regulations [16] were met. Besides the monitoring of the SRM transitions, additional identification criteria were used in the quantitation process: (a) the LC retention time of the compounds (2% of the retention time in the standard) and (b) the relative abundances of the two selected analyte SRM transitions in the sample had to be within 20% with respect to those in the analytical standard (see Table 1).

3.2.2. Optimization of LC conditions

To optimize the chromatographic separation, a series of preliminary experiments were performed, testing as organic mobile phase methanol, acetonitrile, or mixtures thereof, and water with 10 mM ammonium acetate/acetic acid or formic acid as the aqueous phase. The best separation was achieved by using acetonitrile as organic phase and water with 0.1% formic acid as the aqueous phase. For diclofenac, its metabolites and its nitro-derivates, acetonitrile provided higher sensitivity as methanol. The elution gradient was optimized in order to achieve maximum separation and sensitivity for the target compounds. Fig. 1 shows (−)-ESI SRM chromatograms and in particular the chromatographic separation of the hydroxylated metabolites of DCF obtained by comparison of two elution gradients A (Fig. 1A) and B (Fig. 1B). Gradient B afforded chromatographic separation of DCF-gluc and 5-OH-DCF. The better sensitivity for 4′,5-diOH-DCF could be achieved with gradient B.

3.2.3. Optimization of solid-phase extraction

Two different sorbents were tested for the preconcentration of the target analytes from wastewater samples: Oasis HLB, which assures good recovery of compounds in a wide range of polarities, and Oasis MAX, a mixed-mode polymer with reversed-phase and anion-exchange functionalities, which provides high selectivity for acidic compounds. A commonly applied SPE procedure for the simultaneous extraction of compounds with acid, basic and neutral properties relies on the use of Oasis HLB cartridges at sample pH 8.0 [17–19]. For the extraction of acidic analytes, Lindqvist et al. [20] acidified samples before SPE with Oasis HLB cartridges, Oasis MAX cartridges were used for improving the extraction of acidic metabolites and TPs [21]. Table 2 compares the three extraction protocols tested: (A) Oasis HLB at sample pH 8.0; (B) Oasis HLB with sample acidification pH 2, and (C) Oasis MAX at sample pH of 8.0.

Table 3 shows the recoveries as well as method detection limits (MDLs) and method quantification limits (MQLs) in influent and effluent wastewater samples applying the three different extraction protocols. The extraction efficiencies were determined for WWI and WWE samples ($n = 5$) spiked at 600 ng L^{-1} and 200 ng L^{-1}, respectively. Recoveries were determined by comparing the concentrations obtained after the SPE procedure, with the initial spiking levels, corrected by the background levels measured in unspiked blanks extracted in parallel. Owing to the acidic nature of the majority of analytes (see pK_a values in Table S-1), their deprotonated forms were expected to be poorly retained at pH 8.0 by sorbents with mostly hydrophobic interactions. However, the additional hydrophilic component of the sorbent allowed to obtain satisfactory extraction. The hydroxylated metabolites of DCF showed lower recoveries than the parent compound (Table 3). In order to retain all acidic compounds in their neutral form, in method B, the sample pH was adjusted to 2. This, however, resulted in recoveries in treated wastewater exceeding 100%. Finally, the use of Oasis MAX cartridges did not significantly improve extraction efficiencies of the target compounds as compared to Oasis HLB without sample acidification. With the exception of DCF-gluc, the

Table 3
Method performance parameters: linearity (linear correlation coefficients, r^2), recoveries (RSD), method detection and quantification limits (MDL and MQL in ng L^{-1}) in WWI and WWE.

Compound	Linearity (r^2)	IDL (pg injected)	Matrix effects %		Method A						Method B						Method C					
					% recovery (RSD)		MDL		MQL		% recovery (RSD)		MDL		MQL		% recovery (RSD)		MDL		MQL	
			WWI	WWE	WWI	WWE	WWI	WWE	WWI	WWE	WWI	WWE	WWI	WWE	WWI	WWE	WWI	WWE	WWI	WWE	WWI	WWE
DCF	0.9997	0.3	53	18	84 (±2)	90 (±10)	0.6	0.3	2.0	1.0	81 (±24)	>150 (±23)	1.2	2.2	3.9	7.5	40 (±6)	95 (±24)	0.5	0.4	1.7	1.27
4′-OH-DCF	0.9996	0.01	87	81	20 (±9)	30 (±6)	0.8	0.3	2.6	1.0	39 (±25)	81 (±9)	2.9	1.7	10	5.5	30 (±92)	23 (±10)	1.2	0.8	4.2	2.6
5-OH-DCF	0.9998	–	72	61	40 (±12)	24 (±7)	0.6	0.1	1.9	0.3	103 (±28)	>150 (±14)	4.7	2.6	16	8.6	79 (±20)	117 (±11)	2.0	1.5	6.8	5.0
4′, 5-diOH-DCF	0.9996	0.3	86	81	20 (±9)	34 (±12)	2.9	1.8	10	6	35 (±11)	78 (±24)	4	8	14	25	22 (±81)	13 (±23)	6.4	1.3	21.4	4.4
5-OHD-DCF	1.0000	1.6	57	17	63 (±5)	62 (±9)	1.2	0.5	4.1	1.8	81 (±27)	346 (±3)	5.9	4.5	17.2	15.0	95 (±8)	71 (±5)	3.1	1.6	10.4	5.3
DCF-Gluc	0.9996	0.3	79	59	11 (±55)	17 (±8)	26	3	88	9	–	>150 (±1)	276.4	26.6	921.4	80.5	29 (±36)	12 (±26)	33.0	5.2	110.0	17.4
TP339	1.0000	1.0	77	32	32 (±4)	60 (±5)	0.2	0.1	0.6	0.5	43 (±5)	99 (±11)	0.9	0.4	2.8	1.2	24 (±4)	36 (±12)	2.0	0.4	7	1.3
TP323	0.9998	0.2	52	22	93 (±9)	101 (±2)	0.8	0.5	2.5	1.7	100 (±18)	137 (±6)	2.9	0.8	9.8	2.5	56 (±4)	127 (±5)	1.1	0.3	3.5	0.9
LMX (surr)	0.9998	0.9	55	25	90 (±7)	81 (±5)	0.5	0.2	1.6	0.8	28 (±3)	80 (±3)	3	0.8	9	2.7	17 (±15)	40 (±6)	0.5	0.2	1.7	0.7
SMT d6 (surr)	0.9998	0.6	57	65	111 (±34)	118 (±3)	0.9	0.2	3.1	0.8	103 (±7)	98 (±6)	1.6	0.9	5	3.2	101 (±4)	46 (±11)	0.8	0.5	2.7	1.6

recoveries ranged from 20 to 84% (influent) and from 24 to 101% (effluent) for Method A; from 35 to 103% (influent) and from 78 to >150% (effluent) for Method B; 22–95% (influent) and 13–127% (effluent) for Method C. DCF-gluc showed the lowest recoveries in all methods tested, with the exception of effluent processed according to Method B, where recoveries were >150%. As the best compromise, Method A was selected for extracting samples. The overall method precision, expressed as relative standard deviation (RSD), was satisfactory, with RSD between 2 and 12%. Due to the low recovery and high RSD of DCF-Gluc in WWI, 11 (±55) %, the levels of this compound in WWI samples must be considered as semi-quantitative.

Regarding sensitivity, method quantification limits (MQL) were calculated from spiked wastewater ($n = 5$) as the minimum detectable amount of analyte with a signal-to-noise ratios of 3 and 10, respectively (see Table 3 for MDL). For Method A the MQL, ranged from 0.6 to 10 ng L^{-1} and from 0.3 to 1.8 ng L^{-1} for wastewater influent (WWI) and wastewater effluent (WWE), respectively (given the high MDL and MQL of DCF-gluc, regardless matrix and extraction method, this compound is not included in the ranges mentioned before).

The precision of the instrument was determined as relative standard deviation (RSD), using repeated injections ($n = 5$) of a 100 μg L^{-1} standard solution during the same day (repeatability) and on different days (reproducibility). The RSD achieved were lower than 12 and 25% for intra- and interday analysis, respectively. Regarding quantitative performance in terms of dynamic range, the linear response generally covered three orders of magnitude. Regarding the use of Oasis HLB cartridges, in general, the extraction of the analytes from samples at pH of 8.0 (Method A) was more acceptable yielding the higher recoveries and better MDLs and MQLs for almost all compounds. Therefore the SPE conditions tested in Method A are those preferably used for the extraction of pharmaceuticals, their human metabolites and their transformation products from aqueous real samples.

3.3. Matrix effects

To evaluate the degree of ion suppression or enhancement caused by the presence of matrix components in the LC eluent, the peak areas from the analysis of spiked influent and effluent wastewater extracts (after substracting the peak areas corresponding to the native analytes present in the sample) were compared with peak areas from matrix-free solutions spiked at the same concentration (100 ng mL^{-1}). The percentage of matrix effect (% ME) corresponding to each analyte was calculated as the $(A - B)/B \times 100$ [22], where A is the peak area of the standard spiked after extraction into WW effluent and influent extracts and B corresponds to the peak area obtained in a standard neat solution [23]. The signal is enhanced if the % ME > 100, whereas the signal is suppressed if the % ME < 100. All compounds were subject to ion suppression, since all ME values were below 100% (Table 3). The percentage of ion suppression ranged from 52 to 87% and from 17 to 81%, for WWI and WWE, respectively (Table 3). More pronounced matrix effects were observed in WWI which could be explained by the more complex matrix. In view of these results, it was important to use structurally similar internal standards to correct for ion suppression. In order to compensate for matrix effects, isotopically labeled compounds were used as internal standards and added to the analytical extracts before the LC–MS/MS analysis. To demonstrate that the internal standards were suitable for compensating the matrix effects, we compared calibration curves prepared in wastewater extracts and pure water (0.1–100 ng mL^{-1}) [22,23]. Identical slopes (see Fig. S-4) confirmed that calibration curves in pure water could be used for wastewater analysis when corrected by the signal intensity of the internal standard.

Table 4
Concentrations of the target compounds (ng L^{-1}): (a) influent and (b) effluent samples from ten urban WWTP.

	DCF	4′-OH-DCF	5-OH-DCF	4′,5-diOH-DCF	5-OHD-DCF	DCF-gluc[a]	TP339	TP323
(a) WW influent								
Besòs	754	4840	ND	716	73	1030	ND	ND
Montcada	829	6000	ND	ND	91	345	ND	ND
Vilafranca	523	2830	ND	ND	49	188	ND	ND
Granollers	624	3030	ND	ND	63	393	ND	ND
Girona	414	2600	417	255	51	ND	ND	ND
Teià	719	5740	ND	983	81	418	ND	ND
Riu Sec	722	5800	ND	847	109	1260	ND	ND
Tarragona	417	2300	ND	ND	41	ND	ND	ND
St Feliu	1080	4830	ND	746	100	ND	ND	ND
Rubí	841	5640	ND	1030	66	ND	ND	ND
(b) WW effluent								
Besòs	887	2220	755	ND	74	41	ND	ND
Montcada	642	1870	ND	229	57	21	ND	ND
Vilafranca	504	1410	ND	129	43	ND	ND	4
Granollers	842	1670	ND	105	23	ND	ND	36
Girona	189	585	180	12	16	ND	ND	ND
Teià	597	898	565	ND	49	13	20	105
Riu Sec	1150	2610	525	190	111	ND	ND	ND
Tarragona	331	598	ND	25	23	ND	28	67
St Feliu	720	727	290	ND	35	ND	ND	20
Rubí	675	1360	ND	212	53	38	29	29

ND: not detected.
[a] Semiquantitative results.

3.4. Analysis of the target analytes in wastewater samples

To demonstrate the applicability of the developed method, WWI and WWE samples from ten Catalan WWTPs were analyzed (see Fig. S-3). The samples were pre-concentrated by SPE Method A. Levels of the target compounds were corrected by multiplying the concentration of each compound by its corresponding recovery factor which are shown in Table 3. The results are summarized in Table 4. DCF was detected in all samples at levels in the range from 417 ng L^{-1} to 1080 μg L^{-1} in WWI and 331 ng L^{-1} to 1150 μg L^{-1} in WWE. The hydroxylated metabolite 4′-OH-DCF was present in the WWI and the WWE at higher levels than those determined for its parent compound, with a range from 3000 to 6000 ng L^{-1} and from 585 to 2610 ng L^{-1}, respectively. On the other hand, 5-OH-DCF was detected in only one influent sample at 417 ng L^{-1} and in five out of the ten effluent samples at concentrations from 180 up to 755 ng L^{-1} because it is a minor metabolite. The dihydroxylated metabolite (4′,5-diOH-DCF) was detected more frequently than the monohydroxylated (5-OH-DCF), being detectable in six and seven out of the ten WWI and WWE samples, respectively. Levels of 4′,5-diOH-DCF in WWI were in the range from 255 to 1028 ng L^{-1}, whereas in WWE they were considerably lower ranging from 12 to 229 ng L^{-1}. On the contrary, the lactam 5-OHD-DCF was detected in all samples at concentrations that did not exceed 111 ng L^{-1}. As far DCF-gluc is concerned, it was detected in six out of the ten WWI while in WWE it was only detected in four samples. The levels of DCF-gluc in Table 4 are of orientative nature because retention of this compound on the SPE cartridge is suboptimal. Despite the low recoveries of DCF-gluc in spiked WW samples, the substantially higher values determined in untreated sewage suggested hydrolytic cleavage during passage through WWTP.

In humans DCF is excreted as DCF-gluc (15%), 4′ OH-DCF (30%), 4′,5-diOH-DCF (15%) and 5-OH-DCF (10%) [6]. Therefore, metabolites of DCF should be expected to occur at higher levels than DCF. The levels of the hydroxylated metabolites determined in influent samples are in agreement with the excretion pattern of the parent drug. Regarding the concentration ranges of 4′-OH-DCF and 5-OH-DCF in the effluent samples of the present study, were higher than those observed by Stülten et al. [10]. They analyzed monohydroxylates 4′-OH-DCF, 5-OH-DCF, and the lactam 4-OHD-DCF in WWE samples from six German WWTPs but no influent samples were measured. As for DCF, concentrations in the German WWE were higher than those determined in our study. The average concentration ratios between hydroxy metabolites and DCF in the German WWE were 0.7 for 4′-OH-DCF and 0.15 for 5-OH-DCF [10]. In the present study the ratios were 2.2 for 4′-OH-DCF and 0.72 for 5-OH-DCF. These values are in agreement with those reported by Langford and Thomas [8], where DCF concentrations in two Norwegian WWE were also relatively low compared to the hydroxy-metabolite concentration. Moreover, elimination rates of compounds should be considered for comparison of metabolite-to-parent ratios in WWE, due to different operating conditions and designs of the WWTPs. In the present study several metabolites of DCF could also be detected in WWI. Interestingly, the concentrations of 4′-OH-DCF exceed that of DCF in all samples with a mean ratio of concentrations of 6.3. These results are in line with the aforementioned expectation, based on excretion pattern.

On the other hand, DCF-gluc is prone to undergo enzymatic hydrolysis in the sewer system [7], giving rise to DCF. This hypothesis would be in agreement with the conclusions of the studies of D'Ascenzo et al. [24] and Göbel et al. [25] who investigated the release of sulfamethoxazole and ethinylestradiol from glucuronide conjugates in the sewer or in the WWTP. Besides, Pérez and Barceló [7] suggested the enzymatic hydrolysis of the pharmaceutical aceclofenac (hydroxyacetic acid ester of DCF) and the acyl glucuronide of DCF, as a source of DCF formed in the biological wastewater treatment, thus they can contribute to the unexpectedly high levels of DCF in effluent samples. Furthermore, in dermal applications only 6% of DCF was absorbed while the rest was washed off [26]. Together with direct disposal of DCF-containing medication into the sewage system, these are potential sources of DCF.

Regarding the microbial TPs, neither compound was detectable in any of the WWI samples. TP323, in turn, was detected in six out of the nine WWE samples at concentrations ranging from 4 to 105 ng L^{-1}, while TP339 was only determined in three WWE at levels of 20 and 29 ng L^{-1}. These findings are in agreement with those obtained in the study of the presence of nitrated TPs of acetaminophen (APAP) in a French WWTP [27]. There, none of the

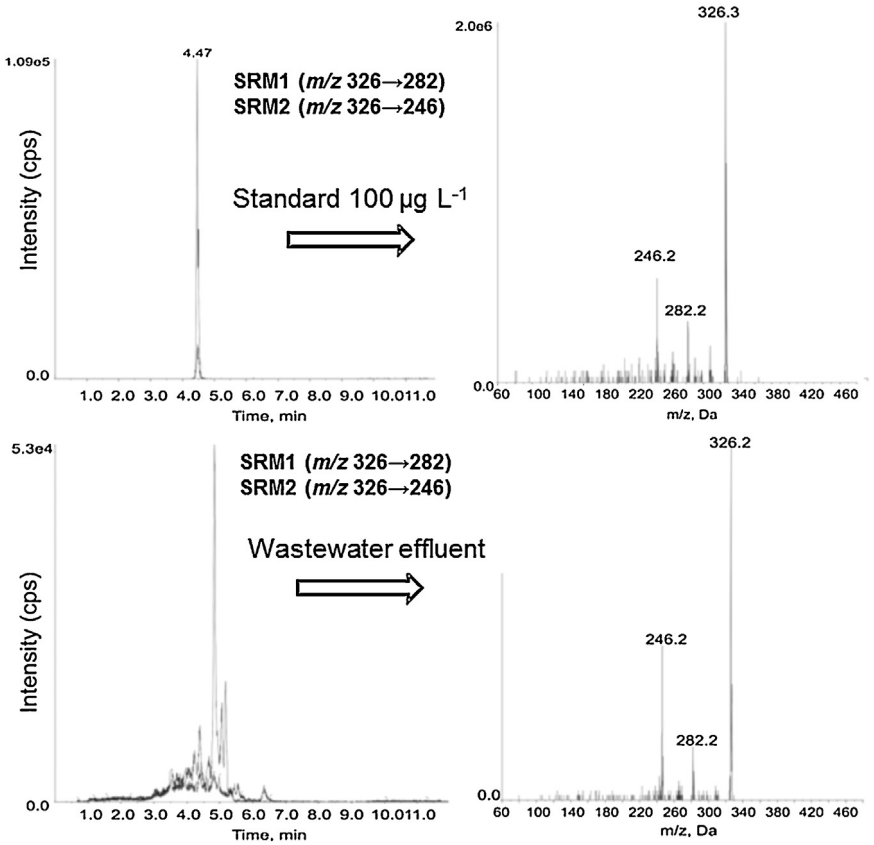


Fig. 2. Example of an IDA experiment performed for the determination of the transformation product 4′,5-diOH-diclofenac in (a) a standard and (b) an urban effluent.

nitrated TPs were determined in WWI samples, while 3-nitro-APAP and 5-nitro-APAP were determined at levels ranging from 28 to 320 ng L^{-1}. Although nitration was observed as a minor transformation pathway of APAP in nitrifying activated sludge, nitrated derivatives were formed in the WWTP and ultimately reached the receiving surface waters.

3.5. Confirmation by instrument-dependant acquisition

In the IDA experiment, an MS survey scan was used to generate a peak list of all ions present, of which one or several ions are automatically selected based on user-defined criteria and then submitted to an EPI scan. This cycle is repeated throughout the chromatographic run generating a large amount of informative data. In this study, the survey scan was the SRM acquisition, the dependent scan consisted of three EPI scans at collision energies of 20, 35 and 50 eV. Finally, EPI scans obtained from standards solutions were manually matched against the spectra obtained from the data-dependent EPI scans of the real samples. The IDA experiments were performed to confirm the presence of some of the target analytes that showed low intensity, such as nitroderivates of diclofenac or analytes with one SRM transition. Metabolites and TPs were expected to be found at low concentrations and therefore performing IDA experiments allowed for unequivocal identification of the target compounds. Fig. 2 shows an example of how an IDA experiment was performed for the determination of 4′,5-diOH-DCF in a WWE sample. In Fig. 2A and B, the extracted ion chromatograms (XIC) for the two SRM transitions recorded for a standard and a wastewater effluent sample are shown. The confirmation of the presence of 4′,5-diOH-DCF with the EPI spectra corresponding to the precursor ion mass of m/z 326 at retention time of 4.47 min showed a good match.

4. Conclusions

The developed multi-residue analytical method, based on offline SPE-LC–MS/MS allowed the simultaneous detection of eight compounds. The method afforded detection limits in the low ng L^{-1} range and good precision for wastewaters, thus providing a reliable and robust tool for routine analysis of such an ubiquitous pharmaceutical as is DCF, its main human metabolites and its microbial nitration/nitrosation transformation products in wastewater samples. Application of the method to the analysis of effluent and influent wastewaters demonstrated the occurrence of the metabolites and transformation products of diclofenac in such matrixes, in the ng L^{-1} range. To the authors' knowledge, this is the first evidence of the occurrence of microbial nitration/nitrosation transformation products of DCF in WWTPs. These results corroborate our previous work in which DCF was transformed to nitroso/nitro compounds in lab scale reactors through biological reactions. The knowledge of the presence of metabolites and transformation products in the aquatic environment is still scarce and more studies would be needed to evaluate the overall fate of selected pharmaceuticals in WWTPs. As well as elucidation and determination of unknown metabolites and transformation products, toxicity assessment on these compounds would be equally relevant, in order to define their potential toxicological effects on aquatic ecosystems.

Acknowledgements

This work has been funded by the Spanish Ministry of Economy and Competitiveness through the project SCARCE (Consolider-Ingenio 2010 CSD2009-00065). It reflects only the authors' views and the European Community is not liable for any use that may

be made of the information contained therein. The mass spectrometry team, R. Chaler. D. Fanjul and M. Comesaña, is thanked for providing support in the handling and maintenance of the equipments. SP acknowledges the contract from the Ramón y Cajal Program of the Spanish Ministry of Economy and Competitiveness. B. Z. acknowledges the Marie Curie Actions ITN CSI:Environment PITN-GA-2010-264329 for the Early Stage Researcher contract and funding.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chroma.2014.04.058.

References

[1] J.V. Willis, M.J. Kendall, R.M. Flinn, D.P. Thornhill, P.G. Welling, Eur. J. Clin. Pharmacol. 16 (1979) 405.
[2] J.V. Willis, M.J. Kendall, D.B. Jack, Eur. J. Clin. Pharmacol. 18 (1980) 415.
[3] J.R. Kenny, J.L. Maggs, X. Meng, D. Sinnott, S.E. Clarke, B.K. Park, A.V. Stachulski, J. Med. Chem. 47 (2004) 2816.
[4] M. Jiskra, J. Hollender, Biogeochemistry and Pollutant Dynamics, 2007, pp. 21.
[5] K.M. Onesios, J.T. Yu, E.J. Bower, Biodegradation 20 (2009) 441.
[6] H. Stierlin, J.W. Faigle, A. Sallmann, W. Kung, W.J. Richter, H.P. Kriemler, K.O. Alt, A. Winkler, Xenobiotica 9 (1979) 601.
[7] S. Pérez, D. Barceló, Anal. Chem. 80 (2008) 8135.
[8] K. Langford, K.V. Thomas, J. Environ. Monit. 13 (2011) 416.
[9] M. Scheurell, S. Franke, R.M. Shah, Hühnerfuss, Chemosphere 77 (2009) 870.
[10] D. Stülten, M. Zülke, M. Lamshöft, M. Spiteller, Sci. Total Environ. 405 (2008) 310.
[11] M.J. Bailey, R.J. Dickinson, Chem. Biol. Interact. 145 (2003) 117.
[12] Directive 2013/39/EU of the European Parliament and of the Council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy.
[13] S.A. Fleming, M.D. Ridges, A.W. Jensen, J. Label. Compd. Radiopharm. 38 (1996) 13.
[14] M.A. Zolfigol, F. Shirini, C.A. Ghorbani, A. Taqian-Nasab, H. Keypour, S.J. Salehzadeh, J. Chem. Res. Synop. (2000) 420.
[15] S.R. Hitchcock, G.P. Nora, C. Hedberg, D.M. Casper, L.S. Buchanan, M.D. Squire, D.X. West, Tetrahedron 56 (2000) 8799.
[16] CCL-3 Drinking water priority contaminant list for regulatory decision making and information collection September 2009 US Environmental Protection Agency (EPA) http://water.epa.gov/scitech/drinkingwater/dws/ccl/ccl3.cfm
[17] F. Sacher, F.T. Lange, H.J. Brauch, I. Blankenhorn, J. Chromatogr. A 938 (2001) 199.
[18] S. Castiglioni, R. Bagnati, D. Calamari, R. Fanelli, E. Zuccato, J. Chromatogr. A 1092 (2005) 206.
[19] A.M. Stolker, W. Niesing, E.A. Hogendoorn, F.M. Versteegh, R. Fuchs, U.A.T. Brinkman, Anal. Bioanal. Chem. 378 (2003) 955.
[20] N. Lindqvist, T. Tuhkanen, L. Kronnerg, Water Res. 39 (2005) 2219.
[21] A. Agüera, L.A. Pérez Estrada, I. Ferrer, E.M. Thurman, S. Malato, A.R. Fernández-Alba, J. Mass Spectrom. 40 (2005) 908.
[22] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 75 (2003) 3019.
[23] A. Kruve, I. Leito, Anal. Methods 5 (2013) 3035.
[24] G. D'Ascenzo, A. Di Corcia, A. Gentili, R. Mancini, R. Mastropasqua, M. Nazzari, et al., Sci. Total Environ. 302 (2003) 199.
[25] A. Göbel, A. Thomsen, C.S. McArdell, A. Joss, W. Giger, Environ. Sci. Technol. 39 (2005) 3981.
[26] P. Schrey, M. Wilhelm, Wasser 94(a) (1999) 1 (in German).
[27] S. Chiron, E. Gomez, H. Fenet, Environ. Sci. Technol. 44 (2010) 284.

Supplementary content

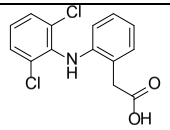
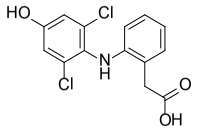
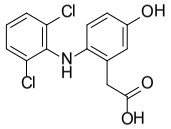
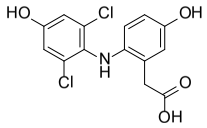
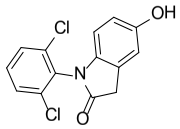
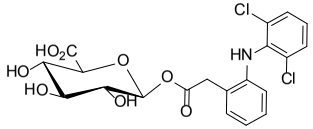
Synthesis of metabolites and transformation products

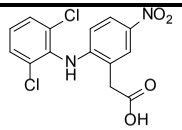
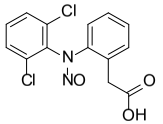
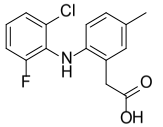
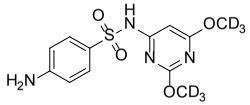
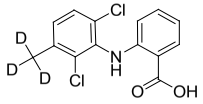
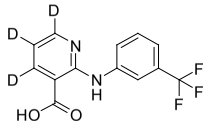
The standards were prepared following already described synthesis procedures [1, 2, 3]. When anhydrous reaction conditions were required, the methodology proposed by Burfield and Smithers was followed [4].

Human metabolites. The preparation of hydroxylated derivates of diclofenac (and evein their dehydrated amides) has been already described [5]. Thus, the methodology proposed was followed to obtain: 1-(2,6-dichlorophenyl)-5-hydroxyindolin-2-one and 4',5-dihydroxydiclofenac (2-[(2´,6´-dichlorine-4'-hydroxyphenyl)amino)]-5-hydroxyphenyl-acetic acid). On the basis of Ullman coupling, the substrates 2,6-dichlorine-4-methoxyaniline and iodinemethoxydimethylamide (previously prepared), were selected as starting products***. Figure S-2(a)*** shows the synthetic route of 4',5-dihydroxydiclofenac. This coupling provided a single product the diarilic structure with both functionalities at each one of the aromatic rings. The basic hydrolysis of the dimethoxyderivate, lead to obtain the 2-[(2´,6´-dichlorine-4'-methoxiphenyl)amino)]-5-methoxtphenylcarboxilic acid. The final step, the gradual addition of BBr_3 at a temperature of 0 ºC provided the expected acid product. ***Figure S-3(a)*** shows the synthetic route of ***5-OH-DCF-lactam***. When the starting product 3-metoxyfenil-*N*,*N*-dimetilacetamide (previously prepared) reacted with the *N*-iodinesuccinimide in acetonitrile, the (6-iodine-3-methoxy)phenyl-*N*,*N*-dimethylacetamide was regioselectively obtained. Once more, the Ullman coupling of the 6-iodine derivate with the 2,6-dichlorineaniline provided the diarylic compound with the protected alcohol at the 5 position. The corresponding carboxilic acid of the diarylamine, was obtained after basic treatment and the further addition of BBr_3 at 0 ºC lead to obtain a mix of compounds. Among these products, the resulting amide of the ciclation process of 5-hidroxydiclofenac was successfully purified and characterized as follows: reaction extracts were dryed over $MgSO_4$, filtrated and concentrated under evaporation at low pressure. Reactions were followed by thick layer chromatography. Compounds were eluted using different solvent mixtures of methylene-methanol and hexane-ethyl acetate. TLC plates were observed by UV radiation at 254 nm after or by immersion in an ethanolic solution of phosphomolibdic

acid (5 %). Chromatographic separation and purification of reaction products was achieved using columns packed with silica gel (35-70 μm). Compounds were eluted using different solvent mixtures of methylene-methanol and hexane-ethyl acetate depending on the polarity of compounds.

Table S-1: Properties of target compounds

Target Compound	Class	CAS number	Molecular structure	Molecular formula	Molecular weight	pKa[e]
Diclofenac[a] (DCF)	Anti-inflammatory drug	15307-86-5		$C_{14}H_{11}Cl_2NO_2$	296.1486	4.0
4' -Hydroxydiclofenac[a] (4'-OH-DCF)	Metabolite (hydroxylation)	64118-84-9		$C_{14}H_{11}Cl_2NO_3$	312.1480	3.8
5 -Hydroxydiclofenac[b] (5-OH-DCF)	Metabolite (hydroxylation)	69002-84-2		$C_{14}H_{11}Cl_2NO_3$	312.1480	3.8
4',5-Dihydroxydiclofenac (4',5-diOH-DCF)	Metabolite (hydroxylation)	69002-86-4		$C_{14}H_{11}Cl_2NO_4$	328.1474	3.6
5-OH-DCF-lactam (5-OHD-DCF)	Metabolite (lactam)	-		$C_{14}H_9Cl_2NO_2$	294.1328	
Diclofenacglucuronide1-β-O-acyl [b] (DCF-gluc)	Metabolite (glucuronidation)	64118-81-6		$C_{20}H_{19}Cl_2NO_2$	472.2728	3.2

TP339	Transformation product (nitrification)	-		$C_{14}H_{10}Cl_2N_2O_3$	341.1462	3.4
TP323	Transformation product (nitrification)	-		$C_{10}H_{10}Cl_2N_2O_3$	325.1468	3.5
Surrogates						
LMX	Anti-inflammatory drug (withdrawn)	220991-20-8		$C_{15}H_{13}ClFNO_2$	293.7206	4.1
Sulfadimethoxine-d_6[a] (SDM-d_6)				$C_{12}H_8D_6N_4O_4S$	316.3659	1.95
Internal Standard						
Mefenamic acid-d_3 (MFA-d_3)		-		$C_{15}H_{12}NO_2D_3$	244.3036	3.9
Niflumic acid-d_5 (NFA-d_5)		-		$C_{13}H_4D_5F_3N_2O_2$	287.2365	1.9

[a]Sigma Aldrich; [b] Toronto Research Chemicals; [c]Dr. Ehrenstorfer;[d] Santa Cruz Biotechnology; [e] estimated with ChemAxon.com

Table S-2: Characterization of chemically synthetized compounds

Ion	Elemental composition	Calculated mass [*m/z*]	Measured mass [*m/z*]	Error [ppm]	DBE
4',5-diOH-DCF					
^{1}H RMN (400 MHz, CD_3OD): 6.85 (1H, s), 6.69 (1H, d, *J* = 3), 6.52 (1H, dd, J_1 = 3, J_2 = 8.4), 6.22 (1H, d, *J* = 8.4), 3.67 (2H, s, $ArCH_2$). **^{13}C RMN** (100 MHz, $CDCl_3$): 175.8 (CO), 155.2 (C), 152.8 (C), 137.7 (C), 132.0 (C), 131.8 (C), 127.3 (C), 119.4 (CH), 118.4 (CH), 116.9 (CH), 115.3 (CH), 39.1 (CH_2).					
$[M+H]^+$	$C_{14}H_{12}NO_4Cl_2$	328.01379	328.01450	2.165	8.5
m/z 310	$C_{14}H_{10}NO_3Cl_2$	310.00323	310.00320	-0.081	9.5
m/z 282	$C_{13}H_{10}NO_2Cl_2$	282.00831	282.00839	-0.282	8.5
m/z 246	$C_{13}H_9NO_2Cl$	246.03163	246.03160	-0.133	9.5
m/z 210	$C_{13}H_8NO_2$	210.05496	210.05493	-0.119	10.5
m/z 182	$C_{12}H_8NO$	182.06004	182.06003	-0.167	9.5
$[M-H]^-$	$C_{14}H_{10}NO_4Cl_2$	325.999	325.99936	1.120	9.5
m/z 282	$C_{13}H_{10}NO_2Cl_2$	282.00941	282.00946	0.187	8.5
m/z 246	$C_{13}H_9NO_2Cl$	246.03273	246.03275	3.895	10.5
m/z 210	$C_13H_8NO_2$	210.05605	210.05687	-0.119	10.5
5-OHD-DCF					
^{1}H RMN (400 MHz, $CDCl_3$): 7.50 (1H, s), 7.48 (1H, s), 7.36 (1H, t, J = 8), 6.88 (1H, d, J = 1), 6.66 (1H, dd, J_1 = 2, J_2 = 8), 6.25 (1H, d, J = 8), 3.75 (2H, s, $ArCH_2$). **^{13}C RMN** (100 MHz, $CDCl_3$): 173.7 (CO), 152.2 (C), 136.6 (C), 135.4 (C), 130.7 (CH), 129.0 (CH), 125.6 (C), 114.1 (CH), 113.0 (CH), 109.6 (CH), 36.1 (CH_2).					
$[M+H]^+$	$C_{14}H_{10}NO_2Cl_2$	294.00831	294.00803	-0.954	9.5
m/z 259	$C_{14}H_{10}NO_2Cl$	259.03946	259.03952	0.240	10.0
m/z 231	$C_{13}H_{10}NOCl$	231.04454	231.04458	0.159	9.0
m/z 148	$C_8H_6NO_2$	148.03931	148.03936	0.372	6.5
$[M-H]^-$	$C_{14}H_8NO_2Cl_2$	291.99376	291.99387	10.5	0.386
m/z 228	$C_{13}H_7NOCl$	228.02216	228.02246	10.5	1.294
m/z 146	$C_8H_4NO_2$	146.02475	146.02452	7.5	-1.587
TP339					

^{1}H RMN (400 MHz, $CDCl_3$): 8.17 (1H, d, *J* = 2.5), 8.02 (1H, dd, *J* = 9.0, 2.5), 7.43 (1H, s), 7.41 (1H, s), 7.31 (1H, bs), 7.16 (1H, t, J= 7.5), 6.46 (1H, d, *J* = 9.0), 3.88 (2H, s).

^{13}C RMN (100 MHz, $CDCl_3$): 177.5 (CO), 149.0 (C), 141.2 (C), 135.2 (C), 131.7 (C), 129.3 (CH), 127.5 (C), 126.7 (C), 124.9 (CH), 121.4 (C), 115.7 (CH), 38.6 (CH_2).

$[M+H]^+$	$C_{14}H_{11}N_2O_4Cl_2$	341.00904	*341.00879*	0.729	9.5
m/z 322	$C_{14}H_9N_2O_3Cl_2$	322.99847	*322.99838*	-0.291	10.5
m/z 295	$C_{13}H_9N_2O_2Cl_2$	295.00356	*295.00366*	-0.341	9.5
m/z 260	$C_{13}H_9N_2O_2Cl$	260.03471	*260.03470*	-0.026	10.0
m/z 214	$C_{13}H_9NCl$	214.04180	*214.04172*	-0.390	9.0
m/z 179	$C_{13}H_9N$	179.07295	*179.07281*	-0.787	10.0
$[M-H]^-$	$C_{14}H_9N_2O_4Cl_2$	338.99448	338.99444	-0.134	10.5
m/z 295	$C_{13}H_9N_2O_2Cl_2$	295.00466	295.00470	0.148	9.5
m/z 259	$C_{13}H_8N_2O_2Cl$	259.02798	259.0274	2.271	9.5
m/z 229	$C_{13}H_8NOCl$	229.0300	229.03026	5.968	10.5

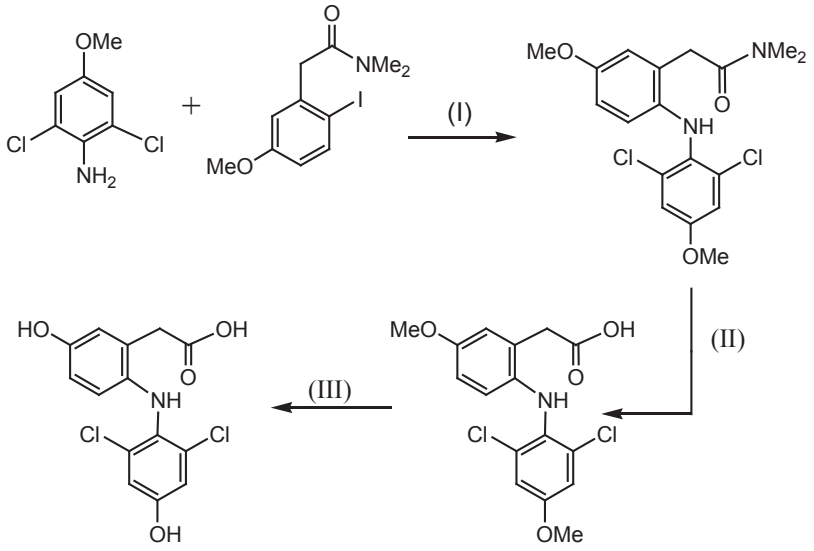
TP323 (85:15 rotamers mixture)

^{1}H RMN (400 MHz, $CDCl_3$): 7.53 (1H, dd, *J* = 7.5, J2= 1.5), 7.46 (1H, d, *J* = 1.5), 7.44 (2H, bs), 7.44-7.26 (4H, m), 7.02 (1H, dd, J1= 7.5, J2= 1.5), 3.94 (2H, s), 3.50 (0.3H, s).

^{13}C RMN (100 MHz, $CDCl_3$): 176.2 (CO), 139.0 (C), 134.3 (C), 133.5 (C), 131.7 (C), 129.7 () 129.3 (CH), 128.5 (C), 128.1 (C), 128.0 (CH), 122.6 (C), 38.5 (CH_2).

$[M+H]^+$	$C_{14}H_{11}NO_2Cl_2$	295.01614	295.01638	0.829	9.0
m/z 277	$C_{14}H_9NOCl_2$	277.00557	277.00575	0.647	10.0
m/z 242	$C_{14}H_9NOCl$	242.03672	242.03675	0.132	10.5
m/z 214	$C_{13}H_9NCl$	214.04180	214.04180	-0.017	9.5
$[M-H]^-$	$C_{14}H_9N_2O_3Cl_2$	322.99957	322.99966	-2.264	9.5
m/z 279	$C_{13}H_9N_2OCl_2$	279.00974	279.00911	-2.264	9.5
m/z 249	$C_{13}H_9NCl_2$	249.01175	249.01160	-0.615	9.0
m/z 245	$C_{13}H_8NO_2Cl$	245.0249045	245.02501	0.430	10.0
m/z 209	$C_{13}H_7NO_2$	209.04823	209.04898	3.603	11.0

Figure S-1. (a) Synthesis route of 4',5-dihydroxy-diclofenac. Reactives and conditions: (I) Cu act., CuI, K_2CO_3, toluene, reflux; (II) NaOH aquoeus, EtOH, 80 °C, Ar. (III) BBr_3, DCM, 0°.



(b) (-)-ESI-MS-product ion spectrum of 4',5-diOH-DCF, ($[M-H]^-$ *m/z* 326) acquired at normalized collision energy of 35.

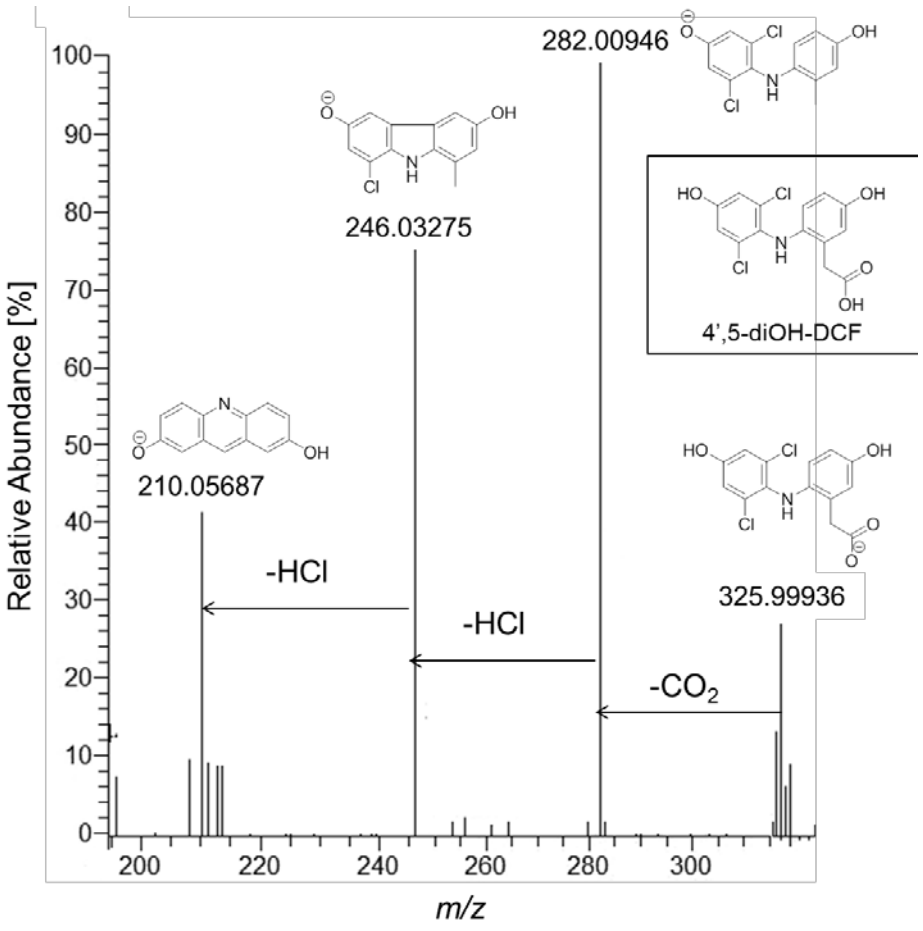
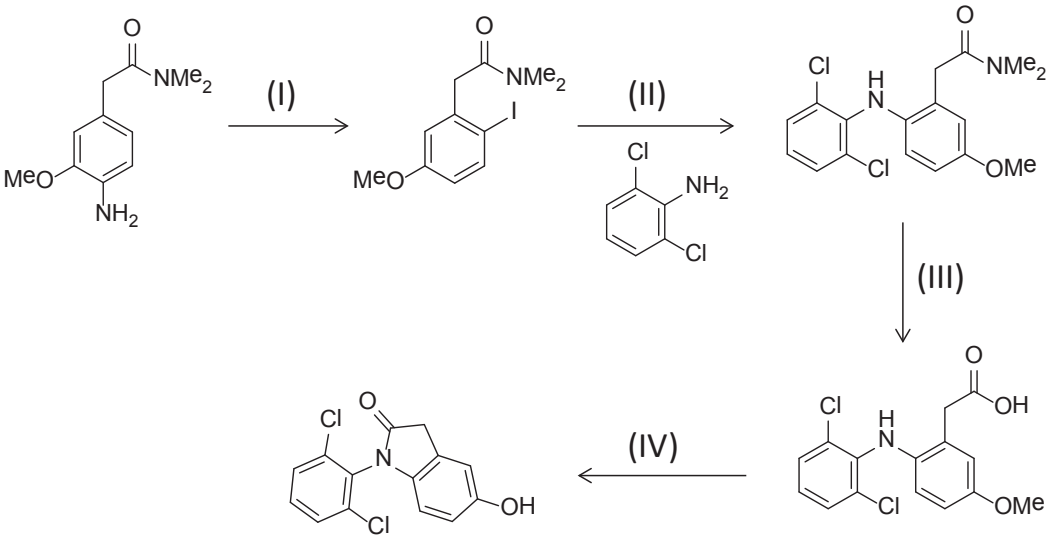


Figure S-2. (a) Synthetic route of 5-OH-DCF-lactam. Reactives and conditions: (I) *N*-Iodosuccinimide, CH_3CN, reflux; (II) Cu act., CuI, K_2CO_3, toluene, reflux; (III) NaOH aquoeus, EtOH, 80 °C, Ar. (IV) BBr_3, DCM, 0°.



(b) (+)-ESI-MS-product ion spectrum of 5-OH-DCF-lactam, ($[M+H]^+$ *m*/*z* 294) acquired at normalized collision energy of 35.

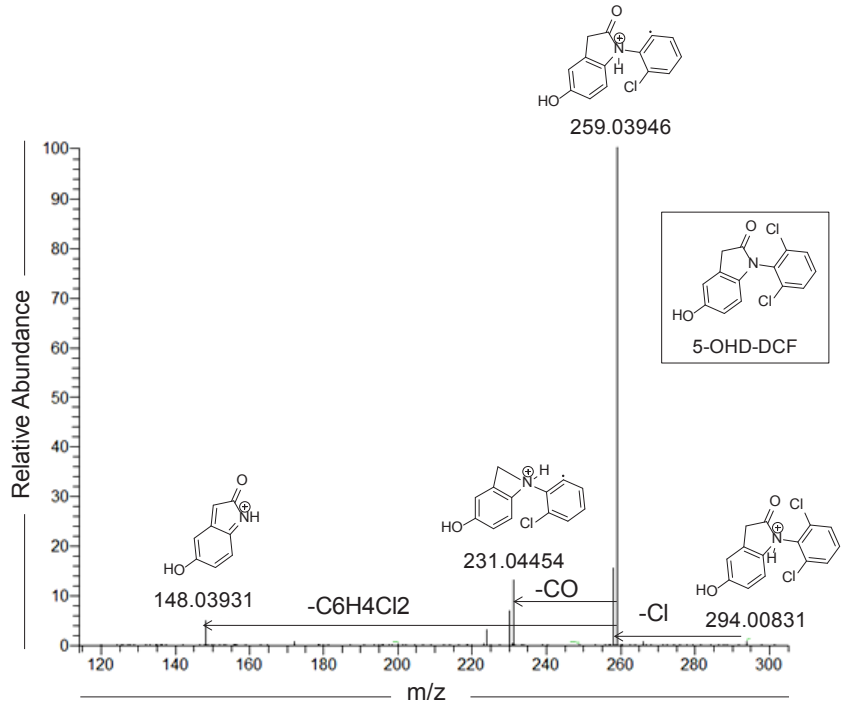


Figure S-3. WWTPs located in Catalonia (NE, Spain) sampled for screening purposes.

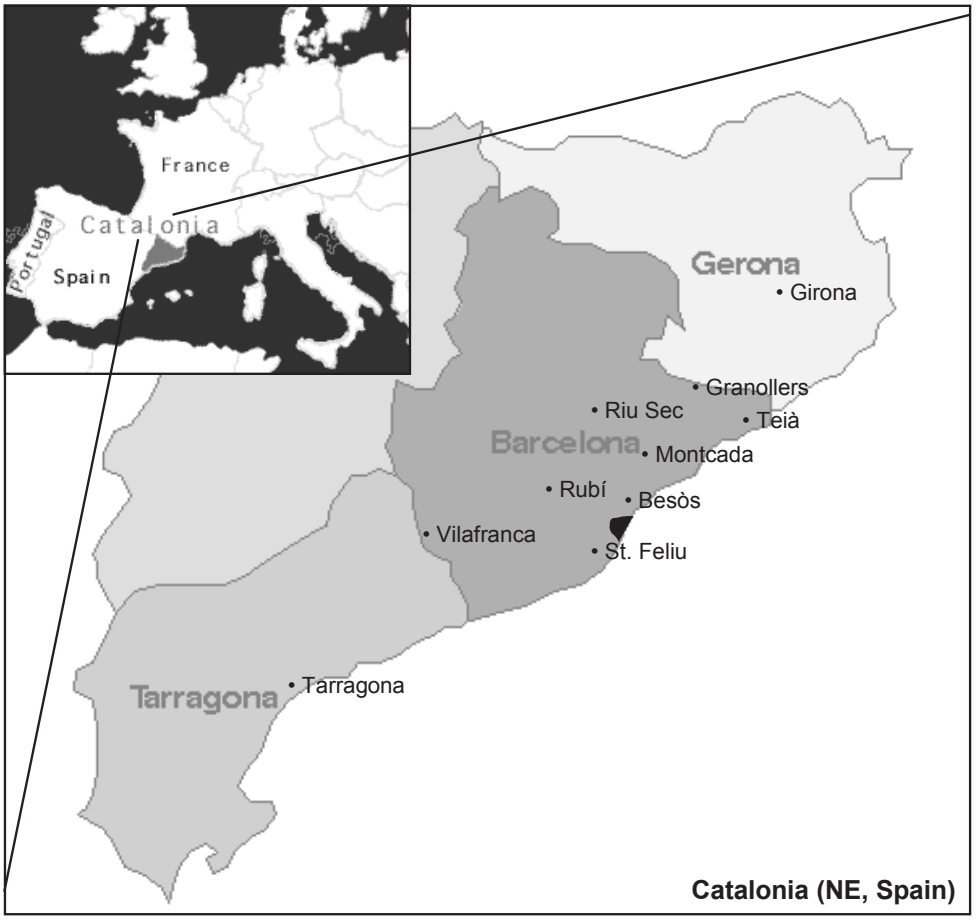
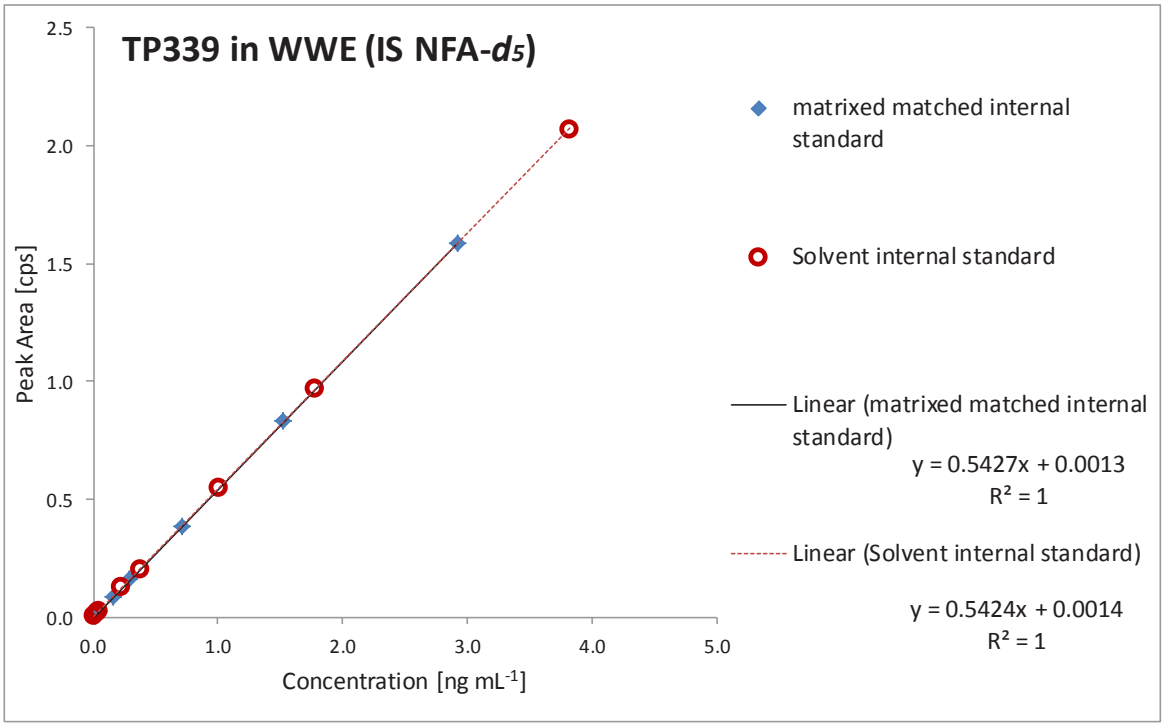
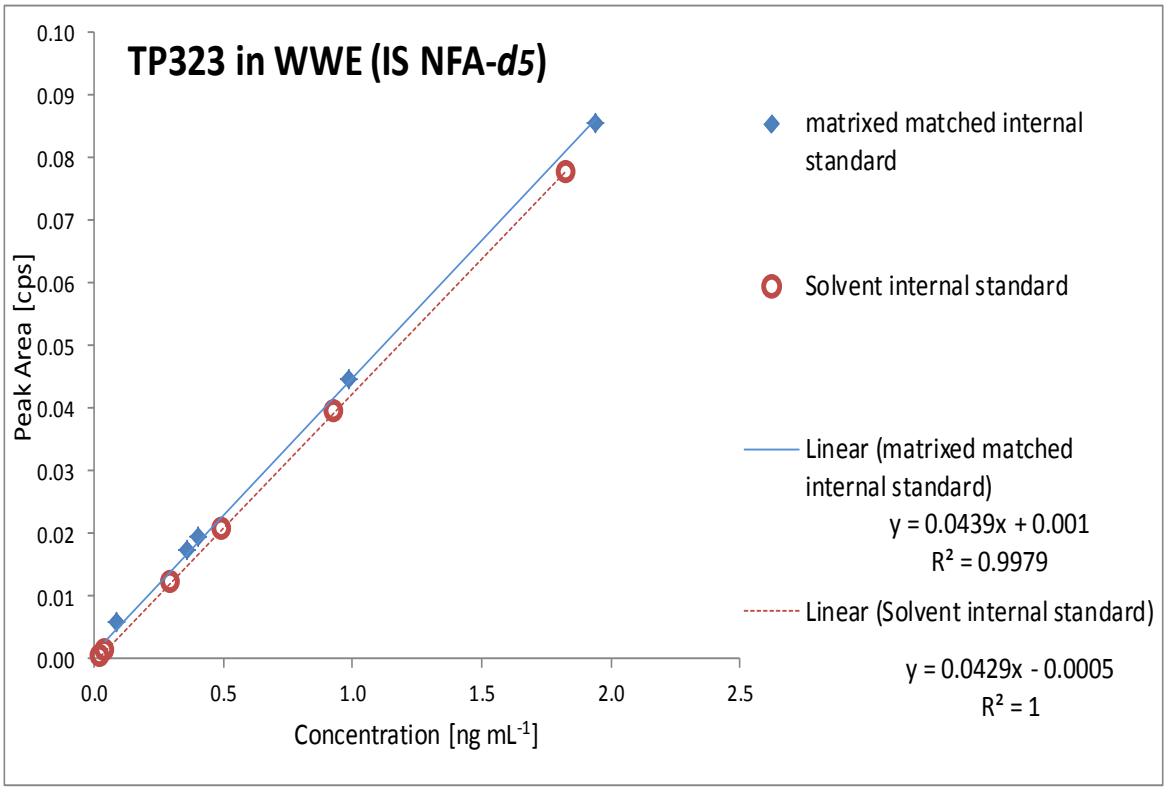


Figure S-4. Matrix effects correction by the internal standard calibration method. Examples: **(a)** TP 339 in WWE, % ME = 100.0553; **(b)** TP323 in WWE, % ME = 102.3310 and **(c)** 5-OH-DCF in WWI, % ME = 99.8829.

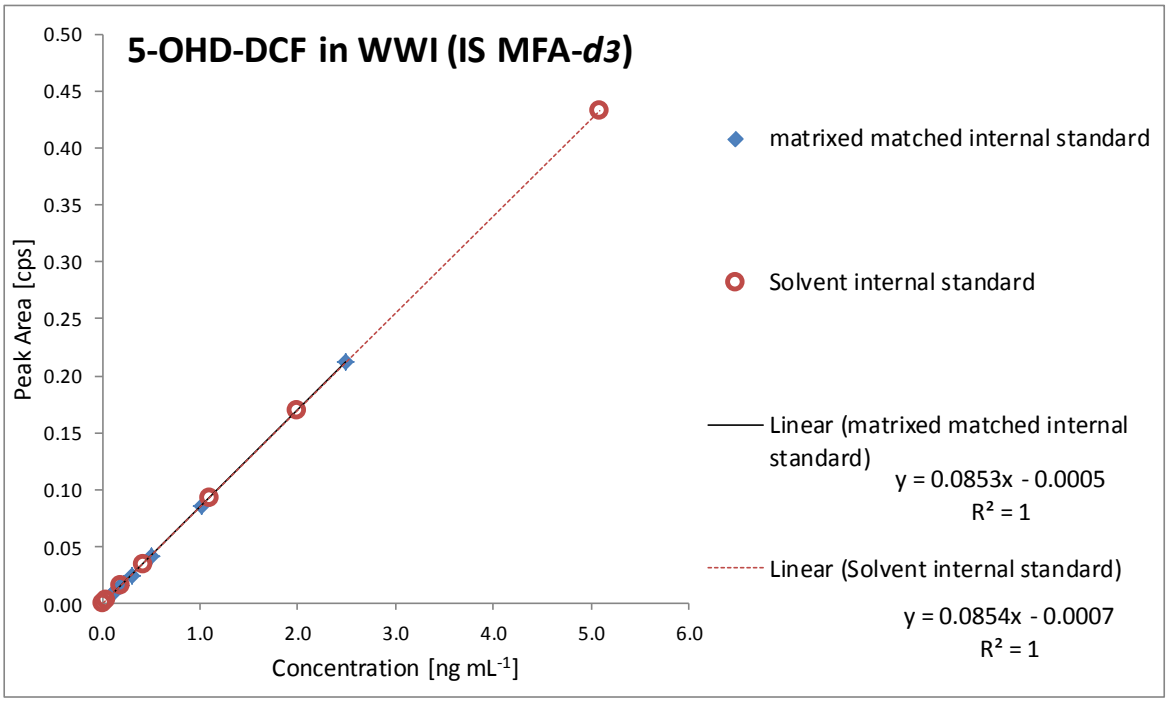
(a)



(b)



(c)



REFERENCES

[1] G.N. Bollenback, J.W. Long, D.G. Benjamin, J.A. Lindquist, J. Am. Chem. Soc. 77*(*1955*)* 3310.
[2] E.M. Hodnett, G. Prakash, J. Amirmoazzami, J. Med. Chem. 21*(*1978*)* 11.
[3] S.A. Fleming, M.D. Ridges, A.W. Jensen, J. Labelled Compd. Radiopharm. 38(1996) 13.
[4] D.R. Burfield, R.H. Smithers, J. Org. Chem., 48 (1983) 2420.
[5] J.R. Kenny, J.L. Maggs, X. Meng, D. Sinnott, S.E. Clarke, B. K. Park, A. V. Stachulski, J. Med. Chem. 47(2004), 2816.

3.3. Article: "Behaviour of diclofenac and other structurally related nonsteroidal anti-inflammatory drugs (NSAIDs) in nitrifying lab scale batch-reactors"

Behavior of diclofenac and other structurally related nonsteroidal anti-inflammatory drugs (NSAIDs) in nitrifying activated sludge of WWTPs

Victoria Osorio[1], Sandra Pérez[1*], Damià Barceló[1,2]

[1] Water and Soil Quality Research Group, IDAEA-CSIC, c/Jordi Girona 18-26, 08034 Barcelona (Spain).

[2] Catalan Institute of Water Research, ICRA Catalan Institute for Water Research- ICRA, C/Emili Grahit, 101, Edifici H2O, Parc Científic i Tecnològic de la Universitat de Girona, E-17003 Girona (Spain)

*Corresponding author:

Sandra Pérez

IDAEA-CSIC

Water and Soil Quality Research Group

Jordi Girona 18-26

Barcelona 08034, Spain

E-mail: spsqam@idaea.csic.es

Phone: ++34-93 400 6100

Fax: ++34-93 204 5904

1. Introduction

Due to demographic, social and economic factors, the production and use of pharmaceuticals in industrialized societies have increased to considerable levels. For instance, non-steroidal anti-inflammatory drugs and antimicrobials are produced annually worldwide in the range of kilotons. Non-steroidal anti-inflammatory drugs (NSAIDs) are a large group of chemically heterogeneous drugs that are used primarily to treat inflammation, mild to moderate pain, and fever. After their use in human medicine, 30-90% passes through the human body completely unchanged. They may then reach surface waters via hospital and municipal sewage. One of the anti-inflammatory drugs with the highest consumption rates is diclofenac (DCF) which has been frequently detected at high levels in influents and effluents samples from wastewater treatment plants (WWTPs). In full-scale WWTP relying on continuous activated sludge (CAS) treatment, removal efficiencies for DCF are widely varying, [7-80] %, rendering it difficult to identify common patterns in their biotransformation. As a matter of fact, DCF is in the EU watch list of organic pollutants in surface waters [1]

Compared to the amount of data dealing with the distribution of pharmaceutical residues in the environment, very little evidence has been published as regards metabolic pathways in complex microbial communities like those encountered in the aeration tank of the activated sludge treatment. And even less has been published, using novel approaches based on high resolution mass spectrometry. One of the few examples described in the literature is our previous work on DCF [2], in which nitroso (NO-DCF or TP324) and nitro (NO_2-DCF or TP340) derivatives were tentatively identified using time-of-flight mass spectrometry. During CAS treatment, the most important process that takes place in the nitrifying activated sludge treatment is related with nitrogen removal. This microbially driven process consists of two main steps which are nitrification and denitrification. In these steps reactive nitrogen species are generated which are believed to be involved in the formation of TPs containing nitro or nitroso groups such as nitroacetaminophen [3]. The formation of this compound has recently been reported for nitrifying activated sludge. Moreover, the presence of the TPs of DCF was determined in effluents from Catalan WWTPs at concentrations from 4 to 105 ng L^{-1} [4]. Given the potential ecotoxicological risks of nitrated derivatives, the generation of these compounds is a highly environmentally relevant matter of concern.

In this context, the present study aimed at investigating still uncovered aspects of the fate of NSAIDs in WWTP. Thus, biodegradation experiments were conducted controlled laboratory settings in order to gain further insight into the biodegradability and metabolic pathways of five selected NSAIDS: diclofenac (phenylacetic acid), mefenamic acid (MEF), tolfenamic acid (TOLF), meclofenamic acid (MEC) and flufenamic acid (FLUF) (anthranilic acids). In addition, the structurally related non-chlorinated compound 2-anilinophenylacetic acid (APAA), not present in the wastewater samples, was studied in order to better understand the degradability of the

NSAIDs. Samples from the bioreactor were analyzed for the presence of stable intermediates by high-resolution mass spectrometry (HR-MS).

2. Experimental

2.1. Chemicals and standards

Diclofenac was purchased from Sigma-Aldrich (Steinheim, Germany), while the following were obtained from Toronto Research Chemicals (Toronto, Canada): APAA, mefenamic acid, tolfenamic acid, meclofenamic acid and flufenamic acid.

Allylthiourea, sodium azide and ammonium acetate were provided by Sigma-Aldrich (Steinheim, Germany). Formic acid (98%-100%), acetic acid (98-100%), ammonia hydroxide (36%) and $^{15}NH_4$-N ammonia hydroxide (36%) were purchased from Merck (Darmstadt, Germany). All solvents used (methanol, acetonitrile and water) were purchased from Fisher Scientific (Geel, Belgium). Calibration of the Q-Exactive was done with ESI negative ion calibration solution from Thermo Scientific (Dresden, Germany).

2.2. Biodegradation in laboratory-batch reactors

DCF and the related NSAIDs were added at 10 mgL^{-1} to amber 1 L glass bottles loaded with 1000 mL of mixed liquor freshly collected from the aeration tanks of the WWTP Rubi. Bubbling of air through Teflon tubing into the test medium provided continuous aeration of the system and ensured suspension of the sludge particulate matter (5 gL^{-1}). For isotope labelled biodegradation experiments, two batch reactors amended with mixed liquor and spiked with 10 mg/L of DCF were run in parallel. One reactor was daily enriched with NH_4-N while NH_4-^{15}N was added to the other one, as well as a third reactor, which was not supplemented with any kind of ammonia. Biologically inactive control batch-reactors were run in parallel under identical substrate conditions. The inhibition of the biochemical activity of the microbial community of the sludge through addition of sodium azide (10 mgL^{-1}) allowed accounting for possible abiotic removal mechanisms. For the monitored period, the pH of the mixed liquor in the batch-reactors was maintained at 7.4±0.3, while the ambient temperature was 20-22 ºC.

2.3. Identification of TPs

To identify the structures of the NSAIDs TPs in the bioreactor an ultra-performance liquid chromatography (UPLC)-/(-)-electrospray ionization (ESI)-Q Exactive Orbitrap-MS was used. Samples from the biodegradation experiments were separated on a Waters Acquity BEH C18 column (50 × 2.1 mm, 1.7-µm particle size) equipped with precolumn (5 × 2.1 mm) of the same packing material. The mobile phases were (A) 10 mM aqueous ammonium acetate/ acetic acid (pH 5.8) and (B) acetonitrile. Exact mass measurements of the parent compounds and its bio-TPs obtained from the biodegradation samples were carried out in full-scan and product ion modes.

3. Results and discussion

3.1. Addition of $^{15}NH4$-N to the reactor spiked with DCF

By addition of stable isotope-labeled $^{15}NH_4$-N in bioreactor, +1 Da mass shift was expected due to incorporation of $^{15}NO_2$ and ^{15}NO into DCF molecule. MS spectra observed for NO_2-DCF (TP340) and isotope-labeled ^{15}N NO_2-DCF (TP340), allowed to confirm position of NO_2 group in the molecule (figure 1).

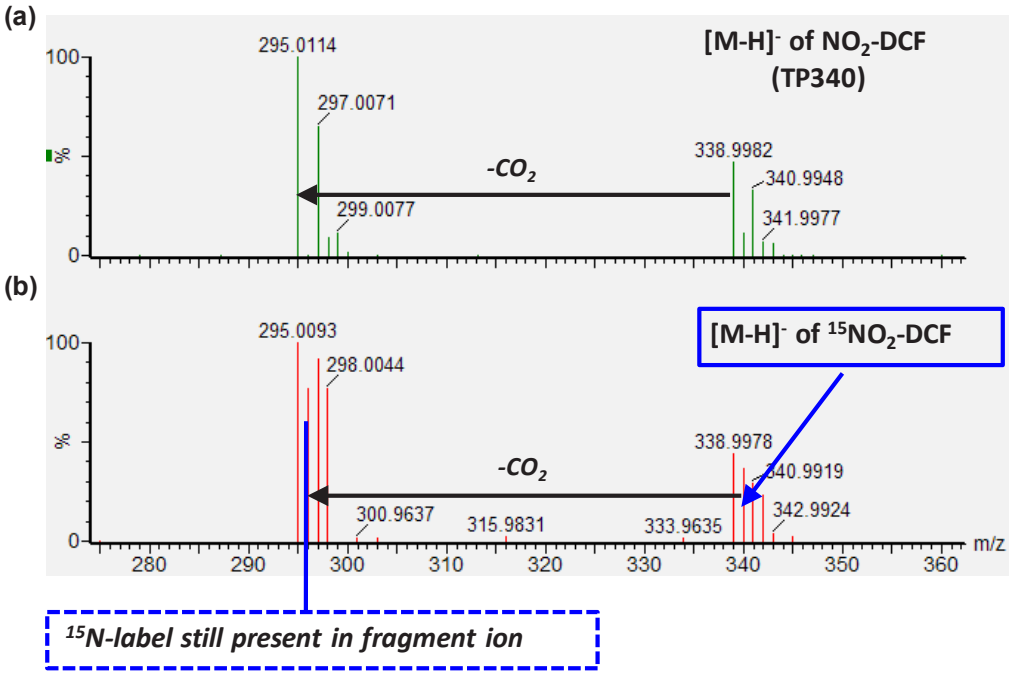


Figure 1. (-)ESI-MS of (a) NO_2-DCF (TP340) from a bioreactor amended with NH_4-N and (b) ^{15}N NO_2-DCF (TP340) from a bioreactor amended with $^{15}NH_4$-N.

3.2. Degradation of non-chlorinated structurally related compound (APAA)

Structure elucidation by UPLC-ESI-MS tentatively identified it as corresponding to nitrosation of the hydroxyl group in the carboxylic acid moiety (TP256) (figure 2). The (-)-ESI-MS/MS spectrum of APAA (figure 2) shows a deprotonated molecule at m/z 226.0885 confirming the molecular composition of $C_{14}H_{12}NO_2$. The loss of 44 Da (CO2) from the deprotonated molecule resulted in the formation of a major fragment ion at m/z 182.1004. The (-)-ESI-MS/MS spectrum of TP256 (figure 2) shows a deprotonated molecule at m/z 255.0771 confirming the molecular composition of $C_{14}H_{12}N_2O_4$. The fragment ion appeared at m/z 181.0936 that was formed by concurrent loss of COONO. As it was observed previously observed for TP324 [2], such MS/MS pattern suggested that the introduction of a nitroso group to the molecule of APAA occurred by substitution of a hydrogen atom in the carboxylic moiety. Regarding the conditions leading to the generation of the nitrosated microbial metabolites TP256 and TP324 in the batch-reactor spiked with APAA and DCF respectively, as well as TP340 it seems reasonable to postulate a link to nitrifying wastewater bacteria which is a first stage of the microbiological process bringing about

oxidation of ammonia to nitrite, followed by further oxidation of the nitrite to nitrate. Although the contribution of nitrifying bacteria to the biomass in the mixed microbial community of the activated sludge tank in WWTPs is less than 5 %, the operational conditions of the lab-scale reactors were favourable for the growth of nitrifiers in terms of oxygen supply, temperature, and pH of the mixed liquor. Irrespective of mechanistic aspects and the interest in identifying the bacteria responsible for APAA and DCF conversion in the present work, it needs to be stressed that TP256 and TP324 are the result of biotransformation rather than biodegradation as the chemical modification does, unfortunately, not imply a break-up of the core structure.

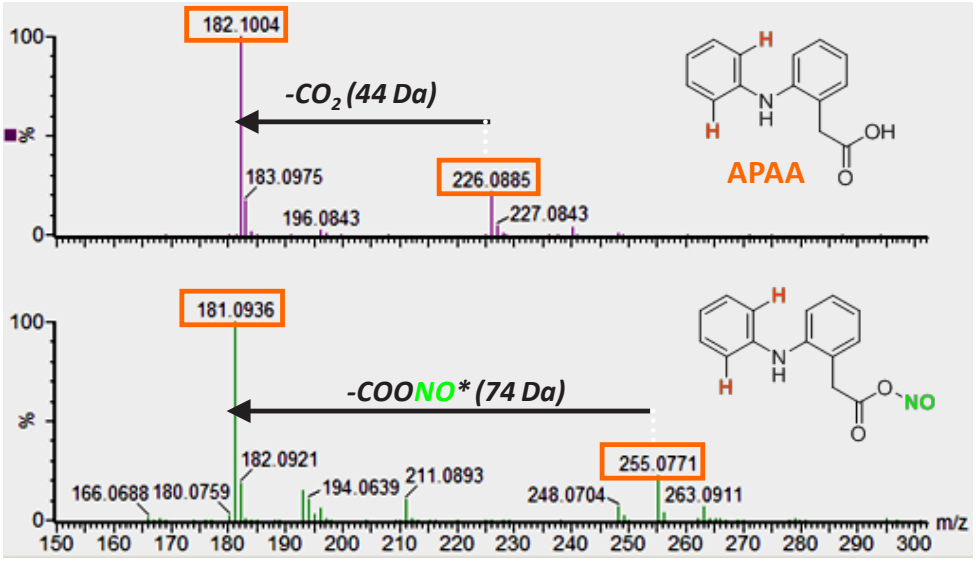
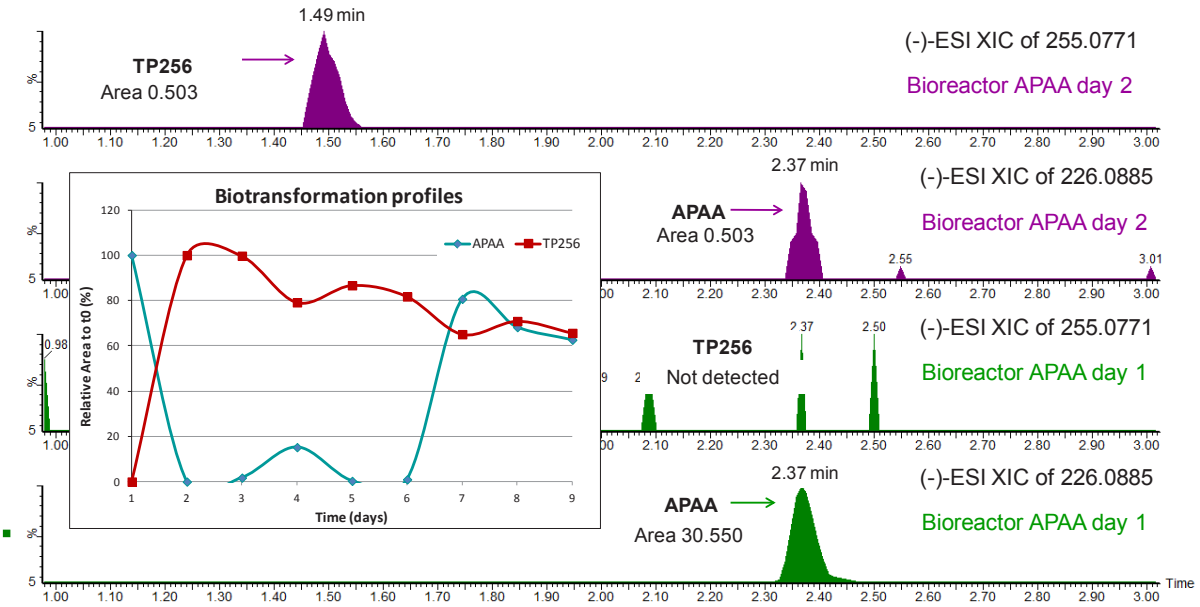


Figure 2. (-)-ESI MS/MS spectra and chemical structures of APAA and its tentatively proposed nitroso derivative TP256.

Non-chlorinated compound APAA degraded faster than DCF (see figure 3). After one day of experiment, almost 100% of the initial amount of APAA (227 Da) had disappeared with concomitant formation of a nitrosated derivative (figure 3a). On the other hand, concentrations of DCF remained almost unchanged during the biodegradation experiment and the formation of TP340 and TP324 was not observed until respective fourth and fifth days of bioreaction (figure 3b).This apparent relatively poor biodegradability of DCF is in agreement with the low removal efficiencies in WWTPs operating with CAS treatment reported for this drug [5]. The higher biodegradation rate observed for APAA, relative to DCF could be explained by steric hindrance of the chlorine atoms in DCF molecule that would reduce its reactivity towards other molecules such as NO radicals. However, although the formation of nitro and nitroso TPs of DCF and APAA was confirmed, their biotransformation profiles were not proportional. This was specially noticed in the case of APAA, suggesting that this compound might be partly mineralized and/or biotransformed into other unknown products. It has been demonstrated that the biodegradation of DCF increases with the solids retention time of the CAS treatment (i.e. from <15% up to 70% when SRT= 150 days) [6]. The authors conjectured that longer SRT would allow the increase of the bacterial community diversity and the development with ability to degrade DCF. Therefore we hypothesize

that the low biodegradability of DCF and formation of TP324 and TP340 could be due to the combination of steric effects and poor bacterial diversity which were strongly affected by the short duration of the biodegradation experiments. Moreover, despite APAA degraded faster, a recovery of the compound was observed from the seventh day of treatment, while TP256 started to decrease from day 4. These observations highlighted the need to conduct further investigations on the biotransformation mechanisms of DCF and other related structures in the activated sludge.

(a)



(b)

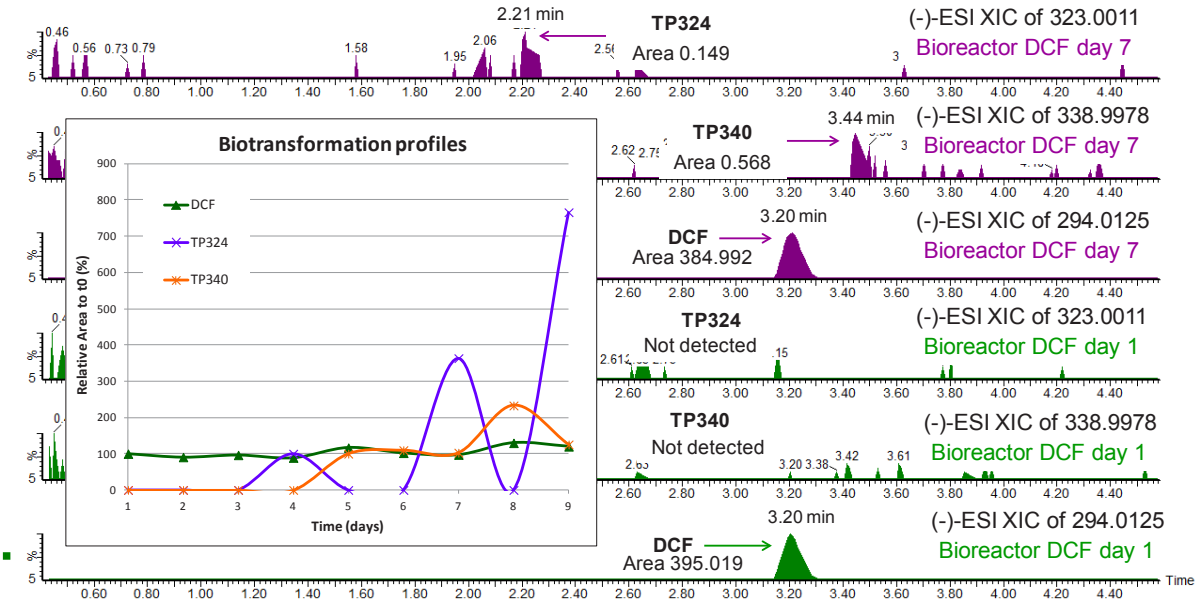


Figure 3. Biotransformation profiles and extracted ion chromatograms acquired in negative ionization mode in bioreactor samples collected at different days of parent compounds and their TPs: (a) APA Aand TP256 and (b) DCF, TP340 and TP324.

3.3. Degradation of the related NSAIDs

The use of HR-MS for the analysis of such complex samples allowed the detection and identification of TPs of the NSAIDs tested in the bioreactor samples. Initially, full scan and MS^n experiments were conducted for the suspect screening of extracts collected daily from the bioreactors. Figure 4 shows the MS and MS/MS spectra acquired for parent compounds in standard solutions and TPs identified in bioreactor samples corresponding to the day 36 of experiment. Afterwards, several procedures for structure elucidation were followed. Several TPs could be identified by the assignment of elemental formulae based on exact mass and the interpretation of characteristic product ion spectra allowed to identify the most likely sites of structural modifications in these TPs (see table 1 and figures 4 and 5). Particularly, TP324 and TP340 were identified in bioreactors solely fortified with DCF; while for MEC TP324M and two isomers of TP340M were confirmed. Similarly, TP270 and two isomers of TP286 were identified in bioreactors spiked with MEF. Diversely, four TPs of TOLF and FLUF were identified in their respective bioreactors, namely TP290 and three isomers of TP306 for TOLF; while for FLUF these were TP310 and three isomers of TP326.

Table 1. Characterization of parent compounds and elucidated nitro and nitroso TPs. Fragmentation studies were performed in (-)-ESI mode.

(a)

Ion	Elemental composition	Calculated mass [*m/z*]	Measured mass [*m/z*]	Error [ppm]	DBE
$[M-H]^-$		**DCF**			
m/z 294	$C_{14}H_{10}Cl_2NO_2$	294.00831	294.01012	2.424	9.5
m/z 250	$C_{13}H_{10}Cl_2N$	250.01848	250.01886	-2.872	8.5
m/z 214	$C_{13}H_9ClN$	214.04180	214.04203	-4.227	9.5
m/z 178	$C_{13}H_8N$	178.06513	178.06573	-2.767	10.5
$[M-H]^-$		**TP324**			
m/z 323	$C_{14}H_9Cl_2N_2O_3$	322.99957	322.9947	-0.312	10.5
m/z 279	$C_{13}H_9Cl_2N_2O$	279.00974	279.00960	-0.508	9.5
m/z 249	$C_{13}H_9Cl_2N$	249.01175	249.01143	-1.1297	9.0
m/z 245	$C_{13}H_8NO_2Cl$	245.02491	245.02411	1.234	10.0
m/z 228	$C_{13}H_7ONCl$	228.02107	228.02067	-1.746	10.5
m/z 209	$C_{13}H_7NO_2$	209.04823	209.04831	5.645	11.0
m/z 200	$C_{12}H_7NCl$	200.02615	200.02562	-2.667	9.5
m/z 181	$C_{12}H_7ON$	181.05222	181.05119	-5.664	10.0
$[M-H]^-$		**TP340**			
m/z 338	$C_{14}H_9Cl_2N_2O_4$	338.99339	338.99357	-2.701	10.5
m/z 295	$C_{13}H_9Cl_2\ N_2O_2$	295.00466	295.00416	-1.682	9.5
m/z 259	$C_{13}H_8ClN_2O_2$	259.02798	259.01146	-4.473	10.5
m/z 229	$C_{13}H_8ClNO$	229.02889	229.02933	-2.881	10.0

(b)

Ion	Elemental composition	Calculated mass [*m/z*]	Measured mass [*m/z*]	Error [ppm]	DBE
$[M-H]^-$		**MEC**			
m/z 294	$C_{14}H_{10}Cl_2NO_2$	294.00831	294.00982	1.404	9.5
m/z 258	$C_{14}H_9ClNO_2$	258.03163	258.03191	-3.176	10.5
m/z 250	$C_{13}H_{10}Cl_2N$	250.01848	250.01887	-2.832	8.5
m/z 214	$C_{13}H_9ClN$	214.04180	214.04179	-1.110	9.5
m/z 178	$C_{13}H_8N$	178.06513	178.06553	-3.890	10.5
$[M-H]^-$		**TP324M**			
m/z 323	$C_{14}H_9Cl_2N_2O_3$	322.99957	322.99879	-2.418	10.5
m/z 258	$C_{14}H_9ClNO_2$	258.03272	258.03199	-2.866	10.5
m/z 214	$C_{13}H_9ClN$	214.04180	214.04197	-4.347	9.5
$[M-H]^-$		**TP340M IS1**			
m/z 338	$C_{14}H_9Cl_2N_2O_4$	338.99339	338.99328	-3.556	10.5
m/z 295	$C_{13}H_9Cl_2N_2O_2$	295.00466	295.00386	-2.699	9.5
m/z 259	$C_{13}H_8ClN_2O_2$	259.02798	259.02863	2.515	10.5
m/z 229	$C_{13}H_8ClNO$	229.02889	229.02919	-3.493	10.0
$[M-H]^-$		**TP340M IS2**			
m/z 338	$C_{14}H_9Cl_2N_2O_4$	338.99339	338.99430	-0.547	10.5
m/z 295	$C_{13}H_9Cl_2N_2O_2$	295.00466	295.00479	0.453	9.5
m/z 259	$C_{13}H_8ClN_2O_2$	259.02798	259.02768	-1.153	10.5
m/z 229	$C_{13}H_8ClNO$	229.02889	229.03063	-2.795	10.5

(c)

Ion	Elemental composition	Calculated mass [*m/z*]	Measured mass [*m/z*]	Error [ppm]	DBE
$[M-H]^-$		**FLUF**			
m/z 280	$C_{14}H_9F_3NO_2$	280.05799	280.05887	-0.773	9.5
m/z 236	$C_{13}H_9F_3N$	236.06816	236.06895	-1.302	8.5
m/z 216	$C_{13}H_8F_2N$	216.06193	216.06223	-3.698	9.5
m/z 196	$C_{13}H_7FN$	196.05570	196.05599	-4.136	10.5
$[M-H]^-$		**TP310**			
m/z 309	$C_{14}H_8F_3N_2O_3$	309.04815	309.04929	0.129	10.5
m/z 279	$C_{14}H_8F_3NO_2$	279.05016	279.05117	-0.328	10.0
m/z 234	$C_{13}H_7F_3N$	234.05251	234.05331	-1.270	9.5
$[M-H]^-$		**TP326 IS1**			
m/z 325	$C_{14}H_8F_3N_2O_4$	325.04307	325.04350	-2.045	10.5
m/z 281	$C_{13}H_8F_3N_2O_2$	281.05324	281.05463	1.048	9.5
m/z 263	$C_{13}H_6F_3N_2O$	263.04267	263.04343	-1.296	10.5
m/z 250	$C_{13}H_7F_3NO$	250.04743	250.04866	0.553	9.5
m/z 141	$C_7H_3OF_2$	141.01465	141.015573	-1.237	5.5
$[M-H]^-$		**TP326 IS2**			
m/z 325	$C_{14}H_8F_3N_2O_4$	325.04307	325.04360	-1.737	10.5
m/z 281	$C_{13}H_8F_3N_2O_2$	281.05324	281.05478	1.582	9.5
m/z 263	$C_{13}H_6F_3N_2O$	263.04267	263.04342	-1.334	10.5
m/z 250	$C_{13}H_7F_3NO$	250.04743	250.04867	0.593	9.5
m/z 251	$C_{13}H_8F_3NO$	251.05525	251.05683	1.925	9.0
m/z 230	$C_{13}H_6F_2NO$	230.04120	230.04136	-4.058	10.5
m/z 141	$C_7H_3F_2O$	141.01465	141.01559	-1.096	5.5
$[M-H]^-$		**TP326 IS3**			
m/z 325	$C_{14}H_8F_3N_2O_4$	325.04307	325.04336	-2.475	10.5
m/z 281	$C_{13}H_8F_3N_2O_2$	281.05324	281.05479	1.617	9.5
m/z 261	$C_{13}H_7F_2O_2N_2$	261.04701	261.04799	-1.600	10.5
m/z 250	$C_{13}H_7F_3NO$	250.04743	250.04875	0.913	9.5
m/z 251	$C_{13}H_8F_3NO$	251.05525	251.05684	1.964	9.0
m/z 230	$C_{13}H_6F_2NO$	230.04120	230.04128	-4.406	10.5

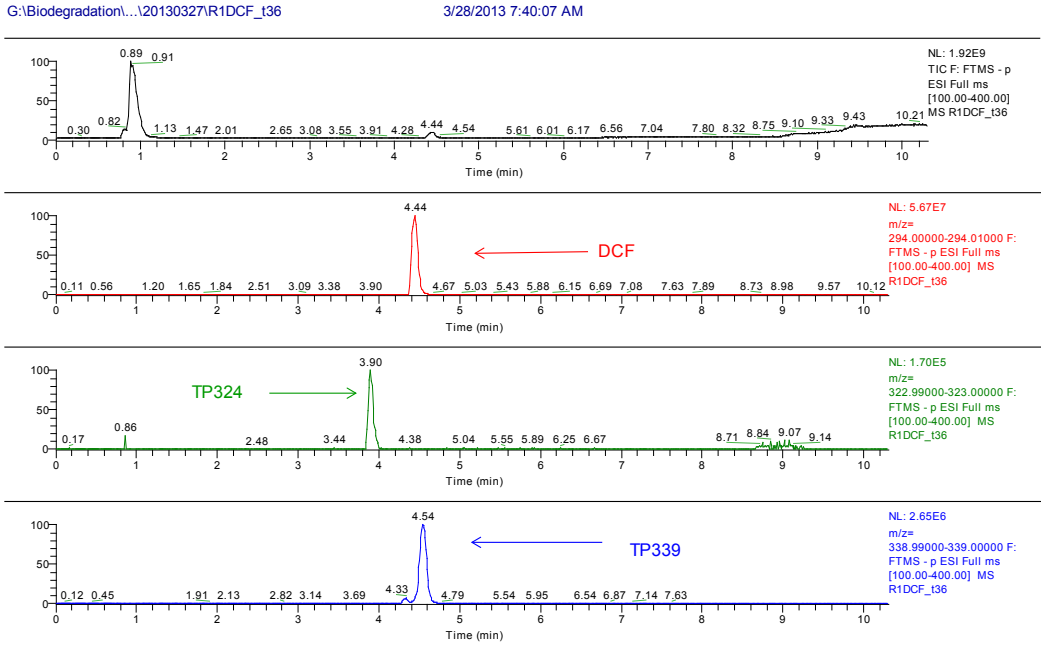
(d)

Ion	Elemental composition	Calculated mass [*m/z*]	Measured mass [*m/z*]	Error [ppm]	DBE
$[M-H]^-$		**TOLF**			
m/z 260	$C_{14}H_{11}ClNO_2$	260.04728	260.04847	0.348	9.5
m/z 216	$C_{13}H_{11}ClN$	216.05745	216.05763	-4.260	8.5
m/z 196	$C_{13}H_{10}NO$	196.07569	196.07668	-0.547	9.5
m/z 178	$C_{13}H_8N$	178.06513	178.06542	-4.508	10.5
$[M-H]^-$		**TP290**			
m/z 289	$C_{14}H_{10}ClN_2O_3$	289.03745	289.03906	1.788	10.5
m/z 258	$C_{14}H_9ClNO_2$	258.03163	258.03369	3.722	10.5
m/z 214	$C_{13}H_9ClN$	214.04180	214.04195	-4.440	9.5
m/z 178	$C_{13}H_8N$	178.06513	178.06494	-0.186	10.5
$[M-H]^-$		**TP306 IS1**			
m/z 305	$C_{14}H_{10}ClN_2O_4$	305.03236	305.03310	-1.173	10.5
m/z 261	$C_{13}H_{10}ClN_2O_2$	261.04253	261.04371	0.312	9.5
m/z 243	$C_{13}H_8ClN_2O$	243.03198	243.03302	-0.181	10.5
m/z 230	$C_{13}H_9ClNO$	230.03672	230.03792	0.457	9.5
$[M-H]^-$		**TP306 IS2**			
m/z 305	$C_{14}H_{10}ClN_2O_4$	305.03236	305.03311	-1.140	10.5
m/z 261	$C_{13}H_{10}ClN_2O_2$	261.04253	261.04372	0.350	9.5
m/z 243	$C_{13}H_8ClN_2O$	243.03198	243.03309	0.107	10.5
m/z 230	$C_{13}H_9ClNO$	230.03672	230.03792	1.760	9.5
m/z 225	$C_{13}H_9N_2O_2$	225.06585	225.06683	-0.357	10.5
$[M-H]^-$		**TP306 IS3**			
m/z 305	$C_{14}H_{10}ClN_2O_4$	305.03236	305.03341	-0.157	10.5
m/z 261	$C_{13}H_{10}ClN_2O_2$	261.04253	261.04371	0.542	9.5

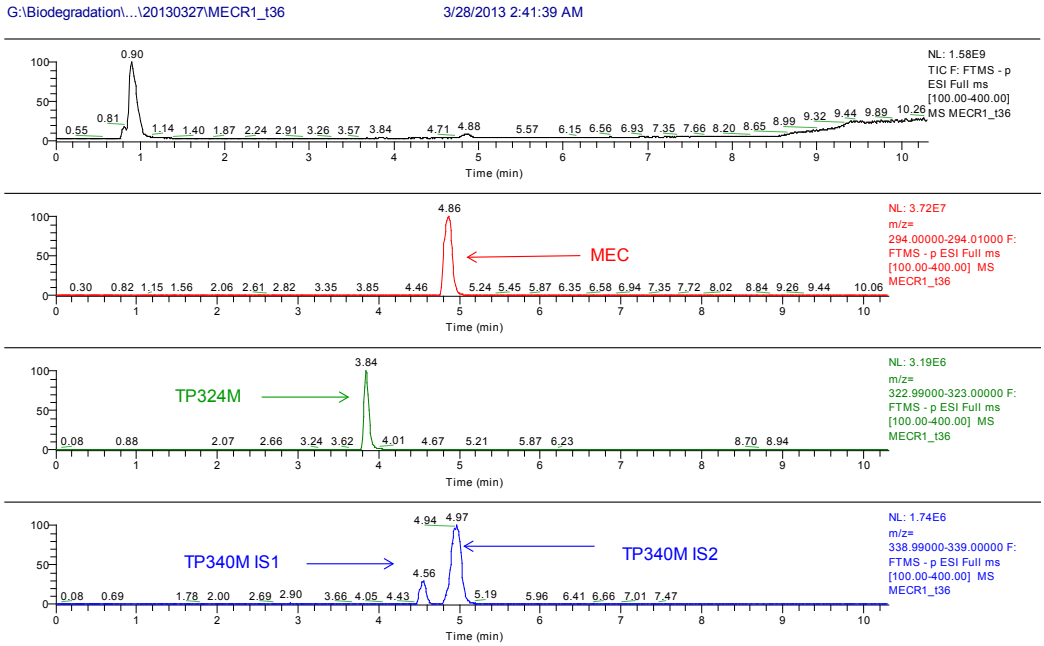
(e)

Ion	Elemental composition	Calculated mass [*m/z*]	Measured mass [*m/z*]	Error [ppm]	DBE
$[M-H]^-$		**MEF**			
m/z 240	$C_{15}H_{14}NO_2$	240.10191	240.10212	-3.674	9.5
m/z 196	$C_{14}H_{14}N$	196.11208	196.11233	-4.298	8.5
m/z 180	$C_{13}H_{10}N$	180.08078	180.08157	-1.681	9.5
$[M-H]^-$		**TP270**			
m/z 269	$C_{15}H_{13}N_2O_3$	269.09207	269.09277	-1.470	10.5
m/z 238	$C_{15}H_{12}NO_2$	238.08626	238.08795	2.512	10.5
m/z 194	$C_{14}H_{12}N$	194.09643	194.09717	-1.817	9.5
m/z 179	$C_{13}H_9N$	179.07295	179.07349	-3.114	10.0
$[M-H]^-$		**TP286 IS1**			
m/z 285	$C_{15}H_{13}N_2O_4$	285.08698	285.08777	-1.088	10.5
m/z 241	$C_{14}H_{13}N_2O_2$	241.09715	241.09874	2.028	9.5
m/z 223	$C_{14}H_{11}N_2O$	223.08659	223.08798	1.361	10.5
m/z 211	$C_{14}H_{13}NO$	211.09917	211.09929	-4.606	9.0
$[M-H]^-$		**TP286 IS2**			
m/z 285	$C_{15}H_{13}N_2O_4$	285.08698	285.08780	-1.088	10.5
m/z 241	$C_{14}H_{13}N_2O_2$	241.09715	241.09811	-0.585	9.5
m/z 223	$C_{14}H_{11}N_2O$	223.08659	223.08798	1.361	10.5
m/z 211	$C_{14}H_{13}NO$	211.09917	211.09941	-4.038	9.0

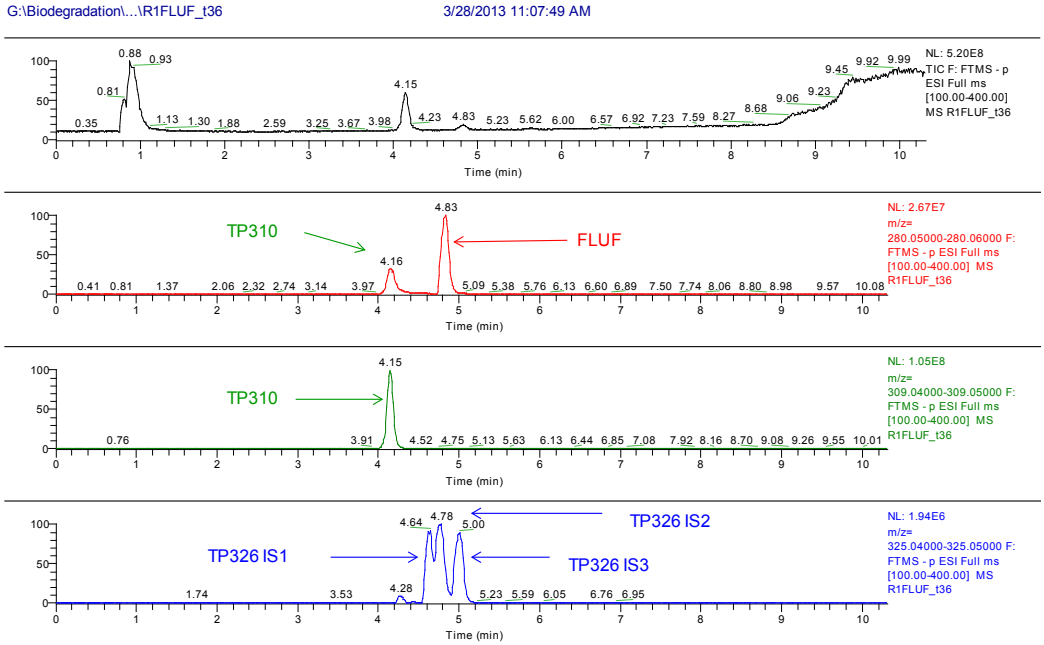
(a)



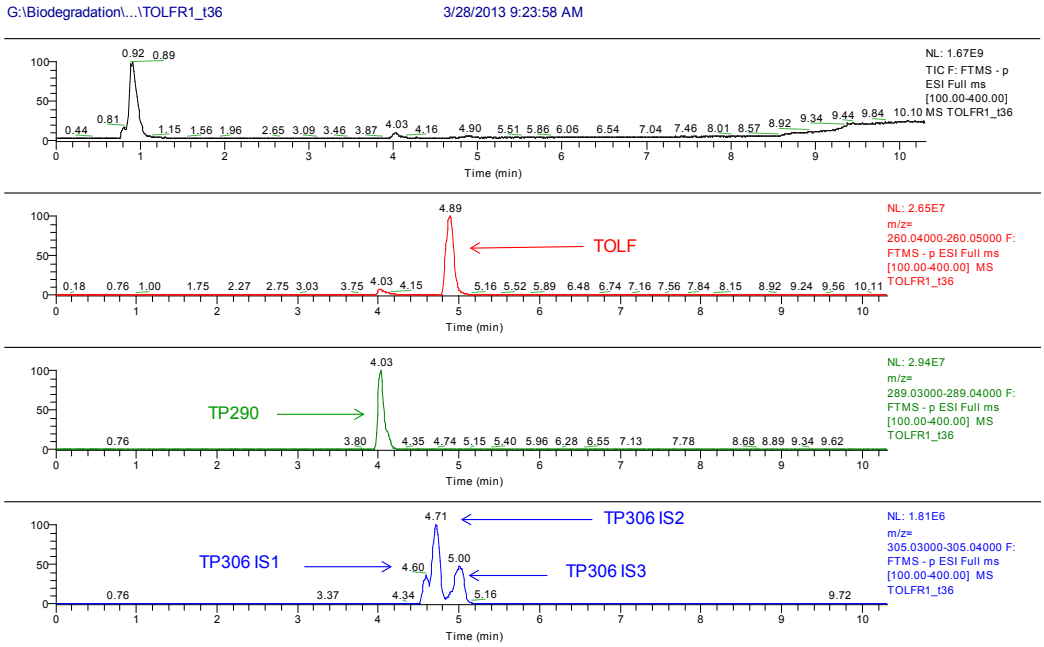
(b)


G:\Biodegradation\...\20130327\MECR1_t36
3/28/2013 2:41:39 AM
NL: 1.58E9
TIC F: FTMS - p ESI Full ms [100.00-400.00] MS MECR1_t36
MEC
NL: 3.72E7
m/z= 294.00000-294.01000 F: FTMS - p ESI Full ms [100.00-400.00] MS MECR1_t36
TP324M
NL: 3.19E6
m/z= 322.99000-323.00000 F: FTMS - p ESI Full ms [100.00-400.00] MS MECR1_t36
TP340M IS1
TP340M IS2
NL: 1.74E6
m/z= 338.99000-339.00000 F: FTMS - p ESI Full ms [100.00-400.00] MS MECR1_t36
Time (min)

(c)


G:\Biodegradation\...\R1FLUF_t36
3/28/2013 11:07:49 AM
NL: 5.20E8
TIC F: FTMS - p ESI Full ms [100.00-400.00] MS R1FLUF_t36
TP310
FLUF
NL: 2.67E7
m/z= 280.05000-280.06000 F: FTMS - p ESI Full ms [100.00-400.00] MS R1FLUF_t36
TP310
NL: 1.05E8
m/z= 309.04000-309.05000 F: FTMS - p ESI Full ms [100.00-400.00] MS R1FLUF_t36
TP326 IS1
TP326 IS2
TP326 IS3
NL: 1.94E6
m/z= 325.04000-325.05000 F: FTMS - p ESI Full ms [100.00-400.00] MS R1FLUF_t36
Time (min)

(d)



(e)

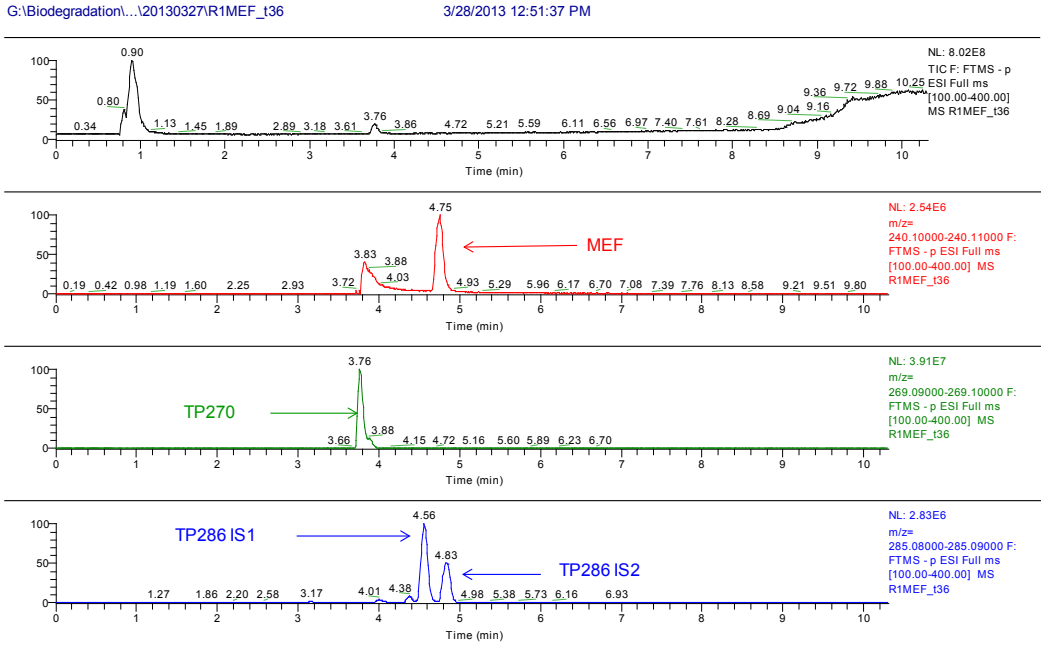
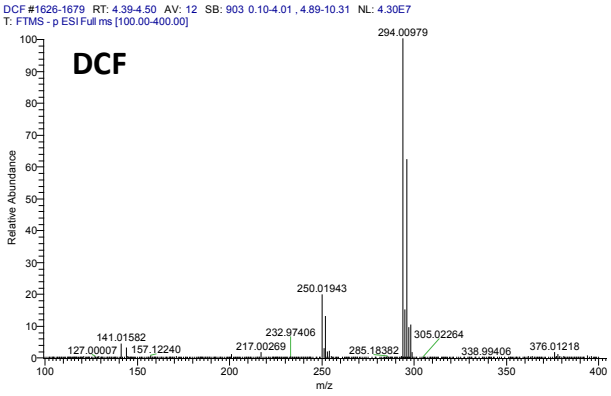
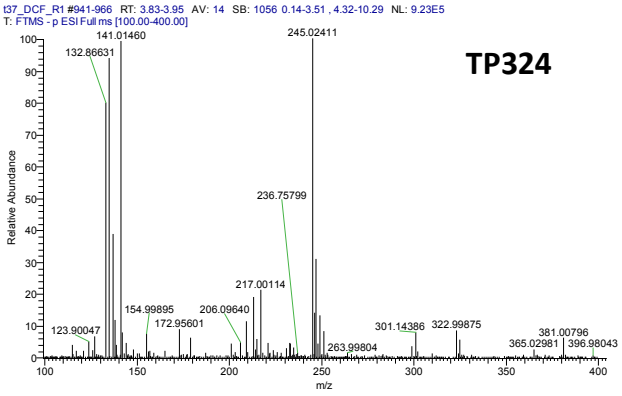


Figure 4. Total Ion Chromatograms (TIC) and Extracted Ion Chromatograms (XIC) acquired by HRMS in (-)-ESI mode of parent NSAIDs and TPs detected in samples collected from activated sludge bioreactors: (a) DCF, (b) MEC, (c) FLUF, (d) TOLF and (e) MEF.

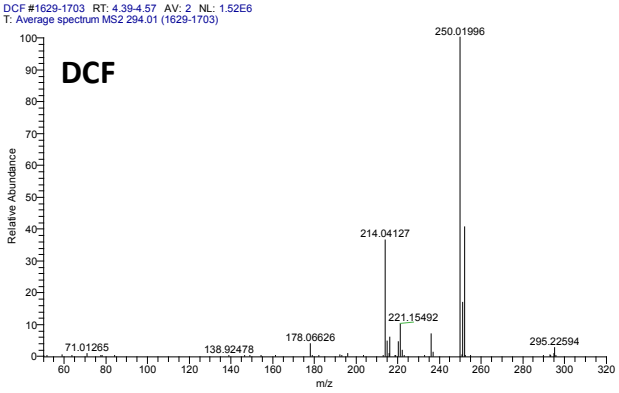
(a)

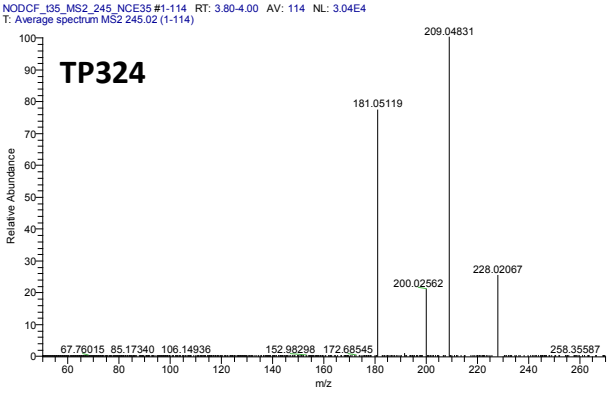
Ful scan



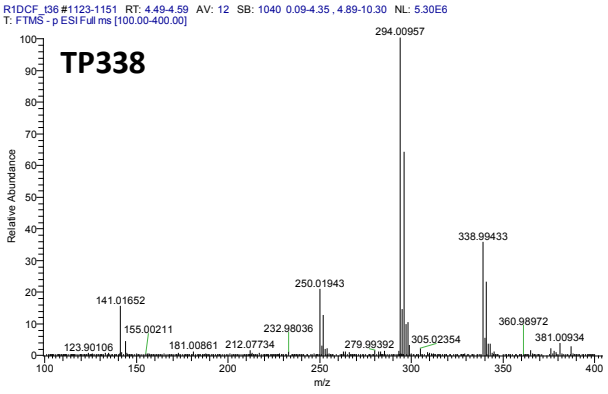


MS²

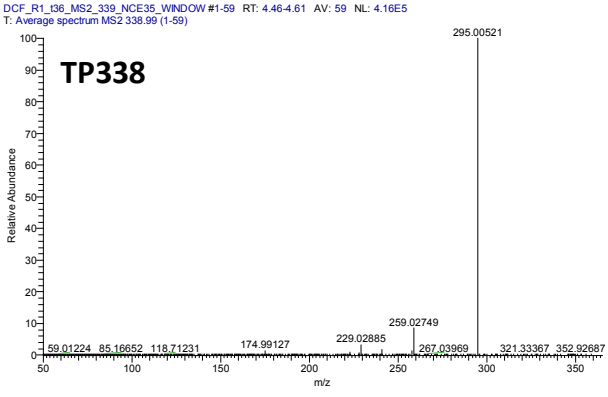




Ful scan

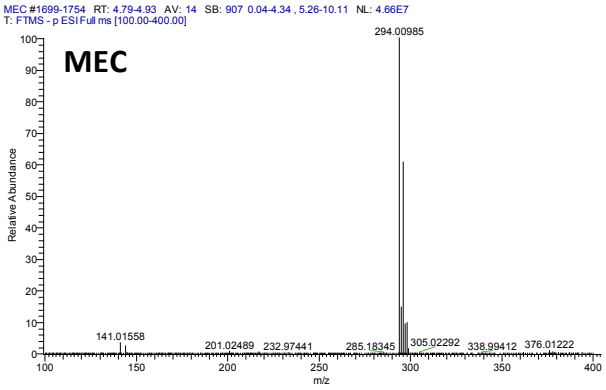


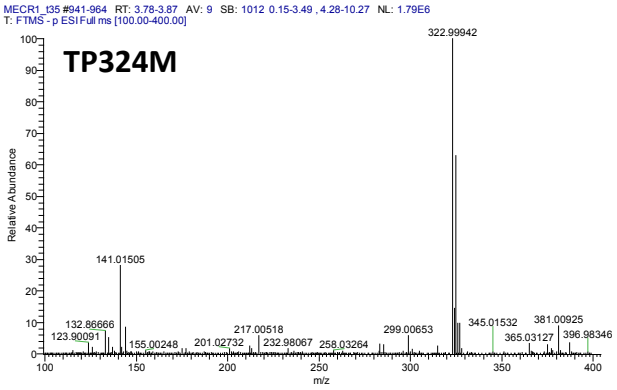
MS²



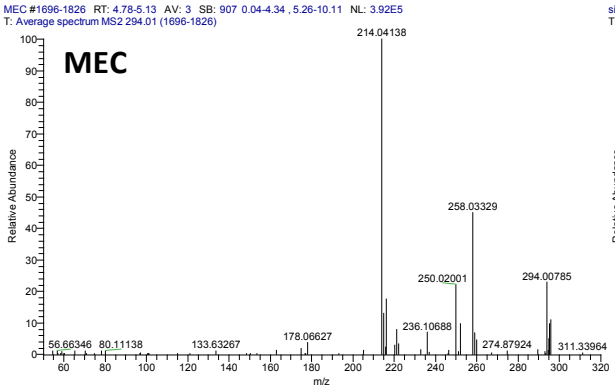
(b)

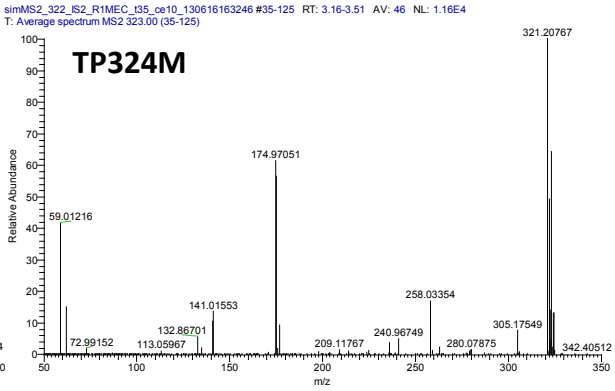
Ful scan



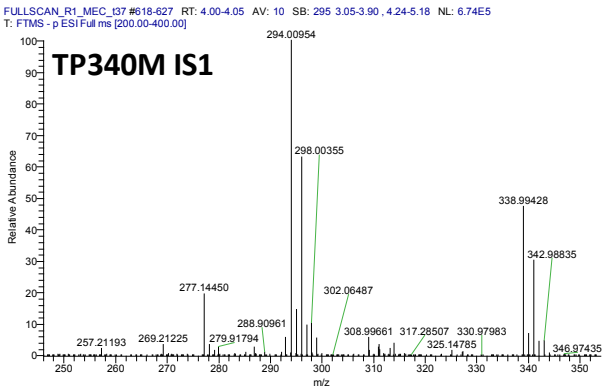


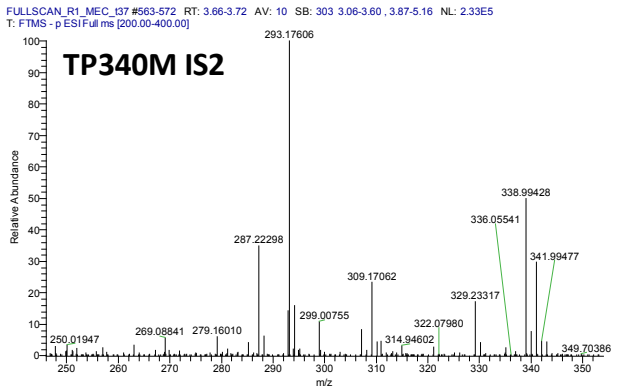
MS²



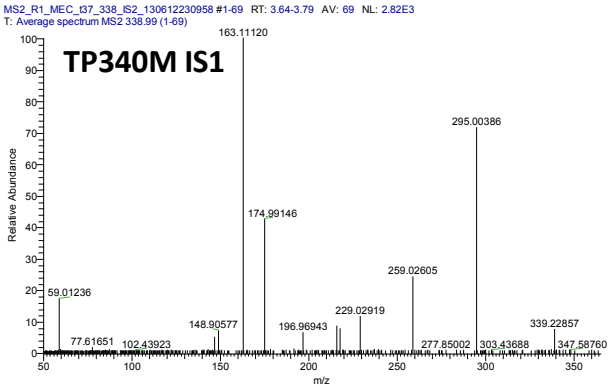


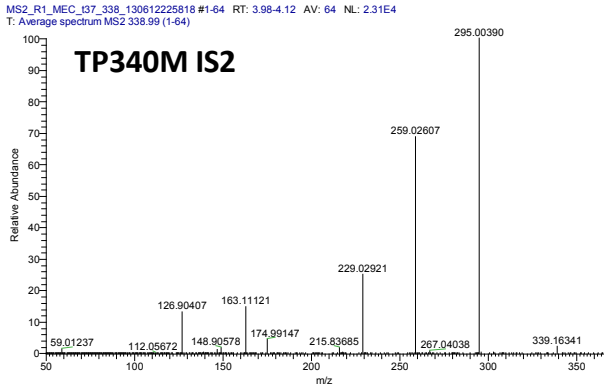
Ful scan





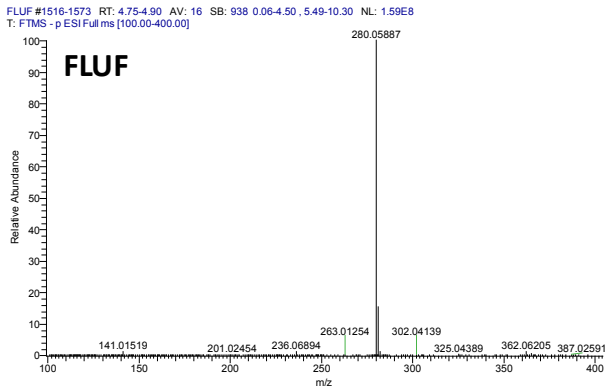
MS²

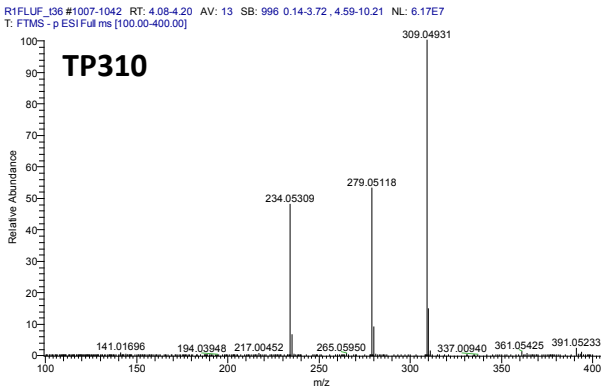




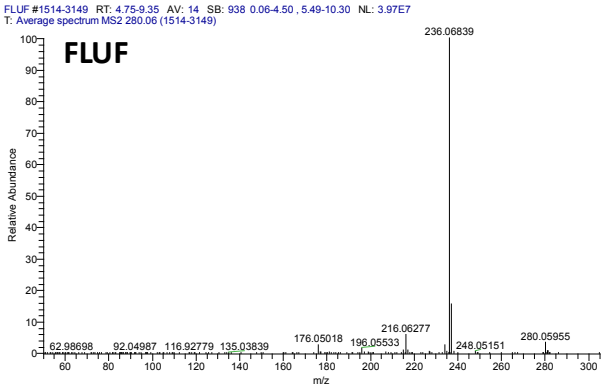
(c)

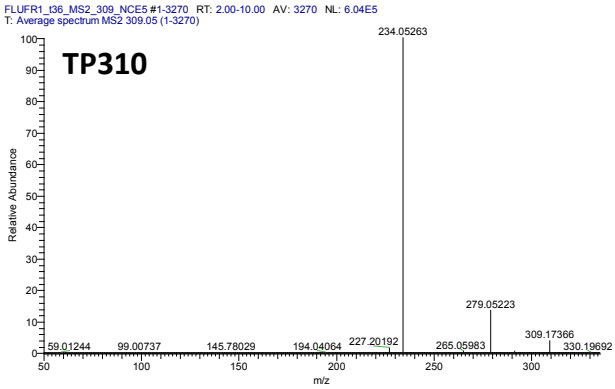
Ful scan



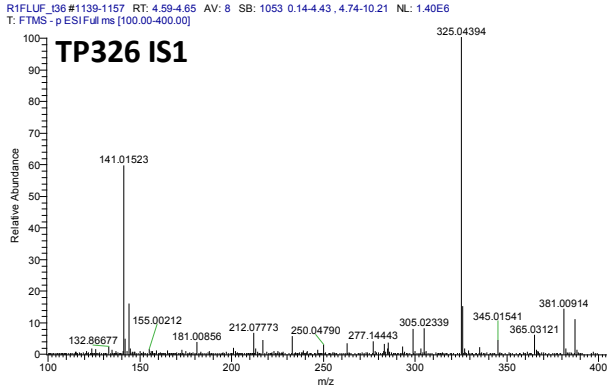


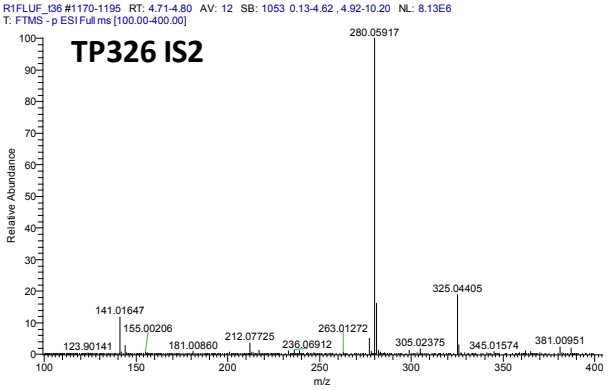
MS²



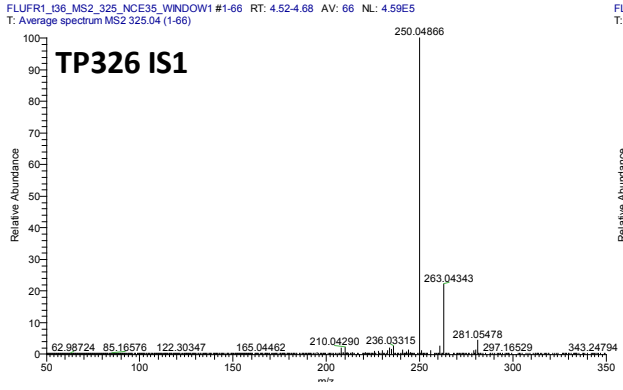


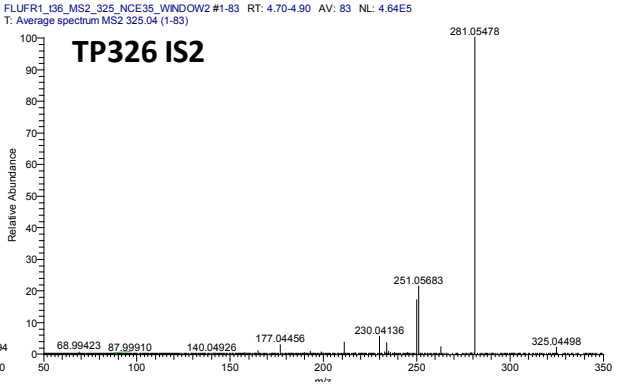
Ful scan



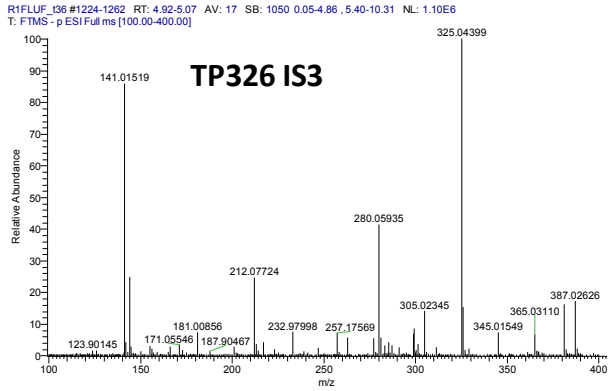


MS²

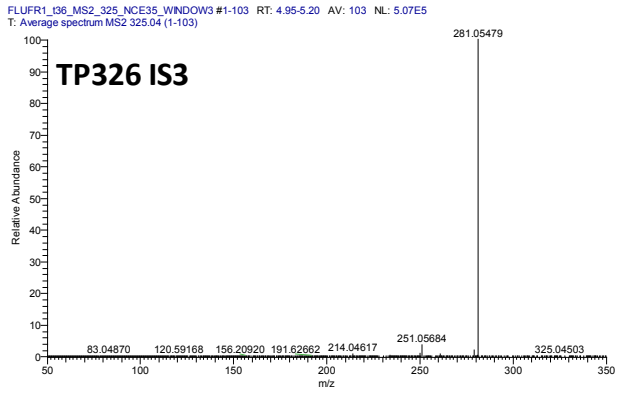




Ful scan

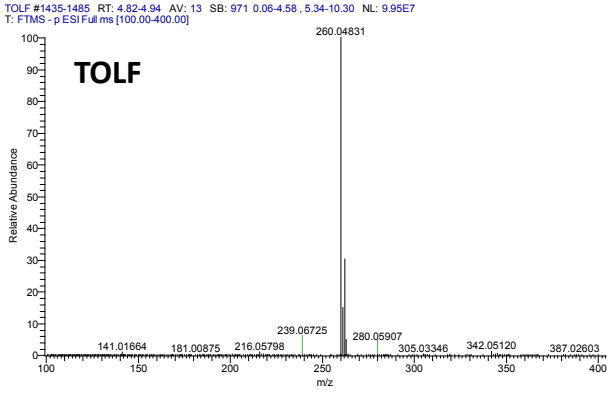


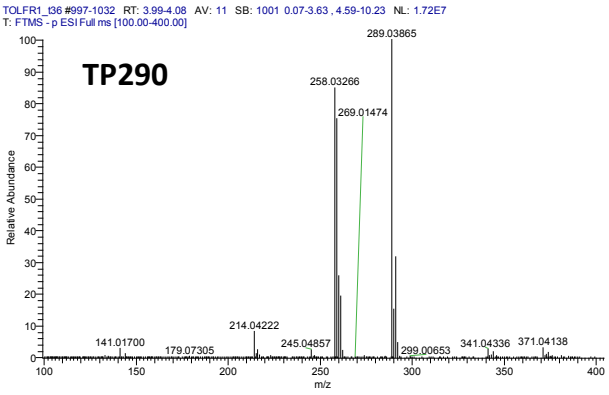
MS^2



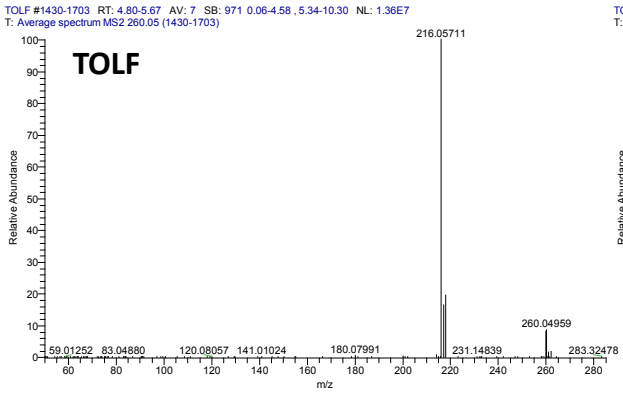
(d)

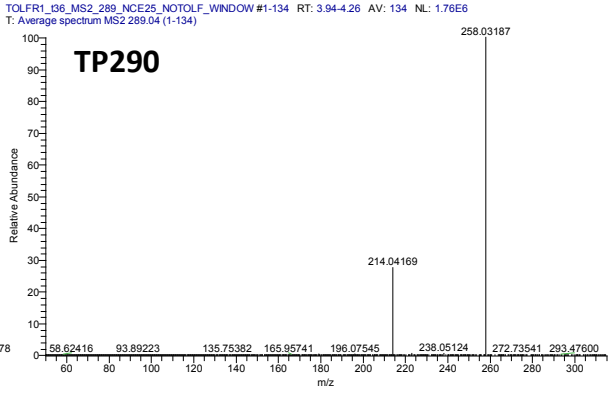
Ful scan



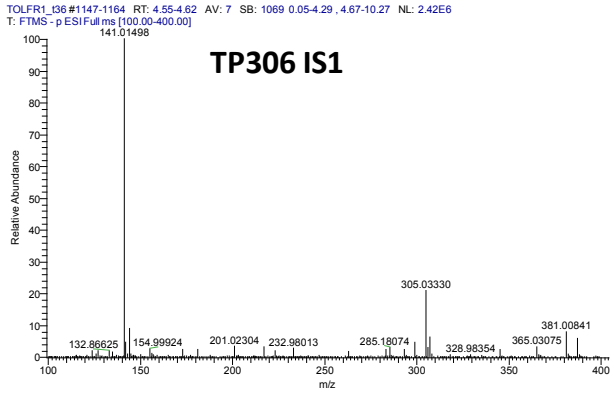


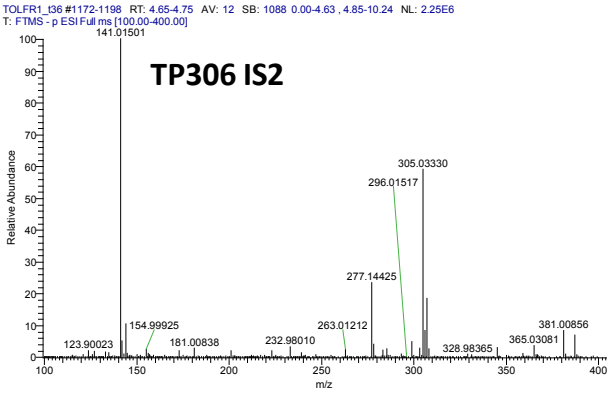
MS^2



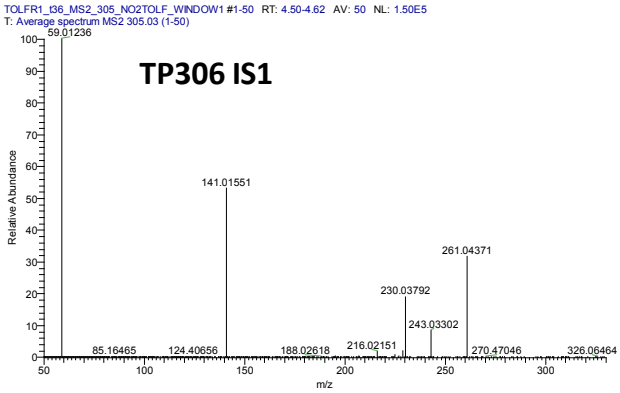


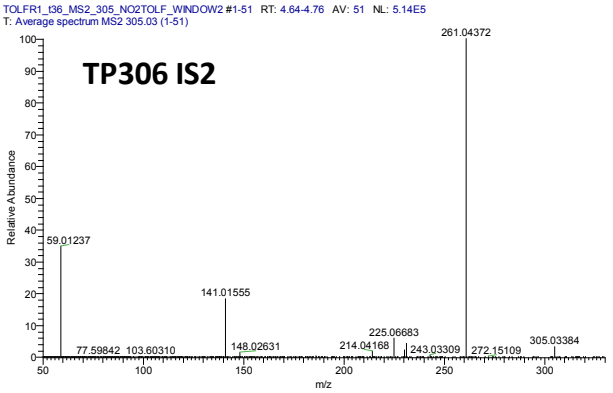
Ful scan



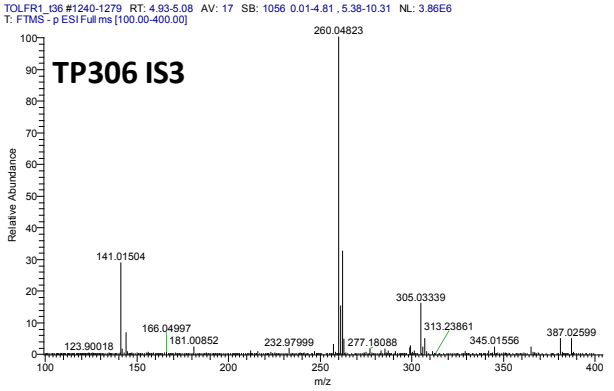


MS²

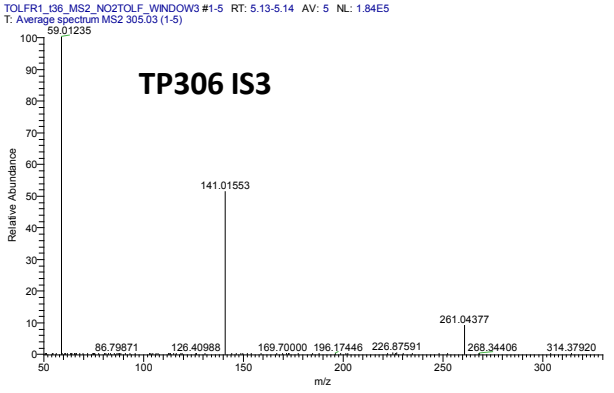




Ful scan

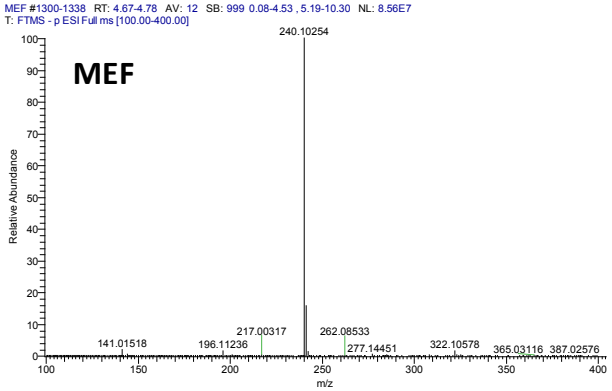


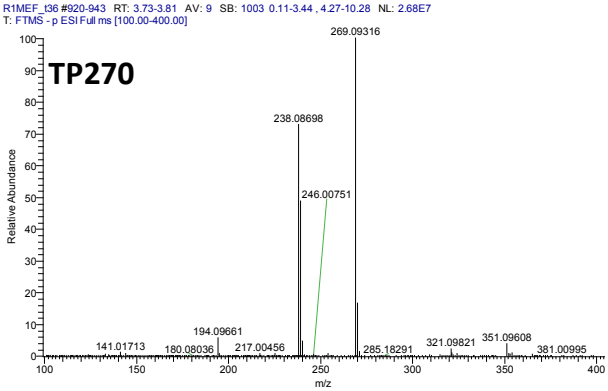
MS²



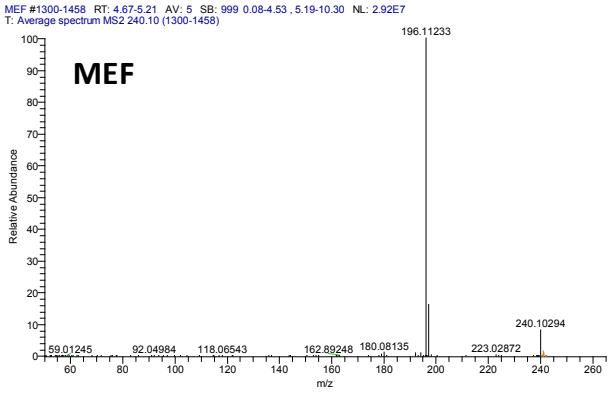
(e)

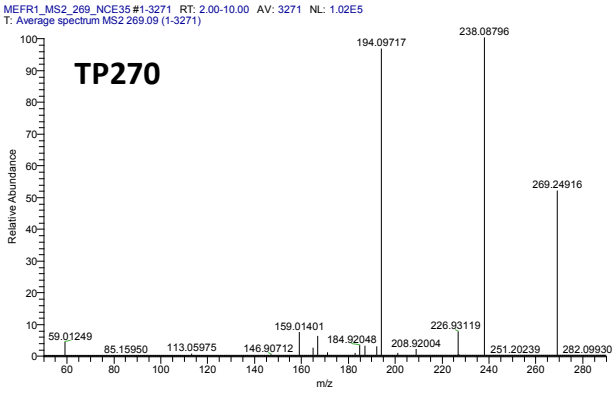
Ful scan



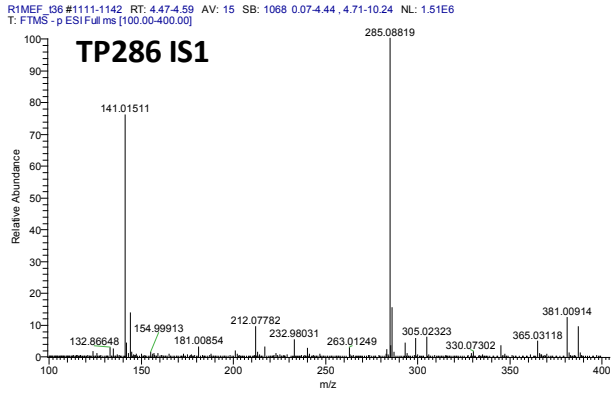


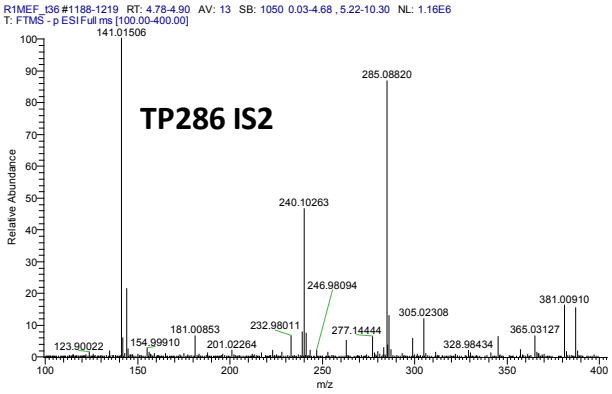
MS²



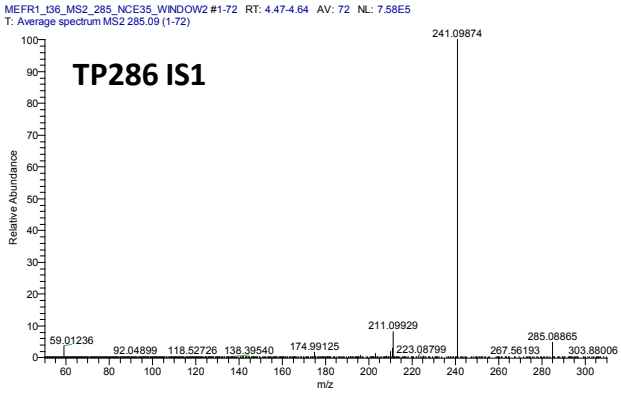


Ful scan





MS²



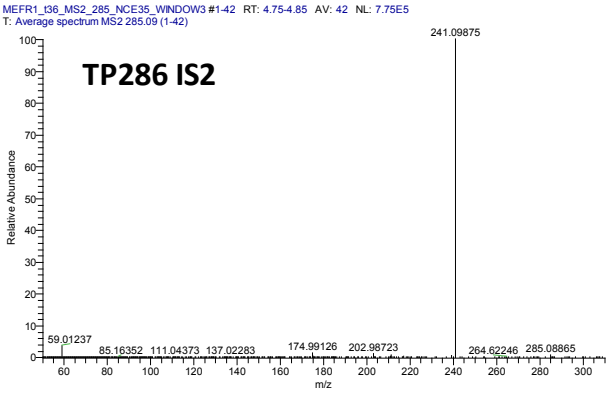


Figure 5. Full scan and MS2 spectra acquired by HRMS in (-)-ESI mode of parent NSAIDs and TPs detected in samples collected from activated sludge bioreactors: (a) DCF, (b) MEC, (c) FLUF, (d) TOLF and (e) MEF.

Besides relative isotope abundances and isotope spacing provided additional confirmation of those TPs including chlorine atoms like DCF structure such as MEC and TOLF. As it can be noticed in figure 5, MEC, which is indeed a positional isomer of diclofenac displayed the same isotopic pattern than DCF.

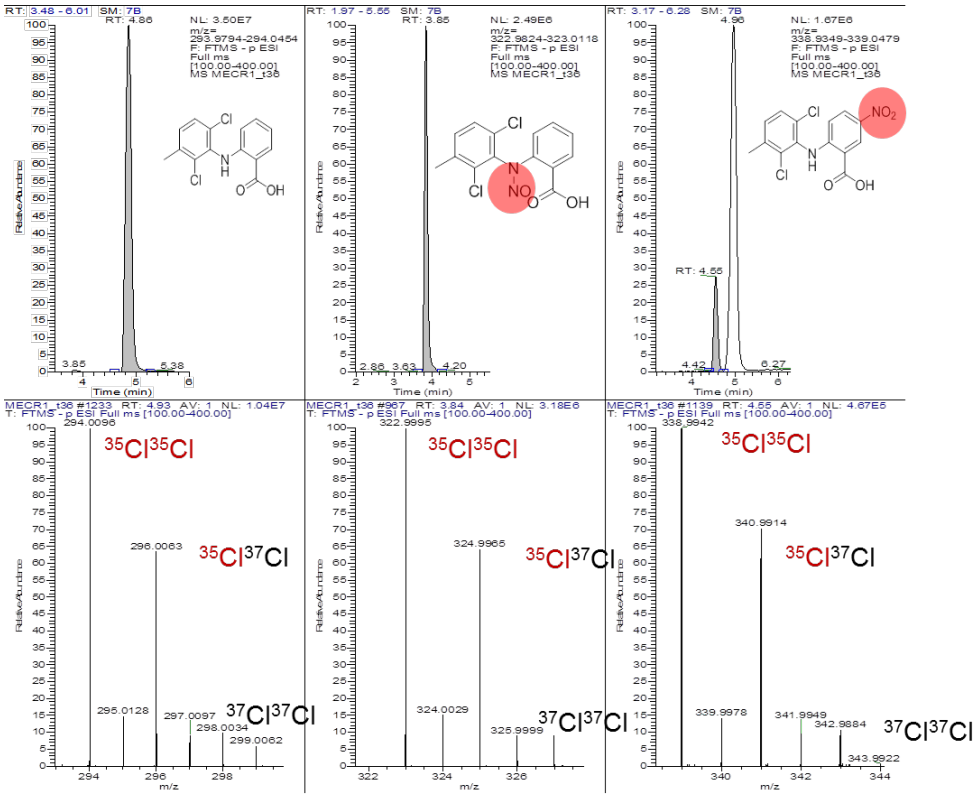
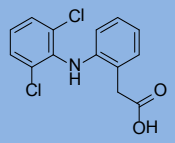
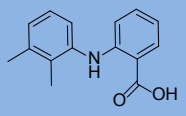
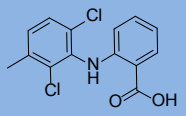
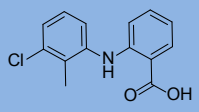
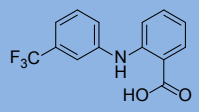
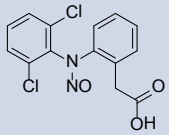
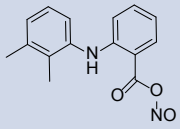
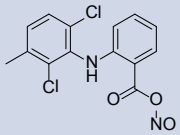
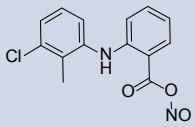
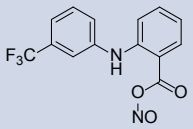
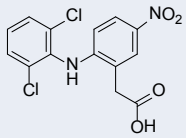
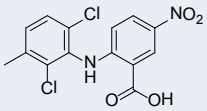
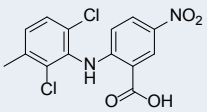
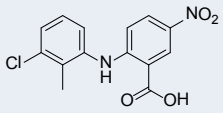
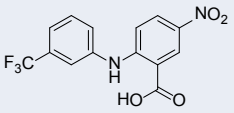


Figure 5. Meclofenamic acid (a) XIC of m/z 294, m/z 323 and m/z 339 (b) MS spectra of [M-H]- of 294,0088, 322,9690 and 338,9940.

Using these approaches, MEF, TOLF, MEC and FLUF, could be unequivocally proved to produce nitro and nitroso compounds in a similar manner as reported earlier for DCF. Table 2 shows the proposed structures, chemical formula and molecular weight for the nitro and nitroso TPs identified in the activated sludge bioreactors, as well as the corresponding to their parent compounds.

Table 2. Proposed chemical structures, formulae and molecular weight of nitro and nitroso TPs identified in bioreactors fortified separately with DCF, MEC, TOLF, FLUF and MEF.

Diclofenac	Mefenamic acid	Meclofenamic acid	Tolfenamic acid	Flufenamic acid
 C14H11Cl2NO2 296.1486	 C15H15NO2 241.1103	 C14H11Cl2NO2 296.1486	 C14H12ClNO2 261.0557	 C14H10F3NO2 281.0664
TP324  C14H10Cl2N2O3 325.1468	TP270  C15H14N2O3 270.2833	TP324M  C14H10Cl2N2O3 325.1468	TP290  C14H11ClN2O3 290.7017	TP310  C14H9F3N2O3 310.2281
TP340  C14H10Cl2N2O4 341.1462	TP286  C15H14N2O4 286.2827	TP340M  C14H10Cl2N2O4 341.1462	TP306  C14H11ClN2O4 306.7011	TP326  C14H9F3N2O4 326.2275

Similar degradation profiles were observed for all NSAIDs tested. In all instances, concentration profiles without any marked decline of the spiked test compound(s) was observed for the control reactors.

4. Conclusions

In this work we have reported the discovery of unusual microbial nitration/nitrosation TPs of related NSAIDs of DCF in reactors amended with mixed liquor of WWTP. These results are consistent with our previous hypothesis of DCF transformation through biological reactions to generate these TPs in lab-scale reactors and also with the first evidence of the occurrence of the nitration/nitrosation TPs of DCF in WWTPs, reported in other work of our group [4]. The knowledge of the presence of metabolites and TPs in the aquatic environment is still scarce and more studies would be needed to evaluate the fate of selected pharmaceuticals in WWTP. Besides elucidation and characterization of unknown metabolites and TPs, toxicity assessment on these compounds would be equally relevant, in order to define their potential toxicological effects on aquatic ecosystems.

Acknowledgements

The work presented in this abstract was supported by the Spanish Ministry of Economy and Competitiveness, SCARCE (CSD-2009-00065). SP acknowledges the contract from the Ramón y Cajal Program of the Spanish Ministry of Economy and Competitiveness. Thermofisher are also acknowledged for their technical support with the Orbitrap software.

References

[1] DIRECTIVE 2013/39/EU OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy.

[2] Pérez S., Barceló D., 2008, Anal. Chem., 80; 8135–8145

[3] Chiron S., Gómez E., Nefenet H., 2010, Environ. Sci. Technol., 44: 284–289.

[4] Osorio V., Marta Imbert-Bouchard Bozo Zonja, Abad J.L., Pérez S., Barceló D., 2014, J Cromatogr. 1347, 63-71

[5] Onesios, K.M., Yu, J.T., Bouwer, E.J., 2009, Biodegradation 20, 441-466.

[6] Fernandez-Fontaina, E., Omil, F., Lema, J.M., Carballa, M., 2012, Wat. Res. 46(16), 5434-5444.

Chapter 4

Study of the occurrence and modeling of pharmaceuticals of Iberian waste water treatment plants and rivers.

Chapter 4

Study of the occurrence and modeling of pharmaceuticals of Iberian waste water treatment plants and rivers.

4.1. Introduction

This chapter reports the assessment of the presence of PhACs in SW and sediments from four Iberian River basins characterized by a high anthropogenic pressure. The chapter is divided in three sub-sections, each one presenting the successive publications (Osorio et al., 2012a; 2012b; 2015) evaluating the spatial and temporal distribution of PhACs and the factors affecting their occurrence (i.e. hydrological conditions and human and animal uses) applying modeling approaches and statistical tools.

4.2. Article: "Pharmaceuticals on a sewage impacted section of a Mediterranean River (Llobregat River, NE Spain) ahd their relationship with hydrological conditions."

Environ Sci Pollut Res (2012) 19:1013–1025
DOI 10.1007/s11356-011-0603-4

RESEARCH ARTICLE

Pharmaceuticals on a sewage impacted section of a Mediterranean River (Llobregat River, NE Spain) and their relationship with hydrological conditions

Victoria Osorio · Sandra Pérez · Antoni Ginebreda · Damià Barceló

Received: 16 March 2011 / Accepted: 29 August 2011

Abstract
Introduction Mediterranean rivers are characterized by a high flow variability, which is strongly influenced by the seasonal rainfall. When water scarcity periods occur, water flow, and dilution capacity of the river is reduced, increasing the potential environmental risk of pollutants. On the other hand, floods contribute to remobilization of pollutants from sediments. Contamination levels in Mediterranean rivers are frequently higher than in other European river basins, including pollution by pharmaceutical residues. Little attention has been paid to the transport behavior of emerging contaminants in surface waters once they are discharged from WWTP into a river. In this context, this work aimed to relate presence and fate of emerging contaminants with hydrological conditions of a typical Mediterranean River (Llobregat, NE Spain).
Methods River fresh water samples were collected twice a week over a period of 5 weeks at three sampling points. Sixty-six pharmaceutical compounds belonging to different therapeutical classes were analyzed by LC-MS/MS.
Results and discussion Positive and negative correlations between the concentrations of the target analytes and hydrological variables like river flow and dissolved organic matter were observed pointing out the relevance of different hydrological phenomena like dilution effects or sediment re-suspension. Sensitivity calculations showed that the majority of compounds were sensitive to flow variations

Keywords Pharmaceuticals · Occurrence · Flow · Dissolved organic carbon · Mediterranean river

Responsible editor: Philippe Garrigues

Electronic supplementary material The online version of this article (doi:10.1007/s11356-011-0603-4) contains supplementary material, which is available to authorized users.

V. Osorio · S. Pérez · A. Ginebreda · D. Barceló
Department of Environmental Chemistry, IDAEA-CSIC,
Jordi Girona 18-26,
Barcelona 08034, Spain

D. Barceló
Catalan Institute of Water Research, ICRA Catalan Institute for Water Research- ICRA, C/Emili Grahit, 101, Parc Científic i Tecnològic de la Universitat de Girona,
17003 Girona, Spain

D. Barceló
King Saud University,
P.O. Box 2455, 11451 Riyadh, Saudi Arabia

S. Pérez (✉)
Department of Environmental Chemistry, IDAEA-CSIC,
Jordi Girona 18-26,
Barcelona 08034, Spain
e-mail: spsqam@idaea.csic.es

1 Introduction

Global hydrological change is a result of climate change, land use change, water transfers, and river engineering. These impacts manifest themselves in changes to fluxes of water and related material with global consequences in terms of soil erosion, carbon transfer and storage, nutrients, pollutants, and sediment supply to ocean, biodiversity of continental aquatic systems, as well as for the sustainability of human development. For instance, water scarcity periods result in reduced water flow and dilution capacity, increasing the potential environmental risk of pollutants. In that sense, several studies reported an increasing frequency and intensity of extreme hydrological events (New et al. 2001;

Huntington 2006; Hirabayashi et al. 2008) over the past decades.

The Mediterranean region is predicted to undergo severe alterations in flow regime not only because a decrease in days of precipitation, but also an increase in days of heavy rains. Besides, regional climatic models for southern Europe predict a raise in frequency and duration of heat waves and heavy rainfall events during summer and stress that the Mediterranean region might be especially vulnerable to global change (Sánchez et al. 2004; Giorgi and Lionello 2008). As a result, more extreme and unpredictable hydrological events, such as flooding and drought, as well as higher and more variable temperatures, are expected, thus creating novel environmental conditions in the freshwater ecosystems in this region (Acuña and Tockner 2010). Besides, these rivers present heavy contamination due to continuing human pressure from extensive urban, industrial and agricultural activities, affecting the resources and the ecosystem. For that reason, contamination levels in Mediterranean rivers are quite frequently higher than in any other European basins (Ginebreda et al. 2010).

A good example is the Llobregat River (Catalonia, NE Spain), suffering from low flows during normal conditions (5 m^3/s) and extraordinary peak events (maximum recorded of 2,500 m^3/s) that periodically reset the system. In addition, the river receives the effluent discharges of more than 55 WWTPs, and at some points especially at drought periods, the effluents may represent almost 100% of the total flow of the river. This statement can explain the high levels of emerging organic contaminants detected on the river, increasing together with the augment of WWTPs and population pressure when moving downstream along the river (Céspedes et al. 2005; Huerta-Fontela et al. 2008). Furthermore, the Llobregat River provides drinking water to the large city of Barcelona.

Around 3,000 different PhACs (Richardson and Ternes 2005) of different therapeutic classes are used in human medicine in the European Union. Regarding Spain, it was the ninth largest world market in 2010, whereas, it took the fifth position in Europe's top pharmaceuticals market (IMS Health; www.farmaindustria.es). The main route of PhACs into the aquatic environment is the excretion by humans and the direct disposal through domestic wastewater. Despite its previous treatment in WWTPs, depending on the treatment efficiency and chemical properties of the compound, they are able to reach surface and ground waters. In the worst-case scenario, they have even been detected in finished drinking water (Benotti et al. 2009). More than 150 PhACs have been identified in surface (Gros et al. 2010), ground, and even drinking waters. Levels of PhACs detected in WWTP effluents are in the range of μg/L, whereas in river and groundwater, the levels are much lower, generally in the ng/L range. Compounds more frequently studied in the aquatic environment are the antibiotics, with several families included: macrolides (erythromycin), fluoroquinolones (ofloxacin and ciprofloxacin), sulfonamides (sulfamethoxazole), penicillins (amoxicillin), metronidazol, and trimethoprim. Other therapeutic groups are the analgesics and anti-inflammatories (like diclofenac, ibuprofen, naproxen, acetylsalicylic acid, and paracetamol), as well as the β-blocker atenolol, the lipid regulators (gemfibrozil and benzafibrate), the antiepileptic carbamazepine, and antidepressants (diazepam, fluoxetine, and paroxetine; Petrovic et al. 2010).

When entering river waters, PhACs present in effluent wastewaters are generally diluted at levels of one order of magnitude lower than the formers. These compounds are transported by water and can adsorb to suspended particulate matter and accumulate in sediments. After adsorption, chemicals can be remobilized by re-suspension or desorption. Also, PhACs may be removed from surface waters under natural degradation processes. Aquatic microcosm studies (Lam et al. 2004, 2005) showed that photodegradation was the most significant loss process with hydrolysis and microbial degradation being insignificant. However, this process may be less important when light is attenuated by high concentration of dissolved organic matter and suspended particles. Furthermore, effectiveness of all these processes is highly influenced by seasonal variation of environmental factors such as sunshine time, temperature, or precipitation.

The levels of PhACs have been shown to either decrease (Kolpin et al. 2000) or increase (Boyd et al. 2004) with increasing river flow and rainfall and to develop no significant correlations with concentration of suspended particulate matter (Shala and Foster 2010). PhACs are distributed in the water phase; the number of drugs, with high variable chemical properties, is large enough to consider their potential partition in water between suspended solids and sediments, and aqueous phase. The presence of some PhACs in suspended solids has been reported (Matamoros and Bayona 2006) and recent studies have suggested aquatic colloids in the natural environment as more powerful sorbents of PhACs than suspended solids and aquatic sediments (Maskaoui and Zhou 2010 and Yang et al. 2011). On these basis, the objectives of this study were to (1) determine the concentrations of PhACs in a selected section of the Llobregat and (2) correlate the results with hydrological river parameters such as flow and dissolved organic carbon (DOC) obtained aiming to gain further information about the behavior of PhACs in a sewage impacted section of a Mediterranean River course and how they can be affected by the principal events of the hydrological climate change, namely floods and droughts.

Environ Sci Pollut Res (2012) 19:1013–1025

2 Materials and methods

2.1 Pharmaceutical standards

The standards (see Table S-1 in Supporting Material) were purchased from Jescuder (Rubí, Spain), Sigma-Aldrich (Steinheim, Germany), LGC Promochem (London, UK), and Cerilliant (Texas, USA). Isotopically labeled compounds were used for internal standard calibration. ^{13}C-phenacetin, fluoxetine-d_5, and flumequine were provided by Sigma-Aldrich (Steinheim, Germany), sulfathiazole-d_4 from Toronto Research Chemicals (Canada), diazepam-d_5 and Phenobarbital-d_5 from Cerilliant (Texas, USA), and atenolol-d_7, carbamazepine-d_{10}, ibuprofen-d_3 from CDN isotopes (Quebec, Canada), and mecoprop-d_3 from Dr. Ehrenstorfer (Augsburg, Germany). All standards were of purity grade (>90%). Stock standard solutions were prepared on a weight basis in methanol, except fluoroquinolones which were dissolved in a mixture of water/methanol (1:1) with 0.2% HCl. After preparation, standards were stored at −20°C. Fresh stock solutions of antibiotics were prepared monthly due to their limited stability while stock solutions for the rest of substances were renewed every 3 months. Due to their different solubilities, codeine, furosemide, butalbial, pentobarbital, and phenobarbital were dissolved in acetonitrile while diazepam and lorazepam were obtained as solutions in methanol at 1 mg/mL. All PhACs were mixed by appropriate dilution of individual stock solutions in methanol/water (1:3). Working standard solutions were prepared daily (Gros et al. 2009). Glass fiber filters (47 mm) Whatman GF/F (0.7-μm of porus size) and 0.45 μm nylon membrane filters, used for pre-treatment of samples, were provided by Teknokroma (Barcelona, Spain). Solid-phase extraction (SPE) was carried out with cartridges Oasis HLB (6 mL, 200 mg) from Waters (Milford, MA), using a Baker vacuum system (J.T. Baker, Deventer, The Netherlands).

2.2 Site of study and sampling

The Llobregat River is the second longest river in Catalonia (NE Spain), with a total length of 156 km and covers a catchment area of approximately 4,957 km^2. The hydrology of the Llobregat River is characterized by a high variable flow, which is strongly influenced by seasonal rainfall. The mean annual precipitation is 3,330 Hm^3 and it has an annual average discharge of 693 Hm^3. Figure S-1 is a historical diagram of the Llobregat River flow recorded at Sant Joan Despí. The year-round hydraulic conditions shown in this figure are characterized by several peak flow events that have varied river flow from 50 m^3/s on May 2004 to 1 m^3/s on March 2008. The maximum peak flow recorded at 90 m^3/s on April 2000 followed by a drastic fall down to 10 m^3/s is a clear example of strength of seasonal rainfall effects on Llobregat River. Its watershed is heavily populated with more than three million inhabitants living therein. Together with its two main tributaries, the River Cardener and the River Anoia, the Llobregat is subjected to a heavy anthropogenic pressure. The river receives extensive urban and industrial wastewater discharges (137 Hm^3/year; 92% coming from wastewater treatment plants) as well as surface runoff from agricultural areas that cannot be diluted by its natural flow (0.68–6.5 m^3/s basal flow). Forty-eight percent of these point sources are located in the studied area. On Fig. 1 are indicated the main WWTPs distributed along the Llobregat system. Those that are more important, indicated with bigger points, correspond to major flow treated and consequently higher effluent volume discharged to the river (Table S-2). A river system containing multiple discharge points, such as Llobregat, creates the potential to produce a constant loading of PhACs over its course. Besides, the Llobregat River is one of main drinking water sources of Barcelona with a nearly 30% of its discharge being used for drinking water. Furthermore, the middle part of the basin receives natural salt slurries from salt formations and mining operations, which have caused an increase in water salinity downstream. Therefore, this typical Mediterranean River turns into an illustrative example of overexploited river, with water temporality being caused by a mixture of natural and human-driven components.

Three different sampling sites were selected along 36 km of the river's course, from the middle and lower part of the Llobregat main channel. Since the sampling points were two sites downstream a DOCinant WWTP and one site upstream, these sites were part of a pollution gradient. The first sampling point CB, located in Castellbell (see Fig. 1), is the least polluted of the three sampling points studied. Following the foresaid gradient, the second sampling site ABR (Abrera), located in Abrera, is a densely inhabited area receiving urban and industrial wastewater inputs. The third sampling site SJD is located in Sant Joan Despí and, according to the monitoring data of the Water Authority (Catalonian Water Agency), is the most polluted one. Sampling was performed in October–November 2009, covering one of the most relevant periods in the system in terms of hydrology. This period was characterized by low flow conditions derived from long dry season (almost 4 months) but with a typical short flood in response to the first rainfall event after summer (Fig. 2). River water samples were collected twice a week over a period of 4 weeks and a half, from the middle of the river along the water column. Five hundred milliliters of amber PET bottles previously rinsed with ultrapure water was filled with composite water samples obtained by mixing collected at different depths of the river, following a stratified

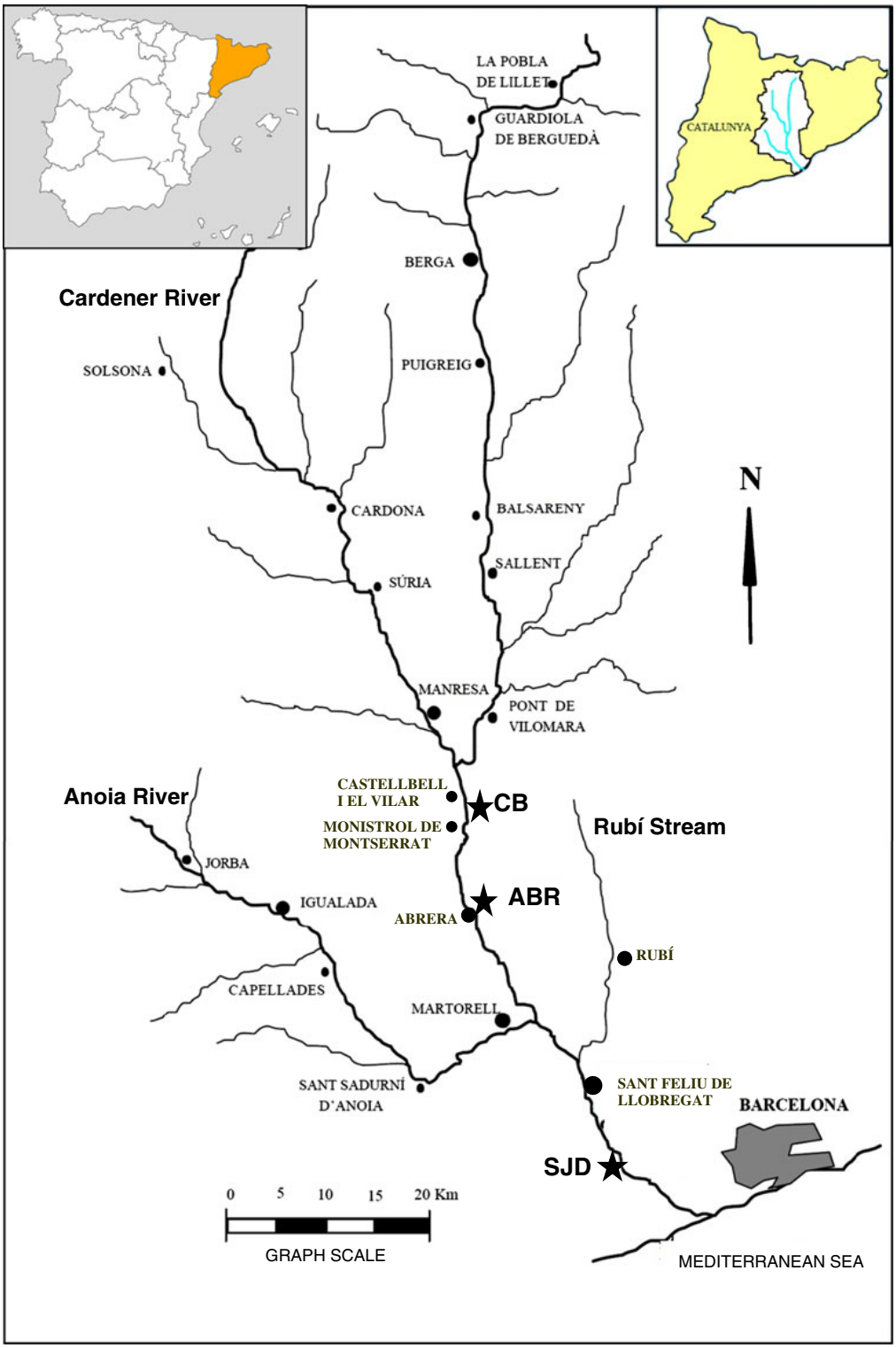


Fig. 1 Llobregat River. Map of the basin indicating the sampling sites: *filled star* Castellbell (*CB*) upstream after junction with its tributary river Cardener, Abrera (*ABR*) before junction with Anoia River, and Sant Joan Despí (*SJD*) downstream the river. Main WWTPs (*filled circle*) indicated along the Llobregat river and its tributaries, Anoia river, Cardener river, and Rubí stream. (The more important WWTPs, in terms of treated flow (m^3/day), are indicated with *bigger points*)

sampling strategy. Bottles were placed in a cooler (at 4°C) during transport to the laboratory and samples were immediately pre-treated.

2.3 Analysis of PhACs

The concentrations of 66 PhACs, belonging to different therapeutic groups (see Table S-1), were determined in surface waters using a multiresidue analytical method based on LC-MS/MS after solid-phase extraction (Gros et al. 2009). All water samples (500 mL) were filtered through a 0.7-μm glass fiber filters followed by a 0.45-μm nylon membrane filters in a Millipore glass vacuum filter holder. An aqueous solution of 5% Na_2EDTA was added to achieve a final concentration of 0.1%. Within 48 h, the samples were extracted by SPE, the cartridges were rinsed with

Environ Sci Pollut Res (2012) 19:1013–1025

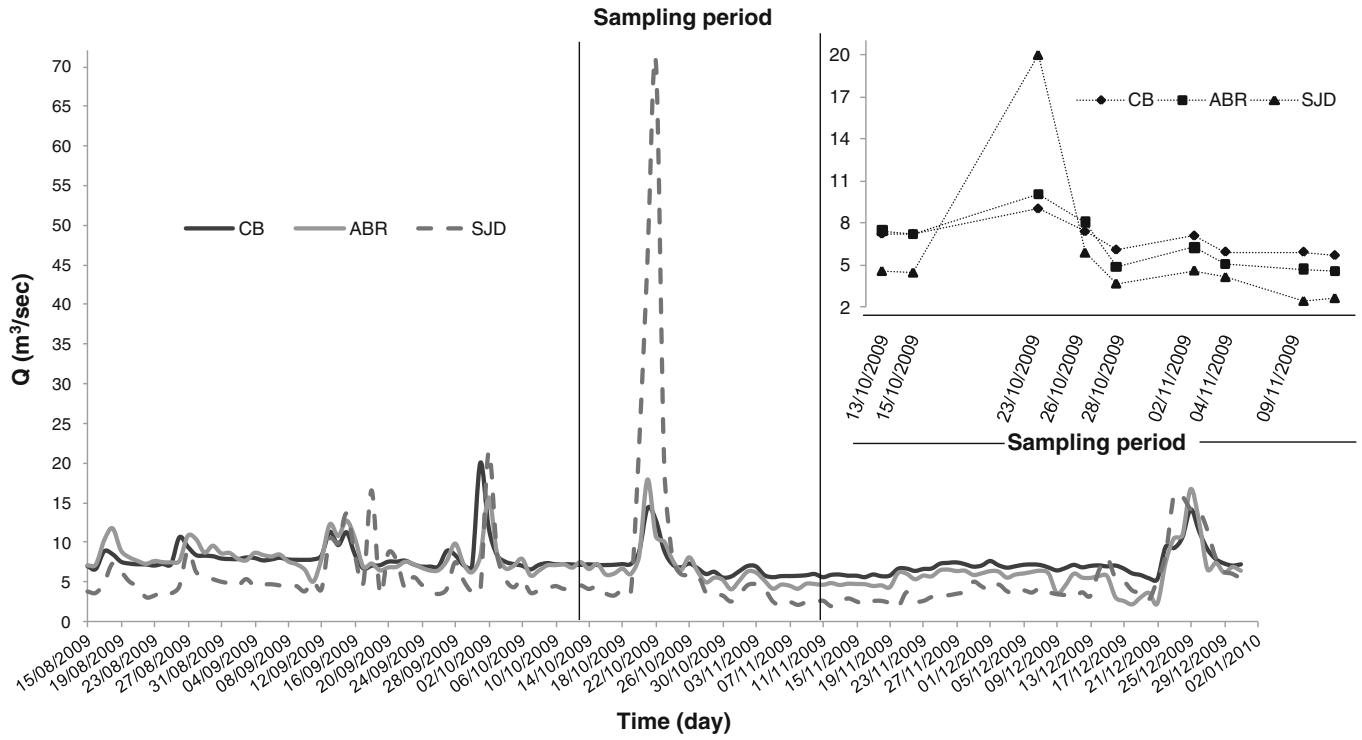


Fig. 2 Discharge (m^3/s) of the three sampling sites studied (*CB* Castebell, *ABR* Abrera, *SJD* Sant Joan Despí) registered between 15 August 2009 and 2 January 2010. *Vertical lines* specify sampling period. Average discharge (m^3/s) of the three sampling points during the sampling campaign is represented in the graph (*on the top right corner*; data: Catalan Water Agency data base)

5 mL of HPLC grade water, and were dried under vacuum for 15–20 min. After elution with 2×4 mL of methanol, the extracts were evaporated to dryness under a gentle stream of nitrogen and reconstituted with 1 mL of methanol/water (1:3). For internal standard calibration, 10 μL of a 1-mg/L standard mixture of the isotopically labeled compounds was added to the final analytical sample.

Instrumental analysis was performed by liquid chromatography, using an Agilent HP 1,100 HPLC (Palo Alto, CA, USA) system, coupled to a 4,000 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer operating with a Turbo Ion Spray Source (Applied Biosystems-Sciex, Foster City, CA, USA). Target compounds were separated with a Purospher Star RP-18 end-capped column (125×2.0 mm, particle size 5 μm) preceded by a C18 guard column (4×4.5 μm), both supplied by Merck (Darmstadt, Germany). Depending on the mode of analysis, different mobile phases were used. For the negative ionization mode, a mixture of acetonitrile/methanol (1:1, *v*/*v*; eluent A) and HPLC grade water (eluent B) at flow rate 0.2 mL/min was used. The elution gradient started with 20% eluent A, increasing to 80% in 20 min, raising to 90% in 4 min and then, back to initial conditions within 3 min. The column was re-equilibrated for 15 min before another injection with a total time for chromatographic analysis of 42 min. For analysis in positive ionization mode, acetonitrile (eluent A) and HPLC grade water with 0.1% formic acid (eluent B) were used. The elution gradient started with 5% eluent A, increasing to 95% in 25 min, raising to 100% in 5 min and then, back to initial conditions within 5 min. The column was re-equilibrated for 10 min and chromatographic analysis lasted 45 min. The sample injection volume was 20 μL in all chromatographic methods. Quantification of PhACs was carried out in Selected Reaction Monitoring mode monitoring two transitions per analyte.

2.4 Hydrological variables

Flow data of the three sampling sites were obtained from the public website of the Water Authority (http://www.gencat.cat/aca/) that records measures of the Catalan watershed every 5 min and aggregated at daily intervals. Flow values (represented as *Q* hereafter) used in this study were calculated as the average of *Q* data of sampling date, the days before and after (Fig. 2).

DOC was also obtained from the same source. However, only data for sampling site ABR were available. Consequently, studies relating concentration of PhACs with DOC were only considered in this point of the river.

2.5 Sensitivity

In order to have a quantitative insight of the relationship between pharmaceutical levels and hydrological variables

of the Llobregat River, the concentrations of each compound X (represented as C_X) were correlated with the aforementioned hydrological variables, namely Q (m^3/s) and DOC (mg/L) registered on the same sampling site and day. For that purpose, the respective correlation r and relative sensitivity coefficients s of C_X vs Q and C_X vs DOC were calculated. Sensitivity coefficients may be defined in several ways; however for the purposes of the present study, they are defined for each sampling site as follows (MacLeod et al. 2002):

$$s_{XY} = \frac{\sigma_X}{\sigma_Y} \cdot \frac{\mu_Y}{\mu_X} = \frac{CV_X}{CV_Y}$$

Where X refers to the compound and Y to the hydrological variables Q (flow) and DOC σ and μ are their standard deviations and averages, respectively. Relative sensitivity coefficients may be equivalently defined as the ratio of the coefficients of variation CV.

3 Results and discussion

3.1 Occurrence of PhACs in the Llobregat River

In the present study, a total number of 66 different commonly prescribed drugs were monitored. They included different therapeutic classes (Table S-1), namely, analgesics and antiinflamatories (NSAIDs) (11), lipid regulators and cholesterol lowering (6), psychiatric drugs (5), anti-ulcers (histamine H2-receptor antagonist) (3), antihistaminics (1), barbiturates (3), beta-blockers (9), antibiotics tetracyclines (2), antibiotics macrolides (8), antibiotics sulfonamides (2), antibiotics fluoroquinolones (5), antibiotics others (5), bronchodilators beta agonists (1), antihypertensives (1), diuretics (1), cancer treatment (1), antifungals (1), and antidiabetic (1). Target compounds were selected according to the information found in the literature on the occurrence and ubiquity in the aquatic environment, being wastewater effluents discharged to river waters the major contributors (Gros et al. 2010), as well as their high human use and consuABRion worldwide (Petrovic et al. 2006). Detection frequencies, expressed as percentages, are shown in Table S-1 of supporting information. Sixty-two of the 66 compounds studied were present in at least one of the samples analyzed; among these compounds, only butalbial was detected in 89% of the samples from SJD, whereas the rest of PhACs were detected in all the samples. Only four of these compounds were determined below the limit of quantification (mefenamic acid, chloroamphenicol, nifuroxazide, and loratidine only in ABR). Compounds not detected in all sampling campaign were betaxolol, danofloxacin, doxiciline, and clenbuterol. Seven of the target compounds detected presented maximum concentrations above 500 ng/L. These compounds were the analgesics ibuprofen in CB, acetaminophen in CB, and ABR and naproxen in SJD and the anti-depressant lorazepam, the β-blocker metoprolol, and the antibiotic tetracycline in SJD. The highest mean concentration was observed only for the antibiotic sulfonamide sulfamethoxazole in SJD sampling site, which exceeded 1 μg/L. This profile is consistent with studies carried out in other representative Iberian river, as it is the Ebro (Gros et al. 2009). In general, the maximum levels of PhACs monitored were in SJD while MT and CB presented minor concentrations being CB slightly less polluted (Fig. 3). Far from expected natural attenuation of pollutants (Fono et al. 2006), target compounds were detected following an increasing gradient together with number of WWTPs distributed along the river section studied. As mentioned in the "Site of study and sampling" section, this highlights the impact of effluents from WWTPs as significant contributors to the PhACs presence in the Llobregat River. The increasing levels of compounds along the three samplings points agree with the positive gradient of WWTPs effluents discharge along the river section studied and contribution of Anoia River and Rubí stream. In the case of the last one mentioned, when high precipitation events occur, untreated WWTPs effluents are discharged to Llobregat River, owing to bypass WWTP treatment.

Regarding therapeutic families, NSAIDs were the most ubiquitous and highest concentrated therapeutic group along the river section studied, which were determined in the range of 700–1,700 ng/L. After that, arrangement of the more representative groups varies from one sampling point to another. However, after NSAIDs, the most concentrated groups were psychiatric drugs, antibiotics, beta-blockers, lipid regulators, and cholesterol lowering. With the exception of NSAIDs, target compounds were generally detected at below μg/L range in SJD whereas in MT and CB their concentration rarely exceeded 300 ng/L. Levels of concentrations in the river section studied ranged from 0.01 to 1,500 ng/L. Considering the values reported for other Mediterranean rivers like the Ebro (Kuster et al. 2008; Gros et al. 2007; Comoretto and Chiron 2005), where concentrations ranged from 0.1 to 0.6 μg/L, which appear quite high. Nevertheless, the results make sense if one takes into account the high demographic pressure exerted in the low part of the Llobregat basin, together with the limited dilution capacity of this river (Ginebreda et al. 2010). In addition, mean concentrations of PhACs in the Llobregat River described by Muñoz et al. (2009) ranged from 0.02 to 2.28 μg/L were quite higher than those recorded in this study. This statement can be explained in terms of dilution factor. In the previous study (Muñoz et al. 2009), the sampling of Llobregat River was carried out under low flow periods, while the present study was conducted in fall

Environ Sci Pollut Res (2012) 19:1013–1025

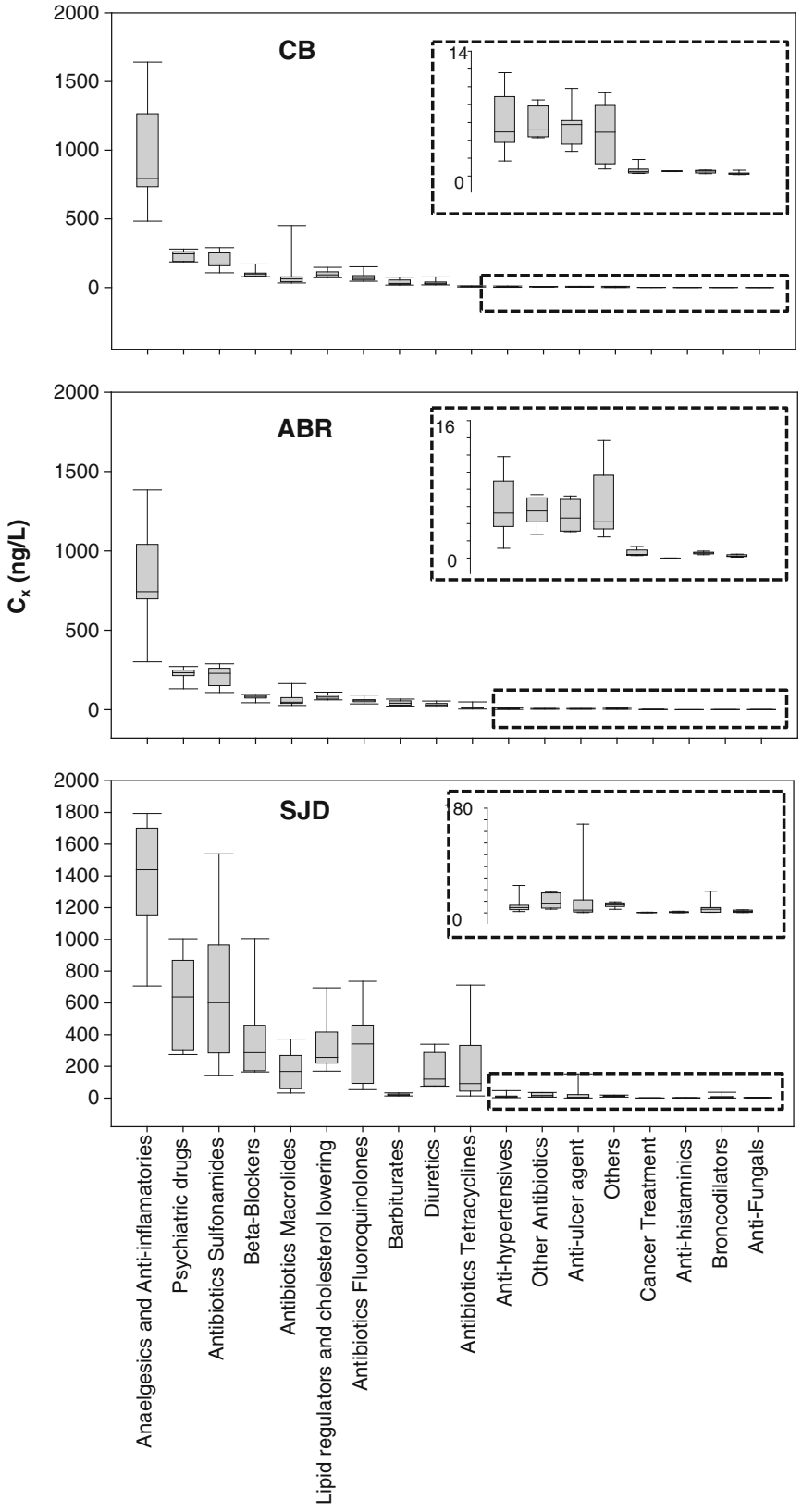


Fig. 3 Box plot indicating concentration ranges and average values of the target compounds monitored, classified by therapeutic groups of pharmaceuticals in the three sampling points studied. Each box plot includes a number of measures which corresponds to the sum of individual compound levels of each therapeutic group, along the Llobregat river section studied, for all the sampling campaign

season including a peak flow event, which clearly contributed to dilution of compounds targeted. Regarding therapeutic groups, the pharmaceutical products observed in the Llobregat River, closely matched those identified by the Spanish National System as those most consumed. There are mostly, analgesics and anti-inflammatories, antibiotics, physiquiatric drugs, β-blockers, and lipid regulators. As for compounds individually, maximum concentrations were

found for diclofenac, acetaminophen, lorazepam, and sulfamethoxazole, which showed average concentrations upper to 250 ng/L. These can be explained by their high human consuABRion rate being acetaminophen the most consumed, as well as their resistance to biodegradation in conventional WWTPs (Gros et al. 2009) in case of diclofenac, lorazepam, and sulfamethoxazole. As a matter of fact, the occurrence of PhACs in Llobregat River and their relationship with hydrological dynamics are expected to present a high variability and a wide range of results.

3.2 Relationship of flow and levels of PhACs

The individual concentration (C_X) of each one of the 66 target compounds were correlated with Q for all the sampling days and sampling points. The results presented a wide range of variability on their behavior which is difficult to interpret. In order to better focus our discussion and due to the extensive volume of compounds, the selection of the compounds was done by ones which presented concentration levels above their limit of detection. The final list of 23 selected compounds was then subsequently used for further study and are hereby discussed (Fig. 4).

Values of r (C_X/Q) obtained for CB sampling point ranged from −0.77 to 0.66. Lorazepam and fluoxetine presented the best correlation with Q. Forty-eight percent of the compounds showed a negative relationship with Q, meaning that their levels decreased when Q increased whereas concentrations of the other 52% of PhACs increased when the flow increased (Fig. 4). Regarding MT, it was observed a general improvement for r (C_X/Q) values. Correlation parameters were in the range of [−0.84, 0.68] for C_X/Q. Cimetidine showed the best relationship with Q, obtaining r (C_X/Q)=−0.84, whereas fluoxetine, enrofloxacine, and ciprofloxacin presented all of them the positive correlation value of r (C_X/Q)=0.68. It was observed the opposite response to Q for 57% of PhACs studied. As for SJD, results were in r (C_X/Q) ranged from −0.66 for gemfibrozil to 0.63 for butalbial. A remarkable percentage of drugs (83%) showed a negative response to increases of Q, which means that for almost all the compounds selected for this study their concentration decreases as a consequence of flow increase. In general, all compounds showed the same response respect to Q meaning that part of the drugs studied responded negatively to Q and the rest behaved equally positively. In view of these results, a tentative interpretation can be extrapolated that there is a predominance of the dilution factor caused by the increase of flow over remobilization. This behavior is likely to occur especially for the more polar (soluble) compounds. Nevertheless, all PhACs studied did not behave equally, as expected if it is considered that their fate varies and so do the governing mechanisms. Concerning to the more concentrated PhACs determined, lorazepam and sulfamethoxazole presented always negative correlations with Q, having the best correlation coefficients in CB (r (C_X/Q)=−0.77) and in ABR (r (C_X/Q)=−0.77), respectively. As for analgesics and anti-inflammatories, the most ubiquous therapeutic group, acetaminophen, generally showed the same positive response to Q of along the three sampling points. On the contrary, diclofenac was the compound showing the most changing

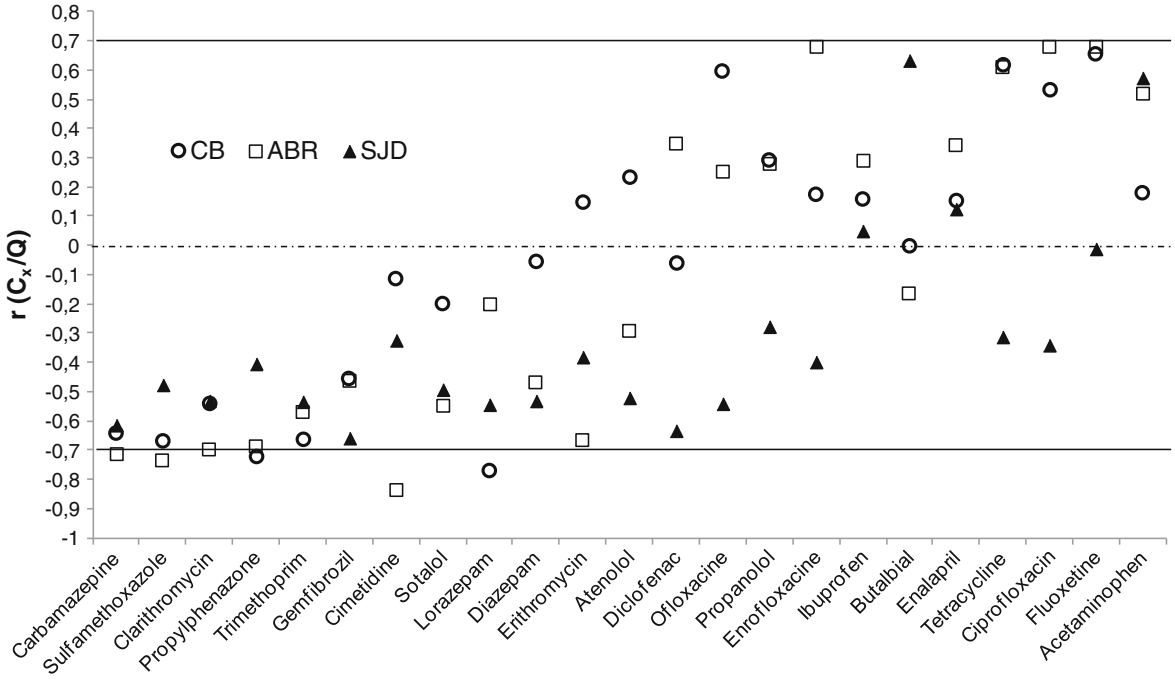


Fig. 4 Values of r (C_X/Q) of pharmaceuticals selected, along the three sampling points studied

Environ Sci Pollut Res (2012) 19:1013–1025

response to flow along the three sampling points, with a negative relationship with Q in sites CB and SJD, but a positive response to Q in MT. However, diclofenac obtained only one significant value of r (C_X/Q)=−0.63 in SJD and being the rest of r (C_X/Q)=−0.06 in CB and r (C_X/Q)=0.35 in ABR. Since diclofenac is readily photo-transformed (Tixier et al. 2003), this factor may contribute to variability observed for this chemical. In order to discern a trend for each compound along the river section studied, compounds selected for the study were ordered based on their average correlation values along the three sampling points, from the compound with the best negative relationship to the best positively correlated. Figure 4 shows PhACs arranged with respect to r (C_X/Q) from carbamazepine to acetaminophen, while in it can be appreciated three groups of compounds differentiated by marked trends.

a. Compounds with negative values of r (C_X/Q). These are from carbamazepine to diazepam. Along the studied section course of the river, concentration of these compounds decrease due to an increase of Q.
b. PhACs that response positively to Q. Only acetaminophen presents a positive value of r (C_X/Q). In the singular case of this compound, its concentration raises with the increase of Q for all the three sampling points.
c. Negative and positive relationships of C_X with Q without any clear pattern of behavior. All these compounds presented changing responses, like those mentioned before for diclofenac.

Complementary to that, it is also shown on Fig. S-2 from supporting information, C_X with Q relationships of representative compound having the best correlations negative (group a) and positive (group b), sulfamethoxazole and propylphenazone, and fluoxetine enrofloxacine for the sampling point ABR.

Significant reduction of elimination rates (60–70% reduction) of PhACs from WWTPs were reported when rainfall events occurred (Ternes 1998). As a consequence, an increased flow in the WWTPs tributaries of the river section studied, could result in increased water concentrations of the target compounds, for instance acetaminophen, due to reduced residence time in the WWTPs. Regarding PhACs classified in group b, for instance fluoxetine and enrofloxacine, increase of their concentration as long as flow raise can be explained in terms of sediment re-suspension, since some PhACs are expected to deposit in sediments; or suspended particulate matter and colloids re-mobilization have since been demonstrated to be a very important sink and as a carrier of PhACs in the aquatic systems. This statement agrees with the several reports of occurrence of PhACs in sediments (Kwon and Armbrust 2006; Zhou et al. 2011) and suspended particulate matter and colloids (Maskaoui and Zhou 2010; Yang et al. 2011).

3.3 Correlation of levels PhACs versus DOC

Association of solutes to DOC increases the amount of chemical in the aqueous phase thus facilitating transport of these compounds through surface water of the river (Tolls 2001). In order to complement the study of behavior of PhACs in the river section studied, DOC was considered. Owing to available data, as mentioned in the "Hydrological variables" section, only ABR sampling site and six sampling days were studied. Since the amount of data is different than the corresponding for studies with Q, results of this study cannot be properly compared with those obtained for PhACs responses to Q. However, PhACs relationship with DOC was used an atteABR to discern some additional trend and establish a possible relationship with Q.

Prior to discussing the relationships among concentrations C_X and hydrological variables Q and DOC, it is worth noting the well-known correlation between the last two parameters (Fig. S-3).There is an observed opposite (negative) correlation displayed for ABR sampling point, showing the general decrease of DOC with increasing Q. Equal negative correlations have been reported for DOC (Tao 1998; Hejzlar et al. 2003), increase in flow raises the release of DOC from the bottom sediment, but at the same time diluted the DOC in water. C_X of each one of the 66 target compounds was correlated with DOC for six sampling days in ABR. Results obtained (Fig. 5) presented a wide range of positive values from ciprofloxacin with r (C_X/DOC)=0.08 to enalapril with r (C_X/DOC)=0.08. Therefore, concentration of PhACs increases with DOC, which also means that high DOC increments mobility of the chemicals in the aqueous phase. The compounds best correlated with DOC were enalapril, propanolol, fluoxetine, ibuprofen, and ofloxacine, all with values upper than the environmentally acceptable; r (C_X/DOC)>0.70. 52% of PhACs presented r>0.70, while butalbial, acetaminophen, and erythromycin showed poor correlation with DOC having the following values r (C_X/DOC)=0.08, r (C_X/DOC)=0.27, and r (C_X/DOC)=0.29, respectively. In view of these high relationships, the same co-variation reported for DOC and organic pollutants (Tao 1998) is expected and confirmed for levels of PhACs and DOC.

In order to compare responses of PhACs to both hydrological parameters, Q and DOC, Fig. 5 shows respective correlation coefficients, arranged equally to Fig. 4. According to the opposite relationship between Q and DOC (Fig. S-3), the 57% of compounds negatively correlated with Q agrees with their positive response to DOC. However, the concentration of the remaining 43% PhACs increases with DOC,

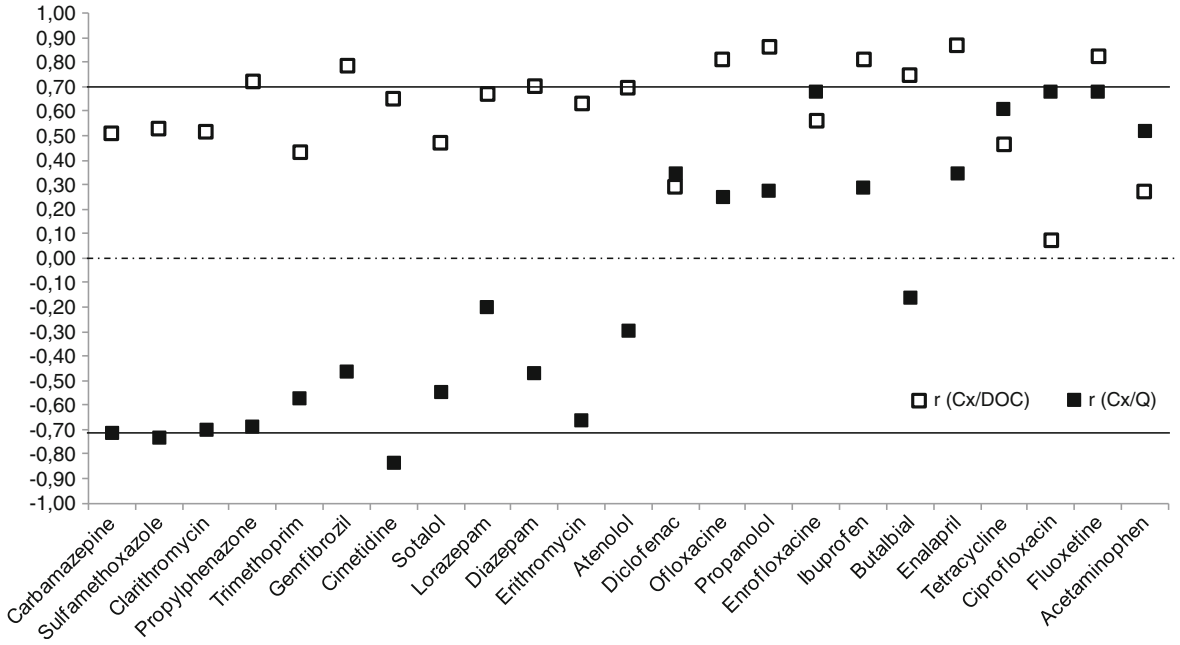


Fig. 5 Relationship of levels of pharmaceuticals with flow compared to correlation with dissolved organic matter at ABR

but also does with Q; this would mean than DOC should increase with Q. This inconsistency could be explained in terms on solid re-mobilization. As mentioned in the "Relationship of flow and levels of PhACs" section, concentration of PhACs expected to be present on solid phase could increase their levels in aqueous phase as a cause of sediment re-suspension with higher flow. Therefore, fraction of PhACs adsorbed to solids would transfer to aqueous phase, reflecting an increase of concentration. Once associated to DOC, mobility of PhACs transferred from solid phase would increase and higher transport of these chemicals downstream would be expected. Regardless, DOC and Q negative relationship remains constant.

Among these compounds, sulfamethoxazole, propylphenazone, fluoxetine, and enrofloxacine are the most representative. The respective correlation coefficients of levels of these compounds with DOC were, r (C_X/DOC)=0.53, r (C_X/DOC)=0.72, r (C_X/DOC)=0.83, and r (C_X/DOC)=0.56 (Fig. S-4). Together with compound relationships with flow, it can be sensed that levels of sulfamethoxazole decrease with DOC dropping due to an increase of flow. This is consistent with the dilution factor of PhACs and DOC due to a raise of flow. On the other hand, fluoxetine, which is always positively well correlated with DOC, presents increasing levels while flow raise. This behavior supports what was mentioned before, considering the constant negative correlation between DOC and Q, and the reported presence of this pharmaceutical in solid phase, higher concentrations of fluoxetine were detected as flow increased as a likely consequence of sediment re-suspension. Regarding enrofloxacine and propylphenazone, the high correlation coefficient of the last one reinforces the theory of dilution of PhACs when river flow increases.

Concerning the more concentrated PhACs determined, lorazepam, carbamazepine, acetaminophen, and diclofenac, they presented the respective correlation coefficients with DOC r (C_X/DOC)=−0.67, r (C_X/DOC)=−0.51, r (C_X/DOC)=−0.27, and r (C_X/DOC)=−0.29; while, relationships with Q were r (C_X/DOC)=−0.20, r (C_X/DOC)=−0.71, r (C_X/DOC)=0.52, and r (C_X/DOC)=0.35. Although good correlation values were obtained with DOC, the corresponding correlation coefficients with Q were less acceptable, so that, co-variation of levels of these compounds with both hydrological variables was considered not worthy.

3.4 Sensitivity of PhACs to flow and DOC

With the aim of gaining more information about the relationships studied before, there were estimated sensitivity (s) parameters of the same compounds. Figure 6 shows sensitivity of C_X of the 23 selected drugs to Q and DOC calculated for sampling point ABR. Compounds were arranged equally than correlation coefficients shown in Fig. 4. Regarding the flow, PhACs presented s (C_X/Q) values in the range of 0.33 for tetracycline to −1.43 for lorazepam. Concerning PhACs, which are more discussed in prior section, sulfamethoxazole, propylphenazone (negatively correlated with flow), and enrofloxacine (positively correlated), arrangement for these compounds were from the less sensitive enrofloxacine (s (C_X/Q)=0.44), then the slightly higher sensitive fluoxetine (s (C_X/Q)=0.48), followed by

Environ Sci Pollut Res (2012) 19:1013–1025

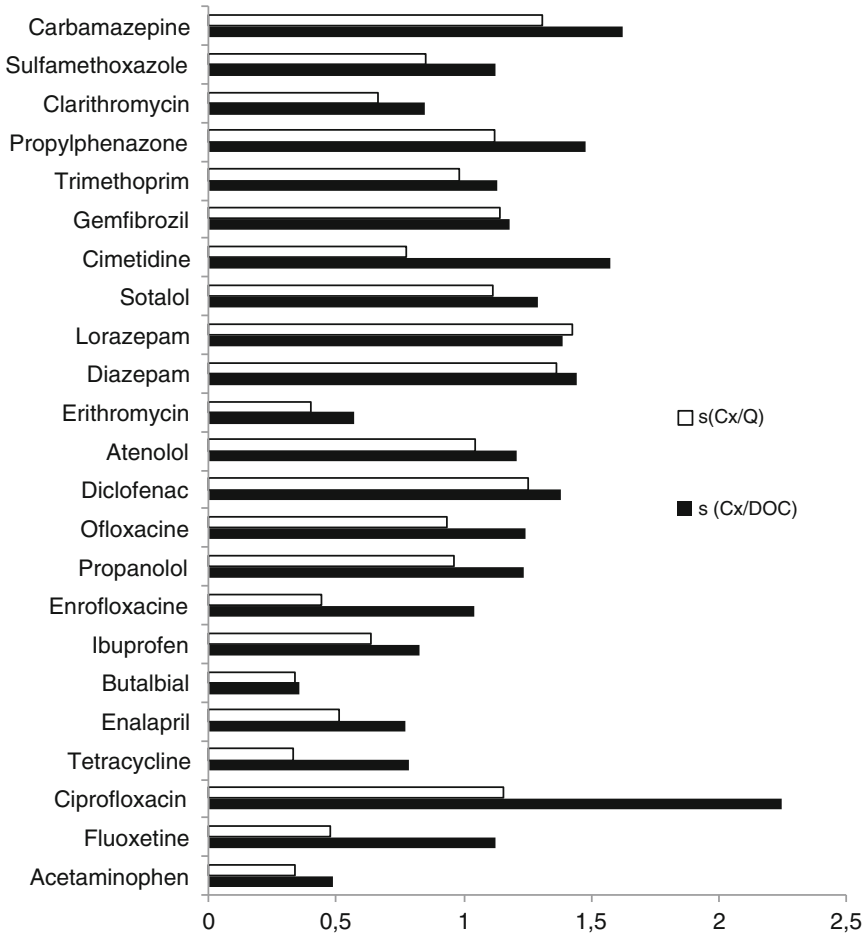


Fig. 6 Values of sensitivity of pharmaceuticals to both flow and dissolved organic matter at ABR

sulfamethoxazole (s (C_X/Q)=0.85), and the more sensitive propylphenazone (s (C_X/Q)=1.12).

The comparison between sensitivity values show that PhACs are more sensitive to DOC than to Q, suggesting the importance of response of PhACs to organic matter. In some cases, s (C_X/DOC) is especially higher than s (C_X/Q), for instance ciprofloxacine, with s (C_X/DOC)=2.24, carbamazepine (s (C_X/DOC)=1.62), and cimetidine (s (C_X/DOC)=1.62). However, in the singular case of lorazepam, this is more sensitive to Q (s (C_X/Q)=1.43) than to DOC (s (C_X/DOC)=1.38). If compounds are considered individually, it can be found that arrangements of sensitivity to both hydrological parameters are almost the same.

DOC dynamics as a function of changes in climatic and hydrologic conditions have been reported to vary historically and seasonally (Hejzlar et al. 2003). The concentration of DOC is influenced by climatic and hydrologic conditions, especially seasonal and long-term changes of temperature and runoff components and long-term changes in the atmospheric deposition of acidity (loading of soils with mineral acids is known to cause decreased leaching of DOC; Tipping and Hurley 1988). Thus, more factors involved in observed response of PhACs to DOC and Q should be analyzed in order to gain further information about PhACs behavior under hydrological conditions of the Llobregat River.

4 Conclusions

The present study has reported several pharmaceutical products in the Llobregat River at concentrations higher than those cited in other studies. Maximum concentrations were found for diclofenac, acetaminophen, lorazepam, and sulfamethoxazole, which showed average concentrations, up to 250 ng/L. This data can be explained by their high human consuABRion rate, as well as their resistance to biodegradation in conventional WWTPs with secondary treatment. Out of 66 target analytes, a total of 63 compounds were detected in 85% of samples analyzed. The general raise of pharmaceutical concentrations has also been detected during the sampling campaign which can be correlated with the occurrence of the high flow increase. Contradictory findings for some compounds to the flow increment were observed. Information which correlates concentration of pollutants with river flow is still scarce and therefore further attention should be paid to this matter.

Since several PhACs are expected to sorb to suspended solids and colloids by several mechanisms and being this sorption one of the key processes controlling the transport and fate of this compounds. Future work will be based on determination of partition coefficients of PhACs in both the solid and aqueous phases of the river system as well as factors involved in their mechanisms of distribution. Results suggest complex interactions between pollution sources, transport and degradation of PhACs in the highly dynamic Llobregat system. Other environmental factors should be taken into account like photo- and biodegradation which can affect fate of PhACs in surface waters.

Acknowledgements This work has been supported by the Spanish Ministry of Science and Innovation [projects Cemagua CGL2007-64551/HID and Consolider-Ingenio 2010 Scarce CSD2009-00065]. It reflects the authors' views only. The European Union (EU) is not liable for any use that may be made of the information contained in it. Merck is acknowledged for the gift of LC columns. SP acknowledges the contract from the Ramón y Cajal Program of the Spanish Ministry of Science and Innovation.

References

Acuña V, Tockner K (2010) The effects of alterations in temperature and flow regime on organic carbon dynamics in Mediterranean river networks. Glob Ch Biol 16:2638–2650

Benotti MJ, Trenholm RA, Vanderford BJ, Holady JC, Standford BD, Snyder SA (2009) PhACs and endocrine disrupting compounds in U.S. drinking water. Environ Sci Technol 43:597–603

Boyd RG, Palmeri MJ, Zhang S, Grimm AD (2004) PhACs and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) in stormwater canals and bayou St. John in New Orleans, Louisiana, USA. Sci Tot Environ 333:137–148

Céspedes R, Lacorte S, Raldúa D, Ginebreda A, Barceló D, Piña B (2005) Distribution of endocrine disruptors in the Llobregat River basin (Catalonia, NE Spain). Chem 61(11):1710–1719

Comoretto L, Chiron S (2005) Comparing pharmaceutical and pesticide loads into a small Mediterranean river. Sci Tot Env 349(1–3):201–210

Fono LJ, Kolodziej EP, Sedlak DL (2006) Attenuation of wastewater-derived contaminants in an effluent-DOCinated river. Environ Sci Technol 40:7257–7262

Ginebreda A, Muñoz I, Alda ML, Brix R, López-Doval J, Barceló D (2010) Environmental risk assessment of PhACs in rivers: relationships between hazard indexes and aquatic macroinvertebrate diversity indexes in the Llobregat River (NE Spain). E Int 36:153–162

Giorgi F, Lionello P (2008) Climate change projections for the Mediterranean region. Glob Plan Ch 63:90–104

Gros M, Petrovic M, Barceló D (2007) Wastewater treatment plants as a pathway for aquatic contamination by pharmaceuticals in the Ebro river basin (northeast of Spain). Environ Toxicol Chem 26:1553–1562

Gros M, Petrovic M, Barceló D (2009) Tracing PhACs residues of different therapeutic classes in environmental waters by using liquid chromatography/quadrupole-linear ion trap mass spectrometry and automated library searching anal. Chem 81:898–912

Gros M, Petrovic M, Ginebreda A, Barceló D (2010) Removal of PhACs during wastewater treatment and environmental risk assessment using hazard indexes. E Int 36:15–26

Hejzlar J, Dubrovsky M, Buchtelec J, Ruzicka M (2003) The apparent and potential effects of climate change on the inferred concentration of dissolved organic matter in a temperate stream (the Malse River, South Bohemia). Sci Tot Env 310:143–152

Hirabayashi Y, Kanae S, Emori S et al (2008) Global projections of changing risks of floods and droughts in a changing climate. Hydrol Sci J 53:754–772

Huerta-Fontela M, Galcerán MT, Ventura F (2008) Stimulatory drugs of abuse in surface waters and their removal in a conventional drinking water treatment plant. Environ Sci Technol 42 (18):6809–6816

Huntington TG (2006) Evidence for intensification of the global water cycle: review and synthesis. J Hydrol 319:83–95

Kolpin DW, Furlong ET, Meyer MT, Thruman EM, Zaugg SD, Barber LB, Buxton H (2000) PhACs, hormones and other wastewater contaminants in U.S. streams. Environ Sci Technol 32:2498–2506

Kuster M, López de Alda MJ, Hernando MD, Martín-Alonso J, Barceló D (2008) Analysis and occurrence of pharmaceuticals, estrogens, progestogens and polar pesticides in sewage treatment plant effluents, river water and drinking water in the Llobregat river basin (Barcelona, Spain) J Hydrol 358(1–2):112–123

Kwon J-K, Armbrust KL (2006) Laboratory persistence and fate of fluoxetine in aquatic environments. Environ Toxicol Chem 25 (10):2561–2568

Lam MW, Young CJ, Brain RA, Johnson DJ, Hanson MA, Wilson CJ, Richards SM, Solomon KR, Mabury SA (2004) Aquatic persistence of eight PhACs in a microcosm study. Environ Toxicol Chem 23:1431–1440

Lam MW, Young CJ, Mabury SA (2005) Aqueous photochemical reaction kinetics and transformations of fluoxetine. Environ Sci Technol 39:513–522

MacLeod M, Fraser AJ, Mackay D (2002) Evaluating and expressing the propagation of uncertainty in chemical fate and bioaccumulation models. Environ Toxicol Chem 21(4):700–709

Maskaoui K, Zhou JL (2010) Colloids as a sink for certain PhACs in the aquatic environment. Environ Sci Pollut Res 17:898–907

Matamoros V, Bayona JM (2006) Elimination of pharmaceuticals and personal care products in subsurface flow constructed wetlands. Environ Sci Technol 41(23):8171–8177

Muñoz I, López-Doval JC, Ricart M, Villagrassa M, Brix R, Geiszinger A, Ginebreda A, Guash H, de Alda ML, Romani AM, Sabater S, Barceló D (2009) Bridging levels of PhACs in river water with biological community structure in the Llobregat River basin (NE, Spain). Environ Toxicol Chem 28:2706–2714

New M, Todd M, Hulme M et al (2001) Precipitation measurements and trends in the twentieth century. International J Climat 21:1899–1922

Petrovic M, Gros M, Barceló D (2006) Multi-residue analysis of PhACs in wastewater by mass spectrometry. J Chromatogr 1124:68–81

Petrovic M, Postigo C, de Alda ML, Ginebreda A, Gros M, Radjenovic J, Barceló D (2010) Water scarcity in the Mediterranean: perspectives under global change. Hdb Env Chem 8:1978–228

Richardson SD, Ternes TA (2005) Water analysis: emerging contaminants and current issues. Anal Chem 77:3807–3838

Sánchez EC, Gallardo MA, Gaertner A (2004) Future climate extreme events in the Mediterranean simulated by a regional climate model: a first approach. Glob Plan Ch 44:163–180

Shala L, Foster GD (2010) Surface water concentrations and loading budgets of PhACs and other DOCestic-use chemicals in an urban

watershed (Washington, DC, USA). Arch Environ Contam Toxicol 58:551–561

Tao S (1998) Spatial and temporal variation in DOC in the Yichun River. China Wat Res 32(7):2205–2210

Ternes T (1998) Occurrence of drugs in German sewage treatment plants and rivers. W Ress 32(11):3245–3260

Tipping E, Hurley MA (1988) A model of solid–solution interactions in organic solid, based on complexation properties of humic substances. J Soil Sci 39:505–519

Tixier C, Singer HP, Oellers S, Müller SR (2003) Occurrence and fate of carbamazepine, clofibric acid, diclofenac, ibuprofen, ketoprofen and naproxen in surface waters. Environ Sci Technol 37:1061–1068

Tolls J (2001) Sorption of veterinary pharmaceuticals in soils: a review. Env Sci Technol 35(17):3397–3406

Yang Y, Fu J, Peng H, Hou L, Liu M, Zhou JL (2011) Occurrence and phase distribution of selected PhACs in the Yangtze Estuary and its coastal zone. J Hazard Mater 190:588–596

Zhou L-J, Ying G-G, Zhao J-L, Yang J-F, Wang L, Yang B, Liu S (2011) Trends in the occurrence of human and veterinary antibiotics in sediments of the Yellow River, Hai River and Liao River in northern China. Environ Pollut 159:1877–1885

SUPPORTING INFORMATION

	CB					ABR					SJD				
	Min C.	Max C.	Ave.	St. Dev.	Freq.	Min C.	Max C.	Ave.	St. Dev.	Freq.	Min C.	Max C.	Ave.	St. Dev.	Freq.
Analgesics and antiinflamatories															
Ketoprofen (a)	14.1	115.0	56.1	35.3	100	38.2	165.0	38.0	33.0	100	96.1	258.5	93.8	75.4	100
Naproxen (a)	55.7	193.9	116.1	46.3	100	68.7	398.9	110.4	39.5	100	169.5	633.4	181.1	55.1	100
Ibuprofen (a)	98.9	502.9	254.3	136.9	100	10.8	111.7	248.0	112.4	100	15.4	108.6	404.6	185.8	100
InDOCethacin (b)	12.6	29.6	20.1	5.1	100	88.7	167.2	27.4	31.8	100	110.6	460.3	42.1	29.5	100
Diclofenac (a)	106.3	213.8	145.7	30.3	100	88.7	167.2	128.6	29.6	100	66.1	442.6	299.3	131.9	100
Mefenamic acid (b)	<LOQ	<LOQ	<LOQ	<LOQ	100	<LOQ	<LOQ	<LOQ	<LOQ	100	0.9	9.5	3.4	3.0	100
Acetaminophen (b)	20.3	755.2	317.2	264.8	100	22.4	629.8	237.3	201.7	100	66.1	442.6	239.4	134.7	100
Propyphenazone (c)	2.2	4.4	3.5	0.7	100	2.0	4.6	3.5	0.9	100	5.2	35.0	15.2	11.2	100
Phenazone (b)	7.9	15.5	11.4	2.7	100	6.8	13.4	10.5	2.3	100	10.1	94.0	40.8	30.3	100
Phenylbutazone (b)	3.5	6.5	4.9	1.3	100	1.7	5.8	3.6	1.2	100	6.6	50.5	17.0	14.4	100
Codeine (c)	6.6	22.6	14.7	5.4	100	6.4	20.4	13.3	5.1	100	3.8	122.7	48.6	43.1	100
Lipid regulators and cholesterol lowering															
Clofibric acid (b)	1.3	19.1	3.7	5.8	100	1.1	2.0	1.7	0.3	100	6.8	40.1	19.5	10.8	100
Gemfibrozil (a)	11.6	23.3	17.5	4.3	100	9.3	23.4	15.3	3.9	100	12.5	152.0	78.4	45.9	100
Bezafibrate (b)	12.4	42.1	26.0	9.7	100	13.7	41.2	23.6	8.4	100	33.7	217.1	88.5	64.5	100
Fenofibrate (b)	33.4	81.6	45.7	16.0	100	24.0	61.7	35.6	11.0	100	36.6	277.6	137.1	85.8	100
Atorvastatin (c)	0.5	1.1	0.7	0.2	100	0.3	1.3	0.8	0.3	100	1.0	3.2	2.0	0.7	100
Mevastatin (b)	2.7	9.4	4.6	2.1	100	1.0	8.2	4.0	2.1	100	2.5	7.5	5.3	1.9	100
Pysichiatric drugs															
Fluoxetine (b)	12.1	30.2	16.4	5.4	100	8.0	37.5	15.1	9.1	100	13.9	53.5	30.9	13.3	100
Paroxetine (c)	1.6	4.3	2.2	0.8	100	1.6	7.1	4.2	1.7	100	4.6	104.3	26.4	43.0	100
Diazepam (d)	2.0	3.5	2.8	0.4	100	1.6	3.6	2.7	0.6	100	4.3	32.0	14.9	9.8	100
Lorazepam (d)	110.9	201.5	156.6	33.9	100	87.7	187.7	152.1	30.8	100	104.4	643.1	354.8	207.8	100
Carbamazepine (b)	42.1	58.1	50.2	6.1	100	31.3	63.4	50.8	11.2	100	42.2	266.7	159.4	84.5	100
Anti-ulcers (histamine H2-receptor antagonist)															
Famotidine (b)	0.6	1.0	0.8	0.2	100	0.7	1.1	0.9	0.1	100	0.2	6.4	2.6	2.4	100
Ranitidine(b)	1.0	4.7	2.3	1.2	100	0.8	3.3	1.9	1.1	100	0.1	115.8	13.6	38.3	100
Cimetidine (b)	1.2	4.3	2.2	1.0	100	1.2	3.3	2.2	0.8	100	0.2	30.5	8.0	11.2	100
Barbiturates															
Butalbital (d)	2.0	14.6	7.2	5.0	100	2.0	27.3	10.5	9.0	100	2.1	5.0	3.1	1.2	89
Pentobarbital (d)	7.5	39.8	21.1	12.4	100	6.7	45.0	19.1	12.3	100	9.6	16.1	12.4	2.4	100
Phenobarbital (d)	3.5	21.3	10.4	6.2	100	6.3	21.6	11.9	5.3	100	2.7	12.1	6.2	3.2	100
Anti-histaminics															
Loratidine (b)	0.5	0.6	0.5	0.0	100	<LOQ	<LOQ	<LOQ	<LOQ	100	0.2	2.9	1.3	0.9	100
Beta-blockers															
Atenolol (b)	21.0	56.7	36.7	11.5	100	15.9	43.6	32.0	8.8	100	64.7	251.2	142.0	71.8	100
Sotalol (b)	12.8	24.5	18.3	3.2	100	9.1	21.0	16.2	4.2	100	26.6	164.7	79.4	53.8	100
Metoprolol (b)	9.1	16.1	12.8	2.4	100	9.4	17.6	12.6	2.8	100	26.0	535.0	97.7	164.6	100
Propanolol (b)	2.9	21.8	14.5	5.1	100	7.6	25.6	16.2	4.9	100	7.3	68.5	34.1	22.1	100
Timolol (b)	1.5	119.3	18.8	39.3	100	0.9	2.4	1.8	0.4	100	2.1	10.2	6.4	2.8	100
Betaxolol (b)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Carazolol (b)	0.0	0.4	0.2	0.1	100	0.1	0.3	0.2	0.1	100	0.2	1.0	0.5	0.2	100
Pindolol (b)	0.0	0.2	0.1	0.1	100	0.1	0.2	0.1	0.0	100	0.2	0.5	0.3	0.1	100
Nadolol (b)	0.3	0.5	0.4	0.1	100	0.1	0.5	0.3	0.1	100	0.4	1.1	0.6	0.2	100
Cancer treatment															
Tamoxifen (b)	0.3	1.8	0.6	0.5	100	0.3	1.3	0.6	0.4	100	0.2	1.3	0.6	0.3	100
Fungals															

Metronidazole(b)	0.2	0.6	0.3	0.1	100	0.1	0.5	0.3	0.1	100	0.6	5.2	2.7	1.9	100
Antibiotics macrolides															
Erythromycin(b)	1.9	7.3	4.5	1.9	100	1.1	8.7	3.4	2.5	100	0.1	45.2	14.1	15.1	100
Azithromycin (b)	3.6	7.0	6.6	1.1	100	6.9	7.1	7.0	0.1	100	0.0	7.2	6.3	2.4	100
Roxithromycin (b)	0.4	0.9	0.7	0.2	100	0.3	0.8	0.5	0.2	100	0.5	8.1	3.2	2.5	100
Clarithromycin (b)	12.8	54.8	36.1	14.7	100	9.3	48.1	31.1	13.5	100	16.3	232.1	102.3	84.5	100
Tylosin (b)	1.2	4.7	2.7	1.1	100	1.3	3.8	2.6	0.8	100	2.4	30.3	7.6	8.7	100
Josamycin(b)	0.3	0.6	0.4	0.1	100	0.2	0.7	0.5	0.2	100	0.7	3.6	2.0	1.1	100
Spiramycin(b)	4.6	13.2	7.4	2.8	100	4.0	12.5	7.0	2.5	100	6.5	52.8	25.6	16.3	100
Tilmicosin (b)	0.6	370.2	42.0	123.1	100	0.9	95.8	11.5	31.6	100	2.0	95.3	13.4	30.8	100
Antibiotics fluoroquinolones															
Ofloxacin (b)	14.5	85.7	30.1	22.2	100	9.4	31.9	20.2	6.3	100	11.0	296.2	134.0	109.3	100
Ciprofloxacin (b)	19.7	47.6	27.5	8.4	100	18.4	40.2	25.5	6.4	100	24.0	187.9	76.5	55.7	100
Enoxacin (b)	6.7	13.7	9.1	2.3	100	5.3	12.5	9.0	2.0	100	0.7	31.4	17.2	11.1	100
Danofloxacin (b)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Enrofloxacin (b)	1.5	45.1	8.2	13.9	100	1.7	10.5	4.0	2.6	100	5.7	279.9	93.6	96.3	100
Antibiotics tetracyclines															
Tetracycline(b)	3.6	12.6	8.0	2.9	100	3.7	48.6	15.1	13.2	100	13.7	712.4	196.9	227.1	100
Doxycycline (b)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Antibiotics sulfonamides															
Sulfamethoxazole (b)	98.8	282.8	189.7	60.2	100	96.8	284.4	194.6	66.2	100	134.8	1500.0	615.3	461.4	100
Sulfadiazine (b)	3.2	20.2	7.1	5.3	100	1.5	62.7	14.9	19.5	100	5.2	75.2	29.4	21.3	100
Antibiotics others															
Trimethoprim (b)	4.3	8.5	5.9	1.7	100	2.7	7.4	5.4	1.6	100	6.4	35.6	20.0	12.6	100
Chloramphenicol (b)	<LOQ	<LOQ	<LOQ	<LOQ	100	<LOQ	<LOQ	<LOQ	<LOQ	100	<LOQ	<LOQ	<LOQ	<LOQ	100
Clenbuterol (b)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Nifuroxazide (b)	<LOQ	7.7	4.7	2.2	100	1.7	10.6	4.9	3.3	100	<LOQ	12.3	7.3	2.9	100
Flumequine (b)	0.2	1.1	0.4	0.3	100	0.2	1.1	0.4	0.3	100	0.4	0.8	0.6	0.2	100
Broncodilators beta agonists															
Salbutamol (b)	0.3	0.7	0.5	0.1	100	0.4	0.8	0.6	0.1	100	0.9	37.2	8.3	11.4	100
Anti-hypertensives															
Enalapril (b)	1.7	11.6	6.0	3.3	100	1.1	11.8	6.0	3.4	100	2.3	47.1	12.7	13.5	100
Diuretics															
Furosemide (b)	19.4	76.6	35.0	17.4	100	16.5	54.1	30.7	12.1	100	74.8	339.5	170.1	107.6	100
Antidiabetic															
Glibenclamide (b)	0.5	1.9	1.2	0.5	100	0.6	2.0	1.0	0.4	100	1.8	12.6	6.7	4.0	100

Table S-1. Range of concentrations (min., max and average), expressed in ng/L, of pharmaceuticals monitored at the three sampling sites studied, standard deviation and frequency expressed in %. (LOQ : limit of quantification; ND: not detected).

Table S-2. Characteristics of the main WWTP that discharge into the studied section of the Llobregat river.

WWTP	Effluent Discharge Point	Flow Treated (m^3/day)	h-e treated
Manresa	Cardener (before CB)	25.962	118.993
Pont de Vilomara	Llobregat (before CB)	514	3.598
Castellbell i El Vilar	Llobregat (before CB)	2.537	7.146
Monistrol de Montserrat	Llobregat (between CB and MPT)	1.654	9.759
Abrera	Llobregat (after MPT)	15.597	77.985
Rubí	Rubí (between MPT and SJD)	21.865	171.758
Martorell	Anoia (between MPT and SJD)	6.778	46.768
Sant Feliu de Llobregat	Llobregat (between MTP and SJD)	72.000	320.000

h-e equivalent per habitant

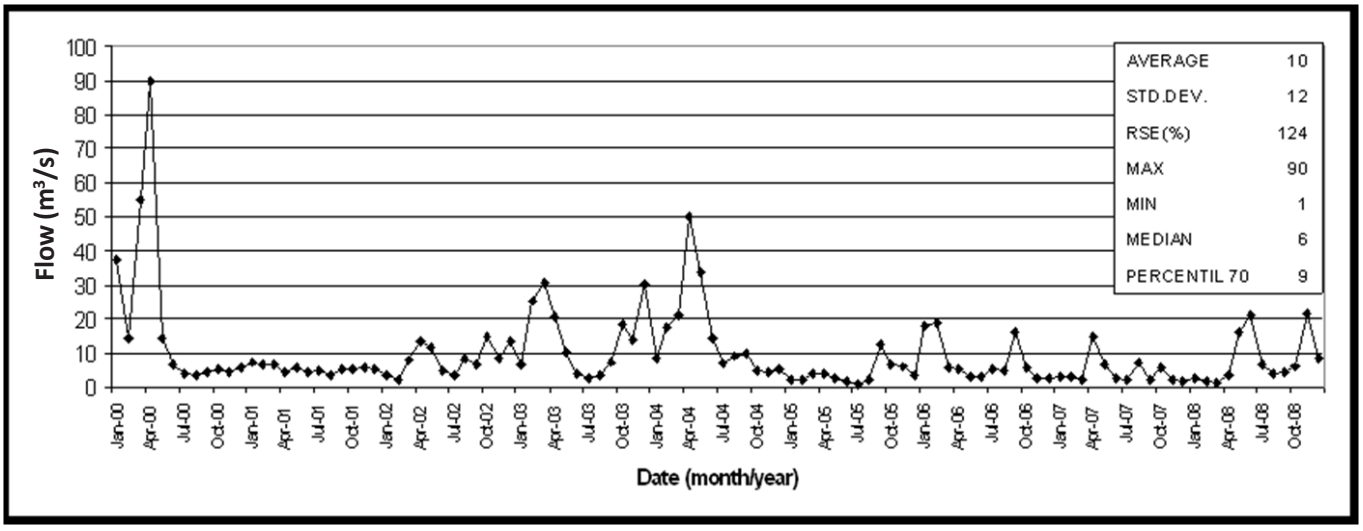
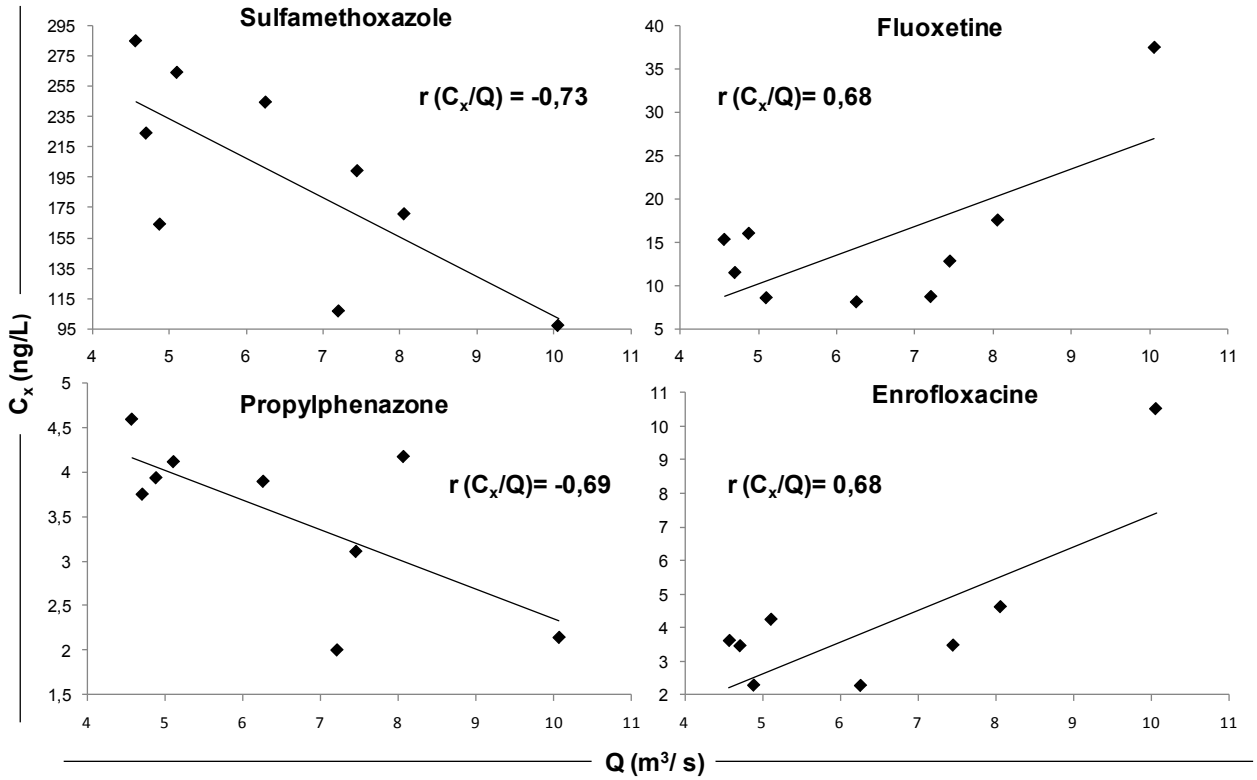
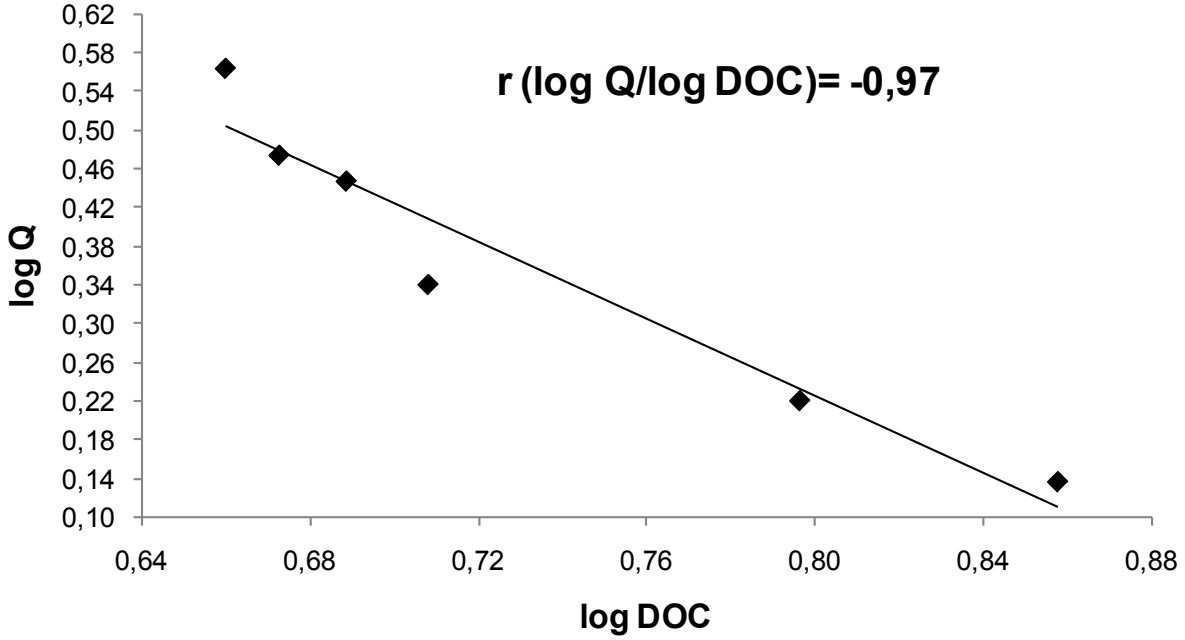


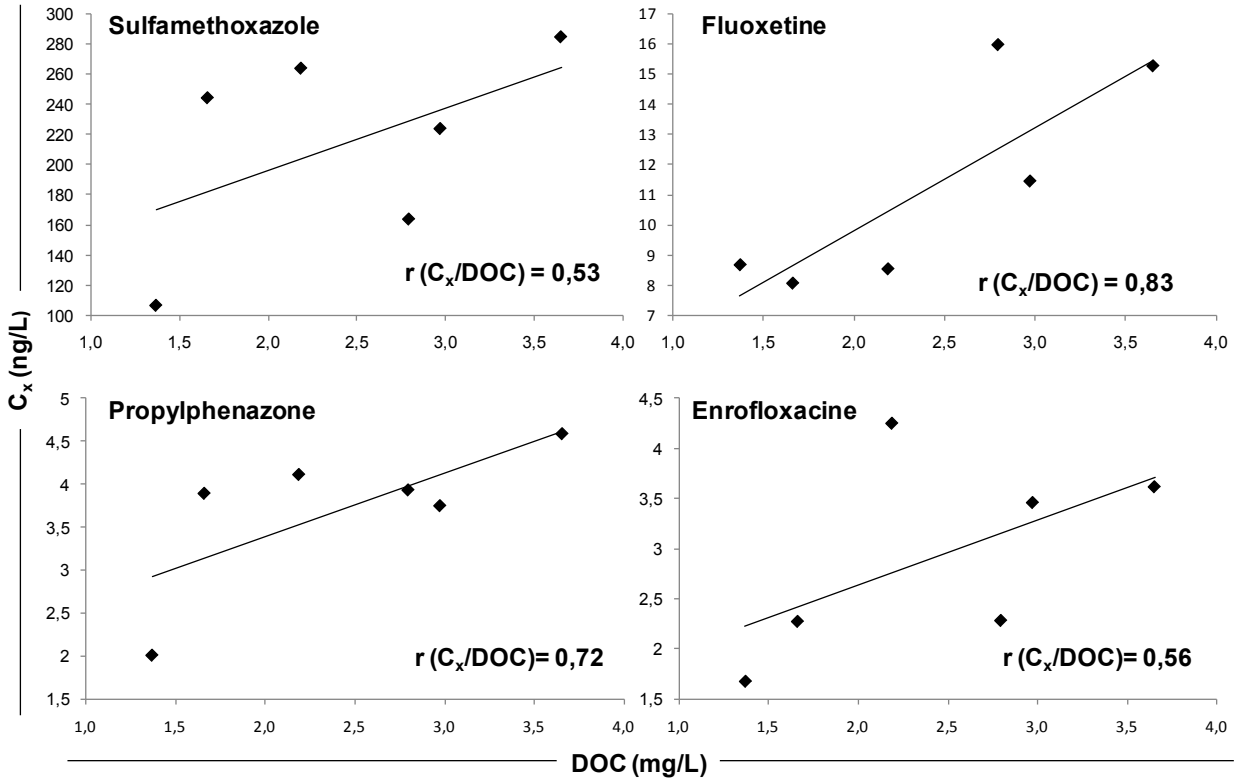
Figure S-1. Historical diagram of the Llobregat River flow recorded at Sant Joan Despí.



FigureS-2. Concentrations (CX) with discharge (Q) relationships of representative pharmaceuticals having the best correlations negative (group a) and positive (group b), sulfamethoxazole and propylphenazone, and fluoxetine enrofloxacine for the sampling point ABR.



FigureS-3. Relationship between log Q and log DOC in ABR and correlation coefficient (on the top). Correlation calculated for six days of the sampling campaign. DOC data corresponding to sampling days 13/10/2009, 23/10/2009 and 26/10/2009 were not recorded.



FigureS-4. Correlation between concentrations of representative pharmaceuticals and both hydrological variables studied: (a) Q (m^3/s) at sampling point ABR for all the sampling campaign and (b) DOC (mg/L) at sampling point ABR for six days of sampling respectively. Relationships are shown for the more representative compounds: sulfamethoxazole and enrofloxacine negatively correlated with flow (a) while positively correlated with DOC (b); and propylphenazone and fluoxetine with a positive response to both hydrological variables.

4.3. Article: "Occurrence and modeling of pharmaceuticals on a sewage-impacted Mediterranean river and their dynamics under different hydrological conditions"

Science of the Total Environment 440 (2012) 3–13

Contents lists available at SciVerse ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Occurrence and modeling of pharmaceuticals on a sewage-impacted Mediterranean river and their dynamics under different hydrological conditions

Victoria Osorio [a], Rafael Marcé [b], Sandra Pérez [a], Antoni Ginebreda [a,*], Jose Luís Cortina [c], Damià Barceló [a,b]

[a] IDAEA-CSIC, Jordi Girona 18-26, Barcelona, Spain
[b] Catalan Institute for Water Research (ICRA), Scientific and Technological Park of the University of Girona, Emili Grahit 101, Girona, Spain
[c] Cetaqua, Water Technology Centre, UPC North Campus, Paseo de los Tilos, 3, Barcelona, Spain

HIGHLIGHTS

► The occurrence of pharmacological compounds in a Mediterranean river at variable hydrological conditions was studied.
► The impact of the flow changes on the concentrations was assessed using relative sensitivity coefficients.
► A plug-flow model was developed to explain the observed variations in the load of the most relevant compounds analyzed.
► The model takes into consideration the circulating flow, the average upstream emissions and an overall decay constant.

ARTICLE INFO

Article history:
Received 11 May 2012
Received in revised form 3 August 2012
Accepted 3 August 2012
Available online 27 September 2012

Keywords:
Pharmaceuticals
Mediterranean River
Occurrence
Hydrological dynamics
Modeling

ABSTRACT

The occurrence of 73 representative pharmacologically active compounds (PhACs) was assessed in a sewage-impacted section of the Llobregat River (NE Catalonia, Spain). This Mediterranean river is characterized by flow rate fluctuations strongly influenced by seasonal rainfall. River flow variations increase the potential environmental risk posed by organic micro-pollutants as their concentrations may increase substantially under low flow conditions. Little is known about the transport behavior of emerging contaminants in surface waters once they are discharged from waste water treatment plants (WWTP) into rivers. This research aimed to study the presence and fate of emerging contaminants under different hydrological conditions by sampling two different sites along the river in different seasons. The highest levels of pharmaceuticals were determined during cold and dry periods. The impact of the flow changes on the concentration of the pharmaceuticals in the river was assessed with the relative sensitive coefficients. Due to expected dilution effects, the response of pharmaceuticals to river flow was negative. Only in a few cases, positive relationships between drug concentrations and flow were detected, suggesting an important role of other hydrological phenomena like sediment re-suspension as well as the source of pollutants. To evaluate the role of other factors influencing PhAC concentrations, a plug-flow model was applied to obtain disappearance constants "k" for a set of selected compounds. Erythromycin presented k values of $-0.15\ h^{-1}$ in both sites being the compound more efficiently removed from the water column. The k values for ibuprofen, furosemide, enrofloxacin, enalapril, acetaminophen, diclofenac and Ketoprofen were between -0.04 and $-0.10\ h^{-1}$ showing less disappearance than erythromycin in the water column. However, other compounds presented k values <0.06, which suggested conservative behavior of these compounds in the water column. This study supports the reliability of the calculated k values for the disappearance of compounds in river waters.

1. Introduction

Pharmacologically active compounds (PhACs) constitute an environmentally relevant group of compounds due to their increasing consumption and their intrinsic biological activity. Around 3000 different compounds belonging to different therapeutic classes are used in human medicine in the European Union (EU), covering a broad range of chemical structures and physico-chemical properties (Richardson and Ternes, 2005). The main route of entry of PhACs into the aquatic environment is through waste water treatment plant (WWTP) effluents because their generally polar nature makes their removal from WWTPs challenging (Conley et al., 2008). Despite physico-chemical and biological treatment, many PhACs are able to reach surface and ground waters. As a consequence, PhACs are now recognized to be widespread pollutants in the aquatic environment (Petrovic et al., 2010). More than 150 PhACs have been identified in surface, ground and even drinking waters (Benotti et al., 2009). Levels of PhACs detected in WWTP effluents are in the range of

* Corresponding author at: IDAEA-CSIC, Department of Environmental Chemistry, Jordi Girona 18-26, Barcelona 08034, Spain.
E-mail address: agmqam@idaea.csic.es (A. Ginebreda).

0048-9697/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scitotenv.2012.08.040

μg/L, whereas they are much lower in river and groundwater, generally in the ng/L range (Gros et al., 2010). Nevertheless, little attention has been paid to the transport behavior of these emerging contaminants in surface waters once they are discharged from WWTP into a river. They are transported by water and may be removed from the dissolved phase through adsorption to suspended particles and may accumulate in sediments. Chemical bounded to sediments can be remobilized by re-suspension (Petrovic et al., 2011). Levels of PhACs can also be reduced by biotic and abiotic (e g. photodegradation) natural degradation processes. However, the efficiency of these processes is highly dependent on seasonally fluctuating environmental factors such as sunlight intensity, water temperature, and stream flow.

The Llobregat River (Catalonia, NE Spain) constitutes a typical example of a Mediterranean behavior (Marcé et al., in press), suffering from low flows during normal conditions (5 m^3/s) and extraordinary peak events (maximum recorded of 2500 m^3/s). In addition, the river receives the effluent discharges of more than 55 WWTPs, and at some places the effluents may represent almost 100% of the total flow, especially during drought periods. This fact can explain the high levels of emerging organic contaminants detected on the river including PhACs, increasing together with the volume of effluent discharged by WWTPs when moving downstream along the river (Ginebreda et al., 2010). Furthermore, according to the predictions of the Intergovernmental Panel on Climate Change (IPCC) (Christensen et al., 2007), such tendency is expected to increase in the medium/long term in the Mediterranean area (Acuña and Tockner, 2010).

As far as contamination is concerned, and as a result of the hydrological situation above described, different physical phenomena may occur at the same time: first, the lack of dilution during water scarcity periods may increase the concentration of pollutants; second, and working in the opposite direction, low flows increase the hydraulic residence time, thus facilitating natural degradation processes (Lam et al., 2004); finally, floods may contribute to remobilization of pollutants from sediments (Petrovic et al., 2011).

In this context, the present study aimed (a) to trace the presence of PhACs in sewage impacted surface waters in the lower course of the Llobregat River as a representative example of a stressed Mediterranean River, and (b) to determine some quantitative relationships between levels of PhACs and flow under different hydrological conditions. To this end, we applied a rough modeling approach based on the plug-flow model as proposed by Pistocchi et al. (2010), in order to have a preliminary quantitative assessment on (a) the load of each pollutant generated by the sewage systems upstream from the point under control, and (b) the overall observed decay of the different compounds in the river channel.

2. Materials and methods

2.1. Basin and site description

The Llobregat River is the second longest river in Catalonia (NE Spain), with a total length of 156 km and a catchment area of 4957 km^2. Its hydrology is characterized by a high variable flow, which is strongly influenced by seasonal rainfall. The mean annual bulk precipitation is 3330 hm^3 and it has an annual average bulk discharge of $693 \cdot 10^6$ m^3. The year-round hydraulic conditions are characterized by several peak flow events that are highly variable, from 50 m^3/s on May 2004, to 1 m^3/s on March 2008 (Figure S-1). The maximum flow recorded in April 2000 (90 m^3/s), followed by a drastic drop down to 10 m^3/s is a clear example of the strength of seasonal rainfall effects on the Llobregat River. Its watershed is densely populated, with more than 3 million inhabitants living therein. Together with its two main tributaries, the River Cardener and the River Anoia, the Llobregat is subjected to a heavy anthropogenic pressure. The Llobregat River is one of the main drinking water sources for Barcelona, with nearly 30% of its discharge being used for drinking water. Furthermore, the middle part of the basin receives natural salt slurries from salt formations and mining activities, which have caused an increase in water salinity downstream. The river receives extensive urban and industrial wastewater discharges ($137 \cdot 10^6$ m^3/year; 92% coming from WWTPs) as well as surface runoff from agricultural areas that cannot be diluted by its natural flow (0.68–6.5 m^3/s basal flow). Forty-eight percent of these point sources are located in the studied area (Fig. 1 and Table S-1). Therefore, this typical Mediterranean River turns into an illustrative example of overexploited river, with high flow variability being caused by a mixture of natural and human-driven components (Marcé et al., in press).

2.2. Sampling

Two sampling sites were selected at the lower reach of the Llobregat River, Abrera (ABR) and Sant Joan Despí (SJD) which were 17 km apart. One sampling point (ABR in Fig. 1) is located in a sparsely populated area in which the Llobregat River receives some urban and industrial wastewater inputs. Another sampling site (SJD) is located in the greater metropolitan area of the city of Barcelona and therefore expected to be more impacted than ABR. In fact, according to the previously existing monitoring data from the Catalan Water Agency (ACA), SJD is the most polluted section of the River. Since previous studies suggested that the levels of PhACs could vary over time depending on the meteorological conditions (Choi et al., 2008; Kolpin et al., 2004), the Llobregat River was sampled during four periods in order to investigate variations in PhAC concentrations under different river flow conditions. With this aim, sampling was performed from October 2009 to July 2010, covering four seasonal periods: Campaign A from 13/10/2009 to 11/11/2009 (Fall), Campaign B from 23/11/2009 to 18/12/2009 (Fall/Winter), Campaign C from 10/03/2010 to 12/04/2010 (Winter/Spring), and Campaign D from 09/06/2010 to 12/07/2010 (Spring/Summer). Campaign A was a sampling period characterized by low flow conditions (mean flows of 6.48 $m^3\ s^{-1}$ in ABR and 5.82 $m^3\ s^{-1}$ in SJD) but with a typical short-lasting flood event (peak flows of 10.06 $m^3\ s^{-1}$ in ABR and 19.98 $m^3\ s^{-1}$ in SJD) in response to the first rainfall event after summer. Campaign B was characterized by steady low flow conditions (mean flows of 5.38 $m^3\ s^{-1}$ in ABR and 4.16 $m^3\ s^{-1}$ in SJD), while campaign C was performed under high steady flow conditions (mean flows of 25.01 $m^3\ s^{-1}$ in ABR and 25.98 $m^3\ s^{-1}$ in SJD). Finally, campaign D started with a severe flood event (peak flows of 215.09 $m^3\ s^{-1}$ in ABR and 111.72 $m^3\ s^{-1}$ in SJD) followed by high flow conditions (mean flow of 49.95 $m^3\ s^{-1}$ in ABR and 34.03 $m^3\ s^{-1}$ in SJD). River water samples were collected twice a week over the four periods (9–13 samples per campaign and monitoring site,) from the thalweg of the river. Composite water samples were collected in 500 mL amber PET bottles that had been pre-rinsed several times with deionized water in the laboratory, and were rinsed with sample water onsite. Bottles were placed in a cooler (at 4 °C) and delivered to the laboratory within 2 h. Samples were immediately pre-treated (filtration) and stored in a refrigerator (−20 °C) until analysis within two days.

2.3. Pharmaceutical standards

We selected 73 PhACs from the major therapeutic groups based on high frequency of usage, physico-chemical properties, and behavior in WWTPs (Gros et al., 2010; Petrovic et al., 2006), (see Table S-2 in Supporting Information). The standards were purchased from Sigma-Aldrich (Steinheim, Germany), Jescuder (Rubí, Spain); LGC Promochem (London, UK) and Cerilliant (Texas, USA). Isotopically labeled compounds were used for internal standard calibration. All standards were of purity grade (>98%). Stock standard solutions were prepared on a weight basis in methanol, with the exception of

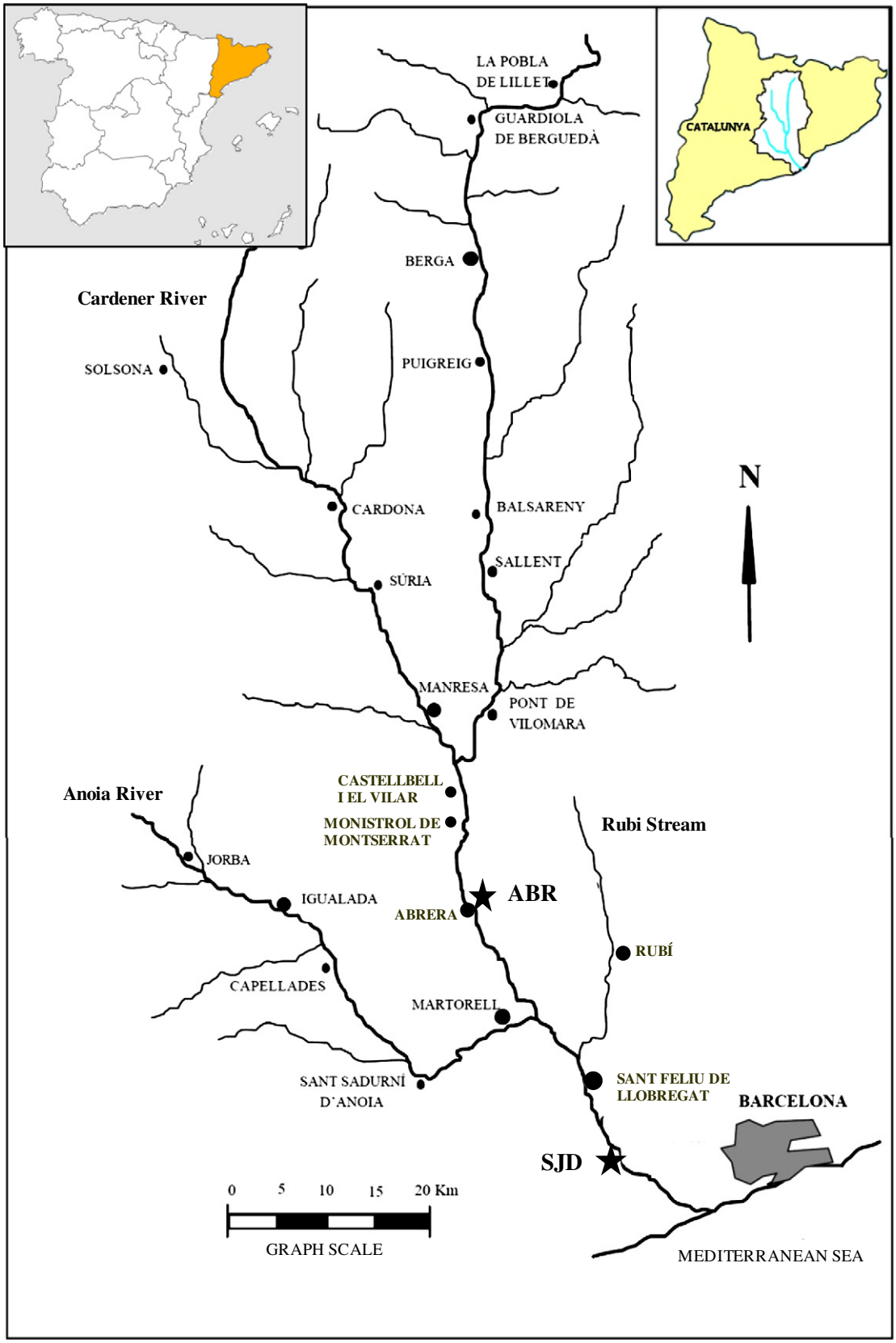


Fig. 1. Llobregat River: map of the basin indicating the sampling sites (stars): Abrera before junction with Anoia River (ABR), and Sant Joan Despí (SJD). Main WWTPs indicated as big full circles along the Llobregat River and its main tributaries, Anoia River, Cardener River and Rubí stream.

fluoroquinolones (water/methanol, (1:1) with 0.2% HCl), and codeine, furosemide, butalbial, pentobarbital, and phenobarbital (acetonitrile). After preparation, standards were stored at −20 °C. Fresh stock solutions of antibiotics were prepared monthly due to their limited stability, while stock solutions for all other substances were prepared freshly every three months. All PhACs were mixed by appropriate dilution of individual stock

solutions in methanol/water (1:3). Working standard solutions were prepared daily (Gros et al., 2009).

2.4. Chemical analysis

Prior filtration, an aqueous solution of 5% Na_2EDTA was added to the samples in order to achieve a final concentration of 0.1%. The 47-mm glass fiber filters Whatman GF/F (0.7-μm pore size) and 0.45 μm nylon membrane filters were used for the pre-treatment of samples (Teknokroma, Barcelona, Spain). The solid-phase extraction (SPE) of the samples of the dissolved phase was carried out with Oasis HLB cartridges (6 mL, 200 mg) from Waters (Milford, MA), using a Baker vacuum system (J.T. Baker, Deventer, The Netherlands).

The concentrations of the 73 PhACs (see Table S-3) were determined using a multi-residue analytical method based on LC-MS/MS after SPE (Gros et al., 2009). Within 48 h, the samples were extracted by SPE. The cartridges were rinsed with 5 mL of HPLC grade water, and dried under vacuum for 15–20 min. After elution with 2×4 mL of methanol, the extracts were evaporated to dryness under a gentle stream of nitrogen and reconstituted with 1 mL of methanol/water (1:3). For internal standard calibration, 20 μL of a 1 mg/L standard mixture of the isotopically labeled compounds was added to the final analytical sample.

Instrumental analysis was performed by LC, using a Symbiosis™ Pico (SP104.002, Spark, Holland), equipped with an auto-sampler and connected in series with a 4000 QTRAP Hybrid Triple Quadrupole – Linear Ion Trap-MS equipped with a Turbo Ion Spray source (Applied Biosystems-Sciex, Foster City, CA, USA). Target compounds were separated on a Purospher Star RP-18 end-capped column (125 mm×2.0 mm, particle size 5 μm) with a C18 guard column (4×2.0 mm), both supplied by Merck (Darmstadt, Germany). Depending on the ionization mode, different mobile phases were used. For the negative ionization mode a mixture of acetonitrile/methanol (1:1, v/v) (eluent A) and HPLC grade water (eluent B) at flow rate 0.2 mL/min was used. The elution gradient started at 20% eluent A, increasing to 80% in 20 min, raising to 90% in 4 min and then, back to initial conditions within 3 min. The column was re-equilibrated for 15 min before the next injection with a total time for chromatographic analysis of 42 min. For analysis in positive ionization mode, acetonitrile (eluent A) and HPLC grade water with 0.1% formic acid (eluent B) were used. The elution gradient started with 5% eluent A, increasing to 95% in 25 min, raising to 100% in 5 min and then, back to initial conditions within 5 min. The column was re-equilibrated for 10 min and chromatographic analysis lasted 45 min. The sample injection volume was 20 μL in all chromatographic methods. Quantification of PhACs was carried out in Selected Reaction Monitoring (SRM) mode monitoring two transitions per analyte (see Table S-2).

2.5. Relationships between PhAC concentration and streamflow

Daily stream flow data measured in the gauge stations located in the two sampling sites were obtained from the public website of the ACA (http://www.gencat.cat/aca/).

Given the dramatic flow changes observed in the Llobregat River during the period covered by the current study, this hydrological parameter can be expected to be one of the most relevant driving factors influencing PhAC concentrations. Therefore, it seems appropriate to investigate their relationship more in detail. For that purpose a representative group of PhACs was selected to study their behavior under river flow fluctuations that occurred during the different monitoring periods. The selection criteria were based on their known consumption, as well as their ubiquity and high concentration levels found in this and other previous studies. In order to have a quantitative insight into the relationship between PhAC levels and the hydrological variability, we calculated both non-parametric Spearman correlations and the relative sensitivity coefficients s of C_i *vs* Q in each sampling site. Sensitivity coefficients may be defined in several ways, however for the purpose of the present study the following approach was used for each sampling site:

$$s_{C_iQ} = \frac{\sigma_{C_i}}{\sigma_Q} \cdot \frac{\mu_Q}{\mu_{C_i}} = \frac{CV_{C_i}}{CV_Q} \quad (1)$$

where C_i refers to the compound concentration, Q refers to the hydrological variable flow, and σ and μ are their standard deviations and averages, respectively.

Relative sensitivity coefficients may be equivalently defined as the ratio of the coefficients of variation (CV). It must be noted that the sensitivity coefficients calculated in this way cannot be interpreted as sensitivity coefficients calculated using a numerical model. In a typical sensitivity exercise using a numerical model all the variability in C would be promoted by variations in Q. But this was not our case, because the variability of both variables may not be fundamentally linked in the observational data.

2.6. Modelling

We modeled the concentration of compound i (C_i) at a given monitoring site, which is assumed to be located downstream to an emission source E_i. In practice, such emission can be associated to the discharge of a single WWTP or more likely the pooled aggregation of several ones. According to the plug-flow model (Chapra, 1997; Pistocchi et al., 2010) C_i is described by:

$$C_i(\xi) = \frac{E_i}{Q} e^{-k_i \tau(\xi)} \quad (2)$$

where ξ refers to the river length between the emission source and the control point; Q is the river flow (assumed uniform throughout the river reach ξ); τ(ξ) is the traveling time (hydraulic residence time) from the emission to the point of measurement, and k_i the first-order decay constant of the chemical which is assumed to embody all the contributing decay processes. Rearranging Eq. (2) and considering E_i a fixed quantity, we can linearly relate the load of a compound at a given monitoring point with k_i:

$$\ln(C_i \cdot Q) = \ln E_i - k_i \cdot \tau(\xi). \quad (3)$$

Considering that in our water quality monitoring points C_i and Q are known quantities, a sound estimate of τ(ξ) will allow us to fit E_i and k by least-squares linear regression using all the data available in each station. The hydraulic residence time can be formulated as:

$$\tau(\xi) = \frac{\xi}{w} \quad (4)$$

where w is the mean water velocity in the reach of length ξ. In our case, ξ is a rather imprecise quantity, since both sampling points (ABR and SJD) receive several (n) WWTP effluents. Consequently, it is not clear which length we should consider in Eq. (4). To overcome this limitation and to account for all point sources upstream each sampling point, we calculated a weighted ξ (ξ^w) considering the distance to every upstream WWTP (ξ_j) and the corresponding annual effluent volume (V_j) as a weighing variable:

$$\xi^w = \frac{\sum_{j=1}^{n} \left(\xi_j \times V_j\right)}{\sum_{j=1}^{n} V_j}. \quad (5)$$

ξ_j was calculated using a digitized river network map in a GIS platform, and ACA supplied V_j values for each WWTP. Finally, water velocity relates with stream flow following:

$$w = f \cdot Q^S \quad (6)$$

where f and S are site specific constants. In our case, representative values for these constants in the watersheds defined by the two sampling points (ABR and SJD) were estimated from information contained in an extensive flood risk study performed by ACA, based on detailed geomorphologic analysis of 1492 river cross sections in the Llobregat River and tributaries.

The use of ξ_j as the characteristic length to calculate $\tau(\xi)$ embodies an important assumption: it is supposed that the emission of a given compound by each WWTP is proportional to its effluent volume. This is roughly the same as suggesting that both consumption per capita and removal efficiency are homogeneous across the basin. Moreover, the use of ξ_j implies that E_i values should not be understood as the actual loads from WWTP's, neither k_i should be interpreted as a site specific decay constant. They must be interpreted as characteristic parameters in an idealized, lumped description of the watershed.

3. Results and discussion

3.1. Occurrence

More than 50 of the 73 target compounds were present in all analyzed samples (Table S-3). Concerning therapeutic groups (Fig. 2), anti-inflammatory drugs were generally the most ubiquitous compounds and the therapeutic group with the highest total concentration along the river section studied and throughout the monitoring campaigns. Levels of anti-inflammatories ranged from 200 to 1100 ng/L in ABR, whereas in SJD the range of concentrations was from 200 to 1800 ng/L. Individual concentrations of the detected compounds were usually within the tens to hundreds of ng/L range. Ibuprofen, acetaminophen and diclofenac were the most concentrated anti-inflammatory drugs, with concentrations in the mid-to-high ng/L range (100–500 ng/L). Despite the high elimination rates in WWTPs reported for ibuprofen, acetaminophen and ketoprofen (Onesios et al., 2009; Jelic et al., 2011), the high concentrations of these compounds in the Llobregat River can be explained by their broad use as analgesics and anti-inflammatories in human medicine as most of them are readily available as over-the-counter drugs. In the case of diclofenac, the low removal rates in WWTPs may be an additional cause (Gros et al., 2009). The frequency of detection and average concentrations of antiinflammatories in the Llobregat river were higher than those observed in other Spanish rivers (Gros et al., 2007; Martin et al., 2011; Fernández et al., 2011) due to the dilution effect in the Spanish rivers with high flow (see Table 1). As for psychiatric drugs, carbamazepine is one of the most prominent drugs with a long history of clinical usage, and it is frequently found in the environment (Petrovic et al., 2010). This drug has proved to be very recalcitrant during wastewater treatment with elimination rates below 25% (Jelic et al., 2011). Levels of carbamezapine (up to 160 ng/L) in the Llobregat River were considerably higher than those reported for other Spanish rivers (Table 1). Lipid regulators are ordinarily applied drugs in clinical practice. Bezafibrate is frequently detected in WWTPs and reported contradictory removals from 36 to 54%, while fenofibrate showed the highest removal (≈100%) (Jelic et al., 2011). In this study, bezafibrate and fenofibrate were detected up to 15,060 and 250 ng/L, respectively. Trends for lipid regulators were the same as those observed for analgesics and antiinflammatories. Hydrochlothiazide presented the highest concentration detected in the Llobregat River (≈1.140 μg/L) and rather higher than the observed in Henares–Jarama–Tajo River system (Table 1). Besides these drugs, β-blockers, which are prescription drugs, were also frequently detected in all the river samples. For instance, atenolol and sotalol were detected at high concentrations with maximums rather higher than those reported by Ginebreda et al. (2010). Since contradictory removal rates during sewage treatment were reported for such compounds (Jelic et al., 2011), occurrence of β-blockers in the Llobregat system cannot be explained in terms of WWTP elimination. Numerous studies confirmed the ubiquity of several antibiotics (i.e. ofloxacin, trimethoprim, sulfamethoxazole and erythromycin) in surface waters, mainly due to their indiscriminate or excessive use and because of low removals in the WWTPs such as <30% for trimethoprim (Jelic et al., 2011). Regarding our study of Llobregat River, trends for antibiotics were similar to those observed for antiinflammatory drugs, with the exception of ofloxacin, which was determined at higher concentrations in the present study.

Comparison of the results of the two sampling points indicated that concentrations of PhACs were significantly higher downstream (SJD) than upstream (ABR). Considering WWTPs as a main pathway for aquatic contamination by PhACs (Gros et al., 2007), it is common to observe higher concentrations downstream of the WWTP effluent outlet compared to the upstream sampling point (Vieno et al., 2005; Gros et al., 2007; Conley et al., 2008). However, some compounds did not follow this general trend. Those analytes which were detected in a high concentration in the surface water samples such as hydrochlorothiazide in campaign A, norfloxacin in campaign B, and gemfibrozil and diclofenac in campaign D, they were present at considerably higher levels in ABR than in SJD. These results suggest that natural attenuation of PhACs occurred in the river by different degradation processes such as photodegradation or biodegradation (Gonçalves et al., 2011).

3.2. Influence of seasonal variation

Comparison of concentration data measured for the different PhAC classes throughout the different sampling campaigns (Fig. 2) revealed a significant degree of variability. This behavior is common to the two sites monitored. Due to WWTP effluent discharge raise, the throughput of pollutants into the aquatic system is increased. Conversely, high flows may contribute to sediment re-suspension and compound re-dissolution. In any case, flow fluctuations are proposed to be one of the most relevant governing factors of variability in the occurrence of PhACs in the river section studied.

The relationship between PhAC concentration and river flow along the sampling period (Fig. 3) for the two sites monitored roughly evidences an opposite trend, which points to an inverse dependence between flow and concentration thus supporting dilution as the main factor. This appraisal is quantitatively supported by their non-parametric correlation using Spearman coefficient (Table S-4). With the exception of erythromycin and others with no significant correlation, the great majority of PhACs selected showed a negative relationship with flow, meaning that their levels decreased when flow increased. Significant values of $r(C_X/Q)$ obtained, ranged from −0.305 (diclofenac) to 0.807 (sulfamethoxazole) in ABR while in SJD correlation coefficients ranged from −0.405 (hydrochlorothiazide) to −0.796 (sulfamethoxazole). These results are in agreement with the expected dilution effects of pollutants as a consequence of increased river flow. In general, better correlation was observed in ABR. This fact can be explained by the higher contribution of WWTP effluent discharge and other anthropogenic pressure to which the river section comprised between ABR and SJD is subjected, and that may play a significant role in the variability of flow dynamics in SJD site and consequent PhAC response. The complex relationship between PhAC concentration and river flow in SJD is reinforced when sensitivity values are also considered (Fig. 4). Selected PhACs were in general more sensitive to flow in the monitoring site SJD, with a mean value of $S(C_X/Q)$ of 1.46 compared to the corresponding one in ABR ($S(C_X/Q) = 0.96$). PhAC concentrations in ABR exhibited $S(C_X/Q)$ values in the range of 0.56 for furosemide to 1.82 for erythromycin

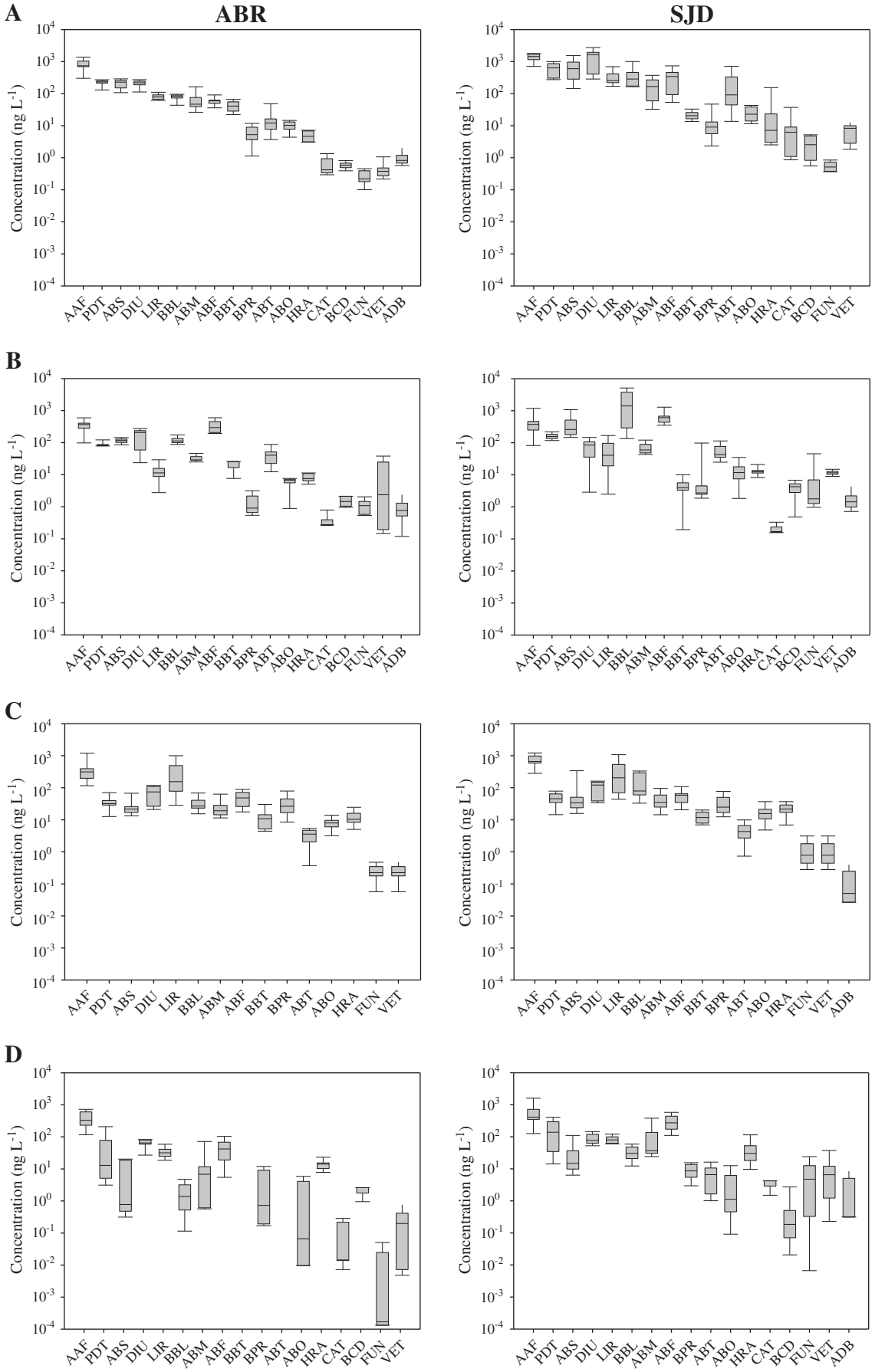

A
ABR
SJD
B
C
D
Concentration (ng L-1)
AAF PDT ABS DIU LIR BBL ABM ABF BBT BPR ABT ABO HRA CAT BCD FUN VET ADB

Table 1
Range of concentrations in ng/L of a selection of the more representative PhACs, determined in surface waters from Spanish river systems, corresponding to previous studies in the Llobregat, Ebro, Henares–Jarama–Tajo; as well as in the present one.

Compound	Llobregat Oct 09–Jul 10 (present study)		Llobregat Jun 05–May 06 (Ginebreda et al., 2010)		Ebro Jun and Nov 05 (Gros et al., 2007)		Henares–Jarama–Tajo Feb 08–Dec 08 (Fernández et al., 2011)	
	Min	Max	Min	Max	Min	Max	Min	Max
Ketoprofen	0.7	224.9	160.0	2710.0	<LOD	144.0	0.3	991.0
Ibuprofen	2.7	868.0	160.0	9890.0	2.0	8.0	6.3	2784.0
Diclofenac	0.4	785.9	80.0	18740.0	<LOD	56.0	0.7	156.0
Acetaminophen	4.4	1059.8	60.0	2420.0	–	–	0.1	43.3
Benzafibrate	0.2	217.1	30.0	15060.0	4.0	37.0	0.3	46.0
Carbamazepine	1.2	266.7	80.0	3090.0	11.0	90.0	0.3	104.0
Atenolol	0.0	251.2	50.0	670.0	160.0	465.0	1.9	334.3
Sotalol	0.1	3552.6	110.0	1820.0	–	–	–	–
Erythromycin	0.0	362.5	10.0	107.0	<LOD	71.0	–	–
Sulfamethoxazole	0.2	1500.0	30.0	11920.0	22.0	169.0	0.1	23.7
Trimethoprim	0.0	35.6	20.0	470.0	10.0	69.0	0.4	23.3
Hydrochlorotiazide	<LOQ	2435.5	–	–	–	–	4.2	960.3
Metoprolol	0.1	3960.0	10.0	180.0	–	–	1.8	26.0
Ofloxacin	<LOD	488.4	160.0	160.0	<LOD	146.0	–	–

while in SJD they varied from $S(C_X/Q) = 0.71$ for ibuprofen to $S(C_X/Q) = 2.76$ for erythromycin. Nevertheless, some PhACs showed the same inconsistencies than those observed in a former study (Osorio et al., 2012), which concluded in the need for taking into account other environmental factors.

Seasonal variations caused by other factors affecting environmental conditions like temperature or UV radiation are supposed to be important sources of variability influencing dynamics and fate of PhACs. Degradation processes (i.e. photodegradation and biodegradation), sorption on sediments, seasonal related specific PhAC human consumption and WWTP operation efficiency (% of PhAC removal) can be some among the possible contributing factors. As far as degradation is concerned, biodegradation and sorption are the two key processes with biodegradation being the dominant one (Ternes et al., 2004; Clara et al., 2005; Urase and Kikuta, 2005). Both mechanisms are temperature dependent. For many compounds, sorption increases with decreasing temperature whereas biodegradation efficiency decreases at lower water temperatures (Hulscher and Cornelissen, 1996). Since efficient nitrogen removal by nitrification process has been associated with high removal of biodegradable pharmaceuticals (Clara et al., 2005), the lack of nitrifying microorganisms during winter, owing to low water temperature (<10 °C) has been proposed as the cause of the poor removal of PhACs in WWTPs observed during winter season (Lacey et al., 2012). Total concentrations of PhACs determined in ABR were up to 2000 ng/L during October/November and slightly higher (2500 ng/L) during November/December-March/April. The minimum levels observed during June/July with 1400 ng/L could be explained by the high temperatures during this season and thus better elimination rates of PhACs in WWTPs, as well as improved natural degradation processes (photodegradation due high UV solar radiation, biodegradation) and decrease of human consumption during this period. This hypothesis is reinforced by the similar behavior observed in SJD sampling point. Higher concentrations were detected in colder periods: 35,000 ng/L and 12,000 ng/L during October/November and November/December respectively; and similar levels up to 3500 ng/L within March/April–June/July. These results were consistent with former research on this field (Martin et al., 2011; Wu et al., 2009) and evaluation studies of performance of WWTPs under seasonal variation (Lacey et al., 2012; Sui et al., 2011). On the other hand, Fernandez et al. (2011) observed an opposite relationship between the occurrence of PhACs and seasonal variation, with drastically higher concentrations in September than in December, which was supposed to be due to pollutant dilution by seasonal rainfall concentrated within the period of October–December. Rainfall can either dilute the concentrations of PhACs in WWTP effluents or concentrate these pollutants when removal efficiency is lowered due to reduced Hydraulic Retention Time (HRT) in WWTPs (Sui et al., 2011).

The joint consideration of all the above influencing factors may be a cumbersome complex task that can be only approached by an appropriate modeling. Feasibility of a given model is usually limited by the available information concerning the required parameterization. In the next section we would like to describe the application and results obtained using a preliminary simple plug-flow model (Pistocchi et al., 2010).

3.3. River plug-flow modeling

Results from fitted models for eight of the 18 tested compounds showed significant negative k values at both sampling sites (Table 2). Apparently, Erythromycin was the tested compound that is more efficiently removed from the water column, with k values around $-0.15\ h^{-1}$ at both sampling sites. Models for Ibuprofen, Furosemide, Enrofloxacin, Enalapril, Acetaminophen, Diclofenac, and Ketoprofen showed k values between -0.04 and $-0.10\ h^{-1}$. k for Spiramycin was also negative in ABR and SJD, but was significant just at the former. Only the model for Sulfamethoxazole was assigned with a positive, significant k value at both sampling sites (Table 2). The model for Sotalol was fitted with a positive k at both sampling sites, but the value was significant only at ABR. Models for the remaining compounds were non-significant for k, suggesting that they were fundamentally conservative in river reaches under the environmental conditions prevailing during this study. In coherence with the occurrence data, Ibuprofen was by a large amount the compound with the largest emission (E, Table 2), followed by Acetaminophen, Erythromycin, Furosemide, and Diclofenac.

First order decay (k) values for the same compound at the different sampling sites were similar (Fig. 5B), which was a remarkable result considering the simplicity of our approach. Only k values for

Fig. 2. Box plot indicating log concentration ranges and average values of the target compounds monitored, classified by therapeutic groups of pharmaceuticals in the two sampling points (ABR and SJD) studied along the four sampling campaigns (A, B, C and D). Each box plot includes a number of measures which corresponds to the sum of individual compound levels, of each therapeutic group, along the Llobregat river section studied, for all the sampling campaign. Analgesics and Antiinflammatories (AAF), Psychiatric Drug Treatment (PDT), Antibiotics Sulfonamides (ABS), Blood Pressure Regulators (BPR), Lipid Regulators (LIR), β-Blockers (BBL), Antibiotics Macrolides (ABM), Antibiotics Fluoroquinoles (ABF), Barbiturates (BBT), Diuretics (DIU), Antibiotics Tetracyclines (ABT), Antibiotics Others (ABO), Histamine H1 and H2 Receptor Antagonists (HRA), Cancer Treatment (CAT), Broncodilators (BCD), Veterinary use (VET), Fungicides (FUN), Antidiabetics (ADB).

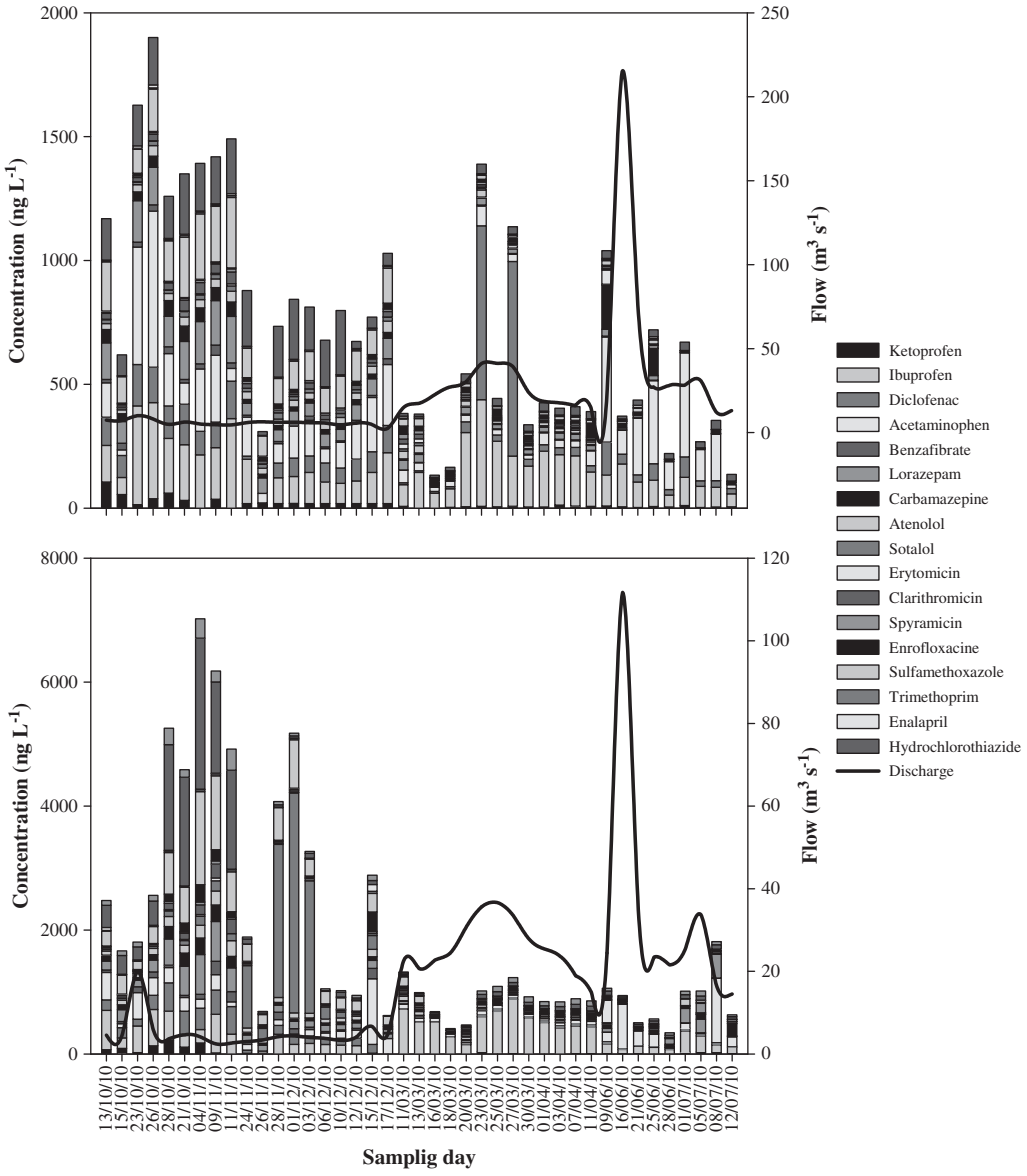


Fig. 3. Concentrations of representative PhACs observed at logarithmic scale in ng/L versus river flow recorded all through the four sampling periods in sampling sites ABR and SJD.

Atenolol, Solatol, Furosemide, and Enrofloxacin were considerably different between sampling sites. On the other hand, models for most compounds showed larger E values in SJD (Fig. 5A), a result anticipated by the occurrence data and the distribution of main WWTPs in the basin. All these results support the validity of our modeling approach and suggest that fitted k and E values can be used as descriptors of aggregate properties of the watershed upstream sampling points.

In general, models with the largest E show more negative k ($r^2 = 0.64$, $p < 0.0001$, $n = 36$) and explain more variability ($r^2 = 0.65$, $p < 0.0001$, $n = 36$). This might suggest that part of the observed dynamics could be altered by lack of precision during model fit of low concentration compounds. However, PhAC mean concentration measured in the river did not correlate with the model parameter values. Therefore, although the influence of precision cannot be ruled out, it did not play a major role on modeling results. Obviously, part of the relationship between E and k relies on the fact that component loads cannot attain negative values and we selected compounds detected during different hydrological conditions for modeling, imposing a lower limit that affects particularly compounds with small E. But it is remarkable that components with large E always showed significant, negative k values with crisp (i.e. high explained variance) relationships between loads and hydraulic residence time (Eq. (3)). However, our study did not strongly support a biogeochemical relationship between E and k because while we found consistent (but weak) differences in E values at both sampling points (Fig. 5A), k values remained basically identical (Fig. 5B). Nevertheless, the potential relationship between E and k suggested in our modeling results deserves further research, because the dependence of k on E would have important environmental implications.

One of the values of this work is that we provided k figures for PhACs in river channels, a kind of information very scarce in the literature. In spite of this, our results agreed with recent findings for Ibuprofen that assigned river k values between -0.07 and $-0.23\ h^{-1}$ (Fono et al., 2006; Lin et al., 2006; Kunkel and Radke, 2011). Our results also compared well with other studies supporting that Bezafibrate is a conservative compound (i.e., $k = 0$) in river channels (Radke et al., 2010; Kunkel

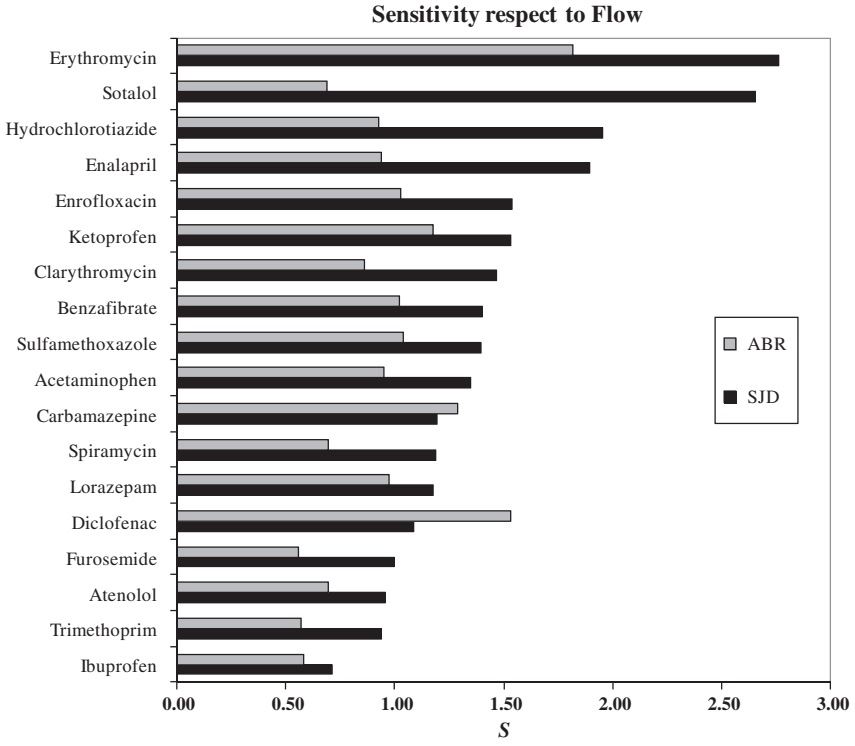


Fig. 4. Sensitivity of compounds'concentration with respect to flow in the two control sites. Sensitivity is defined using Eq. (1) (see text).

and Radke, 2011). Diclofenac has been described both as a conservative compound (Johnson et al., 2007; Kunkel and Radke, 2011) and as a degrading substance ($k \sim -0.15$, Radke et al., 2010), being the latter closer to our modeling results. Finally, Atenolol and Solatol have been described as conservative compounds (Alder et al., 2010), while our modeling results gave near zero to positive k values. Positive k values may be tentatively related to a re-suspension mechanism from the sediments, but this would imply more re-suspension during low-flow periods (i.e. large τ), a difficult claim to maintain. We do not have a definitive explanation for positive k values in part because we cannot guarantee that important assumptions of our model (mainly temporal invariability of consumption per capita and removal efficiency at WWTPs) were not violated to a large extent for these compounds (Atenolol, Solatol, Clarythromycin, and particularly Sulfamethoxazole).

However, it must be acknowledged that information in the literature about the fate of PhACs in river channels is totally inconclusive. For instance, in a recent review Pal et al. (2010) gave k figures for Ibuprofen that are orders of magnitude lower than those reported here and in references above, even after summation of different processes (photodegradation and biodegradation). For Diclofenac only a laconic "rapid" was given. For most compounds not even a single value can be found in the literature. With no doubt, more research is needed in this field, and the simple approach applied in this work may be a convenient option to accumulate data before more detailed empirical studies disentangle the concrete biogeochemical processes at play.

Another problem in the current literature about the fate of PhACs in river channels is that almost all retention metrics are expressed as a first order decay rate (k). In non-engineered systems this may be inconvenient, because k is in fact a volumetric constant that carries the effect of potential varying depths. In absence of conclusive results about the prevalence of the different removal mechanisms (photodegradation, sorption, biodegradation, etc.) in streams, we cannot discard that removal processes are dominated by benthic processes. In this case, the use of k as a descriptor of removal processes is not the best option, because k values are not independent on the particular combination of hydrological and geomorphologic properties of the system under study, turning comparison between different systems problematic. If benthic processes dominate contaminant removal, a much more convenient metric considers decay as a flux across the sediment/water interface, by means of a mass transfer coefficient (vf, m h^{-1}):

$$k = \frac{vf}{h} \tag{7}$$

where h is the water depth in meters. vf is a scale free parameter (Stream Solute Workshop, 1990) that lumps all biogeochemical processes involved in contaminant removal from the water column without the direct effect of the hydrology/geomorphology, contained in h. The generalization of vf as a reference for contaminant removal (or at least the inclusion of h along with k values) would result in a wiser comparison between sites and contrasting hydrological conditions. For the reader's convenience, we reported vf values resolved as in Eq. (3) but solving for vf instead of k (Table 2).

4. Conclusions

Occurrence of pharmaceutical compounds in Mediterranean rivers like the Llobregat is subjected to a pronounced seasonal variation. This fact can be partially explained in terms of the extreme flow changes characteristic of the Mediterranean hydrology. For instance, in the case studied flow peaks during the rainy season exceeded by up to two orders of magnitude with the base river flow directly affecting concentration. However several other concurrent phenomena (eventually operating in opposite directions) like re-suspension, sorption, degradation, the variable hydraulic residence time of the circulating river, or performance of the discharging WWTPs may give rise to a complex pattern, which can be only disentangled

Table 2
Plug-flow model parameters for the two sampling sites considered. Indicated in bold are the compounds showing significant negative $\mathbf{k_i}$ constant. In italics, compounds showing positive, significant $\mathbf{k_i}$ values. $\mathbf{v_f}$ values were added for reference (see text).

Compound	E_i ($g \cdot h^{-1}$)	k_i (h^{-1})	p-Value for k_i ($H_0 = 0$)	Pearson's r	vf ($m \cdot h^{-1}$)
Point #1: ABR					
Ketoprofen	**6.44**	**−0.072**	**<0.001**	**−0.64**	−0.020
Ibuprofen	**251.05**	**−0.106**	**<0.001**	**−0.74**	−0.029
Diclofenac	**6.57**	**−0.029**	**0.040**	**−0.33**	−0.008
Acetaminophen	**19.26**	**−0.054**	**0.002**	**−0.47**	−0.015
Benzafibrate	0.59	−0.002	0.885	−0.02	−0.001
Lorazepam	3.41	−0.007	0.534	−0.10	−0.002
Carbamazepine	1.55	−0.009	0.491	−0.11	−0.002
Atenolol	1.11	0.003	0.873	0.03	0.001
Sotalol	*0.15*	*0.056*	*0.013*	*0.39*	0.016
Erythromycin	**26.04**	**−0.148**	**<0.001**	**−0.76**	−0.040
Clarythromycin	0.29	0.015	0.475	0.11	0.005
Spiramycin	**2.84**	**−0.050**	**<0.001**	**−0.57**	−0.013
Enrofloxacin	**4.98**	**−0.054**	**0.001**	**−0.52**	−0.014
Sulfamethoxazole	*0.82*	*0.039*	*0.002*	*0.48*	0.011
Trimethoprim	0.32	−0.009	0.589	−0.09	−0.002
Enalapril	**1.86**	**−0.071**	**<0.001**	**−0.67**	−0.019
Hydrochlorotiazide	6.22	−0.015	0.313	−0.17	−0.004
Furosemide	**15.50**	**−0.063**	**<0.001**	**−0.63**	−0.017
Point#2: SJD					
Ketoprofen	**1.41**	**−0.040**	**0.016**	**−0.38**	−0.011
Ibuprofen	**103.71**	**−0.096**	**<0.001**	**−0.73**	−0.027
Diclofenac	**18.11**	**−0.071**	**0.003**	**−0.46**	−0.019
Acetaminophen	**25.48**	**−0.062**	**0.001**	**−0.49**	−0.016
Benzafibrate	0.38	−0.016	0.282	−0.17	−0.003
Lorazepam	0.88	0.011	0.594	0.09	0.004
Carbamazepine	1.04	−0.020	0.258	−0.18	−0.005
Atenolol	0.01	0.098	0.065	0.29	0.029
Sotalol	0.21	0.008	0.637	0.08	0.002
Erythromycin	**14.80**	**−0.178**	**<0.001**	**−0.75**	−0.051
Clarythromycin	0.10	0.033	0.309	0.16	0.009
Spiramycin	0.34	−0.027	0.284	−0.17	−0.007
Enrofloxacin	**2.99**	**−0.117**	**0.036**	**−0.33**	−0.032
Sulfamethoxazole	*0.16*	*0.064*	*0.011*	*0.39*	0.020
Trimethoprim	0.19	−0.029	0.428	−0.13	−0.006
Enalapril	**0.60**	**−0.070**	**0.006**	**−0.42**	−0.019
Hydrochlorotiazide	3.82	−0.016	0.238	−0.19	−0.004
Furosemide	**39.35**	**−0.114**	**<0.001**	**−0.85**	−0.032

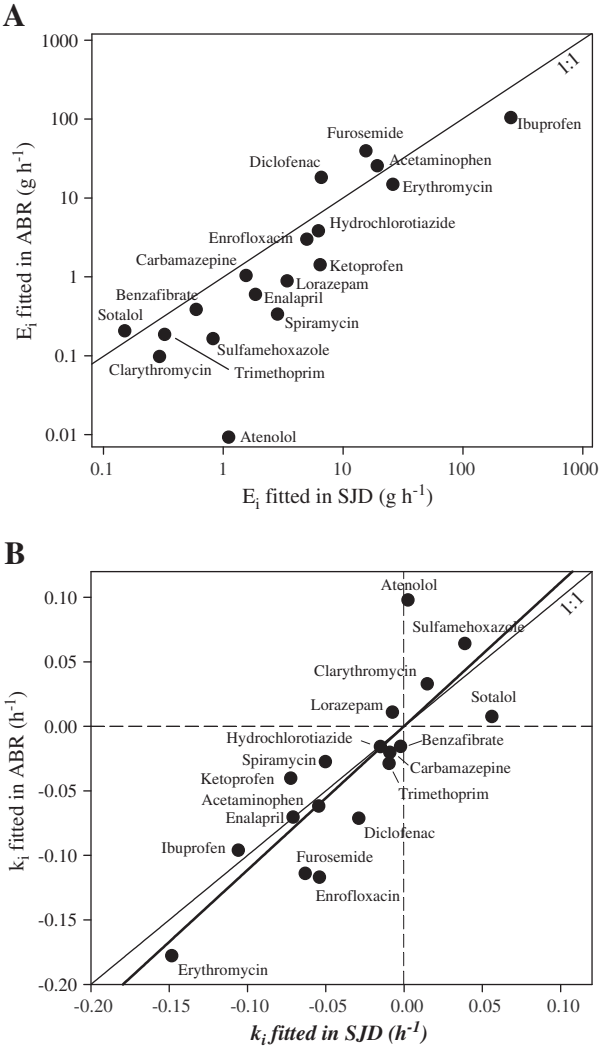


Fig. 5. Relationship between model parameters: (A) E_i and (B) k_i at SJD and ABR sampling stations, for all the modeled compounds. In (A) the 1:1 line is depicted as a thin line, while in (B) the thick line denotes the actual relationship between ABR and SJD values. In (B) the zero value reference was added to both axes as a dashed line.

through an appropriate modeling approach. In that context, we have attempted to develop a preliminary plug-flow model, which has allowed explaining the observed variations in the load of the most relevant compounds analyzed in two river sites in terms of the circulating flow and two compounds' characteristic parameters, i.e. a parameter associated to the average load discharged upstream and another one interpretable as the overall decay constant during the circulating time. The results obtained for the two sites studied show consistency and the proposed method can be thus qualified as potentially useful for management purposes at basin or water-body scale. The concurrent reporting of a volumetric and an areal removal rate is recommended in absence of conclusive results about the prevalence of the different removal mechanisms in river channels.

Acknowledgments

This work has been funded by the Spanish Ministry of Economy and Competitiveness through the projects VIECO (2008 009/RN08/011), SCARCE (Consolider-Ingenio 2010 CSD2009-00065) and CEMAGUA (CGL2007-64551/HID). It reflects only the authors' views and the European Community is not liable for any use that may be made of the information contained therein. Merck is acknowledged for the gift of LC columns. SP acknowledges the contract from the Ramón y Cajal Program of the Spanish Ministry of Economy and Competitiveness.

Prof. Barceló acknowledges King Saud University (Riyadh, Saudi Arabia) for his contract position as a Visiting Professor.

Appendix A. Supplementary material

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2012.08.040.

References

Acuña V, Tockner K. The effects of alterations in temperature and flow regime on organic carbon dynamics in Mediterranean river networks. Glob Change Biol 2010;16:2638–50.

Alder AC, Schaffner C, Majewsky M, Klasmeier J, Fenner K. Fate of beta-blocker human pharmaceuticals in surface water: comparison of measured and simulated concentrations in the Glatt Valley Watershed, Switzerland. Water Res 2010;44(3):936–48.

Benotti MJ, Trenholm RA, Vanderford BJ, Holady JC, Standford BD, Snyder SA. PhACs and endocrine disrupting compounds in U.S. drinking water. Environ Sci Technol 2009;43:597–603.

Chapra S. Surface water-quality modeling. New York: McGraw-Hill Int. Ed; 1997.

Choi K, Kim Y, Jung J, Kim M-H, Kim C-S, Kim N-H. Occurrences and ecological risks of roxithromycin, trimethoprim, and chloramphenicol in the Han River, Korea. Environ Toxicol Chem 2008;27:711–9.

Christensen JH, Hewitson B, Busuioc A, Chen A, Gao X, Held I, et al. Regional climate projections. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL, editors. Climate Change: The physical science basis. Contribution of working group I to the fourth assessment report of the intergovernmental panel on climate change. UK, USA: Cambridge University Press; 2007.

Clara M, Kruzinger N, Strenn B, Gans O, Kroiss H. The solids retention time. A suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. Water Res 2005;39:97-106.

Conley JM, Symes SJ, Schorr MS, Richards SM. Spatial and temporal analysis of pharmaceutical concentrations in the upper Tennessee River basin. Chemosphere 2008;73: 1178–87.

Fernández C, González-Doncel M, Pro J, Carbonell G, Tarazona JV. Occurrence of pharmaceutically active compounds in surface waters of the henares-jarama-tajo system (Madrid, Spain) and potential risk characterization. Sci Total Environ 2011;408: 543–51.

Fono LJ, Kolodziej EP, Sedlak DL. Attenuation of wastewater-derived contaminants in an effluent-dominated river. Environ Sci Technol 2006;40(23):7257–62.

Ginebreda A, Muñoz I, Alda ML, Brix R, López-Doval J, Barceló D. Environmental risk assesment of PhACs in rivers: relationships between hazard indexes and aquatic macroinvertebrate diversity indexes in the Llobregat River (NE Spain). Environ Int 2010;36:153–62.

Gonçalves C, Pérez S, Osorio V, Petrovic M, Alpendurada MF, Barceló D. Photofate of oseltamivir (Tamiflu) and oseltamivir carboxylate under natural and simulated solar irradiation: kinetics, identification of the transformation products, and environmental occurrence. Environ Sci Technol 2011;45(10):4307–14.

Gros M, Petrovic M, Barceló D. Wastewater treatment plants as a pathway for aquatic contamination by pharmaceuticals in the Ebro river basin (Northeast Spain). Environ Chem 2007;26(8):1553–62.

Gros M, Petrovic M, Barceló D. Tracing PhACs residues of different therapeutic classes in Environmental Waters by using Liquid Chromatography/Quadrupole-Linear Ion Trap Mass Spectrometry and Automated Library Searching. Anal Chem 2009;81:898–912.

Gros M, Petrovic M, Ginebreda A, Barceló D. Removal of PhACs during wastewater treatment and environmental risk assessment using hazard indexes. Environ Int 2010;36:15–26.

Hulscher ThEM, Cornelissen G. Effect of temperature on sorption equilibrium and sorption kinetics of organic micropollutants —A review. Chemosphere 1996;32:609–26.

Jelic A, Gros M, Ginebreda A, Cespedes-Sánchez R, Ventura F, Petrovic M, et al. Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment. Water Res 2011;45(3):1165–76.

Johnson AC, Keller V, Williams RJ, Young A. A practical demonstration in modeling diclofenac and propranolol river water concentrations using a GIS hydrology model in a rural UK catchment. Environ Pollut 2007;146(1):155–65.

Kolpin DW, Skopec M, Meyer MT, Furlong ET, Zaugg SD. Urban contribution of pharmaceuticals and other organic wastewater contaminants to streams during differing flow conditions. Sci Total Environ 2004;328:119–30.

Kunkel U, Radke M. Reactive tracer test to evaluate the fate of pharmaceuticals in rivers. Environ Sci Technol 2011;45:6296–302.

Lacey C, Basha S, Morrissey A, Tobin JM. Occurrence of pharmaceutical compounds in wastewater process streams in Dublin, Ireland. Environ Monit Assess 2012;184: 1049–62.

Lam MW, Young CJ, Brain RA, Johnson DJ, Hanson MA, Wilson CJ, et al. Aquatic persistence of eight PhACs in a microcosm study. Environ Toxicol Chem 2004;23:1431–40.

Lin A, Plumlee M, Reinhard M. Natural attenuation of pharmaceuticals and alkylphenol polyethoxylate metabolites during rivertransport: photochemical and biological transformation. Environ Toxicol Chem 2006;25:1458–64.

Marcé R, Honey-Rosés J, Manzano A, Moragas L, Catllar B, Sabater S, et al. The Llobregat River Basin: a paradigm of impaired rivers under climate change threats. In: Sabater S., et al., editors. The Llobregat: the story of a polluted Mediterranean River Hdb Env Chem in press. http://dx.doi.org/10.1007/698_2012_152.

Martin JD, Camacho-Muñoz D, Santos JL, Aparicio I, Alonso E. Monitoring of pharmaceutically active compounds on the Guadalquivir River basin (Spain): occurrence and risk assessment. J Environ Monit 2011;13:2042.

Onesios KM, Yu JT, Bower EJ. Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: a review. Biodegradation 2009;20:441–66.

Osorio V, Pérez S, Ginebreda A, Barceló D. Pharmaceuticals on a sewage impacted section of a Mediterranean River (Llobregat River, NE Spain) and their relationship with hydrological conditions. Environ Sci Pollut Res 2012;19:1013–25.

Pal A, Gin K, Lin A, Reinhard M. Impacts of emerging organic contaminants on freshwater resources: review of recent occurrences, sources, fate and effects. Sci Total Environ 2010;408(24):6062–9.

Petrovic M, Gros M, Barceló D. Multi-residue analysis of PhACs in wastewater by mass spectrometry. J Chromatogr 2006;1124:68–81.

Petrovic M, Postigo C, de Alda ML, Ginebreda A, Gros M, Radjenovic J, et al. Water scarcity in the Mediterranean: Perspectives under Global Change. Handb Environ Chem 2010;8:197–228.

Petrovic M, Ginebreda A, Acuña V, Batalla RJ, Elosegi A, Guasch H, et al. Combined scenarios of chemical and ecological quality under water scarcity in Mediterranean rivers. TrAC 2011;30(8):1268–78.

Pistocchi A, Sarigiannis DA, Vizcaino P. Spatially explicit multimedia fate models for pollutants in Europe: State of the art and perspectives. Sci Total Environ 2010;408:3817–30.

Radke M, Ulrich H, Wurm C, Kunkel U. Dynamics and Attenuation of Acidic Pharmaceuticals along a River Stretch. Environ Sci Technol 2010;44:2968–74.

Richardson SD, Ternes TA. Water analysis: emerging contaminants and current issues. Anal Chem 2005;77:3807–38.

Stream Solute Workshop. Concepts and methods for assessing solute dynamics in stream ecosystems. J North Am Benthol Soc 1990;9:95-119.

Sui Q, Huang J, Deng S, Chen W, Yu G. Seasonal variation in the occurrence and removal of pharmaceuticals and personal care products in different biological wastewater treatment processes. Environ Sci Technol 2011;45:3341–8.

Ternes TA, Herrmann N, Bonerz M, Knacker T, Siegrist H, Joss A. A rapid method to measure the solid–water distribution coefficient (Kd) for pharmaceuticals and musk fragrances in sewage sludge. Water Res 2004;38:4075–84.

Urase T, Kikuta T. Separate estimation of adsorption and degradation pf pharmaceutical substances and estrogens in the activated sludge process. Water Res 2005;39: 1289–300.

Vieno NM, Tuhkanen T, Kronberg L. Seasonal variation in the occurrence of pharmaceuticals in effluents from a sewage treatment plant and in the recipient water. Environ Sci Technol 2005;39:8220–6.

Wu C, Witter JD, Spongberg AL, Czajkowski KP. Occurrence of selected pharmaceuticals in agricultural landscape, western Lake Erie basin. Water Res 2009;43:3407–16.

SUPPORTING INFORMATION

Table S-1. Characteristics of the main WWTP that discharge into the studied section of the Llobregat River.

WWTP	Effluent Discharge Point	Flow Treated (m^3/day)	h-e treated
Manresa	Cardener (before CB)	25.962	118.993
Pont de Vilomara	Llobregat (before CB)	514	3.598
Castellbell i El Vilar	Llobregat (before CB)	2.537	7.146
Monistrol de Montserrat	Llobregat (between CB and MPT)	1.654	9.759
Abrera	Llobregat (after MPT)	15.597	77.985
Rubí	Rubí (between MPT and SJD)	21.865	171.758
Martorell	Anoia (between MPT and SJD)	6.778	46.768
Sant Feliu de Llobregat	Llobregat (between MTP and SJD)	72.000	320.000

h-e equivalent per habitant

Table S-2. Target compounds, identification number (CAS), molecular formula and QqLIT-MS/MS parameters used for quantification (SRM 1) and confirmation (SRM 2 and Rt) of each compound by SRM negative ($[M-H]^-$) and Positive ($[M+H]^+$) ionization.

Therapeutic group	Compounds	CAS number	Molecular formula	Rt (min)	Precursor ion (m/z)	SRM 1	SRM 2
Analgesics and Anti-inflammatories (AAF)	**Ketoprofen** (a)	22071-15-4	$C_{16}H_{14}O_3$	14.9	253 $[M-H]^-$	209	-
	Ibuprofen (a)	15687-27-1	$C_{13}H_{18}O_2$	19.2	205 $[M-H]^-$	161	-
	Indometacine (b)	53-86-1	$C_{19}H_{16}ClNO_4$	20.6	356 $[M-H]^-$	312	214
	Diclofenac (a)	15307-86-5	$C_{14}H_{11}Cl_2NO_2$	19.9	294 $[M-H]^-$	250	-
	Mefenamic acid (b)	61-68-7	$C_{15}H_{15}NO_2$	21.1	240 $[M-H]^-$	196	297
	Acetaminophen (b)	103-90-2	$C_8H_9NO_2$	3.6	150 $[M-H]^-$	107	
	Propiphenazone (c)	479-92-5	$C_{14}H_{18}N_2O$	15.3	231 $[M+H]^+$	56	148
	Phenybutazone (b)	1698-60-8	$C_{10}H_8ClN_3O$	20.7	309 $[M+H]^+$	77	250
	Phenazone (b)	50-33-9	$C_{19}H_{20}N_2O_2$	9.8	189 $[M+H]^+$	56	314
	Codeine (d)	76-57-3	$C_{18}H_{21}NO_3$	7.4	300 $[M+H]^+$	152	130
	Naproxen (a)	22204-53-1	$C_{14}H_{14}O_3$	14.3	229 $[M-H]^-$	185	285
Lipid regulators (LIR)	**Clorifibic acid** (b)	882-09-7	$C_{10}H_{11}ClO_3$	12.9	213 $[M-H]^-$	127	-
	Gemfrobizil	25812-30-0	$C_{15}H_{22}O_3$	24.3	249 $[M-H]^-$	121	180
	Benzafibrate (b)	41859-67-0	$C_{19}H_{20}ClNO_4$	16.7	360 $[M-H]^-$	274	85
	Fenofibrate (b)	49562-28-9	$C_{20}H_{21}ClO_4$	25.2	361 $[M+H]^+$	139	160
	Atorvastatine (c)	134523-00-5	$C_{33}H_{35}FN_2O_5$	19.8	559 $[M+H]^+$	440	154
	Mevastatine (b)	73573-88-3	$C_{23}H_{34}O_5$	21.5	391 $[M+H]^+$	185	169
	Pravastatin	81093-37-0	$C_{23}H_{36}O_7$	14.2	447 $[M+H]^+$	327	576
Psychiatric drugs Treatment (PDT)	**Fluoxetine** (b)	54910-89-3	$C_{17}H_{18}F_3NO$	15.1	310 $[M+H]^+$	44	-
	Paroxetine (c)	61869-08-7	$C_{19}H_{20}FNO_3$	14.4	330 $[M+H]^+$	192	773
	Diazepam (d)	439-14-5	$C_{16}H_{13}ClN_2O$	18.1	285 $[M+H]^+$	193	267
	Lorazepam (d)	846-49-1	$C_{15}H_{10}Cl_2N_2O_2$	15.7	323 $[M+H]^+$	174	-
	Carbamazepine (b)	298-46-4	$C_{15}H_{12}N_2O$	14.7	237 $[M+H]^+$	194	-
Histamine H1 and H2 receptor antagonists (HRA)	**Famotidine** (b)	76824-35-6	$C_8H_{15}N_7O_2S_3$	6.3	338 $[M+H]^+$	189	-
	Ranitidine (b)	66357-35-5	$C_{13}H_{22}N_4O_3S$	6.5	315 $[M+H]^+$	176	159
	Cimetidine (b)	51481-61-9	$C_{10}H_{16}N_6S$	6.3	253 $[M+H]^+$	95	190
	Loratadine (b)	79794-75-5	$C_{22}H_{23}ClN_2O_2$	17.5	383 $[M+H]^+$	337	600
β-Blockers (BBL)	**Atenolol** (b)	29122-68-7	$C_{14}H_{22}N_2O_3$	6.2	267 $[M+H]^+$	145	82
	Sotalol (b)	3930-20-9	$C_{12}H_{20}N_2O_3S$	6.1	273 $[M+H]^+$	213	166
	Metoprolol (b)	37350-58-6	$C_{15}H_{25}NO_3$	10.2	268 $[M+H]^+$	121	244
	Propanolol (b)	525-66-6	$C_{16}H_{21}NO_2$	12.5	260 $[M+H]^+$	116	92
	Timolol (b)	26839-75-8	$C_{13}H_{24}N_4O_3S$	9.8	317 $[M+H]^+$	261	154
	Betaxolol (b)	63659-18-7	$C_{18}H_{29}NO_3$	12.9	308 $[M+H]^+$	116	-
	Carazolol (b)	57775-29-8	$C_{18}H_{22}N_2O_3$	11.8	299 $[M+H]^+$	116	573
	Pindolol (b)	13523-86-9	$C_{14}H_{20}N_2O_2$	8.8	249 $[M+H]^+$	116	201
	Nadolol (b)	42200-33-9	$C_{17}H_{27}NO_4$	8.5	310 $[M+H]^+$	254	-
Cancer Treatment (CAT)	**Tamoxifen** (b)	10540-29-1	$C_{26}H_{29}NO$	19.4	372 $[M+H]^+$	72	-
Fungicides (FUN)	**Metronidazole** (b)	443-48-1	$C_6H_9N_3O_3$	5.8	172 $[M+H]^+$	172	
Antibiotics Macrolids (ABM)	**Erytromicin** (b)	114-07-8	$C_{37}H_{67}NO_{13}$	13.4	734 $[M+H]^+$	158	65
	Azythromicin (b)	83905-01-5	$C_{38}H_{72}N_2O_{12}$	10.9	749 $[M+H]^+$	591	132
	Roxythromycin (b)	80214-83-1	$C_{41}H_{76}N_2O_{15}$	15.1	838 $[M+H]^+$	158	158
	Clarithromicin (b)	81103-11-9	$C_{38}H_{69}NO_{13}$	14.6	748 $[M+H]^+$	591	123
	Tylosin (b)	1401-79-0	$C_{46}H_{77}NO_{17}$	14.1	916 $[M+H]^+$	174	121

	Josamycin (b)	16846-24-15	$C_{42}H_{69}NO_{15}$	15.6	828 $[M+H]^+$	174	189
	Spyramicin (b)	8025-81-8	$C_{43}H_{74}N_2O_{14}$	10.7	843 $[M+H]^+$	174	133
	Tilmicosin (b)	10850-54-0	$C_{46}H_{80}N_2O_{13}$	11.8	869 $[M+H]^+$	696	222
Antibiotics Fluoroquinolones (ABF)	**Ofloxacine** (b)	82419-36-1	$C_{18}H_{20}FN_3O_4$	9.2	362 $[M+H]^+$	261	98
	Ciprofloxacine (b)	85731-33-1	$C_{17}H_{18}FN_3O_3$	9.4	332 $[M+H]^+$	288	201
	Enrofloxacine (b)	93106-60-6	$C_{19}H_{22}FN_3O_3$	9.9	360 $[M+H]^+$	316	147
	Norfloxacin (b)			9.3	320 $[M+H]^+$	302	-
	Enoxacine (b)	74011-58-8	$C_{15}H_{17}FN_4O_3$	8.9	321 $[M+H]^+$	303	261
	Danofloxacin (b)	112398-08-0	$C_{19}H_{20}FN_3O_3$	9.7	358 $[M+H]^+$	340	231
Antibiotics Tetracyclines (ABT)	**Tetracycicline** (b)	60-54-8	$C_{22}H_{24}N_2O_8$	11.8	445 $[M+H]^+$	428	444
	Doxicycline (b)	564-25-0	$C_{22}H_{24}N_2O_8$	9.7	445 $[M+H]^+$	410	124
	Oxytetracycline (b)	79-57-2	$C_{22}H_{24}N_2O_9$	9.2	461 $[M+H]^+$	426	234
	Chlortetracycline (b)	57-62-5	$C_{22}H_{23}ClN_2O_8$	11.4	479 $[M+H]^+$	462	540
Antibiotics Sulfonamides (ABS)	**Sulfamethoxazole** (b)	723-46-6	$C_{10}H_{11}N_3O_3S$	12.5	254 $[M+H]^+$	156	-
	Sulfadiazine (b)	68-35-9	$C_{10}H_{10}N_4O_2S$	7.3	253 $[M+H]^+$	156	259
	Sulfamethazine (b)	57-68-1	$C_{12}H_{14}N_4O_2S$	9.5	279 $[M+H]^+$	186	-
Antibiotics Others (ABO)	**Trimethoprim** (b)	738-70-5	$C_{14}H_{18}N_4O_3$	8.8	291 $[M+H]^+$	230	-
	Chloramphenicol (b)	56-75-7	$C_{11}H_{12}Cl_2N_2O_5$	15.1	323 $[M-H]^-$	152	-
	Nifuroxazide (b)	965-52-6	$C_{12}H_9N_3O_5$	12.8	276 $[M+H]^+$	121	183
Bronchodilators (BCD)	**Salbutamol** (b)	18559-94-9	$C_{13}H_{21}NO_3$	5.7	240 $[M+H]^+$	148	127
Blood pressure Regulators (BPR)	**Enalapril** (b)	75847-73-3	$C_{20}H_{28}N_2O_5$	12.5	377 $[M+H]^+$	234	174
	Lisinopril (b)	83915-83-7	$C_{21}H_{31}N_3O_5$	8.1	406 $[M+H]^+$	84	92
Diuretics (DIU)	**Furosemide** (b)	54-31-9	$C_{12}H_{11}ClN_2O_5S$	13.3	329 $[M-H]^-$	205	85
	Hydrochlorothiazide (b)	58-93-5	$C_7H_8ClN_3O_4S_2$	6.1	296 $[M-H]^-$	78	66
Antidiabetic (ADB)	**Glibenclamide** (b)	10238-21-8	$C_{23}H_{28}ClN_3O_5S$	20.7	494 $[M+H]^+$	369	-
Barbiturics (BBT)	**Phenobarbital** (d)	50-06-6	$C_{12}H_{12}N_2O_3$	14.2	231 $[M-H]^-$	188	-
	Pentobarbital (d)	76-74-4	$C_{11}H_{18}N_2O_3$	18.6	225 $[M-H]^-$	182	154
	Butalbial (d)	77-26-9	$C_{11}H_{16}N_2O_3$	16.6	223 [M-H]-	180	194
Veterinary use (VET)	**Clenbuterol** (b)	37148-27-9	$C_{12}H_{18}Cl_2N_2O$	10.3	277 $[M+H]^+$	203	245
	Flumequine (b)	42835-25-6	$C_{14}H_{12}FNO_3$	15.4	262 $[M+H]^+$	202	-
Internal standards	**Phenobarbital-d5 (IS)** (d)			14.2	236 $[M-H]^-$	193	197
	Diazepam-d5 (IS) (d)			17.6	290 $[M+H]^+$	198	229
	Fluoxetina-d5 (IS) (a)			15.3	315 $[M-H]^-$	153	679
	13C-Fenacetin (IS) (a)			12.7	181 $[M+H]^+$	139	303
	Sulfatiazol-d4 (IS) (e)			8.2	260 $[M+H]^+$	160	115
	Ibuprofen-d3 (IS) (g)			19.1	208 $[M-H]^-$	164	85
	Mecoprop-d3 (IS) (f)			14.8	218 $[M-H]^-$	146	169
	Atenolol-d7 (IS) (g)			6.2	274 [M+H]+	145	255
	Carbamazepina-d10 (g)			14.5	247 [M+H]+	204	-

(a) Sigma-Aldrich (Steinheim, Germany); (b) Jescuder (Rubí, Spain); (c) LGC Promochem (London, UK); (d) Cerilliant (Texas, USA); (e) Toronto Research Chemicals (Canada); (f) Dr. Ehrenstorfer (Augsburg, Germany); (g) CDN isotopes (Quebec, Canada).

Table S-3. Range of concentrations (average, maximum and minimum), expressed in ng/L, of pharmaceuticals monitored at the two sampling sites studied (a) ABR and (b) SJD, standard deviation and frequency expressed in %. (LOQ : limit of quantification; ND: not detected).

(a)

		Campaign A					Campaign B					Campaign C					Campaign D				
		Ave (ng L^{-1})	Max (ng L^{-1})	Min (ng L^{-1})	St dev (ng L^{-1})	Fre (%)	Ave (ng L^{-1})	Max (ng L^{-1})	Min (ng L^{-1})	St dev (ng L^{-1})	Fre (%)	Ave (ng L^{-1})	Max (ng L^{-1})	Min (ng L^{-1})	St dev (ng L^{-1})	Fre (%)	Ave (ng L^{-1})	Max (ng L^{-1})	Min (ng L^{-1})	St dev (ng L^{-1})	Fre (%)
AAF	**Ketoprofen**	38,00	105,29	0,74	33,05	100	18,30	18,95	18,22	0,23	100	5,91	12,11	1,93	2,76	100	5,69	10,55	2,35	2,64	100
	Ibuprofen	248,03	398,90	68,74	112,38	100	111,29	205,44	2,71	55,29	100	192,00	430,57	57,89	101,59	100	98,41	172,66	48,14	38,30	100
	Indometacine	27,37	111,66	10,81	31,85	100	16,30	24,85	4,31	7,59	100	3,64	7,64	1,46	1,80	100	3,14	5,45	1,21	1,40	100
	Diclofenac	128,55	167,16	88,66	29,63	100	63,56	110,04	0,42	33,44	100	132,96	785,93	6,83	272,00	100	49,40	133,06	21,73	38,16	100
	Mefenamic acid	<LOQ	<LOQ	<LOQ	<LOQ	100	3,70	5,49	0,17	1,49	100	0,18	0,98	0,03	0,26	100	1,76	5,96	0,87	1,78	100
	Acetaminophen	237,33	629,76	22,40	201,65	100	131,86	246,27	38,04	66,26	100	35,71	79,53	17,53	18,49	100	216,25	424,45	15,19	147,25	100
	Propiphenazone	3,52	4,59	2,01	0,91	100	0,05	0,11	0,01	0,04	100	1,49	3,53	0,70	0,93	100	0,19	0,38	0,01	0,15	66,67
	Phenybutazone	10,52	13,42	6,83	2,32	100	3,13	4,82	0,44	1,87	100	4,85	11,26	2,43	2,36	100	0,48	1,85	0,17	0,54	100
	Phenazone	3,62	5,81	1,69	1,17	100	nq	nq	nq	nq	nq	1,56	3,57	0,55	0,89	100	<LOQ	<LOQ	<LOQ	<LOQ	100
	Codeine	13,34	20,37	6,37	5,07	100	6,98	13,03	3,45	3,16	100	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq
	Naproxen	110,35	164,98	38,17	39,50	100	nq	nq	nq	nq	nq	26,41	57,74	11,99	13,45	100	17,27	25,25	9,63	5,26	100
LIR	**Clorifibic acid**	1,71	2,02	1,07	0,34	100	1,12	4,74	0,57	1,30	100	0,65	2,00	0,01	0,63	100	0,68	2,32	0,13	0,89	100
	Gemfrobizil	15,33	23,40	9,27	3,88	100	nq	nq	nq	nq	nq	116,71	1114,56	15,84	300,15	100	18,18	33,68	6,81	7,91	100
	Benzafibrate	23,64	41,21	13,65	8,36	100	10,25	23,74	0,28	6,37	100	2,77	5,09	1,28	1,08	100	2,32	3,54	1,76	0,53	100
	Fenofibrate	35,60	61,68	23,96	10,97	100	nq	nq	nq	nq	nq	182,12	611,47	4,30	201,71	100	5,13	11,47	0,35	4,87	100
	Atorvastatine	0,76	1,27	0,33	0,29	100	1,33	2,55	0,73	0,52	100	1,15	2,87	0,30	0,79	100	0,41	1,48	0,03	0,54	100
	Mevastatine	4,00	8,20	1,04	2,12	100	0,53	1,04	0,23	0,23	100	0,30	0,89	0,03	0,26	100	2,94	8,57	0,20	3,20	100
	Pravastatin	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq	6,04	10,38	1,59	3,14	100	3,60	6,14	2,76	1,21	100
PDT	**Fluoxetine**	15,07	37,49	8,05	9,09	100	10,39	17,07	1,42	5,81	100	4,39	12,67	2,19	3,42	100	0,39	1,26	0,01	0,48	100
	Paroxetine	4,22	7,07	1,64	1,68	100	2,15	3,36	1,40	0,58	100	3,43	12,03	1,64	3,26	100	0,36	0,82	0,02	0,28	100
	Diazepam	2,66	3,56	1,56	0,56	100	1,21	1,65	0,20	0,42	100	2,07	5,28	1,02	1,40	100	0,13	0,65	0,00	0,21	100
	Lorazepam	152,09	187,68	87,66	30,83	100	57,24	82,14	46,26	11,47	100	21,52	38,75	6,23	9,01	100	7,48	27,86	0,70	9,77	100
	Carbamazepine	50,79	63,36	31,31	11,21	100	17,48	23,62	14,63	2,82	100	4,26	6,32	1,21	1,60	100	37,43	177,60	1,78	62,60	100
HRA	**Famotidine**	0,87	1,12	0,73	0,13	100	0,58	0,87	0,30	0,20	100	nq	nq	nq	nq	nq	0,30	1,47	0,07	0,46	100
	Ranitidine	1,92	3,35	0,80	1,06	100	3,11	6,27	1,81	1,49	100	12,34	26,86	4,80	6,31	100	2,38	5,71	0,13	1,86	100
	Cimetidine	2,16	3,30	1,18	0,81	100	4,19	8,24	0,57	2,34	100	nq	nq	nq	nq	nq	11,09	18,42	6,39	3,84	100
	Loratadine	<LOQ	<LOQ	<LOQ	<LOQ	100	0,40	0,62	0,22	0,11	100	0,10	0,12	0,08	0,01	100	ND	ND	ND	ND	0
BBL	**Atenolol**	31,97	43,60	15,88	8,82	100	29,57	45,52	24,48	6,09	100	15,56	30,53	4,67	8,85	100	0,06	0,25	0,00	0,10	100
	Sotalol	16,16	21,05	9,14	4,18	100	15,81	24,88	12,73	3,42	100	6,00	11,51	2,47	2,50	100	1,07	3,70	0,08	1,10	100
	Metoprolol	12,60	17,57	9,43	2,85	100	19,69	47,52	3,99	15,87	100	3,49	7,68	1,20	1,76	100	0,67	3,96	0,00	1,38	100
	Propanolol	16,21	25,58	7,64	4,88	100	9,98	21,62	4,97	4,65	100	3,14	8,98	1,68	2,47	100	ND	ND	ND	ND	ND
	Timolol	1,82	2,37	0,95	0,45	100	2,99	6,53	0,90	2,14	100	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq
	Betaxolol	ND	ND	ND	ND	0	6,11	10,64	2,36	2,69	100	2,49	5,30	1,19	1,45	100	ND	ND	ND	ND	ND
	Carazolol	0,19	0,34	0,11	0,07	100	0,41	1,25	0,11	0,38	100	0,71	3,57	0,07	1,23	100	<LOQ	<LOQ	<LOQ	<LOQ	100
	Pindolol	0,12	0,19	0,09	0,03	100	36,18	53,84	21,55	11,91	100	0,85	3,19	0,59	0,71	100	ND	ND	ND	ND	0
	Nadolol	0,34	0,45	0,11	0,10	100	0,52	0,98	0,07	0,28	100	0,76	1,97	0,32	0,51	100	0,03	0,11	0,00	0,04	100
CAT	**Tamoxifen**	0,60	1,34	0,29	0,38	100	0,36	0,83	0,27	0,18	100	nq	nq	nq	nq	nq	0,10	0,28	0,01	0,11	100

<LOQ = Below limit of quantification; ND = Not detected; NQ = Not quantified

Table S-3. Range of concentrations (average, maximum and minimum), expressed in ng/L, of pharmaceuticals monitored at the two sampling sites studied (a) ABR and (b) SJD, standard deviation and frequency expressed in %. (LOQ : limit of quantification; ND: not detected).

FUN	**Metronidazole**	0,27	0,46	0,10	0,12	100	1,14	2,10	0,54	0,52	100	0,25	0,48	0,05	0,14	100	0,01	0,05	0,00	0,02	100
ABM	**Erytromicin**	3,43	8,74	1,10	2,48	100	0,30	0,89	0,04	0,29	100	6,42	19,44	3,33	5,02	100	9,37	57,73	0,47	18,52	100
	Azythromicin	6,97	7,07	6,88	0,06	100	7,72	12,29	4,38	2,79	100	nq	nq	nq	nq	nq					
	Roxythromycin	0,52	0,77	0,31	0,15	100	0,12	0,38	0,02	0,11	100	0,82	3,00	0,21	0,96	100	ND	ND	ND	ND	0
	Clarithromicin	31,07	48,10	9,30	13,48	100	16,23	24,78	8,85	4,99	100	9,12	19,64	3,73	5,09	100	1,50	5,24	0,00	2,11	100
	Tylosin	2,60	3,79	1,32	0,78	100	0,66	1,05	0,34	0,22	100	1,46	6,57	0,78	1,62	100	0,05	0,22	0,00	0,07	100
	Josamycin	0,46	0,71	0,21	0,17	100	0,32	0,49	0,21	0,09	100	0,05	0,34	0,01	0,10	100	0,02	0,05	0,01	0,02	100
	Spyramicin	6,96	12,46	3,96	2,55	100	7,19	9,88	2,71	2,76	100	7,39	20,06	1,74	5,27	100	1,44	8,80	0,04	2,82	100
	Tilmicosin	11,46	95,77	0,87	31,62	100	0,46	0,97	0,33	0,23	100	nq	nq	nq	nq	nq					
ABF	**Ofloxacine**	20,20	31,87	9,37	6,25	100	73,86	159,74	35,86	41,40	100	nq	nq	nq	nq	nq	<LOQ	<LOQ	<LOQ	<LOQ	100
	Ciprofloxacine	25,45	40,24	18,37	6,38	100	44,49	78,96	24,30	21,57	100	nq	nq	nq	nq	nq	3,31	7,43	0,56	2,70	100
	Enrofloxacine	4,02	10,53	1,67	2,63	100	11,00	23,61	0,00	8,22	100	18,83	52,46	5,70	12,77	100	2,27	5,28	0,01	2,12	77,78
	Norfloxacin	nq	nq	nq	nq	nq	170,35	404,80	42,80	127,80	100	32,00	64,77	9,42	17,80	100	33,87	86,05	4,23	25,78	100
	Enoxacine	9,02	12,48	5,32	2,04	100	19,89	30,40	14,14	5,69	100	nq	nq	nq	nq	nq	ND	ND	ND	ND	0
	Danofloxacin	ND	ND	ND	ND	0	19,94	37,68	10,48	8,79	100	nq	nq	nq	nq	nq	5,42	14,21	0,41	4,61	100
ABT	**Tetracycicline**	15,13	48,64	3,68	13,23	100	22,74	42,56	2,26	14,38	100	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq
	Doxicycline	ND	ND	ND	ND	0	6,35	18,16	1,56	6,20	100	nq	nq	nq	nq	nq	<LOQ	<LOQ	<LOQ	<LOQ	100
	Oxytetracycline	nq	nq	nq	nq	nq	11,80	47,84	0,00	13,92	100	nq	nq	nq	nq	nq	ND	ND	ND	ND	0
	Chlortetracycline	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq	3,24	5,63	0,35	1,68	100	<LOQ	<LOQ	<LOQ	<LOQ	100
ABS	**Sulfamethoxazole**	194,63	284,40	96,84	66,15	100	112,80	141,60	81,60	17,29	100	13,49	30,13	4,85	7,60	100	5,93	18,83	0,21	8,16	100
	Sulfadiazine	14,92	62,73	1,54	19,46	100	2,13	3,71	1,34	0,71	100	7,95	30,02	3,54	7,51	100	1,55	6,14	0,06	2,24	100
	Sulfamethazine	nq	nq	nq	nq	nq	1,99	9,55	0,03	2,81	100	5,41	13,48	2,61	3,52	100	0,10	0,46	0,00	0,18	100
ABO	**Trimethoprim**	5,41	7,38	2,73	1,59	100	5,40	7,33	0,00	2,11	100	3,33	4,77	0,54	1,52	100	1,67	5,82	0,01	2,38	100
	Chloramphenicol	<LOQ	<LOQ	<LOQ	<LOQ	100	nq	nq	nq	nq	nq	0,45	1,06	0,36	0,23	100	ND	ND	ND	ND	0
	Nifuroxazide	4,89	10,64	1,66	3,30	100	0,65	1,46	0,39	0,35	100	4,31	10,63	2,25	2,61	100	<LOQ	<LOQ	<LOQ	<LOQ	100
BCD	**Salbutamol**	0,59	0,81	0,39	0,13	100	1,54	2,15	0,96	0,48	100	ND	ND	ND	ND	0	2,22	2,58	0,95	0,59	100
BPR	**Enalapril**	5,95	11,81	1,13	3,37	100	1,37	3,25	0,54	0,94	100	2,50	8,09	1,04	2,20	100	2,73	8,49	0,03	3,40	100
	Lisinopril	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq	30,73	72,24	6,30	21,41	100	1,47	3,38	0,14	1,59	100
DIU	**Furosemide**	30,68	54,14	16,50	12,09	100	23,83	61,31	5,79	16,94	100	45,60	92,93	9,45	28,51	100	38,05	48,71	15,89	10,31	100
	Hydrochlorothiazide	180,37	243,90	84,25	44,51	100	142,98	259,20	15,36	97,10	100	24,86	38,05	8,57	10,45	100	26,09	36,10	10,99	7,50	100
ADB	**Glibenclamide**	0,98	1,98	0,57	0,44	100	0,99	2,45	0,08	0,72	100	ND	ND	ND	ND	0	ND	ND	ND	ND	0
BBT	**Phenobarbital**	10,45	27,28	1,96	8,97	100	18,76	22,92	4,91	7,49	100	12,38	37,84	4,36	8,89	100	ND	ND	ND	ND	0
	Pentobarbital	19,09	44,98	6,73	12,31	100	2,77	3,13	2,71	0,14	100	nq	nq	nq	nq	nq	ND	ND	ND	ND	0
	Butalbial	11,86	21,56	6,30	5,35	100	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq	ND	ND	ND	ND	0
VET	**Clenbuterol**	ND	ND	ND	ND	0	10,04	39,36	0,00	14,60	100	nq	nq	nq	nq	nq	0,24	0,76	0,00	0,25	100
	Flumequine	0,43	1,06	0,22	0,26	100	0,26	0,41	0,03	0,12	100	0,23	2,47	0,03	0,67	100	0,63	1,19	0,07	0,49	100

<LOQ = Below limit of quantification; ND = Not detected; NQ = Not quantified

Table S-3. Range of concentrations (average, maximum and minimum), expressed in ng/L, of pharmaceuticals monitored at the two sampling sites studied (a) ABR and (b) SJD, standard deviation and frequency expressed in %. (LOQ : limit of quantification; ND: not detected).

(b)

		Campaign A					Campaign B					Campaign C					Campaign D				
		Ave (ng L^{-1})	Max (ng L^{-1})	Min (ng L^{-1})	St dev (ng L^{-1})	Fre (%)	Ave (ng L^{-1})	Max (ng L^{-1})	Min (ng L^{-1})	St dev (ng L^{-1})	Fre (%)	Ave (ng L^{-1})	Max (ng L^{-1})	Min (ng L^{-1})	St dev (ng L^{-1})	Fre (%)	Ave (ng L^{-1})	Max (ng L^{-1})	Min (ng L^{-1})	St dev (ng L^{-1})	Fre (%)
AAF	**Ketoprofen**	93,83	224,94	5,22	75,36	100	6,12	7,76	5,88	0,62	88,89	12,26	37,67	3,75	9,67	100	19,95	32,20	8,76	9,09	100
	Ibuprofen	404,60	633,44	169,49	185,83	100	116,94	311,58	4,18	99,66	88,89	507,29	867,95	147,23	184,81	100	141,51	340,69	54,24	95,55	100
	Indometacine	42,08	108,56	15,38	29,46	100	2,75	11,07	1,71	3,12	88,89	5,71	10,66	2,01	3,09	100	8,99	39,19	2,45	11,52	100
	Diclofenac	299,29	460,32	110,59	131,93	100	106,78	162,12	0,87	50,17	88,89	39,05	65,96	12,04	19,78	100	33,69	45,02	20,36	9,46	100
	Mefenamic acid	3,38	9,54	0,93	3,02	100	5,68	14,59	0,65	4,62	88,89	0,45	1,14	0,02	0,35	100	1,09	1,47	0,86	0,19	100
	Acetaminophen	239,40	442,62	66,12	134,66	100	195,68	1059,84	4,45	310,50	100	33,21	59,85	14,42	12,81	100	280,61	1032,49	7,56	349,80	100
	Propiphenazone	15,21	34,96	5,23	11,23	100	0,69	1,87	0,02	0,63	100	1,82	3,92	0,76	0,96	100	<LOQ	<LOQ	<LOQ	<LOQ	100
	Phenazone	40,80	94,00	10,08	30,25	100	4,88	7,47	1,22	2,32	100	24,19	67,71	8,40	18,24	100	65,60	336,40	1,56	115,54	100
	Phenybutazone	17,00	50,48	6,59	14,39	100	nq	nq	nq	nq	nq	2,11	3,89	0,70	0,92	100	2,76	5,42	0,78	1,83	100
	Codeine	48,60	122,72	3,80	43,13	100	15,18	26,01	9,16	5,25	100	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq
	Naproxen	181,06	258,54	96,07	55,14	100	nq	nq	nq	nq	nq	104,44	575,35	20,20	154,13	100	29,18	42,31	15,31	10,27	100
LIR	**Clorifibic acid**	19,48	40,05	6,81	10,78	100	2,24	11,92	0,32	4,09	88,89	4,35	9,38	0,99	2,86	100	1,60	6,34	0,16	1,91	100
	Gemfrobizil	78,44	152,00	12,51	45,91	100	nq	nq	nq	nq	nq	71,53	119,98	26,21	34,72	100	49,17	89,12	18,85	20,89	100
	Benzafibrate	88,49	217,09	33,67	64,53	100	58,64	167,06	0,21	54,24	88,89	6,86	13,08	2,53	3,34	100	3,78	6,05	2,19	1,09	100
	Fenofibrate	137,11	277,60	36,64	85,82	100	nq	nq	nq	nq	nq	250,94	1244,53	3,37	347,49	100	19,25	29,96	6,67	8,70	100
	Atorvastatine	1,97	3,20	1,05	0,71	100	2,84	5,44	1,41	1,10	100	2,38	5,19	0,60	1,45	100	1,84	3,75	1,12	0,84	100
	Mevastatine	5,33	7,52	2,50	1,91	100	0,53	1,36	0,30	0,35	100	0,28	0,95	0,03	0,27	100	3,65	4,73	3,33	0,41	100
	Pravastatin	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq	8,70	13,73	1,15	4,37	100	4,43	6,52	2,54	1,42	100
PDT	**Fluoxetine**	30,92	53,50	13,93	13,27	100	5,48	12,23	1,83	3,14	100	6,69	25,31	2,34	6,53	100	4,12	15,31	0,18	4,95	100
	Paroxetine	26,37	140,34	4,60	42,97	100	2,69	4,26	1,02	1,16	100	3,85	13,40	1,90	3,39	100	2,86	5,39	0,51	1,68	100
	Diazepam	14,89	31,99	4,31	9,85	100	8,00	27,78	1,68	7,65	100	2,87	6,70	1,07	1,69	100	0,59	2,32	0,12	0,68	100
	Lorazepam	354,76	643,08	104,44	207,84	100	102,44	129,17	76,07	16,50	100	26,54	39,82	6,17	10,53	100	106,02	384,29	6,04	138,57	100
	Carbamazepine	159,38	266,69	42,22	84,49	100	41,93	57,08	24,08	8,94	100	7,00	10,10	1,67	2,68	100	56,24	192,76	20,87	59,22	88,89
HRA	**Famotidine**	2,56	6,44	0,20	2,41	100	1,22	2,89	0,28	0,85	100	nq	nq	nq	nq	nq	3,65	12,99	0,02	4,76	100
	Ranitidine	13,58	115,75	0,11	38,33	100	4,30	6,46	0,23	1,89	100	22,12	40,82	5,99	9,34	100	10,66	21,02	0,22	6,31	100
	Cimetidine	7,96	30,46	0,22	11,15	100	6,83	10,83	3,01	2,70	100	nq	nq	nq	nq	nq	13,34	23,60	7,55	5,34	100
	Loratadine	1,33	2,90	0,24	0,92	100	0,63	1,86	0,22	0,51	100	0,11	0,20	0,10	0,04	100	2,34	8,41	0,32	3,05	100
BBL	**Atenolol**	142,02	251,20	64,72	71,84	100	72,34	120,00	44,48	19,95	100	31,78	72,00	7,61	18,39	100	6,38	19,92	0,41	6,62	100
	Sotalol	79,39	164,74	26,62	53,82	100	990,17	3552,64	18,97	1275,26	100	11,81	25,68	3,74	6,14	100	6,27	32,95	0,19	11,03	100
	Metoprolol	97,69	534,96	25,99	164,65	100	885,69	3960,00	19,73	1201,03	100	97,31	280,32	8,36	100,15	100	7,66	37,52	0,13	12,67	100
	Propanolol	34,05	68,54	7,34	22,08	100	9,33	11,42	7,68	1,36	100	3,64	9,55	1,75	2,56	100	3,28	6,40	2,37	1,25	100
	Timolol	6,36	10,16	2,08	2,79	100	2,07	2,80	1,33	0,49	100	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq
	Betaxolol	ND	ND	ND	ND	0	8,05	10,80	4,62	2,32	100	2,56	6,47	1,26	1,80	100	2,30	3,91	1,83	0,77	100
	Carazolol	0,50	0,97	0,17	0,25	100	0,48	1,20	0,21	0,32	100	0,75	3,18	0,07	0,96	100	<LOQ	<LOQ	<LOQ	<LOQ	100
	Pindolol	0,32	0,54	0,16	0,13	100	45,77	165,86	10,51	49,05	100	0,95	2,52	0,59	0,52	100	6,31	6,37	5,82	0,18	100
	Nadolol	0,65	1,10	0,41	0,20	100	0,51	0,70	0,33	0,13	100	0,98	2,61	0,34	0,62	100	0,85	2,77	0,05	0,93	100
CAT	**Tamoxifen**	0,64	1,25	0,24	0,34	100	0,20	0,34	0,16	0,06	100	nq	nq	nq	nq	nq	40,38	115,04	9,69	31,94	100

<LOQ = Below limit of quantification; ND = Not detected; NQ = Not quantified

Table S-3. Range of concentrations (average, maximum and minimum), expressed in ng/L, of pharmaceuticals monitored at the two sampling sites studied (a) ABR and (b) SJD, standard deviation and frequency expressed in %. (LOQ : limit of quantification; ND: not detected).

FUN	**Metronidazole**	2,67	5,19	0,56	1,87	100	7,89	48,80	0,96	14,99	100	1,19	3,98	0,22	1,02	100	0,49	2,73	0,02	0,86	100
ABM	**Erytromicin**	14,10	45,20	0,08	15,05	100	2,05	12,80	0,02	3,83	100	9,28	29,65	3,32	8,79	100	58,09	362,49	12,26	114,36	100
	Azythromicin	6,30	7,21	0,00	2,37	100	16,03	37,46	6,52	11,02	100	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq
	Roxythromycin	3,21	8,11	0,52	2,46	100	0,51	1,15	0,05	0,33	100	1,02	3,11	0,53	0,92	100	1,04	2,34	0,36	0,79	100
	Clarithromicin	102,32	232,13	16,33	84,51	100	26,98	37,05	16,00	6,46	100	16,85	38,27	4,70	9,55	100	1,62	6,23	0,10	2,08	100
	Tylosin	7,63	30,27	2,39	8,72	100	2,68	13,90	0,73	4,03	100	1,97	7,91	0,78	2,12	100	0,71	2,44	0,12	0,97	100
	Josamycin	1,99	3,61	0,74	1,08	100	2,07	5,97	0,42	2,04	100	0,14	0,65	0,01	0,20	100	2,14	11,09	0,01	3,40	100
	Spyramicin	25,60	52,82	6,46	16,31	100	18,39	28,58	10,94	5,16	100	13,66	28,83	2,81	8,25	100	31,65	152,09	2,00	53,94	100
	Tilmicosin	13,35	95,34	1,97	30,75	100	1,00	2,17	0,33	0,59	100	nq	nq	nq	nq	nq	ND	ND	ND	ND	0
ABF	**Ofloxacine**	133,96	296,19	10,97	109,35	100	301,93	488,38	168,90	107,44	100	nq	nq	nq	nq	nq	ND	ND	ND	ND	0
	Ciprofloxacine	76,45	187,88	24,02	55,67	100	103,06	271,04	34,38	82,03	100	nq	nq	nq	nq	nq	11,50	23,78	3,50	6,21	100
	Norfloxacin	nq	nq	nq	nq	nq	46,22	313,21	6,76	94,05	100	27,14	47,09	5,37	12,60	100	12,31	24,36	2,02	8,36	100
	Enoxacine	17,16	31,36	0,73	11,08	100	128,47	235,20	27,36	74,51	100	29,28	64,00	10,40	17,67	100	126,88	400,94	74,83	106,88	100
	Danofloxacin	ND	ND	ND	ND	0	38,46	56,00	23,10	9,15	100	nq	nq	nq	nq	nq	110,76	279,19	6,68	81,91	100
	Enrofloxacine	93,60	279,89	5,72	96,32	100	18,52	51,68	7,09	13,30	100	nq	nq	nq	nq	nq	41,04	129,36	20,48	34,59	100
ABT	**Tetracycicline**	196,89	712,40	13,67	227,11	100	23,49	60,80	9,04	17,35	100	nq	nq	nq	nq	nq	ND	ND	ND	ND	0
	Doxicycline	ND	ND	ND	ND	0	7,93	9,44	6,10	0,79	100	nq	nq	nq	nq	nq	<LOQ	<LOQ	<LOQ	<LOQ	100
	Oxytetracycline	nq	nq	nq	nq	nq	23,59	81,60	1,47	27,77	100	nq	nq	nq	nq	nq	6,61	16,26	1,01	5,21	100
	Chlortetracycline	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq	4,66	11,23	0,58	2,97	100	<LOQ	<LOQ	<LOQ	<LOQ	100
ABS	**Sulfamethoxazole**	615,33	1500,00	134,76	461,41	100	301,76	782,40	139,20	202,63	100	33,87	150,93	5,83	43,39	100	25,17	109,49	3,39	33,33	100
	Sulfadiazine	29,40	75,24	5,23	21,30	100	24,65	106,67	2,78	33,17	100	12,90	41,10	5,48	12,02	100	2,58	9,53	0,18	2,97	100
	Sulfamethazine	nq	nq	nq	nq	nq	54,51	280,90	2,19	88,81	100	30,31	198,26	2,69	59,90	100	1,72	6,78	0,22	2,02	100
ABO	**Trimethoprim**	19,95	35,56	6,39	12,57	100	11,91	33,72	0,00	10,10	100	7,88	20,53	0,70	4,91	100	2,01	6,97	0,09	2,53	100
	Chloramphenicol	<LOQ	<LOQ	<LOQ	<LOQ	100	nq	nq	nq	nq	nq	0,36	0,36	0,36	0,00	100	ND	ND	ND	ND	0
	Nifuroxazide	7,31	12,34	3,24	2,68	88,89	1,75	2,91	0,79	0,73	100	9,13	20,14	2,84	5,47	100	12,50	12,50	12,50	-	100
BCD	**Salbutamol**	8,28	37,20	0,86	11,37	100	4,02	6,92	0,32	1,87	100	ND	ND	ND	ND	ND	3,53	4,23	1,50	0,97	100
BPR	**Enalapril**	12,66	47,09	2,33	13,47	100	13,70	108,00	1,83	33,18	100	4,49	15,26	1,48	4,24	100	6,12	14,58	1,47	5,58	100
	Lisinopril	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq	30,87	71,55	10,08	18,52	100	3,03	8,07	0,62	3,06	100
DIU	**Furosemide**	170,12	339,47	74,79	107,61	100	38,01	86,24	1,60	23,02	88,89	53,63	91,33	13,09	34,13	100	42,50	78,19	22,42	17,63	100
	Hydrochlorothiazide	1136,50	2435,52	209,84	823,86	100	48,47	77,84	0,00	26,51	88,89	47,42	74,42	16,88	21,14	100	49,82	69,30	30,09	16,78	100
ADB	**Glibenclamide**	6,73	12,61	1,85	4,02	100	0,55	2,80	0,10	0,80	100	0,13	0,46	0,03	0,14	100	0,15	0,93	0,00	0,30	100
BBT	**Butalbial**	3,15	4,96	2,13	1,16	88,89	nq	nq	nq	nq	nq	12,54	21,12	6,54	4,79	100	ND	ND	ND	ND	0
	Pentobarbital	12,35	16,09	9,59	2,36	100	1,56	6,45	0,00	2,46	88,89	nq	nq	nq	nq	nq	ND	ND	ND	ND	0
	Phenobarbital	6,19	12,06	2,67	3,22	100	3,48	3,78	1,81	0,67	88,89	nq	nq	nq	nq	nq	ND	ND	ND	ND	0
VET	**Clenbuterol**	ND	ND	ND	ND	0	11,14	14,73	8,63	1,96	100	nq	nq	nq	nq	nq	6,69	24,00	0,00	8,42	100
	Flumequine	0,55	0,85	0,37	0,18	100	1,76	4,48	0,71	1,12	100	0,05	0,20	0,04	0,05	100	9,29	37,54	0,23	11,57	100

<LOQ = Below limit of quantification; ND = Not detected; NQ = Not quantified

Table S-4. Results of spearman correlation test of levels of selected PhACs versus river flow recorded all through the four sampling periods in sampling sites ABR and SJD.

	ABR		SJD	
Compound	r	p	r	p
Ketoprofen	-0.552	<0,001	+	n.s.
Ibuprofen	-	n.s.	+	n.s.
Diclofenac	-0.305	0.049	-0.568	<0,001
Acetaminophen	-	n.s.	-	n.s.
Benzafibrate	-0.696	<0,001	-0.599	<0,001
Lorazepam	-0.772	<0,001	-0.606	<0,001
Carbamazepine	-0.604	<0,001	-0.645	<0,001
Atenolol	-0.713	<0,001	-0.707	<0,001
Sotalol	-0.786	<0,001	-0.732	<0,001
Erytromicin	0.396	0.010	0.348	0.024
Clarithromicin	-0.736	<0,001	-0.656	<0,001
Spyramicin	-0.393	0.010	-0.458	0.002
Enrofloxacine	+	n.s.	-	n.s.
Sulfamethoxazole	-0.807	<0,001	-0.796	<0,001
Trimethoprim	-0.654	<0,001	-0.487	0.001
Enalapril	-	n.s.	-	n.s.
Hydrochlorothiazide	-0.688	<0,001	-0.405	0.009
Furosemide	0.363	0.018	-	n.s.

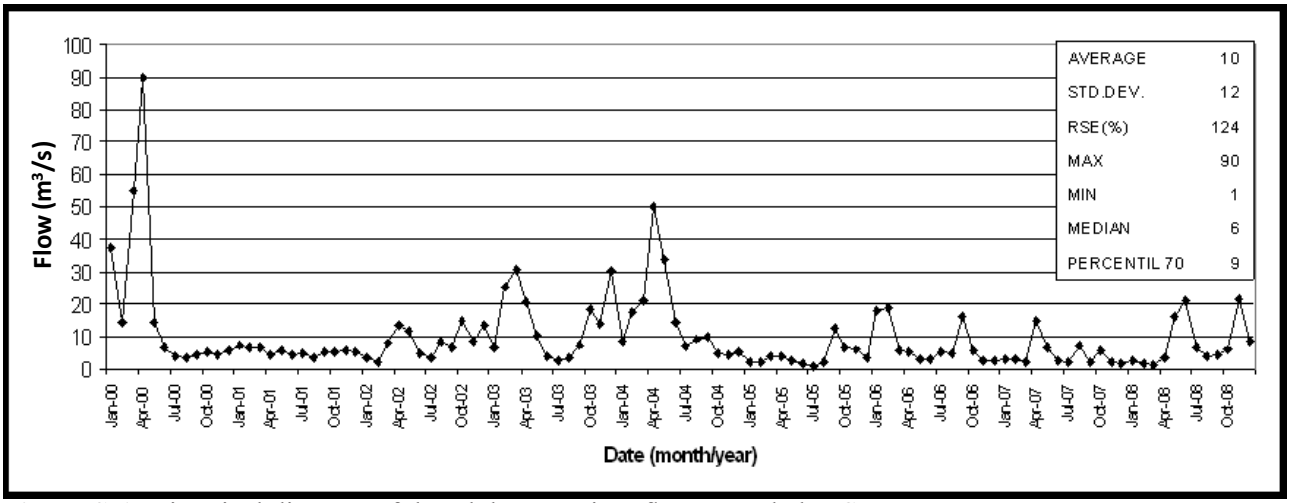


Figure S-1. Historical diagram of the Llobregat River flow recorded at Sant Joan Despí.

4.4. Article: "Concentration and risk of pharmaceuticals in freshwater systems are related to the population density and the livestock units in Iberian Rivers"

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Concentration and risk of pharmaceuticals in freshwater systems are related to the population density and the livestock units in Iberian Rivers

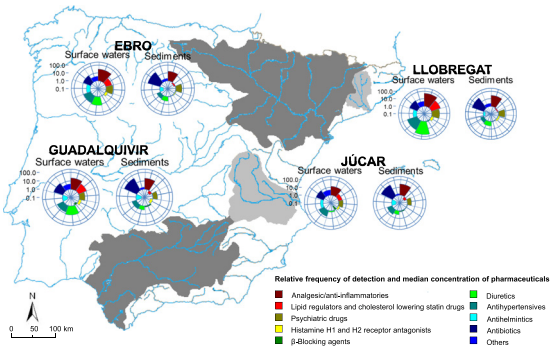
Victoria Osorio [a,1], Aitor Larrañaga [b,1], Jaume Aceña [a], Sandra Pérez [a,*], Damià Barceló [a,c]

[a] Water and Soil Quality Research Group, IDAEA-CSIC, c/ JordiGirona, 18–26, 08034 Barcelona, Spain
[b] Laboratory of Stream Ecology, Dept. of Plant Biology and Ecology, University of the Basque Country, UPV/EHU, PO Box 644, 48080 Bilbao, Spain
[c] Catalan Institute for Water Research (ICRA), Scientific and Technological Park of the University of Girona, Emili Grahit 101, 17003 Girona, Spain

HIGHLIGHTS

- Spatial distribution of pharmaceuticals was assessed across 4 Iberian River basins.
- Ecotoxicological effects of pharmaceuticals to aquatic biota were estimated in SW.
- Hotspots of pharmaceuticals concentration and ecotoxicological risk were identified.
- Concentration and ecotoxicological risk was related to human/animal pressure.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 1 May 2015
Received in revised form 29 June 2015
Accepted 29 June 2015
Available online xxxx

Editor: D. Barcelo

Keywords:
Spatial databases
Mediterranean rivers
Toxic units
Surface waters
Sediments

ABSTRACT

Considerable amounts of pharmaceuticals are used in human and veterinary medicine, which are not efficiently removed during wastewater and slurries treatment and subsequently entering continuously into freshwater systems. The intrinsic biological activity of these non-regulated pollutants turns their presence in the aquatic environment into an ecological matter of concern. We present the first quantitative study relating the presence of pharmaceuticals and their predicted ecotoxicological effects with human population and livestock units. Four representative Iberian River basins (Spain) were studied: Llobregat, Ebro, Júcar and Guadalquivir. The levels of pharmaceuticals were determined in surface water and sediment samples collected from 77 locations along their stream networks. Predicted total toxic units to algae, *Daphnia* and fish were estimated for pharmaceuticals detected in surface waters. The use of chemometrics enabled the study of pharmaceuticals for: their spatial distribution along the rivers in two consecutive years; their potential ecotoxicological risk to aquatic organisms; and the relationships among their occurrence and predicted ecotoxicity with human population and animal farming pressure. The Llobregat and the Ebro River basins were characterized as the most polluted and at highest ecotoxicological risk, followed by Júcar and Guadalquivir. No significant acute risks of pharmaceuticals to aquatic organisms were observed. However potential chronic ecotoxicological effects on algae could be expected at two hot spots of pharmaceuticals pollution identified in the Llobregat and Ebro basins. Analgesics/antiinflammatories, antibiotics and diuretics were the most relevant therapeutic groups across the four river basins. Among them, hydrochlorothiazide and gemfibrozil, as well as azithromycin and ibuprofen were widely spread and concentrated pharmaceuticals in surface waters and sediments, respectively. Regarding their predicted ecotoxicity, sertraline,

* Corresponding author.
E-mail address: spsqam@idaea.csic.es (S. Pérez).
[1] These authors contributed equally to this work.

http://dx.doi.org/10.1016/j.scitotenv.2015.06.143

gemfibrozil and loratidine were identified as the more concerning compounds. Significantly positive relationships were found among levels of pharmaceuticals and toxic units and population density and livestock units in both surface water and sediment matrices.

1. Introduction

Freshwaters receive considerable inputs of non-regulated pollutants like pharmaceutically active compounds (PhACs), which are consumed by human population and used in livestock farming (Kemper, 2008; Awad et al., 2014). Reliable information about PhACs consumption patterns in livestock farming and treatment of humans is scarce but a straightforward approach to indirectly assess them is their determination in PhAC-impacted surface waters. Up to now, the occurrence of more than 200 different PhACs has been reported in lakes, rivers and streams, for instance at concentrations of up to a maximum of 6.5 mg L^{-1} for the antibiotic ciprofloxacin (Petrie et al., 2015; Hughes et al., 2013). Of particular concern are antibiotics, which are used in great quantities in animal farming not only for therapeutic purposes (see Kools et al., 2008), but they are also administered to healthy livestock to promote growth (Van Boeckel et al., 2015). The second important source of PhACs in surface waters is expectedly the human population. The combined effects of improved health standards in developing countries with their rapidly growing populations and of aging populations in industrialized nations are anticipated to lead to an increase in the consumption of PhACs and ultimately their environmental burden. To date most publications on the environmental occurrence of PhACs study their presence in different matrices in conjunction with their spatial and temporal distribution. In many studies the sites with the highest levels of PhACs were located in the vicinity of big cities with high population densities (Fernández et al., 2010).

The intrinsic biological activity of PhACs turns their presence in the aquatic environment into an ecological matter of concern, since, despite intense research over the past 15 years, there are still substantial knowledge gaps in terms of chronic effects on non-target aquatic organisms and the effects on ecosystem functioning and biodiversity loss (Bartelt-Hunt et al., 2011; Hughes et al., 2013). Recently, several studies conducted at laboratory scale showed that some PhACs can act as endocrine disruptors suspected of causing intersex, while the widespread presence of antibiotics has been shown to lead to the selection of antibiotic resistant bacteria in the environment.

The application of chemometrics in environmental studies has facilitated the assessment of a huge volume of data and thus allowing statistically reliable conclusions (Mas et al., 2010). The more recent research on the environmental occurrence of PhACs has been carried out relying on chemometrics (Dai et al., 2015; Jia et al., 2011). The role of livestock and agricultural activities was proposed as a source of antibiotic contamination in the Huangpu River (Jiang et al., 2011). In other studies, however, the use of chemometrics allowed to statistically identify human discharge as the main source of antibiotic sulfonamides and other PhACs to Liaodong Bay and Beiyun River (China) (Jia et al., 2011; Dai et al., 2015). However to the best of our knowledge there are no quantitative studies in the literature relating their presence and their predicted ecotoxicity with human population and livestock. In this context, this study aimed (I) to determine the presence of the contaminants in four main river basins of the Iberian Peninsula, (II) to evaluate their spatial and temporal distribution between water and sediment compartments of the river along the four river basins, (III) to assess the ecotoxicological risk to aquatic organisms related to the PhAC presence in these freshwater systems and to correlate the predicted risk with sources of emission.

2. Materials and methods

2.1. River basins

Four representative Spanish River basins and 77 sampling sites located along their stream networks were studied: Llobregat (15 sites), Ebro (23), Júcar (15) and Guadalquivir (24) river basins (see Fig. S-1 in supporting material). These sampling sites were subjected to very different kind and degree of stresses, with some sites in clean headwater reaches and the others at various positions along the stream network. The Llobregat River (NE, Spain) is 156 km long and drains a 4957 km^2 catchment. This typically Mediterranean river is characterized by a highly variable hydrology, which is strongly influenced by seasonal rainfall. The Ebro River (NC-NE, Spain) is 910 km long and drains an area of 85,534 km^2. Due to its larger size, the river covers contrasting climates thus being characterized by a complex hydrological regime. The Júcar River (E, Spain) is 498 km long and drains a 21,632 km^2 catchment. Its hydrology is typically Mediterranean, with considerable hydrologic variability and rapid alternation of droughts and floods. The Guadalquivir River (S, Spain) is 657 km long and drains a 57,527 km^2 catchment. The entire basin is under a Mediterranean climate, receiving some influence from the Atlantic Ocean in the lowest part. Summer droughts are especially severe as a result of high temperature and lack of rain. These basins are characterized by a high population, agricultural and industrial pressure. As a consequence, water pollution is common all along these Iberian River basins. To test the relationship between the sources of PhACs, i.e. humans and livestock, and the occurrence of PhACs in the water and the sediments, we processed geographic data. Raster layers provided by the Food and Agriculture Organization of the United Nations (FAO, http://www.fao.org) were used to calculate the human population density and the livestock units (LSU) at each of the catchments. For the human population the 2015 estimate of global population map was used with a pixel size of 2.5 arc-minutes. The livestock densities were obtained for 2014 as separate layers for cattle, pigs, sheep, goats and chicken with a pixel size of 0.5 arc-minutes. The density values of those layers were multiplied by the coefficients specified in Eurostat (http://ec.europa.eu/eurostat) for each kind of animal: cattle = 1, pigs = 0.5, sheep and goat = 0.1 and chicken = 0.014. Those multiplied values were summed to obtain a new layer representing the livestock units (LSU), i.e. the cattle-equivalent density of domesticated animals at each pixel. This aggregation is based on the nutritional requirements of the animals, but we used it as an approach to represent the stockbreeding intensity as a source of PhACs in our sites. Both for the population density and for the LSU the average value of the pixels located in the upstream catchment for each of the sampling site was used as descriptor. The subcatchments in the four basins studied (Llobregat, Ebro, Júcar and Guadalquivir) spanned two orders of magnitude in terms of population density (from 1.8 to 208.7 human km^{-2}) and an order of magnitude for LSU (from 10.9 to 147.4 LSU km^{-2}) (Fig. 1). The population density was significantly higher in the catchments of Guadalquivir and Llobregat and lowest in Júcar, with Ebro showing values in between (ANOVA: $F_{3,73}$: 70.37, $p < 0.0001$) (Table S-1). On the other hand, the highest values for LSU were estimated for Llobregat, followed by Ebro and then by Guadalquivir and Júcar. Both variables were uncorrelated to each other (Pearson $r = 0.094$, $p = 0.42$).

2.2. Sampling campaign and sample analysis

Two extensive field campaigns were carried out in autumn 2010 (C1) and 2011 (C2) under different hydrological conditions. The

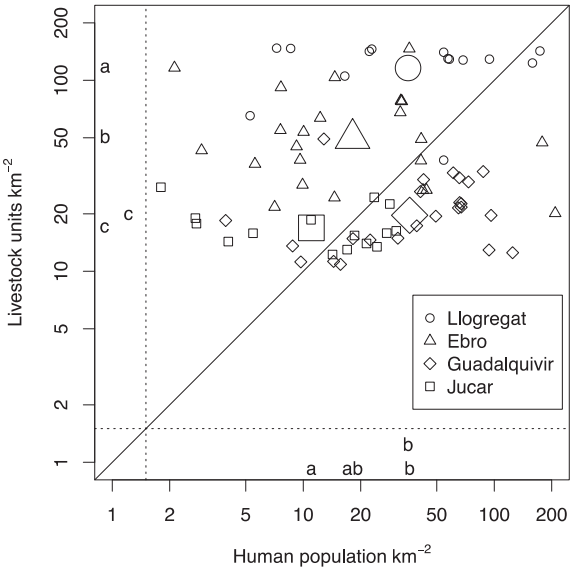


Fig. 1. Average livestock units (Y axis) versus average human population (X axis) calculated for every sub-catchment (km^2) that drained to each location studied: Llobregat (○); Ebro (△), Júcar (□) and Guadalquivir (◇). Geometric averages for population density and livestock unit using all the values at each of the four main basins are also shown. The diagonal line represents the 1:1 relation. Letters placed on the Y axis margin (a, b, c); and the X axis margin (a, b) represent the respective TukeyHSD posthoc differences for livestock units and human population density among the four river basins studied, respectively.

autumn of 2010 was characterized by intense precipitation, which resulted in the high flow of Iberian rivers, while the autumn 2011 was dry and the river flows were low. Grab surface waters (SW) and bed sediments were collected along the four river basins. Amber glass bottles pre-rinsed with ultrapure water were used for sample collection. Bottles were placed in a cooler (at 4 °C) and delivered to the laboratory within 2 days. Samples were immediately pre-treated and stored in a refrigerator (−20 °C) until analysis within one week. Due to logistic issues, SW samples from CAB2 (Júcar) and sediment samples from EBR5 and EBR8 (Ebro); JUC3 and CAB4 (Júcar) were not collected during the first sampling campaign. Over the second sampling campaign, only sediments from EBR8 were not collected. Procedures for analysis of water and sediments samples were previously described elsewhere (Jelic et al., 2009; Gros et al., 2012). Briefly, a) *SW samples* were filtered through 0.7-μm glass fiber filters followed by 0.45-μm nylon membrane filters (Whatman, U.K.). An aqueous solution of 5% Na_2EDTA was added to the SW samples to achieve a final concentration of 0.1% and surrogate standards were spiked at a final concentration of 50 ng L^{-1} in SW. Target compounds were extracted from SW samples by automatic Solid Phase Extraction (SPE) with a GX-271 ASPEC™ system (Gilson, Villiers le Bel, France) using Oasis HLB cartridges (200 mg, 6 mL). SPE cartridges were conditioned with 6 mL of methanol followed by 6 mL of HPLC grade water at a flow rate of 2 mL min^{-1}. 500 mL of SW were loaded onto the cartridge at a flow rate of 1 mL min^{-1}. After sample pre-concentration, cartridges were rinsed with 6 mL of HPLC grade water, at a flow rate of 2 mL min^{-1} and were dried with air for 5 min, to remove excess of water. Finally, analytes were eluted with 6 mL of pure methanol at a flow rate of 1 mL min^{-1}. The final volume of the extract was 1 mL methanol/water (10:90, v/v) and 10 μL of a 1 mg L^{-1} standard mixture of isotopically labeled standards. b) *Sediment samples*. 1 g of lyophilized sediment was spiked in the laboratory with perdeuterated PhACs as surrogate standards at 10 ng L^{-1} (see supporting material) and extracted by pressurized liquid extraction (PLE) using Dionex ASE 350 (Dionex; Sunnyvale, CA). Then, concentrated extracts were diluted in HPLC grade water to a methanol content of <5 vol.% and processed applying the same protocol used for SW samples. Afterwards, a selected list of 76 PhACs (Table S-2) was determined in SW and sediment extracts using a multi-residue analytical method based on ultrahigh performance liquid chromatography coupled to tandem mass spectrometry (UPLC–MS/MS) (Gros et al., 2012) (see supporting material).

2.3. Chemicals and materials

The standards (see Table S-2 in supporting material) were purchased from Sigma-Aldrich (Steinheim, Germany); US Pharmacopeia (USP), European Pharmacopeia (EP), and Toronto Research Chemicals (TRC). Isotopically labeled compounds were used for internal standard calibration and as surrogate standards and were provided by Sigma-Aldrich (Steinheim, Germany), CDN isotopes (Quebec, Canada) and Toronto Research Chemicals (Ontario, Canada). All standards were of purity grade (>90%). Stock standard solutions were prepared on a weight basis in methanol, except ofloxacin and ciprofloxacin, which were dissolved in methanol adding 100 μL of NaOH 1 M, and cefalexin, which was solved in HPLC grade water. After preparation, standards were stored at −20 °C. Fresh stock solutions of antibiotics were prepared every three months while fluoroquinolone antibiotics were prepared monthly due to their limited stability. Stock solutions for the rest of substances were renewed every six months.

2.4. Calculation of toxic units

Toxic units (TU) were estimated on the basis of acute toxicity of PhACs to aquatic organisms. TU values were calculated as the ratio between concentrations and EC_{50} reported and estimated values, for three in vivo bioassays commonly used in environmental toxicology, namely, algae, *Daphnia* and fish (Table S-3). The ecotoxicity of PhACs to these aquatic organisms was assessed in SW of the entire four river basins, thus TU values estimated for each location studied. Acute toxicity values searched on the literature were only available for 55 compounds out of the 76 PhACs analyzed. Consequently, the study of the ecotoxicological effects to aquatic organisms was referred only to these 55 compounds (Table S-3). To assess the ecotoxicological risk of PhACs to aquatic organisms along the four river basins, we summed TU values of each compound at every site, on the basis of the concentration addition model for mixtures of substances (Ginebreda et al., 2014). Since the relative contribution of each PhAC to the ecotoxicity may vary according to its individual toxicity and concentration, to identify the PhACs that were contributing most to the total toxicity of the water at each site we divided the concentration: EC_{50} ratio for each compound by the total TU of the site and gave the result as percentage.

2.5. Statistical methods

ANOVA analyses followed by TukeyHSD pairwise comparisons were performed to compare human population density and LSU across the 4 basins. To avoid using multiple zeroes in the analyses derived from undetected PhACs we used the limits of detection (LOD) and limits of quantification (LOQ) of the analytical procedure (see Table S-4) in the datasets: Undetected compounds and compounds below LOQ were given the corresponding LOD/2 and LOQ/2 value in the datasets. As the distribution of the PhACs was extremely right-skewed and transformations were not able to approach it to normality we opted to use a non-metric Multidimensional Scaling (NMDS) to understand the distribution of the PhACs in the four basins with an ordination analysis. The NMDS was based on rank orders of Euclidean distances of log-transformed values of the PhAC concentrations. Permanova analyses (Euclidean pairwise distances and 10^6 permutations) with basins and sampling campaigns as fixed factors were performed to test for the overall differences of PhACs concentration in SW and sediment samples (Anderson, 2001). The PhACs were then compared among the four basins and the two sampling campaigns by means of ANOVA based on permutation (Anderson, 2001). As multiple univariate analyses were being

performed Bonferroni correction was applied to p-values to control familywise error rate (Dunn, 1961). We wanted to find the sites, basins and campaigns with outlying values above and below the average concentration across all the samples. We assumed that the distribution of the concentration of PhACs to be lognormal (Limpert et al., 2001) (i.e. approximate normal distribution after being log-transformed). Thus, outlying values were extracted from boxplots constructed with log-transformed concentration values. The concentrations above the value of adding 1.5 times the interquartile range to the 75 percentile (i.e. to the 3rd quartile) were considered "outlying high" concentrations. On the contrary, concentrations below 1.5 times the interquartile range below the 1st quartile were considered outlying low values. Thus, the outlying high and low values represent cases that showed outlying values from the distribution of the log-transformed concentrations for each particular PhAC. We counted the number of outlying concentrations observed per basin, site, campaign or PhAC to find the most problematic cases. To test whether the presence of outlying values was consistent across the four basins and the two sampling campaigns we performed a Fisher's exact test (Agresti, 1992). The relationship between mean concentration of PhACs, in the SW and the sediment, and the population density and the LSU were tested by means of linear mixed effect models (LME models) with sampling campaign as a random factor (Pinheiro and Bates, 2000). Mean concentration of PhACs used for human was tested against human population density, whereas mean concentration of PhACs used with livestock was tested against LSU (see Table S2 to see the use of the different PhACs). The relationship between the TU and, the population density and LSU were also tested by means of LME models. To test the effect of the variation of the discharge from the first to the second campaign we computed ratios using discharge and PhAC concentrations. If the discharge was the only relevant factor that varied between the sampling campaigns the discharge C2:C1 ratios and the PhAC concentration C2:C1 ratios would show the opposite trend. We tested this by a Permanova model with C2:C1 ratios of the PhAC concentrations as dependent variable and C2:C1 ratios for the discharge as independent. Both ratios were log-transformed for the analyses and Euclidean distance was used as dissimilarity index. All statistical analyses were performed in R using the package Vegan for NMDS analysis, LME4 for LME models (R Core Team, 2014).

3. Results

3.1. Occurrence of PhACs in water and sediments in the four river basins

The concentration of PhACs in SW varied from the low to high ng L^{-1} range (Table S-5). Llobregat and Ebro rivers were the most polluted in PhACs during C1, with corresponding total levels for the entire basin of 13,022 and 12,028 ng L^{-1}. These concentrations were substantially lower in Guadalquivir and Júcar: of 1702 and 759 ng L^{-1}, respectively. Diversely, Ebro presented the highest levels of drugs all along C2, followed by Llobregat, Guadalquivir and Júcar, with respective total concentrations of 7202, 4948, 4676, and 1638 ng L^{-1}. Among the three most concentrated therapeutics groups, per catchment and campaign, analgesics/antiinflamatories presented the highest average levels in all SW samples (see Table S-5 and also median concentrations averaged for both campaigns in Fig. 2). These levels were the highest in the Llobregat river, 193.88 and 109.21 ng L^{-1} for C1 and C2, respectively; followed by the Ebro, 147.52 and 90.04 ng L^{-1} for C1 and C2, respectively; the Guadalquivir, with respective 18.77 and 63.19 ng L^{-1} for C1 and C2; and the Júcar, with 9.36 and 27.99 ng L^{-1} in C1 and C2, respectively. Other concentrated therapeutic groups along the four river basins were: diuretics, in Llobregat (238.60 and 88.44 ng L^{-1} in C1 and C2, respectively) and Ebro (85.18 and 74.29 ng L^{-1} in C1 and C2, respectively) and Guadalquivir (29.67 ng L^{-1} in C2); antihypertensives in Ebro (94.73 and 43.65 ng L^{-1} in C1 and C2, respectively) and Llobregat (187.14 ng L^{-1} in C1) and Júcar (10.98 ng L^{-1} in C1); lipid

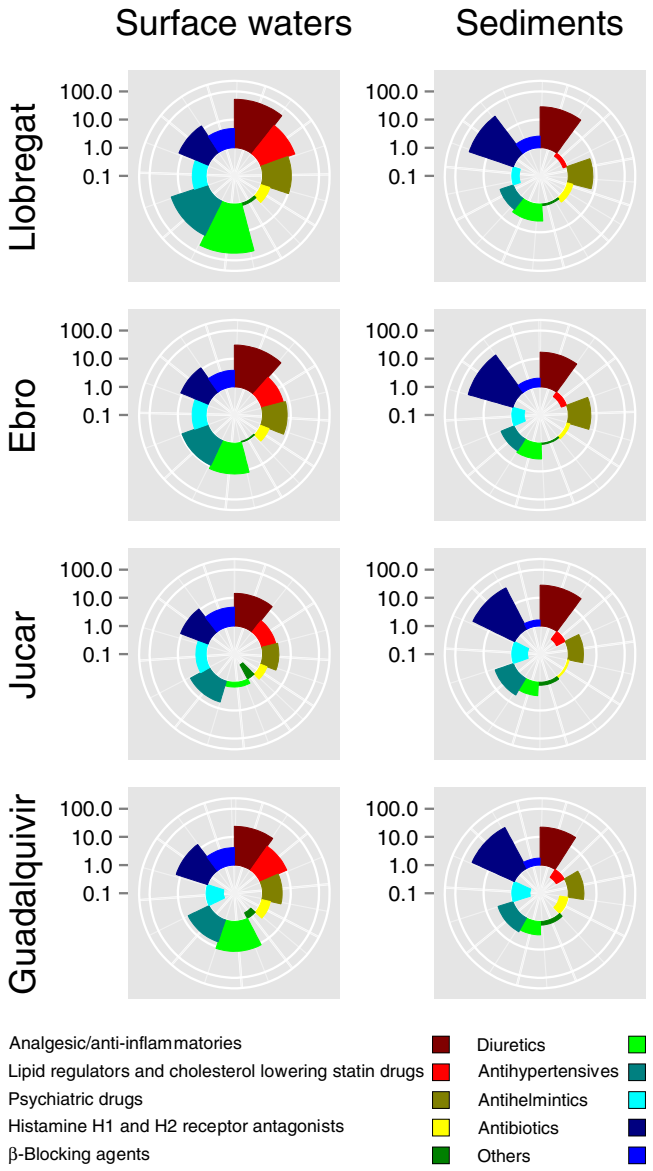


Fig. 2. Relative frequency of detection and median concentration of pharmaceuticals, classified by therapeutic families, in surface waters (left) and sediments (right) of the four river basins assessed: the Llobregat (on top), followed by the Ebro, the Júcar and the Guadalquivir (at the bottom). The circumference of each fan is scaled by the relative proportion of detections. Each point outward on the radial axis represents sequentially 0.1, 1, 10 and 100 ng L^{-1} of the median concentration of pharmaceuticals detected.

regulators/cholesterol lowering drugs in Guadalquivir (11.91 and 41.62 ng L^{-1} in C1 and C2, respectively) and Llobregat (70.76 ng L^{-1} in C2); antibiotics in Júcar (11.28 and 24.99 ng L^{-1} in C1 and C2, respectively) and Guadalquivir (10.98 ng L^{-1} C1); and antihelmintics in Júcar (20.00 ng L^{-1} in C2). The individual compounds averaging highest levels per river basin (and campaign) were: iopromide in Llobregat (373.95 ng L^{-1} in C1), gemfibrozil in Llobregat (70.27 ng L^{-1} in C2) and Guadalquivir (11.46 and 40.98 ng L^{-1} in respective C1 and C2), hydrochlorothiazide in Ebro (72.22 and 61.33 in respective C1 and C2) and thiabendazole (6.31 ng L^{-1} in C1) and metronidazole in Júcar (22.40 ng L^{-1} in C2) (Table S-5). Other PhACs detected at high concentrations were: valsartan, furosemide, ibuprofen, ketoprofen, irbesartan, tetracycline, losartan, naproxen and indomethacine (Table S-5). Regarding their frequency of detection (average of both C1 and C2 is also shown in Fig. 2), about the 60% of the PhACs studied was present in at least half of the SW samples analyzed in both sampling campaigns. The 22% of these compounds were detected in all cases. PhACs were

more frequently detected during C1 than over C2 in SW matrices. During C1 and C2 8 and 31 compounds respectively, were detected in less than 50% of SW samples. The major ubiquity of PhACs in SW was observed in the Llobregat river basin. Ebro, Júcar and Guadalquivir followed the frequency of detection rate for SW. Regarding individual compounds thiabendazole, hydrochlorothiazide and glibenclamide were present in all SW samples.

PhACs were found in sediments at the low ng g^{-1} level (Table S-6). Sediments from Guadalquivir and Ebro were the most concentrated, with respective total values for the entire basin of 1875 and 1596 ng g^{-1} in C1, and 1871 and 1601 ng g^{-1} in C2. Differently, levels determined in Llobregat and Júcar were lower and varied from respective values of 1323 and 1005 ng g^{-1} in C1 to 1051 and 1218 ng g^{-1} in C2. Among the three most concentrated therapeutics groups, per catchment and campaign, antibiotics and analgesics/antiinflamatories averaged the higher concentrations in all sediment samples (see Table S-6 and also median concentrations averaged for both campaigns in Fig. 2). These levels were the highest in Llobregat river, 22.97 and 15.08 ng g^{-1} for C1 and C2, respectively, followed by Ebro, 18.81 and 11.96 ng g^{-1} for C1 and C2, respectively, Júcar, with 21.74 and 33.31 ng g^{-1} in respective C1 and C2; and Guadalquivir, with respective 16.74 and 19.40 ng g^{-1} for C1 and C2. Other concentrated therapeutic groups were: psychiatric drugs in Llobregat (18.02 and 5.26 ng g^{-1} in respective C1 and C2) and Ebro (7.26 and 5.07 ng g^{-1} in respective C1 and C2) and Júcar (4.41 ng g^{-1} in C1), diuretics in Júcar (3.19 ng g^{-1} in C2) and Guadalquivir (3.32 ng g^{-1} in C2) and histamine receptor antagonists in Guadalquivir (6.89 ng g^{-1} in C1) (Table S-5, Fig. 2b). Concerning individual compounds, among the most concentrated (considering average highest levels) all along the catchments and over campaigns we found sertraline (12.08 ng g^{-1} in Llobregat C1), ketoprofen (7.13 ng g^{-1} in Llobregat C2), acridone (3.73 ng g^{-1} in Ebro C1), hydrochlorothiazide (3.01 ng g^{-1} in all cases), tetracycline (5.92 ng g^{-1} in all cases), codeine (11.58 ng g^{-1} in all cases), ibuprofen (12.56 ng g^{-1}) clarithromycin (12.72 ng g^{-1} in all cases) and azithromycin (23.92 ng g^{-1} in all cases). As for their frequency of detection (average of both C1 and C2 is also shown in Fig. 2), about the 60% of the PhACs studied were present in at least half of the sediment samples analyzed in both sampling campaigns. The 18% of these compounds were detected in all cases. PhACs were more frequently detected during C1 than over C2 in sediment matrices. 21 and 30 compounds were detected in less than 50% of sediment samples over C1 and C2, respectively. As it was observed in SW, the major ubiquity of PhACs in sediment matrices was observed in the Llobregat river basin. Júcar, Ebro and Guadalquivir river basins followed the frequency of detection rate. Regarding individual compounds, azithromycin and thiabendazole were the most ubiquitous compounds in sediments.

3.2. PhACs distribution differences over sampling campaigns and sites

The ordination of the sampled sites and the 76 PhACs by NMDS revealed a better fit for SW than for sediments (see lower stress value in Fig. 3). Llobregat and Ebro rivers showed more variability among sites than Júcar and Guadalquivir, in particular when considering SW samples (Fig. 3). The overlapping of C1 (continuous lines in Fig. 3) and C2 (broken lines) polygons for SW pointed out a large similarity between the two sampling campaigns, while temporal differences were clearly observed for sediment. Among the 20 compounds that showed the most differing patterns among sampling sites and campaigns we found atenolol, propyphenazone, phenazone or clarithromycin in SW (with high concentrations in the first campaign for Llobregat and Ebro, Fig. 4) and venlafaxine in sediment samples (with high concentrations in the first campaign for Llobregat, Fig. 5). These compounds differing the most among samples both for SW and sediments belonged to the therapeutic groups of analgesics/antiinflammatories, lipid regulators, psychiatric drugs, β-blocking agents, antihypertensives, x-ray contrast media, antihelmintics and antibiotics. Compounds classified as diuretics, prostatic hyperplasia and to treat asthma drugs were also among the ones showing the highest variations among samples in SW, while in sediments histamine receptor antagonists, synthetic glucocorticoid and calcium channel blocker were varying the most. According to the Permanova taking into account all the PhACs together (Table S-7), PhAC concentrations were significantly different among basins and campaigns, for both SW and sediment samples. Interestingly, a significant interaction between campaigns and basins was also found both for SW and sediment samples. Further univariate ANOVAs based on permutation for individual PhACs (Table S-8) revealed that on average 30% of the total variation in the concentration of PhACs in SW was explained by the factor Basin, 37% by the factor Campaign and a further 24% was explained the interaction between the two factors, with a 9% of the variation left unexplained, on average (Table S-8). For the concentration of PhACs in sediments those percentages were 22%, 50%, 21% and 7%, respectively. For both sample types the percentage of the variation that was able to explain each source of variation varied a lot, from a difference of 59% between the minimum and maximum variation explained for the Basin x Campaign interaction for SW to a difference of 100% for the factor Campaign for sediment samples (Table S-8). Levels of PhACs in SW where generally higher during C1 compared to C2, while sediments followed the opposite trend (Figs. 4 and 5). Nevertheless, the comparison of the C2:C1 ratios for

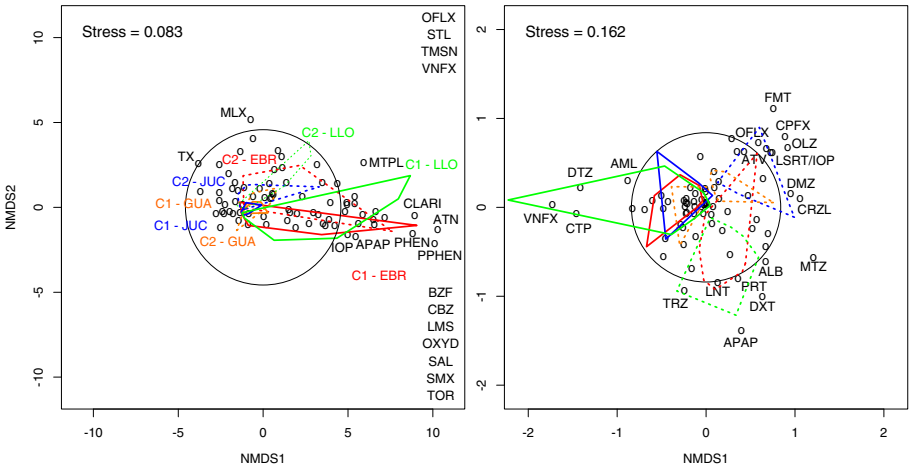


Fig. 3. NMDS ordination of the sites and the pharmaceuticals along the four river basins and the two sampling campaigns in surface waters (left) and sediments (right). The twenty pharmaceuticals that showed the most differing patterns across basins or sampling campaigns are outside the circles. Exact location of the pharmaceuticals in the ordination for the surface water samples is only given for some of them due to space constraints. Code for the short names of the pharmaceuticals can be seen in the supporting material (Table S-1).

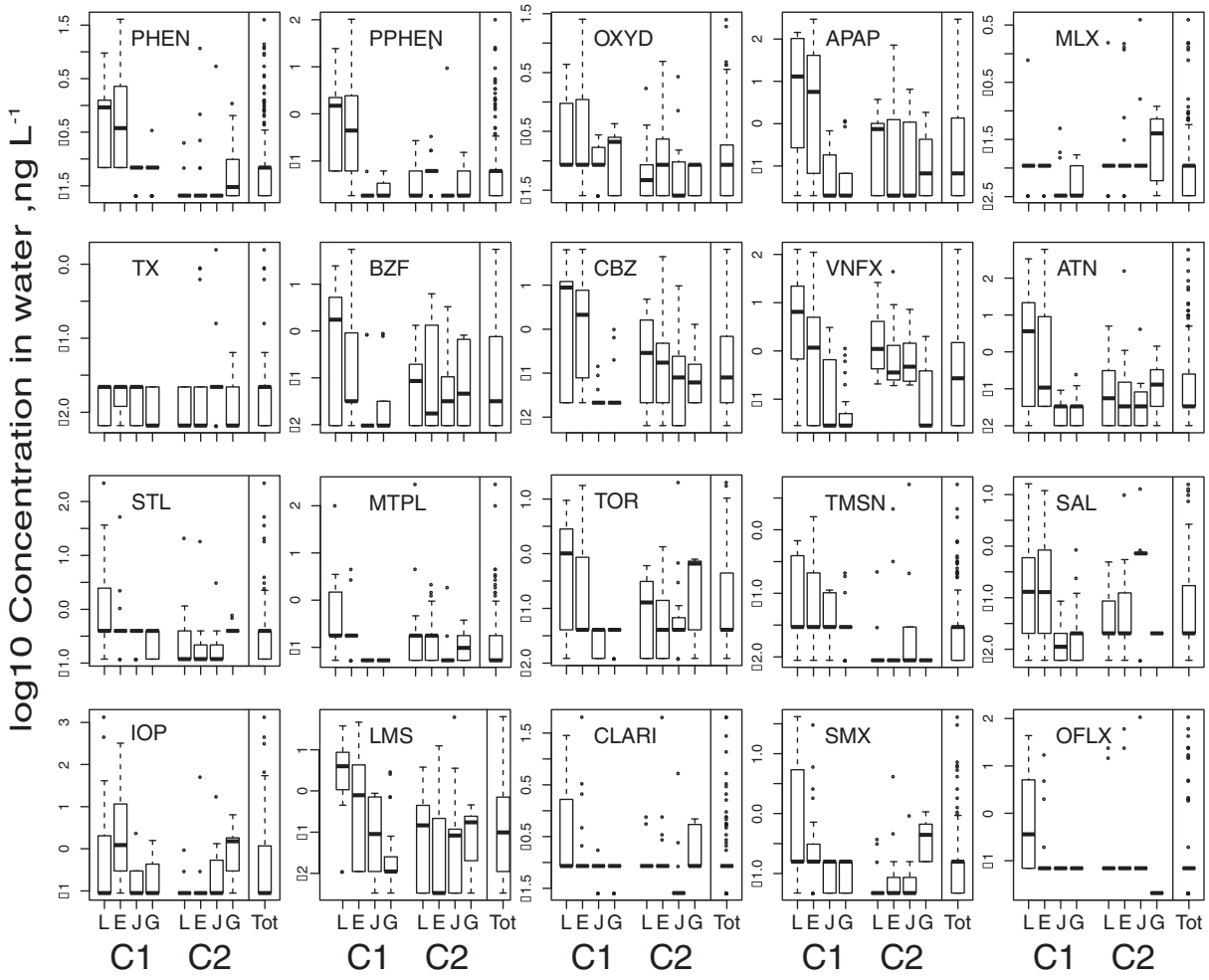


Fig. 4. Concentration boxplots of the twenty pharmaceuticals that showed the most differing pattern across basins or sampling campaigns in surface water samples. Boxplots for each basin at each campaign as well as a boxplot taking into account all the data are shown at each plot. Code for the short names of the pharmaceuticals can be seen in the supporting material (Table S-2).

PhACs and for the discharge with a Permanova did not reveal any significant pattern for SW and for sediment samples (Table S-9). The total levels of PhACs grouped by therapeutic class (Tables S-10 and S-11) for SW and sediments, respectively, show that the Llobregat and Cardener rivers followed a pronounced pollution gradient from headwaters to river mouth, mainly in C1, being LLO7 the most polluted site in both campaigns. By contrast, the most polluted site of the Anoia tributary was ANO2 in both C1 and C2. Within the Ebro river basin, the highest drug concentrations were observed at ARG, HUE and ZAD, whereas the least polluted site was GAL1 during both campaigns. In the Júcar catchment area, the most polluted site was JUC7 while the least polluted sites in both campaigns were CAB5, JUC5 and CAB4. Lastly, the Guadalquivir river basin showed maximum levels of PhACs in GUAA and the minimum levels were detected in GUA9 and GUA1 during both campaigns. Unlike the behavior observed in SW, PhACs did not show any pollution gradient nor any temporal pattern along the sediments of the Llobregat catchment in the different campaigns (see Table S-11).

3.3. Investigation on outlying cases of contamination and assessment of ecotoxicological risk

3.3.1. Outlying cases for each PhAC

Although some PhACs showed a distribution with a limited number of cases that were outside of the general pattern, most of the PhACs did not follow a log-normal distribution (Figs. 4 and 5). For SW only acetaminophen, bezafibrate, carbamazepine, venlafaxine and levamisole fitted to a log-normal distribution when taking into account all SW samples (Fig. 4). On the other hand, acetaminophen, carazolol, amlodipine, losartan, iopromide, albendazole, dexamethasone and metronidazole showed a fit to a log-normal distribution for sediment samples (Fig. 5). The PhACs showing "outlying high" values compared to the overall concentration across the sites and the campaigns were different for SW and sediment samples (Table S-12a). The PhACs showing the highest number of outlying high values in SW were phenazone and propyphenazone, while for sediments the compounds were trazodone and famotidine. On the other end, ibuprofen and pravastatin were among the compounds very frequently found at "outlying low" concentrations in SW, whereas for sediments these were nadolol and tetracycline (Table S-12b). Regarding the number of outlying high values, the Ebro and Júcar were identified as the basins showing the highest (Table S-13a) and the lowest ones (Table S-13b), respectively. On the other hand, Júcar and Ebro accounted for the highest number of outlying low values of PhACs in SW and sediment samples, respectively, whereas Llobregat and Júcar summed the lowest number in SW and sediments samples, respectively (Table S-13a). For SW samples C1 accounted for the highest number of cases with outlying high PhACs concentrations, while for sediments C2 showed the highest number (Table S-14b). On the contrary, C2 in SW and C1 in sediments summed the highest number of outlying low levels of PhACs (Table S-14a). Fisher's exact test revealed that the number of outlying high (Table S-15a) and low (Table S-15b) values of PhAC concentration in each basin varied significantly with the sampling campaign for both SW and sediments. Among the four catchments, the sampling

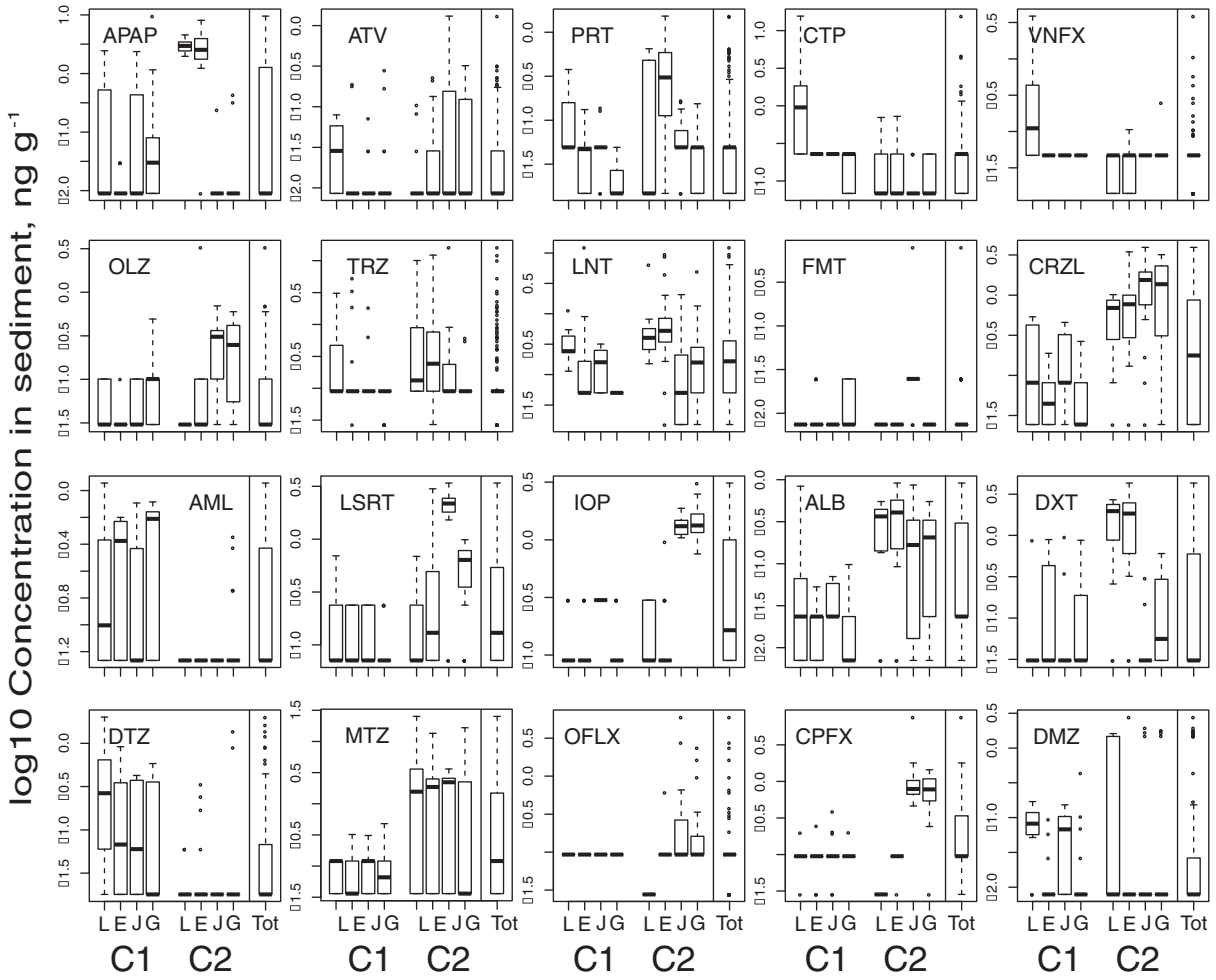


Fig. 5. Concentration boxplots of the twenty pharmaceuticals that showed the most differing pattern across basins or sampling campaigns in sediment samples. Boxplots for each basin at each campaign as well as a boxplot taking into account all the data are shown at each plot. Code for the short names of the pharmaceuticals can be seen in the supporting material (Table S-2).

sites having the highest number of PhACs detected at outlying high concentrations compared to the rest of the sites were ZAD, LLO7 and ANO2 in SW; while LLO7 and CAR4 were among the highest in sediments (Table S-16a). The SW and sediment samples from these sites were specially polluted by a high number of PhACs. Against expectations, all headwater reaches, such as in LLO1, EBR1, GUA1 and especially in JUC1, also showed outlying high levels of PhACs (Table S-16a). On the other end, for the locations close to the river mouth such as JUC8 (in SW samples) and EBR9 (in sediment samples), again against expectations, only a low number of PhACs showed outlying high concentrations. The locations with the most cases of PhACs at outlying low concentrations compared to the rest of the samples were completely different between SW and sediments (Table S-16b). The sites that displayed most outlying low levels of PhACs for SW were CAB5 and JUC5, whereas for sediments these were EBR1, LLO5 and RS. Unexpectedly again, some PhACs were found at outlying low concentrations at low reaches of the basins in SW, specially CAB5 (Table S-16b). Overall, fewer cases of outlying low concentrations of PhACs were detected in comparison to the cases of outlying high concentration of PhACs (Table S-16).

3.3.2. Ecotoxicological risk (toxic units): identification of sites, basins and campaigns associated with outlying high and low values and of compounds responsible for the ecotoxicological risk

TU values were highest for algae and lowest for fish, with *Daphnia* showing values in between (see Fig. 7 and Table S-17). TUs of PhACs to aquatic organisms estimated all over the four river basins spanned from 2.18E − 0.5 to 5.39E − 0.3 for algae, from 5.97E − 06 to 1.52E − 03 for *Daphnia* and from 2.91E − 06 to 8.39E − 04 for fish (Fig. 7 and Table S-17). More in detail, the locations where PhACs showed the minimum estimated ecotoxicological effects to aquatic organisms, per campaign were: CIN1 (3.37E − 05) in C1 and JUC5 (2.18 − 05) in C2 for algae, CIN1 (8.05E − 06 in C1) and CAB5 (5.97E − 06 in C2) for *Daphnia*, and GAL1 (5.17E − 03) in C1 and JUC5 (2.91E − 0.6) in C2 for fish. As for the maximum ecotoxicity observed, LLO7 was the location showing highest TU values in C1 for an all aquatic species (5.39E − 03 for algae, 1.52E − 03 for *Daphnia* and 8.39E − 04 for fish). In C2, the highest risk was shared between ZAD (3.45E − 03 for algae) and LLO7 (5.61E − 04 for *Daphnia* and 4.81E − 04 for fish). Among these locations, the presence of PhACs in LLO7 in C1 posed the highest ecotoxicological risk to all aquatic species (5.39E − 03 for algae, 1.52E − 03 for *Daphnia* and 8.39E − 04 for fish). A similar trend was observed in ZAD in C1, with TU values close to those estimated in LLO7 (4.67E − 03 for algae, 5.61E − 04 for *Daphnia* and 6.19E − 04 for fish). Generally, ecotoxicological effects estimated for PhACs were more important in C1 compared to C2 (TU values averaged 3.94E − 04, 7.84E − 05 and 7.94E − 05 in C1 and 1.98E − 0.4, 7.10E − 05 for corresponding algae, *Daphnia* and fish). As for the river basins, estimated average ecotoxicological risk to aquatic organisms was most relevant in Llobregat (2.50E − 04), closely followed by Ebro (2.28E − 04) and then Guadalquivir (6.35E − 05) and Júcar (3.97E − 05). TU calculated for algae showed the highest number of outlying high values with a total of 13 cases in SW samples (Table S-18). TU based on fish did not show outlying high values (Table S-18), as all the values estimated fitted within the whiskers of the boxplots created with log-

transformed TU values (not shown). Llobregat and Ebro showed the highest number of outlying high TU values, 6 taking into account the three kinds of TUs (Table S-19). The number of outlying high values was higher in C1 (8 cases) than in C2 (5 cases) (Table 20). LLO7 and ZAD were the sites with the highest number of outlying high TU values (4 cases) (Table S-21). No outlying low values were observed for TU. The distribution of outlying high values of TU across basins was not related to the sampling campaign (Fisher's exact test: $p = 0.53$ for SW and $p = 0.15$ for sediments) (Table S-22). The compounds that contributed at least 5% to the total predicted toxicity in the samples were sertraline, erythromycin, losartan and dimetridazole with values of 22, 20, 11 and 6%, respectively, when considering TU based on algae for SW (Table 23). For TU based on *Daphnia* there were again four PhACs reaching the 5%-threshold, namely, sertraline (29%), gemfibrozil (12%), loratidine (10%) and fluoxetine (5%). For TU based on fish gemfibrozil was found to be the PhAC that most contributed to the predicted toxicity of SW samples, 43% on average. Sertraline (11%), loratidine (10) and azithromycin (6) also showed predicted toxicities over 5% of the total TU of the sample (Table 23).

3.4. Relationship of PhACs pollution with population density and livestock units

Significant positive correlations were observed between mean PhACs concentrations in SW and both population density and LSU (mean concentrations of the PhACs that are used in each case, see subscripts in Table S-2), while for sediment a similar significant correlation was only observed for LSU (Fig. 6, Table S-24). For an increase in population density from 10 to 100 the mean PhAC concentration in SW was 4.2 times higher (Fig. 6). For the same increase in LSU the mean PhAC concentrations in SW and sediments were only 3.3 and 1.4 times higher, respectively. Moreover, the relationship between the TUs based on algae, *Daphnia* and fish and the population density and the LSU were all significant (Fig. 7, Table S-25). However, the relationships between TUs (for the three species) and population density were more pronounced than those observed with LSU: for an increase in population density from 10 to 100 the TU were 3.2, 3.3 and 5.4 times higher for algae, *Daphnia* and fish, whereas the same increment of LSU was associated with 2.0, 2.0 and 2.3 times higher predicted toxicity using the same indices (Fig. 7).

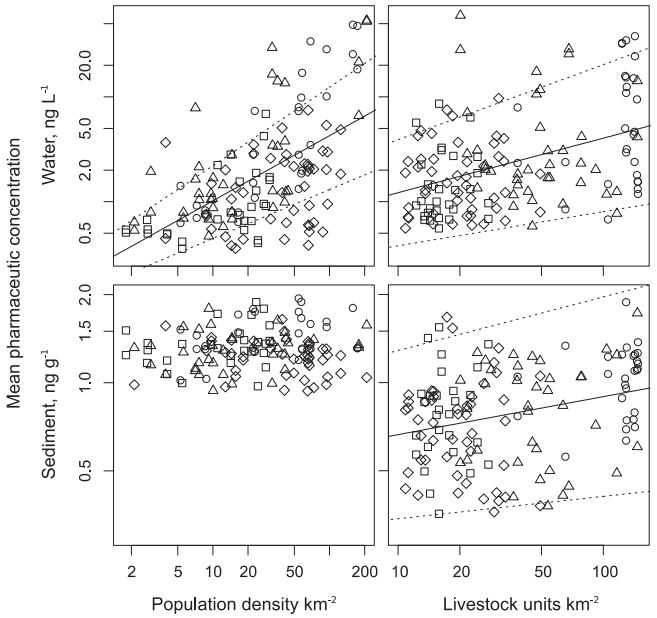


Fig. 6. Relationships of mean concentration of pharmaceuticals in surface waters (top) and sediments (bottom) with population density (left) and livestock units (right). Significant fixed effects of the fitted linear mixed effect models are displayed with continuous lines and 95% confidence intervals with dashed lines. Different symbols are used for the different catchments: Llobregat (○); Ebro (△), Júcar (□) and Guadalquivir (◇).

4. Discussion

4.1. Distribution and outlying cases of PhACs

PhAC concentrations varied four orders of magnitude in SW whereas they were much more constant in sediment. Similar results were previously observed in the Ebro river basin, in US streams, in Valencian wetlands (Spain) and the Túria river basin (Spain) (da Silva et al., 2011; Schultz et al., 2010; Vazquez-Roig et al., 2011, 2012; Carmona et al., 2014). We did not sample the supposedly most pristine points within the selected four basins, but with the extensive sampling in medium and low reaches of these streams we could have a useful measure of the maximum concentrations and estimated toxicity that we could detect in rivers of the Iberian Peninsula. The Llobregat and Ebro catchments displayed the highest ubiquity and concentrations of PhACs in SW, while sediments from Guadalquivir and Ebro were the most polluted ones. The presence of a wide diversity of PhACs had been previously confirmed in the water columns of the Llobregat and Ebro basins (Gros et al., 2007; da Silva et al., 2011; Osorio et al., 2012a, b). However, only three locations had been surveyed for PhACs levels in SW of the Guadalquivir River (Robles-Molina et al., 2014) and, to our knowledge, our study presents the first monitoring of PhACs in the Júcar river basin. The distribution of PhACs varied substantially across the Llobregat and Ebro river basins, in which the highest number of cases of outlying high concentrations and TU values of PhACs in SW were detected. In agreement with previous findings (da Silva et al., 2011; Osorio et al., 2012a,b; Carmona et al., 2014; Vazquez-Roig et al., 2011, 2012) and also following the global trend (Hughes et al., 2013) analgesics/anti-inflammatories, antibiotics, and diuretics were the most concentrated and frequently detected therapeutic groups in both SW and sediment samples. Therefore, our present database, together with previous research, reveals that analgesics/anti-inflammatories, antibiotics and diuretics are widespread and pseudo-persistent therapeutic groups in Spanish freshwater systems. The relevancy of other PhAC families varied across river basins and matrices, which could be due to regionally specific consumption patterns (Ortiz et al., 2013). Hydrochlorothiazide and gemfibrozil as well as azithromycin and ibuprofen were widely spread and concentrated PhACs in SW and sediments, respectively. Similar trends observed for hydrochlorothiazide, gemfibrozil and ibuprofen in published data (da Silva et al., 2011; Vazquez-Roig et al., 2011; Carmona et al., 2014) leads to consider these compounds as pseudo-persistent emerging pollutants in the national aquatic environment. The widely varying physicochemical properties of PhACs play an important role on their partitioning between sediments and the water column (Chen and Zhou, 2014). In agreement with a previous study (Osorio et al., 2012a) SW from the Llobregat catchment followed a PhAC pollution gradient from the headwaters to the river mouth, a pattern that was mimicked by the Cardener sub-catchment. Nevertheless, the remaining river basins did not follow any clear trend, with spots in which high concentration of PhAC spread across the four catchments. The highest levels of PhACs were detected in both SW and sediments of the sites ANO2, LLO7, ZAD and MAG. The locations LLO4 and LLO7 are well-known highly polluted sites of the Llobregat catchment (see MT and SJD corresponding to LLO4 and LLO7 in Osorio et al., 2012ab). Similarly, ZAD and ARG were previously identified as hotspots of PhACs (see T3 and T11 corresponding to ZAD and ARG in da Silva et al., 2011). Other troublesome locations identified were ARG, HUE, JUC7, and GUAA for SW; and LLO4, JUC1, GUA4 and BOR for sediments. On the other hand, the lower levels of PhACs in both SW and sediments were detected LLO1, LLO2, EBR1, GAL1, RS, CAB5, JUC5 and GUA1. On this basis, we would propose the water management

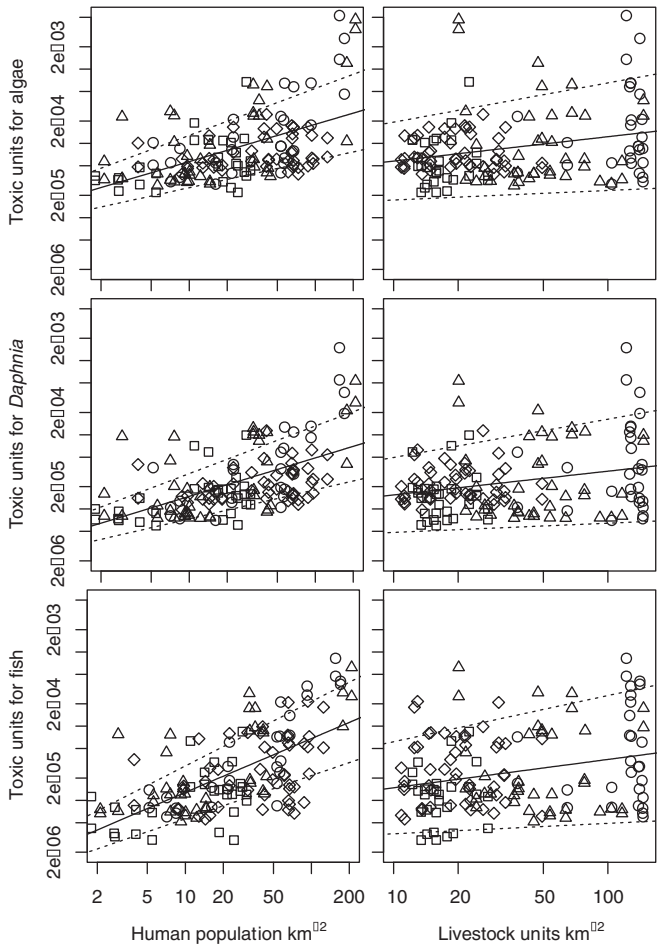


Fig. 7. Relationships of pharmaceutical toxic units for algae (top), *Daphnia* (middle) and fish (bottom) in surface waters with population density (left) and livestock units (right). Significant fixed effects of the fitted linear mixed effect models are displayed with continuous lines and 95% confidence intervals with dashed lines. Different symbols are used for the different catchments: Llobregat (○); Ebro (△), Júcar (□) and Guadalquivir (◇).

authorities to include the monitoring of PhACs in these locations as an indicator of the water quality status.

4.2. Effect of the changes of the discharge

There are studies that explain the seasonality of the concentration of PhACs by means of the more or less intensive use of those PhACs by the population (Moreno-González et al., 2015). Both sediment and water samples are subject to seasonal variations of the concentration of pollutants (Fairbairn et al., 2015). Influents of WWTP have also shown clear hourly and seasonal cycles that were also related to the consumption of those products by human (Coutu et al., 2013). In the work by Coutu et al. (2013) the relationship between PhAC concentration and discharge of the effluent was positive, i.e. the highest loads of PhAC were recorded in the first hours of morning or in winter. Nevertheless, the flow of the WWTP influents depends on the consumption of that water by human, whereas in lotic systems the relationship between PhAC concentration and the natural water discharge should be negative, as the latter dilutes the inputs from WWTP (see Hua et al., 2006; Kumar et al., 2011). Although some of the sites that showed outlying high values (ANO2, LLO7, ZAD, MAG2 for both SW and sediments) repeated from the first (high flows) to the second campaign (low flows), the overall relationship between PhACs and discharge C2:C1 ratios was non-significant. It seems that the hypothesized modulation of PhACs concentration under changing hydrological conditions observed in previous studies (Osorio et al., 2014) is not supported by our data, although we realize that with just two samplings we lack statistical power to be totally confident about any conclusion. The additional factors affecting the variation of PhAC concentration such as natural attenuation processes (mainly photodegradation and biodegradation) (Kümmerer, 2010), route that the PhACs use to reach the river (point emissions from WWTPs for human drugs; diffuse sources for veterinary drugs) and anthropogenic causes (human and animal drug consumption patterns, water use and a changing wastewater treatment efficiency) could counteract their natural dilution by the discharge in SW (Kümmerer, 2010; Vystavna et al., 2012). Besides, sediments can act as a reservoir of PhACs from where these substances can be re-dissolved into the aqueous phase under turbulent flow conditions in the river, thus modifying the concentration of PhACs in both phases of the water column (Nentwig et al., 2004). Moreover, the intrinsic physicochemical properties of PhACs, such as speciation or solubility, combined with the physicochemistry of the freshwater system, such as pH or total suspended solids, can affect the distribution of these substances along the water column, thus contributing to the variability of PhAC levels in SW and sediments (Carmona et al., 2014; Veach and Bernot, 2011).

4.3. Risk-based prioritization of locations and PhACs

Similar to what was observed for PhACs concentrations, the potential risk of PhACs to aquatic organisms increased downstream the Llobregat River, but no clear trend of increasing concern was observed for the remaining basins. The highest total predicted TUs of PhACs in SW were estimated at the sites LLO7, ZAD, MAG2, GUA6, GUA4, MAG1 and JUC7. None of the total TUs calculated at every site for algae, *Daphnia* and fish, exceeded the unit value, thus, according to standard thresholds (Malaj et al., 2014), no acute risk associated with PhACs was observed. However, though only for LLO7 and ZAD, the corresponding total TU values for algae were estimated above ~1E − 03 in both sampling campaigns, evidencing the potential long-term ecotoxicological effects on these primary producers (Malaj et al., 2014). On the other hand, CAB5, JUC5, LLO2, ESE, GUA1 and CIN1 were among the less worrisome locations. Similar findings were reported for the particular study cases of the Llobregat and Ebro river basins (Ginebreda et al., 2014; Damásio et al., 2011; Gros et al., 2010). Ginebreda et al. (2014) observed an increase of total TU estimated for algae and *Daphnia* downstream the Llobregat River as well (see the locations LL2 and LL7 in the referenced work, corresponding to LLO4 and LLO7 in the present study). Damásio et al. (2011) also observed the same trend for *Daphnia* (among other two invertebrate species) (see the locations L2 and L3 of the study cited, corresponding to LLO4 and LLO7 in the present one). Besides, the location LLO7 was also estimated at high risk of chronic ecotoxicological effects in both studies. Diversely to the current study, both works aforementioned assessed the apportionment of other pollutants such as pesticides (Ginebreda et al., 2014) and also metals and alkylphenols (Damásio et al., 2011) to total ecotoxicity. Indeed, Damásio et al. (2011) reported a marginal contribution of PhACs (4%) to the total predicted hazard to invertebrate species; while metals and pesticides accounted for 39% and 54%, respectively. These findings evidence the need to expand the ecotoxicological risk assessment to all kinds of pollutants that might be present in a complex environmental mixture, as it has been recently attempted by Kuzmanović et al. (2015). However, the set of biomarkers applied by Damásio et al. (2011) were not developed to evaluate the effects of PhACs, which indicates that further research on should rely on specific biochemical responses to these substances. The vast number of chemical products that society is using nowadays makes it difficult to find a way to decontaminate every one of them. Alternatively, a clear prioritization using the potential risk of the different chemicals should highlight the critical products among the rest. To create the prioritization the toxicity of the compound, its concentration in nature and the facility to transform into innocuous compounds needs to be taken into account. Our study

cannot address the last issue, but we present extensive data on the concentration of PhACs and estimate their contribution to the total toxicity in the field. In this sense, we have observed very low concentration of erythromycin that following literature seems to be a very toxic compound (VSDB), and on the other hand, we also have seen very high concentrations of atenolol and ketoprofen but their toxicity is very low (ECOTOX; Sanderson et al., 2003). Computing the relative contribution of the different substances to the total toxicity in the locations sampled we have been able to enumerate the critical PhACs in the waters of the catchments of the Iberian Peninsula None of the compounds found to contribute at least 5% to the total predicted toxicity were in agreement with those reported by Damásio et al. (2011). However, as well as the present work, Gros et al. (2010) reported fluoxetine as one of the major contributors to the ecotoxicological risk to *Daphnia* species. Importantly, although our ecotoxicological risk assessment was only focused on PhACs, the compounds identified as principal contributors to total predicted toxicity were classified by Kuzmanović et al. (2015) among the more relevant pollutants of the same River Basins (i.e. sertraline, erythromycin and losartan to algae; sertraline to *Daphnia*; and gemfibrozil for fish). All in all, we propose in essence that sertraline, gemfibrozil and loratidine are the PhACs into which a bigger effort should be concentrated if contamination of freshwater systems by PhACs needs to be controlled.

4.4. Effects of population density and livestock units

Our study shows a significant positive effect of the potential sources of PhACs, i.e. human population and livestock, on the concentration of PhAC in SW and sediments and the TU in SW. These relationships were stronger for SW and especially with the variation with population density. Given the very different use of the PhACs in terms of dosage, target population or seasonality (Ortiz et al., 2013; Veach and Bernot, 2011) it is remarkable to observe these significant relationships between the spatial information of the sources of PhACs and their average concentration and estimated toxicity on rivers. To our knowledge, this relationship has never been empirically proven beforehand, although other studies that find a relationship between the density of the population or the presence of activities involving livestock and the concentration of PhACs in river waters are quite common (Bartelt-Hunt et al., 2011; Murata et al., 2011; Osorio et al., 2012a; Fairbairn et al., 2015). Nevertheless, interestingly, in no case the differences of the population density or the LSU in the catchments are followed by a proportional increase of the concentration of PhAC in SW or in sediments. The highest increment of average PhAC concentration was observed for SW in relation to population density (X4.2 in PhAC concentration for a tenfold increase in population density). Although a higher density of population and LSU is linked to a higher use of PhACs (e.g. Kools et al., 2008) the water consumption also increases (Mekonnen and Hoekstra, 2012; Panagopoulos et al., 2012), diluting in part the PhAC spilled into nature. On the other hand, the activity of microorganisms in the water/sediment interface or the streambed sediments have been seen to be very relevant in the biodegradation of pharmaceuticals (e.g. Radke and Maier, 2014), what might partially explain the lower increment of the concentration of PhACs (about 40% of increase in concentration of PhACs for a tenfold increase in LSU) in sediment samples.

Pollutants can have contrasting effects on the biotic components of ecosystems and on the processes, and thus services, that the biota can drive (Flores et al., 2014). Given the individual toxicity of the PhAC and their concentrations in the field the TUs in our work were highest for algae and lowest for fish, with *Daphnia* showing values in between. This result suggests that toxicity from PhACs would harm the assemblage of primary producers more than other biota. Nevertheless, TUs for *Daphnia* and fish showed a stronger response, i.e. a steeper slope, to the increase of the population density and the LSU, suggesting that whereas the affections on ecosystem processes in which algae are important, as primary production, metabolism and autodepuration, would not change very much with population density of LSU, the assemblages representing the top of the food webs (invertebrates and fish) are going to become more impaired as pollutants are further concentrated in those ecosystems. Among the relevant functions, secondary production will be reduced as invertebrates and vertebrates are affected (Carlisle and Clements, 2003). The important but indirect role of invertebrates and fish in the regulation of other important processes as autodepuration (controlled by herbivory of primary consumers, Libourissen et al., 2005) or organic matter recycling (through the consumption of it or of its consumers, Woodward et al., 2008) makes the understanding of the effects of PhACs on different kind of organisms a critical step to predict alterations on ecosystem processes.

5. Conclusions

With this work we have demonstrated the ubiquity of PhACs in SW and sediments of Iberian rivers, although some sites have shown outlying concentrations of some PhACs and the total concentration of PhACs, which focuses the attention on specific sites and PhACs. Both average concentration of the PhACs and their estimated total toxicity, have shown to be positively related to the population density and the livestock units in the upstream sub-basin, thus responding to the anthropic pressures in the catchments. Although the contribution of the different PhACs to the estimated total toxicity of the SW is site dependent, five compounds (erythromycin, gemfibrozil, loratidine, losartan and sertraline) are responsible for more than 50% of the TU for algae, *Daphnia* or fish, and should therefore be specially addressed when dealing with SW pollution with emergent contaminants. Our study highlights that SW can receive relevant amounts of PhACs that might interfere with the natural organization of the biota and affect ecosystem processes and, thus, services.

Acknowledgments

Authors would like to thank the Spanish Ministry of Economy and Competitiveness for its financial support through the projects SCARCE (Consolider-Ingenio 2010 CSD2009-00065). This work has been supported by the European Union Seventh Framework Programme Funding under Grant agreement no. 603629-ENV-2013-6.2.1 Globaqua and by the Generalitat de Catalunya (Consolidated Research Groups "2014 SGR 418 — Water and Soil Quality Unit" and 2014 SGR 291 — ICRA. SP and AL acknowledge the contract from the Ramón y Cajal Program of the Spanish Ministry of Economy and Competitiveness and the funding program of the Basque Government for Consolidated Research Groups, respectively.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2015.06.143.

References

Agresti, A., 1992. A survey of exact inference for contingency tables. Stat. Sci. 7 (1), 131–153.

Anderson, M.J., 2001. Permutation tests for univariate or multivariate analysis of variance and regression. Can. J. Fish. Aquat. Sci. 58 (3), 626–639.

Awad, Y.M., Kim, S.C., Abd El-Azeem, S.A.M., Kim, K.H., Kim, K.R., Kim, K., et al., 2014. Veterinary antibiotics contamination in water, sediment, and soil near a swine manure composting facility. Environ. Earth Sci. 71 (3), 1433–1440.

Bartelt-Hunt, S., Snow, D.D., Damon-Powell, T., Miesbach, D., 2011. Occurrence of steroid hormones and antibiotics in shallow groundwater impacted by livestock waste control facilities. J. Contam. Hydrol. 123, 94–103.

Carlisle, D.M., Clements, W.H., 2003. Growth and secondary production of aquatic insects along a gradient of Zn contamination in Rocky Mountain streams. J. N. Am. Benthol. Soc. 22 (4), 582–597.

Carmona, E., Andreu, V., Picó, Y., 2014. Occurrence of acidic pharmaceuticals and personal care products in Turia River basin: from waste to drinking water. Sci. Total Environ. 484, 53–63.
Chen, K., Zhou, J.L., 2014. Occurrence and behavior of antibiotics in water and sediments from the Huangpu River, Shanghai, China. Chemosphere 95, 604–612.
Coutu, S., Wyrsch, V., Wynn, H.K., Rossi, L., Barry, D.A., 2013. Temporal dynamics of antibiotics in wastewater treatment plant influent. Sci. Total Environ. 458–460, 20–26.
da Silva, B.F., Jelic, A., López-Serna, R., Mozeto, A.A., Petrovic, M., Barceló, D., 2011. Occurrence and distribution of pharmaceuticals in surface water, suspended solids and sediments of the Ebro river basin, Spain. Chemosphere 85 (8), 1331–1339.
Dai, G., Wang, B., Huang, J., Dong, R., Deng, S., et al., 2015. Chemosphere occurrence and source apportionment of pharmaceuticals and personal care products in the Beiyun River of Beijing, China. Chemosphere 119, 1033–1039.
Damásio, J., Barceló, D., Brix, D., Postigo, C., Gros, M., Petrovic, M., Sabater, S., Guasch, H., de Alda, M., Barata, C., 2011. Are pharmaceuticals more harmful than other pollutants to aquatic invertebrate species: a hypothesis tested using multi-biomarker and multi-species responses in field collected and transplanted organisms. Chemosphere 85 (10), 1548–1554.
Dunn, O.J., 1961. Multiple comparisons among means. J. Am. Stat. Assoc. 56 (293), 52–64.
Fairbairn, D.J., Karpuzcu, M.E., Arnold, W.A., Barber, B.L., Kaufenberg, E.F., Koskinen, W.C., et al., 2015. Sediment–water distribution of contaminants of emerging concern in a mixed use watershed. Sci. Total Environ. 505, 896–904.
Fernández, C., González-Doncel, M., Pro, J., Carbonell, G., Tarazona, J.V., 2010. Occurrence of pharmaceutically active compounds in surface waters of the Henares–Jarama–Tajo river system (Madrid, Spain) and a potential risk characterization. Sci. Total Environ. 408, 543–551.
Flores, L., Banjac, Z., Farré, M., Larrañaga, A., Mas-Martí, E., Muñoz, I., et al., 2014. Effects of a fungicide (imazalil) and an insecticide (diazinon) on stream fungi and invertebrates associated with litter breakdown. Sci. Total Environ. 476–477, 532–541.
Ginebreda, A., Kuzmanovic, M., Guasch, H., de Alda, M., López-Doval, J.C., Muñoz, I., Ricart, M., Romaní, A.M., Sabater, S., Barceló, D., 2014. Assessment of multi-chemical pollution in aquatic ecosystems using toxic units: compound prioritization, mixture characterization and relationships with biological descriptors. Sci. Total Environ. 468, 715–723.
Gros, M., Petrovic, M., Barceló, D., 2007. Wastewater treatment plants as a pathway for aquatic contamination by pharmaceuticals in the Ebro river basin (NE Spain). Environ. Toxicol. Chem. 26 (8), 1553–1562.
Gros, M., Petrović, M., Ginebreda, A., Barceló, D., 2010. Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Environ. Int. 36 (1), 15–26.
Gros, M., Rodríguez-Mozaz, S., Barceló, D., 2012. Fast and comprehensive multi-residue analysis of a broad range of human and veterinary pharmaceuticals and some of their metabolites in surface and treated waters by ultra-high-performance liquid chromatography coupled to quadrupole-linear ion trap tandem mass spectrometry. J. Chromatogr. A 1248, 104–121.
Hua, W.Y., Bennett, E.R., Maio, X.-S., Metcalfe, C.D., Letcher, R.J., 2006. Seasonality effects on pharmaceuticals and s-triazine herbicides in wastewater effluent and surface water from the Canadian side of the upper Detroit River. Environ. Toxicol. Chem. 25 (9), 2356–2365.
Hughes, S.R., Kay, P., Brown, L.E., 2013. Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems. Environ. Sci. Technol. 47 (2), 661–677.
Jelic, A., Petrovic, M., Barceló, D., 2009. Multi-residue method for trace level determination of pharmaceuticals in solid samples using pressurized liquid extraction followed by liquid chromatography/quadrupole-linear ion trap mass spectrometry. Talanta 80 (1), 363–371.
Jia, A., Hu, J., Wu, X., Peng, H., Wu, S., Dong, Z., 2011. Occurrence and source apportionment of sulfonamides and their metabolites in Liaodong Bay and the adjacent Liao River basin, North China. Environ. Toxicol. Chem. 30 (6), 1252–1260.
Jiang, L., Hu, X., Yin, D., Zhang, H., Yu, Z., 2011. Occurrence, distribution and seasonal variation of antibiotics in the Huangpu River, Shanghai, China. Chemosphere 82, 822–828.
Kemper, N., 2008. Veterinary antibiotics in the aquatic and terrestrial environment. Ecol. Indic. 8, 1–13.
Kools, S.A.E., Moltmann, J.F., Knacker, T., 2008. Estimating the use of veterinary medicines in the European union. Regul. Toxicol. Pharmacol. 50, 59–65.
Kumar, V., Nakada, N., Yamashita, N., Johnson, A.C., Tanaka, H., 2011. How seasonality affects the flow of estrogens and their conjugates in one of Japan's most populous catchments. Environ. Pollut. 159, 2906–2912.
Kümmerer, K., 2010. Pharmaceuticals in the environment. Annu. Rev. Environ. Resour. 35, 57–75.
Kuzmanović, M., Ginebreda, A., Petrović, M., Barceló, D., 2015. Risk assessment based prioritization of 200 organic micropollutants in 4 Iberian rivers. Sci. Total Environ. 503, 289–299.
Libourissen, L., Jeppesen, E., Bramm, M.E., Lassen, M.F., 2005. Periphyton–macroinvertebrate interactions in light and fish manipulated enclosures in a clear and a turbid shallow lake. Aquat. Ecol. 39, 23–39.
Limpert, E., Stahel, W.A., Abbt, M., 2001. Log-normal distributions across the sciences: keys and clues. Bioscience 51, 341–352.
Malaj, E., Peter, C., Grote, M., Kühne, R., Mondy, C.P., Usseglio-Polatera, P., Brack, W., Schäfer, R.B., 2014. Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale. PNAS 111 (26), 9549–9554.
Mas, S., de Juan, A., Tauler, R., Olivieri, A.C., Escandar, G.M., 2010. Application of chemometric methods to environmental analysis of organic pollutants: a review. Talanta 80, 1052–1067.
Mekonnen, M.M., Hoekstra, A.Y., 2012. A global assessment of the water footprint of farm animal products. Ecosystems 15, 401–415.
Moreno-González, R., Rodriguez-Mozaz, S., Gros, M., Barceló, D., León, V.M., 2015. Seasonal distribution of pharmaceuticals in marine water and sediment from a Mediterranean coastal lagoon (SE Spain). Environ. Res. 138, 326–344.
Murata, A., Takada, H., Mutoh, K., Hosoda, H., Harada, A., Nakada, N., 2011. Nationwide monitoring of selected antibiotics: distribution and sources of sulfonamides, trimethoprim, and macrolides in Japanese rivers. Sci. Total Environ. 409, 5305–5312.
Nentwig, G., Oetken, M., Oehlmann, J., 2004. Effects of pharmaceuticals on aquatic invertebrates — the example of carbamazepine and clofibric acid. In: Kümmerer, K. (Ed.), Pharmaceuticals in the Environment. Sources, Fate, Effects and Risks, 2nd edition Springer-Verlag, Berlin, Heidelberg.
Ortiz, S.d.G., Pinto, G.P., García, P.E., Irusta, R.M., 2013. Consumption and occurrence of pharmaceutical and personal care products in the aquatic environment in Spain. Sci. Total Environ. 444, 451–465.
Osorio, V., Pérez, S., Ginebreda, A., Barceló, D., 2012a. Pharmaceuticals on a sewage impacted section of a Mediterranean River (Llobregat River, NE Spain) and their relationship with hydrological conditions. Environ. Sci. Pollut. Res. 19, 1013–1025.
Osorio, V., Marcé, R., Pérez, S., Ginebreda, A., Cortina, J.L., Barceló, D., 2012b. Occurrence and modelling of pharmaceuticals on a sewage-impacted Mediterranean river and their dynamics under different hydrological conditions. Sci. Total Environ. 40, 3–13.
Osorio, V., Proia, L., Ricart, M., Pérez, S., Ginebreda, A., Cortina, J.L., Sabater, S., Barceló, D., 2014. Relating natural hydrological variations in a Mediterranean river with micropollutant levels and biofilm functioning. Sci. Total Environ. 472, 1052–1061.
Panagopoulos, G.P., Bathrellos, G.D., Skilodimou, H.D., Martsouka, F.A., 2012. Mapping urban water demands using multi-criteria analysis and GIS. Water Resour. Manag. 26, 1347–1363.
Petrie, B., Barden, R., Kasprzyk-Hordern, B., 2015. A review on emerging contaminants in wastewaters and the environment: current knowledge, understudied areas and recommendations for future monitoring. Water Res. 72, 3–27.
Pinheiro, J.C., Bates, D.M., 2000. Mixed-Effects Models in S and S-PLUS. Springer, New York.
R Core Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
Radke, M., Maier, M.P., 2014. Lessons learned from water/sediment-testing of pharmaceuticals. Water Res. 55, 63–73.
Robles-Molina, J., Gilbert-López, B., García-Reyes, J.F., Molina-Díaz, A., 2014. Monitoring of selected priority and emerging contaminants in the Guadalquivir River and other related surface waters in the province of Jaén, South East Spain. Sci. Total Environ. 479–480, 247–257.
Sanderson, H., Johnson, D.J., Wilson, C.J., Brain, R.A., Solomon, K.R., 2003. Probabilistic hazard assessment of environmentally occurring pharmaceuticals toxicity to fish, daphnids and algae by ECOSAR screening. Toxicol. Lett. 144, 383–395.
Schultz, M.M., Furlong, E.T., Kolpin, D.W., Werner, S.L., Schoenfuss, H.L., Barber, L.B., et al., 2010. Antidepressant pharmaceuticals in two U.S. effluent-impacted streams: occurrence and fate in water and sediment, and selective uptake in fish neural tissue. Environ. Sci. Technol. 44, 1918–1925.
Van Boeckel, T.P., Brower, C., Gilbert, M., Grenfell, B.T., Levin, S.A., Robinson, T.P., 2015. Global trends in antimicrobial use in food animals. Proc. Natl. Acad. Sci. U. S. A. http://dx.doi.org/10.1073/pnas.1503141112.
Vazquez-Roig, P., Andreu, V., Onghena, M., Blasco, C., Picó, Y., 2011. Assessment of the occurrence and distribution of pharmaceuticals in a Mediterranean wetland (L'Albufera, Valencia, Spain) by LC–MS/MS. Anal. Bioanal. Chem. 400 (5), 1287–1301.
Vazquez-Roig, P., Andreu, V., Blasco, C., Picó, Y., 2012. Risk assessment on the presence of pharmaceuticals in sediments, soils and waters of the Pego–Oliva Marshlands (Valencia, eastern Spain). Sci. Total Environ. 440, 24–32.
Veach, A.M., Bernot, M.J., 2011. Temporal variation of pharmaceuticals in an urban and agriculturally influenced stream. Sci. Total Environ. 409, 4553–4563.
VSDB, d. VSDB: Veterinary Substances DataBasehttp://sitem.herts.ac.uk/aeru/vsdb/index.htm.
Vystavna, Y., Huneau, F., Grynenko, V., Vergeles, Y., Celle-Jeanton, H., Tapie, N., Budzinski, H., Le Coustumer, P., 2012. Pharmaceuticals in rivers of two regions with contrasted socio-economic conditions: occurrence, accumulation, and comparison for Ukraine and France. Water Air Soil Pollut. 223 (5), 2111–2124.
Woodward, G., Papantoniou, G., Edwards, F., Lauridsen, R.B., 2008. Trophic trickles and cascades in a complex food web: impacts of a keystone predator on stream community structure and ecosystem processes. Oikos 117, 683–692.

SUPPORTING INFORMATION

Concentration and risk of pharmaceuticals in freshwater systems are related to the population density and the livestock units in Iberian Rivers

Victoria Osorio[1*], Aitor Larrañaga[2*], Jaume Aceña[1], Sandra Pérez[1*], Damià Barceló[1,3]

[1] Water and Soil Quality Research Group, IDAEA-CSIC, c/ JordiGirona, 18-26, 08034 Barcelona, Spain

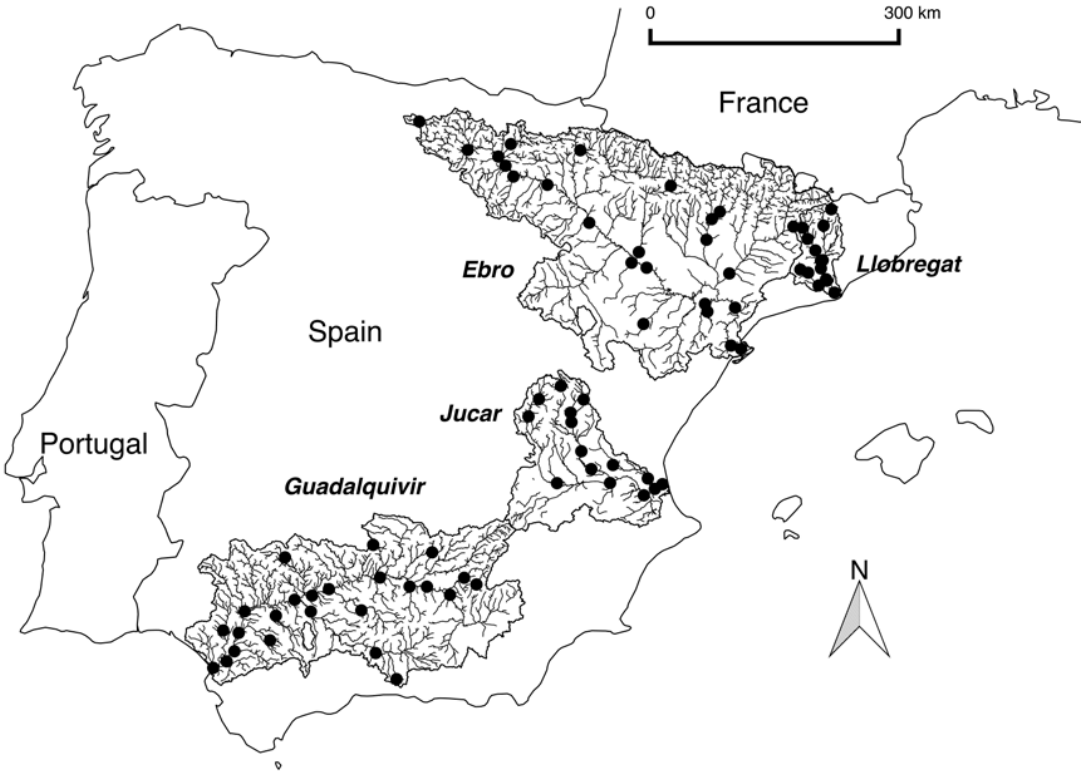
[2] Laboratory of Stream Ecology, Dept. of Plant Biology and Ecology, University of the Basque Country, UPV/EHU, PO Box 644, 48080 Bilbao, Spain

[3] Catalan Institute for Water Research (ICRA), Scientific and Technological Park of the University of Girona, Emili Grahit 101, 17003 Girona, Spain

*These authors contributed equally to this work

Supporting Material

Figure S-1. Locations sampled across the four river basins



2.1. Sampling campaign and sample analysis

Procedures for preparation of water and sediments samples for instrumental analysis were previously described in detail (Jelic et al., 2009; Gros et al., 2012). WWE and SW samples were filtered through 0.7-μm glass fibber filters followed by 0.45-μm nylon membrane filters (Whatman, U.K.). An aqueous solution of 5 % Na_2EDTA was added to achieve a final concentration of 0.1%. To assess the extraction efficiency in each sample processed, water and sediment samples were spiked, prior to pre-concentration and clean-up steps, with an appropriate volume of a standard mixture containing surrogate standards in order to have a concentration of 100 ngL^{-1} in WWE; 50 ngL^{-1} in SW and 10 ngL^{-1} in sediment. The dried sediments were weighted (1 g) and extracted by pressurized liquid extraction (PLE) using Dionex ASE 350 (Dionex; Sunnyvale, CA). The extractions were carried out using a methanol–water mixture (1:2) as extraction solvent, at 1500 psi and 100 ºC in 3 static cycles, each one lasting 5 min. Finally, the extraction cell was flushed with 100% cell volume of fresh solvent. Concentrated extracts were diluted with water in order to reduce the content of methanol (<5 vol %) and processed as water samples for further clean-up.

Target compounds were extracted from WWE and SW samples and sediment extracts by automatic Solid Phase Extraction (SPE) with a GX-271 ASPEC^{TM} system (Gilson, Villiers le Bel, France) using Oasis HLB cartridges (200 mg, 6 mL). SPE cartridges were conditioned with 6 mL of methanol followed by 6 mL of HPLC grade water at a flow rate of 2 $mLmin^{-1}$. 200 mL of WWE, 500 mL of SW and 500 mL of diluted sediment extract were loaded onto the cartridge at a flow rate of 1 $mLmin^{-1}$. After sample pre-concentration, cartridges were rinsed with 6 mL of HPLC grade water, at a flow rate of 2 $mLmin^{-1}$ and were dried with air for 5 min, to remove excess of water. Finally, analytes were eluted with 6 mL of pure methanol at a flow rate of 1 $mLmin^{-1}$. Extracts were evaporated to dryness under a gentle nitrogen stream and reconstituted with 1 mL of methanol/water (10:90, v/v). Finally, 10 μL of a 1 mgL^{-1} standard mixture containing all isotopically labeled standards were added in the extract as internal standard.

Based on published literature about occurrence and distribution in the aquatic environment, a selected list of 76 PhACs (Table S-2), were determined in WWE, SW and sediment extracts using a multi-residue analytical method based on UPLC-MS/MS (Gros et al. 2012). Instrumental analysis was performed by liquid chromatography, using a Waters Acquity Ultra-Performance^{TM} liquid chromatography system (Milford, MA, USA), coupled to a 5500 QTRAP hybrid triple quadrupole-linear ion trap mass spectrometer (Applied Biosystems, Foster City, CA, USA) with a turbo Ion Spray source. Chromatographic separation was carried out an Acquity HSS T3 column (50 mm ×2.1 mm i.d., 1.8 μm particle size) for the compounds analyzed under positive electrospray ionization (PI) and an Acquity BEH C18 column (50 mm×2.1 mm i.d., 1.7 μm particle size) for the ones analyzed under negative electrospray ionization (NI), both purchased from Waters Corporation. Depending on the

mode of analysis, different mobile phases were used. For the analysis in PI mode methanol (eluent A) and 10 mM formic acid/ammonium formate (pH 3.2) (eluent B) at flow rate 0.5 mL/min were used. The elution gradient was: initial conditions 5% A; 0–4.5 min, 5–95% A; 4.5–4.6 min, 100% A; 4.6–6.0 min, 100% A; from 6.0 to 6.1 return to initial conditions; 6.1–6.7, equilibration of the column.For analysis in NI mode, acetonitrile (eluent A) and 5 mM ammonium acetate/ammonia (pH = 8) (B) at a flow rate of 0.6 mL/min were used. The elution gradient was: 0–1.5 min, 0–60% A; 1.5–2.0 min, 100% A; 2.0–3.0 min, 100% A; 3.20 min return to initial conditions; 3.20–3.70 min, equilibration of the column. The sample injection volume was 5 µL. Compound dependent MS parameters and source-dependent parameters were set as described in (Gros et al. 2012). All transitions were recorded by using the Scheduled MRM^{TM} algorithm. Quantification of PhACs was carried out in Multiple Reaction Monitoring (MRM) mode monitoring two transitions per analyte.

2.3. Chemicals and materials

Working standard solutions, containing all pharmaceuticals, were also prepared in methanol/water (10:90, v/v) and were renewed before each analytical run by mixing appropriate amounts of the intermediate solutions. Separate mixtures of isotopically labelled internal standards, used for internal standard calibration, and surrogates, were prepared in methanol and further dilutions were also prepared in a methanol/water (10:90, v/v) mixture. 47-mm glass fibber filters GF/F (0.7-µm of pore size) and 0.45 µm nylon membrane filters, used for pre-treatment of samples were purchased from Whatman (UK). Solid-phase extraction (SPE) was carried out with cartridges Oasis HLB (6 mL, 200 mg) from Waters (Milford, MA, USA). HPLC grade methanol, acetonitrile, water (Lichrosolv), HPLC grade methanol, acetonitrile, water (Lichrosolv) and formic acid 98% were supplied by Merck (Darmstadt, Germany). Ammonium hydroxyde, hydrochloric acid and ethylenediaminetetraacetic acid disodium salt solution (Na2EDTA) at 0.1 molL-1 were from Panreac. Nitrogen for drying was from Abelló Linde S.A. (Spain) and it was of 99.9990% purity. A Milli-Q-Advantage system from Millipore Ibérica S.A. (Spain) was used to obtain HPLC-grade water.

Table S-1. ANOVA analyses for the comparison of population densities and LSU among basins

		DF	SS	MS	F	p
log_{10}(Population density)						
	Basin	3	7.36	2.453	70.368	<0.0001
	Residuals	73	2.545	0.035		
		DF	SS	MS	F	p
log_{10}(LSU)						
	Basin	3	3.196	1.065	5.279	0.0024
	Residuals	73	14.731	0.202		

Table S-2. List of pharmaceuticals analyzed including target compounds classified by their therapeutic activity, human and/or veterinary use and approved/not approved in Spain, internal standards and surrogates; commercial providers indicated with letters (a-e) in brackets; compound name abbreviation; identification number (CAS); molecular formula; and UPLC-QqLIT-MS/MS parameters used for quantification (SRM 1) and confirmation (SRM 2 and Rt) of each compound by SRM negative ($[M-H]^-$) and positive ($[M+H]^+$) ionization.

Therapeutic group/ Compounds	Abbreviation	CAS number	Molecular formula	Precursor ion (m/z)	SRM 1	SRM 2	Rt (min)
Analgesics/anti-inflammatories	AAF						
Phenazone[7] (a)	PHEN	60-80-0	$C_{19}H_{20}N_2O_2$	189 $[M+H]^+$	77	56	2.05
Propyphenazone[7] (b)	PPHEN	479-92-5	$C_{14}H_{18}N_2O$	231 $[M+H]^+$	189	56	3.20
Oxycodone[7] (a)	OXYD	124-90-3	$C_{18}H_{21}NO_4$	316 $[M+H]^+$	298	241	1.45
Codeine[7] (a)	COD	76-57-3	$C_{18}H_{21}NO_3$	300 $[M+H]^+$	152	115	1.36
Acetaminophen[2] (a)	APAP	103-90-2	$C_8H_9NO_2$	150 $[M-H]^-$	107	-	0.56
Ibuprofen[7] (a)	IBU	15687-27-1	$C_{13}H_{18}O_2$	205 $[M-H]^-$	161	-	1.18
Indomethacine[7] (a)	INDO	53-86-1	$C_{19}H_{16}ClNO_4$	356 $[M-H]^-$	312	297	1.27
Diclofenac[1] (a)	DCF	15307-86-5	$C_{14}H_{11}Cl_2NO_2$	294 $[M-H]^-$	250	214	1.25
Ketoprofen[2] (a)	KETO	22071-15-4	$C_{16}H_{14}O_3$	253 $[M-H]^-$	209	-	1.01
Naproxen[2] (a)	NAP	22204-53-1	$C_{14}H_{14}O_3$	229 $[M-H]^-$	170	185	0.96
Piroxicam[7] (a)	PRC	36322-90-4	$C_{15}H_{13}N_3O_4S$	330 $[M-H]^-$	146	266	0.93
Meloxicam[1] (a)	MLX	71125-39-8	$C_{14}H_{13}N_3O_4S_2$	350 $[M-H]^-$	146	286	1.06
Tenoxicam[8] (a)	TX	59804-37-4	$C_{13}H_{11}N_3O_4S_2$	336 $[M-H]^-$	152	172	0.90
Lipid regulators and cholesterol lowering statin drugs	LIR						
Bezafibrate[7] (a)	BZF	41859-67-0	$C_{19}H_{20}ClNO_4$	360 $[M-H]^-$	274	154	1.10
Gemfibrozil[7] (a)	GFZ	25812-30-0	$C_{15}H_{22}O_3$	249 $[M-H]^-$	121	127	1.40
Pravastatin[7] (a)	PARA	81131-70-6	$C_{23}H_{36}O_7$	447 $[M+H]^+$	321	303	1.00
Fluvastatin[7] (b)	FLU	93957-54-1	$C_{24}H_{26}FNO_4$	410 $[M-H]^-$	210	348	1.46
Atorvastatin[7] (b)	ATV	134523-03-8	$C_{33}H_{35}FN_2O_5$	559 $[M+H]^+$	278	397	1.52

Table S-2. *(cont)*

Therapeutic group/ Compounds	Abbreviation	CAS number	Molecular formula	Precursor ion (m/z)	SRM 1	SRM 2	Rt (min)
Psychiatric drugs	PSY						
Fluoxetine[2] (a)	FLX	56296-78-7	$C_{17}H_{18}F_3NO$	310 $[M+H]^+$	44	148	3.47
Norfluoxetine (metabolite) (a)	NFLX	83891-03-6	$C_{16}H_{16}F_3NO$	296 $[M+H]^+$	134	-	2.93
Paroxetine[7] (c)	PRT	110429-35-1	$C_{19}H_{20}FNO_3$	330 $[M+H]^+$	192	123	3.26
Diazepam[1] (a)	DZP	439-14-5	$C_{16}H_{13}ClN_2O$	285 $[M+H]^+$	193	154	3.76
Lorazepam[7] (a)	LRZ	846-49-1	$C_{15}H_{10}Cl_2N_2O_2$	321 $[M+H]^+$	275	303	3.42
Alprazolam[7] (a)	APZ	28981-97-7	$C_{17}H_{13}N_4Cl$	309 $[M+H]^+$	281	205	3.43
Carbamazepine[7] (a)	CBZ	298-46-4	$C_{15}H_{12}N_2O$	237 $[M+H]^+$	194	193	3.19
Acridone (metabolite) (a)	ACRI	578-95-0	$C_{13}H_9NO$	196 $[M+H]^+$	166	167	3.00
Sertraline[7] (c)	SRT	79559-97-0	$C_{17}H_{17}NCl_2$	307 $[M+H]^+$	159	276	3.60
Citalopram[7] (a)	CTP	59729-32-7	$C_{20}H_{21}N_2FO$	325 $[M+H]^+$	109	262	2.90
Venlafaxine[7] (c)	VNFX	99300-78-4	$C_{17}H_{27}NO_2$	278 $[M+H]^+$	58	260	2.75
Olanzapine[7] (b)	OLZ	132539-06-1	$C_{17}H_{20}N_4S$	313 $[M+H]^+$	256	198	1.88
Trazodone[7] (a)	TRZ	25332-39-2	$C_{19}H_{22}ClN_5O$	372 [M+H]+	176	148	2.63
Histamine H1 and H2 receptor antagonists	HRA						
Loratadine[7] (c)	LNT	79794-75-5	$C_{22}H_{23}ClN_2O_2$	383 $[M+H]^+$	337	267	4.37
Desloratadine[7] (a)	DLNT	100643-71-8	$C_{19}H_{19}ClN_2$	311 $[M+H]^+$	259	258	3.16
Ranitidine[7] (a)	RNT	66357-59-3	$C_{13}H_{22}N_4O_3S$	315 $[M+H]^+$	176	130	1.24
Famotidine[7] (a)	FMT	76824-35-6	$C_8H_{15}N_7O_2S_3$	338 $[M+H]^+$	189	256	1.24
Cimetidine[6] (a)	CMT	51481-61-9	$C_{10}H_{16}N_6S$	253 $[M+H]^+$	159	95	1.28
β-Blocking agents	BBL						
Atenolol[7] (a)	ATN	29122-68-7	$C_{14}H_{22}N_2O_3$	267 $[M+H]^+$	145	190	1.22
Sotalol[7] (a)	STL	959-24-0	$C_{12}H_{20}N_2O_3S$	273 $[M+H]^+$	255	133	1.10
Metoprolol[7] (b)	MTPL	56392-17-7	$C_{15}H_{25}NO_3$	268 $[M+H]^+$	133	121	2.20
Propranolol[7] (a)	PRPL	318-98-9	$C_{16}H_{21}NO_2$	260 $[M+H]^+$	116	183	2.86
Nadolol[7] (a)	NDL	42200-33-9	$C_{17}H_{27}NO_4$	310 $[M+H]^+$	254	201	1.88
Carazolol[5] (a)	CRZL	57775-29-8	$C_{18}H_{22}N_2O_3$	299 $[M+H]^+$	116	222	2.52

Table S-2. *(cont)*

Therapeutic group/ Compounds	Abbreviation	CAS number	Molecular formula	Precursor ion (m/z)	SRM 1	SRM 2	Rt (min)
Diuretics	DIU						
Torasemide[7] (c)	TOR	56211-40-6	$C_{16}H_{20}N_4O_3S$	347 $[M-H]^-$	262	196	1.13
Hydrochlorothiazide[1] (a)	HCTZ	58-93-5	$C_7H_8ClN_3O_4S_2$	296 $[M-H]^-$	269	205	0.71
Furosemide[2] (a)	FUR	54-31-9	$C_{12}H_{11}ClN_2O_5S$	329 $[M-H]^-$	285	205	0.97
Antidiabetic	ATD						
Glibenclamide[7] (a)	GLB	10238-21-8	$C_{23}H_{28}ClN_3O_5S$	494 $[M+H]^+$	369	169	4.00
Antihypertensives	AHT						
Amlodipine[1] (c)	AML	111470-99-6	$C_{20}H_{25}ClN_2O_5$	409 $[M+H]^+$	238	294	3.53
Irbesartan[7] (b)	ISRT	138402-11-6	$C_{25}H_{28}N_6O$	427 $[M-H]^-$	193	399	1.28
Losartan[7] (a)	LSRT	124750-99-8	$C_{22}H_{23}ClN_6O$	421 $[M-H]^-$	127	179	1.17
Valsartan[7] (b)	VSRT	137862-53-4	$C_{24}H_{29}N_5O_3$	442 $[M-H]^-$	179	350	0.95
Antiplatelet agent	APT						
Clopidogrel[7] (a)	CLPG	135046-48-9	$C_{16}H_{16}ClNO_2S$	322 $[M+H]^+$	212	184	4.34
Prostatic hyperplasia	PHP						
Tamsulosin[7] (a)	TMSN	106463-17-6	$C_{20}H_{28}N_2O_5S$	409 $[M+H]^+$	228	200	2.45
To treat asthma	AST						
Salbutamol[7] (a)	SAL	18559-94-9	$C_{13}H_{21}NO_3$	240 $[M+H]^+$	148	122	1.20
Anticoagulant	ACG						
Warfarin[3] (a)	WARF	81-81-2	$C_{19}H_{16}O_4$	309 $[M+H]^+$	163	251	3.79
X-ray contrast agent	XCA						
Iopromide[7] (a)	IOP	73334-07-3	$C_{18}H_{24}I_3N_3O_8$	792 $[M+H]^+$	573	300	1.32
Antihelmintics	AHM						
Albendazole[1] (a)	ALB	54965-21-8	$C_{12}H_{15}N_3O_2S$	266 $[M+H]^+$	234	191	3.70
Thiabendazole[3] (a)	TALB	148-79-8	$C_{10}H_7N_3S$	202 $[M+H]^+$	175	131	2.33
Levamisole[3] (a)	LMS	16595-80-5	$C_{11}H_{12}N_2S$	205 $[M+H]^+$	178	91	1.46
Synthetic glucocorticoid	SGC						
Dexamethasone[1] (a)	DXT	50-02-2	$C_{22}H_{29}FO_5$	451 $[M-H]^-$	361	307	1.35

Table S-2. *(cont)*

Therapeutic group/ Compounds	Abbreviation	CAS number	Molecular formula	Precursor ion (m/z)	SRM 1	SRM 2	Rt (min)
Sedation and muscle relaxation	SMR						
Xylazine[4] (a)	XYL	23076-35-9	$C_{12}H_{16}N_2S$	221 $[M+H]^+$	90	77	2.11
Tranquilizers	TQL						
Azaperone[5] (a)	AZPN	1649-18-9	$C_{19}H_{22}FN_3O$	328 $[M+H]^+$	123	95	2.48
Azaperol (metabolite) (a)	AZPL	2804-05-9	$C_{19}H_{24}FN_3O$	330 $[M+H]^+$	121	78	2.25
Calcium channel blocker	CCB						
Diltiazem[7] (c)	DTZ	42399-41-7	$C_{22}H_{26}N_2O_4S$	415 $[M+H]^+$	178	109	3.13
Antibiotics	ATB						
Erythromycin[1] (a)	ERY	59319-72-1	$C_{37}H_{67}NO_{13}$	734 $[M+H]^+$	576	158	3.93
Azithromycin[7] (a)	AZY	83905-01-5	$C_{38}H_{72}N_2O_{12}$	749 $[M+H]^+$	591	116	2.75
Clarithromycin[7] (a)	CLARI	81103-11-9	$C_{38}H_{69}NO_{13}$	748 $[M+H]^+$	158	590	3.72
Tetracycline[1] (a)	TCN	64-75-5	$C_{22}H_{24}N_2O_8$	445 $[M+H]^+$	410	154	1.98
Sulfamethoxazole[1] (a)	SMX	723-46-6	$C_{10}H_{11}N_3O_3S$	254 $[M+H]^+$	92	156	1.98
Trimethoprim[1] (a)	TMP	738-70-5	$C_{14}H_{18}N_4O_3$	291 $[M+H]^+$	230	261	1.73
Metronidazole[1] (a)	MTZ	443-48-1	$C_6H_9N_3O_3$	172 $[M+H]^+$	128	82	1.24
Metronidazole-OH (metabolite) (a)	OH-MTZ	4812-40-2	$C_6H_9N_3O_4$	187 $[M+H]^+$	126	123	0.96
Ofloxacin[1] (a)	OFLX	82419-36-1	$C_{18}H_{20}FN_3O_4$	362 $[M+H]^+$	318	261	1.90
Ciprofloxacin[7] (a)	CPFX	85731-33-1	$C_{17}H_{18}FN_3O_3$	332 $[M+H]^+$	288	245	2.02
Cefalexin[1] (a)	CEF	15686-71-2	$C_{16}H_{17}N_3O_4S$	348 $[M+H]^+$	158	106	1.74
Dimetridazole[4] (a)	DMZ	551-92-8	$C_5H_7N_3O_2$	142 $[M+H]^+$	96	95	1.48
Ronidazole[4] (a)	RNZ	7681-76-7	$C_6H_8N_4O_4$	201 $[M+H]^+$	140	-	1.22
Internal standards							
Ibuprofen-d_3 (a)				208 $[M-H]^-$	164	-	1.17
Indomethacine-d4 (d)				360 $[M-H]^-$	316	-	1.26
Acetaminophen-d_4 (e)				154 $[M-H]^-$	111	-	0.55
Phenazone-d_3 (a)				192 [M+H]+	59	-	2.04
Meloxicam-d_3(a)				353 [M-H]-	289	-	1.05
Carbamazepine-d_{10} (d)				247 $[M+H]^+$	204	-	3.16

Table S-2. *(cont)*

Compounds	Abbreviation	CAS number	Molecular formula	Precursor ion (m/z)	SRM 1	SRM 2	Rt (min)
Bezafibrate-d_6(d)				366 $[M-H]^-$	280	-	1.09
Gemfibrozil-d_6(d)				255 $[M-H]^-$	121	-	1.39
Fluoxetine-d_5 (a)				315 $[M+H]^+$	44	-	3.46
Citalopram-d_4 (d)				329 $[M+H]^+$	113	-	2.89
Venlafaxine-d_6 (e)				284 $[M+H]^+$	64	-	2.74
Diazepam-d_5 (a)				290 $[M+H]^+$	198	-	3.75
Cimetidine-d_3 (d)				256 $[M+H]^+$	95	-	1.26
Atenolol-d_7 (d)				274 $[M+H]^+$	145	-	1.20
Hydrochlorothiazide-d_2 (d)				298 $[M-H]^-$	270	-	0.70
Furosemide-d_5 (e)				334 $[M-H]^-$	290	-	0.96
Amlodipine-d_4 (e)				413 $[M+H]^+$	238	-	3.25
Valsartan-d_8 (d)				442 $[M-H]^-$	179	-	0.95
Sulfamethoxazole-d_4 (e)				258 $[M+H]^+$	160	-	1.96
Warfarin-d_5 (d)				314 $[M+H]^+$	163	-	3.78
Ronidazole-d3 (a)				204 $[M+H]^+$	143	-	1.23
Dexamethasone-d4(d)				395 $[M-H]^-$	363	-	1.34
Xylazine-d_6 (a)				227 $[M+H]^+$	90	-	2.10
Azaperone-d_4 (a)				332 $[M+H]^+$	127	-	2.47
Erythromycin-N,N$^{13}C_2$ (a)				736 $[M+H]^+$	578	-	3.40
Azithromycin-d_3 (e)				752 $[M+H]^+$	594	-	2.69
Ofloxacin-d_3 (a)				365 $[M+H]^+$	160	-	1.90
Verapamil-d_6(e)				461 $[M+H]^+$	165	-	3.12
Surrogates							
Sulfadimethoxine-d_6(a)				317 [M+H]+	162		2.49
Ketoprofen-d_3(a)				256 [M-H]-	212		1.00

[1] PhACs of both human and veterinary use approved in Spain
[2] PhACs of both human and veterinary use but not approved for veterinary use in Spain
[3] PhACs of both human and veterinary use but only approved for veterinary use in Spain
[4] PhACs of only veterinary use but not approved in Spain
[5] PhACs of only veterinary use approved in Spain
[6] PhACs of both human and veterinary use but not approved for any use in Spain (cimetidine was disapproved in 2012 for human use and in 2013 for veterinary use)
[7] PhACs of only human use approved in Spain
[8] PhACs of only human use not approved in Spain

Sources:
Agencia Española del medicamento https://sinaem4.aemps.es/consavetPub/fichasTecnicas.do?metodo=detalleForm
http://www.accessdata.fda.gov/scripts/animaldrugsatfda/index.cfm?gb=1; http://www.inchem.org; http://www.drugs.com/vet/;
http://www.nlm.nih.gov/medlineplus/druginformation.html; http://www.aemps.gob.es/cima/fichasTecnicas.do?metodo=detalleForm ;
http://www.accessdata.fda.gov/scripts/animaldrugsatfda/index.cfm?gb=1

(a) Standards and isotopically labeled standards purchased from Sigma–Aldrich.
(b) Standards provided by the US Pharmacopeia (USP).
(c) Standards acquired from the European Pharmacopeia (EP).
(d) Isotopically labeled standards purchased from CDN isotopes (Quebec, Canada)
(e) Isotopically labeled standards from Toronto Research Chemicals (Ontario, Canada).

Table S-3. EC_{50} reported values, for three *in vivo* bioassays commonly used in environmental toxicology: algae, daphnia and fish (from Kuzmanovic et al., 2015).

Compound	EC_{50} algae (μgL^{-1})	EC_{50} Daphnia (μgL^{-1})	EC_{50} fish (μgL^{-1})	Ref.
Acetaminophen	134000	9200	378000	[1]
Acridone	6738	3419	7817	E
Albendazol	174	1225	2282	E
Alprazolam	1064	2845	2499	E
Amlodipine	6883	8479	4754	E
Amoxicilin	/	/	/	/
Atenolol	190000	205000	1096000	ECOTOX
Atorvastatin	/	/	/	/
Azaperol	/	/	/	/
Azaperone	833	1340	9743	E
Azithromycin	1874	3070	1970	E
Bezafibrate	18000	30000	6000	ECOTOX
Carazolol	2660	60000	2500	[2]
Carbamazepine	85000	76300	35400	ECOTOX
Cefalexin	/	/	/	/
Cimetidine	787	379000	80402	E
Ciprofloxacin	2970	60000	100000	
Citalopram	360	652	4467	E
Clarithromycin	46	3307	17364	
Clopidogrel	/	/	/	/
Codeine	1800	23000	16000	[2]
Desloratidine	26981	49307	75054	E
Dexamethasone	983	21438	23910	E
Diazepam	1249	3129	19307	E
Diclofenac	14500	22000	532000	[1]
Diltiazem	/	/	/	/
Dimetridazole	350	4272	25695	E
Erithromycin	20	30500	61500	VSDB
Famotidine	478143	314690	3594432	E
Fluoxetine	800	510	1700	E
Fluvastatin	1350	5268	287	E
Furosemide	19797	560033	521136	E
Gemfibrozil	4000	4900	900	ECOTOX
Glibenclamide	/	/	/	/
Hidrochlorothiazide	/	/	/	/
Ibuprofen	4000	34000	5000	ECOTOX
Indomethacine	18000	26000	3900	[2]
Iopromide	370000000	7660000000	8650000000	[2]
Irbesartan	/	/	/	/
Ketoprofen	164000	248000	32000	[2]
Levamisol	943	1394	175000	E

Loratidine	62	100	115	E
Lorazepam	1683	44712	49067	E
Losartan	180	2100	2151	E
Meloxicam	184	3994	1392	E
Metformin	/	/	/	/
Metoprolol	8305	9383	81557	E
Metronidazole	40400	1000000	1060000	VSDB
Metronidazole-OH	/	/	/	/
Nadolol	22538	22609	208809	E
Naproxen	137944	121543	193337	E
Norfluoxetine	/	/	/	/
Ofloxacin	2444544	31750	19352000	E
Olanzapine	52515	46786	458553	E
Oxycodone	/	/	/	/
Paroxetine	/	/	/	/
Phenazone	1100	6700	3000	[2]
Piroxicam	289	768	4220	E
Pravastatin	85494	8588	1800	E
Propanolol	/	/	/	/
Propyphenazone	1000	3500	9800	[2]
Ranitidine	66000	63000	1076000	[2]
Ronidazole	1080	19445	242023	E
Salbutamol	/	/	/	/
Sertraline	43	120	408	ECOTOX
Sotalol	/	/	/	/
Sulfamethoxazole	1900	25200	56200	[1]
Tamsulosin	/	/	/	/
Tenoxicam	/	/	/	/
Tetracycline	6000	6000	220000	[1]
Torasemide	/	/	/	/
Trazodone	396	1567	1313	E
Trimethoprim	16000	121000	795000	ECOTOX
Valsartan	3865	44337	88094	E
Venlafaxine	635	1062	7678	E
Warfarin	/	/	/	/
Xylazine	/	/	/	/

[1] M. Grung, T. Källqvist, S. Sakshaug, S. Skurtveit, K.V. Thomas, Environmental assessment of Norwegian priority pharmaceuticals based on the EMEA guideline, Ecotoxicology and Environmental Safety, 71 (2008) 328-340.
[2] H. Sanderson, D.J. Johnson, C.J. Wilson, R.A. Brain, K.R. Solomon, Probabilistic hazard assessment of environmentally occurring pharmaceuticals toxicity to fish, daphnids and algae by ECOSAR screening, Toxicology Letters, 144 (2003) 383-395.

E-ECOSAR Ecological Structure Activity Relationships

VSDB- VSDB: Veterinary Substances DataBase *http://sitem.herts.ac.uk/aeru/vsdb/index.htm*

ECOTOX-ECOTOX Database *http://cfpub.epa.gov/ecotox/*

Table S-4. Limits of detection (LOD) and quantification (LOQ) of the analytical method.

Therapeutic group	Compound	Surface water		Sediment	
		LOD	LOQ	LOD	LOQ
Analgesics/anti-inflammatories					
	Phenazone	0.04	0.14	0.03	0.11
	Propyphenazone	0.04	0.12	0.02	0.08
	Oxycodone	0.05	0.17	0.22	0.74
	Codeine	0.02	0.07	6.95	23.16
	Acetaminophen	0.04	0.13	0.02	0.06
	Ibuprofen	1.17	3.88	7.53	25.11
	Indomethacine	0.09	0.32	0.28	0.94
	Diclofenac	0.61	2.05	0.78	2.58
	Ketoprofen	0.76	2.53	1.19	3.98
	Naproxen	0.19	0.63	0.49	1.63
	Piroxicam	0.02	0.06	0.09	0.30
	Meloxicam	0.01	0.02	0.05	0.15
	Tenoxicam	0.01	0.04	0.39	1.31
Lipid regulators and cholesterol lowering statin drugs					
	Bezafibrate	0.02	0.06	0.05	0.18
	Gemfibrozil	0.04	0.14	0.04	0.13
	Pravastatin	0.12	0.39	0.18	0.61
	Fluvastatin	0.04	0.12	0.13	0.44
	Atorvastatin	0.01	0.02	0.02	0.06
Psychiatric drugs					
	Fluoxetine	0.36	1.19	0.21	0.69
	Norfluoxetine	0.50	1.68	0.08	0.28
	Paroxetine	0.16	0.53	0.03	0.10
	Diazepam	0.05	0.16	0.09	0.31
	Lorazepam	0.27	0.91	0.27	0.91
	Alprazolam	0.02	0.07	0.05	0.16
	Carbamazepine	0.01	0.04	0.04	0.12
	Acridone	0.03	0.09	0.27	0.89
	Sertraline	0.63	2.12	0.69	2.30
	Citalopram	0.02	0.06	0.14	0.46
	Venlafaxine	0.02	0.06	0.03	0.09
	Olanzapine	0.04	0.15	0.06	0.20
	Trazodone	0.03	0.09	0.05	0.18
Histamine H1 and H2 receptor antagonists					
	Loratidine	0.11	0.37	0.03	0.10
	Desloratidine	0.04	0.14	0.04	0.12
	Ranitidine	1.05	3.50	0.03	0.10
	Famotidine	0.09	0.30	0.01	0.05
	Cimetidine	0.09	0.31	0.11	0.35
β-Blocking agents					
	Atenolol	0.02	0.07	0.16	0.53
	Sotalol	0.24	0.79	0.02	0.06
	Metoprolol	0.11	0.35	0.02	0.08
	Propanolol	0.04	0.13	0.04	0.15
	Nadolol	0.07	0.22	0.04	0.14
	Carazolol	0.10	0.40	0.05	0.16

Therapeutic group	Compound	Surface water		Sediment	
		LOD	LOQ	LOD	LOQ
Diuretics					
	Torasemide	0.02	0.08	0.04	0.11
	Hidrochlorothiazide	0.05	0.16	1.80	6.01
	Furosemide	0.45	1.51	0.57	1.91
Antidiabetic					
	Glibenclamide	0.60	1.80	0.60	1.80
Antihypertensives					
	Amlodipine	0.08	0.26	0.11	0.36
	Irbesartan	0.02	0.08	0.03	0.10
	Losartan	0.10	0.34	0.14	0.47
	Valsartan	0.05	0.17	0.09	0.31
Antiplatelet agent					
	Clopidogrel	0.01	0.04	0.04	0.13
Prostatic hyperplasia					
	Tamsulosin	0.02	0.06	0.02	0.05
To treat asthma					
	Salbutamol	0.01	0.04	0.03	0.10
Anticoagulant					
	Warfarin	0.04	0.12	0.14	0.45
X-ray contrast agent					
	Iopromide	0.18	0.59	0.18	0.60
Antihelmintics					
	Albendazol	0.01	0.05	0.01	0.05
	Thiabendazole	0.02	0.06	0.09	0.29
	Levamisol	0.01	0.02	0.05	0.17
Synthetic glucocorticoid					
	Dexamethasone	0.05	0.16	0.06	0.20
Sedation and muscle relaxation					
	Xylazine	0.03	0.11	0.03	0.11
Tranquilizers					
	Azaperone	0.23	0.79	0.14	0.46
	Azaperol	0.32	1.07	0.02	0.07
Calcium channel blocker					
	Diltiazem	0.02	0.05	0.04	0.12
Antibiotics					
	Erithromycin	0.13	0.44	0.68	2.26
	Azithromycin	0.05	0.18	14.35	47.84
	Clarithromycin	0.05	0.17	7.63	25.44
	Tetracycline	3.55	11.83	3.55	11.83
	Sulfamethoxazole	0.09	0.31	0.04	0.14
	Trimethoprim	0.10	0.34	0.02	0.06
	Metronidazole	0.57	1.91	0.07	0.24
	Metronidazole-OH	0.40	1.35	0.22	0.74
	Ofloxacin	0.04	0.14	0.06	0.19
	Ciprofloxacin	0.06	0.19	0.06	0.19
	Cefalexin	0.24	0.80	0.24	0.80
	Dimetridazole	1.50	4.90	0.02	0.05
	Ronidazole	0.83	2.76	0.06	0.19

Table S-5. Maximum (Max), minimum (Min), average (Ave) and total (sum) concentrations of therapeutic groups and individual pharmaceuticals determined in surface waters from each entire river basin over sampling campaigns.

Surface water		Llobregat									
Therapeutic group	Compound	Campaign 1					Campaign 2				
		Max	Min	Ave	Med	Sum	Max	Min	Ave	Med	Sum
Analgesics/anti-inflammatories		628.67	4.45	193.88	90.94	2714.38	507.65	28.42	109.21	46.18	1528.95
	Phenazone	9.53	0.07	1.92	0.92	26.83	0.20	0.07	0.14	0.14	0.27
	Propyphenazone	24.40	0.06	3.03	1.49	42.41	0.27	0.06	0.13	0.06	0.67
	Oxycodone	4.35	0.09	0.67	0.09	9.34	1.73	0.09	0.38	0.09	2.64
	Codeine	44.07	1.08	7.61	3.31	98.97	1.53	0.18	0.65	0.44	2.60
	Acetaminophen	142.89	0.07	48.69	23.09	632.94	3.75	0.73	1.31	0.99	11.76
	Ibuprofen	179.31	1.94	70.54	34.14	846.48	7.46	1.94	3.11	1.94	37.36
	Indomethacine	33.02	0.16	5.50	1.97	71.52	63.72	0.61	12.98	3.56	116.79
	Diclofenac	129.05	1.02	26.04	11.26	312.52	280.00	1.02	35.86	4.87	501.98
	Ketoprofen	153.09	1.27	28.78	17.58	402.92	102.35	22.84	38.40	28.95	537.65
	Naproxen	50.88	0.31	22.42	18.60	268.99	90.53	0.69	27.59	9.63	303.52
	Piroxicam	0.03	0.03	0.03	0.03	0.35	3.92	0.07	1.08	0.69	11.90
	Meloxicam	0.78	0.01	0.08	0.01	0.89	1.58	0.01	0.15	0.01	1.69
	Tenoxicam	0.02	0.02	0.02	0.02	0.22	0.02	0.02	0.02	0.02	0.11
Lipid regulators and cholesterol lowering statin drugs		334.93	0.33	79.90	41.57	1118.59	302.38	2.65	70.76	14.90	990.64
	Bezafibrate	24.55	0.79	7.31	3.02	73.07	1.32	0.08	0.47	0.16	3.74
	Gemfibrozil	302.67	0.07	72.83	39.17	1019.64	301.00	2.25	70.27	14.78	983.74
	Pravastatin	7.82	0.19	1.64	0.64	22.93	0.59	0.19	0.28	0.19	1.98
	Fluvastatin	0.06	0.06	0.06	0.06	0.24	0.70	0.70	0.70	0.70	0.70
	Atorvastatin	2.26	0.01	0.25	0.01	2.71	0.21	0.03	0.10	0.06	0.48
Psychiatric drugs		430.87	1.46	95.20	51.09	1332.87	148.84	1.75	26.94	6.92	377.16
	Fluoxetine	9.46	0.59	3.06	2.35	36.77	4.21	4.21	4.21	4.21	4.21
	Norfluoxetine	3.26	0.84	1.44	0.84	18.74	ND	ND	ND	ND	ND
	Paroxetine	3.41	0.27	0.61	0.27	6.68	3.41	0.27	0.61	0.27	6.68
	Diazepam	35.51	0.08	2.77	0.08	38.73	9.10	0.08	1.87	0.72	22.42
	Lorazepam	187.87	0.46	38.90	22.52	505.66	3.28	0.46	1.61	1.27	12.84
	Alprazolam	4.98	0.04	0.48	0.04	6.72	1.04	0.04	0.20	0.09	2.62
	Carbamazepine	64.04	0.02	13.30	8.88	186.16	4.80	0.02	1.34	0.40	14.79
	Acridone	6.45	0.05	2.01	1.43	28.18	42.73	1.23	6.09	1.60	85.24
	Sertraline	144.87	1.06	25.39	1.06	152.35	42.92	2.47	16.42	3.86	49.26
	Citalopram	31.83	0.03	3.24	0.03	45.37	19.65	0.03	2.97	0.35	35.70
	Venlafaxine	127.62	0.03	21.21	7.62	296.89	26.29	0.21	3.90	1.15	54.61
	Olanzapine	1.64	0.07	0.22	0.07	2.37	5.60	0.07	2.84	2.84	5.67
	Trazodone	4.21	0.04	0.69	0.04	8.26	34.04	0.21	7.56	2.35	83.13
Histamine H1 and H2 receptor antagonists		31.57	0.55	6.67	2.67	93.44	10.60	0.31	3.44	1.82	24.07
	Loratidine	0.19	0.19	0.19	0.19	2.42	3.12	0.24	1.81	2.07	5.43
	Desloratidine	1.62	0.07	0.49	0.29	5.84	2.25	0.07	0.90	0.64	3.60
	Ranitidine	18.44	1.75	4.15	1.75	53.91	6.11	1.62	2.46	1.75	14.73
	Famotidine	0.15	0.15	0.15	0.15	1.37	ND	ND	ND	ND	ND
	Cimetidine	19.42	0.15	2.30	0.69	29.90	0.15	0.15	0.15	0.15	0.31
β-Blocking agents		414.78	0.78	66.43	5.06	929.96	315.41	0.03	25.69	0.56	334.02
	Atenolol	331.58	0.03	40.61	4.87	527.93	5.01	0.03	0.80	0.14	7.16
	Sotalol	223.81	0.40	22.50	0.40	269.96	21.11	0.40	3.98	0.40	23.85
	Metoprolol	102.44	0.18	10.24	0.18	112.64	291.67	0.18	29.80	0.18	297.97
	Propanolol	12.05	0.07	1.30	0.07	13.04	1.36	0.07	0.35	0.07	3.53
	Nadolol	1.67	0.11	0.67	0.50	4.00	0.11	0.11	0.11	0.11	0.11
	Carazolol	0.41	0.20	0.24	0.20	2.40	1.20	0.20	0.70	0.70	1.40

Surface water		Llobregat									
Therapeutic group	Compound	Campaign 1					Campaign 2				
		Max	Min	Ave	Med	Sum	Max	Min	Ave	Med	Sum
Diuretics		816.17	0.12	238.60	206.87	3340.47	459.28	1.36	88.44	25.38	1238.17
	Torasemide	9.43	0.04	2.02	1.38	26.32	0.60	0.04	0.29	0.28	2.89
	Hidrochlorothiazide	793.33	0.08	209.67	204.00	2935.32	304.67	1.36	46.90	9.80	656.55
	Furosemide	205.88	4.59	54.12	12.66	378.82	296.47	8.31	57.87	21.07	578.73
Antidiabetic	Glibenclamide	2.49	0.90	1.01	0.90	14.19	4.61	0.90	1.25	0.90	17.49
Antihypertensives		802.64	0.84	187.14	118.00	2619.92	29.84	0.13	6.64	2.76	92.95
	Amlodipine	23.52	0.31	2.89	1.06	34.65	7.82	3.54	5.27	4.44	15.80
	Irbesartan	141.10	0.04	30.30	12.74	424.22	3.34	0.04	0.72	0.21	10.04
	Losartan	126.88	0.17	36.50	15.78	438.02	6.50	0.84	2.39	1.46	26.33
	Valsartan	698.90	0.35	123.07	84.18	1723.03	20.00	0.09	2.91	0.71	40.78
Antiplatelet agent	Clopidogrel	3.58	0.02	0.58	0.18	8.11	14.49	0.13	2.12	0.42	23.36
Prostatic hyperplasia	Tamsulosin	0.67	0.03	0.25	0.03	3.24	0.22	0.03	0.13	0.13	0.25
To treat asthma	Salbutamol	16.09	0.02	2.00	0.42	21.95	0.49	0.02	0.11	0.02	1.16
Anticoagulant	Warfarin	0.06	0.06	0.06	0.06	0.54	0.22	0.06	0.14	0.14	0.28
X-ray contrast agent	Iopromide	1368.76	0.30	373.95	40.98	1869.73	0.95	0.30	0.63	0.63	1.25
Antihelmintics		50.79	0.06	8.89	5.03	124.51	11.06	2.10	4.08	3.02	57.16
	Albendazol	0.02	0.02	0.02	0.02	0.33	1.79	0.02	0.91	0.91	1.82
	Thiabendazole	12.92	0.03	1.25	0.09	17.53	7.84	2.10	3.46	2.87	48.38
	Levamisol	37.85	0.01	7.62	3.98	106.65	3.79	0.01	0.70	0.18	6.96
Synthetic glucocorticoid	Dexamethasone	4.85	0.52	2.35	2.54	16.45	2.56	1.46	2.01	2.01	4.02
Sedation and muscle relaxation	Xylazine	0.11	0.05	0.06	0.05	0.54	0.27	0.15	0.21	0.21	0.42
Tranquilizers		0.93	0.39	0.86	0.93	12.10	9.37	0.93	1.53	0.93	21.47
	Azaperone	0.39	0.39	0.39	0.39	5.13	7.18	0.39	0.88	0.39	12.31
	Azaperol	0.54	0.54	0.54	0.54	6.97	2.19	0.54	0.65	0.54	9.15
Calcium channel blocker	Diltiazem	31.80	0.16	6.28	2.47	87.97	9.92	2.74	4.19	3.02	29.30
Antibiotics		262.12	10.05	52.55	26.97	735.69	67.55	4.43	20.24	10.66	283.34
	Erithromycin	12.66	0.22	1.85	0.22	25.91	0.46	0.22	0.24	0.22	3.30
	Azithromycin	8.41	0.09	3.76	3.27	45.17	12.20	0.09	1.51	0.51	21.15
	Clarithromycin	28.33	0.09	3.48	0.09	48.72	0.77	0.09	0.17	0.09	2.36
	Tetracycline	17.01	5.92	6.92	5.92	76.17	5.92	5.92	5.92	5.92	71.00
	Sulfamethoxazole	41.51	0.16	5.72	0.16	62.97	0.38	0.16	0.28	0.32	0.85
	Trimethoprim	150.43	0.17	15.75	6.17	220.48	60.22	1.85	8.02	3.26	112.35
	Metronidazole	10.07	0.96	3.41	0.96	30.66	ND	ND	ND	ND	ND
	Metronidazole-OH	3.34	0.67	1.34	0.67	5.36	0.67	0.67	0.67	0.67	2.02
	Ofloxacin	43.55	0.07	6.87	0.98	96.23	24.30	0.07	2.86	0.07	40.08
	Ciprofloxacin	20.00	0.10	1.55	0.10	21.73	15.95	0.10	1.84	0.10	25.69
	Cefalexin	1.28	0.40	0.55	0.40	3.27	0.40	0.40	0.40	0.40	0.40
	Dimetridazole	18.39	4.93	9.78	7.94	87.98	ND	ND	ND	ND	ND
	Ronidazole	1.38	1.38	1.38	1.38	11.03	1.38	1.38	1.38	1.38	4.13

Surface water		Ebro									
Therapeutic group	Compound	Campaign 1					Campaign 2				
		Max	Min	Ave	Med	Sum	Max	Min	Ave	Med	Sum
Analgesics/anti-inflammatories		1364.11	3.96	147.52	28.64	3540.41	678.46	15.54	90.04	29.40	2161.04
	Phenazone	40.72	0.07	3.55	0.39	85.24	11.88	0.07	2.59	0.23	12.95
	Propyphenazone	102.67	0.06	6.61	0.51	152.01	26.36	0.06	1.47	0.06	27.96
	Oxycodone	25.45	0.09	2.70	0.09	59.48	4.88	0.09	0.74	0.09	12.59
	Codeine	64.41	0.49	6.23	2.69	149.57	14.31	0.03	0.94	0.03	16.97
	Acetaminophen	292.78	0.07	34.61	6.54	726.85	71.55	0.76	8.56	1.46	85.63
	Ibuprofen	867.82	1.94	65.79	6.90	1447.33	101.26	1.94	8.03	1.94	184.70
	Indomethacine	27.21	0.16	2.69	0.95	48.49	10.63	0.16	2.90	1.32	40.60
	Diclofenac	96.21	1.02	12.39	1.02	272.66	165.26	1.02	14.68	1.02	352.25
	Ketoprofen	42.84	1.27	11.84	10.43	284.14	356.79	9.40	37.03	16.30	888.74
	Naproxen	114.04	0.31	14.24	1.89	313.32	289.47	0.84	27.17	4.72	516.15
	Piroxicam	0.03	0.03	0.03	0.03	0.67	4.17	0.03	0.82	0.14	15.66
	Meloxicam	0.01	0.01	0.01	0.01	0.24	1.53	0.01	0.21	0.01	4.33
	Tenoxicam	0.02	0.02	0.02	0.02	0.39	0.90	0.02	0.36	0.02	2.51
Lipid regulators and cholesterol lowering statin drugs		353.30	0.26	34.24	7.17	821.68	113.99	2.55	18.49	6.23	443.81
	Bezafibrate	55.64	0.03	4.03	0.03	84.69	6.26	0.03	1.67	1.34	20.04
	Gemfibrozil	293.00	0.07	29.13	6.93	699.18	111.00	2.35	16.81	5.82	403.56
	Pravastatin	10.81	0.19	1.55	0.19	35.56	2.87	0.19	0.40	0.19	6.42
	Fluvastatin	0.06	0.06	0.06	0.06	0.24	0.83	0.06	0.36	0.28	1.45
	Atorvastatin	1.74	0.01	0.09	0.01	2.01	8.64	0.03	1.77	0.71	12.36
Psychiatric drugs		528.87	2.44	51.53	12.70	1236.64	170.28	1.44	24.43	3.25	586.41
	Fluoxetine	17.28	0.59	2.18	0.59	28.29	1.54	0.59	0.91	0.59	2.73
	Norfluoxetine	2.51	0.84	0.93	0.84	17.60	0.84	0.84	0.84	0.84	0.84
	Paroxetine	1.66	0.27	0.62	0.53	9.25	1.66	0.27	0.62	0.53	9.25
	Diazepam	0.08	0.08	0.08	0.08	1.87	6.69	0.08	1.55	0.23	29.54
	Lorazepam	305.62	0.46	29.60	3.98	710.34	52.13	0.46	6.44	1.19	64.36
	Alprazolam	0.74	0.04	0.07	0.04	1.60	6.63	0.04	0.63	0.11	12.67
	Carbamazepine	65.44	0.02	7.49	2.12	179.74	44.26	0.02	2.53	0.24	53.20
	Acridone	4.94	0.05	2.01	2.18	48.31	41.52	1.21	6.28	1.59	150.65
	Sertraline	1.06	1.06	1.06	1.06	14.81	1.06	1.06	1.06	1.06	4.23
	Citalopram	22.37	0.03	1.01	0.03	23.33	12.48	0.03	2.12	0.28	33.94
	Venlafaxine	110.48	0.03	8.29	1.17	198.96	44.95	0.19	3.09	0.36	74.04
	Olanzapine	0.32	0.07	0.09	0.07	1.70	ND	ND	ND	ND	ND
	Trazodone	0.04	0.04	0.04	0.04	0.83	32.64	0.04	8.39	0.63	150.98
Histamine H1 and H2 receptor antagonists		25.95	0.19	3.52	2.20	84.39	8.74	0.19	3.37	1.82	43.85
	Loratidine	5.15	0.19	0.39	0.19	9.43	5.85	0.19	2.78	2.71	22.22
	Desloratidine	0.31	0.07	0.21	0.20	1.50	1.55	0.07	1.06	1.25	5.32
	Ranitidine	20.83	1.75	2.89	1.75	63.61	3.78	1.75	2.00	1.75	16.03
	Famotidine	0.15	0.15	0.15	0.15	3.20	ND	ND	ND	ND	ND
	Cimetidine	4.49	0.15	0.51	0.15	6.66	0.29	0.29	0.29	0.29	0.29
β-Blocking agents		665.89	0.30	33.88	1.12	813.03	183.34	0.03	10.37	0.53	207.38
	Atenolol	605.26	0.03	30.19	0.19	724.66	160.48	0.03	10.91	0.09	163.62
	Sotalol	52.86	0.40	2.90	0.40	63.72	18.41	0.40	3.40	0.40	20.40
	Metoprolol	4.58	0.18	0.51	0.18	10.67	2.18	0.18	0.55	0.18	7.65
	Propanolol	5.04	0.07	0.33	0.07	7.26	1.52	0.07	0.46	0.07	6.89
	Nadolol	0.68	0.11	0.29	0.19	1.72	0.11	0.11	0.11	0.11	0.22
	Carazolol	0.61	0.20	0.23	0.20	5.00	2.69	0.67	1.72	1.94	8.61

Surface water		Ebro									
Therapeutic group	Compound	Campaign 1					Campaign 2				
		Max	Min	Ave	Med	Sum	Max	Min	Ave	Med	Sum
Diuretics		460.38	0.12	85.18	38.73	2044.39	1147.42	1.55	74.29	6.67	1782.97
	Torasemide	17.68	0.04	1.81	0.04	43.47	1.34	0.04	0.32	0.08	4.10
	Hidrochlorothiazide	318.00	0.08	72.22	27.23	1733.30	1146.67	1.17	61.33	3.13	1471.81
	Furosemide	124.71	0.76	26.76	12.70	267.63	132.94	0.76	19.19	4.68	307.06
Antidiabetic	Glibenclamide	1.80	0.90	0.94	0.90	22.50	3.57	0.90	1.19	0.90	28.63
Antihypertensives		457.52	1.58	94.73	28.73	2273.41	959.79	0.13	43.65	2.61	1047.57
	Amlodipine	2.53	0.13	0.87	0.74	20.83	4.86	4.04	4.45	4.46	13.36
	Irbesartan	68.49	0.04	8.58	0.15	205.91	695.89	0.04	30.92	0.13	711.13
	Losartan	220.63	0.17	26.48	4.21	529.53	145.00	0.96	10.71	1.69	171.39
	Valsartan	261.54	0.09	63.21	24.62	1517.14	118.90	0.09	6.32	0.61	151.68
Antiplatelet agent	Clopidogrel	1.84	0.02	0.16	0.02	3.43	8.09	0.02	2.28	0.33	41.00
Prostatic hyperplasia	Tamsulosin	1.61	0.03	0.16	0.03	3.75	2.17	0.32	1.25	1.25	2.49
To treat asthma	Salbutamol	11.91	0.02	1.38	0.16	26.13	9.96	0.02	0.64	0.04	12.17
Anticoagulant	Warfarin	0.06	0.06	0.06	0.06	1.20	0.16	0.06	0.07	0.06	0.52
X-ray contrast agent	Iopromide	320.24	0.30	26.32	1.69	552.65	51.53	0.30	25.91	25.91	51.83
Antihelmintics		135.56	0.06	18.03	3.03	432.73	79.86	2.15	10.95	3.21	262.70
	Albendazol	0.07	0.02	0.03	0.02	0.61	1.91	0.02	0.54	0.02	6.49
	Thiabendazole	129.20	0.03	12.70	0.03	304.92	78.00	2.12	9.94	3.14	238.56
	Levamisol	46.77	0.01	5.30	0.82	127.20	12.38	0.01	1.60	0.23	17.65
Synthetic glucocorticoid	Dexamethasone	2.59	0.48	1.98	2.31	25.69	2.71	1.30	2.06	2.31	12.37
Sedation and muscle relaxation	Xylazine	0.05	0.05	0.05	0.05	0.97	1.63	0.34	0.78	0.58	3.13
Tranquilizers		0.93	0.93	0.93	0.93	22.34	9.00	0.39	2.11	0.93	50.73
	Azaperone	0.39	0.39	0.39	0.39	9.48	6.52	0.39	1.21	0.39	29.06
	Azaperol	0.54	0.54	0.54	0.54	12.87	4.25	0.54	0.94	0.54	21.68
Calcium channel blocker	Diltiazem	42.60	0.03	3.32	1.31	73.14	16.82	2.96	6.34	3.44	31.70
Antibiotics		346.32	2.63	31.62	13.76	758.83	383.58	2.41	25.65	8.72	615.69
	Erithromycin	18.58	0.22	1.29	0.22	29.66	16.28	0.22	1.17	0.22	19.81
	Azithromycin	153.72	0.42	9.09	2.50	218.21	151.90	0.09	7.28	0.57	174.81
	Clarithromycin	65.63	0.09	3.06	0.09	73.46	64.79	0.09	2.83	0.09	67.90
	Tetracycline	27.40	5.92	6.94	5.92	145.73	5.92	5.92	5.92	5.92	112.41
	Sulfamethoxazole	30.71	0.16	2.14	0.16	44.97	4.21	0.16	0.88	0.16	5.31
	Trimethoprim	15.00	0.17	1.49	0.17	35.76	26.61	1.34	2.99	1.89	71.85
	Metronidazole	0.96	0.96	0.96	0.96	15.31	49.13	10.20	29.67	29.67	59.33
	Metronidazole-OH	2.34	0.67	0.95	0.67	5.71	2.26	2.26	2.26	2.26	2.26
	Ofloxacin	17.45	0.07	1.08	0.07	25.99	62.00	0.07	3.66	0.07	87.83
	Ciprofloxacin	16.34	0.10	1.12	0.10	26.86	ND	ND	ND	ND	ND
	Cefalexin	1.28	0.40	0.52	0.40	6.28	0.63	0.63	0.63	0.63	0.63
	Dimetridazole	47.41	2.45	6.06	2.45	121.23	8.02	8.02	8.02	8.02	8.02
	Ronidazole	1.38	1.38	1.38	1.38	9.65	1.38	1.38	1.38	1.38	5.51

Surface water		Júcar									
Therapeutic group	Compound	Campaign 1					Campaign 2				
		Max	Min	Ave	Med	Sum	Max	Min	Ave	Med	Sum
Analgesics/anti-inflammatories		29.27	5.58	11.73	9.36	164.24	101.67	9.97	27.99	15.57	419.84
	Phenazone	0.07	0.07	0.07	0.07	0.76	5.49	5.49	5.49	5.49	5.49
	Propyphenazone	0.06	0.06	0.06	0.06	0.06	9.60	9.60	9.60	9.60	9.60
	Oxycodone	0.28	0.09	0.14	0.09	1.56	2.74	0.09	0.57	0.11	3.99
	Codeine	2.82	1.18	1.79	1.29	21.52	5.33	0.03	0.56	0.03	6.12
	Acetaminophen	0.68	0.07	0.32	0.28	1.62	6.47	1.04	2.24	1.17	11.18
	Ibuprofen	3.91	1.94	2.14	1.94	21.39	5.10	1.94	2.57	1.94	12.87
	Indomethacine	1.55	0.55	0.80	0.69	9.65	2.95	0.16	0.55	0.16	7.21
	Diclofenac	10.13	1.02	2.23	1.02	31.28	11.26	1.02	4.28	2.78	64.17
	Ketoprofen	6.37	1.27	2.87	2.71	40.15	55.80	3.96	15.57	9.86	233.48
	Naproxen	12.21	0.31	2.95	1.86	35.36	17.14	0.31	3.59	2.18	43.10
	Piroxicam	0.23	0.03	0.10	0.08	0.58	5.06	0.40	1.09	0.55	16.34
	Meloxicam	0.05	0.02	0.03	0.02	0.09	4.00	0.01	0.29	0.01	4.31
	Tenoxicam	0.02	0.02	0.02	0.02	0.22	1.59	0.02	0.16	0.02	1.97
Lipid regulators and cholesterol lowering statin drugs		23.51	0.47	5.01	3.77	70.10	17.08	0.45	5.37	3.03	80.48
	Bezafibrate	0.85	0.85	0.85	0.85	1.70	3.31	0.03	0.71	0.07	6.42
	Gemfibrozil	22.10	0.28	4.03	2.46	56.38	9.80	0.19	3.89	2.76	58.35
	Pravastatin	0.56	0.19	0.25	0.19	3.50	1.46	0.19	0.29	0.19	3.79
	Fluvastatin	0.99	0.89	0.92	0.90	4.58	4.19	0.06	2.17	1.67	10.86
	Atorvastatin	0.82	0.01	0.39	0.39	3.94	0.87	0.01	0.13	0.01	1.06
Psychiatric drugs		9.56	1.14	3.98	3.73	55.66	52.04	1.58	10.96	4.31	164.36
	Fluoxetine	0.59	0.59	0.59	0.59	5.95	1.51	1.51	1.51	1.51	1.51
	Norfluoxetine	0.84	0.84	0.84	0.84	10.90	ND	ND	ND	ND	ND
	Paroxetine	1.41	0.27	0.72	0.70	7.24	1.41	0.27	0.72	0.70	7.24
	Diazepam	1.37	0.08	0.31	0.08	2.77	4.57	0.08	1.48	0.46	10.33
	Lorazepam	0.98	0.46	0.63	0.46	6.96	6.25	0.46	1.46	0.95	13.11
	Alprazolam	0.04	0.04	0.04	0.04	0.52	4.61	0.04	0.47	0.09	6.10
	Carbamazepine	0.15	0.02	0.04	0.02	0.52	9.68	0.02	1.33	0.21	11.93
	Acridone	0.31	0.05	0.15	0.16	1.19	18.15	1.18	3.23	1.34	48.40
	Sertraline	1.06	1.06	1.06	1.06	10.58	1.06	1.06	1.06	1.06	1.06
	Citalopram	1.35	0.03	0.30	0.09	2.68	3.23	0.03	0.60	0.29	7.77
	Venlafaxine	3.05	0.03	0.43	0.03	5.99	7.24	0.20	1.29	0.47	19.35
	Olanzapine	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07	0.07
	Trazodone	0.11	0.04	0.06	0.04	0.29	21.84	0.04	3.41	2.20	37.49
Histamine H1 and H2 receptor antagonists		2.62	0.34	1.94	2.09	27.09	9.73	0.15	2.52	1.75	27.67
	Loratidine	0.19	0.19	0.19	0.19	1.68	2.85	2.17	2.51	2.51	5.02
	Desloratidine	0.57	0.49	0.53	0.53	1.06	0.79	0.58	0.68	0.68	1.37
	Ranitidine	1.75	1.75	1.75	1.75	21.00	1.75	1.75	1.75	1.75	15.75
	Famotidine	0.15	0.15	0.15	0.15	1.52	1.25	0.15	0.52	0.15	1.55
	Cimetidine	0.15	0.15	0.15	0.15	1.83	3.98	3.98	3.98	3.98	3.98
β-Blocking agents		1.28	0.03	0.63	0.50	8.14	13.86	0.03	1.95	0.14	17.57
	Atenolol	0.09	0.03	0.04	0.03	0.44	4.24	0.03	0.59	0.08	4.75
	Sotalol	0.40	0.40	0.40	0.40	4.37	3.13	0.40	1.08	0.40	4.32
	Metoprolol	ND	ND	ND	ND	ND	1.89	0.18	1.03	1.03	2.06
	Propanolol	0.68	0.07	0.40	0.52	3.22	0.93	0.29	0.61	0.61	1.22
	Nadolol	0.11	0.11	0.11	0.11	0.11	2.62	0.11	1.36	1.36	2.73
	Carazolol	ND	ND	ND	ND	ND	1.43	1.06	1.25	1.25	2.49

Surface water		Júcar									
Therapeutic group	Compound	Campaign 1					Campaign 2				
		Max	Min	Ave	Med	Sum	Max	Min	Ave	Med	Sum
Diuretics		33.88	0.12	4.32	0.88	60.54	54.81	0.12	7.44	1.65	111.62
	Torasemide	0.04	0.04	0.04	0.04	0.41	20.41	0.04	1.68	0.04	21.88
	Hidrochlorothiazide	25.53	0.08	2.69	0.08	37.67	34.40	0.08	4.94	1.63	74.13
	Furosemide	8.31	0.76	1.87	0.76	22.47	8.28	0.76	3.90	3.28	15.60
Antidiabetic	Glibenclamide	0.90	0.90	0.90	0.90	12.60	4.06	0.90	1.21	0.90	18.19
Antihypertensives		21.56	5.13	10.98	11.24	107.61	85.82	0.45	7.66	1.40	114.96
	Amlodipine	3.23	1.84	2.37	2.05	33.13	ND	ND	ND	ND	ND
	Irbesartan	7.18	0.75	1.55	0.98	18.60	27.40	0.18	2.58	0.51	38.64
	Losartan	7.88	1.85	5.79	7.13	40.51	21.06	1.12	5.43	1.75	27.14
	Valsartan	3.27	0.70	1.28	1.08	15.37	37.36	0.60	3.78	0.85	49.18
Antiplatelet agent	Clopidogrel	0.98	0.02	0.45	0.37	5.79	6.51	0.02	1.43	0.24	11.46
Prostatic hyperplasia	Tamsulosin	0.11	0.03	0.06	0.03	0.73	5.28	0.03	0.80	0.03	5.63
To treat asthma	Salbutamol	0.09	0.02	0.03	0.02	0.21	13.09	0.69	1.68	0.73	21.88
Anticoagulant	Warfarin	0.06	0.06	0.06	0.06	0.36	2.47	2.47	2.47	2.47	2.47
X-ray contrast agent	Iopromide	2.38	0.30	0.82	0.30	3.27	17.61	0.30	4.27	1.17	21.37
Antihelmintics		64.91	0.06	6.90	0.90	95.36	102.91	2.08	20.00	2.62	299.99
	Albendazol	0.44	0.02	0.17	0.02	2.34	1.41	0.02	0.24	0.02	3.12
	Thiabendazole	63.60	0.03	6.31	0.28	88.40	102.80	2.08	15.28	2.44	229.14
	Levamisol	0.88	0.01	0.42	0.60	4.61	63.48	0.01	7.53	0.11	67.73
Synthetic glucocorticoid	Dexamethasone	2.01	1.26	1.55	1.33	18.59	4.35	1.34	2.39	2.29	28.69
Sedation and muscle relaxation	Xylazine	0.20	0.05	0.06	0.05	0.90	1.39	0.34	0.87	0.87	1.73
Tranquilizers		0.93	0.39	0.85	0.93	11.96	7.28	0.93	1.76	0.93	26.44
	Azaperone	0.39	0.39	0.39	0.39	5.53	5.30	0.39	0.93	0.39	14.01
	Azaperol	0.54	0.54	0.54	0.54	6.43	3.70	0.54	0.83	0.54	12.42
Calcium channel blocker	Diltiazem	0.14	0.03	0.05	0.04	0.55	4.58	3.82	4.20	4.20	8.40
Antibiotics		13.02	7.58	11.28	11.29	157.90	112.71	1.67	24.99	9.57	374.84
	Erithromycin	0.46	0.22	0.29	0.22	3.77	4.63	0.22	1.10	0.22	5.50
	Azithromycin	3.06	0.78	1.78	2.22	24.92	33.22	0.09	2.72	0.19	38.02
	Clarithromycin	0.18	0.09	0.09	0.09	1.03	5.35	0.09	1.90	0.25	5.69
	Tetracycline	5.92	5.92	5.92	5.92	76.92	5.92	5.92	5.92	5.92	53.25
	Sulfamethoxazole	0.16	0.16	0.16	0.16	1.26	0.93	0.16	0.35	0.16	1.40
	Trimethoprim	0.49	0.17	0.24	0.17	3.33	5.39	1.51	2.00	1.78	30.05
	Metronidazole	0.96	0.96	0.96	0.96	13.40	65.93	9.87	22.40	10.13	112.00
	Metronidazole-OH	0.67	0.67	0.67	0.67	0.67	ND	ND	ND	ND	ND
	Ofloxacin	0.07	0.07	0.07	0.07	0.97	109.50	0.07	7.36	0.07	110.47
	Ciprofloxacin	1.28	0.10	0.37	0.19	2.96	ND	ND	ND	ND	ND
	Cefalexin	0.40	0.40	0.40	0.40	0.80	1.40	1.40	1.40	1.40	1.40
	Dimetridazole	2.45	2.45	2.45	2.45	19.60	2.45	2.45	2.45	2.45	2.45
	Ronidazole	1.38	1.38	1.38	1.38	8.27	7.72	1.38	2.44	1.38	14.61

Surface water		Guadalquivir									
Therapeutic group	Compound	Campaign 1					Campaign 2				
		Max	Min	Ave	Med	Sum	Max	Min	Ave	Med	Sum
Analgesics/anti-inflammatories		54.96	2.67	18.77	11.38	450.47	190.87	5.03	63.19	55.44	1516.50
	Phenazone	0.35	0.07	0.08	0.07	1.59	1.11	0.04	0.28	0.10	3.41
	Propyphenazone	0.06	0.06	0.06	0.06	0.37	0.15	0.06	0.08	0.06	0.73
	Oxycodone	0.43	0.09	0.27	0.24	4.32	0.09	0.09	0.09	0.09	1.28
	Codeine	2.99	1.17	1.92	1.38	32.71	2.81	0.03	1.53	1.66	30.56
	Acetaminophen	1.19	0.07	0.37	0.07	3.65	1.85	0.07	0.36	0.25	5.73
	Ibuprofen	12.87	1.94	3.26	1.94	45.71	6.97	1.94	2.19	1.94	43.86
	Indomethacine	3.67	0.16	1.29	0.91	23.19	137.44	0.16	25.20	4.83	403.20
	Diclofenac	26.32	1.02	3.40	1.02	81.61	59.58	1.02	12.89	6.20	309.27
	Ketoprofen	7.15	1.27	2.64	1.27	63.44	19.75	3.43	6.08	5.73	146.00
	Naproxen	39.12	0.31	8.04	2.82	193.05	115.79	2.18	30.03	20.35	570.62
	Piroxicam	0.10	0.03	0.03	0.03	0.56	0.03	0.03	0.03	0.03	0.56
	Meloxicam	0.02	0.01	0.01	0.01	0.09	0.12	0.01	0.06	0.06	1.01
	Tenoxicam	0.02	0.01	0.02	0.02	0.17	0.06	0.02	0.03	0.02	0.27
Lipid regulators and cholesterol lowering statin drugs		60.57	0.57	11.91	4.32	285.76	175.09	1.07	41.62	20.34	998.92
	Bezafibrate	0.90	0.03	0.31	0.03	2.77	0.82	0.01	0.45	0.66	6.70
	Gemfibrozil	60.33	0.34	11.46	4.17	275.09	175.00	1.06	40.98	19.19	983.62
	Pravastatin	0.53	0.19	0.22	0.19	4.88	0.88	0.19	0.40	0.43	7.24
	Fluvastatin	1.01	0.06	0.69	0.99	2.06	0.39	0.05	0.20	0.16	0.60
	Atorvastatin	0.77	0.01	0.06	0.01	0.96	0.12	0.01	0.05	0.04	0.77
Psychiatric drugs		11.71	2.24	5.00	3.83	120.12	48.41	0.94	12.12	10.37	290.77
	Fluoxetine	1.20	0.59	0.62	0.59	13.69	4.20	0.59	0.77	0.59	16.09
	Norfluoxetine	0.84	0.84	0.84	0.84	18.44	ND	ND	ND	ND	ND
	Paroxetine	0.95	0.27	0.68	0.75	6.76	0.27	0.27	0.27	0.27	0.80
	Diazepam	0.37	0.08	0.15	0.08	3.10	2.85	0.08	0.71	0.55	15.63
	Lorazepam	2.36	0.46	0.72	0.46	13.72	6.70	0.46	1.93	1.84	44.41
	Alprazolam	0.09	0.04	0.04	0.04	0.95	0.61	0.04	0.33	0.35	7.22
	Carbamazepine	1.01	0.02	0.10	0.02	2.32	1.29	0.02	0.15	0.06	3.52
	Acridone	6.48	0.05	1.34	0.05	25.43	4.82	0.05	1.28	0.83	30.72
	Sertraline	1.06	1.06	1.06	1.06	25.40	2.22	2.22	2.22	2.22	2.22
	Citalopram	0.08	0.03	0.03	0.03	0.51	14.64	0.03	3.56	0.42	74.84
	Venlafaxine	1.15	0.03	0.16	0.03	3.76	1.98	0.03	0.32	0.03	7.67
	Olanzapine	0.07	0.07	0.07	0.07	0.44	0.40	0.07	0.11	0.07	1.37
	Trazodone	3.27	0.03	0.56	0.04	5.61	40.04	0.04	3.75	0.04	86.26
Histamine H1 and H2 receptor antagonists		13.93	3.75	6.96	5.59	167.05	120.44	3.07	19.73	16.60	473.46
	Loratidine	0.19	0.11	0.14	0.11	3.28	10.40	0.19	1.01	0.19	14.08
	Desloratidine	0.04	0.04	0.04	0.04	1.03	6.34	0.07	1.26	0.21	7.58
	Ranitidine	1.75	1.05	1.52	1.75	36.40	49.72	1.75	4.11	1.75	98.53
	Famotidine	0.15	0.09	0.13	0.15	3.17	17.61	0.15	0.89	0.15	21.30
	Cimetidine	0.15	0.09	0.13	0.15	3.06	33.76	0.15	2.17	0.15	41.22
β-Blocking agents		1.10	0.03	0.54	0.63	9.18	2.99	0.40	0.92	0.61	22.08
	Atenolol	0.25	0.03	0.06	0.03	0.97	1.43	0.03	0.27	0.15	6.27
	Sotalol	0.40	0.40	0.40	0.40	5.56	0.79	0.40	0.43	0.40	10.23
	Metoprolol	ND	ND	ND	ND	ND	0.38	0.18	0.19	0.18	2.32
	Propanolol	0.60	0.07	0.13	0.07	1.06	0.99	0.07	0.29	0.18	2.63
	Nadolol	ND	ND	ND	ND	ND	0.11	0.11	0.11	0.11	0.44
	Carazolol	0.20	0.20	0.20	0.20	1.59	0.20	0.20	0.20	0.20	0.20

Surface water		Guadalquivir									
Therapeutic group	Compound	Campaign 1					Campaign 2				
		Max	Min	Ave	Med	Sum	Max	Min	Ave	Med	Sum
Diuretics		94.17	0.84	10.10	3.03	242.29	96.26	3.34	29.67	23.99	712.12
	Torasemide	0.04	0.04	0.04	0.04	0.85	0.79	0.04	0.56	0.67	11.12
	Hidrochlorothiazide	77.33	0.08	5.26	0.08	126.29	51.47	2.58	16.31	10.80	391.48
	Furosemide	18.64	0.76	4.80	2.80	115.15	50.12	0.76	15.48	12.85	309.51
Antidiabetic	Glibenclamide	0.90	0.90	0.90	0.90	21.60	0.90	0.90	0.90	0.90	21.60
Antihypertensives		43.50	0.43	5.43	3.94	130.32	8.77	2.05	4.58	4.47	109.98
	Amlodipine	3.21	0.13	1.49	2.00	35.86	2.55	0.13	1.32	2.22	22.48
	Irbesartan	12.49	0.04	1.20	0.75	28.76	2.42	0.75	1.38	1.19	33.01
	Losartan	7.88	0.17	1.08	0.17	10.80	0.17	0.17	0.17	0.17	4.07
	Valsartan	30.11	0.09	2.61	0.93	54.89	4.46	1.01	2.10	2.01	50.42
Antiplatelet agent	Clopidogrel	1.14	0.02	0.17	0.02	3.27	1.43	0.02	0.58	0.34	12.14
Prostatic hyperplasia	Tamsulosin	0.21	0.03	0.05	0.03	0.98	ND	ND	ND	ND	ND
To treat asthma	Salbutamol	0.86	0.02	0.10	0.02	1.56	0.02	0.02	0.02	0.02	0.49
Anticoagulant	Warfarin	0.06	0.06	0.06	0.06	1.08	0.13	0.06	0.07	0.06	0.43
X-ray contrast agent	Iopromide	1.57	0.30	0.80	0.74	6.40	6.41	0.30	1.86	1.56	39.00
Antihelmintics		3.01	0.05	0.50	0.07	11.93	1.90	0.03	0.36	0.27	8.71
	Albendazol	0.58	0.02	0.09	0.02	2.11	0.08	0.01	0.04	0.02	0.61
	Thiabendazole	0.69	0.03	0.10	0.03	2.50	1.69	0.03	0.17	0.03	3.97
	Levamisol	2.96	0.01	0.39	0.01	7.33	0.46	0.01	0.21	0.20	4.13
Synthetic glucocorticoid	Dexamethasone	2.40	1.27	1.45	1.32	27.52	0.20	0.08	0.09	0.08	0.83
Sedation and muscle relaxation	Xylazine	0.21	0.05	0.06	0.05	1.45	0.05	0.05	0.05	0.05	0.76
Tranquilizers		0.93	0.39	0.89	0.93	21.27	0.93	0.93	0.93	0.93	22.34
	Azaperone	0.39	0.39	0.39	0.39	9.48	0.39	0.39	0.39	0.39	9.48
	Azaperol	0.54	0.54	0.54	0.54	11.79	0.54	0.54	0.54	0.54	12.87
Calcium channel blocker	Diltiazem	0.05	0.03	0.03	0.03	0.25	3.86	0.10	1.83	2.36	42.01
Antibiotics		15.35	4.15	10.98	10.43	263.47	31.77	13.84	21.72	21.90	521.23
	Erithromycin	0.45	0.22	0.24	0.22	5.69	1.81	0.22	0.73	0.22	17.53
	Azithromycin	2.26	0.80	2.00	2.19	48.10	7.62	1.22	5.21	7.02	124.99
	Clarithromycin	0.09	0.09	0.09	0.09	1.80	0.70	0.09	0.25	0.09	5.93
	Tetracycline	5.92	5.92	5.92	5.92	136.08	5.92	5.92	5.92	5.92	142.00
	Sulfamethoxazole	0.16	0.16	0.16	0.16	2.68	1.07	0.16	0.47	0.44	11.39
	Trimethoprim	1.66	0.17	0.26	0.17	5.93	4.78	0.84	2.59	2.91	62.25
	Metronidazole	0.96	0.96	0.96	0.96	15.31	10.07	2.00	4.35	3.74	69.65
	Metronidazole-OH	0.67	0.67	0.67	0.67	2.70	4.08	0.67	2.20	0.67	33.06
	Ofloxacin	0.07	0.07	0.07	0.07	1.67	ND	ND	ND	ND	ND
	Ciprofloxacin	0.53	0.10	0.13	0.10	3.17	2.68	0.26	0.94	0.78	21.58
	Cefalexin	0.40	0.40	0.40	0.40	5.58	ND	ND	ND	ND	ND
	Dimetridazole	2.45	2.45	2.45	2.45	19.60	3.56	2.45	3.00	3.00	6.01
	Ronidazole	1.38	1.38	1.38	1.38	15.16	5.76	1.38	2.98	1.38	26.85

Table S-6. Maximum (Max), minimum (Min), average (Ave) and total (sum) concentrations of therapeutic groups and individual pharmaceuticals determined in sediments from each entire river basin over sampling campaigns.

Sediment		Llobregat									
Therapeutic group	Compound	Campaign 1					Campaign 2				
		Max	Min	Average	Median	Sum	Max	Min	Average	Median	Sum
Analgesics/anti-inflammatories		37.20	4.50	22.97	22.30	321.60	25.71	9.02	15.08	11.82	211.06
	Phenazone	0.06	0.06	0.06	0.06	0.28	0.06	0.06	0.06	0.06	0.17
	Propyphenazone	0.04	0.04	0.04	0.04	0.50	0.04	0.04	0.04	0.04	0.19
	Oxycodone	0.37	0.37	0.37	0.37	5.17	0.37	0.37	0.37	0.37	5.17
	Codeine	11.58	11.58	11.58	11.58	127.39	11.58	11.58	11.58	11.58	57.90
	Acetaminophen	1.22	0.25	0.45	0.29	2.67	2.28	0.98	1.49	1.48	20.87
	Ibuprofen	12.56	12.56	12.56	12.56	87.90	ND	ND	ND	ND	ND
	Indomethacine	0.47	0.47	0.47	0.47	5.15	1.54	0.47	0.60	0.47	4.82
	Diclofenac	1.29	1.29	1.29	1.29	18.09	1.29	1.29	1.29	1.29	18.09
	Ketoprofen	10.14	1.99	4.59	3.15	64.33	10.49	4.92	7.13	7.10	99.88
	Naproxen	0.82	0.82	0.82	0.82	3.26	0.82	0.82	0.82	0.82	0.82
	Piroxicam	0.15	0.15	0.15	0.15	1.93	0.15	0.15	0.15	0.15	2.08
	Meloxicam	0.08	0.08	0.08	0.08	1.00	0.08	0.08	0.08	0.08	1.07
	Tenoxicam	0.66	0.66	0.66	0.66	3.94	ND	ND	ND	ND	ND
Lipid regulators and cholesterol lowering statin drugs		2.34	0.37	0.76	0.62	10.64	6.08	0.37	1.02	0.49	14.33
	Bezafibrate	0.09	0.09	0.09	0.09	0.63	ND	ND	ND	ND	ND
	Gemfibrozil	1.92	0.07	0.30	0.07	4.17	1.25	0.07	0.20	0.07	2.74
	Pravastatin	0.30	0.30	0.30	0.30	3.63	0.30	0.30	0.30	0.30	4.24
	Fluvastatin	0.22	0.22	0.22	0.22	1.97	4.53	0.22	1.04	0.22	7.26
	Atorvastatin	0.03	0.03	0.03	0.03	0.23	0.03	0.03	0.03	0.03	0.09
Psychiatric drugs		127.97	2.35	18.02	7.76	252.29	21.81	1.36	5.26	3.58	73.63
	Fluoxetine	0.34	0.34	0.34	0.34	4.82	0.34	0.34	0.34	0.34	4.82
	Norfluoxetine	0.14	0.14	0.14	0.14	1.67	ND	ND	ND	ND	ND
	Paroxetine	0.19	0.05	0.07	0.05	0.95	0.32	0.05	0.20	0.25	1.19
	Diazepam	0.34	0.16	0.17	0.16	2.38	0.32	0.16	0.18	0.16	1.58
	Lorazepam	0.45	0.45	0.45	0.45	5.00	0.45	0.45	0.45	0.45	0.91
	Alprazolam	0.30	0.08	0.11	0.08	0.95	0.08	0.08	0.08	0.08	0.49
	Carbamazepine	0.12	0.06	0.07	0.06	0.67	0.06	0.06	0.06	0.06	0.19
	Acridone	13.43	0.44	4.68	3.75	65.58	14.38	0.44	3.05	2.03	42.64
	Sertraline	119.28	1.15	12.08	1.15	144.94	4.30	1.15	1.54	1.15	12.36
	Citalopram	7.79	0.23	1.21	0.40	16.88	0.23	0.23	0.23	0.23	0.91
	Venlafaxine	1.94	0.05	0.25	0.05	3.54	0.05	0.05	0.05	0.05	0.38
	Olanzapine	0.10	0.10	0.10	0.10	0.50	ND	ND	ND	ND	ND
	Trazodone	1.54	0.09	0.31	0.09	4.39	5.03	0.09	0.58	0.09	8.17
Histamine H1 and H2 receptor antagonists		1.37	0.05	0.51	0.37	7.10	5.06	0.35	1.74	1.76	24.38
	Loratidine	0.57	0.05	0.18	0.12	2.48	3.21	0.05	0.42	0.20	5.82
	Desloratidine	0.57	0.06	0.29	0.25	2.94	1.90	0.21	1.23	1.45	15.94
	Ranitidine	0.11	0.05	0.09	0.10	0.62	0.05	0.05	0.05	0.05	0.15
	Famotidine	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Cimetidine	0.18	0.18	0.18	0.18	1.06	0.18	0.18	0.18	0.18	2.47
β-Blocking agents		2.14	0.35	0.84	0.60	11.74	1.20	0.15	0.61	0.69	8.56
	Atenolol	0.27	0.27	0.27	0.27	2.67	0.27	0.27	0.27	0.27	1.07
	Sotalol	0.06	0.03	0.04	0.03	0.15	ND	ND	ND	ND	ND
	Metoprolol	0.54	0.04	0.16	0.04	0.66	0.15	0.04	0.05	0.04	0.38
	Propanolol	2.04	0.26	0.53	0.37	6.39	0.37	0.07	0.18	0.20	2.49
	Nadolol	0.07	0.07	0.07	0.07	0.63	0.07	0.07	0.07	0.07	0.77
	Carazolol	0.27	0.08	0.16	0.15	1.24	0.51	0.08	0.30	0.37	3.86

Sediment		Llobregat									
		Campaign 1					Campaign 2				
Therapeutic group	Compound	Max	Min	Average	Median	Sum	Max	Min	Average	Median	Sum
Diuretics		4.47	3.96	4.07	4.01	56.96	4.34	3.30	3.90	3.96	54.57
	Torasemide	0.50	0.05	0.12	0.05	1.49	0.59	0.27	0.35	0.32	3.88
	Hidrochlorothiazide	3.01	3.01	3.01	3.01	42.08	3.01	3.01	3.01	3.01	42.08
	Furosemide	0.96	0.96	0.96	0.96	13.39	0.96	0.96	0.96	0.96	8.61
Antidiabetic	Glibenclamide	0.90	0.90	0.90	0.90	11.70	ND	ND	ND	ND	ND
Antihypertensives		8.07	0.20	1.41	0.44	19.79	0.54	0.05	0.23	0.24	2.56
	Amlodipine	0.57	0.18	0.24	0.18	1.65	ND	ND	ND	ND	ND
	Irbesartan	0.71	0.05	0.24	0.19	3.08	0.15	0.05	0.07	0.05	0.39
	Losartan	0.24	0.24	0.24	0.24	1.42	0.24	0.24	0.24	0.24	1.19
	Valsartan	7.36	0.15	0.97	0.15	13.63	0.37	0.15	0.24	0.23	0.98
Antiplatelet agent	Clopidogrel	0.41	0.07	0.14	0.07	0.87	1.70	0.07	0.25	0.07	2.97
Prostatic hyperplasia	Tamsulosin	0.03	0.03	0.03	0.03	0.37	0.03	0.03	0.03	0.03	0.34
To treat asthma	Salbutamol	0.05	0.05	0.05	0.05	0.45	0.05	0.05	0.05	0.05	0.51
Anticoagulant	Warfarin	1.62	0.23	0.37	0.23	4.83	0.23	0.23	0.23	0.23	3.16
X-ray contrast agent	Iopromide	0.30	0.30	0.30	0.30	0.90	0.30	0.30	0.30	0.30	1.50
Antihelmintics		0.65	0.23	0.30	0.25	4.14	0.66	0.22	0.36	0.34	5.00
	Albendazole	0.42	0.02	0.07	0.02	0.73	0.27	0.07	0.17	0.19	2.08
	Thiabendazole	0.33	0.14	0.16	0.14	2.20	0.44	0.14	0.20	0.14	2.75
	Levamisole	0.09	0.09	0.09	0.09	1.21	0.09	0.09	0.09	0.09	0.17
Synthetic glucocorticoid	Dexamethasone	0.44	0.44	0.44	0.44	0.44	1.35	0.10	0.90	1.04	10.85
Sedation and muscle relaxation	Xylazine	0.27	0.06	0.11	0.06	1.61	0.06	0.06	0.06	0.06	0.55
Tranquilizers		0.26	0.23	0.25	0.26	3.56	0.26	0.04	0.19	0.25	2.69
	Azaperone	0.23	0.23	0.23	0.23	3.19	0.23	0.23	0.23	0.23	2.28
	Azaperol	0.04	0.04	0.04	0.04	0.37	0.04	0.04	0.04	0.04	0.41
Calcium channel blocker	Diltiazem	1.00	0.06	0.30	0.19	3.63	0.06	0.06	0.06	0.06	0.12
Antibiotics		44.73	38.48	43.58	44.50	610.06	55.76	42.99	45.33	44.35	634.58
	Erithromycin	1.13	1.13	1.13	1.13	15.82	1.13	1.13	1.13	1.13	6.78
	Azithromycin	23.92	23.92	23.92	23.92	334.86	23.92	23.92	23.92	23.92	334.86
	Clarithromycin	12.72	12.72	12.72	12.72	178.05	12.72	12.72	12.72	12.72	178.05
	Tetracycline	5.92	5.92	5.92	5.92	71.00	5.92	5.92	5.92	5.92	82.83
	Sulfamethoxazole	0.26	0.07	0.09	0.07	0.87	0.07	0.07	0.07	0.07	0.88
	Trimethoprim	0.03	0.03	0.03	0.03	0.41	0.19	0.03	0.08	0.06	0.76
	Metronidazole	0.12	0.12	0.12	0.12	1.08	12.61	0.30	2.54	1.12	25.39
	Metronidazole-OH	ND	ND	ND	ND	ND	0.37	0.37	0.37	0.37	1.47
	Ofloxacin	0.09	0.09	0.09	0.09	1.30	ND	ND	ND	ND	ND
	Ciprofloxacin	0.10	0.10	0.10	0.10	1.24	ND	ND	ND	ND	ND
	Cefalexin	0.40	0.40	0.40	0.40	4.39	ND	ND	ND	ND	ND
	Dimetridazole	0.08	0.03	0.05	0.04	0.54	0.81	0.73	0.76	0.75	3.05
	Ronidazole	0.10	0.10	0.10	0.10	0.49	0.10	0.10	0.10	0.10	0.49

Sediment		Ebro									
Therapeutic group	Compound	Campaign 1					Campaign 2				
		Max	Min	Average	Median	Sum	Max	Min	Average	Median	Sum
Analgesics/anti-inflammatories		35.93	3.83	18.81	17.47	413.88	36.81	5.41	11.96	9.30	275.17
	Phenazone	0.06	0.06	0.06	0.06	0.33	0.06	0.03	0.05	0.06	0.20
	Propyphenazone	0.04	0.04	0.04	0.04	0.42	0.13	0.02	0.05	0.04	0.42
	Oxycodone	0.37	0.37	0.37	0.37	7.01	0.37	0.37	0.37	0.37	8.49
	Codeine	11.58	11.58	11.58	11.58	162.13	11.58	6.95	10.42	11.58	41.69
	Acetaminophen	0.03	0.03	0.03	0.03	0.09	4.04	0.62	1.68	1.45	36.86
	Ibuprofen	12.56	12.56	12.56	12.56	138.13	12.56	7.53	10.88	12.56	32.65
	Indomethacine	0.47	0.47	0.47	0.47	9.37	0.47	0.28	0.45	0.47	3.56
	Diclofenac	1.29	1.29	1.29	1.29	28.42	1.29	1.29	1.29	1.29	29.71
	Ketoprofen	8.63	1.99	2.42	1.99	53.14	11.10	1.99	4.73	4.30	108.85
	Naproxen	3.38	0.82	1.33	0.82	6.65	0.82	0.49	0.65	0.65	1.30
	Piroxicam	0.15	0.15	0.15	0.15	1.78	0.15	0.15	0.15	0.15	3.27
	Meloxicam	0.08	0.08	0.08	0.08	1.15	0.08	0.08	0.08	0.08	1.61
	Tenoxicam	0.66	0.66	0.66	0.66	5.25	0.66	0.66	0.66	0.66	6.56
Lipid regulators and cholesterol lowering statin drugs		1.00	0.28	0.49	0.37	10.77	0.76	0.37	0.58	0.57	12.72
	Bezafibrate	0.41	0.09	0.20	0.09	0.59	ND	ND	ND	ND	ND
	Gemfibrozil	0.34	0.07	0.09	0.07	1.99	0.26	0.07	0.16	0.19	3.35
	Pravastatin	0.30	0.30	0.30	0.30	6.06	0.30	0.30	0.30	0.30	6.36
	Fluvastatin	0.22	0.22	0.22	0.22	1.75	0.22	0.22	0.22	0.22	2.63
	Atorvastatin	0.19	0.18	0.18	0.18	0.37	0.11	0.03	0.06	0.05	0.37
Psychiatric drugs		29.66	2.26	7.26	5.52	152.43	14.14	2.18	5.07	4.56	111.59
	Fluoxetine	0.34	0.34	0.34	0.34	5.17	0.34	0.34	0.34	0.34	5.86
	Norfluoxetine	0.14	0.14	0.14	0.14	1.11	0.14	0.14	0.14	0.14	1.11
	Paroxetine	0.13	0.05	0.06	0.05	0.57	0.76	0.05	0.23	0.16	4.78
	Diazepam	0.16	0.16	0.16	0.16	3.30	0.16	0.16	0.16	0.16	2.35
	Lorazepam	1.03	0.45	0.54	0.45	3.75	0.45	0.45	0.45	0.45	4.54
	Alprazolam	0.08	0.08	0.08	0.08	0.58	0.08	0.08	0.08	0.08	0.74
	Carbamazepine	0.06	0.06	0.06	0.06	0.43	0.06	0.06	0.06	0.06	0.43
	Acridone	23.92	0.44	3.73	2.15	78.41	5.99	0.44	2.68	2.13	59.06
	Sertraline	10.53	1.15	2.31	1.15	43.82	1.15	1.15	1.15	1.15	17.27
	Citalopram	0.23	0.23	0.23	0.23	4.79	0.23	0.23	0.23	0.23	1.82
	Venlafaxine	0.05	0.05	0.05	0.05	0.99	0.05	0.05	0.05	0.05	0.70
	Olanzapine	0.10	0.10	0.10	0.10	0.10	1.64	0.10	0.36	0.10	2.14
	Trazodone	3.36	0.09	0.47	0.09	9.41	6.10	0.09	0.57	0.09	10.76
Histamine H1 and H2 receptor antagonists		6.56	0.05	0.69	0.34	13.09	7.72	0.40	2.40	1.98	52.75
	Loratidine	6.19	0.05	0.38	0.05	7.95	4.78	0.05	0.80	0.26	16.85
	Desloratidine	0.61	0.06	0.19	0.13	2.90	3.18	0.17	1.49	1.49	32.80
	Ranitidine	0.22	0.05	0.15	0.16	1.38	0.83	0.05	0.25	0.05	0.98
	Famotidine	0.02	0.02	0.02	0.02	0.07	ND	ND	ND	ND	ND
	Cimetidine	0.18	0.18	0.18	0.18	1.94	0.18	0.18	0.18	0.18	2.12
β-Blocking agents		1.52	0.27	0.81	0.79	16.94	3.26	0.22	0.88	0.71	19.46
	Atenolol	0.27	0.27	0.27	0.27	2.94	0.27	0.27	0.27	0.27	2.41
	Sotalol	0.03	0.03	0.03	0.03	0.12	0.25	0.14	0.19	0.19	0.38
	Metoprolol	0.04	0.04	0.04	0.04	0.43	0.60	0.04	0.12	0.04	0.98
	Propanolol	1.04	0.07	0.57	0.69	11.38	0.86	0.07	0.22	0.18	4.66
	Nadolol	0.07	0.07	0.07	0.07	1.12	0.07	0.07	0.07	0.07	1.40
	Carazolol	0.14	0.08	0.09	0.08	0.95	1.73	0.07	0.46	0.39	9.64

Sediment		Ebro									
Therapeutic group	Compound	Campaign 1					Campaign 2				
		Max	Min	Average	Median	Sum	Max	Min	Average	Median	Sum
Diuretics		10.47	3.01	4.05	3.96	85.14	4.28	3.01	3.44	3.30	75.64
	Torasemide	0.05	0.05	0.05	0.05	0.47	0.34	0.28	0.31	0.31	1.87
	Hidrochlorothiazide	3.01	3.01	3.01	3.01	63.12	3.01	3.01	3.01	3.01	66.13
	Furosemide	7.46	0.96	1.54	0.96	21.54	0.96	0.96	0.96	0.96	7.65
Antidiabetic	Glibenclamide	0.90	0.90	0.90	0.90	18.90	ND	ND	ND	ND	ND
Antihypertensives		1.16	0.05	0.58	0.62	12.27	4.36	0.05	0.48	0.27	9.52
	Amlodipine	0.63	0.11	0.42	0.56	6.34	ND	ND	ND	ND	ND
	Irbesartan	0.36	0.03	0.10	0.05	1.11	1.57	0.05	0.21	0.05	2.98
	Losartan	0.24	0.14	0.23	0.24	1.80	1.50	0.24	0.38	0.24	4.17
	Valsartan	0.62	0.15	0.27	0.15	3.01	1.29	0.15	0.40	0.15	2.37
Antiplatelet agent	Clopidogrel	0.07	0.07	0.07	0.07	1.21	0.20	0.07	0.08	0.07	1.61
Prostatic hyperplasia	Tamsulosin	0.03	0.03	0.03	0.03	0.40	0.03	0.03	0.03	0.03	0.47
To treat asthma	Salbutamol	0.05	0.05	0.05	0.05	0.30	0.05	0.05	0.05	0.05	0.66
Anticoagulant	Warfarin	0.23	0.23	0.23	0.23	2.26	0.23	0.23	0.23	0.23	4.52
X-ray contrast agent	Iopromide	0.30	0.30	0.30	0.30	1.20	0.30	0.30	0.30	0.30	1.50
Antihelmintics		6.57	0.14	0.52	0.25	10.85	12.53	0.14	1.01	0.43	22.28
	Albendazol	0.02	0.02	0.02	0.02	0.35	0.45	0.05	0.24	0.23	4.70
	Thiabendazole	6.54	0.14	0.46	0.14	9.72	12.29	0.14	0.80	0.14	17.49
	Levamisol	0.09	0.09	0.09	0.09	0.78	0.09	0.09	0.09	0.09	0.09
Synthetic glucocorticoid	Dexamethasone	0.89	0.33	0.45	0.41	4.03	2.15	0.10	0.98	0.95	19.60
Sedation and muscle relaxation	Xylazine	0.46	0.03	0.10	0.06	2.15	0.06	0.06	0.06	0.06	0.66
Tranquilizers		0.26	0.23	0.25	0.26	5.30	0.26	0.04	0.22	0.26	4.77
	Azaperone	0.23	0.23	0.23	0.23	4.79	0.23	0.23	0.23	0.23	4.10
	Azaperol	0.04	0.04	0.04	0.04	0.52	0.04	0.04	0.04	0.04	0.66
Calcium channel blocker	Diltiazem	0.35	0.06	0.19	0.17	2.53	0.17	0.06	0.10	0.09	0.41
Antibiotics		45.52	31.49	40.12	38.35	842.45	50.03	43.08	44.89	44.78	987.67
	Erithromycin	1.13	1.13	1.13	1.13	23.74	1.13	1.13	1.13	1.13	12.43
	Azithromycin	23.92	23.92	23.92	23.92	502.30	23.92	23.92	23.92	23.92	526.22
	Clarithromycin	12.72	12.72	12.72	12.72	228.92	12.72	12.72	12.72	12.72	279.80
	Tetracycline	5.92	5.92	5.92	5.92	76.92	5.92	5.92	5.92	5.92	130.16
	Sulfamethoxazole	0.07	0.07	0.07	0.07	0.61	0.19	0.07	0.09	0.07	1.82
	Trimethoprim	0.03	0.03	0.03	0.03	0.61	0.18	0.03	0.09	0.09	1.91
	Metronidazole	0.32	0.12	0.16	0.12	0.80	6.73	0.44	1.47	1.11	23.52
	Metronidazole-OH	1.62	0.37	0.99	0.99	1.98	2.29	0.37	0.60	0.37	5.98
	Ofloxacin	0.09	0.09	0.09	0.09	1.95	0.31	0.09	0.10	0.09	2.25
	Ciprofloxacin	0.10	0.10	0.10	0.10	1.81	0.10	0.10	0.10	0.10	2.00
	Cefalexin	0.40	0.40	0.40	0.40	2.39	ND	ND	ND	ND	ND
	Dimetridazole	0.06	0.03	0.04	0.05	0.13	1.39	1.39	1.39	1.39	1.39
	Ronidazole	0.10	0.10	0.10	0.10	0.29	0.10	0.10	0.10	0.10	0.19

Sediment		Júcar									
Therapeutic group	Compound	Campaign 1					Campaign 2				
		Max	Min	Average	Median	Sum	Max	Min	Average	Median	Sum
Analgesics/anti-inflammatories		32.32	3.60	21.74	27.94	282.59	38.69	30.17	33.31	32.78	340.82
	Phenazone	0.06	0.06	0.06	0.06	0.39	0.06	0.06	0.06	0.06	0.61
	Propyphenazone	0.04	0.04	0.04	0.04	0.42	0.04	0.04	0.04	0.04	0.46
	Oxycodone	0.37	0.37	0.37	0.37	4.80	0.37	0.37	0.37	0.37	5.54
	Codeine	11.58	11.58	11.58	11.58	115.81	11.58	11.58	11.58	11.58	150.55
	Acetaminophen	1.18	0.22	0.47	0.23	1.87	0.12	0.12	0.12	0.12	0.12
	Ibuprofen	12.56	12.56	12.56	12.56	100.46	12.56	12.56	12.56	12.56	75.34
	Indomethacine	0.47	0.47	0.47	0.47	5.15	0.47	0.47	0.47	0.47	3.28
	Diclofenac	1.29	1.29	1.29	1.29	14.21	1.29	1.29	1.29	1.29	19.38
	Ketoprofen	5.79	1.99	2.28	1.99	29.69	10.52	1.99	5.13	4.60	77.00
	Naproxen	0.82	0.82	0.82	0.82	2.45	0.82	0.82	0.82	0.82	2.45
	Piroxicam	0.15	0.15	0.15	0.15	1.19	0.15	0.15	0.15	0.15	0.89
	Meloxicam	0.08	0.08	0.08	0.08	0.92	0.08	0.08	0.08	0.08	0.61
	Tenoxicam	0.66	0.66	0.66	0.66	5.25	0.66	0.66	0.66	0.66	4.59
Lipid regulators and cholesterol lowering statin drugs		1.51	0.07	0.49	0.40	6.34	2.47	0.30	0.60	0.39	9.02
	Bezafibrate	ND	ND	ND	ND	ND	0.35	0.27	0.31	0.31	0.62
	Gemfibrozil	0.96	0.07	0.15	0.07	1.96	0.24	0.07	0.11	0.07	0.65
	Pravastatin	0.30	0.30	0.30	0.30	3.63	0.30	0.30	0.30	0.30	4.54
	Fluvastatin	0.22	0.22	0.22	0.22	0.66	1.01	0.22	0.35	0.22	2.10
	Atorvastatin	0.03	0.03	0.03	0.03	0.09	0.65	0.06	0.19	0.08	1.11
Psychiatric drugs		8.88	2.28	4.41	4.37	57.29	16.12	1.08	3.01	1.93	45.10
	Fluoxetine	0.34	0.34	0.34	0.34	4.48	0.34	0.34	0.34	0.34	5.17
	Norfluoxetine	0.14	0.14	0.14	0.14	0.83	0.14	0.14	0.14	0.14	0.97
	Paroxetine	0.05	0.05	0.05	0.05	0.54	0.05	0.05	0.05	0.05	0.64
	Diazepam	0.16	0.16	0.16	0.16	1.57	0.40	0.16	0.20	0.16	2.20
	Lorazepam	0.45	0.45	0.45	0.45	3.64	0.45	0.45	0.45	0.45	0.91
	Alprazolam	0.08	0.08	0.08	0.08	0.82	0.08	0.08	0.08	0.08	0.41
	Carbamazepine	0.06	0.06	0.06	0.06	0.19	ND	ND	ND	ND	ND
	Acridone	5.83	0.44	1.94	1.76	25.20	5.71	0.44	0.89	0.44	13.39
	Sertraline	1.15	1.15	1.15	1.15	13.82	1.15	1.15	1.15	1.15	8.06
	Citalopram	0.23	0.23	0.23	0.23	2.96	0.23	0.23	0.23	0.23	0.68
	Venlafaxine	0.05	0.05	0.05	0.05	0.61	0.05	0.05	0.05	0.05	0.70
	Olanzapine	0.10	0.10	0.10	0.10	0.40	0.35	0.10	0.15	0.10	1.81
	Trazodone	0.92	0.09	0.17	0.09	2.23	8.08	0.09	0.68	0.09	10.15
Histamine H1 and H2 receptor antagonists		0.87	0.05	0.48	0.40	6.27	1.92	0.43	0.90	0.82	13.46
	Loratidine	0.16	0.05	0.08	0.05	1.00	1.03	0.05	0.19	0.07	1.93
	Desloratidine	0.59	0.06	0.30	0.25	3.02	0.91	0.17	0.49	0.52	7.33
	Ranitidine	0.17	0.05	0.10	0.10	0.48	0.19	0.05	0.07	0.05	0.86
	Famotidine	ND	ND	ND	ND	ND	0.40	0.02	0.05	0.02	0.69
	Cimetidine	0.18	0.18	0.18	0.18	1.77	0.18	0.18	0.18	0.18	2.65
β-Blocking agents		1.29	0.36	0.69	0.67	9.00	3.67	0.28	1.51	1.50	22.65
	Atenolol	0.27	0.27	0.27	0.27	2.94	0.27	0.27	0.27	0.27	0.53
	Sotalol	0.03	0.03	0.03	0.03	0.15	0.13	0.03	0.06	0.06	0.76
	Metoprolol	0.04	0.04	0.04	0.04	0.08	0.16	0.04	0.07	0.06	0.84
	Propanolol	0.80	0.07	0.30	0.35	3.86	1.18	0.21	0.56	0.47	8.39
	Nadolol	0.07	0.07	0.07	0.07	0.56	0.15	0.07	0.08	0.07	0.99
	Carazolol	0.23	0.08	0.13	0.08	1.40	1.97	0.08	0.80	0.84	11.13

Sediment		Júcar									
Therapeutic group	Compound	Campaign 1					Campaign 2				
		Max	Min	Average	Median	Sum	Max	Min	Average	Median	Sum
Diuretics		4.01	3.01	3.20	3.06	38.35	3.96	3.01	3.19	3.01	47.79
	Torasemide	0.05	0.05	0.05	0.05	0.37	0.68	0.50	0.58	0.57	1.75
	Hidrochlorothiazide	3.01	3.01	3.01	3.01	36.07	3.01	3.01	3.01	3.01	45.09
	Furosemide	0.96	0.96	0.96	0.96	1.91	0.96	0.96	0.96	0.96	0.96
Antidiabetic	Glibenclamide	0.90	0.90	0.90	0.90	11.70	ND	ND	ND	ND	ND
Antihypertensives		1.77	0.00	0.67	0.25	8.66	4.77	0.00	2.75	3.14	41.30
	Amlodipine	0.62	0.18	0.29	0.18	1.75	ND	ND	ND	ND	ND
	Irbesartan	0.51	0.05	0.16	0.05	0.96	1.63	0.59	1.21	1.25	12.07
	Losartan	0.24	0.24	0.24	0.24	1.42	1.70	0.76	1.12	1.13	14.62
	Valsartan	0.85	0.15	0.45	0.42	4.54	2.14	0.98	1.22	1.14	14.62
Antiplatelet agent	Clopidogrel	0.07	0.07	0.07	0.07	0.40	0.07	0.07	0.07	0.07	0.87
Prostatic hyperplasia	Tamsulosin	0.03	0.03	0.03	0.03	0.26	ND	ND	ND	ND	ND
To treat asthma	Salbutamol	0.05	0.05	0.05	0.05	0.45	0.05	0.05	0.05	0.05	0.76
Anticoagulant	Warfarin	0.23	0.23	0.23	0.23	2.49	ND	ND	ND	ND	ND
X-ray contrast agent	Iopromide	0.30	0.30	0.30	0.30	3.89	0.94	0.30	0.58	0.66	8.68
Antihelmintics		14.01	0.17	1.97	0.25	25.67	1.70	0.14	0.39	0.29	5.88
	Albendazol	0.02	0.02	0.02	0.02	0.31	0.43	0.02	0.15	0.15	1.69
	Thiabendazole	13.90	0.14	1.88	0.14	24.41	1.47	0.14	0.25	0.14	3.75
	Levamisol	0.09	0.09	0.09	0.09	0.95	0.09	0.09	0.09	0.09	0.43
Synthetic glucocorticoid	Dexamethasone	0.48	0.35	0.41	0.41	0.82	0.10	0.10	0.10	0.10	0.20
Sedation and muscle relaxation	Xylazine	0.27	0.06	0.09	0.06	1.14	0.06	0.06	0.06	0.06	0.11
Tranquilizers		0.26	0.23	0.24	0.23	3.11	ND	ND	ND	ND	ND
	Azaperone	0.23	0.23	0.23	0.23	2.96	ND	ND	ND	ND	ND
	Azaperol	0.04	0.04	0.04	0.04	0.15	ND	ND	ND	ND	ND
Calcium channel blocker	Diltiazem	0.21	0.06	0.14	0.18	1.13	ND	ND	ND	ND	ND
Antibiotics		44.95	25.57	41.92	44.24	544.97	51.77	43.08	45.41	45.45	681.08
	Erithromycin	1.13	1.13	1.13	1.13	12.43	ND	ND	ND	ND	ND
	Azithromycin	23.92	23.92	23.92	23.92	310.95	23.92	23.92	23.92	23.92	358.78
	Clarithromycin	12.72	12.72	12.72	12.72	152.62	12.72	12.72	12.72	12.72	190.77
	Tetracycline	5.92	5.92	5.92	5.92	59.17	5.92	5.92	5.92	5.92	88.75
	Sulfamethoxazole	0.07	0.07	0.07	0.07	0.54	0.18	0.07	0.09	0.07	0.86
	Trimethoprim	0.03	0.03	0.03	0.03	0.38	0.09	0.03	0.05	0.03	0.63
	Metronidazole	0.12	0.12	0.12	0.12	0.84	3.61	2.21	2.69	2.56	21.53
	Metronidazole-OH	0.37	0.37	0.37	0.37	1.10	0.37	0.37	0.37	0.37	1.47
	Ofloxacin	0.09	0.09	0.09	0.09	1.20	2.99	0.09	0.39	0.09	5.91
	Ciprofloxacin	0.19	0.10	0.10	0.10	1.14	3.79	0.23	0.65	0.39	9.82
	Cefalexin	0.40	0.40	0.40	0.40	3.59	ND	ND	ND	ND	ND
	Dimetridazole	0.08	0.03	0.05	0.05	0.32	0.97	0.75	0.85	0.84	2.56
	Ronidazole	0.10	0.10	0.10	0.10	0.68	ND	ND	ND	ND	ND

Sediment		Guadalquivir									
Therapeutic group	Compound	Campaign 1					Campaign 2				
		Max	Min	Average	Median	Sum	Max	Min	Average	Median	Sum
Analgesics/anti-inflammatories		33.76	2.83	16.74	17.14	401.75	33.49	3.97	19.40	18.16	465.66
	Phenazone	0.06	0.06	0.06	0.06	0.11	0.06	0.06	0.06	0.06	1.33
	Propyphenazone	0.04	0.04	0.04	0.04	0.38	0.04	0.04	0.04	0.04	0.57
	Oxycodone	0.37	0.37	0.37	0.37	7.75	0.37	0.37	0.37	0.37	8.86
	Codeine	11.58	11.58	11.58	11.58	127.39	11.58	11.58	11.58	11.58	231.61
	Acetaminophen	9.53	0.03	0.75	0.03	12.67	0.22	0.16	0.19	0.19	0.38
	Ibuprofen	12.56	12.56	12.56	12.56	125.57	12.56	12.56	12.56	12.56	75.34
	Indomethacine	2.94	0.47	0.59	0.47	12.31	0.47	0.47	0.47	0.47	4.22
	Diclofenac	3.38	1.29	1.39	1.29	30.51	4.14	1.29	1.55	1.29	31.01
	Ketoprofen	7.23	1.99	3.21	1.99	77.13	12.54	1.99	4.36	3.05	104.63
	Naproxen	0.82	0.82	0.82	0.82	4.08	0.82	0.82	0.82	0.82	0.82
	Piroxicam	0.15	0.15	0.15	0.15	1.34	0.15	0.15	0.15	0.15	2.38
	Meloxicam	0.08	0.08	0.08	0.08	0.54	0.08	0.08	0.08	0.08	1.23
	Tenoxicam	0.66	0.66	0.66	0.66	1.97	0.66	0.66	0.66	0.66	3.28
Lipid regulators and cholesterol lowering statin drugs		0.65	0.07	0.42	0.37	10.14	0.72	0.30	0.45	0.43	10.80
	Bezafibrate	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
	Gemfibrozil	0.16	0.07	0.07	0.07	1.66	0.42	0.07	0.13	0.07	2.67
	Pravastatin	0.30	0.30	0.30	0.30	6.66	0.30	0.30	0.30	0.30	7.27
	Fluvastatin	0.22	0.22	0.22	0.22	1.31	0.22	0.22	0.22	0.22	0.22
	Atorvastatin	0.28	0.03	0.13	0.10	0.51	0.16	0.06	0.09	0.09	0.65
Psychiatric drugs		16.60	2.48	6.57	6.11	157.79	3.44	1.01	2.12	2.15	50.91
	Fluoxetine	0.34	0.34	0.34	0.34	5.86	0.34	0.34	0.34	0.34	8.27
	Norfluoxetine	0.60	0.14	0.23	0.14	1.15	0.14	0.14	0.14	0.14	2.08
	Paroxetine	0.05	0.05	0.05	0.05	0.29	0.05	0.05	0.05	0.05	0.74
	Diazepam	0.16	0.16	0.16	0.16	2.20	0.16	0.16	0.16	0.16	2.35
	Lorazepam	0.45	0.45	0.45	0.45	5.45	0.45	0.45	0.45	0.45	2.27
	Alprazolam	0.08	0.08	0.08	0.08	0.91	0.08	0.08	0.08	0.08	1.40
	Carbamazepine	0.06	0.06	0.06	0.06	1.49	0.06	0.06	0.06	0.06	0.06
	Acridone	10.87	0.44	3.49	3.05	83.69	1.97	0.44	0.56	0.44	13.46
	Sertraline	14.05	1.15	3.71	3.57	48.28	1.15	1.15	1.15	1.15	11.52
	Citalopram	0.23	0.23	0.23	0.23	3.88	0.23	0.23	0.23	0.23	2.05
	Venlafaxine	0.05	0.05	0.05	0.05	1.13	0.12	0.05	0.05	0.05	1.20
	Olanzapine	0.25	0.10	0.11	0.10	1.76	0.30	0.10	0.16	0.10	2.94
	Trazodone	0.09	0.09	0.09	0.09	1.70	0.31	0.09	0.11	0.09	2.55
Histamine H1 and H2 receptor antagonists		17.22	2.72	6.89	6.40	165.35	6.16	1.39	3.02	2.91	72.43
	Loratidine	0.05	0.05	0.05	0.05	1.20	2.49	0.05	0.27	0.10	5.21
	Desloratidine	0.59	0.06	0.17	0.16	2.94	0.78	0.14	0.45	0.43	10.44
	Ranitidine	0.21	0.14	0.18	0.19	0.54	0.15	0.05	0.09	0.11	1.62
	Famotidine	0.02	0.02	0.02	0.02	0.22	ND	ND	ND	ND	ND
	Cimetidine	0.18	0.18	0.18	0.18	2.65	0.18	0.18	0.18	0.18	4.24
β-Blocking agents		1.60	0.42	1.14	1.27	27.44	2.99	0.10	1.51	1.36	34.68
	Atenolol	0.27	0.27	0.27	0.27	5.35	0.27	0.27	0.27	0.27	2.41
	Sotalol	0.03	0.03	0.03	0.03	0.43	0.08	0.03	0.05	0.03	0.82
	Metoprolol	0.04	0.04	0.04	0.04	0.78	0.13	0.04	0.07	0.08	1.27
	Propanolol	1.12	0.07	0.85	0.88	18.75	0.92	0.07	0.53	0.54	12.22
	Nadolol	0.10	0.07	0.07	0.07	1.15	0.07	0.07	0.07	0.07	1.40
	Carazolol	0.25	0.08	0.10	0.08	0.98	1.60	0.08	0.83	0.91	16.58

Sediment		Guadalquivir									
Therapeutic group	Compound	Campaign 1					Campaign 2				
		Max	Min	Average	Median	Sum	Max	Min	Average	Median	Sum
Diuretics		3.96	3.01	3.34	3.06	80.10	5.96	3.01	3.32	3.01	79.75
	Torasemide	0.05	0.05	0.05	0.05	0.32	0.57	0.48	0.53	0.52	3.70
	Hidrochlorothiazide	3.01	3.01	3.01	3.01	72.14	3.01	3.01	3.01	3.01	72.14
	Furosemide	0.96	0.96	0.96	0.96	7.65	2.95	0.96	1.95	1.95	3.91
Antidiabetic	Glibenclamide	0.90	0.90	0.90	0.90	21.60	ND	ND	ND	ND	ND
Antihypertensives		1.09	0.11	0.72	0.77	15.92	1.88	0.29	1.23	1.38	29.56
	Amlodipine	0.82	0.18	0.65	0.66	10.35	0.18	0.18	0.18	0.18	0.90
	Irbesartan	0.34	0.05	0.11	0.05	2.31	0.47	0.05	0.17	0.15	3.32
	Losartan	0.24	0.24	0.24	0.24	0.95	0.49	0.24	0.25	0.24	5.24
	Valsartan	0.66	0.15	0.26	0.15	2.31	1.25	0.95	1.06	1.02	20.09
Antiplatelet agent	Clopidogrel	0.07	0.07	0.07	0.07	1.21	0.44	0.07	0.09	0.07	1.61
Prostatic hyperplasia	Tamsulosin	0.03	0.03	0.03	0.03	0.45	ND	ND	ND	ND	ND
To treat asthma	Salbutamol	0.05	0.05	0.05	0.05	0.10	ND	ND	ND	ND	ND
Anticoagulant	Warfarin	0.23	0.23	0.23	0.23	4.97	ND	ND	ND	ND	ND
X-ray contrast agent	Iopromide	0.30	0.30	0.30	0.30	1.50	1.55	0.30	0.68	0.67	16.36
Antihelmintics		0.66	0.14	0.20	0.14	4.69	1.78	0.14	0.38	0.32	9.07
	Albendazol	0.05	0.02	0.03	0.02	0.28	0.27	0.02	0.13	0.14	2.56
	Thiabendazole	0.53	0.14	0.16	0.14	3.85	1.56	0.14	0.20	0.14	4.88
	Levamisol	0.30	0.09	0.14	0.09	0.56	0.09	0.09	0.09	0.09	1.64
Synthetic glucocorticoid	Dexamethasone	0.88	0.10	0.43	0.36	3.01	0.30	0.10	0.14	0.10	1.72
Sedation and muscle relaxation	Xylazine	0.06	0.06	0.06	0.06	0.66	0.38	0.06	0.11	0.06	0.65
Tranquilizers		0.50	0.23	0.27	0.26	6.56	0.23	0.23	0.23	0.23	5.47
	Azaperone	0.46	0.23	0.24	0.23	5.71	0.23	0.23	0.23	0.23	5.47
	Azaperol	0.04	0.04	0.04	0.04	0.85	ND	ND	ND	ND	ND
Calcium channel blocker	Diltiazem	0.58	0.06	0.32	0.37	2.87	0.69	0.45	0.57	0.57	1.13
Antibiotics		44.7	32.1	40.4	38.5	969.1	53.59	42.90	45.46	44.69	1091.04
	Erithromycin	1.13	1.13	1.13	1.13	23.74	1.13	1.13	1.13	1.13	23.74
	Azithromycin	23.92	23.92	23.92	23.92	574.05	23.92	23.92	23.92	23.92	574.05
	Clarithromycin	12.72	12.72	12.72	12.72	292.52	12.72	12.72	12.72	12.72	305.23
	Tetracycline	5.92	5.92	5.92	5.92	65.08	5.92	5.92	5.92	5.92	142.00
	Sulfamethoxazole	0.07	0.07	0.07	0.07	0.88	0.17	0.07	0.08	0.07	1.79
	Trimethoprim	0.13	0.03	0.03	0.03	0.83	0.09	0.03	0.04	0.03	0.90
	Metronidazole	0.48	0.12	0.21	0.12	2.49	8.28	0.94	2.48	1.20	24.83
	Metronidazole-OH	0.37	0.37	0.37	0.37	1.84	0.37	0.37	0.37	0.37	0.74
	Ofloxacin	0.09	0.09	0.09	0.09	2.22	1.18	0.09	0.20	0.09	4.91
	Ciprofloxacin	0.20	0.10	0.10	0.10	2.39	0.72	0.10	0.41	0.39	9.50
	Cefalexin	0.40	0.40	0.40	0.40	2.39	ND	ND	ND	ND	ND
	Dimetridazole	0.44	0.03	0.14	0.04	0.54	0.90	0.75	0.84	0.85	3.35
	Ronidazole	0.10	0.10	0.10	0.10	0.10	ND	ND	ND	ND	ND

T**able S-7.** Permanova analysis for the concentration of PhACs in the different basins and campaigns.

Surface water	DF	SS	MS	F	p
Basin	3	3687.7	1229.2	8.07	<0.0001
Campaign	1	2348.1	2348.1	15.42	<0.0001
Basin:Campaign	3	3030.1	1010	6.63	<0.0001
Residuals	145	22081.4	152.3		
Total	152	31147.3			
Sediment	DF	SS	MS	F	p
Basin	3	1326.1	442	9.2	<0.0001
Campaign	1	1646.3	1646.3	34.25	<0.0001
Basin:Campaign	3	1235	411.7	8.56	<0.0001
Residuals	140	6729.4	48.1		
Total	147	10936.8			

Table S-8. Univariate ANOVAs by permutation for the PhACs in water and sediment samples. The sources of variations considered were Basin, Campaign and their interaction in all analyses. The percentage of variance explained is detailed for each source of variation and for the residual. The mean, the confidence interval, the minimum and the maximum values for the variance explained for all the PhACs is shown at the bottom of the table. The significant p-values after the correction of Bonferroni are in bold.

Univariate ANOVA by permutation (Surface water)							
	Sources of variation						
PhACs	Basin		Campaign		Basin x Campaign		Residuals
	Var. Exp.(%)	p-value	Var. Exp.(%)	p-value	Var. Exp.(%)	p-value	Var. Exp.(%)
PHEN	23.4	0.0011	47.2	0.0008	25.2	0.0005	4.2
PPHEN	29.5	0.0004	43.1	0.0015	22.8	0.0014	4.5
OXYD	46.0	0.0049	33.6	0.0812	9.7	0.4437	10.6
COD	2.4	0.1028	82.8	**0.0000**	13.6	**0.0000**	1.2
APAP	33.9	**0.0000**	43.4	**0.0000**	20.7	**0.0000**	1.9
IBU	34.9	**0.0000**	47.9	**0.0000**	14.5	0.0012	2.7
INDO	47.0	0.0067	15.1	0.2494	26.8	0.0697	11.1
DCF	34.6	0.0210	40.2	0.0504	15.0	0.2242	10.3
KETO	41.3	**0.0000**	56.7	**0.0000**	1.3	0.1933	0.8
NAP	57.2	0.0123	14.2	0.3242	13.6	0.4364	15.0
PRC	12.1	**0.0002**	75.3	**0.0000**	11.0	**0.0002**	1.6
MLX	7.2	0.7418	70.8	0.0195	7.9	0.6690	14.1
TX	21.2	0.2066	47.9	0.0676	16.9	0.3431	14.1
BZF	35.4	0.0004	27.0	0.0254	32.2	0.0006	5.5
GFZ	58.2	**0.0001**	20.6	0.0924	14.1	0.1206	7.1
PARA	14.8	0.0105	55.8	**0.0001**	25.6	**0.0001**	3.9
FLU	80.1	**0.0000**	5.6	0.4523	5.0	0.6746	9.4
ATV	28.1	0.2331	4.7	1.0000	47.4	0.0619	19.8
FLX	14.7	0.0015	63.6	**0.0000**	19.1	**0.0001**	2.7
NFLX	1.4	0.0145	96.5	**0.0000**	1.7	0.0081	0.4
PRT	59.3	0.0061	14.2	0.3137	12.8	0.4207	13.6
DZP	12.8	0.1442	76.6	0.0008	3.5	0.6911	7.0
LRZ	20.5	**0.0000**	43.7	**0.0000**	33.8	**0.0000**	2.0
APZ	3.2	0.8246	69.3	0.0048	18.0	0.1313	9.5
CBZ	40.9	**0.0000**	36.6	**0.0001**	20.2	**0.0001**	2.3
ACRI	26.9	**0.0000**	64.5	**0.0000**	6.0	0.0854	2.6
SRT	19.6	0.0366	68.6	0.0003	4.4	0.6559	7.4
CTP	11.5	0.2061	66.1	0.0031	14.8	0.1196	7.5
VNFX	71.8	**0.0000**	9.3	0.1542	14.4	0.0251	4.5
OLZ	63.7	0.0259	0.3	1.0000	12.7	0.7557	23.2
TRZ	2.7	0.4126	93.4	**0.0000**	1.1	0.7786	2.8
LNT	25.4	0.1837	56.3	0.0599	2.5	0.9302	15.8
DLNT	46.3	0.0505	3.1	0.6870	32.6	0.1456	18.0
RNT	7.5	0.2295	35.1	0.0083	52.2	**0.0000**	5.2
FMT	36.3	0.0547	0.0	0.9991	44.8	0.0123	18.9
CMT	19.2	0.0604	19.6	1.0000	53.0	**0.0001**	8.2

Table S-8.*(cont.)*

	Univariate ANOVA by permutation (Surface water)						
	Sources of variation						
PhACs	Basin		Campaign		Basin x Campaign		Residuals
	Var. Exp.(%)	p-value	Var. Exp.(%)	p-value	Var. Exp.(%)	p-value	Var. Exp.(%)
ATN	30.5	**0.0001**	41.8	0.0007	23.9	0.0004	3.8
STL	35.1	0.0150	33.9	0.0744	20.4	0.1226	10.5
MTPL	85.3	**0.0001**	0.1	1.0000	1.5	0.9489	13.1
PRPL	48.7	0.1560	4.6	1.0000	18.0	0.6214	28.7
NDL	24.6	0.0522	19.4	0.1807	45.8	0.0022	10.2
CRZL	65.2	0.0047	3.0	0.6605	17.3	0.3271	14.6
TOR	21.1	0.0358	11.8	0.2118	59.7	**0.0000**	7.4
HCTZ	56.9	**0.0000**	0.1	1.0000	39.3	**0.0000**	3.7
FUR	55.7	0.0002	27.0	0.0623	9.6	0.2941	7.7
GLB	18.4	0.2266	60.0	0.0304	8.8	0.5696	12.8
AML	4.9	0.0645	82.1	**0.0000**	11.0	0.0012	2.0
ISRT	12.0	0.1408	45.5	0.0077	36.1	0.0009	6.5
LSRT	41.1	**0.0000**	46.6	**0.0000**	9.5	0.0202	2.8
VSRT	21.7	**0.0000**	50.9	**0.0000**	25.8	**0.0000**	1.5
CLPG	9.3	0.3990	67.7	0.0084	13.5	0.2363	9.4
TMSN	26.1	0.1945	21.7	1.0000	34.9	0.0992	17.3
SAL	30.9	0.0133	0.2	0.8858	60.3	**0.0000**	8.7
WARF	19.5	0.8444	2.7	1.0000	43.1	0.2923	34.8
IOP	11.7	0.1242	32.8	0.0212	49.2	**0.0000**	6.2
ALB	26.4	0.1991	21.2	0.2703	35.3	0.1056	17.1
TALB	41.9	**0.0000**	49.7	**0.0000**	6.1	0.0493	2.3
LMS	25.1	0.0003	48.6	**0.0002**	22.8	0.0003	3.5
DXT	30.0	**0.0000**	49.9	**0.0002**	16.9	0.0016	3.2
XYL	22.3	0.5292	20.2	0.4297	29.2	0.4061	28.2
AZPN	12.1	0.2505	67.2	0.0044	12.0	0.2592	8.7
AZPL	11.8	0.2080	72.3	0.0012	8.1	0.3900	7.8
DTZ	49.7	**0.0000**	0.2	0.8256	45.9	**0.0000**	4.2
ERY	13.3	0.4524	19.9	0.2493	52.2	0.0129	14.6
AZY	26.4	0.0011	31.2	0.0099	37.5	**0.0000**	4.8
CLARI	32.3	0.0970	17.7	0.3075	34.0	0.0945	16.0
TCN	26.7	0.1030	39.0	0.0833	21.6	0.1686	12.7
SMX	15.8	0.0520	36.3	0.0138	41.7	0.0002	6.2
TMP	27.0	**0.0000**	66.2	**0.0000**	5.1	0.0317	1.7
MTZ	28.6	0.0248	6.7	0.3883	55.6	0.0005	9.1
OH-MTZ	35.8	0.0004	10.9	0.1593	47.8	**0.0000**	5.5
OFLX	63.7	0.0016	3.6	0.5883	20.8	0.1578	11.9
CPFX	28.6	0.0373	1.6	1.0000	59.4	0.0005	10.5
CEF	2.9	0.6281	80.4	**0.0000**	11.9	0.0585	4.8
DMZ	8.7	0.0008	79.6	**0.0000**	10.3	**0.0000**	1.4
RNZ	48.4	0.0738	1.9	0.7603	28.9	0.2505	20.8

Table S-8.*(cont.)*

	Univariate ANOVA by permutation (Surface water) Sources of variation						
PhACs	Basin		Campaign		Basin x Campaign		Residuals
	Var. Exp.(%)	p-value	Var. Exp.(%)	p-value	Var. Exp.(%)	p-value	Var. Exp.(%)
Mean	30.1		37.2		23.7		9.0
Confidence Interval	4.3		6.1		3.7		1.6
Min	1.4		0.0		1.1		0.4
Max	85.3		96.5		60.3		34.8
No. Signif. (Bonferroni)		19		21		18	

Table S-8.*(cont.)*

	Univariate ANOVA by permutation (Sediment) Sources of variation						
PhACs	Basin		Campaign		Basin x Campaign		Residuals
	Var. Exp.(%)	p-value	Var. Exp.(%)	p-value	Var. Exp.(%)	p-value	Var. Exp.(%)
PHEN	25.9	**0.0000**	25.7	0.0014	45.9	**0.0000**	2.5
PPHEN	19.2	0.4758	9.8	1.0000	49.6	0.0573	21.4
OXYD	17.4	0.2750	52.1	0.0429	17.4	0.2697	13.1
COD	31.6	0.0005	12.5	0.1271	50.6	**0.0000**	5.3
APAP	23.1	**0.0000**	46.5	**0.0000**	29.8	**0.0000**	0.5
IBU	8.3	0.1426	82.1	**0.0001**	5.1	0.3448	4.5
INDO	26.4	0.0455	31.1	0.0804	32.7	0.0209	9.8
DCF	10.8	0.7344	43.1	0.1800	22.0	0.4460	24.1
KETO	12.2	**0.0000**	85.2	**0.0000**	1.8	0.0588	0.7
NAP	8.1	0.6968	62.0	0.0537	13.2	0.5131	16.6
PRC	37.0	0.0007	37.5	0.0155	19.2	0.0308	6.3
MLX	55.7	**0.0000**	6.2	0.2950	32.4	0.0010	5.7
TX	47.8	0.0025	21.0	0.1364	21.9	0.0748	9.3
BZF	22.8	0.3214	0.0	1.0000	57.2	0.0192	20.1
GFZ	59.2	**0.0001**	5.3	1.0000	28.2	0.0079	7.3
PARA	6.6	0.6509	77.0	0.0103	4.6	0.7880	11.8
FLU	38.0	0.0024	34.2	0.0339	18.6	0.0768	9.3
ATV	12.9	0.2691	56.0	0.0091	21.2	0.0788	9.9
FLX	60.6	**0.0000**	0.3	0.8434	31.1	0.0074	7.9
NFLX	0.8	0.9814	13.7	0.3148	72.2	0.0007	13.2
PRT	28.3	**0.0000**	41.4	**0.0000**	28.0	**0.0000**	2.3
DZP	52.7	0.0108	15.1	0.2991	18.3	0.2720	13.9
LRZ	6.3	0.1355	70.9	**0.0000**	19.4	0.0010	3.4
APZ	23.3	0.2043	8.5	0.4791	53.1	0.0108	15.1
CBZ	13.0	0.0003	68.6	**0.0000**	16.4	**0.0000**	2.0
ACRI	41.2	**0.0000**	44.9	**0.0001**	11.7	0.0021	2.2
SRT	8.9	0.0119	84.1	**0.0000**	4.5	0.1486	2.5
CTP	23.5	**0.0000**	52.9	**0.0000**	22.0	**0.0000**	1.5
VNFX	25.9	**0.0000**	36.4	**0.0000**	33.3	**0.0000**	4.4
OLZ	21.4	0.0015	62.0	**0.0000**	12.3	0.0279	4.3
TRZ	49.7	0.0147	34.0	0.1266	2.0	0.9388	14.3
LNT	31.2	0.0023	54.3	0.0021	8.5	0.2532	6.1
DLNT	7.8	**0.0000**	86.0	**0.0000**	5.6	**0.0000**	0.6
RNT	10.2	0.5119	21.3	0.1936	56.1	0.0016	12.4
FMT	27.2	0.0029	23.9	0.1098	36.0	**0.0000**	12.9
CMT	18.4	0.0024	66.5	**0.0000**	11.6	0.0225	3.5

Table S-8.*(cont.)*

	Univariate ANOVA by permutation (Sediment)						
	Sources of variation						
PhACs	Basin		Campaign		Basin x Campaign		Residuals
	Var. Exp.(%)	p-value	Var. Exp.(%)	p-value	Var. Exp.(%)	p-value	Var. Exp.(%)
ATN	2.0	0.5413	88.4	**0.0000**	6.7	0.0713	2.8
STL	19.3	0.0020	61.7	**0.0000**	15.2	0.0069	3.8
MTPL	2.6	0.8798	68.1	0.0050	19.5	0.1077	9.7
PRPL	42.2	**0.0001**	1.1	0.6712	50.7	**0.0000**	6.0
NDL	7.2	0.0729	82.5	**0.0000**	7.3	0.0702	3.0
CRZL	2.3	0.0408	94.2	**0.0000**	2.7	0.0239	0.8
TOR	16.8	0.0015	77.1	**0.0000**	3.1	0.3899	3.0
HCTZ	26.7	0.0911	30.8	0.0911	26.7	0.0911	15.8
FUR	49.0	**0.0002**	36.8	0.0216	7.2	0.3955	7.0
GLB	0.0	0.2270	99.9	**0.0000**	0.0	0.2329	0.0
AML	5.1	0.0216	91.4	**0.0000**	2.0	0.2900	1.5
ISRT	26.7	**0.0000**	31.4	0.0003	39.5	**0.0000**	2.5
LSRT	16.9	**0.0000**	65.6	**0.0000**	16.9	**0.0000**	0.7
VSRT	25.5	**0.0000**	26.9	0.0022	44.8	**0.0000**	2.8
CLPG	26.4	0.0197	58.1	0.0040	6.7	0.5640	8.8
TMSN	32.0	**0.0000**	48.0	**0.0000**	18.9	**0.0000**	1.2
SAL	79.5	**0.0000**	12.1	0.0320	5.8	0.0811	2.6
WARF	30.7	**0.0000**	48.4	**0.0000**	18.1	**0.0000**	2.7
IOP	24.8	**0.0000**	56.0	**0.0000**	19.0	**0.0000**	0.2
ALB	4.0	0.0150	90.9	**0.0000**	4.0	0.0140	1.1
TALB	33.8	0.0581	6.1	0.5297	46.0	0.0155	14.2
LMS	12.2	0.0085	43.5	**0.0002**	41.2	**0.0000**	3.1
DXT	25.4	**0.0000**	53.9	**0.0000**	19.7	**0.0000**	1.0
XYL	15.1	0.0006	66.2	**0.0000**	16.3	**0.0001**	2.4
AZPN	20.5	**0.0000**	61.3	**0.0000**	17.5	**0.0000**	0.7
AZPL	45.3	**0.0000**	2.5	0.3690	49.2	**0.0000**	3.0
DTZ	5.6	0.1362	79.9	**0.0000**	11.5	0.0105	3.0
ERY	11.6	**0.0000**	75.9	**0.0000**	11.4	**0.0000**	1.1
AZY		1.0000		1.0000		1.0000	
CLARI	13.2	0.3544	60.1	0.0418	13.2	0.4122	13.6
TCN	6.6	0.0540	84.2	**0.0000**	6.6	0.0518	2.6
SMX	1.7	0.4439	92.7	**0.0000**	3.7	0.1178	1.9
TMP	5.7	0.0033	86.8	**0.0000**	6.3	0.0015	1.2
MTZ	1.1	0.5699	95.7	**0.0000**	1.6	0.4174	1.6
OH-MTZ	19.9	0.0159	54.2	0.0020	20.0	0.0165	6.0
OFLX	24.3	0.0219	43.5	0.0133	24.3	0.0178	7.9
CPFX	24.6	**0.0000**	52.2	**0.0000**	22.6	**0.0000**	0.7
CEF	6.0	0.0009	87.1	**0.0000**	6.0	0.0008	1.0
DMZ	13.4	0.1884	74.4	0.0029	3.7	0.7281	8.4
RNZ	37.3	**0.0001**	42.1	0.0030	15.7	0.0241	4.9

Table S-8*.(cont.)*

	Univariate ANOVA by permutation (Sediment) Sources of variation						
PhACs	Basin		Campaign		Basin x Campaign		Residuals
	Var. Exp.(%)	p-value	Var. Exp.(%)	p-value	Var. Exp.(%)	p-value	Var. Exp.(%)
Mean	22.3		50.1		20.9		6.7
Confidence Interval	3.7		6.4		3.7		1.3
Min	0.0		0.0		0.0		0.0
Max	79.5		99.9		72.2		24.1
No. Signif. (Bonferroni)		26		37		23	

Table S-9. Permanova for the comparison of the C2:C1 ratios for PhACs and for the discharge

Surface water	DF	SS	MS	F	p
Discharge ratio	1	276.7	276.6	0.96	0.4105
Residuals	46	13217.3	287.3		
Total	47	13493.9			
Sediment	DF	SS	MS	F	p
Discharge ratio	1	105.6	105.6	0.86	0.5746
Residuals	41	5018.2	122.4		
Total	42	5123.8			

Table S-10. Total concentrations of pharmaceuticals detected in surface waters from each sampling site across basins and over sampling campaigns.
Totals in **bold** and *italic* are the **maximum** and *minimum* concentrations determined in the underlined sampling site of each basin.

Surface water			Analgesics/anti-inflammatories		Lipid regulators and cholesterol lowering statin drugs		Psychiatric drugs		Histamine H1 and H2 receptor antagonists		β-Blocking agents		Diuretics	
Catchment	**Subcatchment**	**Site**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**
Llobregat	Llobregat	LLO1	17.26	28.52	0.33	2.70	9.81	1.95	2.49	1.82	0.87	0.07	0.12	2.29
Llobregat	Llobregat	LLO2	11.73	28.42	6.76	4.63	6.19	1.75	2.56	1.62	0.87	ND	0.12	1.36
Llobregat	Llobregat	LLO3	106.19	39.64	45.22	11.40	44.20	3.86	3.46	ND	6.61	0.03	147.17	20.10
Llobregat	Cardener	CAR1	9.14	29.24	0.54	6.30	3.29	3.31	0.55	ND	0.78	0.18	7.38	2.00
Llobregat	Cardener	CAR2	4.45	33.40	0.48	2.65	1.46	1.99	2.14	ND	0.88	0.18	15.73	1.49
Llobregat	Cardener	CAR3	29.47	40.62	7.79	22.36	10.65	5.50	2.30	ND	2.01	0.36	96.90	24.38
Llobregat	Cardener	CAR4	14.31	68.39	2.15	14.05	57.98	27.05	2.09	1.75	3.51	1.37	388.54	58.79
Llobregat	Llobregat	LLO4	154.24	51.74	44.89	15.75	60.23	6.68	7.89	ND	7.67	0.56	232.05	35.20
Llobregat	Llobregat	LLO5	382.26	52.08	124.15	24.60	73.76	10.29	3.52	ND	14.58	0.28	214.25	26.38
Llobregat	Anoia	ANO1	75.69	30.64	38.25	3.33	38.70	7.17	2.55	0.31	0.87	4.66	223.78	12.19
Llobregat	Anoia	ANO2	628.67	307.72	268.79	247.62	430.87	117.74	31.57	10.60	383.65	8.40	587.96	459.28
Llobregat	Anoia	ANO3	366.11	212.23	175.41	302.38	116.79	31.60	2.78	1.90	65.00	1.46	816.17	121.39
Llobregat	Llobregat	LLO6	426.18	98.66	68.89	60.42	64.73	9.43	5.41	ND	27.88	1.07	199.49	52.31
Llobregat	Llobregat	LLO7	488.67	507.65	334.93	272.46	414.22	148.84	24.12	6.07	414.78	315.41	410.80	421.02
Ebro	Ebro	EBR1	105.45	44.89	1.47	2.76	2.44	2.69	2.24	ND	0.98	0.10	30.71	7.11
Ebro	Oca	OCA	145.12	54.64	12.22	10.99	26.90	3.02	2.09	1.75	4.55	0.03	107.11	23.42
Ebro	Ebro	EBR2	17.40	47.45	4.83	5.52	10.89	2.96	2.24	1.75	0.98	0.24	50.47	1.55
Ebro	Zadorra	ZAD	532.79	678.46	353.30	44.63	528.87	170.28	25.95	4.06	665.89	183.34	460.38	1147.42
Ebro	Ebro	EBR3	75.90	50.73	19.38	7.25	38.94	3.40	3.46	ND	22.98	0.10	100.67	6.16
Ebro	Nájerilla	NAJ	21.99	17.95	0.30	5.88	5.71	2.07	2.09	ND	0.87	1.49	65.77	12.20
Ebro	Arga	ARG	383.04	173.68	130.97	83.32	284.66	28.33	7.36	1.75	51.77	0.71	291.21	145.27
Ebro	Ebro	EBR4	67.47	44.65	28.37	16.47	32.12	4.28	5.52	ND	15.01	0.35	82.96	4.36
Ebro	Ebro	EBR5	49.46	29.78	13.26	9.32	19.65	3.25	2.24	ND	0.98	0.18	46.74	8.40
Ebro	Gállego	GAL1	3.96	21.29	1.14	7.10	4.87	3.25	2.40	ND	1.37	0.28	0.88	2.44
Ebro	Gállego	GAL2	16.59	21.18	2.18	3.79	4.50	1.78	2.40	ND	1.26	1.68	11.36	1.94
Ebro	Huerva	HUE	1364.11	586.49	61.81	113.99	41.98	68.29	2.16	1.82	9.22	2.43	317.14	292.94
Ebro	Ebro	EBR6	207.20	114.72	35.59	55.47	113.23	16.91	4.26	3.18	15.03	1.04	165.80	47.46
Ebro	Martín	MAR	20.78	23.92	8.96	9.60	13.89	1.75	2.09	ND	0.87	0.07	12.97	9.43
Ebro	Ésera	ESE	21.16	26.42	5.30	5.23	5.23	2.70	2.09	ND	0.87	ND	0.12	1.75
Ebro	Cinca	CIN1	10.58	31.50	3.42	4.52	2.63	53.31	0.69	6.31	1.86	1.91	21.27	4.32
Ebro	Cinca	CIN2	18.84	28.36	2.56	5.56	9.04	60.25	2.09	7.14	0.87	2.90	23.84	6.24
Ebro	Ribera Salada	RS	12.90	19.38	0.27	2.55	5.79	1.44	2.09	0.19	0.87	ND	0.12	4.98
Ebro	Segre	SEG	323.37	42.82	112.12	26.71	31.76	61.86	2.24	5.54	11.13	4.03	151.25	12.56
Ebro	Matarranya	MAT	10.85	15.54	1.07	2.58	11.60	2.48	2.09	ND	0.87	ND	72.04	24.37
Ebro	Algars	ALG	54.45	17.06	0.26	2.98	3.98	1.99	0.19	ND	2.73	ND	20.81	1.55
Ebro	Ebro	EBR7	20.06	21.03	8.56	5.13	9.71	84.41	2.09	8.74	0.30	6.07	0.12	3.34
Ebro	Ebro	EBR8	35.29	29.02	5.87	6.26	13.81	3.56	2.09	0.91	0.87	0.28	10.51	11.94
Ebro	Ebro	EBR9	21.63	20.07	8.47	6.20	14.44	2.15	2.24	0.70	0.87	0.18	0.12	1.83

Table S-10 (cont.)

Surface water			Analgesics/anti-inflammatories		Lipid regulators and cholesterol lowering statin drugs		Psychiatric drugs		Histamine H1 and H2 receptor antagonists		β-Blocking agents		Diuretics	
Catchment	**Subcatchment**	**Site**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**
Júcar	Júcar	JUC1	9.98	41.49	4.23	8.21	3.32	47.96	2.05	5.39	0.91	2.48	0.84	6.15
Júcar	Júcar	JUC2	7.57	35.14	3.14	7.03	4.08	4.31	2.05	0.15	0.93	0.03	0.84	1.65
Júcar	Júcar	JUC3	14.11	39.19	4.97	15.48	4.88	10.85	2.24	1.75	0.50	0.10	4.22	9.65
Júcar	Júcar	JUC4	13.21	31.87	5.30	8.18	5.36	7.49	2.05	0.15	0.96	0.03	0.88	2.53
Júcar	Júcar	JUC5	5.58	10.86	0.47	0.45	2.73	2.12	0.49	ND	0.03	ND	1.64	0.65
Júcar	Cabriel	CAB1	8.38	12.19	2.60	1.45	2.09	2.09	2.24	1.75	0.43	ND	0.88	1.55
Júcar	Cabriel	CAB2	NA	12.61	NA	0.97	NA	1.75	NA	1.75	NA	ND	NA	1.41
Júcar	Cabriel	CAB3	6.93	13.52	2.74	0.71	3.51	2.90	2.24	1.75	0.03	0.40	0.88	0.53
Júcar	Cabriel	CAB4	8.74	15.57	0.72	0.63	3.94	3.61	2.09	ND	0.40	ND	0.12	0.12
Júcar	Cabriel	CAB5	8.37	9.97	1.07	0.48	1.14	1.89	0.34	1.75	ND	ND	0.12	0.45
Júcar	Júcar	JUC6	7.87	14.57	3.42	1.09	3.38	1.58	2.55	ND	0.97	ND	0.84	1.09
Júcar	Júcar	JUC7	12.69	31.25	4.34	11.03	4.55	6.60	2.62	1.75	1.28	0.14	2.57	18.79
Júcar	Magro	MAG1	29.27	35.67	23.51	3.03	9.56	11.60	2.09	ND	0.78	0.03	33.88	10.57
Júcar	Magro	MAG2	13.86	101.67	9.46	17.08	4.24	52.04	1.94	9.73	0.43	13.86	8.92	54.81
Júcar	Júcar	JUC8	17.67	14.28	4.13	4.67	2.86	7.59	2.09	1.75	0.50	0.49	3.91	1.67
Guadalquivir	Borosa	BOR	9.18	35.33	7.49	7.55	3.00	4.16	4.45	6.06	0.03	0.87	0.88	16.57
Guadalquivir	Guadalquivir	GUA1	2.67	36.36	0.64	6.19	4.16	2.67	5.60	4.94	0.40	0.61	0.84	4.05
Guadalquivir	Guadiana Menor	GUAM	5.54	14.08	1.96	16.40	2.44	3.34	4.53	5.98	ND	0.75	0.88	11.74
Guadalquivir	Guadalquivir	GUA2	26.97	142.08	7.23	37.03	7.87	13.86	10.15	15.95	0.70	0.61	94.17	24.25
Guadalquivir	Magaña	MAG	10.55	8.48	0.67	1.07	3.65	0.94	5.80	3.07	ND	0.60	15.70	6.42
Guadalquivir	Guadabullón	GUAN	50.70	73.97	40.80	175.09	3.03	19.14	5.18	25.93	0.32	2.32	22.92	61.27
Guadalquivir	Guadalquivir	GUA3	19.71	33.57	4.66	61.53	3.24	16.81	5.52	18.86	0.70	1.34	2.90	31.89
Guadalquivir	Yeguas	YEG	5.88	145.40	2.79	18.02	3.43	16.09	5.58	18.15	ND	0.62	0.88	33.01
Guadalquivir	Guadalmoral	GUAL	6.94	151.76	3.22	14.01	4.46	15.74	6.67	17.83	0.40	0.61	0.88	6.68
Guadalquivir	Guadalquivir	GUA4	17.59	121.66	11.38	37.03	8.24	48.41	10.45	50.47	1.10	1.26	15.88	96.26
Guadalquivir	Picachos	PIC	16.00	62.65	0.99	7.43	2.83	2.80	4.92	4.85	0.43	0.58	7.69	28.85
Guadalquivir	Bembézar	BEM	4.38	18.88	0.57	53.23	2.98	6.70	4.43	8.94	ND	0.47	6.19	23.74
Guadalquivir	Cacín	CAC	4.58	22.63	1.32	12.27	2.24	2.24	3.75	12.54	ND	0.69	0.88	15.82
Guadalquivir	Genil	GEN1	54.96	86.61	38.37	144.37	8.11	22.56	10.40	24.61	0.70	2.99	6.17	42.12
Guadalquivir	Genil	GEN2	20.81	65.61	15.92	56.08	3.22	6.85	5.30	9.09	0.48	1.24	5.51	36.07
Guadalquivir	Guadalquivir	GUA5	18.92	68.54	10.17	22.67	7.86	15.35	10.08	17.25	0.70	0.61	2.95	20.35
Guadalquivir	Corbones	COR	12.21	66.27	3.98	39.64	5.78	5.21	8.00	10.12	0.70	0.55	4.05	48.20
Guadalquivir	Herreros	HER	47.33	76.16	14.20	13.96	2.87	3.67	4.38	5.73	0.12	0.58	12.46	38.13
Guadalquivir	Guadaira	GUAA	43.87	190.87	44.57	173.07	11.71	20.33	13.93	22.38	0.70	1.96	33.48	95.97
Guadalquivir	Guadalquivir	GUA6	46.96	48.23	60.57	48.91	11.08	19.40	13.30	21.64	0.63	0.61	1.28	14.88
Guadalquivir	Guadalquivir	GUA7	10.31	25.52	1.53	43.16	6.51	16.44	8.73	18.68	0.63	0.61	0.88	35.04
Guadalquivir	Guadalquivir	GUA8	4.67	5.03	9.36	6.72	4.69	18.58	6.20	20.82	0.47	0.61	3.11	11.31
Guadalquivir	Guadiamar	GUAR	6.89	8.78	1.41	1.29	2.71	2.61	4.15	120.44	ND	0.40	0.88	6.18
Guadalquivir	Guadalquivir	GUA9	2.85	8.04	1.92	2.24	4.01	6.88	5.52	9.12	ND	0.61	0.84	3.34

Table S-10 (cont.)

Surface water			Antidiabetic		Antihypertensives		Antiplatelet agent		Prostatic hyperplasia		To treat asthma		Anticoagulant		X-ray contrast agent	
Catchment	**Subcatchment**	**Site**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**
Llobregat	Llobregat	LLO1	0.90	0.90	0.84	1.52	0.02	0.13	0.03	0.03	0.07	ND	0.06	ND	ND	ND
Llobregat	Llobregat	LLO2	0.90	0.90	2.39	0.13	0.02	ND	0.03	ND	ND	ND	0.06	ND	ND	ND
Llobregat	Llobregat	LLO3	0.90	0.90	119.31	1.76	0.11	0.22	0.36	ND	0.60	0.02	0.06	ND	2.04	ND
Llobregat	Cardener	CAR1	0.90	0.90	4.65	4.45	0.02	ND	0.03	ND	ND	0.02	ND	ND	ND	ND
Llobregat	Cardener	CAR2	0.90	0.90	10.32	1.57	0.02	ND	ND	ND	ND	0.02	ND	ND	ND	ND
Llobregat	Cardener	CAR3	0.90	0.90	30.48	0.44	0.25	0.16	0.03	ND	0.02	0.02	ND	0.06	ND	ND
Llobregat	Cardener	CAR4	0.90	0.90	21.97	2.46	0.71	1.24	0.03	ND	0.02	0.12	ND	ND	0.30	ND
Llobregat	Llobregat	LLO4	0.90	0.90	139.57	3.06	0.02	0.42	0.67	ND	1.31	ND	0.06	ND	ND	ND
Llobregat	Llobregat	LLO5	0.90	0.90	286.52	4.43	0.47	0.40	0.30	ND	0.52	0.02	0.06	ND	40.98	ND
Llobregat	Anoia	ANO1	0.90	0.90	116.70	1.50	0.11	0.18	0.03	ND	0.42	0.04	0.06	ND	ND	ND
Llobregat	Anoia	ANO2	0.90	4.61	802.64	29.84	1.39	14.49	0.66	0.22	16.09	0.49	0.06	0.22	ND	ND
Llobregat	Anoia	ANO3	0.90	0.90	369.18	16.84	1.03	0.47	0.03	ND	0.02	0.09	ND	ND	ND	ND
Llobregat	Llobregat	LLO6	0.90	0.90	155.73	3.69	0.37	0.51	0.39	ND	0.25	0.02	0.06	ND	1368.76	0.30
Llobregat	Llobregat	LLO7	2.49	2.08	559.62	21.27	3.58	5.13	0.65	ND	2.65	0.30	0.06	ND	457.65	0.95
Ebro	Ebro	EBR1	0.90	0.90	12.38	0.70	0.02	0.06	0.21	ND	ND	0.02	0.06	ND	0.30	ND
Ebro	Oca	OCA	0.90	0.90	223.35	6.23	0.07	0.58	0.03	ND	0.12	0.07	0.06	ND	9.69	ND
Ebro	Ebro	EBR2	0.90	0.90	30.14	0.63	0.02	0.13	0.25	ND	ND	ND	0.06	ND	38.02	ND
Ebro	Zadorra	ZAD	1.80	0.90	457.52	959.79	1.84	2.53	1.61	0.32	11.91	9.96	0.06	0.16	320.24	51.53
Ebro	Ebro	EBR3	0.90	0.90	138.58	3.61	0.10	0.07	0.31	ND	1.13	0.05	0.06	0.06	4.13	ND
Ebro	Nájerilla	NAJ	0.90	0.90	85.49	2.26	0.02	0.04	0.03	ND	0.16	ND	0.06	ND	0.30	ND
Ebro	Arga	ARG	0.90	0.90	225.19	12.16	0.41	0.64	0.22	ND	1.27	0.06	0.06	ND	54.32	ND
Ebro	Ebro	EBR4	0.90	0.90	129.02	1.58	0.14	0.16	0.32	ND	1.14	0.02	0.06	ND	67.65	ND
Ebro	Ebro	EBR5	0.90	0.90	121.07	2.77	0.02	0.07	0.21	ND	0.02	0.02	0.06	ND	0.94	ND
Ebro	Gállego	GAL1	0.90	0.90	4.82	1.98	0.02	0.02	0.03	ND	ND	0.04	ND	ND	0.30	ND
Ebro	Gállego	GAL2	0.90	0.90	13.49	1.24	0.02	ND	0.03	ND	0.02	0.02	ND	ND	0.30	ND
Ebro	Huerva	HUE	0.90	0.90	247.95	12.48	0.09	7.31	0.03	ND	0.62	0.44	0.06	0.06	0.30	ND
Ebro	Ebro	EBR6	0.90	0.90	289.48	7.54	0.29	0.49	0.18	ND	7.54	0.02	0.06	ND	29.32	0.30
Ebro	Martín	MAR	0.90	0.90	10.42	1.81	ND	0.06	0.03	ND	0.17	0.02	0.06	ND	ND	ND
Ebro	Ésera	ESE	0.90	0.90	6.30	1.08	0.02	ND	0.03	ND	0.05	0.02	0.06	ND	ND	ND
Ebro	Cinca	CIN1	0.90	3.12	4.70	1.11	0.02	7.46	0.03	ND	ND	0.26	ND	0.06	0.30	ND
Ebro	Cinca	CIN2	0.90	3.57	4.96	5.71	0.02	8.09	0.03	ND	0.17	0.23	0.06	0.06	1.59	ND
Ebro	Ribera Salada	RS	0.90	0.90	1.58	0.13	0.02	ND	0.03	ND	0.04	0.02	0.06	ND	ND	ND
Ebro	Segre	SEG	0.90	3.04	192.97	6.77	0.21	7.15	0.03	ND	1.40	0.34	0.06	0.06	13.87	ND
Ebro	Matarranya	MAT	0.90	0.90	2.81	1.81	0.02	ND	0.03	ND	0.02	ND	0.06	ND	0.68	ND
Ebro	Algars	ALG	0.90	0.90	2.10	3.16	ND	ND	ND	ND	ND	ND	ND	ND	0.30	ND
Ebro	Ebro	EBR7	0.90	0.90	11.65	3.78	0.02	6.11	0.03	2.17	0.04	0.55	0.06	0.06	1.69	ND
Ebro	Ebro	EBR8	0.90	0.90	28.38	2.44	0.02	0.04	0.03	ND	0.14	0.02	0.06	ND	5.95	ND
Ebro	Ebro	EBR9	0.90	0.90	29.08	6.80	0.02	ND	0.03	ND	0.16	ND	0.06	ND	2.50	ND

Table S-10 (cont.)

Surface water			Antidiabetic		Antihypertensives		Antiplatelet agent		Prostatic hyperplasia		To treat asthma		Anticoagulant		X-ray contrast agent	
Catchment	**Subcatchment**	**Site**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**
Júcar	Júcar	JUC1	0.90	4.06	11.80	1.40	0.74	6.51	0.10	0.21	ND	0.85	ND	ND	ND	ND
Júcar	Júcar	JUC2	0.90	0.90	12.58	0.86	0.75	ND	0.11	0.03	ND	0.76	ND	ND	ND	ND
Júcar	Júcar	JUC3	0.90	0.90	7.31	4.90	0.37	0.51	0.03	0.03	ND	0.73	ND	ND	ND	1.33
Júcar	Júcar	JUC4	0.90	0.90	12.89	3.33	0.80	0.04	0.10	0.03	ND	ND	ND	ND	0.30	0.30
Júcar	Júcar	JUC5	0.90	0.90	2.81	0.52	ND	ND	0.03	ND	0.02	0.70	0.06	ND	ND	ND
Júcar	Cabriel	CAB1	0.90	0.90	5.71	1.25	0.02	ND	0.03	ND	ND	0.71	ND	ND	ND	ND
Júcar	Cabriel	CAB2	NA	0.90	NA	1.23	NA	0.02	NA	ND	NA	0.69	NA	ND	NA	ND
Júcar	Cabriel	CAB3	0.90	0.90	3.92	1.15	0.05	ND	ND	ND	0.02	0.71	0.06	ND	ND	ND
Júcar	Cabriel	CAB4	0.90	0.90	2.68	0.45	0.02	ND	0.03	0.03	0.02	0.72	0.06	ND	2.38	ND
Júcar	Cabriel	CAB5	0.90	0.90	1.84	1.50	0.22	ND	0.03	ND	ND	0.71	ND	ND	ND	ND
Júcar	Júcar	JUC6	0.90	0.90	11.42	1.10	0.74	ND	0.10	ND	ND	0.74	ND	ND	ND	ND
Júcar	Júcar	JUC7	0.90	0.90	12.88	3.44	0.98	0.29	0.11	0.03	0.09	ND	ND	ND	ND	1.17
Júcar	Magro	MAG1	0.90	0.90	12.35	6.50	0.66	0.18	0.03	ND	0.02	0.74	0.06	ND	0.30	ND
Júcar	Magro	MAG2	0.90	2.43	5.41	85.82	0.19	3.86	0.03	5.28	0.02	13.09	0.06	2.47	ND	17.61
Júcar	Júcar	JUC8	0.90	0.90	4.01	1.51	0.25	0.06	ND	ND	0.02	0.73	0.06	ND	0.30	0.96
Guadalquivir	Borosa	BOR	0.90	0.90	4.05	5.20	0.04	0.34	0.03	ND	0.02	0.02	0.06	0.13	ND	1.31
Guadalquivir	Guadalquivir	GUA1	0.90	0.90	2.56	2.35	ND	0.02	ND	ND	0.02	0.02	0.06	ND	ND	0.30
Guadalquivir	Guadiana Menor	GUAM	0.90	0.90	3.52	2.52	0.02	0.34	0.03	ND	ND	0.02	ND	0.06	ND	1.61
Guadalquivir	Guadalquivir	GUA2	0.90	0.90	43.50	3.66	0.02	0.99	0.21	ND	0.06	0.02	0.06	ND	0.30	0.30
Guadalquivir	Magaña	MAG	0.90	0.90	5.30	4.70	0.02	0.26	0.03	ND	0.02	0.02	0.06	0.06	ND	1.46
Guadalquivir	Guadabullón	GUAN	0.90	0.90	6.80	8.77	0.88	1.43	0.03	ND	0.02	0.02	0.06	0.06	ND	1.47
Guadalquivir	Guadalquivir	GUA3	0.90	0.90	1.55	6.76	0.02	1.15	0.19	ND	ND	0.02	0.06	0.06	ND	1.66
Guadalquivir	Yeguas	YEG	0.90	0.90	4.05	6.52	0.02	0.28	ND	ND	0.02	0.02	0.06	0.06	ND	1.68
Guadalquivir	Guadalmoral	GUAL	0.90	0.90	4.11	2.95	0.02	ND	0.03	ND	ND	0.02	ND	ND	ND	0.30
Guadalquivir	Guadalquivir	GUA4	0.90	0.90	13.49	7.02	1.14	1.42	0.10	ND	0.86	0.02	ND	ND	0.30	6.41
Guadalquivir	Picachos	PIC	0.90	0.90	4.31	6.25	0.02	0.29	0.03	ND	0.02	0.02	0.06	ND	ND	1.53
Guadalquivir	Bembézar	BEM	0.90	0.90	3.69	4.50	ND	0.27	0.03	ND	ND	0.02	ND	ND	ND	1.36
Guadalquivir	Cacín	CAC	0.90	0.90	2.90	2.74	ND	0.26	ND	ND	ND	0.02	0.06	ND	ND	1.98
Guadalquivir	Genil	GEN1	0.90	0.90	4.19	6.71	0.02	1.05	0.03	ND	0.02	0.02	0.06	ND	1.15	3.41
Guadalquivir	Genil	GEN2	0.90	0.90	4.94	7.52	0.26	0.88	ND	ND	0.02	0.02	0.06	ND	ND	4.78
Guadalquivir	Guadalquivir	GUA5	0.90	0.90	1.34	2.99	ND	0.33	0.03	ND	0.12	0.02	0.06	ND	0.63	2.25
Guadalquivir	Corbones	COR	0.90	0.90	0.96	3.87	0.02	0.43	0.03	ND	0.04	0.02	0.06	ND	1.57	1.66
Guadalquivir	Herreros	HER	0.90	0.90	5.19	3.45	0.38	0.25	0.03	ND	ND	0.02	ND	ND	ND	ND
Guadalquivir	Guadaira	GUAA	0.90	0.90	1.64	4.91	0.08	1.32	0.03	ND	0.24	0.02	0.06	ND	0.68	1.56
Guadalquivir	Guadalquivir	GUA6	0.90	0.90	1.03	3.00	ND	0.34	0.03	ND	0.02	0.02	0.06	ND	0.99	2.49
Guadalquivir	Guadalquivir	GUA7	0.90	0.90	0.43	4.46	0.02	0.27	0.03	ND	ND	0.02	0.06	ND	0.79	0.30
Guadalquivir	Guadalquivir	GUA8	0.90	0.90	3.91	2.59	0.21	ND	0.03	ND	0.02	0.02	0.06	ND	ND	ND
Guadalquivir	Guadiamar	GUAR	0.90	0.90	3.97	4.48	0.02	0.21	ND	ND	0.02	0.02	0.06	ND	ND	1.20
Guadalquivir	Guadalquivir	GUA9	0.90	0.90	2.90	2.05	0.06	ND	0.03	ND	ND	0.02	ND	ND	ND	ND

Table S-10 (cont.)

Surface water			Antihelmintics		Synthetic glucocorticoid		Sedation and muscle relaxation		Tranquilizers		Calcium channel blocker		Antibiotics	
Catchment	**Subcatchment**	**Site**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**
Llobregat	Llobregat	LLO1	0.06	2.57	ND	ND	0.05	ND	0.93	0.93	0.16	2.74	10.05	51.43
Llobregat	Llobregat	LLO2	0.06	2.25	ND	ND	0.05	ND	0.93	0.93	1.48	ND	12.47	11.08
Llobregat	Llobregat	LLO3	7.24	3.13	2.54	ND	0.05	ND	0.93	0.93	4.88	ND	22.23	10.28
Llobregat	Cardener	CAR1	1.13	2.22	0.63	ND	ND	ND	0.93	0.93	1.77	3.02	15.82	9.57
Llobregat	Cardener	CAR2	1.25	2.10	0.52	ND	ND	ND	0.93	0.93	2.20	ND	13.66	10.43
Llobregat	Cardener	CAR3	1.50	2.35	ND	ND	ND	ND	0.93	0.93	1.48	ND	17.32	4.43
Llobregat	Cardener	CAR4	5.27	4.17	ND	ND	ND	ND	0.39	0.93	2.28	3.02	62.74	10.92
Llobregat	Llobregat	LLO4	9.28	3.02	4.85	ND	0.05	ND	0.93	0.93	1.57	ND	31.71	10.96
Llobregat	Llobregat	LLO5	9.10	3.03	2.47	ND	0.05	ND	0.93	0.93	3.92	2.88	36.90	10.69
Llobregat	Anoia	ANO1	0.51	2.11	ND	ND	0.05	ND	0.93	0.93	2.66	ND	12.91	10.63
Llobregat	Anoia	ANO2	50.79	11.06	ND	2.56	0.11	0.27	0.54	9.37	31.80	9.92	109.74	56.46
Llobregat	Anoia	ANO3	4.80	5.11	ND	ND	ND	ND	0.93	0.93	9.10	2.80	72.53	8.32
Llobregat	Llobregat	LLO6	8.81	3.34	2.61	ND	0.05	ND	0.93	0.93	3.48	ND	55.50	10.59
Llobregat	Llobregat	LLO7	24.71	10.71	2.82	1.46	0.05	0.15	0.93	0.93	21.20	4.92	262.12	67.55
Ebro	Ebro	EBR1	2.81	2.30	2.31	ND	0.05	ND	0.93	0.93	0.20	ND	11.79	8.61
Ebro	Oca	OCA	0.06	2.22	ND	ND	0.05	ND	0.93	0.93	3.18	ND	14.55	8.12
Ebro	Ebro	EBR2	3.25	2.15	2.42	ND	0.05	ND	0.93	0.93	3.24	ND	13.72	8.95
Ebro	Zadorra	ZAD	57.31	18.99	2.54	ND	ND	ND	0.93	0.39	42.60	16.82	346.32	383.58
Ebro	Ebro	EBR3	5.04	2.22	2.38	ND	0.05	ND	0.93	0.93	2.88	ND	27.10	9.37
Ebro	Nájerilla	NAJ	0.06	2.52	ND	ND	0.05	ND	0.93	0.93	0.29	ND	10.03	7.60
Ebro	Arga	ARG	14.49	4.43	2.02	ND	ND	ND	0.93	0.93	2.42	ND	61.78	9.54
Ebro	Ebro	EBR4	4.92	2.85	2.45	ND	0.05	ND	0.93	0.93	3.10	ND	31.22	9.98
Ebro	Ebro	EBR5	4.24	2.98	2.45	ND	0.05	ND	0.93	0.93	0.03	ND	17.71	8.57
Ebro	Gállego	GAL1	0.55	2.31	ND	ND	0.05	ND	0.93	0.93	ND	ND	12.57	3.00
Ebro	Gállego	GAL2	0.64	2.81	ND	1.34	ND	ND	0.93	0.93	ND	ND	4.72	7.90
Ebro	Huerva	HUE	1.86	10.30	ND	ND	0.05	ND	0.93	0.93	4.82	5.48	12.11	44.46
Ebro	Ebro	EBR6	35.44	3.61	2.59	ND	ND	ND	0.93	0.93	1.21	2.96	53.92	8.06
Ebro	Martín	MAR	0.06	3.45	1.43	ND	0.05	ND	0.93	0.93	0.03	ND	13.79	20.62
Ebro	Ésera	ESE	0.06	2.16	1.41	ND	0.05	ND	0.93	0.93	0.03	ND	10.77	3.20
Ebro	Cinca	CIN1	1.11	14.08	0.48	2.38	0.05	0.34	0.93	6.92	1.40	ND	2.63	2.41
Ebro	Cinca	CIN2	0.06	10.18	ND	2.40	0.05	0.45	0.93	9.00	1.69	ND	17.43	9.79
Ebro	Ribera Salada	RS	0.06	2.74	1.42	1.30	0.05	ND	0.93	0.93	0.03	ND	12.99	9.38
Ebro	Segre	SEG	135.56	50.19	ND	2.25	0.05	0.71	0.93	8.37	0.46	3.00	15.21	8.82
Ebro	Matarranya	MAT	3.27	2.20	ND	ND	0.05	ND	0.93	0.93	0.09	ND	12.68	9.27
Ebro	Algars	ALG	1.75	5.24	ND	ND	ND	ND	0.93	0.93	3.69	ND	16.15	2.43
Ebro	Ebro	EBR7	53.23	79.86	1.81	2.71	ND	1.63	0.93	8.37	0.03	3.44	12.10	15.22
Ebro	Ebro	EBR8	52.03	21.96	ND	ND	0.05	ND	0.93	0.93	1.48	ND	13.79	8.40
Ebro	Ebro	EBR9	54.83	10.96	ND	ND	0.05	ND	0.93	0.93	0.27	ND	13.72	8.43

Table S-10 (cont.)

Surface water			Antihelmintics		Synthetic glucocorticoid		Sedation and muscle relaxation		Tranquilizers		Calcium channel blocker		Antibiotics	
Catchment	**Subcatchment**	**Site**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**
Júcar	Júcar	JUC1	1.40	10.25	1.88	2.32	0.05	0.34	0.93	7.06	0.03	ND	12.26	18.12
Júcar	Júcar	JUC2	1.36	2.62	1.90	1.34	0.05	ND	0.93	0.93	ND	ND	12.54	2.80
Júcar	Júcar	JUC3	0.14	3.63	1.27	ND	0.20	ND	0.93	0.93	0.14	ND	9.82	17.90
Júcar	Júcar	JUC4	1.50	66.13	1.88	ND	0.05	ND	0.93	0.93	ND	ND	10.88	75.09
Júcar	Júcar	JUC5	0.06	2.38	1.27	2.29	0.05	ND	0.93	0.93	0.03	ND	11.49	1.67
Júcar	Cabriel	CAB1	0.05	2.24	1.26	2.29	0.05	ND	0.93	0.93	ND	ND	11.01	9.57
Júcar	Cabriel	CAB2	NA	2.21	NA	2.32	NA	ND	NA	0.93	NA	ND	NA	19.48
Júcar	Cabriel	CAB3	0.06	2.08	1.27	2.35	0.05	ND	0.93	0.93	0.08	ND	10.30	7.84
Júcar	Cabriel	CAB4	0.05	2.14	ND	2.28	0.05	ND	0.93	0.93	0.08	ND	10.60	3.00
Júcar	Cabriel	CAB5	0.05	2.18	1.36	2.31	0.05	ND	0.39	0.93	0.06	ND	7.58	1.97
Júcar	Júcar	JUC6	2.57	2.37	1.91	2.27	0.05	ND	0.93	0.93	0.03	ND	12.27	7.90
Júcar	Júcar	JUC7	11.98	102.91	2.01	ND	0.05	ND	0.93	0.93	0.06	ND	12.44	112.71
Júcar	Magro	MAG1	5.20	2.75	1.30	2.27	0.05	ND	0.39	0.93	0.03	3.82	13.02	9.60
Júcar	Magro	MAG2	7.24	20.35	ND	4.35	0.05	1.39	0.93	7.28	0.03	4.58	11.08	83.22
Júcar	Júcar	JUC8	63.68	75.75	1.28	2.29	0.05	ND	0.93	0.93	ND	ND	12.60	3.98
Guadalquivir	Borosa	BOR	0.05	0.36	ND	ND	0.21	0.05	0.93	0.93	ND	2.40	10.33	31.77
Guadalquivir	Guadalquivir	GUA1	1.38	0.04	1.91	0.08	0.05	0.05	0.93	0.93	ND	0.10	10.33	18.45
Guadalquivir	Guadiana Menor	GUAM	0.69	0.29	1.31	ND	0.05	0.05	0.93	0.93	0.03	2.46	10.32	27.25
Guadalquivir	Guadalquivir	GUA2	3.01	0.03	2.40	0.08	0.05	0.05	0.93	0.93	ND	0.10	15.35	17.48
Guadalquivir	Magaña	MAG	0.05	0.25	1.34	ND	0.05	0.05	0.93	0.93	0.03	2.36	11.17	25.78
Guadalquivir	Guadabullón	GUAN	0.09	0.51	1.32	ND	0.05	0.05	0.93	0.93	ND	3.22	12.14	21.85
Guadalquivir	Guadalquivir	GUA3	2.68	0.40	ND	ND	0.05	0.05	0.93	0.93	0.03	3.14	9.59	23.26
Guadalquivir	Yeguas	YEG	0.06	0.24	ND	ND	0.05	0.05	0.39	0.93	ND	2.42	11.47	28.34
Guadalquivir	Guadalmoral	GUAL	0.05	0.03	1.28	0.08	0.05	0.05	0.39	0.93	ND	0.31	10.32	15.61
Guadalquivir	Guadalquivir	GUA4	1.67	0.38	1.91	ND	0.05	ND	0.93	0.93	ND	3.12	9.37	27.01
Guadalquivir	Picachos	PIC	0.13	0.19	1.31	ND	0.05	ND	0.93	0.93	ND	2.28	4.15	25.71
Guadalquivir	Bembézar	BEM	0.05	0.28	1.28	ND	0.05	ND	0.93	0.93	ND	2.48	9.64	24.03
Guadalquivir	Cacín	CAC	0.07	0.23	ND	0.08	0.05	ND	0.93	0.93	ND	2.34	10.53	26.27
Guadalquivir	Genil	GEN1	0.06	0.49	1.40	ND	0.05	ND	0.93	0.93	ND	3.86	9.64	21.94
Guadalquivir	Genil	GEN2	0.26	0.39	1.28	ND	0.05	ND	0.93	0.93	0.05	3.20	9.59	21.17
Guadalquivir	Guadalquivir	GUA5	0.06	0.33	1.38	0.20	0.05	0.05	0.93	0.93	0.03	0.33	14.12	13.84
Guadalquivir	Corbones	COR	0.06	0.24	1.41	ND	0.05	ND	0.93	0.93	0.03	2.42	13.43	24.93
Guadalquivir	Herreros	HER	0.05	0.27	1.32	ND	0.05	ND	0.93	0.93	ND	2.34	12.88	17.81
Guadalquivir	Guadaira	GUAA	0.06	0.27	1.39	ND	0.05	ND	0.93	0.93	0.03	2.48	11.97	25.10
Guadalquivir	Guadalquivir	GUA6	0.06	1.90	ND	0.08	0.05	0.05	0.93	0.93	0.03	0.26	12.84	16.07
Guadalquivir	Guadalquivir	GUA7	0.06	0.93	1.43	0.08	0.05	0.05	0.93	0.93	0.03	0.10	14.11	15.33
Guadalquivir	Guadalquivir	GUA8	0.73	0.35	1.30	0.08	0.05	0.05	0.93	0.93	ND	0.18	10.71	15.11
Guadalquivir	Guadiamar	GUAR	0.25	0.16	1.27	ND	0.05	ND	0.93	0.93	ND	ND	10.30	21.59
Guadalquivir	Guadalquivir	GUA9	0.25	0.15	1.27	0.08	0.05	0.05	0.93	0.93	ND	0.10	9.18	15.53

Table S-10 (cont.)

Surface water			Total	Total
Catchment	**Subcatchment**	**Site**	**C1**	**C2**
Llobregat	Llobregat	LLO1	*44.06*	97.61
Llobregat	Llobregat	LLO2	46.63	*53.07*
Llobregat	Llobregat	LLO3	514.10	92.28
Llobregat	Cardener	CAR1	47.55	62.15
Llobregat	Cardener	CAR2	54.94	55.64
Llobregat	Cardener	CAR3	202.04	102.51
Llobregat	Cardener	CAR4	**563.20**	**195.15**
Llobregat	Llobregat	LLO4	697.88	129.21
Llobregat	Llobregat	LLO5	1195.64	136.90
Llobregat	Anoia	ANO1	515.11	74.60
Llobregat	Anoia	ANO2	**3346.23**	**1290.86**
Llobregat	Anoia	ANO3	2000.78	706.42
Llobregat	Llobregat	LLO6	2390.45	242.16
Llobregat	Llobregat	LLO7	**3426.05**	**1786.90**
Ebro	Ebro	EBR1	175.25	71.08
Ebro	Oca	OCA	550.98	112.91
Ebro	Ebro	EBR2	179.83	73.16
Ebro	Zadorra	ZAD	**3811.86**	**3673.18**
Ebro	Ebro	EBR3	444.93	84.85
Ebro	Nájerilla	NAJ	195.07	53.83
Ebro	Arga	ARG	**1513.03**	**461.70**
Ebro	Ebro	EBR4	473.35	86.53
Ebro	Ebro	EBR5	280.96	67.17
Ebro	Gállego	GAL1	*34.79*	*43.54*
Ebro	Gállego	GAL2	59.33	45.52
Ebro	Huerva	HUE	**2066.14**	**1148.31**
Ebro	Ebro	EBR6	962.97	263.60
Ebro	Martín	MAR	87.45	72.55
Ebro	Ésera	ESE	55.39	44.39
Ebro	Cinca	CIN1	*53.01*	140.01
Ebro	Cinca	CIN2	85.14	159.92
Ebro	Ribera Salada	RS	*40.16*	*43.93*
Ebro	Segre	SEG	993.52	244.23
Ebro	Matarranya	MAT	120.07	60.09
Ebro	Algars	ALG	108.23	*36.23*
Ebro	Ebro	EBR7	123.32	253.49
Ebro	Ebro	EBR8	172.20	86.66
Ebro	Ebro	EBR9	150.33	59.15

Table S-10 (cont.)

	Surface water		Total	Total
Catchment	**Subcatchment**	**Site**	**C1**	**C2**
Júcar	Júcar	JUC1	51.44	162.78
Júcar	Júcar	JUC2	49.76	58.56
Júcar	Júcar	JUC3	52.01	107.88
Júcar	Júcar	JUC4	58.00	**197.00**
Júcar	Júcar	JUC5	*28.61*	*23.47*
Júcar	Cabriel	CAB1	36.59	36.92
Júcar	Cabriel	CAB2	NA	46.27
Júcar	Cabriel	CAB3	33.98	35.77
Júcar	Cabriel	CAB4	*33.82*	*30.38*
Júcar	Cabriel	CAB5	*23.53*	*25.02*
Júcar	Júcar	JUC6	49.94	34.53
Júcar	Júcar	JUC7	**70.48**	**291.94**
Júcar	Magro	MAG1	**133.41**	88.60
Júcar	Magro	MAG2	64.79	**500.93**
Júcar	Júcar	JUC8	**115.24**	117.56
Guadalquivir	Borosa	BOR	41.67	113.97
Guadalquivir	Guadalquivir	GUA1	*32.45*	*78.05*
Guadalquivir	Guadiana Menor	GUAM	33.14	88.71
Guadalquivir	Guadalquivir	GUA2	**213.89**	258.32
Guadalquivir	Magaña	MAG	56.28	*57.35*
Guadalquivir	Guadabullón	GUAN	146.17	**396.94**
Guadalquivir	Guadalquivir	GUA3	52.72	202.33
Guadalquivir	Yeguas	YEG	35.60	272.75
Guadalquivir	Guadalmoral	GUAL	39.72	227.80
Guadalquivir	Guadalquivir	GUA4	95.36	**402.30**
Guadalquivir	Picachos	PIC	44.77	145.27
Guadalquivir	Bembézar	BEM	35.13	146.71
Guadalquivir	Cacín	CAC	*28.22*	101.95
Guadalquivir	Genil	GEN1	137.17	362.58
Guadalquivir	Genil	GEN2	69.60	214.74
Guadalquivir	Guadalquivir	GUA5	70.34	166.93
Guadalquivir	Corbones	COR	54.20	205.39
Guadalquivir	Herreros	HER	103.12	164.20
Guadalquivir	Guadaira	GUAA	**166.31**	**542.05**
Guadalquivir	Guadalquivir	GUA6	**150.77**	179.71
Guadalquivir	Guadalquivir	GUA7	47.44	162.82
Guadalquivir	Guadalquivir	GUA8	47.37	83.29
Guadalquivir	Guadiamar	GUAR	33.81	169.20
Guadalquivir	Guadalquivir	GUA9	*30.72*	*50.03*

Table S-11. Total concentrations of pharmaceuticals detected in sediments from each sampling site across basins and over sampling campaigns.
Totals in **bold** and *italic* are the **maximum** and *minimum* concentrations determined in the underlined sampling site of each basin.

Sediment			Analgesics/anti-inflammatories		Lipid regulators and cholesterol lowering statin drugs		Psychiatric drugs		Histamine H1 and H2 receptor antagonists		β-Blocking agents		Diuretics	
Catchment	**Subcatchment**	**Site**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**
Llobregat	Llobregat	LLO1	4.50	24.27	0.62	0.59	4.28	2.21	0.46	2.40	0.63	1.20	4.01	4.29
Llobregat	Llobregat	LLO2	22.73	23.01	0.47	0.37	15.24	4.84	0.21	1.66	0.57	0.68	4.01	4.27
Llobregat	Llobregat	LLO3	29.66	9.54	0.71	0.37	8.42	1.76	0.91	0.94	0.87	0.15	4.01	4.25
Llobregat	Cardener	CAR1	13.04	11.52	0.71	0.40	4.01	3.91	0.05	2.00	0.55	0.70	4.01	3.59
Llobregat	Cardener	CAR2	21.88	12.12	0.37	0.37	2.35	2.94	0.28	1.87	0.44	0.95	4.01	3.34
Llobregat	Cardener	CAR3	18.41	9.02	0.62	0.87	6.25	21.81	0.22	0.35	0.58	0.34	4.01	3.47
Llobregat	Cardener	CAR4	15.97	21.18	0.46	0.70	25.37	4.85	0.28	2.24	2.14	1.17	4.29	3.96
Llobregat	Llobregat	LLO4	31.92	25.71	0.59	0.37	19.18	9.36	0.81	0.80	1.47	0.22	4.14	4.26
Llobregat	Llobregat	LLO5	28.89	20.19	0.62	0.59	3.21	3.01	0.83	0.43	0.58	0.15	4.01	4.23
Llobregat	Anoia	ANO1	37.20	10.81	0.68	0.62	10.90	2.37	0.56	2.23	0.73	0.77	4.47	3.33
Llobregat	Anoia	ANO2	29.18	13.79	0.47	0.40	3.49	1.36	0.12	1.18	0.52	0.35	3.96	3.30
Llobregat	Anoia	ANO3	16.62	10.85	1.15	0.37	14.54	3.25	0.17	1.01	0.71	0.26	3.96	4.34
Llobregat	Llobregat	LLO6	33.55	9.11	0.84	2.24	7.09	3.99	0.83	2.23	0.35	0.74	4.01	3.96
Llobregat	Llobregat	LLO7	18.07	9.95	2.34	6.08	127.97	7.97	1.37	5.06	1.58	0.86	4.01	3.96
Ebro	Ebro	EBR1	35.93	9.34	0.74	0.52	3.25	4.61	0.36	0.40	0.39	0.27	4.01	3.01
Ebro	Oca	OCA	16.47	5.50	0.28	0.59	4.28	2.46	0.79	1.01	0.42	0.49	3.96	3.01
Ebro	Ebro	EBR2	28.48	9.45	0.59	0.37	8.43	4.52	0.13	1.61	0.92	0.70	3.01	4.28
Ebro	Zadorra	ZAD	3.83	6.71	0.37	0.70	6.64	14.14	0.71	1.86	0.27	0.59	4.01	3.96
Ebro	Ebro	EBR3	28.52	8.06	0.59	0.43	4.03	9.79	0.55	2.23	0.85	0.82	4.01	3.01
Ebro	Nájerilla	NAJ	16.68	6.96	0.46	0.76	7.90	4.96	6.56	3.48	0.50	1.74	4.01	3.96
Ebro	Arga	ARG	17.60	7.47	0.37	0.62	3.10	5.96	0.84	2.82	0.49	0.22	4.01	3.01
Ebro	Ebro	EBR4	29.30	12.69	0.59	0.59	ND	6.37	ND	0.44	ND	0.49	ND	3.01
Ebro	Ebro	EBR5	NA	7.84	NA	0.37	NA	3.73	NA	2.16	NA	0.72	NA	3.01
Ebro	Gállego	GAL1	17.65	36.15	0.37	0.59	3.67	6.18	0.29	1.81	0.54	3.26	4.01	3.01
Ebro	Gállego	GAL2	15.55	9.63	0.37	0.56	8.12	4.44	0.05	3.78	0.79	1.21	3.96	3.96
Ebro	Huerva	HUE	4.94	NA	0.78	NA	5.73	NA	0.21	NA	1.14	NA	5.67	NA
Ebro	Ebro	EBR6	17.44	8.62	0.37	0.56	29.66	4.03	0.27	7.72	1.02	1.13	3.01	3.01
Ebro	Martín	MAR	27.89	9.52	0.37	0.74	11.34	4.63	0.38	1.59	1.15	0.54	3.96	3.35
Ebro	Ésera	ESE	27.97	18.62	0.55	0.73	2.73	6.46	0.46	1.01	1.15	0.53	10.47	3.30
Ebro	Cinca	CIN1	28.30	4.85	1.00	0.52	9.43	2.77	0.28	1.65	1.10	0.76	3.96	3.31
Ebro	Cinca	CIN2	18.58	17.54	0.37	0.56	5.52	7.82	0.05	3.42	1.27	1.50	3.01	3.01
Ebro	Ribera Salada	RS	7.71	11.46	0.37	0.44	16.42	2.39	0.33	2.10	1.52	0.68	3.96	3.33
Ebro	Segre	SEG	15.77	6.35	0.65	0.57	2.26	6.18	0.05	1.59	0.77	0.78	3.01	3.96
Ebro	Matarranya	MAT	4.82	7.91	0.37	0.50	5.79	2.18	0.45	0.61	0.96	0.29	3.01	3.29
Ebro	Algars	ALG	16.29	17.65	0.37	0.74	4.37	2.23	0.34	2.59	0.79	0.89	3.06	3.96
Ebro	Ebro	EBR7	16.68	8.62	0.59	0.53	ND	3.38	ND	6.44	ND	1.16	ND	3.96
Ebro	Ebro	EBR8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ebro	Ebro	EBR9	17.49	8.73	0.28	0.74	ND	2.37	ND	2.42	ND	0.68	ND	3.96

Table S-11 (cont.)

Sediment			Analgesics/anti-inflammatories		Lipid regulators and cholesterol lowering statin drugs		Psychiatric drugs		Histamine H1 and H2 receptor antagonists		β-Blocking agents		Diuretics	
Catchment	**Subcatchment**	**Site**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**
Jucar	Júcar	JUC1	29.34	29.23	1.51	2.47	3.99	2.49	0.80	1.39	0.60	0.81	3.01	3.69
Jucar	Júcar	JUC2	29.34	37.10	0.40	0.53	3.29	2.85	0.87	1.47	0.67	3.67	3.06	3.01
Jucar	Júcar	JUC3	NA	32.30	NA	0.30	NA	1.92	NA	0.83	NA	1.55	NA	3.01
Jucar	Júcar	JUC4	15.93	15.98	0.37	0.67	3.13	2.56	0.81	0.97	0.66	2.18	3.06	3.01
Jucar	Júcar	JUC5	3.60	30.95	0.59	0.36	3.03	1.23	0.47	0.67	0.86	1.50	3.01	3.01
Jucar	Cabriel	CAB1	28.83	23.58	0.37	0.30	4.55	1.93	0.05	1.92	0.70	2.30	3.06	3.01
Jucar	Cabriel	CAB2	29.09	3.69	0.37	0.30	6.04	1.42	0.18	0.67	0.71	1.04	4.01	3.01
Jucar	Cabriel	CAB3	29.09	19.11	0.40	0.52	4.37	1.08	0.37	0.65	0.36	1.21	4.01	3.01
Jucar	Cabriel	CAB4	NA	7.98	NA	0.30	NA	1.16	NA	0.43	NA	0.59	NA	3.96
Jucar	Cabriel	CAB5	15.98	30.52	0.07	0.39	2.28	1.31	0.40	0.82	1.29	1.96	ND	3.01
Jucar	Júcar	JUC6	32.32	16.41	0.59	0.30	3.29	1.57	0.81	0.48	0.44	0.54	3.06	3.51
Jucar	Júcar	JUC7	27.94	17.36	0.37	0.37	4.81	2.65	0.68	0.63	0.42	0.28	3.01	3.01
Jucar	Magro	MAG1	17.88	22.40	0.46	0.59	8.88	16.12	0.39	0.91	0.85	1.91	3.01	3.57
Jucar	Magro	MAG2	17.92	20.50	0.37	0.79	4.94	2.61	0.16	0.72	0.59	1.39	3.01	3.01
Jucar	Júcar	JUC8	5.34	33.71	0.49	0.84	4.70	4.20	0.29	0.88	0.84	1.72	3.06	3.01
Guadalquivir	Borosa	BOR	31.11	26.82	0.65	0.53	16.60	3.16	17.22	4.17	1.37	2.19	3.01	3.57
Guadalquivir	Guadalquivir	GUA1	32.46	17.71	0.59	0.30	3.41	2.22	4.23	2.53	0.42	0.10	3.01	3.49
Guadalquivir	Guadiana Menor	GUAM	20.55	17.48	0.59	0.37	5.14	1.20	5.32	1.56	1.29	0.28	3.01	3.52
Guadalquivir	Guadalquivir	GUA2	33.76	16.21	0.62	0.37	3.35	2.57	3.60	3.49	0.42	2.20	3.06	3.01
Guadalquivir	Magaña	MAG	4.12	18.62	0.37	0.54	7.62	1.56	7.91	2.13	1.23	1.00	3.01	3.52
Guadalquivir	Guadabullón	GUAN	5.11	16.68	0.54	0.43	3.46	3.01	3.71	6.16	1.38	1.34	3.01	3.56
Guadalquivir	Guadalquivir	GUA3	33.44	17.02	0.62	0.59	3.35	1.45	3.60	2.94	0.59	2.12	3.06	3.57
Guadalquivir	Yeguas	YEG	6.93	21.64	0.37	0.37	6.13	2.80	6.54	3.86	1.31	2.48	3.06	3.01
Guadalquivir	Guadalmoral	GUAL	28.57	28.07	0.59	0.37	6.29	1.38	6.46	1.56	1.45	0.00	3.01	3.01
Guadalquivir	Guadalquivir	GUA4	28.08	22.00	0.37	0.45	6.08	2.36	6.33	3.52	0.98	2.57	3.96	3.01
Guadalquivir	Picachos	PIC	4.98	22.96	0.37	0.48	8.61	2.93	8.83	4.04	0.94	2.63	3.06	3.01
Guadalquivir	Bembézar	BEM	4.19	4.93	0.07	0.39	8.50	2.85	8.76	3.78	1.42	2.37	3.01	5.96
Guadalquivir	Cacín	CAC	18.63	6.64	0.37	0.43	2.48	1.01	2.72	1.39	1.26	0.48	3.06	3.01
Guadalquivir	Genil	GEN1	19.02	15.98	0.28	0.37	3.74	1.48	4.11	2.02	0.79	0.41	3.96	3.52
Guadalquivir	Genil	GEN2	15.88	16.90	0.37	0.57	3.15	2.68	3.49	3.38	1.34	1.16	3.96	3.01
Guadalquivir	Guadalquivir	GUA5	17.99	3.97	0.37	0.45	4.01	3.44	4.27	4.96	1.54	1.85	3.06	3.01
Guadalquivir	Corbones	COR	4.34	21.66	0.37	0.47	12.25	1.66	12.50	2.88	1.60	2.99	3.01	3.01
Guadalquivir	Herreros	HER	8.27	21.50	0.37	0.37	6.47	1.13	6.83	1.66	1.21	0.64	3.01	3.01
Guadalquivir	Guadaira	GUAA	15.40	14.84	0.37	0.54	13.82	2.09	14.28	2.80	1.35	1.36	3.01	3.01
Guadalquivir	Guadalquivir	GUA6	4.58	15.76	0.37	0.30	3.70	2.61	3.81	3.20	1.30	0.75	3.96	3.96
Guadalquivir	Guadalquivir	GUA7	18.46	27.92	0.37	0.37	9.95	1.06	10.21	1.44	0.80	0.37	3.96	3.01
Guadalquivir	Guadalquivir	GUA8	16.29	28.35	0.37	0.59	6.19	1.80	6.48	2.84	0.99	2.33	3.96	3.01
Guadalquivir	Guadiamar	GUAR	2.83	33.49	0.37	0.43	5.44	3.07	5.86	4.04	1.07	1.97	3.96	3.01
Guadalquivir	Guadalquivir	GUA9	26.74	28.50	0.46	0.72	8.05	1.40	8.26	2.08	1.40	1.10	3.96	3.01

Table S-11 (cont.)

Sediment			Antidiabetic		Antihypertensives		Antiplatelet agent		Prostatic hyperplasia		To treat asthma		Anticoagulant		X-ray contrast agent	
Catchment	**Subcatchment**	**Site**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**
Llobregat	Llobregat	LLO1	0.90	ND	0.20	0.05	ND	0.07	0.03	0.03	0.05	0.05	0.23	0.23	ND	0.30
Llobregat	Llobregat	LLO2	0.90	ND	0.20	ND	ND	0.07	0.03	0.03	0.05	ND	0.51	0.23	ND	0.30
Llobregat	Llobregat	LLO3	0.90	ND	8.07	0.24	0.07	0.07	0.03	0.03	0.05	0.05	0.23	0.23	ND	ND
Llobregat	Cardener	CAR1	0.90	ND	0.44	0.42	ND	0.07	0.03	0.03	ND	0.05	0.45	0.23	ND	ND
Llobregat	Cardener	CAR2	ND	ND	0.20	ND	ND	0.07	0.03	0.03	0.05	0.05	1.62	0.23	ND	ND
Llobregat	Cardener	CAR3	0.90	ND	0.34	0.05	ND	0.51	0.03	0.03	ND	0.05	0.23	0.23	ND	ND
Llobregat	Cardener	CAR4	0.90	ND	0.45	0.24	0.19	0.15	0.03	0.03	ND	0.05	0.23	0.23	ND	ND
Llobregat	Llobregat	LLO4	0.90	ND	1.89	0.54	0.07	0.07	0.03	0.03	0.05	ND	0.23	0.23	0.30	ND
Llobregat	Llobregat	LLO5	0.90	ND	1.55	0.24	ND	ND	0.03	0.03	0.05	0.05	0.23	0.23	ND	0.30
Llobregat	Anoia	ANO1	0.90	ND	0.52	0.20	0.07	0.07	0.03	ND	ND	0.05	ND	0.23	ND	ND
Llobregat	Anoia	ANO2	0.90	ND	0.33	0.15	ND	ND	0.03	0.03	ND	0.05	0.23	0.23	ND	0.30
Llobregat	Anoia	ANO3	0.90	ND	0.38	0.00	0.07	0.07	0.03	0.03	0.05	ND	0.23	0.23	ND	ND
Llobregat	Llobregat	LLO6	0.90	ND	1.33	0.05	ND	0.07	0.03	0.03	0.05	ND	0.23	0.23	0.30	ND
Llobregat	Llobregat	LLO7	0.90	ND	3.88	0.39	0.41	1.70	0.03	0.03	0.05	0.05	0.23	0.23	0.30	0.30
Ebro	Ebro	EBR1	0.90	ND	0.18	0.53	0.07	ND	0.03	0.03	ND	ND	ND	0.23	ND	ND
Ebro	Oca	OCA	0.90	ND	1.00	0.20	ND	0.07	0.03	0.03	0.05	ND	0.23	0.23	0.30	ND
Ebro	Ebro	EBR2	0.90	ND	0.94	ND	0.07	0.07	0.03	0.03	ND	0.05	0.23	0.23	ND	0.30
Ebro	Zadorra	ZAD	0.90	ND	1.01	0.25	ND	0.20	0.03	0.03	0.05	0.05	0.23	0.23	ND	ND
Ebro	Ebro	EBR3	0.90	ND	0.05	0.24	0.07	0.07	ND	0.03	ND	0.05	0.23	0.23	ND	0.30
Ebro	Nájerilla	NAJ	0.90	ND	0.53	0.08	0.07	0.07	ND	0.03	0.05	0.05	0.23	0.23	0.30	ND
Ebro	Arga	ARG	0.90	ND	0.80	0.29	0.07	0.07	0.03	0.03	0.05	0.05	0.23	0.23	ND	ND
Ebro	Ebro	EBR4	ND	ND	ND	0.24	ND	ND	ND	ND	ND	0.05	ND	0.23	ND	0.30
Ebro	Ebro	EBR5	NA	ND	NA	0.76	NA	0.07	NA	ND	NA	ND	NA	0.23	NA	ND
Ebro	Gállego	GAL1	0.90	ND	ND	4.36	ND	0.19	0.03	0.03	ND	0.05	0.23	ND	ND	0.30
Ebro	Gállego	GAL2	0.90	ND	0.64	0.05	0.07	0.07	0.03	0.03	ND	0.05	0.23	0.23	ND	ND
Ebro	Huerva	HUE	0.90	NA	1.03	NA	0.07	NA	0.03	NA	ND	NA	ND	NA	0.30	NA
Ebro	Ebro	EBR6	0.90	ND	1.16	0.05	0.07	0.07	0.03	ND	ND	0.05	ND	ND	ND	ND
Ebro	Martín	MAR	0.90	ND	0.56	0.20	0.07	0.07	ND	0.03	ND	0.05	ND	0.23	ND	ND
Ebro	Ésera	ESE	0.90	ND	0.68	0.39	0.07	0.07	ND	0.03	ND	ND	ND	0.23	ND	ND
Ebro	Cinca	CIN1	0.90	ND	0.82	0.29	0.07	0.07	0.03	0.03	ND	ND	ND	0.23	ND	ND
Ebro	Cinca	CIN2	0.90	ND	0.62	0.05	0.07	0.07	ND	0.03	ND	0.05	ND	0.23	ND	ND
Ebro	Ribera Salada	RS	0.90	ND	0.19	0.24	0.07	0.07	0.03	0.03	ND	ND	ND	0.23	ND	ND
Ebro	Segre	SEG	0.90	ND	0.18	0.35	0.07	0.13	ND	0.03	ND	0.05	ND	0.23	0.30	0.30
Ebro	Matarranya	MAT	0.90	ND	0.98	0.39	0.07	ND	0.03	0.03	ND	ND	ND	0.23	ND	ND
Ebro	Algars	ALG	0.90	ND	0.15	0.29	0.07	0.07	0.03	0.03	0.05	ND	0.23	0.23	ND	ND
Ebro	Ebro	EBR7	ND	ND	ND	ND	ND	0.07	ND	0.03	ND	ND	ND	0.23	ND	ND
Ebro	Ebro	EBR8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ebro	Ebro	EBR9	ND	ND	0.43	0.29	ND	0.07	ND	ND	ND	0.05	ND	0.23	ND	ND

Table S-11 (cont.)

Sediment			Antidiabetic		Antihypertensives		Antiplatelet agent		Prostatic hyperplasia		To treat asthma		Anticoagulant		X-ray contrast agent	
Catchment	**Subcatchment**	**Site**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**
Jucar	Júcar	JUC1	0.90	ND	1.27	4.77	ND	0.07	0.03	ND	0.05	0.05	0.23	ND	0.30	0.30
Jucar	Júcar	JUC2	0.90	ND	1.39	3.59	0.07	0.07	0.03	ND	0.05	0.05	0.23	ND	0.30	0.30
Jucar	Júcar	JUC3	NA	ND	NA	2.29	NA	0.07	NA	ND	NA	0.05	NA	ND	NA	0.71
Jucar	Júcar	JUC4	0.90	ND	1.15	2.87	0.07	0.07	0.03	ND	0.05	0.05	0.23	ND	0.30	0.30
Jucar	Júcar	JUC5	0.90	ND	0.00	1.20	ND	0.07	0.03	ND	0.05	0.05	ND	ND	0.30	0.30
Jucar	Cabriel	CAB1	0.90	ND	0.00	0.00	ND	0.07	0.03	ND	0.05	0.05	0.23	ND	0.30	0.30
Jucar	Cabriel	CAB2	0.90	ND	0.15	2.07	ND	0.07	ND	ND	ND	0.05	0.23	ND	0.30	0.94
Jucar	Cabriel	CAB3	0.90	ND	0.15	3.79	ND	0.07	0.03	ND	ND	0.05	0.23	ND	0.30	0.61
Jucar	Cabriel	CAB4	NA	ND	NA	2.00	NA	ND	NA	ND	NA	0.05	NA	ND	NA	0.80
Jucar	Cabriel	CAB5	0.90	ND	1.06	1.09	ND	0.07	ND	ND	ND	0.05	0.23	ND	0.30	0.74
Jucar	Júcar	JUC6	0.90	ND	1.10	3.78	0.07	ND	0.03	ND	0.05	0.05	0.23	ND	0.30	0.68
Jucar	Júcar	JUC7	0.90	ND	1.77	3.14	0.07	0.07	0.03	ND	0.05	0.05	0.23	ND	0.30	0.66
Jucar	Magro	MAG1	0.90	ND	0.25	3.58	0.07	0.07	0.03	ND	0.05	0.05	0.23	ND	0.30	0.56
Jucar	Magro	MAG2	0.90	ND	0.15	3.63	ND	0.07	ND	ND	0.05	0.05	0.23	ND	0.30	0.75
Jucar	Júcar	JUC8	0.90	ND	0.20	3.52	0.07	0.07	0.03	ND	ND	0.05	ND	ND	0.30	0.74
Guadalquivir	Borosa	BOR	0.90	ND	1.09	1.66	0.07	0.07	0.03	ND	ND	ND	ND	ND	ND	0.30
Guadalquivir	Guadalquivir	GUA1	0.90	ND	1.05	0.95	ND	ND	0.03	ND	ND	ND	0.23	ND	ND	0.30
Guadalquivir	Guadiana Menor	GUAM	0.90	ND	0.85	1.40	0.07	ND	ND	ND	0.05	ND	ND	ND	0.30	0.65
Guadalquivir	Guadalquivir	GUA2	0.90	ND	1.01	1.25	0.07	0.07	0.03	ND	ND	ND	0.23	ND	ND	0.69
Guadalquivir	Magaña	MAG	0.90	ND	0.61	1.51	0.07	ND	0.03	ND	ND	ND	0.23	ND	ND	0.61
Guadalquivir	Guadabullón	GUAN	0.90	ND	0.78	1.43	0.07	0.44	0.03	ND	ND	ND	0.23	ND	0.30	0.30
Guadalquivir	Guadalquivir	GUA3	0.90	ND	0.48	0.60	0.07	0.07	0.03	ND	0.05	ND	0.23	ND	ND	0.92
Guadalquivir	Yeguas	YEG	0.90	ND	0.98	0.39	0.07	0.07	0.03	ND	ND	ND	0.23	ND	ND	0.84
Guadalquivir	Guadalmoral	GUAL	0.90	ND	0.75	1.24	0.07	ND	0.03	ND	ND	ND	0.23	ND	ND	0.70
Guadalquivir	Guadalquivir	GUA4	0.90	ND	0.62	1.49	ND	0.07	0.03	ND	ND	ND	0.23	ND	ND	0.72
Guadalquivir	Picachos	PIC	0.90	ND	ND	0.61	ND	0.07	0.03	ND	ND	ND	0.23	ND	ND	0.95
Guadalquivir	Bembézar	BEM	0.90	ND	0.11	1.88	ND	0.07	ND	ND	ND	ND	0.23	ND	ND	0.98
Guadalquivir	Cacín	CAC	0.90	ND	0.77	1.37	ND	ND	0.03	ND	ND	ND	0.23	ND	ND	0.65
Guadalquivir	Genil	GEN1	0.90	ND	0.34	1.01	0.07	0.07	0.03	ND	ND	ND	0.23	ND	ND	1.55
Guadalquivir	Genil	GEN2	0.90	ND	0.87	1.61	0.07	0.07	0.03	ND	ND	ND	0.23	ND	0.30	0.60
Guadalquivir	Guadalquivir	GUA5	0.90	ND	0.97	1.56	0.07	0.16	ND	ND	ND	ND	0.23	ND	ND	0.30
Guadalquivir	Corbones	COR	0.90	ND	0.77	1.68	0.07	0.07	ND	ND	ND	ND	0.23	ND	ND	0.84
Guadalquivir	Herreros	HER	0.90	ND	0.20	0.29	0.07	ND	0.03	ND	ND	ND	0.23	ND	0.30	0.58
Guadalquivir	Guadaira	GUAA	0.90	ND	0.37	1.88	0.07	0.07	ND	ND	ND	ND	0.23	ND	ND	0.71
Guadalquivir	Guadalquivir	GUA6	0.90	ND	0.67	1.51	ND	0.07	ND	ND	ND	ND	0.23	ND	ND	0.30
Guadalquivir	Guadalquivir	GUA7	0.90	ND	ND	1.26	0.07	ND	0.03	ND	ND	ND	0.23	ND	ND	1.25
Guadalquivir	Guadalquivir	GUA8	0.90	ND	0.69	1.44	0.07	0.07	0.03	ND	ND	ND	0.23	ND	ND	0.30
Guadalquivir	Guadiamar	GUAR	0.90	ND	0.91	0.35	0.07	0.07	ND	ND	ND	ND	0.23	ND	ND	0.68
Guadalquivir	Guadalquivir	GUA9	0.90	ND	1.04	1.19	0.07	0.07	0.03	ND	ND	ND	0.23	ND	0.30	0.65

Table S-11 (cont.)

Sediment			Antihelmintics		Synthetic glucocorticoid		Sedation and muscle relaxation		Tranquilizers		Calcium channel blocker		Antibiotics	
Catchment	**Subcatchment**	**Site**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**
Llobregat	Llobregat	LLO1	0.23	0.39	ND	1.35	0.06	0.06	0.23	0.26	0.06	ND	44.49	45.50
Llobregat	Llobregat	LLO2	0.23	0.33	ND	0.94	0.06	ND	0.23	0.04	ND	ND	44.54	44.92
Llobregat	Llobregat	LLO3	0.28	0.22	ND	0.44	0.06	0.06	0.26	0.26	0.21	ND	44.48	44.39
Llobregat	Cardener	CAR1	0.25	0.49	ND	1.03	0.17	0.06	0.26	0.04	0.06	ND	43.93	46.50
Llobregat	Cardener	CAR2	0.23	0.32	ND	1.06	0.06	0.06	0.23	0.26	0.06	ND	43.99	43.80
Llobregat	Cardener	CAR3	0.25	0.23	ND	ND	0.18	ND	0.26	0.23	0.06	0.06	44.61	46.14
Llobregat	Cardener	CAR4	0.23	0.66	ND	1.20	0.18	0.06	0.26	0.26	1.00	ND	44.73	45.51
Llobregat	Llobregat	LLO4	0.52	0.34	ND	ND	0.27	0.06	0.26	0.23	0.32	ND	38.51	43.62
Llobregat	Llobregat	LLO5	0.25	0.23	ND	0.10	0.18	ND	0.26	0.23	0.19	ND	38.48	44.30
Llobregat	Anoia	ANO1	0.25	0.38	ND	1.24	0.06	0.06	0.26	0.26	0.06	ND	44.55	43.48
Llobregat	Anoia	ANO2	0.25	0.26	0.44	0.61	0.06	0.06	0.26	0.26	ND	ND	44.61	42.99
Llobregat	Anoia	ANO3	0.25	0.22	ND	0.52	0.06	ND	0.23	0.04	0.60	ND	44.09	55.76
Llobregat	Llobregat	LLO6	0.25	0.37	ND	1.17	0.19	0.06	0.26	0.26	0.18	ND	44.51	43.84
Llobregat	Llobregat	LLO7	0.65	0.57	ND	1.18	0.06	0.06	0.26	0.04	0.82	0.06	44.52	43.83
Ebro	Ebro	EBR1	0.25	0.45	ND	ND	0.06	ND	0.23	0.23	0.06	ND	44.02	45.11
Ebro	Oca	OCA	0.25	0.21	0.41	0.31	0.06	ND	0.26	0.23	0.17	ND	44.61	45.14
Ebro	Ebro	EBR2	0.14	0.32	0.40	0.92	0.06	0.06	0.26	0.04	0.17	ND	43.90	45.34
Ebro	Zadorra	ZAD	0.25	0.64	ND	0.93	0.06	0.06	0.26	0.26	0.20	ND	44.09	44.01
Ebro	Ebro	EBR3	0.17	0.42	0.89	1.35	0.46	ND	0.23	0.04	ND	ND	31.77	44.31
Ebro	Nájerilla	NAJ	0.25	0.57	0.42	2.08	0.22	ND	0.23	0.04	0.06	0.17	31.49	45.98
Ebro	Arga	ARG	0.25	0.40	ND	0.30	0.34	ND	0.26	0.26	0.18	ND	44.59	45.15
Ebro	Ebro	EBR4	ND	0.19	ND	0.10	0.13	ND	ND	0.23	ND	ND	ND	43.59
Ebro	Ebro	EBR5	NA	0.69	NA	1.10	NA	0.06	NA	0.26	NA	ND	NA	44.67
Ebro	Gállego	GAL1	0.25	0.14	0.38	0.10	0.06	0.06	0.23	0.26	ND	ND	44.37	43.08
Ebro	Gállego	GAL2	0.43	0.82	ND	2.15	0.24	0.06	0.26	0.26	0.34	ND	37.98	45.29
Ebro	Huerva	HUE	0.17	NA	ND	NA	0.06	NA	0.26	NA	ND	NA	37.98	NA
Ebro	Ebro	EBR6	0.14	0.66	0.43	1.87	0.06	0.06	0.26	0.26	ND	ND	43.90	45.56
Ebro	Martín	MAR	0.17	0.54	ND	0.98	0.03	ND	0.26	0.26	0.35	ND	37.98	44.40
Ebro	Ésera	ESE	0.14	0.21	ND	0.32	0.03	0.06	0.26	0.26	ND	ND	45.52	50.03
Ebro	Cinca	CIN1	0.17	0.32	ND	0.87	0.03	ND	0.26	0.26	0.35	ND	38.31	45.26
Ebro	Cinca	CIN2	0.14	0.57	0.33	1.97	0.03	ND	0.26	0.26	0.35	0.12	37.98	44.88
Ebro	Ribera Salada	RS	6.57	0.36	ND	1.12	0.03	0.06	0.26	0.26	ND	0.06	38.05	44.84
Ebro	Segre	SEG	0.17	12.53	ND	ND	0.03	ND	0.26	0.04	0.17	ND	37.98	44.73
Ebro	Matarranya	MAT	0.14	0.28	0.33	0.10	0.03	0.06	0.26	0.23	ND	ND	38.35	44.40
Ebro	Algars	ALG	0.25	0.31	ND	0.81	0.03	0.06	0.23	0.26	ND	ND	43.96	44.59
Ebro	Ebro	EBR7	ND	1.02	ND	1.24	0.06	0.06	ND	0.26	ND	ND	ND	44.03
Ebro	Ebro	EBR8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ebro	Ebro	EBR9	ND	0.65	ND	0.96	0.06	0.06	ND	0.26	ND	0.06	ND	43.27

Table S-11 (cont.)

Sediment			Antihelmintics		Synthetic glucocorticoid		Sedation and muscle relaxation		Tranquilizers		Calcium channel blocker		Antibiotics	
Catchment	**Subcatchment**	**Site**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**	**C1**	**C2**
Jucar	Júcar	JUC1	0.25	0.25	ND	ND	0.06	ND	0.23	ND	0.17	ND	44.52	51.77
Jucar	Júcar	JUC2	0.25	0.36	ND	ND	0.27	0.06	0.23	ND	0.19	ND	44.24	47.51
Jucar	Júcar	JUC3	NA	0.37	NA	0.10	NA	ND	NA	ND	NA	ND	NA	45.74
Jucar	Júcar	JUC4	0.25	0.40	ND	ND	0.06	ND	0.23	ND	0.19	ND	44.95	43.50
Jucar	Júcar	JUC5	0.25	0.14	ND	0.10	0.06	ND	0.26	ND	0.06	ND	44.52	43.33
Jucar	Cabriel	CAB1	0.25	0.29	ND	ND	0.12	ND	0.23	ND	0.06	ND	43.38	47.67
Jucar	Cabriel	CAB2	0.25	0.21	ND	ND	0.06	ND	0.23	ND	ND	ND	43.32	47.24
Jucar	Cabriel	CAB3	0.25	0.14	ND	ND	0.06	ND	0.23	ND	ND	ND	25.57	43.21
Jucar	Cabriel	CAB4	NA	0.14	NA	ND	NA	ND	NA	ND	NA	ND	NA	46.01
Jucar	Cabriel	CAB5	0.17	0.58	0.35	ND	0.06	ND	0.26	ND	ND	ND	38.47	43.42
Jucar	Júcar	JUC6	0.25	0.14	0.48	ND	0.12	ND	0.26	ND	0.19	ND	44.49	46.01
Jucar	Júcar	JUC7	14.01	0.25	ND	ND	0.13	ND	0.26	ND	0.21	ND	44.02	43.26
Jucar	Magro	MAG1	0.17	0.39	ND	ND	0.06	0.06	0.23	ND	0.06	ND	44.52	45.45
Jucar	Magro	MAG2	0.25	0.49	ND	ND	0.06	ND	0.23	ND	ND	ND	38.45	43.08
Jucar	Júcar	JUC8	9.03	1.70	ND	ND	0.06	ND	0.23	ND	ND	ND	44.51	43.89
Guadalquivir	Borosa	BOR	0.17	0.39	ND	0.10	ND	0.06	0.26	0.23	ND	0.69	38.42	45.92
Guadalquivir	Guadalquivir	GUA1	0.66	0.23	ND	ND	0.06	ND	0.26	0.23	0.17	ND	44.51	47.33
Guadalquivir	Guadiana Menor	GUAM	0.25	0.25	ND	0.30	0.06	ND	0.26	0.23	ND	ND	43.90	44.08
Guadalquivir	Guadalquivir	GUA2	0.25	0.38	ND	ND	0.06	0.06	0.26	0.23	0.06	ND	44.12	45.94
Guadalquivir	Magaña	MAG	0.46	0.29	ND	0.10	ND	ND	0.23	0.23	ND	ND	44.22	45.22
Guadalquivir	Guadabullón	GUAN	0.17	0.33	ND	0.10	ND	ND	0.26	0.23	ND	ND	43.90	44.30
Guadalquivir	Guadalquivir	GUA3	0.17	1.78	0.10	0.10	0.06	ND	0.26	0.23	0.06	ND	44.51	44.30
Guadalquivir	Yeguas	YEG	0.14	0.40	0.52	0.30	ND	0.06	0.26	0.23	0.44	ND	43.97	51.48
Guadalquivir	Guadalmoral	GUAL	0.17	0.23	ND	ND	ND	ND	0.26	0.23	ND	ND	38.17	44.71
Guadalquivir	Guadalquivir	GUA4	0.14	0.43	ND	0.10	ND	0.06	0.26	0.23	ND	ND	44.21	53.59
Guadalquivir	Picachos	PIC	0.17	0.23	ND	ND	ND	0.06	0.26	0.23	ND	ND	36.85	44.59
Guadalquivir	Bembézar	BEM	0.14	0.41	0.88	ND	0.06	ND	0.26	0.23	ND	ND	36.85	45.48
Guadalquivir	Cacín	CAC	0.14	0.14	ND	0.10	0.06	ND	0.26	0.23	ND	ND	38.17	43.94
Guadalquivir	Genil	GEN1	0.14	0.28	ND	ND	0.06	ND	0.26	0.23	ND	ND	37.98	45.28
Guadalquivir	Genil	GEN2	0.14	0.47	ND	0.10	0.06	ND	0.26	0.23	0.37	ND	38.10	44.21
Guadalquivir	Guadalquivir	GUA5	0.14	0.50	ND	0.10	0.06	ND	0.26	0.23	0.42	ND	37.77	44.67
Guadalquivir	Corbones	COR	0.14	0.35	ND	0.21	0.06	ND	0.26	0.23	0.58	ND	39.04	44.48
Guadalquivir	Herreros	HER	0.14	0.25	ND	ND	ND	ND	0.26	0.23	0.39	ND	38.57	42.90
Guadalquivir	Guadaira	GUAA	0.14	0.23	0.47	ND	ND	ND	0.26	0.23	ND	ND	38.05	44.44
Guadalquivir	Guadalquivir	GUA6	0.14	0.19	0.35	ND	ND	ND	0.26	0.23	ND	ND	38.35	44.06
Guadalquivir	Guadalquivir	GUA7	0.14	0.25	0.35	0.10	0.06	0.38	0.50	0.23	0.37	0.45	44.13	45.24
Guadalquivir	Guadalquivir	GUA8	0.17	0.39	0.36	ND	0.06	ND	0.26	0.23	ND	ND	44.74	45.22
Guadalquivir	Guadiamar	GUAR	0.14	0.35	ND	ND	ND	ND	0.26	0.23	ND	ND	38.42	45.53
Guadalquivir	Guadalquivir	GUA9	0.17	0.31	ND	ND	ND	ND	0.26	0.23	ND	ND	32.10	44.13

Table S-11 (cont.)

		Sediment	Total	Total
Catchment	**Subcatchment**	**Site**	**C1**	**C2**
Llobregat	Llobregat	LLO1	*60.97*	**83.24**
Llobregat	Llobregat	LLO2	89.97	81.67
Llobregat	Llobregat	LLO3	99.21	*62.99*
Llobregat	Cardener	CAR1	*68.86*	71.01
Llobregat	Cardener	CAR2	*75.81*	67.46
Llobregat	Cardener	CAR3	76.96	**83.38**
Llobregat	Cardener	CAR4	96.70	82.48
Llobregat	Llobregat	LLO4	**101.46**	**85.83**
Llobregat	Llobregat	LLO5	80.27	74.30
Llobregat	Anoia	ANO1	**101.24**	*66.09*
Llobregat	Anoia	ANO2	84.85	*65.31*
Llobregat	Anoia	ANO3	84.03	76.94
Llobregat	Llobregat	LLO6	94.92	68.34
Llobregat	Llobregat	LLO7	**207.44**	82.31
Ebro	Ebro	EBR1	**90.47**	*65.36*
Ebro	Oca	OCA	74.47	*59.47*
Ebro	Ebro	EBR2	88.66	68.18
Ebro	Zadorra	ZAD	62.91	75.18
Ebro	Ebro	EBR3	73.32	71.92
Ebro	Nájerilla	NAJ	70.86	70.96
Ebro	Arga	ARG	74.11	67.43
Ebro	Ebro	EBR4	*30.02*	68.50
Ebro	Ebro	EBR5	NA	65.56
Ebro	Gállego	GAL1	72.97	**100.22**
Ebro	Gállego	GAL2	69.97	72.39
Ebro	Huerva	HUE	59.25	0.00
Ebro	Ebro	EBR6	**98.71**	74.46
Ebro	Martín	MAR	85.42	76.14
Ebro	Ésera	ESE	**90.94**	69.04
Ebro	Cinca	CIN1	85.01	73.70
Ebro	Cinca	CIN2	69.50	75.90
Ebro	Ribera Salada	RS	76.41	*62.46*
Ebro	Segre	SEG	62.57	**80.04**
Ebro	Matarranya	MAT	56.49	70.77
Ebro	Algars	ALG	71.11	65.57
Ebro	Ebro	EBR7	*17.32*	71.70
Ebro	Ebro	EBR8	NA	NA
Ebro	Ebro	EBR9	*18.26*	**87.42**

Table S-11 (cont.)

		Sediment	Total	Total
Catchment	**Subcatchment**	**Site**	**C1**	**C2**
Jucar	Júcar	JUC1	**87.24**	**97.30**
Jucar	Júcar	JUC2	85.77	**100.56**
Jucar	Júcar	JUC3	NA	89.25
Jucar	Júcar	JUC4	72.35	72.56
Jucar	Júcar	JUC5	*57.98*	82.92
Jucar	Cabriel	CAB1	83.11	81.43
Jucar	Cabriel	CAB2	85.84	*60.70*
Jucar	Cabriel	CAB3	*66.31*	73.44
Jucar	Cabriel	CAB4	NA	*63.41*
Jucar	Cabriel	CAB5	*61.80*	83.95
Jucar	Júcar	JUC6	**88.96**	73.47
Jucar	Júcar	JUC7	**99.21**	*71.72*
Jucar	Magro	MAG1	78.32	**95.66**
Jucar	Magro	MAG2	67.60	77.07
Jucar	Júcar	JUC8	70.04	94.30
Guadalquivir	Borosa	BOR	**110.89**	**89.84**
Guadalquivir	Guadalquivir	GUA1	**92.00**	75.39
Guadalquivir	Guadiana Menor	GUAM	82.54	71.31
Guadalquivir	Guadalquivir	GUA2	91.78	76.44
Guadalquivir	Magaña	MAG	71.01	75.34
Guadalquivir	Guadabullón	GUAN	*63.84*	78.30
Guadalquivir	Guadalquivir	GUA3	91.57	75.70
Guadalquivir	Yeguas	YEG	71.88	87.91
Guadalquivir	Guadalmoral	GUAL	86.94	81.50
Guadalquivir	Guadalquivir	GUA4	**92.19**	**90.60**
Guadalquivir	Picachos	PIC	65.23	82.78
Guadalquivir	Bembézar	BEM	65.37	*69.34*
Guadalquivir	Cacín	CAC	69.08	*59.38*
Guadalquivir	Genil	GEN1	71.92	72.20
Guadalquivir	Genil	GEN2	69.52	75.00
Guadalquivir	Guadalquivir	GUA5	72.05	*65.21*
Guadalquivir	Corbones	COR	76.11	80.51
Guadalquivir	Herreros	HER	67.25	72.55
Guadalquivir	Guadaira	GUAA	88.72	72.18
Guadalquivir	Guadalquivir	GUA6	*58.61*	72.93
Guadalquivir	Guadalquivir	GUA7	90.51	83.32
Guadalquivir	Guadalquivir	GUA8	81.77	86.56
Guadalquivir	Guadiamar	GUAR	*60.46*	**93.21**
Guadalquivir	Guadalquivir	GUA9	83.96	83.39

Table S-12. Pharmaceuticals more frequently detected at:
In bold the cases than accumulate more than 50% of the cases

(a) "outlying high" concentrations

Surface waters		Sediments	
PhAC	Number of cases	PhAC	Number of cases
PHEN	56	**TRZ**	72
PPHEN	48	**FMT**	52
TMSN	42	**CEF**	32
CEF	37	**PRX**	32
IBU	34	**OH.MT**	30
OH-MTZ	34	**DMZ**	28
MLX	32	**NAP**	23
ERY	29	**NDL**	23
FLU	29	**RNZ**	23
ATN	28	**ATV**	20
CLARI	27	**DTZ**	16
MTPL	24	VNFX	13
SMX	24	CTP	12
NDL	22	OFLX	12
PRPL	21	XYL	10
VSRT	19	TALB	8
PRX	18	DCF	7
OFLX	16	LNT	6
STL	16	OLZ	6
PARA	15	CLPG	4
APZ	11	FLX	4
OXYD	10	FLU	2
SAL	10	FUR	2
DZP	9	GFZ	2
GLB	9	INDO	2
LRZ	9	APZ	1
AZPL	8	AZPN	1
CMT	8	CPFX	1
AZPN	7	PARA	1
FLX	7	PRPL	1
XYL	6	SRT	1
TX	5	WARF	1
IOP	4		
RNT	4		
TOR	4		
AZY	3		
DCF	2		
SRT	2		
TCN	2		
ISRT	1		

(a). *(cont)*

Surface waters		Sediments	
PhAC	Number of cases	PhAC	Number of cases
KETO	1		
WARF	1		

(b) "outlying low" concentrations

Surface waters		Sediments	
PhAC	Number of cases	PhAC	Number of cases
IBU	35	**NDL**	35
PARA	25	**TCN**	26
OFLX	24	**AZPN**	23
TCN	21	FLX	18
ERY	19	TRZ	18
CLARI	18	OFLX	14
VSRT	7	PRPL	14
AZPL	5	VNFX	13
AZY	3	DCF	8
AZPN	1	PARA	8
		HCTZ	1

Table S-13. River basins whith more and less cases of PhACs more frequently detected at:
In bold the cases than accumulate more than 50% of the cases

(a) "outlying high" concentrations

Surface waters		Sediments	
Basin	Number of cases	Basin	Number of cases
Ebro	291	**Ebro**	129
Llobregat	220	**Llobregat**	125
Guadalquivir	105	Guadalquivir	103
Jucar	78	Jucar	91

(b) "outlying low" concentrations

Surface waters		Sediments	
Basin	Number of cases	Basin	Number of cases
Jucar	55	Ebro	54
Guadalquivir	54	Guadalquivir	54
Ebro	30	Llobregat	39
Llobregat	19	Jucar	31

Table S-14. Campaigns whith more and less cases of PhACs more frequently detected at:

(a) "outlying high" concentrations

Surface waters		Sediments	
Campaign	Number of cases	Campaign	Number of cases
C1	422	C2	253
C2	272	C1	195

(b) "outlying low" concentrations

Surface waters		Sediments	
Campaign	Number of cases	Campaign	Number of cases
C2	112	C1	98
C1	46	C2	80

Table S-15. Comparison among river basins and campaigns of cases of PhACs detected at:

(a) "outlying high" concentrations

		Surface waters		Sediments	
Basin	Campaign	Number of cases	p-value	Number of cases	p-value
Ebro	C1	193	<0.0001	45	<0.0001
Ebro	C2	98		84	
Gua	C1	31		44	
Gua	C2	74		47	
Llo	C1	176		29	
Llo	C2	44		74	
Juc	C1	22		77	
Juc	C2	56		48	

(b) "outlying low" concentrations

		Surface waters		Sediments	
Basin	Campaign	Number of cases	p-value	Number of cases	p-value
Ebro	C1	7	0.113	28	<0.0001
Ebro	C2	23		26	
Gua	C1	20		45	
Gua	C2	34		9	
Llo	C1	8		11	
Llo	C2	11		28	
Juc	C1	11		14	
Juc	C2	44		17	

Table S-16. Sampling sites whith more and less cases of PhACs more frequently detected at:
In bold the cases than accumulate more than 50% of the cases

(a) "outlying high" concentrations

Site	Number of cases	Site	Number of cases
ZAD	**67**	**LLO7**	**24**
LLO7	**47**	**CAR4**	**18**
ANO2	**44**	**JUC2**	**13**
MAG2	**34**	**JUC8**	**13**
LLO6	**25**	**LLO4**	**13**
ANO3	**24**	**ANO3**	**11**
HUE	**23**	**ARG**	**11**
ARG	**22**	**BOR**	**11**
SEG	**22**	**NAJ**	**11**
EBR7	**20**	**CAB1**	**10**
EBR6	**19**	**JUC1**	**10**
LLO5	19	**EBR6**	**9**
EBR4	17	**GUA7**	**9**
LLO4	17	**LLO3**	**9**
JUC1	15	**MAG1**	**9**
EBR3	14	**COR**	**8**
CIN2	13	**EBR4**	**8**
EBR5	13	**EBR7**	**8**
LLO3	13	**CAR3**	**7**
CIN1	12	**EBR1**	**7**
GUA4	10	**GAL2**	**7**
OCA	10	JUC4	7
CAR4	9	LLO2	7
GUAN	9	ZAD	7
EBR1	8	CAR1	6
EBR2	8	CIN2	6
ANO1	7	EBR3	6
EBR8	6	GAL1	6
GUA2	6	GUA3	6
GUA3	6	GUA8	6
GUA9	6	JUC6	6
GUAA	6	JUC7	6
EBR9	5	LLO6	6
GEN1	5	SEG	6
GUA1	5	ANO1	5
GUA6	5	ANO2	5
GUAL	5	CAB5	5
LLO1	5	EBR2	5
BOR	4	GUA1	5

(a) *(cont.)*

Surface waters		Sediments	
Site	Number of cases	Site	Number of cases
GEN2	4	GUA4	5
GUA5	4	HUE	5
GUA7	4	JUC3	5
JUC4	4	JUC5	5
JUC7	4	LLO1	5
MAG1	4	LLO5	5
NAJ	4	MAG2	5
ALG	3	OCA	5
CAB5	3	BEM	4
CAC	3	CAB2	4
CAR1	3	CAR2	4
COR	3	CIN1	4
GUA8	3	EBR5	4
GUAM	3	GEN1	4
GUAR	3	GUA2	4
HER	3	GUAN	4
JUC2	3	GUAR	4
JUC6	3	MAR	4
LLO2	3	PIC	4
PIC	3	CAB4	3
CAR2	2	EBR9	3
CAR3	2	ESE	3
ESE	2	GUAA	3
JUC5	2	HER	3
JUC8	2	CAB3	2
MAG	2	GEN2	2
YEG	2	GUA5	2
BEM	1	GUA6	2
CAB1	1	GUA9	2
CAB2	1	RS	2
CAB4	1	ALG	1
GAL1	1	CAC	1
JUC3	1	MAG	1
MAR	1	MAT	1
RS	1	YEG	1

(b) "outlying low" concentrations

Surface waters		Sediments	
Site	Number of cases	Site	Number of cases
CAB5	**7**	**EBR1**	**7**
JUC5	**7**	**LLO5**	**6**
CAB4	**5**	**RS**	**6**
GUAL	**5**	**ESE**	**5**
MAG	**5**	**GEN1**	**5**
ARG	**4**	**ANO3**	**4**
CAB1	**4**	**CAB4**	**4**
CAB3	**4**	**CAB5**	**4**
JUC1	**4**	**CAR3**	**4**
JUC2	**4**	**EBR2**	**4**
PIC	**4**	**GUA6**	**4**
ALG	**3**	**GUA7**	**4**
ANO2	**3**	**GUAM**	**4**
BEM	**3**	**GUAR**	**4**
CAC	**3**	**JUC7**	**4**
CAR1	**3**	**MAT**	**4**
CIN1	**3**	**ALG**	**3**
ESE	**3**	**ANO2**	**3**
GAL2	**3**	**BEM**	**3**
GUA1	**3**	**BOR**	**3**
GUA8	3	**CAR1**	**3**
GUA9	3	**CIN2**	**3**
JUC3	3	EBR3	3
JUC7	3	EBR6	3
JUC8	3	GEN2	3
MAG1	3	GUA1	3
NAJ	3	GUA4	3
ANO3	2	GUAL	3
BOR	2	LLO2	3
CAB2	2	LLO3	3
CAR2	2	LLO4	3
CAR3	2	LLO6	3
CAR4	2	MAG2	3
CIN2	2	PIC	3
COR	2	SEG	3
GAL1	2	CAB1	2
GEN1	2	CAB3	2
GEN2	2	CAR2	2
GUAM	2	CIN1	2
GUAN	2	EBR4	2

(b) *(cont.)*

Surface waters		Sediments	
Site	Number of cases	Site	Number of cases
GUAR	2	GUA2	2
HER	2	GUA3	2
JUC4	2	JUC1	2
JUC6	2	JUC2	2
MAG2	2	JUC5	2
MAR	2	LLO7	2
OCA	2	OCA	2
YEG	2	ANO1	1
ANO1	1	ARG	1
EBR1	1	CAB2	1
EBR4	1	CAC	1
GUA2	1	CAR4	1
GUA3	1	COR	1
GUA4	1	EBR9	1
GUA5	1	GAL1	1
GUA6	1	GAL2	1
GUA7	1	GUA5	1
GUAA	1	GUA9	1
LLO2	1	GUAA	1
LLO3	1	GUAN	1
LLO4	1	HER	1
LLO7	1	HUE	1
ZAD	1	JUC3	1
		JUC4	1
		JUC6	1
		JUC8	1
		LLO1	1
		MAG1	1
		NAJ	1
		YEG	1
		ZAD	1

Table S-17. Total TU values of pharmaceuticals detected in surface waters from each sampling site across basins and over sampling campaigns. Totals in **bold** and *italic* are the **maximum** and *minimum* values determined in the underlined sampling site of each basin.

Catchment	Subcatchment	Site	TU Algae		TU Daphnia		TU Fish	
			C1	C2	C1	C2	C1	C2
Llobregat	Llobregat	LLO1	1.05E-04	5.19E-05	3.68E-05	9.64E-06	1.41E-05	8.13E-06
Llobregat	Llobregat	LLO2	*5.58E-05*	*2.88E-05*	1.77E-05	*7.18E-06*	1.53E-05	8.25E-06
Llobregat	Llobregat	LLO3	2.36E-04	4.09E-05	5.94E-05	1.07E-05	7.32E-05	1.73E-05
Llobregat	Cardener	CAR1	5.61E-05	3.10E-05	*1.07E-05*	8.25E-06	*6.13E-06*	1.11E-05
Llobregat	Cardener	CAR2	8.71E-05	3.56E-05	1.30E-05	7.55E-06	8.00E-06	*6.87E-06*
Llobregat	Cardener	CAR3	8.40E-05	4.04E-05	1.42E-05	1.25E-05	1.45E-05	2.93E-05
Llobregat	Cardener	CAR4	9.32E-05	9.38E-05	2.00E-05	3.55E-05	8.64E-06	2.89E-05
Llobregat	Llobregat	LLO4	2.11E-04	5.51E-05	6.71E-05	1.50E-05	7.47E-05	2.44E-05
Llobregat	Llobregat	LLO5	6.61E-04	6.49E-05	1.30E-04	1.88E-05	2.16E-04	3.47E-05
Llobregat	Anoia	ANO1	2.15E-04	1.24E-04	3.43E-05	4.05E-05	6.22E-05	1.89E-05
Llobregat	Anoia	ANO2	2.65E-03	4.71E-04	3.80E-04	1.98E-04	4.12E-04	3.74E-04
Llobregat	Anoia	ANO3	6.66E-04	1.80E-04	1.33E-04	9.35E-05	2.68E-04	3.54E-04
Llobregat	Llobregat	LLO6	5.93E-04	8.09E-05	1.11E-04	3.02E-05	1.42E-04	7.69E-05
Llobregat	Llobregat	LLO7	**5.39E-03**	**1.39E-03**	**1.52E-03**	**5.61E-04**	**8.39E-04**	**4.81E-04**
Ebro	Ebro	EBR1	4.88E-05	3.05E-05	1.95E-05	7.64E-06	1.17E-05	7.03E-06
Ebro	Oca	OCA	2.70E-04	3.22E-05	4.48E-05	9.40E-06	4.42E-05	1.44E-05
Ebro	Ebro	EBR2	6.03E-05	3.10E-05	1.64E-05	8.33E-06	1.20E-05	1.23E-05
Ebro	Zadorra	ZAD	**4.67E-03**	**3.45E-03**	**5.32E-04**	**2.71E-04**	**6.19E-04**	**2.51E-04**
Ebro	Ebro	EBR3	3.48E-04	4.66E-05	4.61E-05	9.76E-06	4.36E-05	1.23E-05
Ebro	Nájerilla	NAJ	9.53E-05	3.07E-05	1.98E-05	7.88E-06	1.05E-05	8.04E-06
Ebro	Arga	ARG	1.21E-03	1.07E-04	2.12E-04	4.03E-05	2.84E-04	9.91E-05
Ebro	Ebro	EBR4	3.18E-04	3.61E-05	4.97E-05	1.11E-05	5.40E-05	2.10E-05
Ebro	Ebro	EBR5	1.57E-04	4.03E-05	3.04E-05	9.17E-06	3.09E-05	1.33E-05
Ebro	Gállego	GAL1	3.88E-05	3.90E-05	8.72E-06	7.99E-06	*5.17E-06*	1.19E-05
Ebro	Gállego	GAL2	4.05E-05	2.86E-05	8.61E-06	7.63E-06	6.77E-06	7.67E-06
Ebro	Huerva	HUE	6.20E-04	2.57E-04	1.23E-04	1.00E-04	2.77E-04	1.74E-04
Ebro	Ebro	EBR6	5.86E-04	1.15E-04	1.08E-04	4.36E-05	8.19E-05	8.81E-05
Ebro	Martín	MAR	6.75E-05	3.01E-05	1.87E-05	8.92E-06	1.78E-05	1.45E-05
Ebro	Ésera	ESE	3.90E-05	*2.70E-05*	1.11E-05	*7.54E-06*	1.17E-05	9.45E-06
Ebro	Cinca	CIN1	*3.37E-05*	2.29E-04	*8.05E-06*	9.51E-05	7.17E-06	7.77E-05
Ebro	Cinca	CIN2	1.04E-04	2.37E-04	2.03E-05	9.44E-05	1.20E-05	7.68E-05
Ebro	Ribera Salada	RS	5.71E-05	3.26E-05	1.60E-05	8.08E-06	7.59E-06	6.65E-06
Ebro	Segre	SEG	3.80E-04	2.35E-04	8.23E-05	9.94E-05	1.75E-04	9.61E-05
Ebro	Matarranya	MAT	4.17E-05	3.77E-05	1.07E-05	7.64E-06	6.91E-06	*6.23E-06*
Ebro	Algars	ALG	6.08E-05	3.61E-05	1.47E-05	7.54E-06	5.80E-06	7.44E-06
Ebro	Ebro	EBR7	6.32E-05	2.36E-04	1.80E-05	1.10E-04	1.83E-05	8.70E-05
Ebro	Ebro	EBR8	8.69E-05	6.12E-05	2.13E-05	1.80E-05	1.74E-05	1.88E-05
Ebro	Ebro	EBR9	9.41E-05	4.86E-05	2.14E-05	1.53E-05	1.97E-05	1.71E-05

Table S-17.*(cont)*

Catchment	Subcatchment	Site	TU Algae		TU Daphnia		TU Fish	
			C1	C2	C1	C2	C1	C2
Jucar	Júcar	JUC1	1.10E-04	1.89E-04	1.87E-05	7.12E-05	1.52E-05	6.31E-05
Jucar	Júcar	JUC2	1.03E-04	2.53E-05	1.88E-05	8.72E-06	1.41E-05	1.19E-05
Jucar	Júcar	JUC3	6.48E-05	4.92E-05	1.82E-05	1.65E-05	1.34E-05	3.27E-05
Jucar	Júcar	JUC4	1.12E-04	1.11E-04	1.94E-05	5.86E-05	1.65E-05	2.39E-05
Jucar	Júcar	JUC5	5.00E-05	*2.18E-05*	1.40E-05	6.11E-06	6.97E-06	2.91E-06
Jucar	Cabriel	CAB1	3.64E-05	3.18E-05	1.00E-05	7.20E-06	1.12E-05	4.21E-06
Jucar	Cabriel	CAB2	NA	3.23E-05	NA	7.43E-06	NA	3.59E-06
Jucar	Cabriel	CAB3	*3.43E-05*	2.28E-05	9.30E-06	7.03E-06	8.08E-06	3.24E-06
Jucar	Cabriel	CAB4	4.76E-05	2.79E-05	1.40E-05	6.66E-06	7.46E-06	3.50E-06
Jucar	Cabriel	CAB5	3.94E-05	2.19E-05	*8.74E-06*	*5.97E-06*	*6.85E-06*	*2.93E-06*
Jucar	Júcar	JUC6	1.09E-04	3.04E-05	1.84E-05	7.12E-06	1.40E-05	3.70E-06
Jucar	Júcar	JUC7	**1.16E-04**	4.11E-05	2.02E-05	1.58E-05	1.34E-05	1.68E-05
Jucar	Magro	MAG1	7.51E-05	5.36E-05	**2.66E-05**	1.51E-05	**3.33E-05**	1.21E-05
Jucar	Magro	MAG2	3.65E-05	**6.75E-04**	1.16E-05	**9.89E-05**	1.52E-05	**8.43E-05**
Jucar	Júcar	JUC8	5.77E-05	4.29E-05	1.59E-05	1.14E-05	1.20E-05	9.89E-06
Guadalquivir	Borosa	BOR	4.90E-05	4.58E-05	1.50E-05	1.22E-05	1.40E-05	1.56E-05
Guadalquivir	Guadalquivir	GUA1	6.27E-05	1.27E-04	1.39E-05	1.17E-05	*5.58E-06*	1.80E-05
Guadalquivir	Guadiana Menor	GUAM	5.11E-05	4.33E-05	1.35E-05	1.41E-05	8.09E-06	2.60E-05
Guadalquivir	Guadalquivir	GUA2	7.57E-05	1.66E-04	2.18E-05	3.61E-05	1.58E-05	7.96E-05
Guadalquivir	Magaña	MAG	6.17E-05	3.87E-05	1.48E-05	*9.75E-06*	7.47E-06	7.79E-06
Guadalquivir	Guadabullón	GUAN	6.34E-05	1.28E-04	2.29E-05	6.25E-05	5.24E-05	**2.16E-04**
Guadalquivir	Guadalquivir	GUA3	5.75E-05	7.91E-05	1.77E-05	3.11E-05	1.38E-05	8.18E-05
Guadalquivir	Yeguas	YEG	4.82E-05	1.01E-04	1.40E-05	3.98E-05	8.56E-06	3.54E-05
Guadalquivir	Guadalmoral	GUAL	4.96E-05	1.65E-04	1.43E-05	3.52E-05	9.38E-06	5.82E-05
Guadalquivir	Guadalquivir	GUA4	**1.21E-04**	1.58E-04	2.29E-05	4.81E-05	2.62E-05	8.08E-05
Guadalquivir	Picachos	PIC	4.72E-05	*3.85E-05*	*1.33E-05*	1.15E-05	6.69E-06	1.52E-05
Guadalquivir	Bembézar	BEM	*4.72E-05*	5.41E-05	1.35E-05	2.20E-05	6.11E-06	6.77E-05
Guadalquivir	Cacín	CAC	4.78E-05	6.03E-05	1.35E-05	2.02E-05	6.29E-06	2.74E-05
Guadalquivir	Genil	GEN1	6.56E-05	1.18E-04	2.52E-05	5.38E-05	5.11E-05	1.80E-04
Guadalquivir	Genil	GEN2	5.36E-05	6.16E-05	1.63E-05	2.52E-05	2.28E-05	7.26E-05
Guadalquivir	Guadalquivir	GUA5	6.14E-05	1.58E-04	1.85E-05	2.85E-05	1.90E-05	4.43E-05
Guadalquivir	Corbones	COR	5.95E-05	6.10E-05	1.68E-05	2.12E-05	1.18E-05	5.21E-05
Guadalquivir	Herreros	HER	5.80E-05	4.13E-05	1.73E-05	1.29E-05	2.16E-05	2.23E-05
Guadalquivir	Guadaira	GUAA	7.89E-05	1.17E-04	2.93E-05	5.64E-05	5.97E-05	2.09E-04
Guadalquivir	Guadalquivir	GUA6	7.39E-05	1.73E-04	**3.03E-05**	4.15E-05	**7.60E-05**	7.12E-05
Guadalquivir	Guadalquivir	GUA7	5.85E-05	1.63E-04	1.62E-05	3.39E-05	8.87E-06	5.55E-05
Guadalquivir	Guadalquivir	GUA8	5.18E-05	1.66E-04	1.55E-05	3.28E-05	1.61E-05	1.44E-05
Guadalquivir	Guadiamar	GUAR	4.88E-05	**2.45E-04**	1.38E-05	**1.14E-04**	7.52E-06	9.83E-05
Guadalquivir	Guadalquivir	GUA9	4.85E-05	1.34E-04	1.38E-05	1.56E-05	9.92E-06	*7.44E-06*

Table S-18. Aquatic organisms with more outlying TU values

Surface waters	
TU-type	Number of cases
TU-Algae	7
TU-Daphnia	6
TU-Fish	0

Table S-19. River basins with more outlying TU values

Surface waters	
Basin	umber of cases
Llobregat	6
Ebro	6
Jucar	1
Guadalquivir	0

Table S-20. Campaigns with more outlying TU values

Surface waters	
Campaign	Number of cases
C1	8
C2	5

Table S-21. Sampling sites with more outlying TU values

Surface waters	
Site	Number of cases
LLO7	4
ZAD	4
ANO2	2
ARG	2
MAG2	1

Table S-22. Comparison among river basins and campaigns of cases of outlying TU values

		Surface waters	
Basin	Campaign	Number of cases	p-value
Ebro	C1	4	0.5338
Ebro	C2	2	
Gua	C1	0	
Gua	C2	0	
Llo	C1	4	
Llo	C2	2	
Juc	C1	0	
Juc	C2	1	

Table S-23. Contribution of individual pharmaceuticals to the total TU of every sample analized.

Surface waters											
Algae				Daphnia				Fish			
PhAC	Mean	Min	Max	PhAC	Mean	Min	Max	PhAC	Mean	Min	Max
SRT	**22.0**	**0.2**	**72.2**	**SRT**	**29.1**	**0.5**	**79.3**	**GFZ**	**42.7**	**0.5**	**94.5**
ERY	**19.8**	**0.3**	**67.9**	**GFZ**	**12.0**	**0.0**	**65.7**	**SRT**	**11.3**	**0.1**	**56.3**
LSRT	**11.2**	**0.1**	**61.8**	**LNT**	**10.0**	**0.1**	**91.4**	**LNT**	**10.0**	**0.1**	**92.0**
DMZ	**5.8**	**0.2**	**46.2**	**TCN**	**5.2**	**0.1**	**14.0**	**AZY**	**6.2**	**0.0**	**46.8**
LNT	4.9	0.0	68.4	FLX	4.8	0.2	20.7	LSRT	4.7	0.0	45.4
GFZ	4.7	0.0	41.8	LSRT	4.8	0.0	30.2	IBU	4.3	0.0	62.6
CLARI	4.5	0.1	40.8	VNFX	4.2	0.0	31.6	FLU	2.6	0.0	61.1
TRZ	3.8	0.0	64.0	CTP	3.9	0.0	68.5	TRZ	2.5	0.0	37.7
VSRT	1.9	0.0	22.0	AZY	3.7	0.0	24.0	KETO	2.3	0.1	13.6
VNFX	1.8	0.0	16.5	TRZ	2.7	0.0	53.2	INDO	2.1	0.0	60.5
CTP	1.7	0.0	24.5	ACRI	2.4	0.0	11.1	FLX	1.7	0.0	7.5
AZY	1.7	0.0	9.9	LMS	2.3	0.0	77.7	AML	1.4	0.0	7.2
IBU	1.5	0.0	35.0	AZPN	2.0	0.0	5.6	ACRI	1.2	0.0	6.1
LRZ	1.4	0.1	23.9	DMZ	1.9	0.0	15.7	PARA	1.0	0.0	7.3
TCN	1.4	0.0	4.3	APAP	1.5	0.0	38.7	CTP	0.6	0.0	22.8
DXT	1.3	0.0	10.7	PRC	1.4	0.0	16.9	VNFX	0.6	0.0	4.1
RNZ	1.2	0.0	11.6	DCF	1.1	0.0	8.6	DMZ	0.4	0.0	6.6
PRC	1.1	0.0	16.8	VSRT	0.7	0.0	9.9	COD	0.4	0.0	2.5
LMS	1.0	0.0	60.6	IBU	0.7	0.0	20.7	VSRT	0.4	0.0	8.7
AZPN	0.9	0.0	3.4	AML	0.6	0.0	5.1	AZPN	0.3	0.0	1.4
COD	0.8	0.0	3.8	INDO	0.5	0.0	15.0	PHEN	0.3	0.0	4.8
FLX	0.8	0.0	5.2	DZP	0.4	0.0	2.7	PRC	0.3	0.0	4.7
ALB	0.5	0.0	6.5	NAP	0.3	0.0	3.9	CRZL	0.3	0.0	4.2
DCF	0.5	0.0	4.9	CLARI	0.3	0.0	7.2	DXT	0.3	0.0	3.3
FUR	0.5	0.0	4.2	PPHEN	0.3	0.0	5.5	BZF	0.3	0.0	4.5
CMT	0.4	0.0	17.5	KETO	0.3	0.0	2.1	APZ	0.2	0.0	3.1
ACRI	0.4	0.0	2.6	OFLX	0.3	0.0	21.8	LRZ	0.2	0.0	8.9
DZP	0.3	0.0	2.3	ALB	0.3	0.0	3.5	ALB	0.2	0.0	3.1
MLX	0.3	0.0	11.5	RNZ	0.3	0.0	2.5	TCN	0.2	0.0	0.8
PPHEN	0.2	0.0	2.2	COD	0.2	0.0	1.9	NAP	0.2	0.0	1.5
AML	0.2	0.0	1.7	DXT	0.2	0.0	1.8	CBZ	0.1	0.0	3.7
PHEN	0.2	0.0	1.9	LRZ	0.2	0.0	4.2	PPHEN	0.1	0.0	1.7
TMP	0.2	0.0	1.0	APZ	0.2	0.0	2.1	MLX	0.1	0.0	4.6
SMX	0.2	0.0	0.9	PARA	0.2	0.0	1.2	DZP	0.1	0.0	0.5
CPFX	0.2	0.0	5.6	PHEN	0.1	0.0	1.5	CLARI	0.1	0.0	1.5
INDO	0.2	0.0	4.6	MTPL	0.1	0.0	5.5	FUR	0.1	0.0	0.8
APZ	0.1	0.0	2.6	FLU	0.1	0.0	4.8	APAP	0.0	0.0	1.7
FLU	0.1	0.0	6.3	RNT	0.1	0.0	0.7	DCF	0.0	0.0	0.3
KETO	0.1	0.0	0.8	TMP	0.1	0.0	0.7	ERY	0.0	0.0	0.4
NAP	0.1	0.0	1.1	BZF	0.1	0.0	0.9	LMS	0.0	0.0	1.5
CRZL	0.1	0.0	0.4	ERY	0.1	0.0	0.5	RNZ	0.0	0.0	0.3

(a) for surface water samples (cont.)

Surface waters											
Algae				Daphnia				Fish			
PhAC	Mean	Min	Max	Pharm	Mean	Min	Max	PhAC	Mean	Min	Max
MTZ	0.1	0.0	1.5	FUR	0.1	0.0	0.5	SMX	0.0	0.0	0.2
MTPL	0.0	0.0	2.5	CBZ	0.1	0.0	0.7	CPFX	0.0	0.0	1.1
RNT	0.0	0.0	0.3	SMX	0.0	0.0	0.3	MTPL	0.0	0.0	0.7
BZF	0.0	0.0	0.4	MLX	0.0	0.0	1.4	CMT	0.0	0.0	0.4
APAP	0.0	0.0	1.1	CPFX	0.0	0.0	1.5	TMP	0.0	0.0	0.1
CBZ	0.0	0.0	0.2	ATN	0.0	0.0	0.6	MTZ	0.0	0.0	0.3
DLNT	0.0	0.0	0.1	NDL	0.0	0.0	0.2	RNT	0.0	0.0	0.1
PARA	0.0	0.0	0.0	CRZL	0.0	0.0	0.1	DLNT	0.0	0.0	0.1
ATN	0.0	0.0	0.1	MTZ	0.0	0.0	0.1	ATN	0.0	0.0	0.1
NDL	0.0	0.0	0.0	DLNT	0.0	0.0	0.1	NDL	0.0	0.0	0.1
OLZ	0.0	0.0	0.0	OLZ	0.0	0.0	0.1	OLZ	0.0	0.0	0.0
OFLX	0.0	0.0	0.1	CMT	0.0	0.0	0.1	OFLX	0.0	0.0	0.0
FMT	0.0	0.0	0.0	FMT	0.0	0.0	0.0	FMT	0.0	0.0	0.0
IOP	0.0	0.0	0.0	IOP	0.0	0.0	0.0	IOP	0.0	0.0	0.0

Table S-24. Linear mixed model output for the relationship between average PhAC concentration and population density and LSU. All three variables were Ln-transformed for the analysis. P-values were obtained by likelihood ratio tests.
Only PhAC used either for human or livestock were considered each time.

Surface waters	DF	SS	MS	F	p	Slope
Population density	1	70.247	70.247	71.606	<0.0001	0.622
LSU	1	27.188	27.188	27.303	<0.0001	0.521

Sediments	DF	SS	MS	F	p	Slope
Population density	1	0.027	0.027	1.104	0.293	
LSU	1	1.656	1.656	22.709	<0.0001	0.13

Table S-25. Linear mixed model output for the relationship between TU and population density and LSU for water samples. All three variables were Ln-transformed for the analysis. P-values were obtained by likelihood ratio tests.

Algae	DF	SS	MS	F	p	Slope
Population density	1	45.747	45.747	59.786	<0.0001	0.483
LSU	1	7.15	7.15	9.345	0.003	0.268

Daphnia	DF	SS	MS	F	p	Slope
Population density	1	48.615	48.615	70.661	<0.0001	0.499
LSU	1	6.572	6.572	9.552	0.002	0.257

Fish	DF	SS	MS	F	p	Slope
Population density	1	95.433	95.433	104.879	<0.0001	0.704
LSU	1	8.418	8.418	9.251	0.002	0.291

Chapter 5

Risk of pharmaceuticals on freshwater ecosystems

Chapter 5
Risk of pharmaceuticals on freshwater ecosystems

5.1. Introduction

This chapter presents the contribution to the knowledge about the ecotoxicological risk that PhACs may pose to aquatic ecosystems. The chapter is divided in two sub-sections. The first sub-section includes the publication (*submitted to Journal of Hazardous Materials*) where the individual and combined acute toxicity of PhACs and other relevant micropollutants to *D. magna* and *V. fischeri* are assessed. The second sub-section includes the publication (Osorio et al., 2014a) where the impact of changing PhACs levels and water flow conditions on the structure and function of river biofilms is studied. Additionally, though the publication reporting the ecotoxicological risk assessment of PhACs to *D. magna*, *V. fischeri* and fish along four Iberian River basins (Osorio et al., 2015) is not included in this chapter, the subsequent findings are discussed in chapter 6 together with the results presented in this chapter.

5.2. Article: "Investigating the formation and toxicity of nitrogen transformation products of diclofenac and sulfamethoxazole in wastewater treatment plants"

Elsevier Editorial System(tm) for Journal of Hazardous Materials

Manuscript Draft

Manuscript Number:

Title: Investigating the formation and toxicity of nitrogen transformation products of diclofenac and sulfamethoxazole in wastewater treatment plants

Article Type: Research Paper

Keywords: Pharmaceuticals;
nitrification/denitrification;
Vibrio fischeri;
Daphnia magna;
toxicity

Corresponding Author: Dr. Sandra Perez Solsona,

Corresponding Author's Institution: IDAEA-CSIC

First Author: Victoria Osorio

Order of Authors: Victoria Osorio; Josep Sanchis; Jose Luis Abad; Antoni Ginebreda; Marinel.la Farré; Sandra Perez Solsona; Damia Barceló

Abstract: Diclofenac (DCF) and sulfamethoxazole (SMX) are highly consumed pharmaceuticals and detected at high concentrations in efluents from wastewater treatment plants (WWTPs) with conventional treatment since they are not completely eliminated. However, when microbial mediated nitrification/denitrification processes was taking place in the nitrifying activated sludge the degradation of DCF was enhanced and DCF biotransformed into its nitrogen-derivatives (NO-DCF and NO2-DCF) (Pérez and Barceló, 2008). SMX was also transformed under denitrification conditions in water/sediment batch reactors into NO2-SMX and Des-SMX (Nödler et al., 2012). We focused our research in the detection of the four TPs from DCF and SMX in waste waters (WW) and their receiving surface waters (SW). Nitrifying/denitrifying-derivatives of DCF and SMX were detected for the first time in both WW and SW at one order of magnitude lower than their parent compounds. The relationships observed among levels of NO-DCF, NO2-DCF (NO2-SMX and Des-SMX were only detected in one WWTP sample) and nitrogen-species determined in the effluents, the hydraulic retention time and the solids retention time of the WWTPs temptativelly suggested that nitrification/denitrification processes are involved in the nitration and nitrosation of diclofenac during the biological WW treatment. The acute toxicity of compounds of study to Daphnia magna and Vibrio fischeri was assessed both individually and in mixtures with other compounds of environmental concern. Individual effects in culture medium showed these compounds as not harmful and not toxic. However, the synergism effects observed in mixtures evidenced that the contribution of these compounds to the overall toxicity of complex environmental samples, such as WW and receiving SW, should not be dismissed.

September 19, 2015

Statement of novelty of the manuscript

Investigating the formation and toxicity of nitrogen transformation products of diclofenac and sulfamethoxazole in wastewater treatment plants

In recent years a small number of reports on the occurrence of nitrated and nitrosated organic contaminants in wastewater treatment plants (WWTP) have been published suggesting that such species are actually formed during sewage treatment. In a previous publication from our group we could demonstrate the presence of nitro- and nitroso-diclofenac in treated effluent from Spanish WWTPs. Here we extended these studies to sewage-impacted surface water and also included nitro and desamino-sulfoxazole in our monitoring program. For the first time, these unusual transformation products were shown to be present in water samples from WWTPs and rivers. As part of an ecotoxicological assessment, their toxicity was measured in a panel of standard assays of aquatic organism. Only nitro-diclofenac proved to be more toxic than the parent compound.

Investigating the formation and toxicity of nitrogen transformation products of diclofenac and sulfamethoxazole in wastewater treatment plants

Victoria Osorio[1], Josep Sanchís[1], Jose Luís Abad[2], Antoni Ginebreda[1], Marinella Farré[1], Sandra Pérez[1]*, Damià Barceló[1,3]

[1]Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain

[2] RUBAM-IIQAC-CSIC, Jordi Girona 18-26, Barcelona, Spain

[3]Catalan Institute for Water Research (ICRA), Scientific and Technological Park of the University of Girona, Emili Grahit 101, Girona, Spain

*Corresponding author:
Sandra Pérez Solsona
IDAEA-CSIC
Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain
Jordi Girona 18-26
Barcelona 08034, Spain
E-mail: spsqam@idaea.csic.es

Keywords

Pharmaceuticals, nitrification/denitrification, *Vibrio fischeri*, *Daphnia magna,* toxicity

ABSTRACT

Diclofenac (DCF) and sulfamethoxazole (SMX) are highly consumed pharmaceuticals and detected at high concentrations in efluents from wastewater treatment plants (WWTPs) with conventional treatment since they are not completely eliminated. However, when microbial mediated nitrification/denitrification processes was taking place in the nitrifying activated sludge the degradation of DCF was enhanced and DCF biotransformed into its nitrogen-derivatives (NO-DCF and NO_2-DCF) (Pérez and Barceló, 2008). SMX was also transformed under denitrification conditions in water/sediment batch reactors into NO_2-SMX and Des-SMX (Nödler et al., 2012). We focused our research in the detection of the four TPs from DCF and SMX in waste waters (WW) and their receiving surface waters (SW). Nitrifying/denitrifying-derivatives of DCF and SMX were detected for the first time in both WW and SW at one order of magnitude lower than their parent compounds. The relationships observed among levels of NO-DCF, NO_2-DCF (NO_2-SMX and Des-SMX were only detected in one WWTP sample) and nitrogen-species determined in the effluents, the hydraulic retention time and the solids retention time of the WWTPs temptativelly suggested that nitrification/denitrification processes are involved in the nitration and nitrosation of diclofenac during the biological WW treatment. The acute toxicity of compounds of study to *Daphnia magna* and Vibrio fischeri was assessed both individually and in mixtures with other compounds of environmental concern. Individual effects in culture medium showed these compounds as not harmful and not toxic. However, the synergism effects observed in mixtures evidenced that the contribution of these compounds to the overall toxicity of complex environmental samples, such as WW and receiving SW, should not be dismissed.

INTRODUCTION

Diclofenac (DCF) is an over-the-counter nonsteroidal anti-inflammatory pain-relieving drug (NSAID). The antibiotic sulfonamide drug sulfamethoxazole (SMX) is extensively used in both human and veterinary medicine. Both compounds were categorized as Class 1: high priority drugs in the common list of pharmaceuticals relevant to the water cycle (The Global Water Research Coalition, 2004). As a matter of fact, DCF has been recently included in the EU Commission watch list of organic pollutants in surface waters (SW) (Directive 2013/39/EU). DCF and SMX mainly enter into the aquatic environment through effluent discharge from wastewater treatment plants (WWTPs) receiving human wastes (Osorio et al., 2014; Gros et al., 2010). As a consequence of their high consumption rates (García et al., 2006; Lázaro et al., 2010), DCF and SMX have been frequently detected in wastewater influent (WWi) and effluent (WWe) samples from Spanish WWTPs at levels of 200-1100 ngL^{-1} (Osorio et al., 2014; Díaz-Cruz et al., 2008). In full-scale WWTP relying on conventional activated sludge (CAS) treatment, removal rates of DCF and SMX are widely varying, [7-80] % and [0-98] % (Onessios et al., 2009), making it difficult to assess the extent of biotransformation.

Even though the occurrence of DCF and SMX in the aquatic environment has been widely studied (Nödler et al., 2012) very little is known about their fate in WWTPs when operating under nitrifying activated sludge (NAS) conditions. This microbial-driven process consists on two main steps which are nitrification and denitrification. During these processes reactive nitrogen species are generated (Chiron et al., 2010) which may be involved in the formation of transformation products (TPs) such as the nitrosation and nitration derivatives of DCF (NO-DCF and NO_2-DCF, respectively) (Pérez et al., 2008; Osorio et al., 2014) and nitration reaction of acetaminophen (TP 3-nitro-acetaminophen) (Chiron et al., 2010). In addition, denitrifying bacteria are also a potential source of reactive nitrogen species (Nödler et al., 2012). The abiotic formation of nitrifying/denitrifying derivatives of DCF (NO-DCF and NO_2-DCF) and SMX (NO_2-SMX and Des-SMX) was observed in anoxic water/sediment batch experiments under denitrifying conditions (Nödler et al 2012; Barbieri et al., 2012). Considering that nitrification and denitrification processes occur during the biological treatment step, the formation of NO_2-SMX and Des-SMX in the activated sludge of WWTPs was conjectured. On the other hand, increases in nitrogen concentration (mainly ammonium, NH_4^+-N) were observed in Spanish streams receiving WW effluent (WWe) discharges (Martí et al., 2004). These large inputs of NH_4^+-N from WWTPs were further related with the hot spots for microbial nitrification observed in WWe impacted streams (Merseburger et al., 2005).

On the basis of these findings, the possibility of microbially mediated biotransformation of DCF and SMX and the presence of their TPs in WWe and receiving SW was hypothesized. Despite the pseudo-persistence of DCF and SMX in the aquatic environment, these drugs are unlikely to pose a risk to aquatic species, at least in terms of acute toxicity, since their

environmental concentrations are in the range of 3 to 7 orders of magnitude lower than EC_{50} values for several aquatic organisms (i.e. algae, daphnids, fisch) (Fent et al., 2006). However, in exposed aquatic organisms, the lack of data of the ecotoxicity of TPs is a major unaddressed area. Moreover, individual chemicals can interact with each other resulting in additive or synergistic mixture effects. In addition, TPs may contribute to the risk posed by the parent compound since they often exhibit the same mode of action. Consequently, TPs are regarded as concentration-additives in mixtures and their potential synergistic effects are also expected (Escher et al., 2011). In this context, in order to gain further insight into the fate of DCF and SMX in WWTPs during the NAS treatment, the objectives of this study were: (1) to determine the presence of the nitrifying/denitrifying derivatives of DCF and SMX in samples from different WWTPs and receiving SW; (2) to investigate on the relationship between inorganic nitrogen (N)-species in WWTP and the levels of TPs detected in wastewaters (WW) as well as in SW; and (3) to assess the ecotoxicological risk of these TPs for aquatic ecosystems by measuring the individual and combined acute toxicity to *Daphnia magna* and *Vibrio fischeri*.

MATERIAL AND METHODS

Pharmaceutical standards and chemicals

All pharmaceutical standards were of high purity grade (>90%) unless stated otherwise. Compounds with letter *a* (in Table A.1) were kindly supplied by Sigma Aldrich (Steinheim, Germany) and those with letter *b* from Toronto Research Chemicals (Toronto, Canada). Isotopically labeled compounds, used as internal standards, were mefenamic acid-d_3 (MFA-d_3) purchased from Toronto Research Chemicals (Toronto, Canada), sulfamethoxazole-d_4 from Dr. Ehrenstorfer (Augsburg, Germany); niflumic acid-d_5 provided by Santa Cruz Biotechnologies (Santa Cruz, Canada); and sulfadimethoxine-d_6, used as a surrogate was provided by Sigma Aldrich (Steinheim, Germany). Individual stock solutionsand isotopically labeled internal standard solutions were prepared on a weight basis in methanol. After preparation, standards were stored at -20°C. A mixture of all target compounds was prepared by appropriate dilution of individual stock solutions in methanol/water (5:95, v/v). Working standard solutions, also prepared in methanol/water (5:95, v/v) mixture, were renewed before each analytical run. Working solutions were prepared in amber glass vials while standard mixtures were prepared in volumetric flasks. A separate mixture of isotopically labeled internal standards, used for internal standard calibration, was prepared in methanol and further diluted in methanol:water (5:95, v/v). HPLC grade methanol, acetonitrile, water (Lichrosolv), hydrochloric acid (37%) and formic acid (98%) were supplied by Merck (Darmstadt, Germany). Ammonium hydroxide was from Fluka and ammonium acetate salt (99%) was from Sigma-Aldrich (Steinheim, Germany). Nitrogen for evaporating the solvent (99.99% of purity) was from Air Liquide (Spain).

Sampling sites and WWTPs characteristics

A sampling campaign was carried out in seven selected WWTPs in November 2012 (Figure A.1) Samples were collected hourly on a weekday (24h) to build a 1-L composite sample. The desigr and operating characteristics of the WWTPs are shown in Table A.2. Out of the seven WWTPs studied, the WWTPs 3, 5, 6 and7 discharge their effluents into fresh water systems while the other three do it into coastal waters. With regards to SW samples, three discrete freshwater samples were taken in the Llobregat River basin (Llobregat River and Riera de Rubí) 24 hours after sampling the WWTPs, at approximately 500 m from these WWe discharge points (Figure A.1). This distance was selected according to the reported availabilities of N-species and high rates of nitrification along the recieving WWe inputs (Merbt et al., 2011; Martí et al., 2004). Two samples, SW5 and SW6, were collected up and down the Riera de Rubí, receiving the effluent inputs located at 3100 and 300 m from WWTP5 and WWTP6, respectively. The third fresh water sampling (SW7) was carried out in the lower course of the Llobregat River after the effluent discharge point of WWTP7, placed 3600 m from the WWTP. The Riera de Rubí stream and the Llobregat River are highly influenced by anthropogenic pressure receiving extensive urban and industrial wastewater discharges as well as surface runoff from agricultural areas. According to flow data, monthly recorded by the Catalan Water Agency for the last ten years, the Riera de Rubí and the Llobregat River show flow averages of 0.88 and 11 $m^3 s^{-1}$, respectively. WWi, WWe and SW samples were collected in 1 L amber glass bottles previously rinsed with ultrapure water, heated overnight at 400ºC and rinsed again with sample water onsite. Samples were placed in a cooler at 4ºC and delivered to the laboratory within 2 h. Samples were immediately filtred with Nylon filters of 0.45 µm size mesh (Whatman, Maidstone, England) and stored in a refrigerator (-20 ºC) until analysis within two days.

Sample pretreatment and solid phase extraction

Clean-up and pre-concentration step was carried out within 48 h by solid phase extraction (SPE) using a vacuum system (J.T. Baker, Deventer, The Netherlands) and Oasis HLB (200 mg, 6 mL) cartridges (Waters Corporation,Milford, MA, USA). In order to evaluate the efficiency of the SPE process, recovery tests were carried out in WW and SW matrices. WW and SW samples were spiked prior to the extraction with standard mixtures containing the target analytes at two levels: 0.2 and 0.6 μgL^{-1} and 0.05 and 0.1 μgL^{-1}, respectively. After conditioning of the cartridges with 5 mL of methanol and 5 mL of HPLC grade water, the sample (WWe: 200 mL; WWi: 100 mL; SW: 500 mL) were loaded at a flow rate of approximately 5 $mL\ min^{-1}$. Afterwards, the cartridges were rinsed with 5 mL of HPLC grade water and dried under vacuum for 20 min. After elution with 2x4 mL of methanol, the extracts were evaporated to dryness under a gentle stream of nitrogen and reconstituted with 1000 µL of 5 % methanol. For internal standard calibration, 25 µL of a 1000 μgL^{-1} standard mixture containing internal standards (MFA-d_3 and NFA-d_5 for (-)-ESI mode and NFA-5

d_5, for (+)-ESI mode) were added to the final extracts. Since the internal standards were added prior to injection into the LC, the final concentrations were calculated by dividing the recovery (WWi and WWe samples spiked with target analytes (n=3)) with the concentration obtained by internal calibration. Additionally, two standards (Lumiracoxib, LMX for (-)-ESI mode and SDM-d_6 for (+)-ESI mode) were added to all the samples before the extraction at a concentration level of 50 µg L^{-1} and used as surrogates.

Analysis of nitrogen species

The levels of of ammonium (NH_4^+-N), nitrate (NO_3^--N) and nitrite (NO_2^--N) were analysed in WW and SW samples with the Automatic Continuous Flow Analyser Futura (Alliance Instruments).The analytical methods were officially compliant to international certification or standards recommendation bodies such as ISO, AOAC or US EPA (see more details in Supplementary material).

Analysis of target compounds

A previously developed analythical method (Osorio et al., 2014) was modified and applied to the determination of DCF, SMX and their nitro derivatives in WW and SW samples. LC-ESI-(QqLIT) MS/MS analysis was performed using a Symbiosis™ Pico (SP104.002, Spark, The Netherlands), equipped with an autosampler and connected in series with a 4000 QTRAP QqLIT-MS, equipped with a Turbo Ion Spray source (Applied Biosystems-Sciex, Foster City, CA, USA). Chromatographic separation was achieved as described by Osorio et al. (2014), with a Hypersil Gold PFP endcapped column C18 (50×2.1 mm, particle size 3 µm) precedeed by a Hypersil Gold PFP guard cartridge (10 × 2.1 *µ*m, 3 µm), both supplied by Thermo (San Jose, CA, USA). MS/MS instrumental parameters and method quality parameters are summarized in Tables A.3- A.4.

Toxicity assays

In order to assess the acute toxicity of nitro TPs of DCF, two standardized acute toxicity tests, the 48h immobilization of *Daphnia magna* (Directive 92/69/EEC) and the bioluminescence inhibition of the marine bacteria *Vibrio fischeri* (ISO 1994) were carried out. Individual stock solutions of the target compounds were prepared in their respective culture medium and test solutions were prepared in a range of concentrations of 0.01-100 mgL^{-1} Four-parameter equations were fitted and the 50 percent effective concentration (EC_{50}), the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) were calculated.

In addition, the possible synergistic or antagonistic effects of binary mixtures, composed of DCF, SMX or NO-DCF and other organic pollutants such as a surfactant, nonylphenol (NPL); three pesticides, malathion (MLT), diuron (DRN) and glyphosate; and a bactericide, triclosan, (TCS)) that are frequently detected in the environment, were studied. The mixtures to be tested were

prepared to obtain the concentration producing the 50 % of inhibition according to a simply additive model. That means (EC_{10}:EC_{40}, EC_{25}:EC_{25} and EC_{40}:EC_{10}, EC_{10}, EC_{25} and EC_{40}) of each substance for each test.

The tests were performed according to standard protocols and always working in triplicates. Very briefly, the protocols were as follows *D. magna epphipia* were incubated at 21 ± 1 ºC under continuous illumination of 6000 lx. in standard freshwater. Immediately after hatching, the daphnids were fed with *spirulina* algae and after 2 h the test was initiated. The daphnids were exposed during 48 h at 20 ±1 ºC in the dark, to a range of test compound concentrations from cero to the maximum of inhibition that was possible to measure in each case.

To carry out the test based on the bioluminescence inhibition of *V. fischeri* the Microtox 500 Analyzer (SDIX) was used. The bacterial reagent was reconstituted 15 min. prior to the tests. The assays were performed at 15 °C in 2 % saline solution. The bioluminescence inhibition was measured after 30 min of incubation.

RESULTS AND DISCUSSION

Levels of nitrogen species

Table A.5 shows levels of inorganic N-species determined in WWi and WWe and receiving SW samples. WWTP1 received the highest input of NH_4^+-N (62.3 mgL^{-1}) while WWTP7 collected the minimum load (42.7 mgL^{-1}). Average levels of NH_4^+-N, NO_2^--N and NO_3^--N determined in the WWi samples were 53.8, 0.012 and 0.017 mgL^{-1}. The maximum and minimum concentrations of NH_4^+-N determined in WWe samples were those collected from WWTP2 and WWTP3, respectively. The average levels of N-species in the seven effluents were 32.6 mgL^{-1} of NH_4^+-N; 1.67 mg L^{-1} of NO_2^--N and 12.4 mgL^{-1} of NO_3^--N. As expected, the concentration of NH^{4+}-N was generally higher in the influents compared to the effluents and the average NH_4^+-N removal was 40%. On the contrary, levels of NO_2^--N and NO_3^--N were higher in the effluents and the average NO-N (NO_2^--N + NO_3^--N) formed was 99%. These results confirmed that most of the input of inorganic nitrogen is nitrifyied through the wastewater treatment. Regarding the SW samples, levels of N-species were not as high as those observed in 15 downstreams of WWTPs over Catalonia (NE Spain) (Martí et al., 2004). The average levels of NH_4^+-N and NO_3^--N reported in the work cited previously were ~94 and ~59 mgL^{-1} respectively; while we found concentrations of NH_4^+-N and NO_3^--N averaging 15 and 11 mgL^{-1}. On the other hand, Merbt et al. (2011) measured average concentrations of 0.8 and 2 mgL^{-1} of NH_4^+-N and NO_3^--N, respectively. These contrast findings could be mainly explained by the dilution capacity of the receiving stream. Additional factors affecting to a lesser extent could be the different distances from the WWe and the SW collection site, the varying N-species loads from WWe inputs and the nitrification activity along the receiving stream.

Occurrence of DCF, SMX & nitro TPs in wastewater and receiving surface water

The occurrence of DCF, SMX and their TPs determined in seven WWTPs and three receiving SW is summarized in Table 1. DCF and SMX were detected in all WWi samples at the high ngL^{-1} range (48-999 ngL^{-1}) and only SMX exceeded this level in WWTP2 with a concentration of 1218 ngL^{-1}. On average, the input of SMX (815 ngL^{-1}) to the WWTP was higher than the one for DCF (536 ngL^{-1}). This difference does not correspond to that frequently observed in the literature, since DCF is generally found in WWi at higher concentrations (500-7500 ngL^{-1}) than SMX (100-4000 ngL^{-1}) (Jelic et al., 2012). On the contrary, outputs of DCF (669 ngL^{-1}) were higher than those for SMX (322 ngL^{-1}), which was in agreement with general concentration ranges (200-1400 ngL^{-1} for DCF and 50-800 ngL^{-1} for SMX) reviewed by Jelic et al. (2012). Thus, while the concentration of SMX was substantially lower after the WWTP, DCF levels in the WWe did not varied significantly respective to their WWi samples. Different findings were reported by Gros et al. (2010), who observed higher inputs of DCF) and SMX compared to the outputs in other WWTPs. Our main goal was not to assess the elimination of DCF and SMX, but to attempt to gain knowledge on the presence of microbial TPs of DCF and SMX in WW samples in order to better understand the fate of DCF and SMX in WWTPs. The TPs were detected in WWI at a concentration range of [MDL-36 ngL^{-1}]. NO_2-DCF and NO_2-SMX were the most frequently detected ones, being present in four and three out of the seven WWi samples analyzed. NO-DCF was detected in one influent of sample from WWTP6 and NO_2-DCF in WWTP2 while Des-SMX was not found in any of the WWi. These results suggest the formation of DCF and SMX TPs in the collecting sewage system. Recently, it has been demonstrated that PhACs and their TPs can experiment natural attenuation and further transformation by in-sewer anaerobic biodegradation processes occurring along their fate through the urban WW system (Jelić et al., 2015). In the cited work, the concentrations of diltiazem, citalopram, clarithromycin, bezafibrate and amlodipine were substantially decreased (25-60%) during their pass through a pressurized pipe. However, these TPs were more frequently detected in the effluents: NO-DCF in all samples, NO_2-DCF and NO_2-SMX in four out of the seven WWTPs. As for the TPs of SMX only one treated sewage sample presents levels of NO_2-SMX (WWTP6) or Des-SMX (WWTP2). Levels of these compounds ranged from MDL to 11 ng L^{-1}. The highest ubiquity of these TPs in the effluents relative to the influents could be explained by the known and generally controlled amount of microbial communities present in the NAS. Importantly, the levels of TPs of DCF in WW were in agreement with our previous findings (Osorio et al., 2014). Interestingly, the detection of TPs of SMX after CAS treatment suggested that biotic transformation of SMX can lead to generation of NO_2-SMX and Des-SMX, as observed when biotic reactions occurred in denitrifying water/sediment batch reactors reported by Nödler et al (2012). In addition, since metabolites (i.e. NO_2-SMX) postulated by Mueller et al. (2013) may not be the only TPs of SMX generated during the activated sludge treatment it would be interesting to include NO_2-SMX and Des-SMX in further field studies.

Regarding receiving SW, DCF, SMX and their TPs were detected at respective concentration ranges of 235-847, 48-173 and MDL-18 ngL^{-1} (Table 1) with the exception of NO_2-SMX, which was not found in any of the SW samples. NO-DCF was detected in all SW analyzed, while NO_2-DCF and Des-SMX were present in SWs 6 and 7. Concentrations of DCF and SMX were in the range of those reported previously for the same river basin (Osorio et al., 2012a; 2012b). Des-SMX was determined at the same range of concentrations observed in spring water samples (Nödler et al., 2012). The potential retransformation of NO_2-SMX back to its parent compound (Nödler et al., 2012) might be a reasonable explanation for the absence of this TP in SW. Interestingly, the higher concentrations and frequency of detection of target compounds in SW were determined at the location closest to the WWTP (see section 2.2.), namely SW6 (see Table 1). Detections and levels gradually decreased as long as sampling sites were located further from the WWTP, thus being SW5 followed by SW7. The decrease of concentrations between different sampling locations is not clear because data recorded in this study cannot explain the natural attenuation processes that might occur along the river. However, strong dilution effects could explain the the lowest concentrations were measured in SW7 where river flow is about ten fold higher than the corresponding to SW5 and SW6 locations (see section 2.2).

Relationship between nitrification/denitrification processes occurring in the NAS treatment and the detected N-derivatives

In order to have a better insight on how the nitrification/denitrification process may influence the formation of TPs of DCF and SMX, we explored the relationships between NO-DCF and NO_2-DCF with NO-N species generated after the WW treatment and operational parameters of the WWTPs (see Figures 1 and A.2).

According to Chiron et al. (2010) NO radical (nitric oxide) is one of the key reactive nitrogen species that is produced in both nitrifying and denitrifying process steps. Thus, we evaluated how levels of NO-DCF and NO_2-DCF measured in WWE varied with levels of NO-N species calculated as the sum of NO_3^--N and NO_2^--N species in the WWE (Figures 1a and 1b). The trend observed along the seven WWTPs was a negative relationship between levels of NO-DCF and NO-N species (Figure 1a); while levels of NO_2-DCF were positively related with levels of NO-N species (Figure 1b). In view of the observed opposite behaviour of NO-DCF and NO_2-DCF, we also evaluated the relationship between their concentrations (Figure 1c). Concentrations of both TPs showed oppositive trends along the WWTPs in which both were detected. Although the amount of WWTPs studied and detections of TPs was not enough to evaluate quantitative correlations, these trends observed would suggest the microbialy mediated formation of NO-DCF in a first step followed by its transformation into NO_2-DCF. However, this hypothesis should be tested properly by means of biodegradation experiments in activated sludge batch reactors, in order to elucidate the transformation mechanisms of DCF to its TPs.

In addition, we evaluated the qualitative relationship between levels of N-derivatives of DCF in WWe and the operational parameters of the WWTPs studied namely hydraulic retention time (HRT) and solids retention time (SRT) (see Table A.2). HRT is related with reaction time in the activated sludge tank, thus the longer the HRT is; the higher mineralization or biotransformation of pollutants would be expected. We observed an inverse effect trend on the concentration of NO-DCF, thus suggesting again that this compound undergoes further transformations if enough time is given (Figure A.2a). Though only in two cases, the opposite trend was observed for NO_2-DCF (Figure A.2b), which would support the hypothesis of transformation of NO-DCF into NO_2-DCF or at least suggest that NO_2-DCF would take longer reaction times to be generated. Lastly, SRT is related with the age, amount and diversity of microbial community present in the activated sludge, thus the longer SRT more bacteria involved in nitrification/denitrification processes would be expected. Again, NO-DCF and NO_2-DCF showed negative and positive relationships with SRT (Figure A.2c and A.2.d), which would be in agreement with the hypotheses stated before. Moreover, these findings are in also in accordance with the higher biodegradation rates of DCF and SMX observed at longer SRT (Fernández-Fontaina et al., 2012; García-Galán et al., 2012).

Due to only sporadical detection of quantificable levels of TPs of SMX, no relationships with N-species or operational parameters of WWTPs were explored. Nevertheless, SMX has been proved to undergo microbially mediated biotransformation (Mueller et al., 2013) during the NAS treatment, where heterotrophic and autotrophic nitrifying bacteria have been pointed out to be potentially involved in the generation of TPs such as NO_2-SMX. Thus, in the same line of interest for TPs of DCF, additional biodegradation experiments of SMX would be required to better understand the formation of its TPs in the activated sludge.

Regarding the TPs detected in WWe dominated SW, data acquired cannot reveal wether these derivatives are emitted from the WWTPs via effluent discharge and/or they are also generated via nitrification/denitrification processes that may take place in rivers as well. All in all, we consider that further investigations on this issue should be focused on the presence of these TPs in the freshwater systems.

Acute toxicity assays

Since there is a need to evaluate whether the potential ecotoxicological risk originating from TPs is higher than the one identified for their parent compounds, two acute standardized toxicity assays (*Daphnia magna* and *Vibrio fischeri*) were performed to assess the acute toxicity of DCF, SMX and their TPs.

Observed acute toxicity effects of DCF to *D. magna* of DCF (Table 2, EC_{50} = 53.9 mgL^{-1}) were in agreement with those reported by Cleuvers (2003), who determined an EC_{50} value of 68 mgL^{-1} in the same organisms. The EC_{50} value calculated by Oliveira et al. (2015) doubled the one

we reported (EC_{50} = 123.3 mgL^{-1}). Likewise, the toxicity observed for SMX (EC_{50} = 75.3 mgL^{-1}) dic not match the EC_{50} value of 189.2 mgL^{-1} determined by Kim et al. (2007). Nonetheless, ou resulting EC_{50} values and those reported in the literature were in the same concentration range Likewise, the calculated toxicity levels of DCF and SMX to V*. fischeri* (respective EC_{50} values of 22.9 and >100 mgL^{-1}) were also in the order of those previously reported for DCF (EC_{50} = 11.5 mgL^{-1}) (Ferrari et al., 2003); and for SMX (EC_{50} = 140 mgL^{-1}) (Majewsky et al., 2014). As can be seen in Table 2, the LOEC values were in all the cases several orders of magnitude higher than those concentrations found in the environment. Therefore, it can be confirmed that the studied TPs did not show acute toxicity, e.g. for the *D. magna*, the EC_{50} of DCF and SMX were 53.9 and 86.7 mgL^{-1}, respectively. And, for NO-DCF, NO_2-DCF and Des-SMXEC_{50} were even lower than those for their respective precursors. Only NO_2-DCF, Des-SMX and NO_2-SMX showed to be slightly more toxic to *V. fischeri* than their precursors, DCF and SMX. Similar results were obtained when Majewsky et al. (2014) investigated on the residual antibacterial activity of 11 TPs of natural degradation (biodegradation and photodegradation) of SMX in the aquatic environment with regard to their in vitro growth and luminescence inhibition on *V. fischeri*. Results of individual compound experiments showed that some TPs still exhibit clear antibacterial effects. After 30 min of exposure of *V. fischeri*, the EC_{50} value of SMX was 140 mgL^{-1}; while NO_2-SMX was 3 times more toxic upon luminescence emission (EC_{50} = 48 mgL^{-1}). Importantly, this behaviour, was also observed with other TPs e.g. NO-SMX.

The EU-Directive 93/67/EEC (Commission of the European Communities, 1996), classifies substances according to their EC_{50} value as follows: < 1 mgL^{-1} very toxic to aquatic organisms; 1-10 mgL^{-1} toxic to aquatic organisms; and 10-100 mgL^{-1} harmful to aquatic organisms. According to this, none of the compounds studied were considered as toxic to aquatic organisms. However, DCF, NO_2-DCF and SMX were harmful to *D. magna*; while for *V. fischeri* the harmful compounds were DCF, TP339, Des-SMX and NO2-SMX. On the other hand, SMX and Des-SMX were not harmful to *V. fischeri*; while NO-DCF was not harmful to any of the species tested.

In Figures 2 and 3, the study of potential synergistic or antagonistic effects of DCF, SMX and NO-DCF as well as with other contaminants in mixtures is summarized. Since NO-DCF was demonstrated not to pose any relevant threat to aquatic organisms, we decided to investigate on its potential to increase the toxicity of a mixture. As can be seen in Figure2, in most of the cases synergism was the predominant effect, indicating the need to further assess the combination of mixtures. Similarly, synergistic effects of DCF and SMX have been observed in combinations with other pharmaceuticals. For instance, a mixture of DCF and ibuprofen was slightly more toxic to *D. magna* than individual pharmaceuticals, although each individual component was present in a concentration below its individual NOEC (Cleuvers et al., 2003). Synergistic effects were also observed for a mixture of SMX and trimethoprim in algae (Yang et al., 2008). Therefore, although

NO-DCF did not showed acute toxicity, at some concentrations can increase the effects of other toxicants in the same environmental compartments.

While DCF and SMX and their derivatives did not show individually acute toxicity, they show synergistic effects with other relevant contaminants. There is still a gap of information about the combined effects of complex mixtures, in particular considering the presence of TPs.

CONCLUSIONS

To our knowledge, we reported the first evidence of the presence of TPs of DCF and SMX in WW effluent and in SW impacted with WWe. The detection of TPs of DCF and SMX in WWi suggested that microbial transformation processes occur along the collecting sewage system. The qualitative relationships evaluated (among NO-DCF, NO_2-DCF and NO-N determined at the WWe, HRT and SRT of the WWTP) suggested the tentative biotransformation of DCF and SMX into their TPs as a consequence of nitrification/denitrification processes in the activated sludge. To our knowledge, we evaluated for the first time the acute toxicity to *D. magnia* and *V. fischeri* of TPs of DCF and SMX as well as of mixtures of these with other PhACs of emerging concern. Overall, the concentration and associated acute toxicity to D*. magna* and *V. fischeri* of TPs were lower than the corresponding to their parent compounds. Nevertheless,the observed synergism of NO-DCF with other substances of environmental concern evidenced that these TPs should not be disregarded in assessment of such complex mixtures like those found in WW highly impacted SW. In consequence, we would propose these TPs to be included in environmental studies as a complement to understand the occurrence, fate and behavior of DCF and SMX in the engineered systems and the aquatic environment.

Appendix A. Supplementary material

Supplementary data associated with this article shows Tables A.1-A.5 and Figures A.1-A.4.

Acknowledgements

This work has been funded by the Spanish Ministry of Economy and Competitiveness through the project SCARCE (Consolider-Ingenio 2010 CSD2009-00065). It reflects only the authors' views and the European Community is not liable for any use that may be made of the information contained therein. VO acknowledges all WWTPs supervisors for supplying samples, Elena Martínez from IDAEA-CSIC (Barcelona, Spain) for her technical support during sampling and Roser Ventura from CEAB-CSIC (Blanes, Spain) for nutrients analysis. SP acknowledges the contract from the Ramón y Cajal Program of the Spanish Ministry of Economy and Competitiveness.

REFERENCES

Barbieri, M., Carrera, J., Ayora, C., Sanchez-Vila, X., Licha, T., Nödler, K., Osorio, V., Pérez, S., Köck-Schulmeyer, M., López de Alda, M., Barceló, D., 2012. Formation of diclofenac and sulfamethoxazole reversible transformation products in aquifer material under denitrifying conditions: Batch experiments. Science of the Total Environment 426, 256-63.

Chiron, S., Gómez, E., Nefenet, H., 2010. Environmental Science and Technology 44, 284-289.

Clara, M., Kreuzinger, N., Strenn, B., Gans, O., Kroiss, H., 2005. The solids retention time a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. Water Research 39, 97-106.

Cleuvers, M., 2003. Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects. Toxicology Letters 142 (3), 185-194.

Commission of the European Communities, Technical guidance document in support of commission directive 93/67/EEC on risk assessment assessment for new notified substances and commission regulation (EC) No. 1488/94 on risk assessment for existing substances. Part II. Environmental Risk Assessment, Office for Official Publications of the European Communities, Luxembourg, 1996.

Commission of the European Communities, Methods for determination of ecotoxicity; Annex V, C.2, Daphnia, acute toxicity to Daphnia, L. 383A, EEC Directive 92/69/EEC, 1992, p. 172.

Díaz-Cruz, M.S., García-Galán, M.J., Barceló , D., 2008. Highly sensitive simultaneous determination of sulfonamide antibiotics and one metabolite in environmental waters by liquid chromatography-quadrupole linear ion trap-mass spectrometry. Journal of Chromatography A 1193, 50–59.

Directive 2013/39/EU of the European parliament and of the council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy.

Escher, B.I., Fenner, K., 2011. Recent advances in environmental risk assessment of transformation products. Environmental Science and Technology 45, 3835-3847.

Fent, K., Weston, A., Caminada, D., 2006. Ecotoxicology of human pharmaceuticals. Aquatic Toxicology. 76, 122-159.

Fernandez-Fontaina, E., Omil, F., Lema, J. M., Carballa M., 2012. Influence of nitrifying conditions on the biodegradation and sorption of emerging micropollutants. Water Research. 46(16), 5434-5444.

Forrez, I., Boon, N., Verstraete, W., Carballa, M., 2011. Municipal wastes. Biodegradation of Micropollutants and Prospects for Water and Wastewater Biotreatment. In: Murray Moo-Young (ed.), Comprehensive Biotechnology, Second Edition, volume 6, pp. 485–494. Elsevier

García-Galán, M.J., Díaz-Cruz, M.S., Barceló, D., 2009. Combining chemical analysis and ecotoxicity to determine environmental exposure and to assess risk from sulfonamides. Trends in Analytical Chemistry 28 (6), 804-819.

García-Galán, M.J., Díaz-Cruz, M.S., Barceló, D., 2012. Removal of sulfonamide antibiotics upon conventional activated sludge and advanced membrane bioreactor treatment. Analytical and bioanalytical chemistry 404(5), 1505-1515.

García, J.P., de Abajo, F.J., 2006. Utilización de antiinflamatorios no esteroides (AINE) en España, 1992-2006. División de Farmacoepidemiología y Farmacovigilancia de la Agencia Española de Medicamentos y Productos Sanitarios.

Gros, M., Petrovic, M., Ginebreda, A., Barceló, D., 2010. Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes. Environmental International 36(1), 15-26.

GWRC The Global Water Research Coalition . Pharmaceuticals and personal care products in the water cycle. An international review, London, UK; 2004.

Hernando, M.D., Petrovic, M., Fernández-Alba, A.R., D. Barceló, 2004. Analysis by liquid chromatography-electrospray ionization tandem mass spectrometry and acute toxicity evaluation for β-blockers and lipid-regulating agents in wastewater samples. Journal of Chromatography A 1046, 133-140.

ISO (1994) ISO 11348-2: Water quality: determination of the inhibitory effect of water samples on the light emission of Vibrio fischeri (luminiscent bacteria test), draft of revised version. ISO, Geneva, Switzerland

Jelić, A., Rodríguez-Mozaz S., Barceló D., Gutiérrez O. 2015. Impact of in-sewer transformation on 43 pharmaceuticals in a pressurized sewer under anaerobic conditions. Water Research 68, 98-108.

Joss, A., Keller, E., Alder, A. C., Göbel, A., McArdell, C. S., Ternes, T. A., Siegrist, H., 2005. Removal of pharmaceuticals and fragrances in biological wastewater treatment. Water Research 39, 3139-3152.

Kim, Y., Choi K., Jung J., Park S., Kim P.G., Park J. 2007. Aquatic toxicity of acetaminophen, carbamazepine, cimetidine, diltiazem and six major sulfonamides, and their potential ecological risks in Korea. Environment International 33(3), 370-375.

Lázaro, E.B., Montero, D.C., 2010: Uso de antibióticos en España División de Farmacoepidemiología y Farmacovigilancia (Agencia Española de Medicamentos y Productos Sanitarios).

Majewsky, M., Wagner, D., Delay, M., r se, Viviane, S.Y., Horn, H. 2014. Antibacterial Activity of Sulfamethoxazole Transformation Products (TPs): General Relevance for Sulfonamide TPs Modified at the para Position. Chemical research in toxicology 27(10), 1821-1828.

Martí, E., Aumatell, J., Gode, J., Poch, M., Sabater, F., 2004. Nutrient retention efficiency in streams receiving inputs from wastewater treatment plants. Journal of Environmental Quality 33, 285-293.

Merbt, S., Auguet, JC., Casamayor, E., Martí, E., 2011. Biofilm recovery in a wastewater treatment plant- influenced stream and spatial segregation of ammonia oxidizing microbial populations. Limnology and Oceanography 56(3), 1054-1064.

Merseburger, G., E. Martí, F. Sabater., 2005. Net changes in nutrient concentrations below a point source input in two streams draining catchments with contrasting land uses. Science of the Tototal Environment 347, 217-229.

Müller, E., Schüssler W., Horn, H., Lemmer, H., 2013. Aerobic biodegradation of the sulfonamide antibiotic sulfamethoxazole by activated sludge applied as co-substrate and sole carbon and nitrogen source. Chemosphere 92(8), 969-978.

Nödler, K., Licha, T., Barbieri, M., Pérez, S., 2012. Evidence for the microbially mediated abiotic formation of reversible and non-reversible sulfamethoxazole transformation products during denitrification. Water Research 46, 2131-2139.

Oliveira, LD., Antunes S.C., Gonçalves F., Rocha O., Nunes, B. 2015. Evaluation of ecotoxicological effects of drugs on Daphnia magna using different enzymatic biomarkers. Ecotoxicology and environmental safety 119, 123-131.

Onesios, K.M., Yu, J.T., Bouwer, E.J., 2009. Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: A review. Biodegradation 20, 441-466.

Osorio, V., Pérez, S., Ginebreda, A., Barceló, D., 2012a. Pharmaceuticals on a sewage impacted section of a Mediterranean River (Llobregat River, NE Spain) and their relationship with hydrological conditions. Environmental Science and Pollution Research 19, 1013-25.

Osorio, V., Marcé, R., Pérez, S., Ginebreda, A., Cortina, J.L., Barceló, D., 2012b. Occurrence and modeling of pharmaceuticals on a sewage impacted Mediterranean river and their dynamics under different hydrological conditions. Science of the Total Environment 440, 3-13.

Osorio, V., Imbert-Bouchard, M., Zonja, B., Abad, J.L., Pérez, S., Barceló, D., 2014. Simultaneous determination of diclofenac, its human metabolites and microbial nitration/nitrosation transformation products in wastewaters by liquid chromatography/quadrupole-linear ion trap mass spectrometry. Journal of Chromatography A 1347, 63-71.

Pérez, S., Eichhorn, P., Aga, D.S., 2005. Evaluating the biodegradability of sulfamethazine, sulfamethoxazole, sulfathiazole, and trimethoprim at different stages of sewage treatment. Environmental Toxicology and Chemistry 24 (6), 1361-1367.

Pérez, S., Barceló, D., 2008. First evidence for occurrence of hydroxylated human metabolites of diclofenac and aceclofenac in wastewater using QqLIT-MS and QqTOF-MS. Analitical Chemistry 80, 8135-8145.

Petrovic, M., Lopez de Alda, M., Diaz-Cruz, S., Postigo, C., Radjenovic, J., Gros, M., Barceló, D., 2009. Fate and removal of pharmaceuticals and illicit drugs in conventional and membrane bioreactor wastewater treatment plants and by riverbank filtration. Philosophical Transactions 367, 3979-4003.

Sacher, F., Ehmann, M., Gabriel, S., Graf, C., Brauch, H.J., 2008. Pharmaceutical residues in the river Rhine – results of a one-decade monitoring programme. Journal of Environmental Monitoring 10, 664-670.

Sanderson, H., Johnson, D.J., Reitsma, T., Brain, R.A., Wilson, C.J., Solomon, K.R., 2004. Ranking and prioritization of environmental risks of pharmaceuticals in surface waters. Regulatory Toxicology and Pharmacology 39, 158-83.

Suárez, S., Ramil, M., Omil, F., Lema, J. M., 2005. Removal of pharmaceutically active compounds in nitrifying–denitrifying plants. Water Science and Technology 52, 9-14.

Suarez, S., Lema, J.M., Omil, F., 2010. Removal of Pharmaceutical and Personal Care Products (PPCPs) under nitrifying and denitrifying conditions. Water Research 44, 3214-3224.

Von der Ohe, P.C., De Deckere, E., Prüß, A., Muñoz, I., Wolfram, G., Villagrassa, M., Ginebreda, A., Hein, M., Brack, W., 2009. Towards an integrated risk assessment of the ecological and chemical status of European river basins. Integrated Environmental Assessment and Management 5(1), 50-61.

Watanabe, N., Bergamaschi, B.A., Loftin, K.A., Meyer, M.T., Harter, T., 2010. Use and environmental occurrence of antibiotics in freestall dairy farms with manured forage fields. Environmental Science and Technology 44 (17), 6591-6600.

Yang, L.H., Ying G.G., Su, H.G., Stauber, J.L., Adams, M.S., Binet, M.T., 2008. Growth-inhibiting effects of 12 antibacterial agents and their mixtures on the freshwater microalga pseudokirchneriella subcapitata. Environmental Toxicology and Chemistry 27(5), 1201-1208.

FIGURE CAPTIONS

Figure 1. Relationships between levels of NO-DCF (a) and NO_2-DCF (b) detected in WWE with NO-N levels generated after WW treatment calculated as the sum of NO_2^--N and NO_3^--N measured in WWE; and relationship between levels of NO-DCF and NO_2-DCF detected in WWE(c).

Figure 2. Synergistic/antagonistic effects of: **(a)** a mixture of DCF and SMX on the inhibition tests with *D. magna* **(b)** binary mixtures of DCF with NPL, TCS, MLT, DRN and GPT on the inhibition tests with *D. Magna*; **(c)** combinations of DCF, SMX and NO-DCF on the inhibition tests with *V. Fischeri.;* **(d)** binary mixtures of DCFwith NPL, TCS, MLT, DNR and GPT on the inhibition tests with with *V. Fischeri* and **(e)** binary mixtures of NO-DCF with SMX, NPL, TCS, MLT, DNR and GPT on the inhibition tests with with *V. Fischeri.* (NPL: Nonylphenol; TCS: Triclosan; MLT: Malathion; GPT: Glyphosate).

Table 1. Levels of target compounds determined in the ng L-1 range, in WWi and WWe from WTTPs 1-7and SW receiving WWe discharge from WTTPs 5-7

Sampling site	Matrix	Target Compound					
		DCF	NO_2-DCF	NO-DCF	SMX	NO_2-SMX	Des-SMX
WWTP1	WWi	587	<MQL	<MDL	888	<MQL	<MDL
	WWe	717	4.9	6.3	327	<MQL	<MDL
WWTP2	WWi	540	7.1	<MDL	1218	36.4	<MDL
	WWe	669	4.6	1.8	425	<MQL	11.4
WWTP3	WWi	545	<MDL	<MDL	795	<MDL	<MDL
	WWe	603	3.6	7.8	320	<MQL	<MDL
WWTP4	WWi	476	<MDL	<MDL	701	<MDL	<MDL
	WWe	562	4.0	1.1	243	<MDL	<MDL
WWTP5	WWi	572	<MQL	<MDL	894	<MDL	<MDL
	WWe	999	<MDL	6.2	534	<MDL	<MDL
WWTP6	WWi	575	4.75	8.64	790	<MDL	<MDL
	WWe	659	<MDL	3.55	292	9.65	<MDL
WWTP7	WWi	454	<MDL	<MDL	420	<MQL	<MDL
	WWe	475	<MDL	2.47	115	<MDL	<MDL
SW5	SW	847	<MQL	5.6	173	<MDL	8.0
SW6	SW	711	2.6	15.9	79	<MDL	17.7
SW7	SW	235	<MDL	0.8	48	<MDL	<MDL

<MQL: below the method quantification limit; <MDL: below the method detection limit.

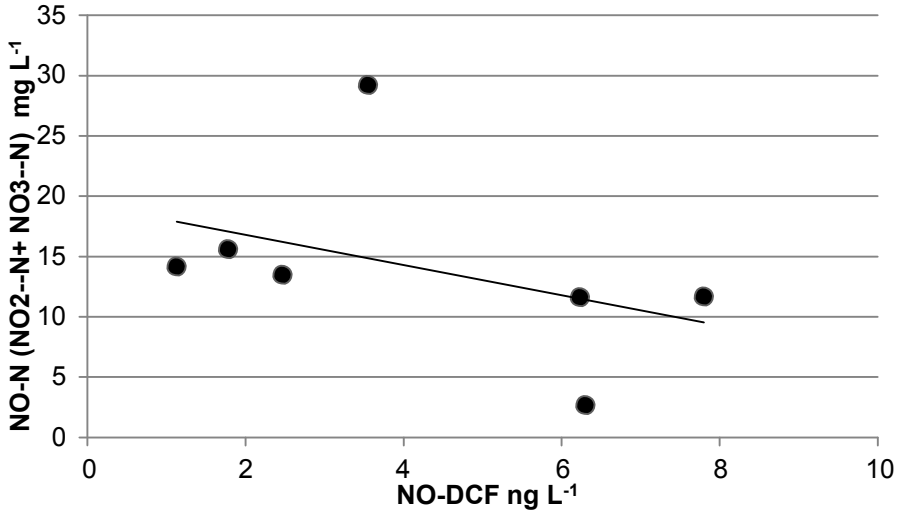
Table 2. Toxicity of DCF, SMX and their TPs to *D.magna* and *V.fischeri.*

Compound	D. *magna*				V. *fischeri*			
	NOEC (mgL^{-1})	LOEC (mgL^{-1})	EC_{20} (mgL^{-1})	EC_{50} (mgL^{-1})	NOEC (mgL^{-1})	LOEC (mgL^{-1})	EC_{20} (mgL^{-1})	EC_{50} (mgL^{-1})
DCF	5.0	24.9	38.2	53.9	5.0	7.1	10.7	22.9
NO_2-DCF	20.0	73.4	80.3	86.7	0.01	1.8	4.9	11.7
NO-DCF	≥100	>100	>100	>100	15.0	45.1	66.0	>100
SMX	0.5	20.2	41.8	75.3	10.0	48.4	109	>100
Des-SMX	≥100	>100	>100	>100	1.0	62.5	76.9	89.3
NO_2-SMX	na	na	na	na	5.0	5.0	14.8	41.4

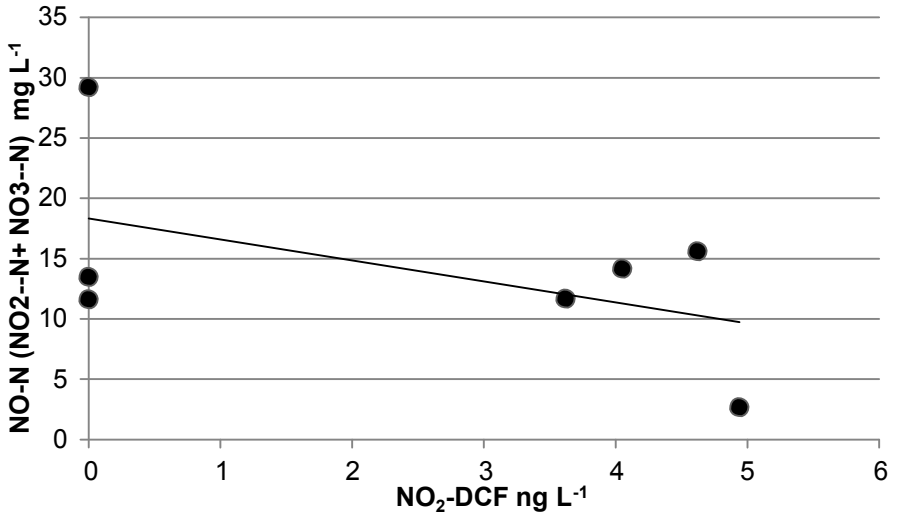
na: not assessed

Figure 1

(a)


NO-N (NO2--N+ NO3--N) mg L-1
NO-DCF ng L-1
0
5
10
15
20
25
30
35
0
2
4
6
8
10

(b)


NO-N (NO2--N+ NO3--N) mg L-1
NO2-DCF ng L-1
0
5
10
15
20
25
30
35
0
1
2
3
4
5
6

(c)

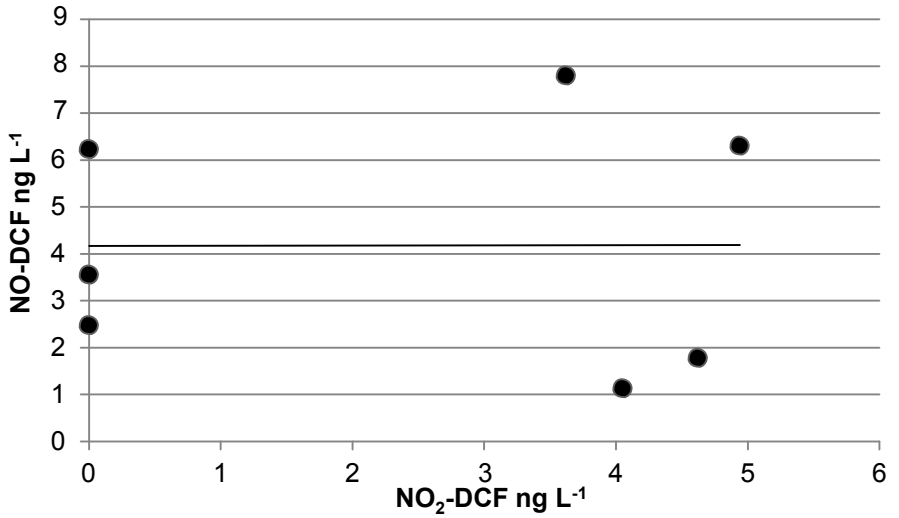
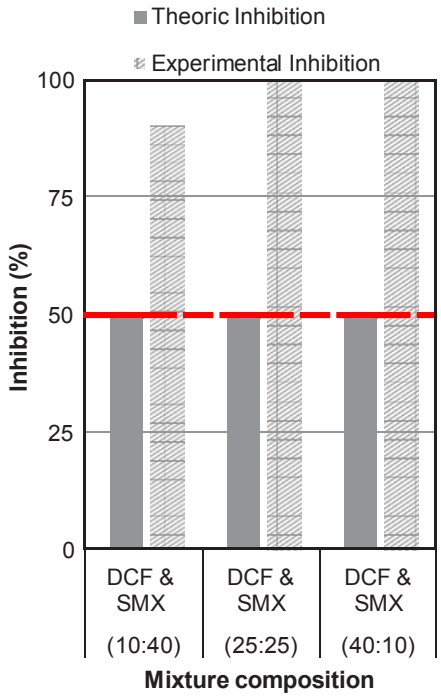
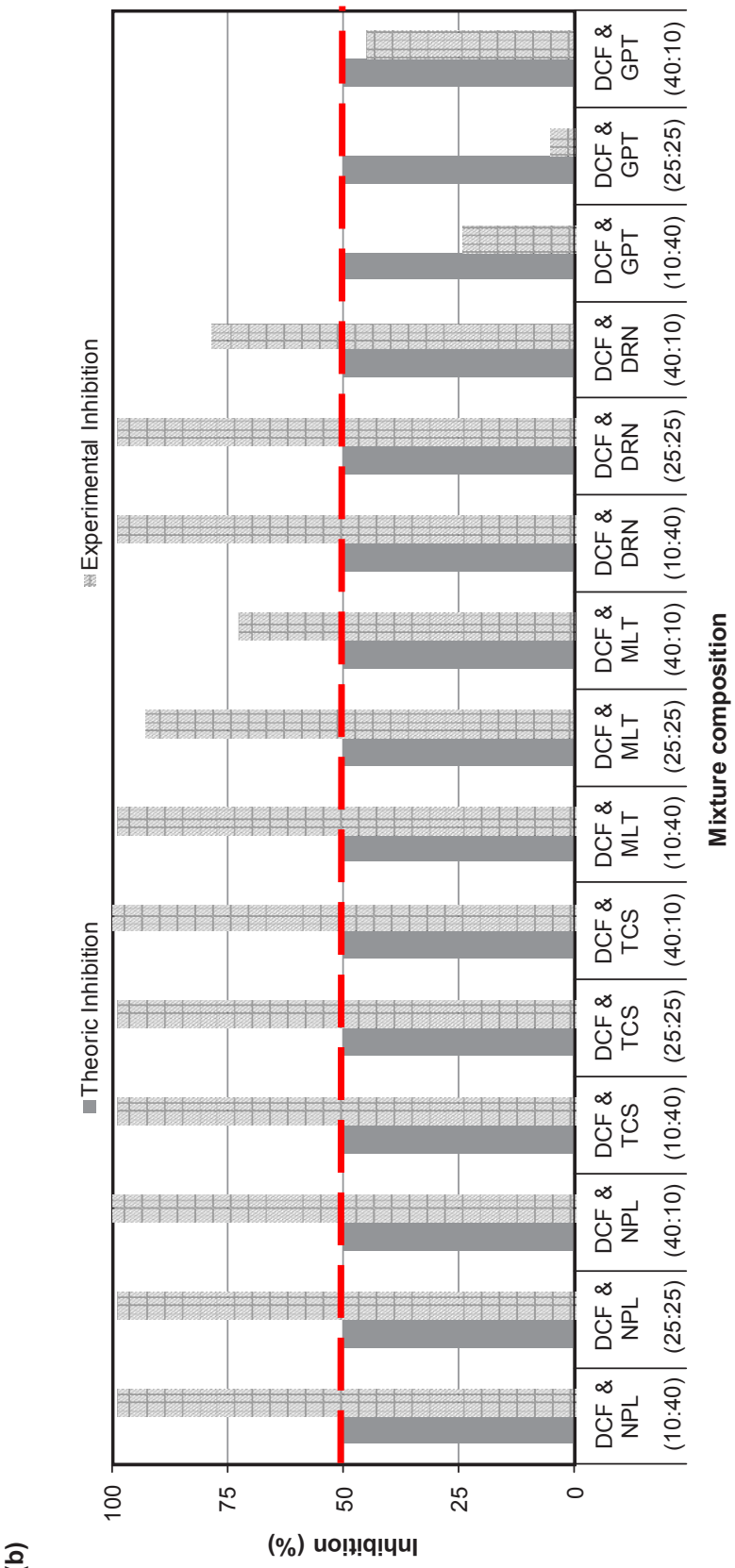
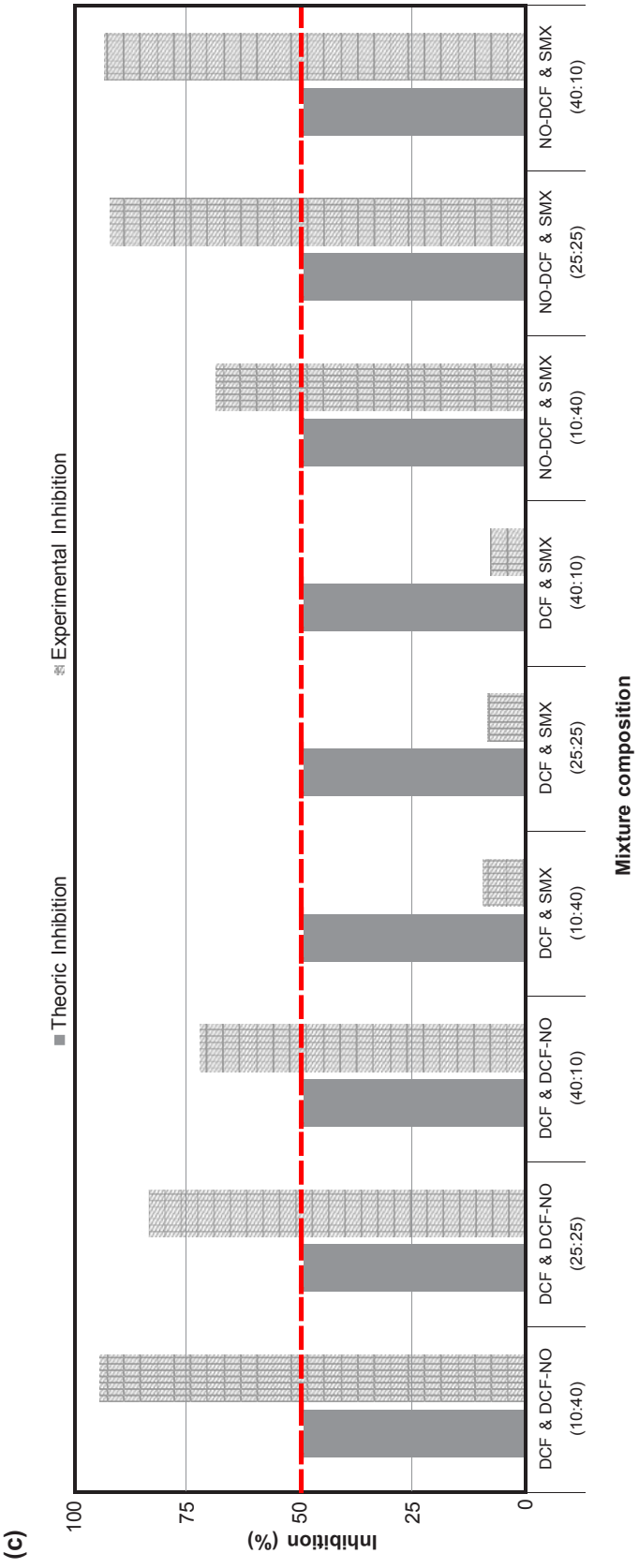

NO-DCF ng L-1
NO2-DCF ng L-1
0
1
2
3
4
5
6
7
8
9
0
1
2
3
4
5
6

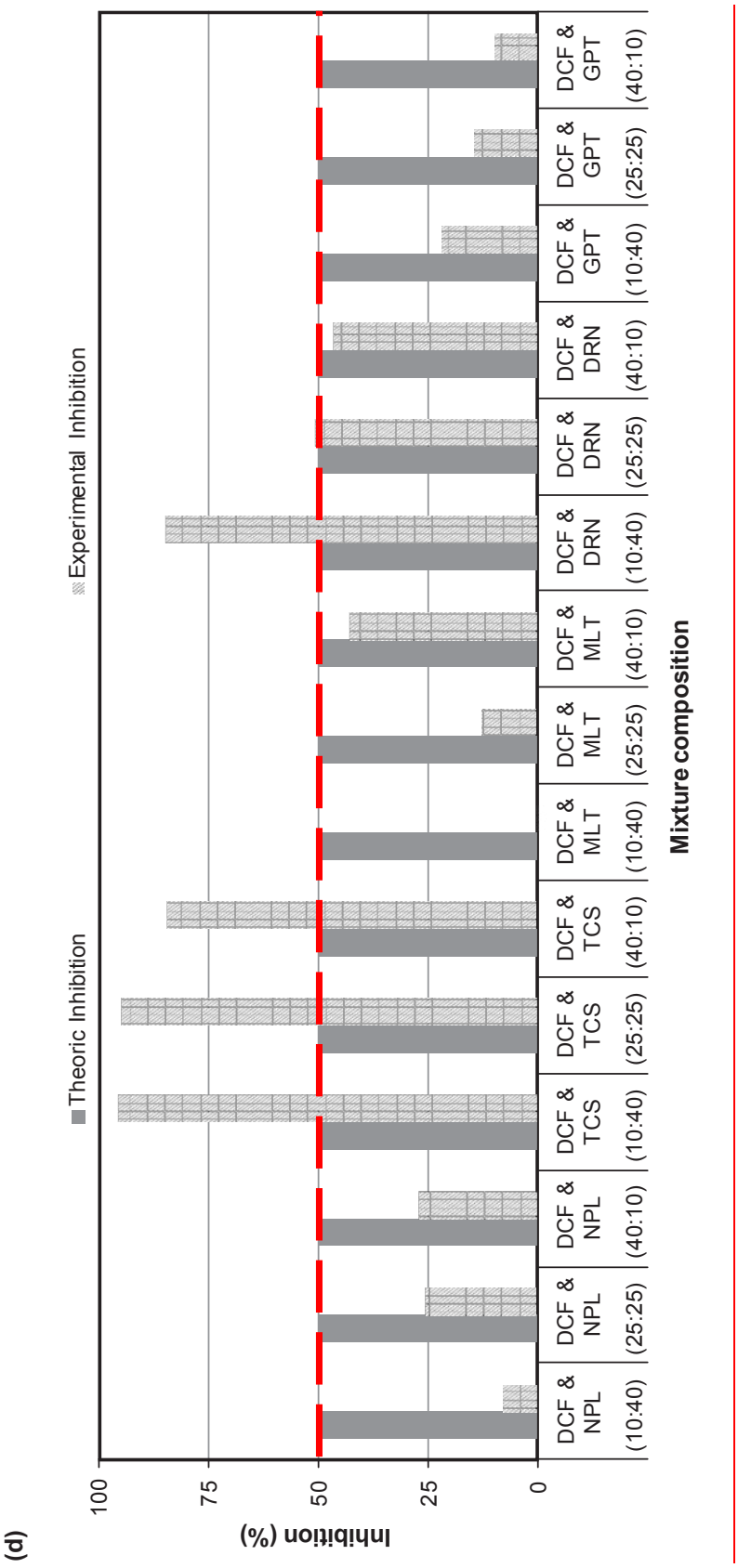
Figure 2.

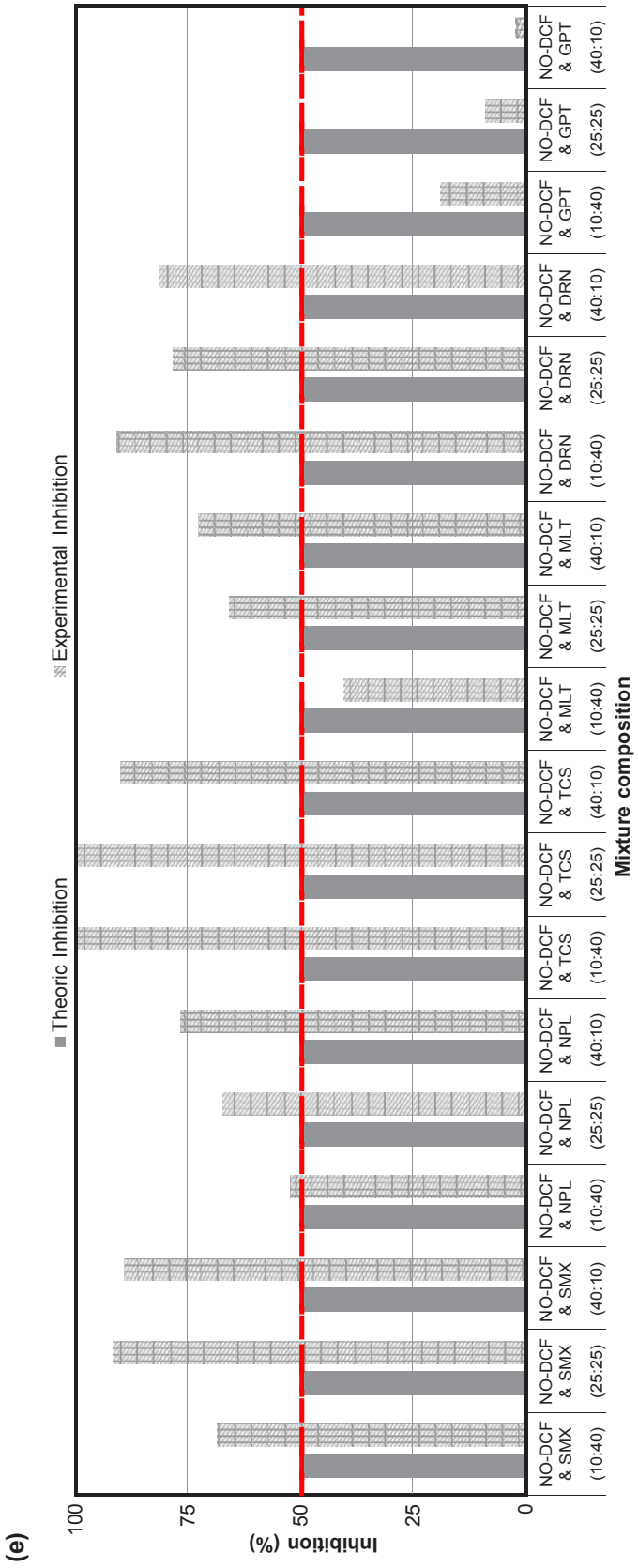
(a)


Theoric Inhibition
Experimental Inhibition
100
75
50
25
0
Inhibition (%)
DCF & SMX (10:40)
DCF & SMX (25:25)
DCF & SMX (40:10)
Mixture composition


(b)
Inhibition (%)
100
75
50
25
0
Theoric Inhibition
Experimental Inhibition
DCF & NPL (10:40)
DCF & NPL (25:25)
DCF & NPL (40:10)
DCF & TCS (10:40)
DCF & TCS (25:25)
DCF & TCS (40:10)
DCF & MLT (10:40)
DCF & MLT (25:25)
DCF & MLT (40:10)
DCF & DRN (10:40)
DCF & DRN (25:25)
DCF & DRN (40:10)
DCF & GPT (10:40)
DCF & GPT (25:25)
DCF & GPT (40:10)
Mixture composition


(c)
Theoric Inhibition
Experimental Inhibition
Inhibition (%)
100
75
50
25
0
DCF & DCF-NO (10:40)
DCF & DCF-NO (25:25)
DCF & DCF-NO (40:10)
DCF & SMX (10:40)
DCF & SMX (25:25)
DCF & SMX (40:10)
NO-DCF & SMX (10:40)
NO-DCF & SMX (25:25)
NO-DCF & SMX (40:10)
Mixture composition


(d)
Theoric Inhibition
Experimental Inhibition
Inhibition (%)
100
75
50
25
0
DCF & NPL (10:40)
DCF & NPL (25:25)
DCF & NPL (40:10)
DCF & TCS (10:40)
DCF & TCS (25:25)
DCF & TCS (40:10)
DCF & MLT (10:40)
DCF & MLT (25:25)
DCF & MLT (40:10)
DCF & DRN (10:40)
DCF & DRN (25:25)
DCF & DRN (40:10)
DCF & GPT (10:40)
DCF & GPT (25:25)
DCF & GPT (40:10)
Mixture composition


(e)
Theoric Inhibition
Experimental Inhibition
Inhibition (%)
0
25
50
75
100
Mixture composition
NO-DCF & SMX (10:40)
NO-DCF & SMX (25:25)
NO-DCF & SMX (40:10)
NO-DCF & NPL (10:40)
NO-DCF & NPL (25:25)
NO-DCF & NPL (40:10)
NO-DCF & TCS (10:40)
NO-DCF & TCS (25:25)
NO-DCF & TCS (40:10)
NO-DCF & MLT (10:40)
NO-DCF & MLT (25:25)
NO-DCF & MLT (40:10)
NO-DCF & DRN (10:40)
NO-DCF & DRN (25:25)
NO-DCF & DRN (40:10)
NO-DCF & GPT (10:40)
NO-DCF & GPT (25:25)
NO-DCF & GPT (40:10)

SUPPLEMENTARY MATERIAL

Investigating the formation and toxicity of nitrogen transformation products of diclofenac and sulfamethoxazole in wastewater treatment plants

Victoria Osorio[1], Josep Sanchís[1], Jose Luís Abad[2], Antoni Ginebreda[1], Marinella Farré[1], Sandra Pérez[1]*, Damià Barceló[1,3]

[1]Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain

[2] RUBAM-IIQAC-CSIC, Jordi Girona 18-26, Barcelona, Spain

[3]Catalan Institute for Water Research (ICRA), Scientific and Technological Park of the University of Girona, Emili Grahit 101, Girona, Spain

*Corresponding author:

Sandra Pérez Solsona

IDAEA-CSIC

Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Jordi Girona 18-26, 08034 Barcelona, Spain

Jordi Girona 18-26

Barcelona 08034, Spain

E-mail: spsqam@idaea.csic.es

Appendix A

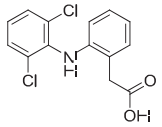
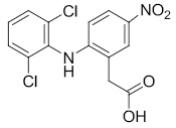
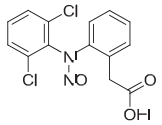
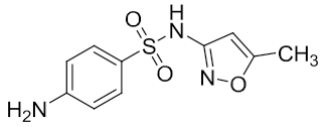
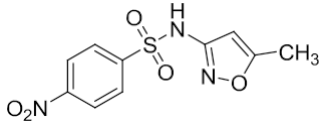
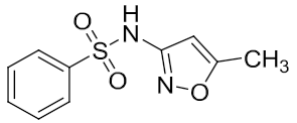
Synthesis & characterization of standards:

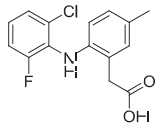
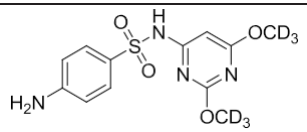
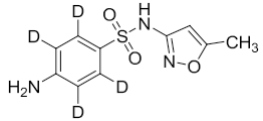
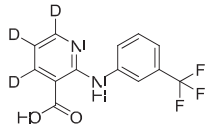
Nitro derivates of DCF were synthesized following reported synthesis pathways for similar structures and used as standards for method optimization and quantitative analysis as described by Osorio et al. (2014). The identity and purity of the TPs were confirmed by ^{1}H-NMR and ^{13}C-RMN spectroscopy and by accurate mass measurements obtained from UPLC/ESI- (LTQ-Orbitrap XL) High Resolution MS/MS analysis of target compounds, carried out in full-scan and product ion scan mode Osorio et al. (2014). The transformation product 4-nitro-N-(5-methylisoxazol-3-yl)-benzenesulfonamide (4-Nitro Sulfamethoxazole) was synthesized as described by Rieder et al. (1988). The transformation product N-(5-methylisoxazol-3-yl)-benzenesulfonamide (Desamino Sulfamethoxazole) was shynthetyzed applying the modifications to the previously mentioned methodology, proposed by Nödler et al. (2012).

Analysis of nitrogen species

NO_3^- and NO_2^- were measured by colorimetric spectroscopy. Nitrites reacted with sulfanilamide and *N*-(1-naphthyl)ethylenediamin in acid conditions to give a pink coloured diazo complex.Colorimetric measures were performed at 520-540 nm. NO_3^- were analysed as NO_2^- by reduction into NO_2^- in a copper/cadmium column. NH^{4+} levels were analysed by fluorescence spectroscopy with a detector equipped with a 360 nm black fluorescence lamp, a 370 nm excitation filter and a long pass emission filter. The method was based on the reaction of ammonia with orthophtaldialdehyde and sulfite. The limits of detection of the analytical method were 3.84 µg L^{-1}, 4.83µg L^{-1} and 1.18 µg L^{-1} for NH^{4+}, NO_3^- and NO_2^-, respectively.

Table A.1. Target compounds and their physico-chemical properties.

Target Compound	Class	Molecular structure	Molecular formula	Molecular weight	pKa[e]	log k_{ow}[e]	log D_{ow} (pH 8.0)[e]
Diclofenac[a] (DCF)	Antiinflammatory drug		$C_{14}H_{11}Cl_2NO_2$	296.1486	4.00	4.26	0.85
NO_2-DCF (TP339)[b]	Transformation product (nitrification)		$C_{14}H_{10}Cl_2N_2O_3$	341.1462	3.42	4.20	0.71
NO-DCF (TP323)[b]	Transformation product -(nitrification)		$C_{10}H_{10}Cl_2N_2O_3$	325.1468	3.52	4.20	0.72
Sulfamethoxazole[a] (SMX)	Sulfonamide bacteriostatic antibiotic drug		$C_{10}H_{11}N_3O_3S$	253.2776	1.97 6.16	0.79	-0.11
Nitro-Sulfamethoxazole (NO_2-SMX)	Bio-Transformation (denitrification)		$C_{10}H_9N_3O_5S$	283.2606	5.70	1.56	0.63
Desamino-Sulfamethoxazole (Des-SMX)	Bio-Transformation product (denitrification)		$C_{10}H_{10}N_2O_3S$	238.2630	5.84	1.62	0.7
Surrogate							

Lumiracoxib[c] (LMX)	Antiinflammatory drug (withdrawn)		$C_{15}H_{13}ClFNO_2$	293.7206	4.11	4.31	0.94
Sulfadimethoxine-d_6[a] (SDM-d_6)	(Isotopically labelled compound)		$C_{12}H_8D_6N_4O_4S$	316.3659	1.95	1.26	0.52
Internal Standard							
Sulfamethoxazole-d_4[d] (SMX-d_4)	(isotopically labelled compound)		$C_{10}H_7D_4N_3O_3S$	257.3023	1.97 6.16	0.79	-0.11
Niflumic acid d5[de] (NFA-d_5)	(isotopically labelled compound)		$C_{13}H_4D_5F_3N_2O_2$	287.2365	1.88	4.63	1.23

[a]Sigma Aldrich; [b] Pérez and Barceló, 2008; Osorio et al., 2014;[c] Toronto Research Chemicals; [d]Dr, Ehrenstorfer (Augsburg, Germany);[e] Santa Cru Biotechnology; [e] estimated with ChemAxon (www.chemaxon.com)

Table A.2.

WWTP Code	WWTP location	Treatment type	Tertiary treatment applied	Flow treated $m^3\ day^{-1}$	h-e treated equivalent population	WW type	BOD_5 [mgO_2/L]	COD [mgO_2/L]	HRT [days]	SRT [days]	N-removal control (NH_4^+/NO_3^-)
WWTP1	TEIÀ - MARESME SUD	biologic	NO	22.500	131.250	I	280.00	nd	nd	nd	nd
						E	15.00	nd			
WWTP2	MATARÓ	biologic	NO	57.000	451.250	I	640.00	950.00	0.29	7.80	YES
						E	16.00	70.00			
WWTP3	GRANOLLERS	biologic with N and P removal	NO	30.000	121.500	I	290.50	nd	0.22	nd	nd
						E	5.00	nd			
WWTP4	PRAT DE LLOBREGAT	biologic with N and P removal	YES	420.000	2.275.000	I	233.00	561.00	0.33-0.42	26.00	YES
						E	2.00	24.00			
WWTP5	TERRASSA	biologic with N and P removal	NO	75.000	400.000	I	400.00	717.00	0.51	11.20	YES
						E	nd	nd			
WWTP6	RUBÍ	biologic with N removal	NO	27.000	135.000	I	260.00	398.00	0.30	46.20	nd
						E	11.00	36.00			
WWTP7	SANT FELIU DE LLOBREGAT	biologic with N and P removal	YES	64.000	373.333	I	214.50	525.00	0.32	10.00	nd
						E	3.50	69			

h-e: inhabitants equivalent

I: wastewater influent

E: wastewater effluent

BOD5: Biological Oxigen Demand

CO: Chemical Oxigen Demand

Table A.3. Optimized QqLIT-MS/MS Parameters by SRM negative and positive ionization (*precursor ion in the (-)-ESI corresponds to the deprotonated molecule, while in (+)-ESI corresponds to the protonated molecule. In case of TP323, precursor ion corresponds to* $[M\text{-}NO+H]^+$).

Compound	Rt (min)	Precursor ion (*m/z*)	DP	Product ion 1	CE/CXP	Product ion 2 (*m/z*)	CE/CXP	SRM ratio (SRM1/SRM2)
				Analyzed in negative mode				
DCF[(1)]	5.27	294	30	214	30/11	250	16/13	17
TP339[(1)]	5.55	339	25	259	32/20	295	19/15	30
LMX (Surr)[(1)]	5.29	292	35	212	32/15	248	16/11	8.7
NFA-d_5(IS 1)	6.01	286	45	242	28/13	-	-	-
				Analyzed in positive mode				
SMX[(1)]	4.78	254	71	156	23/12	92	43/16	1.2
Des-SMX[(1)]	5.40	239	71	77	53/12	131	25/10	2.1
4-NO_2 -SMX	5.92	284	76	189	35/14	75	67/12	1.3
TP323[(2)]	6.28	295	71	242	27/14	214	39/16	1.3
SDM-d_6(Surr)[(2)]	5.17	317	61	162	33/8	92	47/14	1.02
SMX-*d4* (IS 1)	4.77	258	66	160	23/12	-	-	-
NFA-d_5(IS 2)	6.97	288	61	270	35/16	-	-	-

(Surr: surrogate SPE control standard; IS 1: internal standard ; IS 2: internal standard 2 and numbers in superscript indicate which internal standard was used for ion suppression correction).

Table A.4. Method performance parameters: linearity (linear correlation coefficients, r^2), recoveries (RSD), method detection and quantification limits in WWi, WWe and SW.

Compound	linearity (r^2)	IDL (pg injected)	% recovery (RSD)			MDL (ng L^{-1})			MQL (ng L^{-1})		
			WWi	WWe	SW	WWi	WWe	SW	WWi	WWe	SW
DCF	0.9997	0.3	84 (±2)	90 (±10)	60 (±6)	0.6	0.1	0.05	2.2	0.2	0.2
NO_2-DCF	1.0000	1.0	32 (±4)	60 (±5)	73 (±9)	1.2	0.3	0.6	4.1	0.9	2.1
NO-DCF	0.9998	0.2	93 (±9)	101 (±2)	74(±4)	0.4	0.1	0.1	1.5	0.5	0.2
SMX	0.9999	0.1	103 (±14)	94 (±6)	138 (±5)	0.8	0.4	0.1	2.7	1.4	0.2
Des -SMX	0.9999	1.8	112 (±1)	127 (±4)	94(±18)	7.4	1.9	0.8	25	6.4	2.5
NO_2- SMX	1.0000	2.0	112 (±4)	100 (±6)	151 (±17)	8.3	2.3	1.2	28	7.7	3.8
LMX (Surr)	0.9998	0.9	90 (±7)	81 (±5)	73 (±8)	0.8	0.4	0.3	2.6	1.3	0.9
SDM-*d6* (Surr)	0.9998	0.6	111 (±34)	118 (±3)	135 (±10)	0.4	0.2	0.1	1.2	0.6	0.3

Table A.5. Concentration of N-species: NH_4^+-N, NO_3^--N, and NO_2^--N range in WWi and WWe from seven WWTPs and WWe receiving SW from WWTPs 5-7.

(a)

Sample	Matrix	N-species (mgL^{-1})		
		NH_4^+-N	NO_3^--N	NO_2^--N
WWTP1	WWi	62.3	0.01	0.02
	WWe	70.5	2.1	0.56
WWTP2	WWi	69.1	0.02	0.01
	WWe	43.5	10.9	4.6
WWTP3	WWi	51.4	0.02	0.01
	WWe	0.8	10.7	1.01
WWTP4	WWi	51.9	0.01	0.01
	WWe	15.9	13.5	0.62
WWTP5	WWi	53.6	0.01	0.01
	WWe	18.5	10.6	1.0
WWTP6	WWI	45.84	0.02	0.01
	WWe	44.7	27.8	1.4
WWTP7	WWi	42.61	0.03	0.01
	WWe	33.9	10.9	2.5
SW5	SW	17.9	8.4	4.6
SW6	SW	16.1	13.9	10.0
SW7	SW	9.8	9.9	0.65

Figure A.1. Map of the WWTPs and the WW effluent receiving stream/river sites studied.

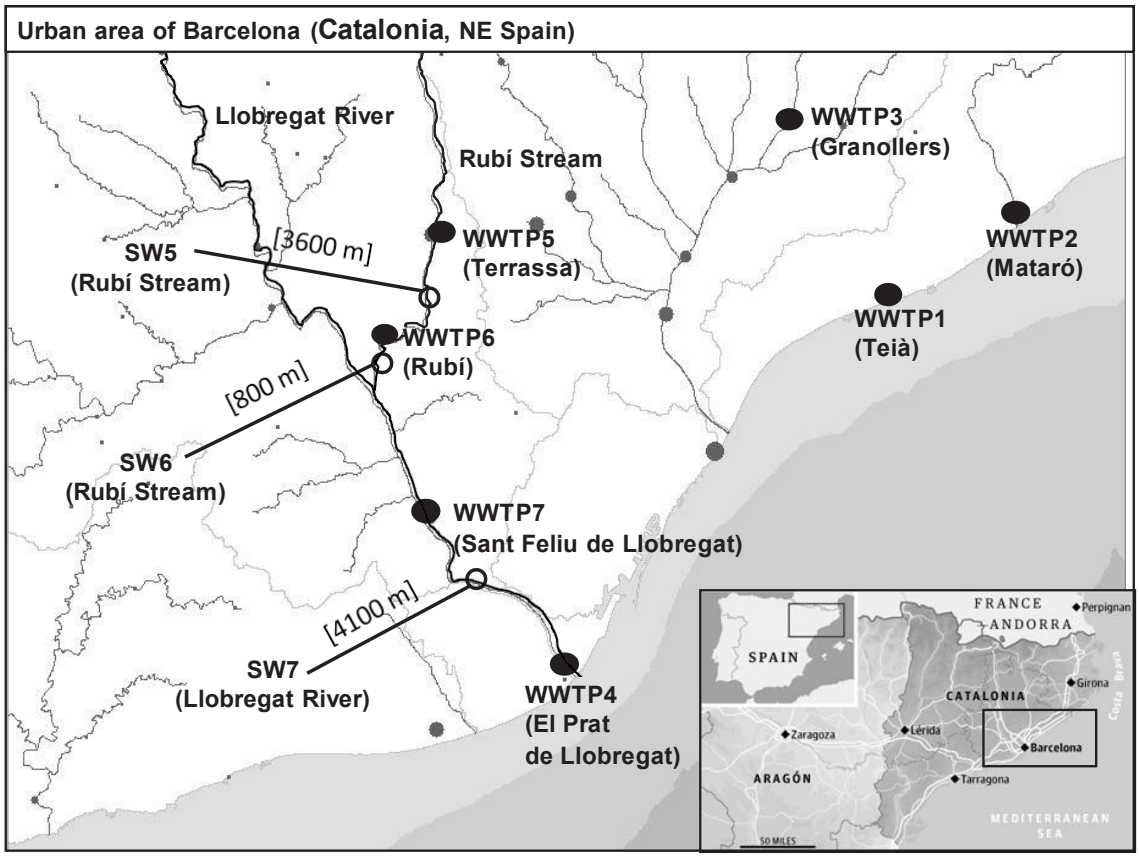
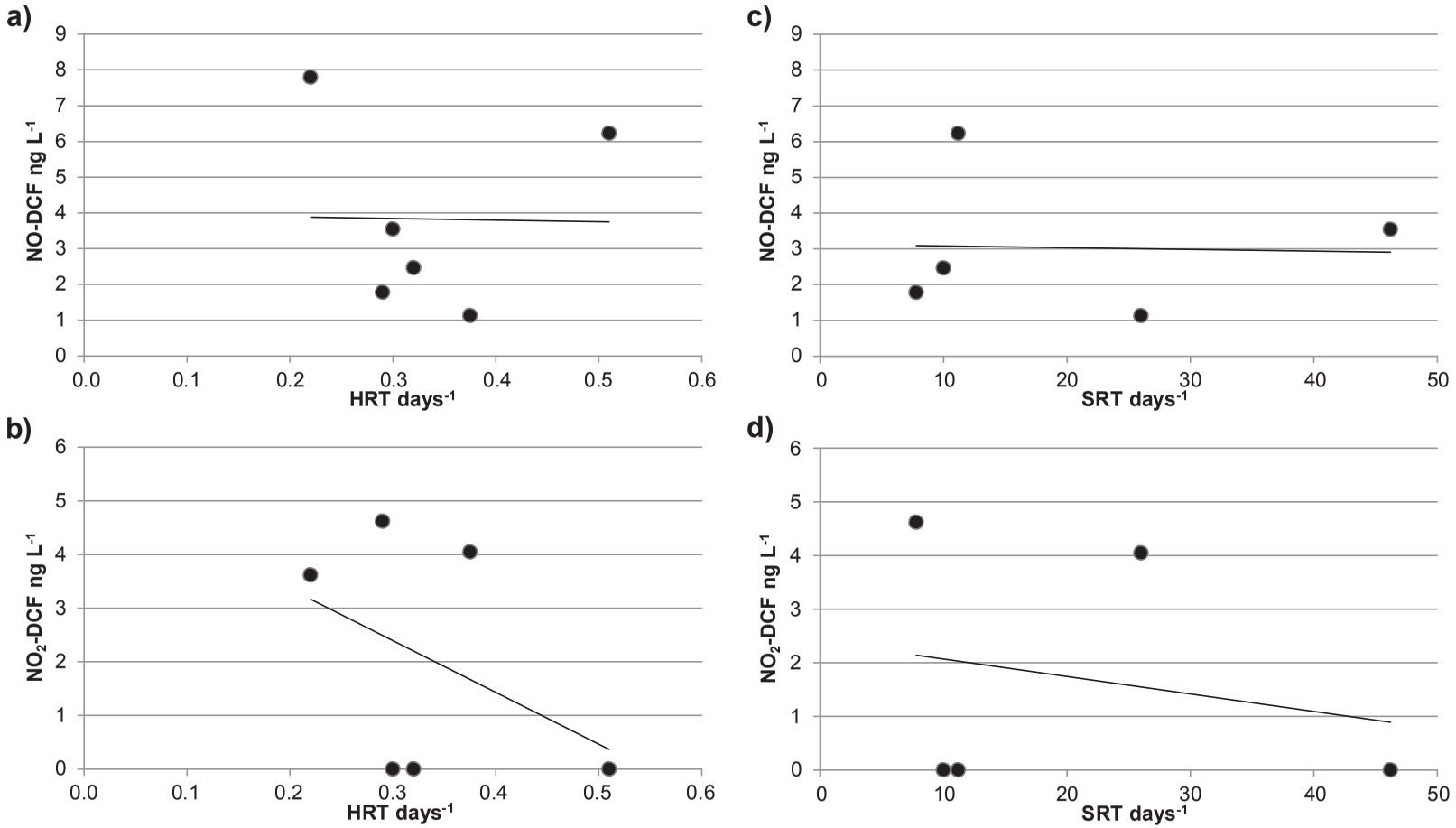


Figure A.2. Relationships between levels of NO-DCF (a and c) and NO_2-DCF (b and d) measured in WWE with SRT and HRT of the WWTPs studied.



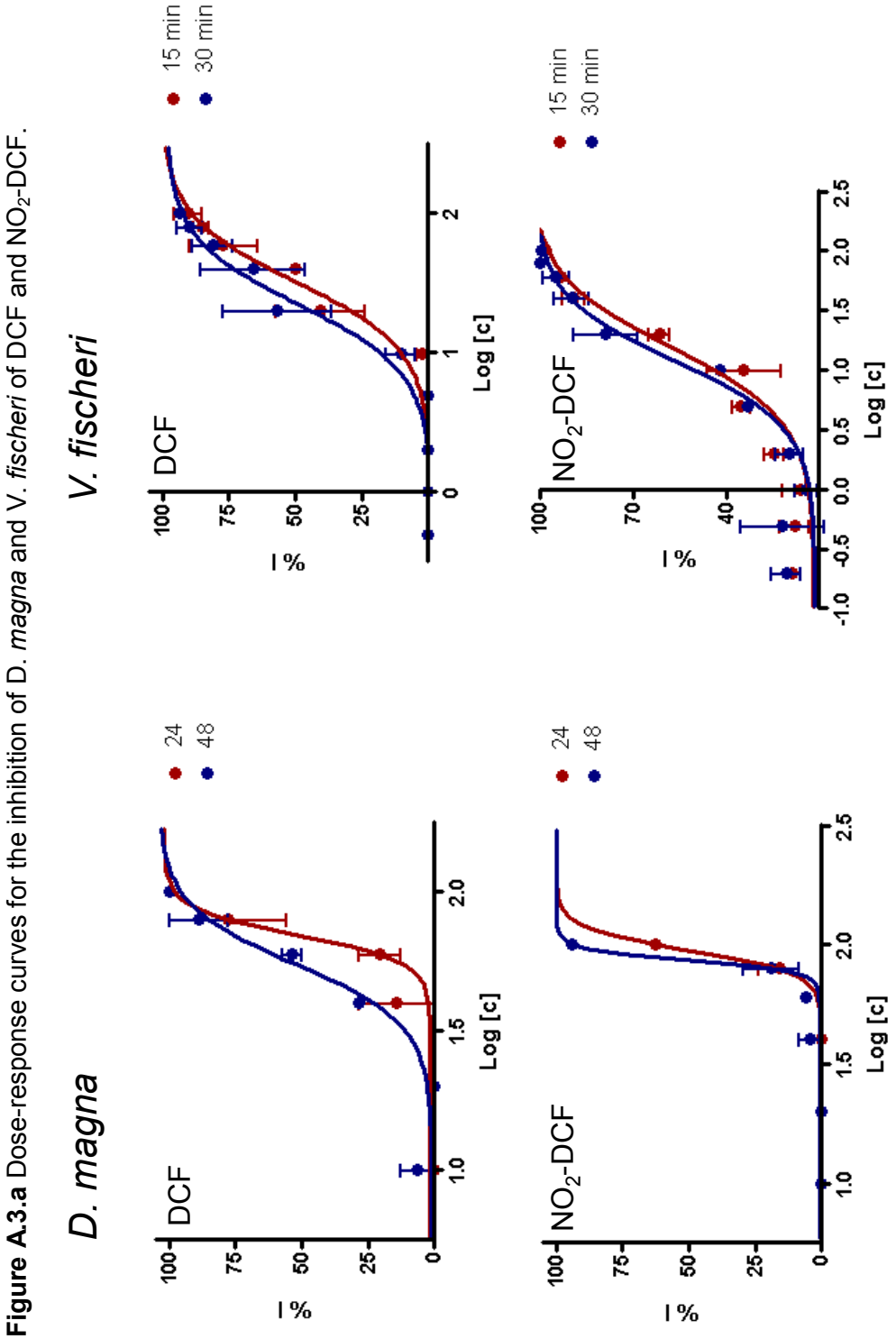


Figure A.3.a Dose-response curves for the inhibition of D. *magna* and V. *fischeri* of DCF and NO_2-DCF.

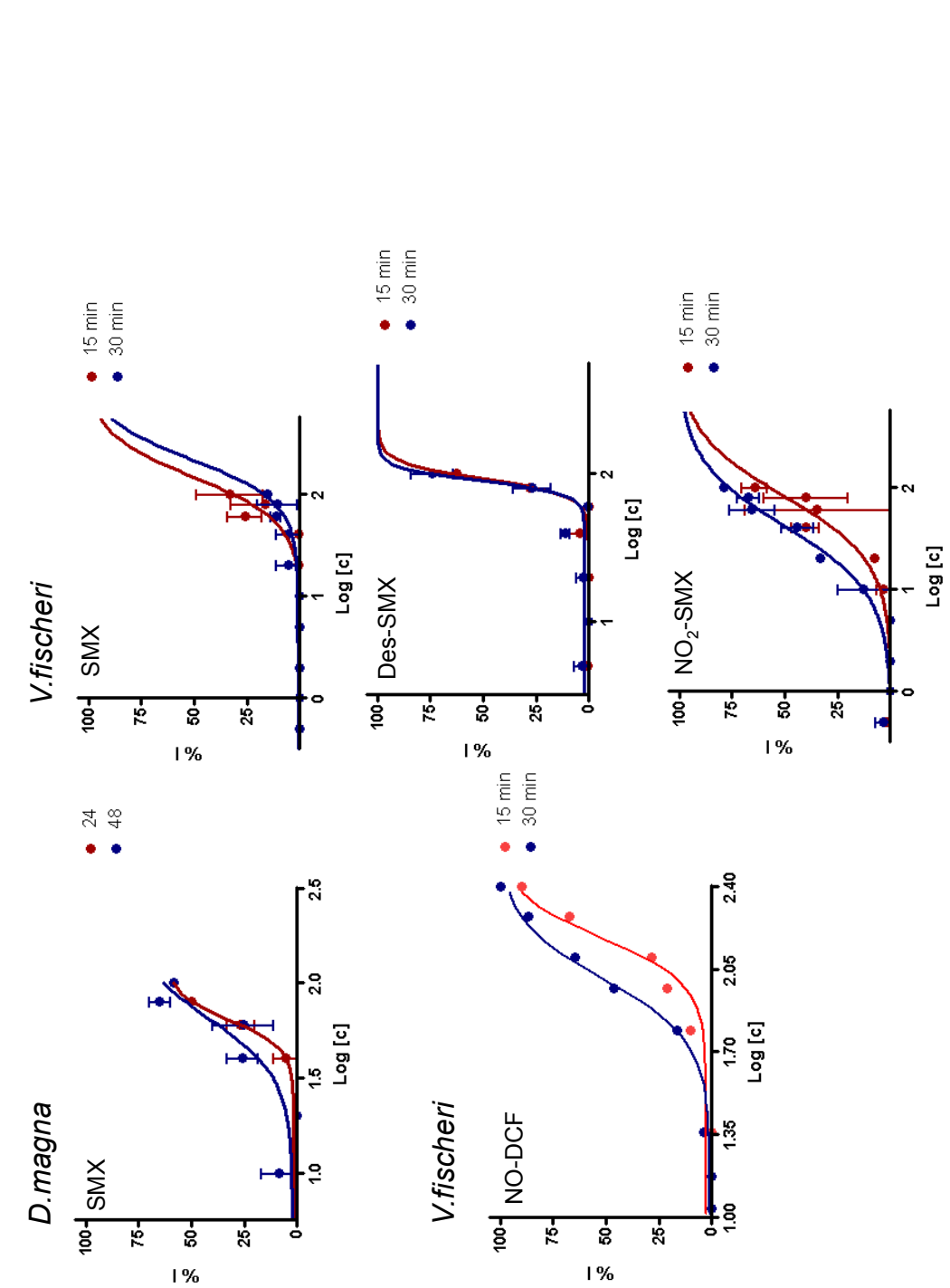


Figure A.3b. Dose-response curves for *D. magna* and *V. fischeri* of SMX, NO-DCF, Des-SMX and NO_2-SMX.

5.3. Article: "Hydrological variation modulates pharmaceutical levels and biofilm responses in a Mediterranean river"

Science of the Total Environment 472 (2014) 1052–1061

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Hydrological variation modulates pharmaceutical levels and biofilm responses in a Mediterranean river

Victoria Osorio [a], Lorenzo Proia [b], Marta Ricart [b], Sandra Pérez [a,*], Antoni Ginebreda [a], Jose Luís Cortina [c], Sergi Sabater [b,d], Damià Barceló [a,d]

[a] IDAEA-CSIC, Jordi Girona 18-26, Barcelona, Spain
[b] Institute of Aquatic Ecology, University of Girona, Girona, Spain
[c] Cetaqua, Water Technology Centre, UPC North Campus, Paseo de los Tilos, 3, Barcelona, Spain
[d] Catalan Institute for Water Research (ICRA), Scientific and Technological Park of the University of Girona, Emili Grahit 101, Girona, Spain

HIGHLIGHTS

- Effects of flow changes on pharmaceuticals (PhACs) concentration were evaluated.
- Higher PhACs levels downstream confirmed a pollution gradient along the river.
- Dilution of PhACs occurred after a flash flood event and restored within two weeks.
- Effects of PhACs on biofilms were evaluated and related to flow regime variations.
- PhAC and biofilm relationship was potentially altered after a flood event.

ARTICLE INFO

Article history:
Received 14 August 2013
Received in revised form 26 October 2013
Accepted 11 November 2013
Available online 17 December 2013

Keywords:
Pharmaceuticals
Biofilms
River
Hydrological variation

ABSTRACT

The Llobregat is a Mediterranean river that is severely impacted by anthropogenic pressures. It is characterized by high flow variability which modulates its chemical and biological status. The present work evaluates the effects of flow changes on the concentration of pharmaceutically active compounds (PhACs) and their relationship to cellular parameters of river biofilms. To this end, at two selected sampling sites at the lower course of the Llobregat river, surface water samples were collected twice a week over two hydrologically different periods exhibiting low and high river flows. Higher levels of PhACs were detected at the downstream sampling site. Irrespective of the flow regime, analgesics, anti-inflammatories and lipid regulators were the most abundant substances at both sampling sites with total concentrations of up to1000 ng/L and 550 ng/L at the upstream and downstream sites, respectively. Antibiotics (fluoroquinolones) and psychiatric treatment drugs were also detected at high levels in the second campaign achieving concentrations of up to 500 ng/L. The principal component analysis (PCA) performed with the PhACs concentrations of the two campaigns revealed differences in the various therapeutic groups depending on sampling site and period. After a flash flood event during the second sampling period, dilution of PhACs occurred, but their average concentrations measured before the flood were restored within two weeks. For the majority of compounds, PhAC concentrations displayed an inverse relationship with river discharge The effects of water containing different concentrations of PhACs on biofilm communities were evaluated and related to flow regime variations. Translocation of biofilm communities from a less to a more polluted site of the river demonstrated an increase in bacteria mortality in the translocated biofilms. After the flood, extracellular peptidase activity and chlorophyll-*a* concentration were significantly reduced, and biofilm growth rate was significantly lower.

1. Introduction

The Water Framework Directive (WFD, 2000/60/CE) establishes the basis to regulate the water bodies in Europe with the aim of conserving, protecting and improving their quality and their sustainable use. Under this Directive, all European surface water bodies are entitled to reach a good ecological and chemical status by 2015. The WFD goes far beyond the traditional concept of water quality, and launched a comprehensive water ecological status assessment based on structural communities by using biological, hydro-morphological and physical–chemical elements. Appropriate metrics for each one of these elements have been developed and applied by the member states, taking into consideration the diverse

* Corresponding author at: IDAEA-CSIC, Department of Environmental Chemistry, Jordi Girona 18-26, Barcelona 08034, Spain. Tel.: +34 93 4006100x5310.
E-mail address: spsqam@idaea.csic.es (S. Pérez).

0048-9697/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.scitotenv.2013.11.069

bio-geographical characteristics of each region. Whereas the separate characterization of each element has been more or less satisfactory, the understanding of their respective interrelations and cross-effects remains largely unknown and is still matter of research. This fact is particularly obvious in Mediterranean rivers, which are subject to highly variable hydrologic conditions giving rise to extreme events such as severe droughts and flash-floods (Gasith and Resh, 1999). The system's response (ecological and physical–chemical) to such variations is still poorly understood.

The Llobregat River is an example of a Mediterranean river subjected to heavy anthropogenic pressures (urban, industrial and agricultural), including both point and diffuse pollution, and water abstraction for human use (Sabater et al., 2012). The Llobregat suffers from high flow fluctuations associated to seasonal rainfall that may cause temporary alterations of the base flow by factors of up to 100 or more (Marcé et al., 2012). Owing to the strategic relevance of this river to the population in the watershed (ca. 3 million inhabitants, including the city of Barcelona and its metropolitan area) that make use of its waters, it is of particular interest to understand how much the local hydrology affects the dynamics of pollutants and their potential effects on organisms. The studies developed so far concerns the deployment of the WFD through the application of biological metrics based on macroinvertebrates, diatoms, macrophytes, and fish fauna (Munné et al., 2012a). However, these studies mostly refer to structural responses, and neglect the functional responses of the biota to pollutants. The benthic microbial biofilms can play an important role in freshwater ecosystems for organic matter re-mineralization and inorganic nutrient fluxes (Proia et al., 2012a). In rivers and streams, biofilms are the first to interact with dissolved substances and can integrate the effects of environmental conditions over extended periods of time. Because of this, the behavior of biofilms can be used to detect the effects of disturbances on the ecosystem and have been widely used for routine monitoring, as "early warning systems" after disturbances (Sabater et al., 2007).

Recent studies have already analyzed the effect of certain emerging contaminants on the biological communities in the Llobregat River (Muñoz et al., 2009; Damásio et al., 2010; Ricart et al., 2010). Among these, the pharmaceutically active compounds (PhACs) have been shown to pollute the water of the Llobregat River as a result of the intense and ever increasing human activities (agricultural, industrial and urban) (González et al., 2012). PhACs are a group of chemically bioactive substances and they are produced worldwide on a 100,000 t scale. In the European Union (EU), around 3000 different PhACs are used in human medicine, and many studies have revealed their presence in wastewaters, as well as in surface, ground, and drinking waters (Petrovic and Barceló, 2009). These compounds in surface waters have been reported up to levels from ng/L to μg/L (Osorio et al., 2012a,b). Their presence can be attributed to their partial removal during their treatment in Waste Water treatment Plants (WWTPs), which can be pointed out as the main source of these micro-pollutants into the river (Gros et al., 2010). Since PhACs are intrinsically bioactive compounds and are continuously supplied into surface waters, it is necessary understanding their effects on biological communities, and hence on aquatic ecosystems, resulting from long-term low-dose exposure. It is especially urgent to investigate their response under different hydrological conditions, which may affect both their concentration in the waters as well as the structure of the receiving communities.

The present study was carried out along a section of the Llobregat River which receives the outflow from several WWTPs. Interestingly, a recent research on the exposure of an Italian river to PhACs, the Po river basin, collecting intense and continuous effluent discharge as well, was considered as the worst realistic and representative Italian case scenario to estimate the level of contamination in surface water bodies (Ferrari et al., 2011). In general, the detected PhACs were found to be present in the Po surface waters at levels below 100 ng/L and WWTPs were confirmed as point sources of pollution. Previous studies performed in the Llobregat river water have already suggested that levels of PhACs could vary over time depending on the hydrological conditions (Choi at al., 2008; Kolpin et al., 2004; Tamtam et al., 2008). For instance, Tamtam et al. (2008) observed higher inputs of norfloxacin from French WWTPs discharging into the Seine River and rapid attenuation along the stream during low flow conditions, while sulfamethoxazole inputs were increased and its dissipation was slower under high flow conditions. Similarly, cimetidine was detected in U.S. streams at higher concentrations during low flow periods (Kolpin et al., 2004). On the contrary, Choi et al. (2008) observed the same compound at higher levels under high flow conditions. Therefore, the impact of changing contaminant levels and water flow conditions on the structure and function of river biofilms was analyzed in order to understand the relative influence of hydrology and PhACs on the biological communities in the river. To this aim, experiments that consisted in transferring biofilm communities from less to more polluted sites were performed in the two periods. The hypothesis to be tested was that the chemical and biological descriptors would quickly respond to the changing water flow conditions, the PhACs influencing more the biofilms under basal flow conditions than in the aftermath of a flood event. We based our prediction on the high sensitivity of biofilms to the bioactive compounds and environmental factors, and we used biofilm translocation to further emphasize its sensitivity.

2. Materials and methods

2.1. Study area

The Llobregat River basin is located in Catalonia (NE Spain), and spans from the Pyrenees to the Mediterranean Sea with a total length of 156 km covering a catchment area of 4957 km^2 (Fig. 1). Climate in the basin is Mediterranean with a strong seasonal fluctuation in temperature and rainfall, which mainly occurs in spring and autumn (Marcé et al., 2012). The mean annual bulk precipitation in the river is 3330 Mm^3 with an annual average bulk discharge of 693 Mm^3. The difference between maximum and minimum annual precipitation is higher than 550 mm (Marcé et al., 2012).

The Llobregat River, together with its two main tributaries, the River Cardener and the River Anoia (Fig. 1), constitutes an example of highly populated, impacted, and severely exploited area in the Mediterranean region. The complex hydrology of the Llobregat is mainly due to the several infrastructures for human exploitation of the river such as reservoirs, dams, weirs, connections, derivations, withdrawals and returns that are scattered all over the basin.

This human modification of the landscape is especially evident at the middle–lower part of the basin (Fig. 1), where up to 45 weirs (once every ~2 km) are distributed along the main channel. Moreover, the mining activities and salt formations located in several areas of the middle section of the river (i.e. Sallent, Fig. 1), have caused an increase in water salinity downstream exceeding water quality standards. In addition, the highest withdrawal of superficial waters for human consumption is done along the middle–lower section of the river. The tree drinking water treatment plants (DWTP) located along this area (namely, Abrera, Terrassa and Sant Joan Despí, Fig. 1) serve a large region including Barcelona. The uptake of water is so elevated that the river is nearly exhausted downstream.

The urban and industrial wastewater discharges account for 137 Mm^3/year, the 92% coming from wastewater treatment plants (WWTPs) as well as surface runoff from agricultural areas that cannot be diluted by its natural flow (0.68–6.5 m^3/s basal flow). Up to 64 WWTPs serve more than 2 million people in the Llobregat basin, accounting for 850,929 m^3 of sewage treated per day. Most of the WWTPs include biological treatment, the 20% of them with capacity to eliminate phosphorus and nitrogen, but only five of them apply some kind of tertiary treatment. Forty-eight percent of these WWTPs are located in the area studied (Fig. 1 and Table A.1).

A volume of water from the Llobregat river is treated in the DWTP located close to this river (it accounts 205 hm^3/year while

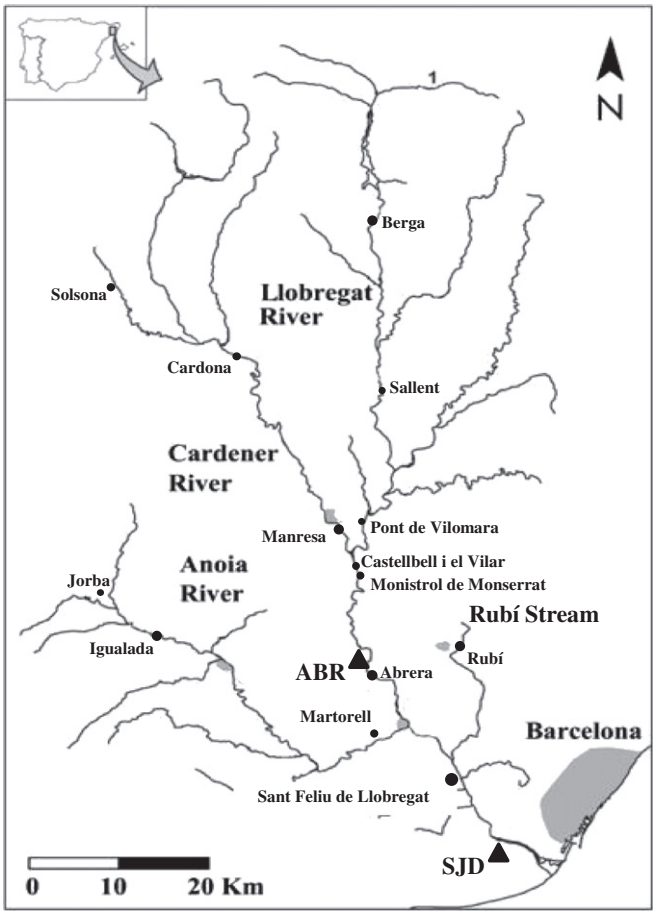


Fig. 1. Llobregat River: map of the basin indicating the sampling sites (full triangles): Abrera before junction with Anoia River (ABR), and Sant Joan Despí (SJD). Main WWTPs indicated as big full circles along the Llobregat River and its main tributaries, Anoia River, Cardener River and Rubí stream. Black stars indicated the main drinking water treatment plants located near, Abrera, Terrassa and Sant Joan Despi.

the total stream flow near the mouth is ~600 hm^3/year). Nearly 25% of the river water is used at the DWTPs located in Abrera and Sant Joan Despí (Marcé et al., 2012). Therefore, the Llobregat River basin is the main watersource to supply the Barcelona Metropolitan Area.

2.2. Hydrology measurements

Daily stream flow data from gauging stations located in the two sampling sites are available at the public website of the Catalan Water Agency (ACA), (http://www.gencat.cat/aca/) (Figure A.1).

2.3. Chemistry

2.3.1. Sampling and sample preparation

Two sampling sites were selected at the lower course of the Llobregat River. These were Abrera (ABR) and Sant Joan Despí (SJD), located 17 km apart. The first sampling point (Fig. 1) is located in a sparsely populated area in which the Llobregat River receives urban and industrial wastewater inputs. The second sampling site (SJD) is located in the greater metropolitan area of the city of Barcelona and therefore expected to be generally more polluted than ABR. According to the previously existing monitoring data from the Catalan Water Agency (ACA) and other studies (Ginebreda et al., 2010), SJD is the most polluted section of the River. Sampling was performed during the Winter/Spring season (March 3rd 2010 to April 12th 2010) and the Spring/Summer season (June 9th 2010 to July 12th 2010). River water samples were collected twice a week over the two periods (9–13 samples per campaign and monitoring site) from the bank of the river. Water samples were collected in 500 mL amber PET bottles that had been pre-rinsed several times with deionized water in the laboratory, and were rinsed three times with sample water onsite. Bottles were placed in a cooler (at 4 °C) and delivered to the laboratory within 2 h. Samples were immediately pre-treated (filtration) and stored in a refrigerator (−20 °C) until analysis within two days.

2.3.2. Analysis of pharmaceuticals

The determination of 73 PhACs belonging to different therapeutic groups (see Table A.2), in surface waters, was performed using a multi-residue analytical method based on SPE–LC–MS/MS (Gros et al., 2009). All water samples (500 mL) were filtered through 0.7-μm glass fiber filters, followed by 0.45-μm nylon membrane filters in a Millipore glass vacuum filter holder. An aqueous solution of 5% Na_2EDTA was added to achieve a final concentration of 0.1%. Within 48 h, the samples were extracted by SPE, the cartridges rinsed with 5 mL of HPLC grade water, dried under vacuum for 15–20 min. After elution with 2 × 4 mL of methanol, the extracts were evaporated to dryness under a gentle stream of nitrogen and reconstituted with 1 mL of methanol/water (1:3). For internal standard calibration, 10 μL of a 1 mg/L standard mixture of the isotopically labeled compounds was added to the final analytical sample. Instrumental analysis was performed by liquid chromatography, using a Symbiosis™ Pico (SP104.002, Spark, Holland), equipped with an auto-sampler and connected in series with a 4000 QTRAP Hybrid Triple Quadrupole–Linear Ion Trap mass spectrometer equipped with a Turbo Ion Spray source (Applied Biosystems–Sciex, Foster City, CA, USA). Target compounds were separated 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). Depending on the mode of analysis, different mobile phases were used. For the negative ionization mode a mixture of acetonitrile/methanol (1:1, v/v) (eluent A) and HPLC grade water (eluent B) at flow rate 0.2 mL/min was used. The elution gradient started with 20% eluent A, increasing to 80% in 20 min, raising to 90% in 4 min and then, back to initial conditions within 3 min. The column was re-equilibrated for 15 min before another injection with a total time for chromatographic analysis of 42 min. For analysis in positive ionization mode, acetonitrile (eluent A) and HPLC grade water with 0.1% formic acid (eluent B) were used. The elution gradient started with 5% eluent A, increasing to 95% in 25 min, raising to 100% in 5 min and then, back to initial conditions within 5 min. The column was re-equilibrated for 10 min and chromatographic analysis lasted 45 min. The sample injection volume was 20 μL in all chromatographic methods. Quantification of PhACs was carried out in Selected Reaction Monitoring (SRM) mode monitoring two transitions per analyte (see Table A.2).

2.3.3. Analysis of physicochemical parameters

Conductivity, temperature, pH and dissolved oxygen were measured with appropriate multi-parameter sensor probes (HACH LANGE GMBH, Germany) (Table 1). Water samples were collected for nutrient content measurement. All water samples were filtered (nylon membrane filters, 0.2 μm; Whatman, Maidstone, UK) prior to analysis. Soluble reactive phosphate was measured following the method of Murphy and Riley (1962). Samples for anions and cations analysis were stored frozen until analysis by ion-chromatography (761 Compact IC, METROHM, Herisau, Switzerland).

2.4. Biofilm study

2.4.1. Experimental design

A field experiment was designed to determine the biofilm responses to the PhACs mixture at ABR and SJD. Biofilms were grown on artificial substrata (unglazed glass tiles 1 cm^2) fixed on methacrylate support,

Table 1
Physicochemical parameters measured at two sampling sites: Abrera (ABR) and Sant Joan Despí (SJD). DO = Dissolved Oxygen; T = Temperature. Mean values and standard deviation (shown in italic and with parenthesis) are reported.

	Campaign 1		Campaign 2	
	ABR	SJD	ABR	SJD
Conductivity (μS cm^{-1})	1276.8 (204.1)	1299.3 (87.4)	946.0 (127.0)	1066.5 (148.8)
DO (mg L^{-1})	9.4 (2.6)	9.4 (1.7)	7.6 (2.4)	7.2 (2.3)
T (°C)	11.2 (2.6)	11.7 (3.2)	21.0 (2.0)	22.2 (0.9)
pH	8.3 (0.0)	8.5 (0.4)	8.1 (0.2)	8.2 (0.1)
P-PO_4 (mg L^{-1})	0.09 (0.04)	0.13 (0.08)	0.18 (0.06)	0.12 (0.05)
N-NO_3 (mg L^{-1})	16.5 (2.0)	16.1 (2.6)	9.5 (2.9)	9.0 (2.5)

suspended in field mesocoms (as shown in Figure A.2). These field mesocosms were placed on the river bank and received water directly and continuously from the river. Biofilms sampling before translocation was carried out at days 8 and 22. After this period, biofilms were translocated to the site with higher pollution (ABR to SJD (A ➔ S)). Translocated and control biofilm replicates were collected from the artificial substrata at days 2 and 9 after translocation (days 24 and 31 from the onset of the experiment, respectively).

2.4.2. Biofilm metrics

2.4.2.1. Bacterial cell viability. Live and dead bacteria, identified as intact cells and membrane-compromised cells respectively, were stained using the LIVE/DEAD® Bacteria Viability Kit L7012 (BacLight™, Molecular Probes, Invitrogen L7012). Colonized glass substrata were sonicated (<60 s, sonication bath at 40 W and 40 kHz, Selecta) and scraped (sterile silicone cell scrapper, Nunc) to obtain a biofilm suspension. Samples were then diluted with pre filtered-sterilized water from the mesocosms, and 2 mL subsamples were incubated with 3 μL of 1:1 mixture of SYTO 9 and propidium iodide, for 15 to 30 min in the dark. At the end of the incubation, samples were filtered through a 0.2 μm black polycarbonate filters (Nuclepore, Whatman). Filters were then dried, placed on a slide with mounting oil (Molecular Probes) and counted by epifluorescence microscopy (Nikon E600, 1000× in immersion oil). Green and red (live and dead, respectively) bacteria cells were counted in 20 random fields per filter.

2.4.2.2. Extracellular enzyme activities. The extracellular activities of the enzymes leucine-aminopeptidase (EC 3.4.11.1), alkaline phosphatase (EC 3.1.3.1-2) and β-D-1,4-glucosidase (EC 3.2.1.21) in the biofilms were measured by fluorescence spectrometry immediately after collection, by using the fluorescent-linked substrates L-leucine-4-methyl-7-coumarinylamide (Leu-AMC, Sigma-Aldrich), 4-methylumbelliferyl-phosphate (MUF-P, Sigma-Aldrich) and 4-methylumbelliferyl β-D-glucopyranoside (4-MUF β-D-glucoside, Sigma-Aldrich), as described by Proia et al. (2012b).

2.4.2.3. Chlorophyll-a density. On each sampling day, one glass slide was collected and the chlorophyll-*a* was extracted with 90% acetone for 12 h. Sonication during 2 min (40 W power, 40 kHz frequency, SELECTA, Spain) improved the pigment extraction. The chlorophyll-*a* concentration was measured by spectrophotometric measurements (UV 1800 Shimadzu) following the method of Jeffrey and Humphrey (1975).

2.4.2.4. In vivo chlorophyll fluorescence measurements. The chlorophyll fluorescence emission was measured with the PhytoPAM (Pulse Amplitude Modulated) fluorometer (Heinz Walz GmbH), which uses a set of light-emitting diodes that excite chlorophyll using four different wavelengths (470, 520, 645, and 665 nm). For each glass slide sampled from each glass jar, three fluorescence measurements were performed in order to represent small scale heterogeneity of biofilm. All the measurements were based on the procedure described by Serra et al. (2009). The photosynthetic efficiency (Yeff) and capacity (Ymax) of PSII were measured based on the fluorescence signal recorded at 665 nm and given as relative units of fluorescence. The minimum fluorescence level of the dark adapted samples was used as an estimation of autotrophic biomass. This estimation was based on the fluorescence recorded at the four different excitation wavelengths (F1 at 470 nm, F2 at 520 nm, F3 at 645 nm, and F4 at 665 nm). F1 is linked to the green algae, whereas F2 is mostly related to that of diatoms. The F3 signal is related to cyanobacteria and the F4 signal is related to the whole algal community (Ricart et al., 2010).

2.4.2.5. Statistical analysis. Multivariate Principal Component Analysis (PCA) was applied to the two different dataset (PhAC concentrations and biofilm metrics) in order to explore the variability of biological and chemical variables in the different conditions and sites. The PhACs dataset was previously log10(x + 1) transformed, while biological data were pre square-root transformed. All the multivariate analyses were performed using the CANOCO software version 4.5 (ter Braak and Smilauer, 1998).

The biofilm changes between the two campaigns were analyzed by a one way analysis of variance (ANOVA) with repeated measures, using the different metrics at each sampling site and setting the sampling campaign as the fixed factor. Differences in the measured biofilm descriptors and responses to translocations were also tested daily using one way analysis of variance (ANOVA), in which the sampling site was set as the fixed factor. Effects of translocation were also analyzed using a post-hoc Tukey's b test. The relation between biological metrics and PhACs concentrations (therapeutic groups) was analyzed using Spearman correlation test. For all these analysis statistical significance was set at $p = 0.05$ and analyses were performed using SPSS Version 15.0.

3. Results

3.1. Hydrological variability

The first sampling campaign (autumn–spring) was characterized by steady flow conditions, with mean flows of 25.0 ± 10 m^3 s^{-1} in ABR and 26.0 ± 6.6 m^3 s^{-1} in SJD (Figure S-1). Higher water flows characterized the second sampling campaign (spring–summer), with mean flows of 50.0 ± 64.7 m^3 s^{-1} in ABR and 34.0 ± 29.9 m^3 s^{-1} in SJD, and that at day 9 registered a strong flood event (peak flow of 215.1 m^3 s^{-1} in ABR and 111.7 m^3 s^{-1} in SJD).

3.2. Occurrence of pharmaceuticals and response to hydrological variations

The occurrence of selected PhACs in the two sites is summarized in Table A.2 (Appendix A. Supplementary material). The concentration of detected compounds was usually within the tens to hundreds of ng/L range. The levels and loads (calculated with river discharge) of therapeutic groups of PhACs studied along the two sampling campaigns and the two sampling points are shown in Fig. 2.

PhAC concentrations roughly follow an inverse relationship with river flow discharge for the majority of compounds, this being reflected on negative correlation coefficients (Spearman, $p \leq 0.01$; Fig. 3) for the most representative compounds as well as for the sum of all in the two sites. While in ABR 13 (73%) of the most relevant compounds show a negative correlation coefficient with water flow, this number increased

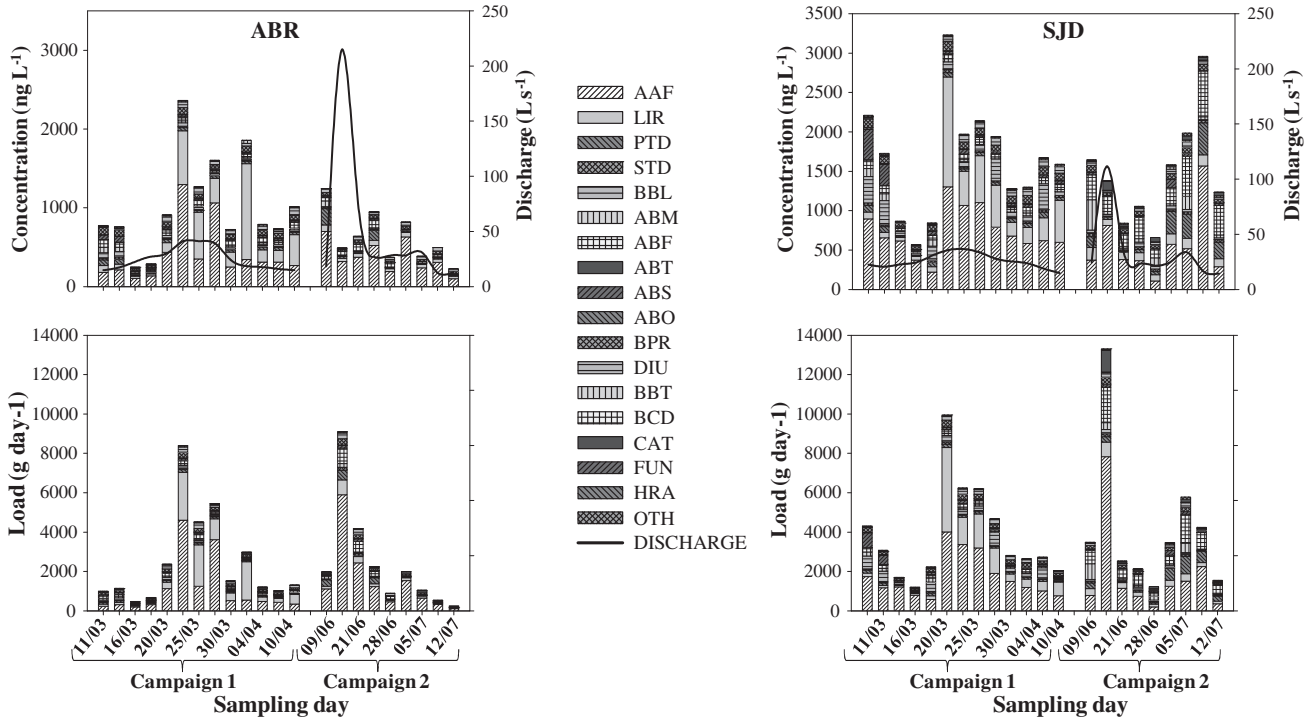


Fig. 2. Levels and loads (calculated with river discharge) of therapeutic groups of pharmaceuticals, represented by aggregated bars, monitored in the two sampling points (ABR and SJD) along the two sampling campaigns (1 and 2) and plotted together with river flow recorded for each day of sampling. Each column includes a number of measures which corresponds to the sum of individual compound levels and loads, of each therapeutic group. Analgesics and Antiinflammatories (AAF), Lipid Regulators (LIR) Psychiatric Drugs Treatment (PTD), Stomach Treatment Drugs (STD), β-Blockers (BBL), Antibiotics Macrolides (ABM), Antibiotics Fluoroquinoles (ABF), Antibiotics Tetracyclines (ABT), Antibiotics Sulfonamides (ABS), Antibiotics Others (ABO), Blood Pressure Regulators (BPR) , Diuretics (DIU), Barbiturates (BBT), Broncodilators (BCD), Cancer Treatment (CAT), Fungicides (FUN), Histamine H1 and H2 Receptor Antagonists (HRA) and others (OTH). The axis on the right is for daily measurements of discharge represented by the dark continuous line in the graph.

to 15 (83%) in SJD, indicating that their concentrations decreased with higher water flow. Few exceptions seem to contradict the general case, erythromycin being the most relevant, while others like ibuprofen or furosemide show a mixed behavior depending on the site considered, and 2 compounds (enrofloxacin and enalapril) had statistically non-significant correlation coefficients.

In the low water flow campaign, a total of 54 of the 73 compounds targeted were present in all samples from ABR sampling point. Gemfibrozil, diclofenac, fenofibrate and ibuprofen were determined at concentrations in the range of 117–192 ng/L, but the remaining PhACs were detected in the lower ng/L range (<50 ng/L). Similarly, 55 of PhACs analyzed were detected in all SJD samples. Only metoprolol,

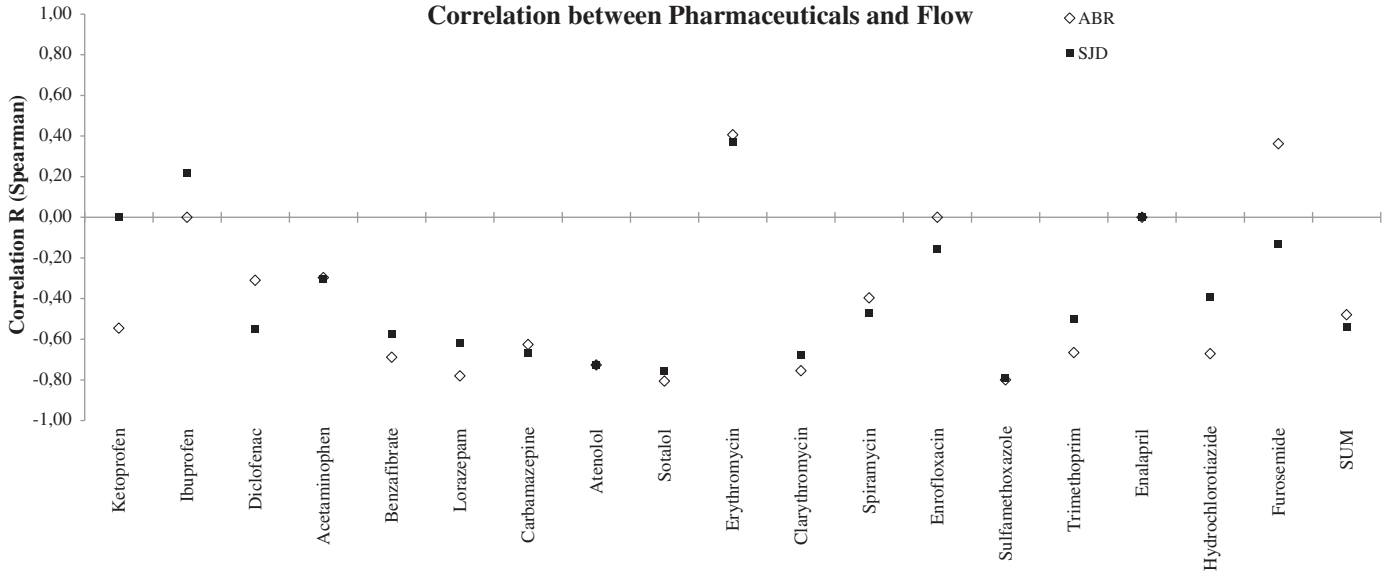


Fig. 3. Spearman correlation coefficients between river flow discharge and some selected PhAC concentrations obtained in the two sites ABR (◊) and SJD (■) during the whole period monitored.

fenofibrate, naproxen and ibuprofen were present at levels of hundreds of ng/L (97–507 ng/L), being the last one the most concentrated. As in ABR, the remaining PhACs were present at concentrations lower than the 50 ng/L. The same therapeutic groups, analgesics and anti-inflammatories and lipid regulators, showed similar importance in the two sampling points. Lipid regulators were the most concentrated in ABR (100–500 ng/L), followed by analgesics and anti-inflammatories (200–400 ng/L). By contrast, analgesics and anti-inflammatories were the most concentrated in SJD (600–1000 ng/L), followed by lipid regulators (100–550 ng/L). Concentrations of remaining PhACs were lower than the 200 ng/L in ABR, as well as in SJD, except for β-blockers, which were up to the 350 ng/L.

In the higher water flow campaign, up to 56 PhACs of all those analyzed were detected in all ABR samples. Only Ibuprofen and Acetaminophen were detected at concentrations higher than the 50 ng/L (98 and 216 ng/L, respectively). On the other hand, 62 compounds targeted were present in all SJD samples. In this campaign there were generally more compounds determined at levels higher than 50 ng/L. Nevertheless, ibuprofen and acetaminophen were equally the most concentrated (182 and 281 ng/L, respectively). By therapeutic groups, analgesics and anti-inflammatories were the more abundant in the two sampling points, accounting for 200–600 ng/L in ABR and 300–700 ng/L in SJD. The antibiotics fluoroquinolones and psychiatric treatment drugs achieved concentrations up to 500 ng/L in SJD. The remaining PhACs therapeutic groups were lower than the 200 ng/L both in ABR and SJD.

The PCA performed with the PhACs concentrations of the two campaigns revealed differences in different therapeutic groups depending on sampling site and period (Fig. 4). The first axis of the PCA explained the 61.1% of the variability and separated the concentrations of PhACs downstream (SJD) than upstream (ABR) in the two campaigns. The second axis explained the 15% of the variability and discriminated the tendencies of each therapeutic group in each campaign. In particular, lipid regulators (LIR) were more concentrated while psychiatric drugs (PDT) and antibiotics fluoroquinolones (ABF) resulted more concentrated in the second one (Fig. 4).

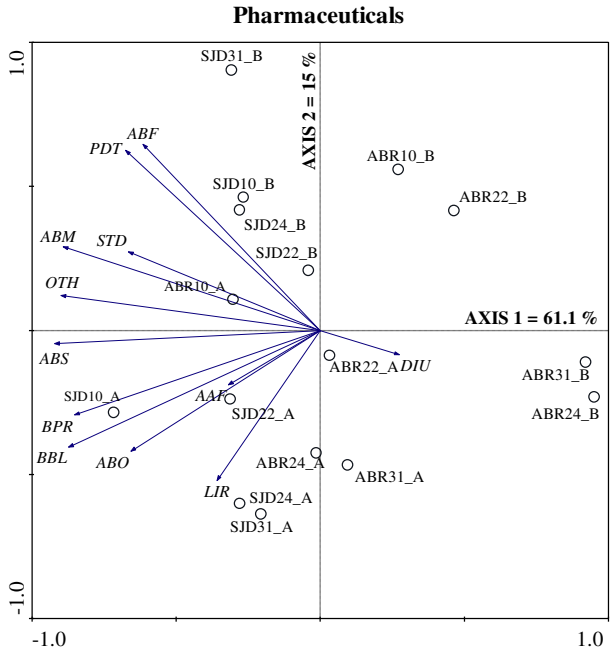


Fig. 4. Principal component analysis of levels of PhACs along both sampling sites and for both sampling campaigns. 10, 22, 24, 31 = Sampling days; ABR = Abrera, SJD = Sant Joan Despí; A = campaign 1; B = campaign 2.

3.3. Biofilms response to translocation and to the flood event

The biofilm development and responses to translocation differed between the two campaigns. The biofilms showed higher autotrophic biomass (chlorophyll-*a*) and heterotrophic activity (extracellular enzyme activities) in the first campaign than in the second one (Fig. 5). In particular, the β-glucosidase and phosphatase activities were higher in the first campaign at both sampling sites (repeated measures ANOVA, $p < 0.05$), while peptidase activity was significantly higher in the first campaign only in SJD biofilms (Fig. 5, repeated measures ANOVA, $p < 0.05$). The accrual of biofilm biomass (calculated as the increase of chlorophyll-*a* in time and per site) was lower in the second campaign (Fig. 6; $p < 0.001$). The ABR biofilm accrual rate was of 0.02 ± 0.002 μg Chla cm^{-2} day^{-1} in the second campaign and of 0.5 ± 0.07 μg Chla cm^{-2} day^{-1} in the first one. The SJD biofilms had an accrual rate of 0.44 ± 0.06 μg Chla cm^{-2} day^{-1} in the first campaign, almost two times higher than in the second one (0.25 ± 0.05 μg Chla cm^{-2} day^{-1}). The behavior observed indicated the different biofilm development between the first sampling campaign and the second one. Interestingly, a flood event occurred the day before the first biofilm sampling of the second campaign.

The translocation from less (ABR) to more (SJD) polluted site affected differently biofilm structure and function depending on the sampling campaign. In general, chlorophyll-*a*, bacterial density and extracellular peptidase activity of biofilms were the parameters that significantly changed in response to translocations. In particular, chlorophyll-*a* significantly decreased in biofilms translocated to SJD during the first campaign, but did not respond in the second one (Fig. 7). The biofilms growth in SJD showed a lower proportion of live bacteria in at each sampling date, and biofilms translocated to this site experienced a significant increase of bacterial mortality. This behavior in the bacterial survival was observed in both campaigns (Fig. 7). Finally, only in the second campaign, the biofilms grown in SJD showed significantly lower extracellular peptidase activity than in ABR and a significant decrease of this activity was measured in samples translocated from ABR to SJD (A ➔ S) both 2 and 9 days after translocation (Fig. 7).

4. Discussion

The occurrence of extended periods of low water flow combines with floods in Mediterranean rivers, to produce an unsteady hydrological template for the solutes and the biota. High water flow episodes are associated to heavy rain events and cause sediment re-suspension, altered biogeochemical patterns, and effects on the organisms. These episodes in the lower Llobregat River can account for up to 170 m^3 s^{-1} (once every 2–10 years), or occasionally up to 800 m^3 s^{-1} (once every 10–50 years) (Munné et al., 2012b). The peak flow reported in this study (around 200 m^3 s^{-1}) produced important effects both on the dynamics of PhACs and in the biomass and activity of biofilms.

The target compounds were generally detected at levels in the range of 10–100 ng/L. These results are in agreement with previous findings (Ferrari et al., 2011; Gros et al., 2007). The respective concentrations of PhACs in the two sites did not follow a natural attenuation (Fono et al., 2006). Instead, the targeted PhACs increased downstream, corresponding to the growing number of WWTPs in the studied section. This observation reinforces those elsewhere (Vieno et al., 2005; Gros et al., 2007; Conley et al., 2008) which propose WWTPs as the main source of emerging contaminants in receiving waters (Gros et al., 2007; Ferrari et al., 2011). The low removal rate during the WWTPs processes can be one of the causes of their relevance (Petrovic et al., 2010), but low dilution capacity of the river is also relevant. The higher levels of gemfibrozil and diclofenac in ABR (upstream site) with respect to the lower site during the second sampling campaign (Table S-3) support the relevance of the respective dilution capacity in the two sites (Ellis, 2006; Osorio et al., 2012b).

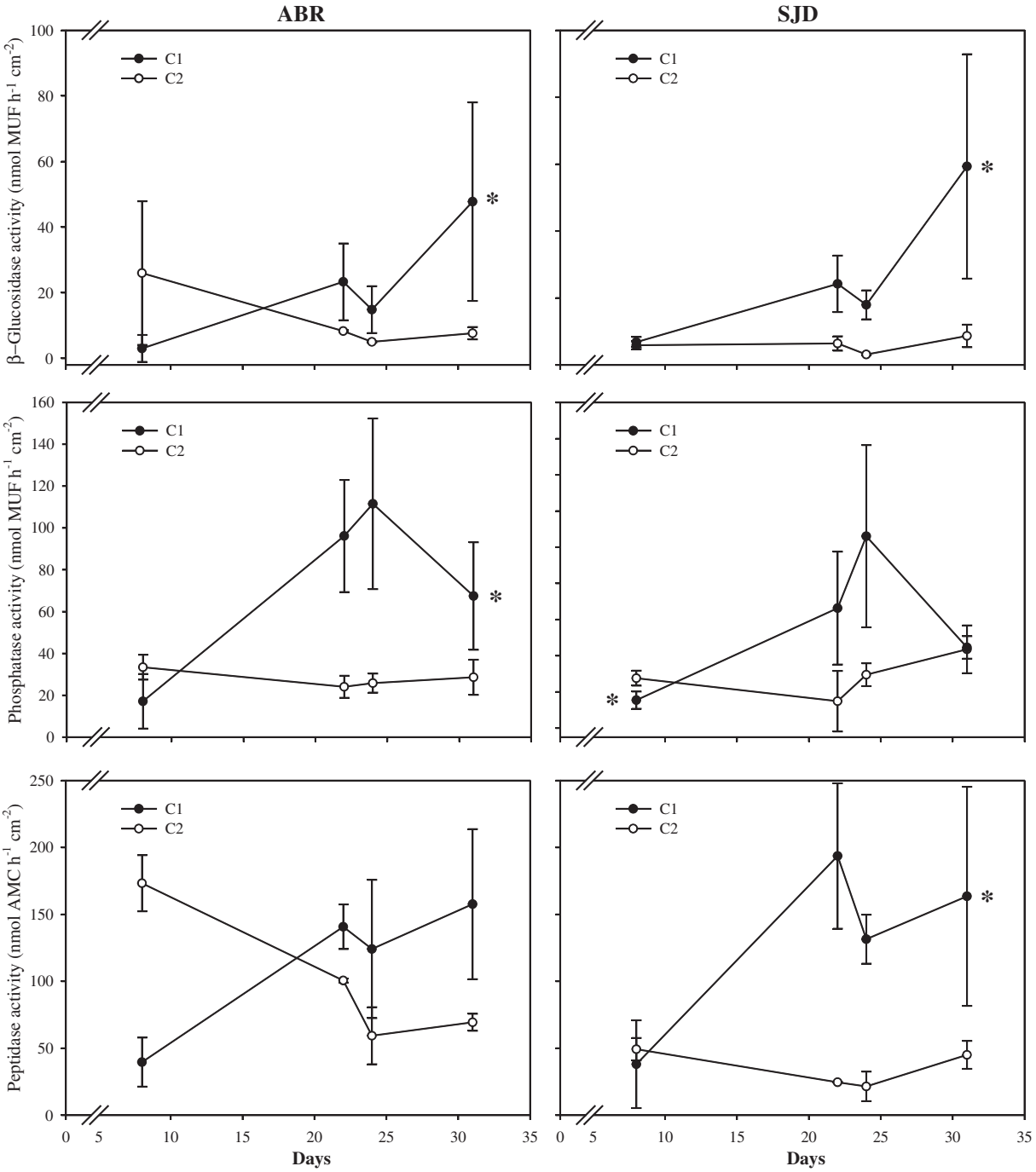


Fig. 5. Patterns of extracellular activity of β-Glucosidase (β-Glu), Alkaline-Phosphatase (APase) and Leucine-AminoPeptidase (LAmP) of biofilms grown at Abrera (ABR) and Sant Joan Despí (SJD) during campaigns 1 (C1) and 2 (C2). Values are means ± standard deviation (n = 3). Stars represent significant differences analyzed by one-way repeated measure of variance (ANOVA) setting sampling campaign as fixed factor. Statistical significance was set at p = 0.05.

The relative contribution of WWTP effluent discharge to the total river flow increased in parallel to the flow decline, favoring the increase of pollutants into the aquatic system. Conversely, high flow episodes may contribute to the sediment re-suspension and compounds re-dissolution. The flood in the Llobregat caused both dilution and re-mobilization of PhACs as a consequence of increased river flow and higher turbulence. This was also observed with other organic contaminants in the Ebro River (Gómez-Gutiérrez et al., 2006), when PCBs, DDTs and HCB inputs were associated to spate periods that caused an increase in suspended particulate matter associated to runoff and sediment re-suspension.

Rainfall events may also affect the sewage system performance due to the lack of separate pluvial networks, which may cause overflow in the system seriously decreasing the overall removal efficiency of pollutants (Sidhu et al., 2013; Kim et al., 2012). As a consequence, PhACs input in the receiving waters may be higher (Sui et al., 2011; Choi et al., 2008; Tamtam et al., 2008). This trend was confirmed when loads of pollutants were calculated (Fig. 2 (b, d)). The occurrence of therapeutic groups of PhACs, as well as their individual concentrations and loads, can vary depending upon the specific site and hydrological conditions (Osorio et al., 2012a,b). Dilution adds to other factors governing the concentration levels of pollutants. Other sources of variability include changes of

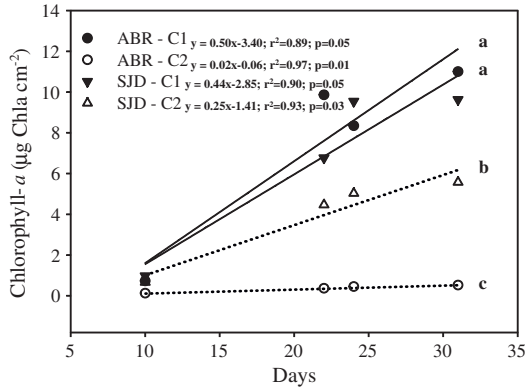


Fig. 6. Growth of biofilm calculated as the increase of chlorophyll-*a* in time at the different sampling sites and in both campaigns. The one-way analysis of variance ANOVA showed significant differences between growth rates. The results of the post hoc test Tukey-b performed are reported in figure, and separated significantly growth rates. Statistical significance was set at $p = 0.05$.

temperature, sediment remobilization, and seasonal and local use of certain drugs (Matthies et al., 2004). In addition, the diverse operating conditions of the WWTPs distributed along the river section studied, can contribute to the differences observed in the occurrence of PhACs between sampling sites. Our results are in agreement with the preponderance of dilution effects of pollutants as a consequence of increased river flow resulting on a decrease of concentrations, which are reflected on negative correlation coefficients (see Section 3.2). In general, slightly better correlations were observed in ABR. This fact can be explained by the higher contribution of WWTP effluent discharge and other anthropogenic pressure to which the river section comprised between ABR and SJD is subjected, and that may play a significant role in the variability of flow dynamics in SJD site and consequent PhAC response.

The continuous release of these compounds from WWTPs generates a downstream increase that is maintained under different hydrological conditions and that can therefore have long-term consequences for biological communities, with important implications for freshwater ecosystems.

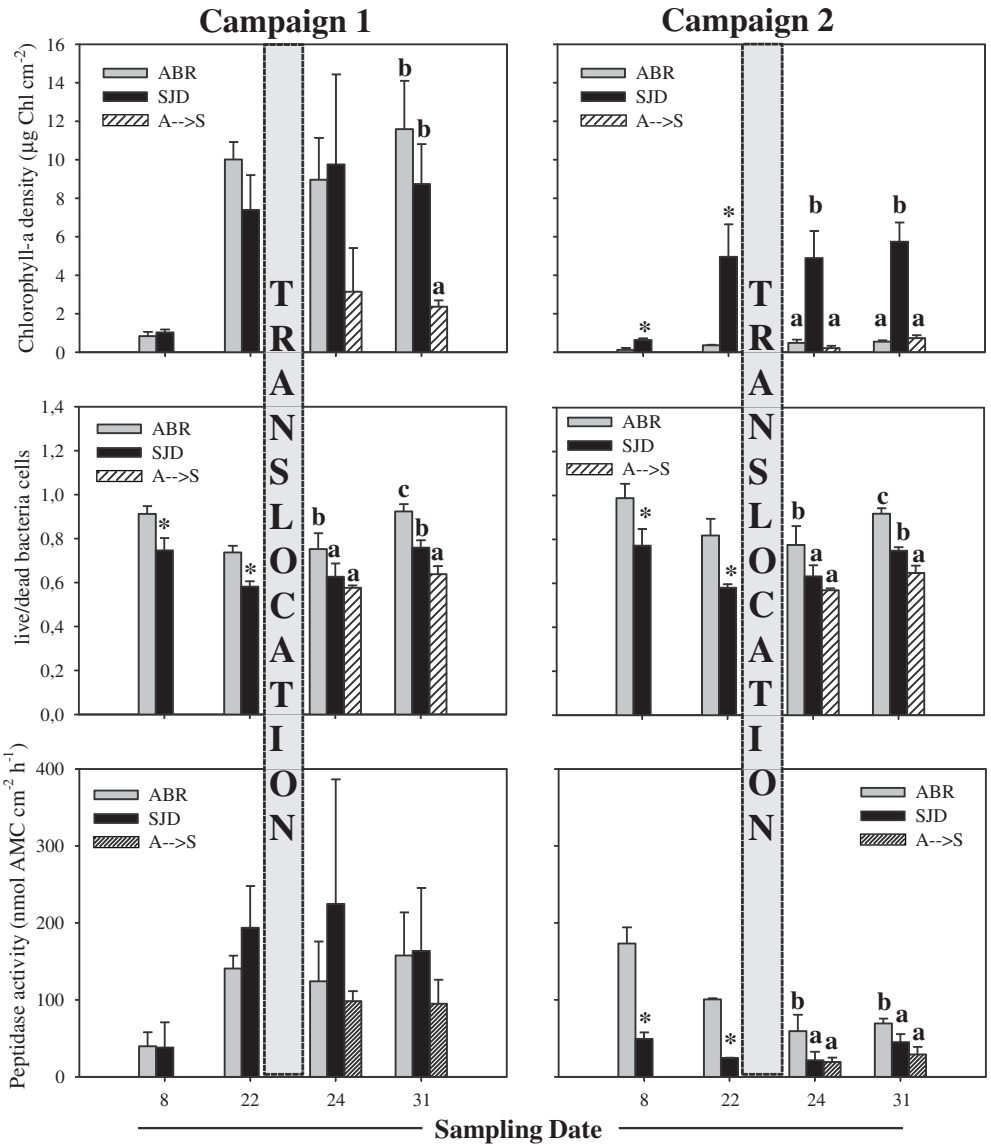


Fig. 7. Biofilms' chlorophyll-*a*, live/dead bacteria ratio and extracellular peptidase activity responses to translocation. Values in bars are mean ± standard deviation ($n = 3$). Stars represent significant differences analyzed daily by one-way analysis of variance (ANOVA) setting sampling site as fixed factor. Letters represent results of post hoc Tukey-b test performed for the samplings after translocation (days 24 and 31). Statistical significance was set at $p = 0.05$.

The potential consequences on the biota when low flow conditions co-occur with high nutrient concentration can be the development of thicker biofilms with higher biomass (Petrovic et al., 2011). Instead, floods can act as "cleaners" of colonized biofilms, partially or totally restarting the colonization of the substrata by the microorganisms. Our study indicated that the flood event occurring during the first phases of colonization (9 days after colonization started) had important consequences on the biofilm structure and functioning. The reduced accrual rates of biofilms after the flood event were result of the flood shear stress on colonized biofilms, and indicated the slower biofilm development and recovery after this event. The extracellular enzyme activities were reduced, decreasing the biofilm capability to decompose and mineralize organic molecules (Proia et al., 2012a). Biofilms showed therefore a reduced capacity of processing organic matter after the flood event.

The biofilm response to translocations was expressed as significant changes in the chlorophyll-*a*, bacterial density and peptidase activity. Chlorophyll-*a* significantly decreased in biofilms translocated to SJD during the first campaign but did not change in the second one. This chlorophyll-*a* response during the base flow campaign might be attributed to the negative effects of the pollutants measured in SJD. Chlorophyll-*a*, was significantly correlated with some PhACs therapeutic groups (psychiatric treatment drugs: $r = -0.786$, $p = 0.021$; antibiotics macrolides: $r = -0.833$, $p = 0.010$; antibiotics fluoroquinoles: $r = -0.857$, $p = 0.007$; antibiotics sulfonamides: $r = -0.762$, $p = 0.028$). Several drugs used for psyquiatric treatment show both chronic and acute toxicity on aquatic organisms (Fent et al., 2006). In particular, fluoxetine, has been described as the most toxic compound ($EC_{50} = 24\ \mu g\ L^{-1}$) of psyquiatric treatment drugs for green algae (Brooks et al., 2003). In previous observations (Osorio et al., 2012a,b), fluoxetine, as well as other potentially toxic compounds, have been reported in Llobregat River surface waters at concentrations similar to EC_{50} values reported in literature. It cannot be excluded that the co-occurrence of low concentrations of a huge number of priority and emerging pollutants, not measured in this study but occurring in Llobregat surface waters (Ricart et al., 2010; Proia et al., 2013a, 2013b), could also contribute to the biofilm response.

The decrease of chlorophyll-*a* in response to the translocation from ABR to SJD was not observed in the second campaign. The dilution effect and the decreased biofilm accrual rate in response to the extreme flood occurring in the second campaign can account for this difference. In fact, the negative correlation between chlorophyll-*a* and the therapeutic groups observed in the first campaign did not exist in the second. While under base-flows conditions PhACs (and other potentially toxic compounds) may affect the autotrophic compartment of river biofilms, flood events may override the potential effect of pollutants even though toxicants concentration is still relevant. This result confirms our hypothesis and is supported by the general behavior of biofilm functioning in the two campaigns. Also , the general heterotrophic capacity of the biofilm was significantly lower in biofilms during the second campaign supporting the hypothesis of an important effect after the flood, masking possible relationships between PhACs and biofilm responses.

The biofilms grown in SJD showed a lower proportion of live bacteria in each sampling date, and biofilms translocated to this site experienced a significant increase of bacterial mortality. This response in the bacterial survival was observed in the two campaigns, indicating the presence of harmful factors on bacteria independent from the flood event. The increased bacterial mortality in biofilm transferred from ABR to SJD, after only two days of translocation in the two campaigns may be related with the increasing concentration of all antibiotics groups in SJD. In this study, the negative correlation between antibiotics macrolids and the number of live bacteria ($r < -0.753$, $p < 0.05$) observed in the two campaigns stresses and confirms this possibility. Similarly, Proia et al. (2013b) found increased bacterial mortality in biofilm transferred from less to more antibiotic-polluted waters in Llobregat River. The authors reported significant correlations between biofilm bacteria responses and antibiotics levels in river water (Proia et al., 2013b). It has been shown that antibiotics—either as single compounds or in mixtures—can have numerous detrimental effects on aquatic life, including direct toxicity to aquatic microbes, even at low concentrations (Hernando et al., 2006). Antibiotics are bioactive against natural bacterial communities, and their presence may lead to short-term physiological alterations, including altered metabolic functions (e.g. biomass production, respiration, and excretion of extracellular enzyme activities), cell death, and long-term changes in microbial biomass or in community composition (Proia et al., 2013b). Other PhACs belonging to other therapeutic groups, and frequently detected at relevant concentrations in Llobregat River (i.e. the β-blocker propanolol, Bonnineau et al., 2010), have been described to affect the heterotrophic compartment of the biofilm. In conclusion, the bacterial compartment of river biofilms was affected mainly by the higher levels of antibiotics in SJD irrespective to the changes determined by the flood event. The mentioned bacterial responses were similar in the two campaigns. This statement confirmed a sensitivity of the bacteria compartment of the biofilm to antibiotics, despite the dilution effects associated to flood conditions.

5. Conclusions

Differences observed in the PhAC concentrations and on the response of biofilms in changing river water flows stress the importance that hydrological variations have on the ecological and chemical status of Mediterranean Rivers. Our study revealed that the differences among biofilms developed in different flow conditions were more relevant than those between sampling sites. This difference was highlighted by the use of translocation of biofilm communities between sites, along the pollution gradient. This evidence suggests that the flood event registered played a principal role in the development of biological communities in Llobregat River. Nevertheless this study also revealed that some potential negative effects of certain groups of PhACs (i.e. antibiotics) on biota (i.e. bacteria) may be maintained under different hydrological scenarios. Thus, disentangling the combined effects of hydrological and pollution changes on the aquatic ecosystem requires combined efforts in analytical chemistry, hydrology and ecology.

Acknowledgments

This work has been funded by the Spanish Ministry of Economy and Competitiveness through the projects VIECO (2008 009/RN08/011), SCARCE (Consolider-Ingenio 2010 CSD2009-00065) and CEMAGUA (CGL2007-64551/HID). It reflects only the authors' views and the European Community is not liable for any use that may be made of the information contained therein. Merck is acknowledged for the gift of LC columns. SP acknowledges the contract from the Ramón y Cajal Program of the Spanish Ministry of Economy and Competitiveness. Prof. Barceló acknowledges King Saud University (Riyadh, Saudi Arabia) for his contract position as Visiting Professor. This work was partly supported by the Generalitat de Catalunya (Consolidated Research Group: Water and Soil Quality Unit 2009-SGR-965).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.scitotenv.2013.11.069.

References

Bonnineau C, Guasch H, Proia L, Ricart M, Geiszinger A, Romaní AM, et al. Fluvial biofilms: a pertinent tool to assess β-blockers toxicity. Aquat Toxicol 2010;96:225–33.

Brooks BW, Foran CM, Richards SM, Weston JW, Turner PK, Stanley JK, et al. Aquatic toxicology of fluoxetine. Toxicol Lett 2003;142(3):169–83.

Choi K, Kim Y, Jung J, Kim MH, Kim CS, Kim NH. Occurrences and ecological risks of roxithromycin, trimethoprim, and chloramphenicol in the Han River, Korea. Environ Toxicol Chem 2008;27:711–9.
Conley JM, Symes SJ, Schorr MS, Richards SM. Spatial and temporal analysis of pharmaceutical concentrations in the upper Tenessee River basin. Chemosphere 2008;73(8):1178–87.
Damásio J, Navarro-Ortega A, Tauler R, Lacorte S, Barceló D, Soares A, et al. Identifying major pesticides affecting bivalve species exposed to agricultural pollution using multi-biomarker and multivariate methods. Ecotoxicology 2010;19:1084–94.
Ellis JB. Pharmaceutical and personal care products (PPCPs) in urban receiving waters. Environ Pollut 2006;144:184–9.
Fent K, Weston A, Caminada D. Ecotoxicology of human pharmaceuticals. Aquat Toxicol 2006;76:122–59.
Ferrari F, Gallipoli A, Balderacchi M, Ulaszewska MM, Capri E, Trevisan M. Exposure of the main Italian river basin to pharmaceuticals. J Toxicol 2011:989270.
Fono LJ, Kolodziej EP, Sedlak DL. Attenuation of wastewater-derived contaminants in an effluent-dominated river. Environ Sci Technol 2006;40(23):7257–62.
Gasith A, Resh VH. Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events. Annu Rev Ecol Syst 1999;30:51–81.
Ginebreda A, Muñoz I, Alda ML, Brix R, López-Doval J, Barceló D. Environmental risk assessment of pharmaceuticals in rivers: relationships between hazard indexes and aquatic macroinvertebrate diversity indexes in the Llobregat River (NE Spain). Environ Int 2010;36:153–62.
Gómez-Gutiérrez AI, Jover E, Bodineau L, Albaigés J, Bayona JM. Organic contaminant loads into the Western Mediterranean Sea: Estimate of Ebro River inputs. Chemosphere 2006;65(2):224–36.
González SA, López-Roldán R, Cortina JL. Presence and biological effects of emerging contaminants in Llobregat River basin: a review. Environ Pollut 2012;161:83–92.
Gros M, Petrovic M, Barceló D. Wastewater Treatment Plants as a pathway for aquatic contamination by pharmaceuticals in the Ebro river basin (Northeast Spain). Environ Chem 2007;26(8):1553–62.
Gros M, Petrovic M, Barceló D. Tracing PhACs residues of different therapeutic classes in Environmental Waters by using liquid chromatography/quadrupole-linear ion trap mass spectrometry and automated library searching. Anal Chem 2009;81:898–912.
Gros M, Petrovic M, Ginebreda A, Barceló D. Removal of PhACs during wastewater treatment and environmental risk assessment using hazard indexes. Environ. Int. 2010;36:15–26.
Hernando MD, Mezcua M, Fernandez-Alba AR, Barceló D. Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments. Talanta 2006;69:334–42.
Jeffrey S, Humphrey GF. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. Biochemie und Physiologie der Pflanzen 1975;167:191–4.
Kim H, Han M, Young Lee J. The application of an analytical probabilistic model for estimating the rainfall–runoff reductions achieved using a rainwater harvesting system. Sci Total Environ 2012;424:213–8.
Kolpin DW, Skopec M, Meyer MT, Furlong ET, Zaugg SD. Urban contribution of pharmaceuticals and other organic wastewater contaminants to streams during differing flow conditions. Sci Total Environ 2004;328:119–30.
Marcé R, Honey-Rosés J, Manzano A, Moragas L, Catllar B, Sabater S. The Llobregat River Basin: a paradigm of impaired rivers under climate change threats. In: Sabater S, Ginebreda A, Barceló D, editors. The Llobregat: the story of a polluted Mediterranean river. Hdb. Env. Chem.Berlin Heidelberg: Springer-Verlag; 2012. p. 1–26.
Matthies M, Berding V, Beyer A. Probabilistic uncertainty analysis of the European Union system for the evaluation of substances multimedia regional distribution model. Environ Toxicol Chem 2004;23:2494.
Munné A, Tirapu L, Solà C, Olivella L, Vilanova M, Ginebreda A, et al. Comparing chemical and ecological status in Catalan Rivers. Analysis of river quality status following the Water Framework Directive. In: Guasch H, Ginebreda A, Geiszinger A, editors. Emerging and priority pollutants in rivers: bringing science into river management plans. Hdb. Env. Chem.Berlin Heidelberg: Springer-Verlag; 2012a. p. 243–66.
Munné A, Solà C, Tirapu L, Barata C, Rieradevall M, Prat N. Human pressure and its effects on water quality and biota in the Llobregat River. In: Sabater S, Ginebreda A, Barceló D, editors. The Llobregat: the story of a polluted Mediterranean river. Hdb. Env. Chem.Berlin Heidelberg: Springer-Verlag; 2012b. p. 1–26.
Muñoz I, López-Doval JC, Ricart M, Villagrassa M, Brix R, Geiszinger A, et al. Bridging levels of PhACs in river water with biological community structure in the Llobregat River basin (NE, Spain). Environ Toxicol Chem 2009;28:2706–14.
Murphy J, Riley JP. A modified single solution method for the determination of phosphate in natural waters. Analytica Chimica Acta 1962;27:31–6.
Osorio V, Pérez S, Ginebreda A, Barceló D. Pharmaceuticals on a sewage impacted section of a Mediterranean River (Llobregat River, NE Spain) and their relationship with hydrological conditions. Environ Sci Pollut Res 2012a;19:1013–25.
Osorio V, Marcé R, Pérez S, Ginebreda A, Cortina JL, Barceló D. Occurrence and modeling of pharmaceuticals on a sewage-impacted Mediterranean river and their dynamics under different hydrological conditions. Sci Total Environ 2012b;440:3–13.
Petrovic M, Barceló D, editors. Analysis, fate and removal of pharmaceuticals in the water cycle. Amsterdam: Elsevier; 2009.
Petrovic M, Postigo C, de Alda ML, Ginebreda A, Gros M, Radjenovic J, et al. Water scarcity in the Mediterranean: perspectives under global change. Handb. Environ. Chem. 2010;8:228–1978.
Petrovic M, Ginebreda A, Acuña V, Batalla RJ, Elosegi A, Guasch H, et al. Combined scenarios of chemical and ecological quality under water scarcity in Mediterranean rivers. TrAC 2011;30(8):1268–78.
Proia L, Cassiò F, Pascoal C, Tlili A, Romaní AM. The use of attached microbial communities to assess ecological risks of pollutants in river ecosystems. The role of heterotrophs. In: Guasch H, Ginebreda A, Geiszinger A, editors. Emerging and priority pollutants in rivers: bringing science into river management plans. Berlin Heidelberg: Springer Verlag; 2012a. p. 55–83.
Proia L, Vilches C, Boninneau C, Kantiani L, Farré M, Romaní AM, et al. Drought episode modulates the response of river biofilm to triclosan. Aquat Toxicol 2012b;127:36–45.
Proia L, Osorio V, Soley S, Köck-Schulmeyer M, Pérez S, Barceló D, et al. Effects of pesticides and pharmaceuticals on biofilms in a highly impacted river. Environ Pollut 2013a;178:220–8.
Proia L, Lupini G, Osorio V, Pérez S, Barceló D, Schwartz T, et al. Response of biofilm bacterial communities to antibiotic pollutants in a Mediterranean river. Chemosphere 2013b;92:1126–35.
Ricart M, Guasch H, Barceló D, Brix R, Conceição MH, Geiszinger A, et al. Primary and complex stressors in polluted Mediterranean rivers: pesticide effects on biological communities. J Hydrol 2010;383:52–61.
Sabater S, Guasch H, Ricart M, Romaní AM, Vidal G, Klünder C, et al. Monitoring the effect of chemicals on biological communities. The biofilm as an interface. Anal Bioanal Chem 2007;387:1425–34.
Sabater S, Ginebreda A, Barceló D. The Llobregat. The story of a polluted Mediterranean riverHdb. Env. Chem. Berlin Heidelberg: Springer-Verlag; 2012.
Serra A, Corcoll N, Guasch H. Copper ac cumulation and toxicity in fluvial periphyton: the influence of exposure history. Chemosphere 2009;74:633–41.
Sidhu JPS, Ahmed W, Gernjak W, Aryal R, McCarthy D, Palmer A, et al. Sewage pollution in urban stormwater runoff as evident from the widespread presence of multiple microbial and chemical source tracking markers. Sci Total Environ 2013;463–464:488–96.
Sui Q, Huang J, Deng S, Chen W, Yu G. Seasonal variation in the occurrence and removal of pharmaceuticals and personal care products in different biological wastewater treatment processes. Environ Sci Technol 2011;45:3341–8.
Tamtam F, Mercier F, Le Bot B, Eurin J, Tuc Dinh Q, Clément M, et al. Occurrence and fate of antibiotics in the Seine River in various hydrological conditions. Sci Total Environ 2008;393:84–95.
ter Braak CJF, Smilauer P. CANOCO reference manual and user's guide to Canoco for Windows: software for Canonical Community Ordination (Version 4). Ithaca, New York: Microcomputer Power; 1998352.
Vieno NM, Tuhkanen T, Kronberg L. Seasonal variation in the occurrence of pharmaceuticals in effluents from a sewage treatment plant and in the recipient water. Environ Sci Technol 2005;39:8220–6.
Water Framework Directive (Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy) European Parliament and Council Article 175(1) OJL; 2000. p. 1–73. (22 December).

Appendix A. Supplementary material

Table A.1. Characteristics of the main WWTP that discharge into the studied section of the Llobregat River.

WWTP	Effluent Discharge Point	Flow Treated (m^3/day)	h-e treated
Manresa	Cardener (before CB)	25.962	118.993
Pont de Vilomara	Llobregat (before CB)	514	3.598
Castellbell i El Vilar	Llobregat (before CB)	2.537	7.146
Monistrol de Montserrat	Llobregat (between CB and MPT)	1.654	9.759
Abrera	Llobregat (after MPT)	15.597	77.985
Rubí	Rubí (between MPT and SJD)	21.865	171.758
Martorell	Anoia (between MPT and SJD)	6.778	46.768
Sant Feliu de Llobregat	Llobregat (between MTP and SJD)	72.000	320.000

h-e equivalent per habitant

Table A.2. Target compounds, identification number (CAS), molecular formula and QqLIT-MS/MS parameters used for quantification (SRM 1) and confirmation (SRM 2 and Rt) of each compound by SRM negative ($[M-H]^-$) and Positive ($[M+H]^+$) ionization.

Therapeutic group	Compounds	CAS number	Molecular formula	Rt (min)	Precursor ion (m/z)	SRM 1	SRM 2
Analgesics and Anti-inflammatories (AAF)	**Ketoprofen** (a)	22071-15-4	$C_{16}H_{14}O_3$	14.9	253 $[M-H]^-$	209	-
	Ibuprofen (a)	15687-27-1	$C_{13}H_{18}O_2$	19.2	205 $[M-H]^-$	161	-
	Indometacine (b)	53-86-1	$C_{19}H_{16}ClNO_4$	20.6	356 $[M-H]^-$	312	214
	Diclofenac (a)	15307-86-5	$C_{14}H_{11}Cl_2NO_2$	19.9	294 $[M-H]^-$	250	-
	Mefenamic acid (b)	61-68-7	$C_{15}H_{15}NO_2$	21.1	240 $[M-H]^-$	196	297
	Acetaminophen (b)	103-90-2	$C_8H_9NO_2$	3.6	150 $[M-H]^-$	107	
	Propiphenazone (c)	479-92-5	$C_{14}H_{18}N_2O$	15.3	231 $[M+H]^+$	56	148
	Phenybutazone (b)	1698-60-8	$C_{10}H_8ClN_3O$	20.7	309 $[M+H]^+$	77	250
	Phenazone (b)	50-33-9	$C_{19}H_{20}N_2O_2$	9.8	189 $[M+H]^+$	56	314
	Codeine (d)	76-57-3	$C_{18}H_{21}NO_3$	7.4	300 $[M+H]^+$	152	130
	Naproxen (a)	22204-53-1	$C_{14}H_{14}O_3$	14.3	229 $[M-H]^-$	185	285
Lipid regulators (LIR)	**Clorifibic acid** (b)	882-09-7	$C_{10}H_{11}ClO_3$	12.9	213 $[M-H]^-$	127	-
	Gemfrobizil	25812-30-0	$C_{15}H_{22}O_3$	24.3	249 $[M-H]^-$	121	180
	Benzafibrate (b)	41859-67-0	$C_{19}H_{20}ClNO_4$	16.7	360 $[M-H]^-$	274	85
	Fenofibrate (b)	49562-28-9	$C_{20}H_{21}ClO_4$	25.2	361 $[M+H]^+$	139	160
	Atorvastatine (c)	134523-00-5	$C_{33}H_{35}FN_2O_5$	19.8	559 $[M+H]^+$	440	154
	Mevastatine (b)	73573-88-3	$C_{23}H_{34}O_5$	21.5	391 $[M+H]^+$	185	169
	Pravastatin	81093-37-0	$C_{23}H_{36}O_7$	14.2	447 $[M+H]^+$	327	576
Psychiatric drugs Treatment (PDT)	**Fluoxetine** (b)	54910-89-3	$C_{17}H_{18}F_3NO$	15.1	310 $[M+H]^+$	44	-
	Paroxetine (c)	61869-08-7	$C_{19}H_{20}FNO_3$	14.4	330 $[M+H]^+$	192	773
	Diazepam (d)	439-14-5	$C_{16}H_{13}ClN_2O$	18.1	285 $[M+H]^+$	193	267
	Lorazepam (d)	846-49-1	$C_{15}H_{10}Cl_2N_2O_2$	15.7	323 $[M+H]^+$	174	-
	Carbamazepine (b)	298-46-4	$C_{15}H_{12}N_2O$	14.7	237 $[M+H]^+$	194	-
Histamine H1 and H2 receptor antagonists (HRA)	**Famotidine** (b)	76824-35-6	$C_8H_{15}N_7O_2S_3$	6.3	338 $[M+H]^+$	189	-
	Ranitidine (b)	66357-35-5	$C_{13}H_{22}N_4O_3S$	6.5	315 $[M+H]^+$	176	159
	Cimetidine (b)	51481-61-9	$C_{10}H_{16}N_6S$	6.3	253 $[M+H]^+$	95	190
	Loratadine (b)	79794-75-5	$C_{22}H_{23}ClN_2O_2$	17.5	383 $[M+H]^+$	337	600
β-Blockers (BBL)	**Atenolol** (b)	29122-68-7	$C_{14}H_{22}N_2O_3$	6.2	267 $[M+H]^+$	145	82
	Sotalol (b)	3930-20-9	$C_{12}H_{20}N_2O_3S$	6.1	273 $[M+H]^+$	213	166
	Metoprolol (b)	37350-58-6	$C_{15}H_{25}NO_3$	10.2	268 $[M+H]^+$	121	244
	Propanolol (b)	525-66-6	$C_{16}H_{21}NO_2$	12.5	260 $[M+H]^+$	116	92
	Timolol (b)	26839-75-8	$C_{13}H_{24}N_4O_3S$	9.8	317 $[M+H]^+$	261	154
	Betaxolol (b)	63659-18-7	$C_{18}H_{29}NO_3$	12.9	308 $[M+H]^+$	116	-
	Carazolol (b)	57775-29-8	$C_{18}H_{22}N_2O_3$	11.8	299 $[M+H]^+$	116	573
	Pindolol (b)	13523-86-9	$C_{14}H_{20}N_2O_2$	8.8	249 $[M+H]^+$	116	201
	Nadolol (b)	42200-33-9	$C_{17}H_{27}NO_4$	8.5	310 $[M+H]^+$	254	-
Cancer Treatment (CAT)	**Tamoxifen** (b)	10540-29-1	$C_{26}H_{29}NO$	19.4	372 $[M+H]^+$	72	-
Fungicides (FUN)	**Metronidazole** (b)	443-48-1	$C_6H_9N_3O_3$	5.8	172 $[M+H]^+$	172	
Antibiotics Macrolids (ABM)	**Erytromicin** (b)	114-07-8	$C_{37}H_{67}NO_{13}$	13.4	734 $[M+H]^+$	158	65
	Azythromicin (b)	83905-01-5	$C_{38}H_{72}N_2O_{12}$	10.9	749 $[M+H]^+$	591	132

	Roxythromycin (b)	80214-83-1	$C_{41}H_{76}N_2O_{15}$	15.1	838 $[M+H]^+$	158	158
	Clarithromicin (b)	81103-11-9	$C_{38}H_{69}NO_{13}$	14.6	748 $[M+H]^+$	591	123
	Tylosin (b)	1401-79-0	$C_{46}H_{77}NO_{17}$	14.1	916 $[M+H]^+$	174	121
	Josamycin (b)	16846-24-15	$C_{42}H_{69}NO_{15}$	15.6	828 $[M+H]^+$	174	189
	Spyramicin (b)	8025-81-8	$C_{43}H_{74}N_2O_{14}$	10.7	843 $[M+H]^+$	174	133
	Tilmicosin (b)	10850-54-0	$C_{46}H_{80}N_2O_{13}$	11.8	869 $[M+H]^+$	696	222
Antibiotics Fluoroquinolones (ABF)	**Ofloxacine** (b)	82419-36-1	$C_{18}H_{20}FN_3O_4$	9.2	362 $[M+H]^+$	261	98
	Ciprofloxacine (b)	85731-33-1	$C_{17}H_{18}FN_3O_3$	9.4	332 $[M+H]^+$	288	201
	Enrofloxacine (b)	93106-60-6	$C_{19}H_{22}FN_3O_3$	9.9	360 $[M+H]^+$	316	147
	Norfloxacin (b)			9.3	320 $[M+H]^+$	302	-
	Enoxacine (b)	74011-58-8	$C_{15}H_{17}FN_4O_3$	8.9	321 $[M+H]^+$	303	261
	Danofloxacin (b)	112398-08-0	$C_{19}H_{20}FN_3O_3$	9.7	358 $[M+H]^+$	340	231
Antibiotics Tetracyclines (ABT)	**Tetracycicline** (b)	60-54-8	$C_{22}H_{24}N_2O_8$	11.8	445 $[M+H]^+$	428	444
	Doxicycline (b)	564-25-0	$C_{22}H_{24}N_2O_8$	9.7	445 $[M+H]^+$	410	124
	Oxytetracycline (b)	79-57-2	$C_{22}H_{24}N_2O_9$	9.2	461 $[M+H]^+$	426	234
	Chlortetracycline (b)	57-62-5	$C_{22}H_{23}ClN_2O_8$	11.4	479 $[M+H]^+$	462	540
Antibiotics Sulfonamides (ABS)	**Sulfamethoxazole** (b)	723-46-6	$C_{10}H_{11}N_3O_3S$	12.5	254 $[M+H]^+$	156	-
	Sulfadiazine (b)	68-35-9	$C_{10}H_{10}N_4O_2S$	7.3	253 $[M+H]^+$	156	259
	Sulfamethazine (b)	57-68-1	$C_{12}H_{14}N_4O_2S$	9.5	279 $[M+H]^+$	186	-
Antibiotics Others (ABO)	**Trimethoprim** (b)	738-70-5	$C_{14}H_{18}N_4O_3$	8.8	291 $[M+H]^+$	230	-
	Chloramphenicol (b)	56-75-7	$C_{11}H_{12}Cl_2N_2O_5$	15.1	323 $[M-H]^-$	152	-
	Nifuroxazide (b)	965-52-6	$C_{12}H_9N_3O_5$	12.8	276 $[M+H]^+$	121	183
Bronchodilators (BCD)	**Salbutamol** (b)	18559-94-9	$C_{13}H_{21}NO_3$	5.7	240 $[M+H]^+$	148	127
Blood pressure Regulators (BPR)	**Enalapril** (b)	75847-73-3	$C_{20}H_{28}N_2O_5$	12.5	377 $[M+H]^+$	234	174
	Lisinopril (b)	83915-83-7	$C_{21}H_{31}N_3O_5$	8.1	406 $[M+H]^+$	84	92
Diuretics (DIU)	**Furosemide** (b)	54-31-9	$C_{12}H_{11}ClN_2O_5S$	13.3	329 $[M-H]^-$	205	85
	Hydrochlorothiazide (b)	58-93-5	$C_7H_8ClN_3O_4S_2$	6.1	296 $[M-H]^-$	78	66
Antidiabetic (ADB)	**Glibenclamide** (b)	10238-21-8	$C_{23}H_{28}ClN_3O_5S$	20.7	494 $[M+H]^+$	369	-
Barbiturics (BBT)	**Phenobarbital** (d)	50-06-6	$C_{12}H_{12}N_2O_3$	14.2	231 $[M-H]^-$	188	-
	Pentobarbital (d)	76-74-4	$C_{11}H_{18}N_2O_3$	18.6	225 $[M-H]^-$	182	154
	Butalbial (d)	77-26-9	$C_{11}H_{16}N_2O_3$	16.6	223 [M-H]-	180	194
Veterinary use (VET)	**Clenbuterol** (b)	37148-27-9	$C_{12}H_{18}Cl_2N_2O$	10.3	277 $[M+H]^+$	203	245
	Flumequine (b)	42835-25-6	$C_{14}H_{12}FNO_3$	15.4	262 $[M+H]^+$	202	-
Internal standards	**Phenobarbital-d5 (IS)** (d)			14.2	236 $[M-H]^-$	193	197
	Diazepam-d5 (IS) (d)			17.6	290 $[M+H]^+$	198	229
	Fluoxetina-d5 (IS) (a)			15.3	315 $[M-H]^+$	153	679
	13C-Fenacetin (IS) (a)			12.7	181 $[M+H]^+$	139	303
	Sulfatiazol-d4 (IS) (e)			8.2	260 $[M+H]^+$	160	115
	Ibuprofen-d3 (IS) (g)			19.1	208 $[M-H]^-$	164	85
	Mecoprop-d3 (IS) (f)			14.8	218 $[M-H]^-$	146	169
	Atenolol-d7 (IS) (g)			6.2	274 [M+H]+	145	255
	Carbamazepina-d10 (g)			14.5	247 [M+H]+	204	-

(a) Sigma-Aldrich (Steinheim, Germany); (b) Jescuder (Rubí, Spain); (c) LGC Promochem (London, UK); (d) Cerilliant (Texas, USA); (e) Toronto Research Chemicals (Canada); (f) Dr. Ehrenstorfer (Augsburg, Germany); (g) CDN isotopes (Quebec, Canada).

Table A.3. Range of concentrations (average, maximum and minimum), expressed in ng/L, of pharmaceuticals monitored at the two sampling sites studied (a) ABR and (b) SJD, standard deviation and frequency expressed in %. (LOQ : limit of quantification; ND: not detected).

(a)

		Campaign 1					Campaign 2				
		Ave ($ng\ L^{-1}$)	Max ($ng\ L^{-1}$)	Min ($ng\ L^{-1}$)	St dev ($ng\ L^{-1}$)	Fre (%)	Ave ($ng\ L^{-1}$)	Max ($ng\ L^{-1}$)	Min ($ng\ L^{-1}$)	St dev ($ng\ L^{-1}$)	Fre (%)
AAF	**Ketoprofen**	5,91	12,11	1,93	2,76	100	5,69	10,55	2,35	2,64	100
	Ibuprofen	192,00	430,57	57,89	101,59	100	98,41	172,66	48,14	38,30	100
	Indometacine	3,64	7,64	1,46	1,80	100	3,14	5,45	1,21	1,40	100
	Diclofenac	132,96	785,93	6,83	272,00	100	49,40	133,06	21,73	38,16	100
	Mefenamic acid	0,18	0,98	0,03	0,26	100	1,76	5,96	0,87	1,78	100
	Acetaminophen	35,71	79,53	17,53	18,49	100	216,25	424,45	15,19	147,25	100
	Propiphenazone	1,49	3,53	0,70	0,93	100	0,19	0,38	0,01	0,15	66,67
	Phenybutazone	4,85	11,26	2,43	2,36	100	0,48	1,85	0,17	0,54	100
	Phenazone	1,56	3,57	0,55	0,89	100	<LOQ	<LOQ	<LOQ	<LOQ	100
	Codeine	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq
	Naproxen	26,41	57,74	11,99	13,45	100	17,27	25,25	9,63	5,26	100
LIR	**Clorifibic acid**	0,65	2,00	0,01	0,63	100	0,68	2,32	0,13	0,89	100
	Gemfrobizil	116,71	1114,56	15,84	300,15	100	18,18	33,68	6,81	7,91	100
	Benzafibrate	2,77	5,09	1,28	1,08	100	2,32	3,54	1,76	0,53	100
	Fenofibrate	182,12	611,47	4,30	201,71	100	5,13	11,47	0,35	4,87	100
	Atorvastatine	1,15	2,87	0,30	0,79	100	0,41	1,48	0,03	0,54	100
	Mevastatine	0,30	0,89	0,03	0,26	100	2,94	8,57	0,20	3,20	100
	Pravastatin	6,04	10,38	1,59	3,14	100	3,60	6,14	2,76	1,21	100
PDT	**Fluoxetine**	4,39	12,67	2,19	3,42	100	0,39	1,26	0,01	0,48	100
	Paroxetine	3,43	12,03	1,64	3,26	100	0,36	0,82	0,02	0,28	100
	Diazepam	2,07	5,28	1,02	1,40	100	0,13	0,65	0,00	0,21	100
	Lorazepam	21,52	38,75	6,23	9,01	100	7,48	27,86	0,70	9,77	100
	Carbamazepine	4,26	6,32	1,21	1,60	100	37,43	177,60	1,78	62,60	100
HRA	**Famotidine**	nq	nq	nq	nq	nq	0,30	1,47	0,07	0,46	100
	Ranitidine	12,34	26,86	4,80	6,31	100	2,38	5,71	0,13	1,86	100
	Cimetidine	nq	nq	nq	nq	nq	11,09	18,42	6,39	3,84	100
	Loratadine	0,10	0,12	0,08	0,01	100	ND	ND	ND	ND	0
BBL	**Atenolol**	15,56	30,53	4,67	8,85	100	0,06	0,25	0,00	0,10	100
	Sotalol	6,00	11,51	2,47	2,50	100	1,07	3,70	0,08	1,10	100
	Metoprolol	3,49	7,68	1,20	1,76	100	0,67	3,96	0,00	1,38	100
	Propanolol	3,14	8,98	1,68	2,47	100	ND	ND	ND	ND	ND
	Timolol	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq
	Betaxolol	2,49	5,30	1,19	1,45	100	ND	ND	ND	ND	ND
	Carazolol	0,71	3,57	0,07	1,23	100	<LOQ	<LOQ	<LOQ	<LOQ	100
	Pindolol	0,85	3,19	0,59	0,71	100	ND	ND	ND	ND	0
	Nadolol	0,76	1,97	0,32	0,51	100	0,03	0,11	0,00	0,04	100
CAT	**Tamoxifen**	nq	nq	nq	nq	nq	0,10	0,28	0,01	0,11	100
FUN	**Metronidazole**	0,25	0,48	0,05	0,14	100	0,01	0,05	0,00	0,02	100
ABM	**Erytromicin**	6,42	19,44	3,33	5,02	100	9,37	57,73	0,47	18,52	100
	Azythromicin	nq	nq	nq	nq	nq					
	Roxythromycin	0,82	3,00	0,21	0,96	100	ND	ND	ND	ND	0
	Clarithromicin	9,12	19,64	3,73	5,09	100	1,50	5,24	0,00	2,11	100
	Tylosin	1,46	6,57	0,78	1,62	100	0,05	0,22	0,00	0,07	100
	Josamycin	0,05	0,34	0,01	0,10	100	0,02	0,05	0,01	0,02	100
	Spyramicin	7,39	20,06	1,74	5,27	100	1,44	8,80	0,04	2,82	100
	Tilmicosin	nq	nq	nq	nq	nq					
ABF	**Ofloxacine**	nq	nq	nq	nq	nq	<LOQ	<LOQ	<LOQ	<LOQ	100
	Ciprofloxacine	nq	nq	nq	nq	nq	3,31	7,43	0,56	2,70	100
	Enrofloxacine	18,83	52,46	5,70	12,77	100	2,27	5,28	0,01	2,12	77,78
	Norfloxacin	32,00	64,77	9,42	17,80	100	33,87	86,05	4,23	25,78	100

	Enoxacine	nq	nq	nq	nq	nq	ND	ND	ND	ND	0
	Danofloxacin	nq	nq	nq	nq	nq	5,42	14,21	0,41	4,61	100
ABT	**Tetracycicline**	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq
	Doxicycline	nq	nq	nq	nq	nq	<LOQ	<LOQ	<LOQ	<LOQ	100
	Oxytetracycline	nq	nq	nq	nq	nq	ND	ND	ND	ND	0
	Chlortetracycline	3,24	5,63	0,35	1,68	100	<LOQ	<LOQ	<LOQ	<LOQ	100
ABS	**Sulfamethoxazole**	13,49	30,13	4,85	7,60	100	5,93	18,83	0,21	8,16	100
	Sulfadiazine	7,95	30,02	3,54	7,51	100	1,55	6,14	0,06	2,24	100
	Sulfamethazine	5,41	13,48	2,61	3,52	100	0,10	0,46	0,00	0,18	100
ABO	**Trimethoprim**	3,33	4,77	0,54	1,52	100	1,67	5,82	0,01	2,38	100
	Chloramphenicol	0,45	1,06	0,36	0,23	100	ND	ND	ND	ND	0
	Nifuroxazide	4,31	10,63	2,25	2,61	100	<LOQ	<LOQ	<LOQ	<LOQ	100
BCD	**Salbutamol**	ND	ND	ND	ND	0	2,22	2,58	0,95	0,59	100
BPR	**Enalapril**	2,50	8,09	1,04	2,20	100	2,73	8,49	0,03	3,40	100
	Lisinopril	30,73	72,24	6,30	21,41	100	1,47	3,38	0,14	1,59	100
DIU	**Furosemide**	45,60	92,93	9,45	28,51	100	38,05	48,71	15,89	10,31	100
	Hydrochlorothiazide	24,86	38,05	8,57	10,45	100	26,09	36,10	10,99	7,50	100
ADB	**Glibenclamide**	ND	ND	ND	ND	0	ND	ND	ND	ND	0
BBT	**Phenobarbital**	12,38	37,84	4,36	8,89	100	ND	ND	ND	ND	0
	Pentobarbital	nq	nq	nq	nq	nq	ND	ND	ND	ND	0
	Butalbial	nq	nq	nq	nq	nq	ND	ND	ND	ND	0
VET	**Clenbuterol**	nq	nq	nq	nq	nq	0,24	0,76	0,00	0,25	100
	Flumequine	0,23	2,47	0,03	0,67	100	0,63	1,19	0,07	0,49	100

(b)

		Campaign 1					**Campaign 2**				
		Ave ($ng\ L^{-1}$)	**Max ($ng\ L^{-1}$)**	**Min ($ng\ L^{-1}$)**	**St dev ($ng\ L^{-1}$)**	**Fre (%)**	**Ave ($ng\ L^{-1}$)**	**Max ($ng\ L^{-1}$)**	**Min ($ng\ L^{-1}$)**	**St dev ($ng\ L^{-1}$)**	**Fre (%)**
AAF	**Ketoprofen**	12,26	37,67	3,75	9,67	100	19,95	32,20	8,76	9,09	100
	Ibuprofen	507,29	867,95	147,23	184,81	100	141,51	340,69	54,24	95,55	100
	Indometacine	5,71	10,66	2,01	3,09	100	8,99	39,19	2,45	11,52	100
	Diclofenac	39,05	65,96	12,04	19,78	100	33,69	45,02	20,36	9,46	100
	Mefenamic acid	0,45	1,14	0,02	0,35	100	1,09	1,47	0,86	0,19	100
	Acetaminophen	33,21	59,85	14,42	12,81	100	280,61	1032,49	7,56	349,80	100
	Propiphenazone	1,82	3,92	0,76	0,96	100	<LOQ	<LOQ	<LOQ	<LOQ	100
	Phenazone	24,19	67,71	8,40	18,24	100	65,60	336,40	1,56	115,54	100
	Phenybutazone	2,11	3,89	0,70	0,92	100	2,76	5,42	0,78	1,83	100
	Codeine	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq
	Naproxen	104,44	575,35	20,20	154,13	100	29,18	42,31	15,31	10,27	100
LIR	**Clorifibic acid**	4,35	9,38	0,99	2,86	100	1,60	6,34	0,16	1,91	100
	Gemfrobizil	71,53	119,98	26,21	34,72	100	49,17	89,12	18,85	20,89	100
	Benzafibrate	6,86	13,08	2,53	3,34	100	3,78	6,05	2,19	1,09	100
	Fenofibrate	250,94	1244,53	3,37	347,49	100	19,25	29,96	6,67	8,70	100
	Atorvastatine	2,38	5,19	0,60	1,45	100	1,84	3,75	1,12	0,84	100
	Mevastatine	0,28	0,95	0,03	0,27	100	3,65	4,73	3,33	0,41	100
	Pravastatin	8,70	13,73	1,15	4,37	100	4,43	6,52	2,54	1,42	100
PDT	**Fluoxetine**	6,69	25,31	2,34	6,53	100	4,12	15,31	0,18	4,95	100
	Paroxetine	3,85	13,40	1,90	3,39	100	2,86	5,39	0,51	1,68	100
	Diazepam	2,87	6,70	1,07	1,69	100	0,59	2,32	0,12	0,68	100
	Lorazepam	26,54	39,82	6,17	10,53	100	106,02	384,29	6,04	138,57	100
	Carbamazepine	7,00	10,10	1,67	2,68	100	56,24	192,76	20,87	59,22	88,89
HRA	**Famotidine**	nq	nq	nq	nq	nq	3,65	12,99	0,02	4,76	100
	Ranitidine	22,12	40,82	5,99	9,34	100	10,66	21,02	0,22	6,31	100

	Cimetidine	nq	nq	nq	nq	nq	13,34	23,60	7,55	5,34	100
	Loratadine	0,11	0,20	0,10	0,04	100	2,34	8,41	0,32	3,05	100
BBL	**Atenolol**	31,78	72,00	7,61	18,39	100	6,38	19,92	0,41	6,62	100
	Sotalol	11,81	25,68	3,74	6,14	100	6,27	32,95	0,19	11,03	100
	Metoprolol	97,31	280,32	8,36	100,15	100	7,66	37,52	0,13	12,67	100
	Propanolol	3,64	9,55	1,75	2,56	100	3,28	6,40	2,37	1,25	100
	Timolol	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq
	Betaxolol	2,56	6,47	1,26	1,80	100	2,30	3,91	1,83	0,77	100
	Carazolol	0,75	3,18	0,07	0,96	100	<LOQ	<LOQ	<LOQ	<LOQ	100
	Pindolol	0,95	2,52	0,59	0,52	100	6,31	6,37	5,82	0,18	100
	Nadolol	0,98	2,61	0,34	0,62	100	0,85	2,77	0,05	0,93	100
CAT	**Tamoxifen**	nq	nq	nq	nq	nq	40,38	115,04	9,69	31,94	100
FUN	**Metronidazole**	1,19	3,98	0,22	1,02	100	0,49	2,73	0,02	0,86	100
ABM	**Erytromicin**	9,28	29,65	3,32	8,79	100	58,09	362,49	12,26	114,36	100
	Azythromicin	nq	nq	nq	nq	nq	nq	nq	nq	nq	nq
	Roxythromycin	1,02	3,11	0,53	0,92	100	1,04	2,34	0,36	0,79	100
	Clarithromicin	16,85	38,27	4,70	9,55	100	1,62	6,23	0,10	2,08	100
	Tylosin	1,97	7,91	0,78	2,12	100	0,71	2,44	0,12	0,97	100
	Josamycin	0,14	0,65	0,01	0,20	100	2,14	11,09	0,01	3,40	100
	Spyramicin	13,66	28,83	2,81	8,25	100	31,65	152,09	2,00	53,94	100
	Tilmicosin	nq	nq	nq	nq	nq	ND	ND	ND	ND	0
ABF	**Ofloxacine**	nq	nq	nq	nq	nq	ND	ND	ND	ND	0
	Ciprofloxacine	nq	nq	nq	nq	nq	11,50	23,78	3,50	6,21	100
	Norfloxacin	27,14	47,09	5,37	12,60	100	12,31	24,36	2,02	8,36	100
	Enoxacine	29,28	64,00	10,40	17,67	100	126,88	400,94	74,83	106,88	100
	Danofloxacin	nq	nq	nq	nq	nq	110,76	279,19	6,68	81,91	100
	Enrofloxacine	nq	nq	nq	nq	nq	41,04	129,36	20,48	34,59	100
ABT	**Tetracycicline**	nq	nq	nq	nq	nq	ND	ND	ND	ND	0
	Doxicycline	nq	nq	nq	nq	nq	<LOQ	<LOQ	<LOQ	<LOQ	100
	Oxytetracycline	nq	nq	nq	nq	nq	6,61	16,26	1,01	5,21	100
	Chlortetracycline	4,66	11,23	0,58	2,97	100	<LOQ	<LOQ	<LOQ	<LOQ	100
ABS	**Sulfamethoxazole**	33,87	150,93	5,83	43,39	100	25,17	109,49	3,39	33,33	100
	Sulfadiazine	12,90	41,10	5,48	12,02	100	2,58	9,53	0,18	2,97	100
	Sulfamethazine	30,31	198,26	2,69	59,90	100	1,72	6,78	0,22	2,02	100
ABO	**Trimethoprim**	7,88	20,53	0,70	4,91	100	2,01	6,97	0,09	2,53	100
	Chloramphenicol	0,36	0,36	0,36	0,00	100	ND	ND	ND	ND	0
	Nifuroxazide	9,13	20,14	2,84	5,47	100	12,50	12,50	12,50	-	100
BCD	**Salbutamol**	ND	ND	ND	ND	ND	3,53	4,23	1,50	0,97	100
BPR	**Enalapril**	4,49	15,26	1,48	4,24	100	6,12	14,58	1,47	5,58	100
	Lisinopril	30,87	71,55	10,08	18,52	100	3,03	8,07	0,62	3,06	100
DIU	**Furosemide**	53,63	91,33	13,09	34,13	100	42,50	78,19	22,42	17,63	100
	Hydrochlorothiazide	47,42	74,42	16,88	21,14	100	49,82	69,30	30,09	16,78	100
ADB	**Glibenclamide**	0,13	0,46	0,03	0,14	100	0,15	0,93	0,00	0,30	100
BBT	**Butalbial**	12,54	21,12	6,54	4,79	100	ND	ND	ND	ND	0
	Pentobarbital	nq	nq	nq	nq	nq	ND	ND	ND	ND	0
	Phenobarbital	nq	nq	nq	nq	nq	ND	ND	ND	ND	0
VET	**Clenbuterol**	nq	nq	nq	nq	nq	6,69	24,00	0,00	8,42	100
	Flumequine	0,05	0,20	0,04	0,05	100	9,29	37,54	0,23	11,57	100

<LOQ = Below limit of quantification; ND = Not detected; NQ = Not quantified

Figure A.1. Flow measurements recorded at study sites ABR and SJD for each sampling day of the two monitoring campaigns.

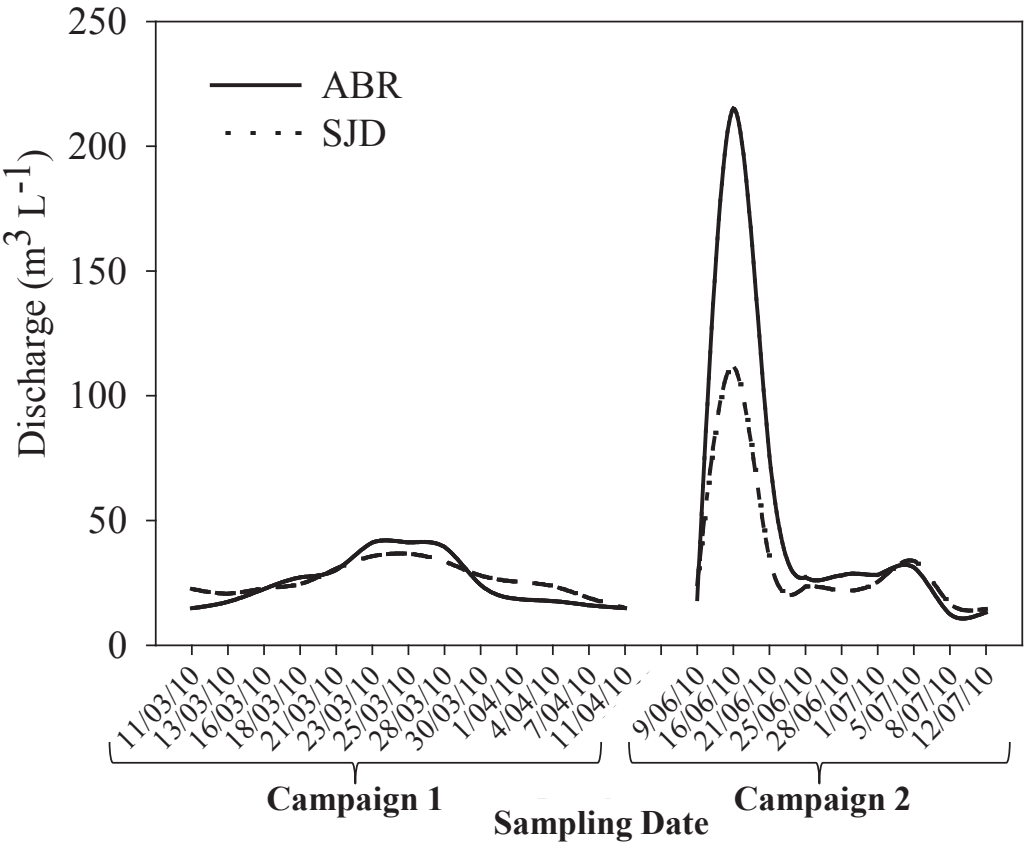
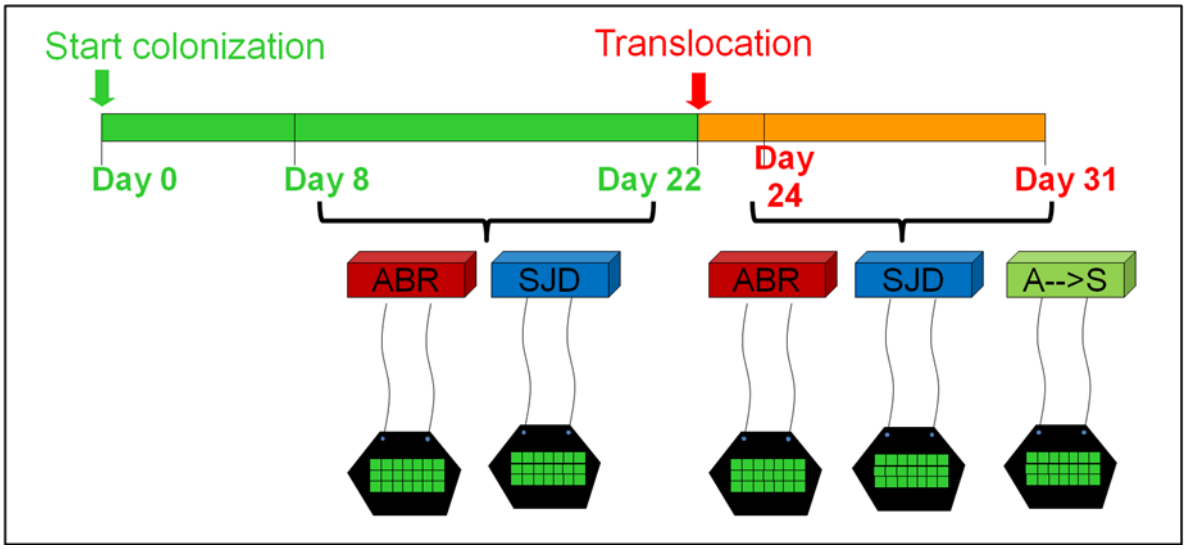


Figure A.2. Experimental design for biofilms translocation



Chapter 6

General discussion

Chapter 6. General Discussion

The present chapter aims bridging this thesis' findings with the most recent literature. Overall, it has been shown within this thesis that PhACs are widely spread micropollutants in the aquatic environment and may be toxic to aquatic ecosystems. The following sub-sections will discuss in detail the results of the three main lines of study conducted in this thesis, namely the analysis, occurrence and effects of PhACs in the aquatic environment.

6.1. Analysis and identification of PhACs and their TPs in WWTPs and receiving SW

The principal source of entrance of PhACs in the aquatic environment are WWTPs. In many cases, the polarity combined with the low microbial degradability as well as other phyisicochemical properties of some PhACs and their TPs result in inefficient elimination in WWTPs and as a consequence they can be detected in treated effluents. DCF and SMX are two widely detected PhACs leading as well to several human metabolites. While their poor degradability under CAS treatment in WWTPs was demonstrated, one study reported (Pérez and Barceló, 2008) that the removal of DCF can be enhanced by promoting the growth of nitrifying bacteria in batch reactors. Two nitrosation/nitration TPs of DCF were identified in that study. Part of the work of the present thesis was to evaluate the presence of these two TPs and others of SMX, in real WWTP samples. First, an analytical method was developed using off-line SPE followed by LC-(ESI)-MS/MS for the analysis of DCF and SMX, their human metabolites and their nitrifying/denitrifying TPs in WWTPs and receiving SW. The developed analytical method enabled to report quantitative levels of DCF and its main human metabolites (4'-OH-DCF, 5-OH-DCF, 4',5-diOH-DCF, 5-OHD-DCFand DCF-gluc) and nitrosation/nitration derivatives (TP323 or NO-DCF, TP 339 or NO_2-DCF) in WWi and WWe (see chapter 3, section 3.2). The occurrence of these compounds was thoroughly discussed in the publication included in this thesis (Osorio et al., 2014b). However, it is worth to note some important findings. Human metabolites were found at higher concentrations in WWi relative to WWe, which was also in agreement with levels of 4'-OH-DCF and 5-OH-DCF recently reported by Larsson et al. (2014). In this thesis, the removal rate of these metabolites was not evaluated due to the inappropriate planning of sampling campaigns such as wrong sample collection and without considering the HRT of the WWTPs. However it was noticed that DCF is not further microbially metabolized into these compounds during the activated sludge treatment. Regarding, the biotransformation of DCF in WWTPs, its nitrosation/nitration TPs were detected and quantified for the first time in the present thesis.

Further, the attention was focused on the TPs of PhACs formed during nitrification/denitrification processes occurring in the NAS treatment in WWTPs. SMX and its nitrifying/denitrifying derivatives (NO_2-SMX and Des-SMX) were also detected in WW and SW (see chapter 5, section 5.2, Osorio et al., *submitted*). Several novel findings can be mentioned: (i) NO_2-SMX and Des-SMX previously identified by Nödler et al. (2012) in GW were measurable in WW and SW; and (ii) nitrosation/nitration TPs of DCF were present in SW. Based on the findings of Chiron et al. (2010) investigating the nitrosation of acetaminophen, the hypothesis in this thesis was that $NO^{\cdot}$ radical species generated during the nitrification/denitrification process may be involved in the formation of nitrifying/denitrifying TPs of DCF and SMX. Although the present study was conducted in a few WWTPs, the relationships observed between levels of nitrifying/denitrifying TPs of DCF

and SMX and some WWTP operational parameters such as HRT and SRT; supported the initial hypothesis on the implication of $NO^{\cdot}$ on the formation of these derivatives. Moreover, the levels of nitrifying/denitrifying TPs detected in WWi and SW led to consider that microbial mediated biotransformation of DCF and SMX might also take place along the collecting sewage system. Indeed, Jelić et al. (2015) demonstrated the in-sewer microbial transformation under anaerobic conditions of several PhACs. A high decrease in concentrations (25-60%) of diltiazem, citalopram, clarithromycin, bezafibrate and amlodipine was observed during their pass through the pressurized pipe. Besides, the authors calculated negative removals for SMX (-66±15%) and irbesartan (-58±25%) in sewers, caused by the conversion of conjugates back to their parent compounds in sewer. Another explanation could be the formation of these TPs via human metabolism of DCF and SMX, their entry into WWTPs through excreta and their subsequent release into receiving SW due to their incomplete removal after WW treatment. For instance, NO_2-SMX was reported as a minor human metabolite of SMX (Bonvin et al., 2012). Thus, further studies should be carried out in order to clarify the source of these derivatives. With this in mind, it was set out to gain further insights into the biotransformation mechanisms of related DCF compounds under NAS treatment in WWTPs (see chapter 3, section 3.3). Thus, controlled biodegradation experiments in batch reactors amended with NAS-mixed liquor from WWTP were conducted. DCF and other NSAIDs with analogous chemical structure were used as model compounds. From these experiments it was observed that no TPs were formed in control abiotic reactors, thus suggesting that a biotic reaction was involved in the formation of nitrosation/ nitration TPs. As the growth of AOB in the batch reactors was favored by maintaining high ammonium concentrations (pH control), it was hypothesized that nitrosation/ nitration TPs originate from NH_3 oxidation by AOB. In order to demonstrate the last hypothesis, stable isotope-labeled $^{15}NH_4$-N was added to the bioreactors amended with DCF. A mass shift of +1 Da expected due to incorporation of ^{15}NO and $^{15}NO_2$ groups into the DCF molecule was observed. Regarding the biodegradation of related NSAIDS (i.e. meclofenamic acid, mefenamic acid, tolfenamic acid and flufenamic acid) this study reported the first evidence of their nitrosation/nitration TPs. The biotransformation rates of parent compounds to their derivatives were generally low possibly due to steric hindrance and/or low microbial biomass. Several studies assessed the effect of operating conditions (e.g. SRT, amount of AOB, concentration of pollutants) in the activated sludge on biotransformation and/or removal of micropollutants in WWTPs (e.g. Suárez et al., 2010; Fernández-Fontaina et al., 2012). After Pérez and Barceló work (2008), very few studies attempted to understand the underlying microbial processes involved in micropollutants biotransformation reactions occurring in WWTPs (Chiron et al., 2010; Helbling et al., 2012; Tran et al., 2013). The lessons learnt from these works are that PhACs can be biotransformed through metabolism by heterotroph microbes or through co-metabolism by AOB and AOA; and that AOB are involved in the generation of NO radicals during the nitrification and denitrification processes. The complete elucidation of the reaction mechanism of the microbial mediated biotransformation process of DCF and its related compounds into nitro and nitroso TPs was not achieved. Thereby, future research by Dr. Sandra Pérez's group would address: (i) the study of the reaction mechanisms of generation of nitrogen reactive species and the incorporation of NO and NO_2 groups into the drug molecule; (ii) the characterization of the diversity and activity of the microbial community in the activated sludge; and (iii) the effects of varying operational conditions of the WWTP on the process.

Importantly, TPs themselves can undergo further transformations. For instance, the cleavage of glucuronides and sulfates to convert back to their parent compounds was reported by Stadler et al. (2012). Unfortunately, this phenomenon was not confirmed when levels of DCF and its glucuronide were measured in

in WWi and WWe during this thesis (Osorio et al., 2014b), probably due to a wrong sampling campaign (see above). However, the conversion of nitro TPs of DCF and SMX (i.e. NO_2-DCF and NO_2-SMX) in denitrifying soil-aquifer systems was observed (Barbieri et al., 2012). This work was conducted in collaboration with Manuela Barbieri (IDAEA-CSIC), and thus the corresponding publication is not included in this thesis. Briefly, the authors investigated the removal mechanisms of DCF and SMX in soil-aquifer processes occurring during artificial recharge of groundwater. Batch experiments were carried out in aquifer material amended with DCF and SMX at environmental concentrations. The observed biotic formation of nitro derivatives of DCF and SMX (the same nitration derivatives identified in the batch reactors amended with sewage sludge in the present thesis) was related to the presence of nitrite generated during denitrification process. However, the reaction mechanism of biotransformation of DCF and SMX and how nitrites were involved could not be described. Interestingly, these nitro-TPs converted back to their parent compounds, what was also previously observed by Nödler et al. (2012) during abiotic denitrification of SMX.

Even though the fate and behavior of PhACs under CAS treatment was not investigated in this thesis, an important contribution to understand their degradation pathways using AOPs was provided. Within the frame of a scientific collaboration with Despo Fatta-Kassinos and Irene Michael (University of Cyprus) the elucidation of TPs of ibuprofen, DCF and trimethoprim generated during the application of solar photo-Fenton, TiO_2 photocatalysis driven by UV-A or simulated solar irradiation, sonolysis, and UV-A photocatalysis integrated with ultrasound irradiation (sonophotocatalysis) to several aqueous matrices at pilot-scale (Michael et al., 2012; Michael et al., 2014b) was conducted. Briefly, twenty-one, seven and ten TPs of trimethoprim, ibuprofen and DCF, respectively, were tentatively identified and attributed to the consecutive attack of hydroxyl radicals (HO•) paralleled with the degradation of the primary compounds. Their degradation pathways were proposed: (i) trimethoprim was transformed by hydroxylation, demethylation and cleavage reactions; (ii) ibuprofen underwent mainly decarboxylation, demethylation and hydroxylation reactions; and (iii) the oxidation of DCF mainly proceeded by oxidation and hydroxylation reactions. The poor mineralization of the three compounds together with their high rate of transformation and the presence of some hydroxylated recalcitrant TPs evidenced that further research is needed before implementing AOPs in WWTPs. Furthermore, other studies reported TPs (e.g. Zwiener et al., 2002; Hernández et al., 2011; Ahmed et al., 2012) which were tentatively characterized as having the same chemical structures known as human metabolites (e.g 1-OH-ibuprofen and carboxy-ibuprofen; 14-OH-clarithromycin; 4'-OH-DCF and 5-OH-DCF, respectively). Interestingly, NO_2-SMX was also identified among the TPs of SMX formed after the application of an ATT based on the sulphate radical reactions (Ahmed et al., 2012). These findings would suggest that diverse processes taking place in the aquatic environment might be involved in the transformation of PhACs.

Different bullet points summarize the issue about TPs: (i) their presence in WW and SW; (ii) their formation by different pathways; and (iii) they can be prone to undergo further reactions such as conversion to the active parent compound. Therefore, in this thesis emphasis was put on the evaluation of the transformation pathways of those not yet studied PhACs and TPs. A given TP can be generated after (i) human metabolism; (ii) biotic transformation mainly mediated by AOB in the activated sludge; and/or (iii) abiotic transformation mediated by HO• in AOP. Moreover, from the present thesis it can be concluded that there is a need to include TPs in thorough monitoring studies in order to improve the understanding of PhACs pathways in the aquatic environment. For example, for a detailed description of the fate of PhACs in WWTPs their TPs have to be

included in the mass balance. This approach, would help to understand the discrepancies in the evaluation of removal efficiencies of PhACs in WWTPs (see table 1.2 in chapter 1).

6.2. Occurrence of PhACs and their TPs and their temporal and spatial fate along WWTPs and Iberian river basins

Regarding the levels of PhACs reported in WW and SW (see section 3.2; and section 5.2), concentrations of DCF remained quite similar along their way from the input to the WWTP until the discharge of the treated effluent into the river; while levels of SMX gradually decreased (Osorio et al., 2014b; Osorio et al. *submitted*). Although WW treatment has been proved to efficiently remove certain PhACs, the emitted concentrations of recalcitrant PhACs and their TPs are still a matter of concern as regards to the impact into receiving freshwater ecosystems.

Aiming to gain knowledge on the presence of PhACs in the aquatic environment, monitoring studies were conducted across Iberian river basins. The initial attention was drawn to SW from WWe impacted sections of the Llobregat River (NE Spain) (see sections 4.2, 4.2 and occurrence data in section 5.2). Afterwards, the study of the occurrence of PhACs was extended to the whole catchment of four major Iberian river basins: Llobregat, Ebro, Júcar and Guadalquivir (see section 4.4). In addition to SW, river bed sediments were assessed. In all studies, a large list of up to 96 PhACs (see table A.1 in annex) were analysed in samples collected during extensive sampling campaigns. In the first study published (Osorio et al., 2012a) a selected list of 66 PhACs was studied along a section of the Llobregat river. Afterwards, 7 extra compounds were included to the previous list to study up to 73 PhACs in the same river (Osorio et al., 2012b; Osorio et al., 2014a). Finally, the most recent study conducted along four Iberian river basins included an updated list of 76 PhACs (Osorio et al., 2015). For the comprehensive analysis of the large volume of data obtained in each study, several approaches were applied, namely: (i) statistics, from simple correlation and sensitivity analysis, to ANOVAs, PCAs and NMDS; and (ii) modeling ("plug-flow" model), from simple equations to more complex tools such as GREAT-ER. The results of these works have been thoroughly discussed in their corresponding publications (Osorio et al., 2012a; 2012b; 2014a; 2015). However, a general discussion tackling the key findings is addressed below.

With respect to the occurrence of therapeutic classes in SW, analgesics and antiinflamatories were undoubtedly the most ubiquitous and most abundant drugs. In the case of sediments, this predominance was shared with antibiotics. Other relevant families in SW were antibiotics, lipid regulators and lowering cholesterol stating drugs and antihypertensives, whereas diuretics and psychiatric drugs were both ubiquitous in SW and sediments. As for individual compounds the most detected and concentrated in SW were as follows: ibuprofen, DCF, naproxen, indomethacine, ketoprofen, acetaminophen, iopromide, carbamazepine, lorazepam, gemfibrozil, bezafibrate, valsartan, irbesartan, losartan, hydrochlorothiazide, furosemide, atenolol, tetracycline, ofloxacin, thiabendazole and metronidazole. Sertraline, ketoprofen, hydrochlorothiazide, tetracycline, codeine, ibuprofen, clarithromycin and azithromycin were detected at higher levels in sediments. Hydrochlorothiazide, gemfibrozil, ibuprofen and azithromyzin were the most ubiquitous in SW and sediments at relative similar concentrations. These findings were in agreement with the data reported by Carmona et al., 2014 but opposite to the findings of Löffler et al., (2005) who reported that ibuprofen showed no significant

affinity for sediment, due to its physicochemical properties (see table A.1 in annex). These observations reflect the complex distribution processes of PhACs between the water/sediment phases. In addition, it also reveals that PhACs partitioning in water compartment is not only dependent on their physicochemical properties such as solubility, but it also depends on the conditions of the aquatic system, namely: (i) the physicochemistry, such as pH and composition of SPM; (ii) hydrology including river flow regime; and (iii) morphology, such as sediment bed topography (e.g. ripples).

The observed temporal and spatial distribution of the studied PhACs along the river basins may be the consequence of several factors or a combination of them, namely: (i) high human and animal consumption patterns; (ii) high percentage of excretion of un-metabolized drug; (iii) direct flush of non-consumed drugs into sewage system; (iv) low removal in WWTPs; (v) re-transformation of TPs back to the active compound; and (vi) natural attenuation in rivers.

For example, analgesics and antiinflammatories, lipid regulators and cholesterol lowering statin drugs, psychiatric drugs and antihypertensives were among the mostly sold therapeutic groups in Spain during 2010 (see figure 1.3 in chapter 1). Besides, acetaminophen, ibuprofen and lorazepam were among the top sales. Even though it cannot be assumed a direct correlation between sales data and consumption rates, the high environmental levels of some therapeutic groups and individual PhACs observed in this thesis closely matched with those identified by the Spanish National System as those mostly sold (SNS, 2012). It is a challenging task to relate occurrence of PhACs to consumption trends in Spain since updated and detailed data on medical prescriptions of drug products is unavailable. However, predicted consumption rates of PhACs (Ortiz et al., 2013) are reliable data to relate with their environmental occurrence. Therefore, the potential relationships between levels of PhACs measured in SW and sediments on the one hand with the population density and on the other hand with livestock units in the four river basins were examined in the present thesis (Osorio et al., 2015). Similarly, a recent study carried out in freshwater systems in Taiwan, correlated the spatial distribution of PhACs with the principal source contributors (i.e. domestic inputs from human use, antibiotics inputs from animal-use and medication inputs from hospital-use) in the catchments (Jiang et al., 2015). However, the relationship between PhACs profile and human/animal pressures of the areas studied were merely descriptive (Jiang et al., 2015). Whereas, in this thesis region-specific quantitative relationships among levels of PhACs, density of population and livestock were evaluated (Osorio et al., 2015). Furthermore, a significant positive correlation between human population and livestock density with the concentration of PhACs in SW and sediments was demonstrated. Still, this relation was not proportional (i.e. levels of PhACs did not increase in the same order than human density or livestock) evidencing the contribution of other anthropogenic and natural factors on the variability of the levels of PhACs in the aquatic environment. For instance, during this thesis, a gradient of PhACs pollution increasing downstream at the Llobregat River together with the number of WWTPs was observed. WWe were regarded as the main emission source of PhACs to SW and the principal cause of this pollution gradient (Osorio et al., 2012a; 2012b; 2015). In fact, this assumption has been recently confirmed in highly urbanized regions across China (Wang et al., 2015). In this study, PCA applied to 34 PhACs measured in several urban river samples as well as discharging WWe revealed the contribution of the WWTPs distributed along the rivers monitored to PhACs pollution observed in their SW. Moreover untreated WWe was identified as the main source of PhACs in the Beiyun River (China) (Dai et al., 2015). In the cited work, the influence of untreated and treated WW

on the PhACs contamination in the Beiyun River was quantitatively demonstrated (67%) applying PCA-MLR analysis on levels of 15 PhACs measured in WWi, WWe of the WWTPs distributed along the river and SW.

These observations agreed that in river basins characterized by heavy anthropogenic pressure, such as the Llobregat, the expected natural attenuation of PhACs along the river course might be counteracted by the continuous entry of PhACs via the dominant WWe discharge. Nevertheless, although this effect might not occur in less densely populated and industrialized regions, such as rural areas; WWe discharge still represents the major burden of PhACs to receiving SW (Nebot et al., 2015). Therefore, the assessment of the spatial distribution of PhACs all along the river basins should also include less anthropized areas. On the other hand, some of the compounds studied in this thesis (e.g. hydrochlorothiazide, gemfibrozil, norfloxacin and DCF) behaved oppositely with decreasing concentrations downstream the water course (Osorio et al., 2012b). Moreover, it was impossible to describe any clear trend on the behavior of PhACs along the remaining river basins assessed in this thesis (Osorio et al., 2015). These findings evidenced the complexity of factors influencing the variable behavior of PhACs.

Among these, natural biodegradation and photodegradation processes may play an important role on the attenuation of PhACs in the river water. As it was already mentioned in section 1.7.2 (chapter 1), the biodegradation activity of microorganisms in the water/sediment interface or the river bed sediments is relevant (e.g. Li et al; 2015). For example, the fate of 19 PhACs during experiments performed in bench-scale flume simulating the boundary conditions of the hyporheic zone (i.e. the region beneath and alongside the stream bed) (Li et al., 2015). The persistence of PhACs was reported to span from readily degradable (DT_{50} = 1.8 days (e.g. acetaminophen, ibuprofen) to not degradable (chlorthalidone, and fluconazole). Besides, the authors identified the formation of 11 TPs (carbamazepine- 10, 11-epoxide, metoprolol acid) (Li et al., 2015). Natural photodegradation of PhACs has been widely investigated (Schulze et al., 2010; Gonçalves et al., 2011; Bonvin et al., 2012; Zonja et al., 2015). In collaboration with Carlos Gonçalves (IAREN-University of Porto), the photodegradation pathways and rates of the antivirals oseltamivir ester and oseltamivir carboxylate (Tamiflu) under artificial and natural solar irradiation were evaluated. Simulated solar irradiation at lab-scale was proved to photodegradate oseltamivir carboxylate and oseltamivir ester in about 150 and 15 days, respectively. However, the identified photo-TPs were more recalcitrant towards further photodegradation than their parent compounds. Importantly, this natural attenuation process was demonstrated to occur in the field, since half-lives of some TPs, were detectable in the Ebro River (NE Spain).

Another key factor for attenuation is the dilution effect which is dependent on river flow and water anthropogenic uses. In view of that, the aim of this thesis was to determine to what extent the levels of PhACs were related to river flow and to identify as well which compounds were more sensitive to dilution effects at different flow regimes. Thus, the correlations between measured PhAC concentrations and recorded river flow over one month sampling campaigns in the Llobregat River (9-13 samples) were evaluated (Osorio et al., 2012a; 2012b). Results, allowed to classify the selected PhACs (66 and 18, respectively out of the 96 PhACs studied in this thesis) in three categories according to the correlation of concentration to river flow by: (i) positively related and thus not affected by dilution effects (only acetaminophen); (ii) negatively related and thus affected by dilution effects (e.g. SMX); and (iii) positively and negatively related depending

on the location studied and thus similarly related to additional factors (e.g. DCF). In addition, PhACs were classified according to their sensitivity to river flow, which allowed to identify those compounds being more sensitive to dilution effects (enrofloxacine < furosemide < ibuprofen < fluoxetine < SMX <propylphenazone < erythromycin). Moreover, the importance of DOC as a factor influencing the behavior of PhACs in rivers was examined. Thus, the relationships between PhAC concentrations and DOC in SW were established (Osorio et al., 2012a). Environmentally acceptable positive correlations between concentrations and DOC for the majority of PhACs were observed. Besides, PhACs showed a higher sensitivity to DOC, as compared to the one determined for river flow. These findings were indeed expected, since association of solutes to DOC increases with the amount of chemical in the aqueous phase (Tolls, 2001). However, despite the negative correlation between DOC and river flow, several PhACs showed positive correlations with both parameters in the work presented in this thesis. The phenomenon of sediments re-suspension under turbulent flow regime in rivers was hypothesized as a good explanation for the observed increasing concentrations of PhACs with both DOC and river flow. When this process happens, a certain fraction of compounds adsorbed to the sediment is transferred to the aqueous phase, thus modifying the water/sediment partitioning of the compound. Thus, sediments and SPM can be a sink and an additional source of PhACs to SW during seasonal peaks in river flow or under certain circumstances such as dredging or flood events.

The Llobregat River is characterized by highly variable hydrological conditions and seasonal rainfall. The expected effects of this temporal variability on the behavior of PhACs in the river were investigated. To that aim, the lower course of the Llobregat River was monitored during four months, one in each of the four seasons of the year (Osorio et al., 2012b). Overall, results revealed that PhACs were more concentrated in SW during dry and cold periods corresponding to fall and winter seasons, whereas they were less concentrated in rainy and warm periods corresponding to spring and summer seasons, respectively. Taking into account the aforementioned factors affecting the fluctuations of levels of PhACs in rivers, the seasonal trends of PhACs levels can be explained. During fall and winter PhAC concentrations in SW where higher due to: (i) high human/animal consumption; (ii) low temperatures and thus reduced removal efficiency in WWTPs and the freshwater column; (iii) less dilution efficiency due to lower water flow; and (iv) less intensity of solar radiation and thus slower photodegradation rates. Obviously due to a higher degradation, lower concentrations of PhACs in SW during spring and summer seasons were detected during this thesis. In a similar seasonal study carried out in the Beiyun River (China), higher levels of PhACs were observed in late winter/early spring (Dai et al., 2015). In the cited work, the concentrations of the 60% of PhACs analyzed were higher in the dry season (March), with median concentrations 2.6 times greater than other seasons. This observed spring peak concentration was explained by two principal factors: low flow (from November to April is the typical low water season in Beijing) and cold-water temperature (the microbial activity and thus PhACs biodegradation, decay during periods of cold weather like March when average water temperature was 9.6ºC) (Dai et al., 2015). Another work reported dilution effects on PhAC concentrations in WWi from urban WWTPs in China after a rainfall episode (Sui et al., 2015). In the cited study, mean concentrations of ten PhACs measured in the WWi decreased by 5–76 % after rainfall due to the dilution of raw sewage by rainwater, which infiltrated into the sewer system. In the WWTPs located in the suburb area, the increased flow of WWi led to decreased removal efficiencies of some compounds. For instance, the removal efficiencies of trimethoprim and metoprolol decreased from 78 and 58% to 21 and 29%, respectively, after the flood event. On the contrary, the influence of rainfall did not affected the levels of PhACs in urban WWTPs, which was

explained by the probably almost unchanged influent flow, good removal performance, or the use of a bypass system to pump the incoming extra sewage to receiving waters (Sui et al., 2015). Unfortunately Sui et al. (2015) did not analyze the receiving SW to evaluate to what extent levels of PhACs in the river were affected by rainfall by passing the WWTPs. The effects of a flash-flood event, which occurred during a heavy rainfall episode, on levels of PhACs in SW were evaluated in the Llobregat River during this thesis (Osorio et al., 2012b; 2014a). While dilution effects were observed upstream the river, concentrations of PhACs increased downstream at the river after the flash-flood event. Though WWe were not monitored, the concentration effects were hypothesized to be explained by the decreased removal efficiencies of PhACs in WWTPs after the flash-flood episode. Importantly, the findings of Sui et al. (2015) support the hypotheses made on this thesis. On the other hand, the natural dilution effects of PhACs levels observed in the upper course of the Llobregat after the rainfall might not be counteracted due to lower amount of WWTPs distributed along the river and thus, the lesser contribution of WWe discharge.

Even though the efforts made to assess the occurrence and behavior of PhACs along rivers and the plausible explanations to the observed trends provided, a holistic understanding combining all the above mentioned factors is still lacking. Therefore, a rough “plug-flow” model was applied in this thesis with the aim to facilitate the evaluation of the role of factors influencing the fluctuation of PhAC concentrations downstream the river (Osorio et al., 2012b). Hence, the concentration of 14 PhACs was modelled at two monitoring sites of the Llobregat River, located downstream to an emission source that was assumed to be associated to the discharge of a pooled aggregation of several WWTPs. According to the “plug-flow” model described by Pistocchi et al. (2010) the following parameters were considered: (i) the river length between the emission source and the control point, which was a weighed value including the distance to every upstream WWTP and the corresponding annual effluent discharge; (ii) the river flow; (iii) the hydraulic residence time from the emission to the point of measurement (calculated as a weighing variable considering the distance to every upstream WWTP using a digitized river network in a GIS platform and the corresponding annual effluent volume); and (iv) the first-order decay constant (k) of the PhAC which was assumed to embody all the contributing attenuation processes (i. e. biodegradation, photodegradation, dilution, sorption to suspended solids and sediments). Importantly, the validity of the modeling approach was demonstrated considering similar k values for a given PhAC at both locations. In addition, the calculated emissions of PhACs were higher at the location downstream the river. These findings were in agreement with the higher levels of PhACs determined, that could be explained by the higher amount of WWTPs discharging their WWe along the lower course of the river. Consequently, fitted k and emission values were proposed to be used as reliable descriptors of aggregate properties of the watershed upstream sampling points. Furthermore, results from fitted models allowed the estimation of attenuation trends of PhACs. For example, furosemide, enrofloxacin, enalapril, acetaminophen, DCF, and ketoprofen showed k values between −0.04 and −0.10 h^{-1}. Interestingly, erythromycin was identified to be apparently more rapidly removed from the water course (k≈ −0.15 h^{-1}); while other compounds showed more conservative behavior. A few PhACs such as SMX showed positive k values. Owing to the assumptions made in building this simple model, a conclusive explanation could not be provided. Possible reasons were proposed: higher human consumption trends of this drug, higher emissions from WWTPs due to lower removal rates; or less efficient attenuation in rivers or even sediments re-suspension effects. Nonetheless, the approach applied in this thesis was regarded as a reliable tool for the prediction of the fate of PhACs in the aquatic environment.

More recently, the in-stream attenuation of 75 PhACs in 4 river segments of the Ebro River was assessed in order to evaluate the variability of attenuation rates among different PhACs as well as among river segments differing in environmental conditions (Acuña et al., 2015). The authors observed that attenuation was highly variable among PhACs and river segments, but none of the considered physicochemical properties proved to be relevant in determining the mean attenuation rates. Interestingly, they found that the log K_{ow} influenced the variability of rates among river segments, which was explained by its effect on sorption to sediments and suspended particles, thus influencing the balance between the different attenuation mechanisms (biotransformation, phototransformation, sorption, and volatilization). The important conclusion of the mentioned study was that all the natural attenuation processes involving PhACs may undergo along their fate on river courses as well as dilution effects should be considered as important when aiming to predict concentrations in freshwater ecosystems.

Keeping these considerations and previous findings in mind, within the framework of the SCARCE project, the collaboration with Joana Aldekoa and Félix Francés (Politecnic University of Valencia) was set out to implement the model GREAT-ER in the Llobregat River basin, in order to study the behavior of DCF. To that end, the concentrations of DCF measured across the Llobregat River basin (Osorio et al., 2015) were used to develop and calibrate the model by estimating the accuracy of DCF predicted concentrations (Aldekoa et al., 2013). The geo-referenced model predicted the spatial pattern of DCF concentration across the river network. In addition, the model was able to estimate concentration values in most sampling points with an acceptable error (i.e. the minimum root mean square error obtained was of 3.9 ng L^{-1}; while the average error for all measured concentrations was 28.1 ng L^{-1}), thus proving to be a more accurate model than a previously developed one (Alder et al., 2010). As confirmed in other studies (Whelan et al., 1999; Johnson et al., 2007; Ort et al., 2009), it was demonstrated that the GREAT-ER model would be a useful tool for simulating PhAC concentrations in rivers and thus providing a better understanding of their fate along the water course. The application of a modeling approach to environmental studies would ultimately benefit the assessment of water quality and as a consequence the management of water resources. In view of the previous results on GREAT-ER, it can be considered that when measured PhACs concentration data is unavailable, predicted data could be as reliable as measured data in the field, reducing the costs and efforts of the large and long-term monitoring studies (i.e. Navarro-Ortega et al., 2012; 2015). Unfortunately, the accuracy of predicted data is affected by rather limited and uncertain available data for the calibration of models, such as hydrological variables, removal rates and emissions of PhACs from WWTPs, natural attenuation rates of PhACs and human/animal consumption trends. Furthermore, predicted data requires to be compared with measured data to confirm its validity (Celle-Jeanton et al., 2014). At this point of time there is no need, however, to analize all PhACs in the environment. Instead, the efforts should be focused on the assessment of those compounds ecotoxicologically relevant to the aquatic environment. To that aim a selection criterion, such as described for ERA procedures in section 1.9 (chapter 1), would be needed in an initial step. Afterwards PhACs would be rated with a certain degree of relevance, to finally come to a selected or prioritized list of those considered more important according to the criteria applied. The WFD pioneered these approaches releasing lists of priority hazardous substances, priority substances, and more recently, the watch list of concerning substances; which are under permanent revision and thus periodically updated (Directive 2013/39/EU and Decision 2015/495/EU). Prioritization is also being included in current research on PhACs in the aquatic environment (Riva et al., 2015; Daouk et al., 2015). Several prioritizing criteria

can be considered for limiting the number of PhACs to be studied in the aquatic environment, namely: (i) the likeliness of their occurrence; (ii) sales volumes or consumption data; (ii) metabolic and excretion rates after human/animal consumption; (iii) fate in WWTPs; (iv) persistence in freshwater systems; and (v) risk for environmental human health (e.g. toxicity or bioaccumulation) (Riva et al., 2015; Daouk et al., 2015). It can be considered that the wider are the selection criteria, the more realistic would be the list of priority PhACs in the aquatic environment.

On the whole, research on the occurrence, fate and behavior of PhACs and their TPs in the aquatic environment has steadily spanned mainly due to the development of sensitive analytical techniques capable of measuring these substances at trace and ultra-trace levels. Many PhACs have been demonstrated to be pseudo-persistent in the aquatic environment, mainly due to high human/animal continuous consumption, incomplete removal in WWTPs and limited alternatives in the industry. Until recently, the efforts to prevent the entry of PhACs in the aquatic environment have been only directed to the improvement of WW treatment technologies and, to a lesser extent, to the collection of unused medicines (Daughton, 2013). Importantly, the scientific community is drawing their attention to upstream preventive measures targeting PhACs prescription. Thus, the issue of PhACs is being addressed by different sections of their life cycle, from its design to release (Daughton, 2014; Daughton and Ruhoy, 2013). Interestingly a recent work has described a Bayesian network based on socioecological impact assessment of a set of measures aimed at reducing the entry of metformin and metoprolol in the aquatic environment (Brandmayr et al., 2015). The measures investigated were selected across three sectors: public health market, environmental politics and drug design innovation. The results of the model allowed the identification of a spectrum of measures that should be implemented in order to reduce the emission of these PhACs in the aquatic environment (e.g. improved drug removal in WWTPs, prescription of alternative drugs, preventive health measures, drug disposal, healthcare consulting and improved drug bioavailability).

Nonetheless, the ongoing continuous release of PhACs from WWTPs generally triggers a downstream increase in receiving rivers that can be steady under different hydrological conditions and that can therefore have long-term consequences for biological communities. For instance, the effects of WWe as the principal source of PhACs, regarded as stressors, and nutrients, considered as subsidizers; were assessed on river biofilms and ecosystem metabolism in one river segment following a pollution gradient from a WWTP (Aristi et al., 2015). The study concluded that WWe can alter the balance between autotrophic and heterotrophic processes and produce spatial discontinuities in ecosystem functioning along rivers as a consequence of the mixed contribution of stressors and subsidizers.

6.3. Ecotoxicological effects of PhACs and hydrology as relevant stressors of the aquatic ecosystems

Concerned about the consequences of the pseudo-persistence of PhACs in the aquatic environment from an ecological point of view, this thesis also contributed to the knowledge of the effects of PhACs on aquatic ecosystems (see chapter 5). The first publication included in this chapter (Osorio et al., *submitted*; in section 5.2) presented the results of the acute toxicity assessment of DCF, SMX and their nitrifying/denitrifying

derivatives on aquatic organisms (i.e. *D. magna* and *V. fischeri*). Calculated LOECs values for DCF and SMX revealed that, at the general levels that these PhACs are present in the aquatic environment, they are not expected to pose any ecotoxicological risk to aquatic species such as *D. magna* and *V. fischeri* after short-term exposure. Overall, the results of this thesis were in accordance with literature. For instance, toxicity of DCF and SMX to *D. magna* was in the same concentration order than that observed by Cleuvers (2003) and Kim et al. (2007).

While the ecotoxicological effects of PhACs are relatively well documented compared to other substances of emerging concern (Farré et al., 2008; Brausch et al., 2012; Vásquez et al., 2014) there is a substantial gap of information referred to the potential threats of TPs of PhACs to aquatic ecosystems. In the past, only sparse studies included the toxicity assessment of TPs such as that conducted by Henschel et al. (1997), who demonstrated the acute adverse effects of active metabolites of PhACs (e.g. salycilic acid and clofibric acid) towards non-target organisms (e.g. *D. magna*, algae and bacteria). Importantly, the contribution to the knowledge of the ecotoxicity of TPs of drugs has gradually increased in the recent years (e.g. Rosal et al., 2010; Majewsky et al., 2014; and Rubirola et al., 2014). For example, among the PhACs and metabolites tested, Rosal et al. (2010) reported the highest toxicity to *V. fischeri* of fenofibric acid (i.e. EC_{50} = 1.7 mg L^{-1}), the metabolite of the lipid regulator fenofibrate. Similarly, Rubirola et al. (2014) evaluated the potential acute toxicity to *V. fischeri* of metoprolol and its TPs identified after CAS treatment in lab scale batch reactors. Importantly, the ecotoxicity studies conducted by the authors revealed that the metabolite O-desmethylmetoprolol exhibited more acute toxicity (i.e. EC_{50} =18 mg L^{-1}) than its parent compound (i.e. EC_{50} = 65 mg L^{-1}). As a matter of fact, the main interest of the study presented in this thesis was to find out whether the nitrifying/denitrifying TPs of DCF and SMX were more toxic to sensitive aquatic species, or not. The comparison of toxicity values to *V. fischeri* determined for SMX, Des-SMX and NO_2-SMX revealed that TPs displayed higher toxicological effects than their parent drug (e.g. EC_{50} values for SMX was >>100 mg L^{-1}; while for Des-SMX and NO_2-SMX these were 89.3 and 41. 4 mg L^{-1}). Moreover, NO_2-DCF (EC_{50} =11.7 mg L^{-1}) did also display higher toxicity to *V. fischeri* than DCF (EC_{50} = 22.9 mg L^{-1}). However, calculated LOECs for TPs were at several orders higher than the concentrations determined in WW and receiving SW. For instance LOECs of Des-SMX and NO_2-DCF in *V. fischeri* were respectively 48.4 and 1.8 mg L^{-1}; while respective levels determined in WWe were 11.4 and 3.62-4.94 ng L^{-1} and 8.04-17.7 and <MQL-2.64 ng L^{-1} in SW (Osorio et al., *submitted*).

The consequences of long-term exposure to DCF and SMX are still limited or entirely unexplored as is the case of their nitrifying/denitrifying derivatives. Particularly, a few examples were discussed by Oliveira et al. (2015) who did also report no significant reproductive impairment for *D. magna* exposed to DCF concentrations ranging from 29.5 to 72 mg L^{-1}. On the other hand, Sarma et al. (2013) observed the opposite response for the rotifer *Plationus patulus* and the cladoceran *Moina macrocopa* at DCF exposure levels of 1.56-25 mg L^{-1}. Likewise, Lee et al. (2011) reported a significant reduction of population grow rate of *D. magna* exposed to DCF at concentrations of 0.93–25 mg L^{-1}. In addition, they reported a chronic EC_{50} value of 23.8 mg L^{-1}, which is several orders of magnitude higher than levels of DCF detected in the aquatic environment and thus chronic effects of DCF were not expected to occur in the aquatic environment. Nevertheless, more studies that support these observations should be needed, as well as the inclusion of other non-target aquatic

organisms (e.g. algae, fish) to come to a definitive conclusion about the potential long-term effects of DCF, and likewise any PhAC, to aquatic ecosystems.

Moreover the co-occurrence of PhACs together with their TPs in complex environmental samples such as WW and collecting SW, can lead to additive and synergistic or antagonistic effects on non-target aquatic organisms (Farré et al., 2008; Ginebreda et al., 2014; Vásquez et al., 2014; Backhaus et al., 2014). For example, low-dose effects of PhACs were observed for a mixture of 10 quinolone antibiotics and also for a 12 drugs mixture with different modes of action (Backhaus et al., 2000a; 2000b). Even mixtures of only comparatively few compounds often show a similar pattern. A mixture of fluoxetine and clofibric acid killed more than 50% of a daphnia population after an exposure of 6 days, although the individual components were only present at concentrations that did not induce significant effects (Flaherty and Dodson, 2005). Also in binary mixtures it was observed that trimethoprim shifts the concentration response curve of SMX and sulfadiazine by a factor of 4-5 towards higher toxicities, even if present only at its NOEC (Egucci et al., 2004). Similarly, clear synergistic effects to algae were observed for mixtures of flumequine+erythromycin and oxytetracycline+flumequine by (Christensen et al., 2006). Acording to these evidences, greater ecotoxicological effects than predicted can be expected when assessing the toxicity associated to mixtures of PhACs, with both similar and dissimilar modes of action, and with other pollutants of the aquatic environment as well. In fact, synergistic, and to a lesser extent antagonistic, effects were observed within this thesis when DCF, SMX and NO-DCF were mixed with other environmentally relevant compounds (i.e. nonylphenol, malathion, diuron, glyphosate and triclosan) (Osorio et al., *submitted*). These findings are in agreement with the available evidences that have mainly reported synergistic effects for binary mixtures of PhACs (Backhaus et al., 2014). To the author's knowledge, the effects of synergistic or antagonistic interactions among PhACs and their TPs are a rarely investigated field.

Nevertheless, the possible biotransformation of PhACs during WW treatment has been recently considered in ecotoxicological assessment studies of treated WW (Michael et al., 2012; 2014b; Czech et al., 2014). These investigations have been mainly leaded to the implementation of ATTs in WWTPs. For instance, Michael et al. (2012; 2014b) performed biolumiscence inhibition assays on *V. fischeri* and inmobilization tests on *D. magna* to evaluate to what extent the toxicity associated to WW was reduced after the application of a solar AOP treatment. As it has been previously described, the authors conducted photodegradation experiments on simulated WWe containing individual PhACs (i.e. ibuprofen, DCF and trimethoprim) in reactors at lab scale. They assessed the toxicity profile of each drug in WW during the application of the advanced treatment and attributed the varying effects observed to the TPs that were generated at different stages of the process. The authors concluded that the intermediate TPs generated during the oxidation of trimethoprim did not exhibit any toxic effects to *V. fischeri*. As for DCF and ibuprofen, they demonstrated the capacity of sonophotocatalysis treatment to reduce the initial toxicity of these PhACs towards *D. magna* yielding 20% and 40% immobilization, respectively, at the end of the treatment.

Importantly, a recent research on the ecotoxicological effects of PhACs in the aquatic environment has encompassed the potential contribution of natural photo-TPs to the whole toxicity of a given environmental mixture (Wang et al., 2014). For instance, despite that solar photodegradation has long been considered a significant natural attenuation process of PhACs in SW, and thus decreasing their ecological risk, Wang et

al. (2014) identified for the first time the increased toxicity to *V fischeri* of an irradiated mixture of 27 PhACs (e.g. SMX, ofloxacin, trimethoprim, ibuprofen and DCF) in SW at environmental concentrations. Interestingly, since the compounds included in the mixture had been previously reported to undergo photo-transformation (Chowdhury et al., 2011; Jiao et al., 2008; Li et al., 2011; Lin et al., 2013; Trovó et al., 2009; Wang and Lin, 2012), they attributed the higher toxicity of the irradiated SW to the synergistic effects of photo-TPs of PhACs generated. These findings revealed the lack of comprehension of the environmental implications of natural transformation of PhACs and the risk posed by the subsequent TPs to the aquatic ecosystem.

All in all, although certain PhACs, and their TPs, may not pose any ecotoxicological threat to aquatic species under short-term exposure, their contribution to the whole toxicity of complex environmental mixtures should be evaluated. Moreover, to unveil their magnitude and biological significance on freshwater ecosystems the possible interactions (i.e. synergistic and antagonistic effects) among PhACs, other contaminants of environmental concern and their TPs that might occur in the water bodies should be assessed. Therefore, understanding the fate of mixtures of pharmaceutical compounds and their chronic effects on aquatic organisms is a challenge for water management agencies.

Following up with the exploration of ecotoxicological effects on non-target organisms associated to the presence of complex mixtures of PhACs in the aquatic environment, a risk assessment of 55 PhACs determined along four Iberian river basins was conducted (Osorio et al., 2015, in section 4.4). To that aim, the concentration addition model for mixtures of substances (Ginebreda et al., 2014) combined with laboratory-based toxicity data was used to calculate total TUs of PhACs present in every sample collected along the water course. Individual TU values estimated for the selected PhACs revealed no significant acute risks to the tested aquatic organisms. However potential chronic ecotoxicological effects on algae could be expected at two hot spots of PhACs pollution identified in the Llobregat and Ebro basins (i.e. LLO7 and ZAD, respectively). At the region-specific level, the Llobregat and the Ebro river basins were characterized as at highest ecotoxicological risk, followed by Júcar and Guadalquivir. Hot spots of ecotoxicological risk were identified in every basin (e.g. LLO7 in Llobregat; ZAD in Ebro; GUA6 in Guadalquivir; and JUC7 in Júcar). On the other hand, the less polluted locations with lower risk were also identified (e.g. CAB5 in Júcar; LLO2 in Llobregat; ESE in Ebro; and GUA1 in Guadalquivir). The need for prioritization of PhACs has already been mentioned. For that reason, the relative contribution of the different substances to the total toxicity in the locations sampled was calculated in order to list the priority PhACs at the Iberian Peninsula catchments. In addition, considering that the relative contribution of each PhAC to the ecotoxicity may vary according to its individual toxicity and concentration, the compounds that were contributing most to the total toxicity of the water at each site were identified. Sertraline, erythromycin, losartan, dimetridazole, loratidine and fluoxetine contributed at least 5% to the total toxicity of the SW sample. Among these, sertraline, gemfibrozil and loratidine were regarded as the compounds of emerging concern in these river basins (Osorio et al., 2015).

The characterization of PhACs by their potential ecotoxicological risk to non-target aquatic organisms was performed in numerous studies (e.g. Hernando et al., 2006; Ginebreda et al., 2010; Gros et al., 2010; Damásio et al., 2011; Pereira et al., 2015; Johnson et al., 2015; Kuzmanović et al., 2015; de Castro-Català et al., 2015a; 2015b). Importantly, Hernando et al. (2006) applied for the first time a preliminary approach, to characterize the environmental risk for PhACs (i.e. antibiotics, analgesics and antiinflammatories,

lipid regulators, β-blockers, antiepileptics and steroid hormones) most frequently detected in WWe, SW and sediments at the global scale (Hernando et al., 2006). To that aim, occurrence data collected from literature was used to calculate HQs based on acute toxicity data on aquatic organisms (bacteria, algae and invertebrates). High risk was suspected to be induced in WWe for the following drugs: antibiotics (erythromycin), antiinflammatories (ibuprofen, naproxen, DCF, ketoprofen), lipid regulators agents (gemfibrocil, clofibric acid), β-blockers (propanolol, metoprolol) and antiepileptics (carbamazepine). High risk was also suspected in SW for antiinflammatories (ibuprofen, naproxen, DCF, ketoprofen) and antiepileptics (carbamazepine). Reported concentrations in sediments for these drug residues were not suspected to induce risk in this compartment (Hernando et al., 2006). More recently, an ERA of eleven PhACs was conducted in WWi and WWe from Portuguese WWTPs by means of HQs to different aquatic organisms (i.e. algae, daphnids and fish) (Pereira et al., 2015). According to their estimated HQ values (>1), ciprofloxacin, bezafibrate, gemfibrozil, simvastatin and DCF; adverse effects were expected to aquatic organisms. A similar study conducted at a larger scale, aimed to determine the potential ecotoxicological risk associated to antibiotics (i.e. ciprofloxacin, SMX, trimethoprim and erythromycin) present throughout European rivers (Johnson et al., 2015). Levels of antibiotics were estimated from reviewed available data on national consumption rates, excretion and WW treatment removal rates. As both predicted and observed WWe concentrations were below reported effect levels for the most sensitive aquatic organisms, a direct toxicity in rivers was not expected. However, predicted and observed river concentrations for ciprofloxacin and erythromycin were closest to effect levels in aquatic biota (2 orders of magnitude lower), followed by SMX (3 orders of magnitude lower). In view of these results, ciprofloxacin and erythromycin were regarded by the authors as PhACs of concern to the aquatic ecosystems (Johnson et al., 2015).

None of the total TUs calculated in the work presented in this thesis at every site of the Iberian catchments assessed for algae, Daphnia and fish, exceeded the unit value (Osorio et al., 2015). Therefore, according to standard thresholds (Malaj et al., 2014), no acute risk associated with PhACs was observed. However, though only for LLO7 and ZAD, the corresponding total TU values for algae were estimated above ~1E − 03 in both sampling campaigns, evidencing the potential long-term ecotoxicological effects on this primary producers (Malaj et al., 2014). Though the likeliness of acute toxicity effects of PhACs was considered as minor in all these studies, their contribution to the mixture toxicity as well as their potential long-term effects needs further studies. Thus, further risk considerations should be considered concerning to chronic effects of PhACs. These results highlight the importance of conducting such monitoring and ERA studies to support future prioritization measures by water authorities.

A critical issue for any of study that strives to analyze the impact of PhAC mixtures in the field, is to distinguish effect-directed links between the compounds present in a given compartment of the environment (e.g. water column or be sediments) and field observed ecotoxicological effects. This challenging task has been tackled by the use of correlation based methods, employing translocation experiments and advanced chemical analytical surveys. For instance, Muñoz et al. (2009) investigated the correlation between the occurrence of 21 PhACs and benthic community structure. The authors observed adverse effects on the diatom diversity at one of the polluted sites, but no significant overall correlation between diatom biodiversity and PhAC concentrations. However, such a correlation was found between the occurrence of indomethacin, propranolol, atenolol and ibuprofen and the abundance and biomass of several benthic invertebrates

(Chironomus and Tubifex). Diversely, Ginebreda et al. (2010) based their mixture risk assessment on the addition of hazard indices, following the concentration addition model, and found a good correlation between in situ invertebrate biodiversity and the sum of HQs for daphnids.

Interestingly, de Castro-Català et al. (2015b) conducted an ERA of sediments collected from diverse locations of the same river basins. Differently from the study presented in this thesis, they assessed the toxicity of the sample, which provided a more realistic and appropriate estimation of the ecotoxicological risks at the sites studied. The authors calculated TU values from acute pore water tests (*V. fischeri, Pseudokirchneriella subcapitata and D. magna*) and whole-sediment exposure tests (*V. fischeri, Chironomus riparius*) and evaluated the invertebrate community composition (multivariate analyses) to detect short and long-term responses of the organisms. The combination of the different approaches allowed to detect ecotoxicological effects in organisms and to identify the main contributors to the toxicity in these multi-stressed rivers. Furthermore, the adverse effects observed by the authors on the target aquatic organisms with a large amount of chemicals measured in the locations assessed (e.g. metals, PhACs, pesticides) were correlated. Hot spots of toxicity risk were identified in the cited work at three sites (the downstream sites of the Llobregat (LLO5) and the Júcar (JUC5), and the most upstream site of the Ebro (EBR1)). Besides, organophosphate insecticides and metals were identified as the main contributors responsible for this toxicity, particularly in the whole-sediment tests (de Castro-Català et al., 2015b). These sites were different from those identified in this thesis (Osorio et al., 2015), evidencing the importance of other pollutants present in the mixture that might contribute to the overall toxicity of the sediment. Regarding PhACs, only sediments of the Guadalquivir River, which presented high toxicity for the *V. fischeri* short-term bioassay, were related to antibiotics. However, their contribution to the toxicity of the whole sediment was shared with metals (i.e. Cu, Ni and Hg).

The presence of other contaminants of emerging concern, with different modes of action than PhACs, in complex environmental matrices, and thus their relative contribution to the toxicity of the whole mixture; was already discussed in the publication presented in this thesis (Osorio et al., 2015). Two contemporary studies (Kuzmanović et al. 2015; de Castro-Català et al., 2015a), on Iberian river basins, demonstrated that PhACs were not the most relevant group of contaminants contributing to the whole toxicity of SW matrices. Ten compounds belonging to the groups of organophosphorate insecticides and alkylphenolic endocrine disruption compounds (EDCs) were identified as the main contributors of toxicity to aquatic biota (i.e. algae, *D. magna* and fish) in SW (Kuzmanović et al. 2015). However, the authors identified sertraline, erythromycin and losartan among the main contributors to the whole toxicity of the SW sample for algae; while sertraline was relevant for *D. magna* and gemfibrozil for fish. Furthermore, de Castro-Català et al. (2015a) found that PhACs and EDCs are the most likely chemical families related to benthic invertebrate responses. Furthermore, in the follow-up study (de Castro-Català et al. (2015b), metals and some organophosphorate insecticides were the main contributors to sediment toxicity.

In addition, the predicted ecotoxicity of PhACs to aquatic organisms (i.e. algae, daphnids and fish) was quantitatively related to human population and animal farming pressure within this thesis (Osorio et al., 2015). Significantly positive relationships among TUs of PhACs in SW and population density and livestock units were empirically proved for the first time. TUs for Daphnia and fish showed a stronger response caused

by the increase of population density and livestock units as compared to TUs for algae. However, TUs were highest for algae and lowest for fish, with daphnids showing values in between, suggesting that toxicity from PhACs would harm the assemblage of primary producers more than other biota. Thus, the effects on ecosystem processes in which algae are important, as primary producers, would not change very much with population density or livestock units; while other organisms at the top food webs (i.e. invertebrates and fish) were expected to become more impaired with the increase of PhACs levels. These inconsistencies observed revealed the need to extend the risk characterization of PhACs to the whole food chain of aquatic biota, covering the range of different sensitivities to compounds with different modes of action. Analyses of community level responses to single compounds and their mixtures provide a more realistic impression of the potential range of effects of contaminants and with greater ecological relevance. In this sense, microbial communities make excellent surrogates for the ecosystem as a whole, revealing effects involving all trophic levels, carbon and energy flow, and impacts on biogeochemical cycles vital to overall ecosystem health and function and biodiversity (Lawrence et al., 2012).

As already introduced in chapter 1 (see section 1.8), river biofilms are useful descriptors of the effects of pollutants on freshwater ecosystems. Interestingly, a contribution to the investigation on the effects of PhACs detected in the Llobregat River on fluvial biofilms (Proia et al., 2013a) and more specifically on the effects of antibiotics on the attached bacterial communities (Proia et al., 2013b) was performed. Biofilms were grown under controlled conditions in mesocosms containing water collected from the river at three locations following a pollution gradient. After colonization, the biofilms were translocated from less to more polluted waters and different responses were measured. The translocation from less to more polluted site was the most effective one. Multivariate analysis revealed that analgesics and antiinflammatories significantly affected biofilms responses. In particular, ibuprofen and acetaminophen were associated with negative effects on photosynthesis, and with the decrease of the green algae/cyanobacteria ratio. Since these descriptors are mainly related to autotrophic organisms, these observations suggested that ibuprofen and acetaminophen might affect the structure and function of the autotrophic biofilm community (i.e. green algae, cyanobacteria and diatoms). On the other hand, DCF did not show any relationship with biofilm autotrophic metrics. Instead, the statistical analysis revealed the association of DCF to phosphatase activity. Since phosphatase enzyme in biofilms is mainly produced by bacteria, it was suggested that DCF might affect this heterotrophic community in biofilms both directly or indirectly. These findings were in agreement with those observed by Lawrence et al. (2012). Bacterial communities of biofilms grown in waters from different sites, differed markedly in their structure, but less so in terms of function. Interestingly, the abundance of actinobacteria increased after translocation to the more polluted site and this effect was associated to the higher concentrations of antibiotics. Importantly, species of this bacterial group are natural producers of antibiotics (e.g. the genus Streptomyces produce streptomycin) being therefore intrinsically resistant to them (D'Costa et al., 2011). In addition, biofilms showed increased bacterial mortality, which was hypothesized to be associated to the presence of antibiotics in the water (Proia et al., 2013b). Indeed, there was a significant positive correlation between tetracycline concentration and the proportion of dead bacteria in the biofilms translocated to more polluted waters. As well as the effects on the structure, also bacterial activity was affected after translocation. Indeed, the metabolism capacity of heterotrophic bacteria was observed to decrease. Overall, a significant correlation between antibiotic concentrations and biofilm responses was observed. These findings were not fully in the line of those reported by Yergueau et al. (2012). Diversely, they observed slight changes in bacterial community structure; while the biofilms displayed a variety of functional shifts after a short-term exposure to

environmental concentrations of erythromycin, SMX, sulfamethazine and gemfibrozil. In accordance with the observations in this collaboration (Proia et al., 2013a; 2013b), they did also observe effects on autotrophs (i.e. decrease in cyanobacteria and photosynthetic activity) after exposure to erythromycin and SMX. In addition, they reported several shifts in descriptors related with biofilms uptake of nutrients, such as carbohydrate, nitrogen and phosphor cycling. However, the list of PhACs included in the previously cited studies (Lawrence et al., 2012, Yergueau et al., 2012) was substantially shorter than the one analysed in the studies performed within this thesis, thus limiting the comparison of results.

Considering the observed variation of PhACs concentration in SW due to fluctuations in river flow (Osorio et al., 2012a; 2012b; 2014a), further investigations aimed to evaluate how this modulation affected the biological status of the river. To that end, the effects of flow changes on the concentration of PhACs and their relationship to river biofilms responses were evaluated (Osorio et al., 2014a). This was the third work carried out within the scientific collaboration with Lorenzo Proia (University of Girona), for more details see chapter 5 (section 5.3). Valuably, translocation experiments of biofilm communities from a less to a more polluted site of the river were carried out in mesocosms settled in the field. Interestingly, the relevant flash-flood event that occurred at the beginning of the second study campaign, which corresponded to the initial phases of colonization of biofilms, had important consequences on their structure and functioning. After the flash-flood episode, it was observed that the growth rate of biofilms was significantly reduced indicating lessened accrual rates and subsequent slow recovery. Interestingly, chlorophyll-a, a descriptor related with autotrophic community, significantly decreased in biofilms translocated to the more polluted sites during the first campaign but did not change in the second one. According to the significant negative correlations between chlorophyll-a and some therapeutic groups, it was suggested that the decrease in chlorophyll-a in the more polluted site might be a consequence of the higher levels of PhACs. On the other hand, chlorophyll-a did not response at all to translocation during the second sampling campaign. Since PhACs were substantially diluted due to the flash-flood event, it was suggested that their potential effects on biofilms might be counteracted. Regardless of hydrological conditions, results showed an increase in bacteria mortality in the biofilms translocated to the more polluted site. Besides, it was observed a significant negative correlation between antibiotics macrolides and the amount of live bacteria. These findings supported the hypothesis of the increase of bacteria mortality in river biofilms as a direct consequence of the increase of antibiotic concentrations in SW. Therefore, the bacterial compartment of biofilms demonstrated to keep sensitive to antibiotics in spite of the dilution effects associated to flood conditions.

A similar study assessed the long-term effects of a mixture of selected PhACs at environmental concentrations combined with river flow intermittency in indoor artificial streams (Corcoll et al., 2015). Results of the cited work were regarded as complementary to the findings of this thesis, since the authors investigated on the effects of the subsequent PhACs concentration during dry periods, a phenomenon that had been demonstrated previously within this thesis (Osorio et al., 2012b). In agreement with the findings of this thesis, they observed that biofilms were negatively affected by PhACs, such as changes in the bacterial community structure or the metabolism of green algae and heterotrophs. Besides, flow intermittency also modulated these effects on biofilms. For instance, the algal community became more sensitive to short-term exposure of PhACs during water intermittency, indicating cumulative effects between the two assessed stressors (Corcoll et al., 2015). These effects are in accordance with those observed during this thesis after the flash-flood event for the particular case of the chlorophyll-a, a descriptor related with the algae metabolism (Osorio et

al., 2014a).

The observed effects of PhACs on biofilms structure and function, led to conjecture that the presence of these micropollutants in rivers might cause important alterations in river ecosystem functioning. In fact, river biofilm communities are generally net primary producers very effective in organic matter transformation (Romaní et al., 2004), and are transducers of energy to higher trophic levels (Lamberti, 1996). Moreover, they play a key role on nutrient uptake and remineralisation (House, 2003; Von Schiller et al., 2007), being thus relevant for self-depuration processes occurring in rivers (Pusch et al., 1998). Nevertheless, the co-occurrence of many other trace pollutants, not considered in the study conducted within this thesis (Osorio et al., 2014a) but detected in the Llobregat River (e.g. Köck-Schulmeyer et al., 2012), may also interfere with the observed results. Biofilms responses can be explained by both direct and indirect effects of environmental factors and chemical pollution on community structure and function. Therefore, no conclusive causality between PhACs studied and the effects observed could be stated. Moreover, these findings manifested that the potential environmental risk of PhACs to fluvial ecosystems is subjected to flow regime of the river, and particularly sensitive to flash-flood events or flow intermittence. Overall, the interpretation of the complex interactions among multiple stressors of freshwater ecosystems, such as the continuous load of PhACs or the high variability of hydrological regime, and their combined effects on biofilms; turned out as a cumbersome task. Interactions among different stressors might be mainly non-additive (i.e. synergies or antagonisms) suggesting that multiple stressors may more commonly interact to generate 'ecological surprises' rather than simple additive effects (Darling and Côté, 2008). Determining which specific stressors interact to generate these effects on the aquatic ecosystems and what is the prevalence and magnitude of these interactions remains a challenge for the scientific community. Consequently, the analysis, quantification and prediction of responses to multiple stressors at the community level should be the major targets for future work in the ecotoxicological assessment of the aquatic environment.

The findings of this thesis, together with the observations of other authors aforementioned discussed, have evidenced the importance of integrating chemical, toxicological and ecological disciplines for an appropriate assessment of the fate and risk of PhACs in the aquatic environment.

Chapter 7

Conclusions

Chapter 7

Conclusions and future recommendations

1. In this thesis a novel multi-residue analytical method, based on offline SPE-LC–MS/MS, for the simultaneous determination of DCF, SMX, five of their human metabolites (4'-OH-DCF, 5-OH-DCF, 4',5-diOH-DCF, 5-OHD-DCFand DCF-gluc) and four nitrifying/denitrifying TPs (NO_2-DCF, NO-DCF, NO_2-SMX and Des-SMX) in WW and SW waters was developed and validated. The method was successfully applied to the analysis of WWi, WWe and SW reporting the occurrence of the metabolites and TPs of DCF in the ng L^{-1} range (i.e. [16-5,800] ng L^{-1} for its metabolites and [1-105] ng L^{-1} for its NO-DCF and NO_2-DCF). As for SMX, levels of NO_2-SMX and Des-SMX detected in WWi, WWe and SW ranged from 8 to 36 ng L^{-1}. To our knowledge this is the first time that the presence of nitrosation/nitration TPs of DCF and SMX in WWTPs and WWe-impacted SW is described. The calculated relationships between NO-DCF, NO_2-DCF and N-species (N-NO_2^- and N-NO_3^-) suggested that the biotransformation of DCF into their nitrosation/nitration TPs is associated with the nitrification/denitrification process occurring in the activated sludge. We recommend the inclusion of these TPs in environmental monitoring studies as complementary information to understand the occurrence, fate and behaviour of DCF and SMX in the aquatic environment.

2. During the biodegradation of DCF (Pérez et al., 2008), the incubation of a series of close structural analogs (i.e. 2-anilinophenylacetic acid (APAA), mefenamic acid, tolfenamic acid, meclofenamic acid and flufenamic acid) with WW bacteria produced a number of nitrosation/nitration TPs. This experiment showed as well that the reaction pathway is not unique to DCF. The use of HR-MS/MS allowed the identification of their tentative chemical structures. By addition of stable isotope-labeled $^{15}NH_4$-N in bioreactor, +1 Da mass shift was observed in the MS spectra of isotope-labeled ^{15}N NO_2-DCF (TP340) compared to NO_2-DCF (TP340), the position of an NO_2 group in the molecule could be confirmed. All compounds studied confirmed the formation of the nitrosation/nitration TPs identified by biotic mechanisms.

3. Several PhACs (73 out of the total 96 compounds studied in this thesis) belonging to the most consumed therapeutic groups were determined in SW from a sewage impacted section of the Llobregat River. The selected PhACs were measured over time in four monthly (9-13 samples per campaign) monitoring sampling campaigns. In addition, the temporal variability of levels of PhACs determined over the four seasons (C_X for a given X compound) was related with hydrological factors such as river flow (Q) (during the four campaigns) and DOC (during the first campaign) (i.e. respective correlations r(C_X/Q) and r(C_X/DOC)). Besides, the impact of flow and DOC changes on the concentration of PhACs in the river was assessed by relative sensitive coefficients (s(C_X/Q) and s(C_X/DOC, respectively)). Based on the collected data, the specific conclusions from objectives 3 and 4 were:

   (i) PhACs demonstrated to be widespread micropollutants in the Llobregat River. More than 50 out of the selected 73 target compounds were present in all analyzed samples.

   (ii) Analgesics and antiinflammatories were the most ubiquitous [67-100 % of samples] and concentrated therapeutic groups ([200-1,100] ng L^{-1} in ABR and [200-18,000] ng L^{-1} in SJD). Individual concentrations were usually within the tens to hundreds of ng L^{-1} range.

Ibuprofen, acetaminophen and DCF were the most concentrated anti-inflammatory drugs, with concentrations in the mid-to-high ng L^{-1} range (100–500 ng L^{-1}).

(iii) A gradient of pollution was observed along the section of the river studied. On average, minimum concentrations of PhACs were determined in ABR (~2,000 ng L^{-1}), while the maximum were observed in SJD (~16,800 ng L^{-1}). Such trend was explained by the increasing loads of PhACs from the WWTPs distributed along the section of the river basin assessed.

(iv) Maximum levels of PhACs were determined during cold and dry periods corresponding to autumn and winter seasons. Higher total concentrations of PhACs determined during autumn and winter were up to 2,000 and 2,500 ng L^{-1} in ABR and 35,000 and 12,000 ng L^{-1} in SJD, respectively. On the opposite site, lower levels were determined in spring and summer: only up to 1,400 and 1,200 ng L^{-1} in ABR and similar levels in both seasons up to 3,500 ng L^{-1}. The general minimum levels observed during spring and summer seasons were explained by: dilution effects due to rainfall; high temperatures and thus better elimination rates of PhACs in WWTPs; improved natural degradation processes (photodegradation due high UV solar radiation, biodegradation); and lower human consumption of drugs during this period.

(v) The response of PhACs to river flow was negative (significant values of $r(C_X/Q)$ ranged from −0.305 for DCF to 0.807 for SMX), principally due to expected dilution effects. Only in a few cases, positive relationships between drug concentrations and flow were detected, suggesting an important role of other hydrological phenomena like sediment re-suspension as well as pollutants' sources.

(vi) The response of PhACs to DOC was positive, due to anticipated association of drugs to DOC. These results suggested that, concentration of PhACs increases with DOC, which also means that high DOC increments mobility of the chemicals in the aqueous phase.

(vii) Sensitivity calculations showed that the majority of compounds were sensitive to flow variations. PhACs presented $s(C_X/Q)$ values in the range of 0.33 for tetracycline to −1.43 for lorazepam; while $s(C_X/DOC)$ values ranged from 0.04 for butalbial to 2.40 for ciprofloxacin. Overall, arrangements of sensitivity to both hydrological parameters were the same for all compounds. However, PhACs showed to be more sensitive to DOC than to Q, suggesting the importance of the response of PhACs to organic matter.

4. Modeling was applied aiming to understand the pattern that 14 relevant PhACs described along the river water course after their emission from WWTPs. A "plug-flow" model was successfully developed, which allowed to explain the observed temporal variations in the loads of the selected PhACs analyzed in two locations influenced by WWe discharges. The model described the fate of PhACs in terms of river flow and compound-specific parameters, i.e. PhAC emission (E), associated to the average load discharged upstream and the overall decay constant (k) interpreted as the PhAC attenuation over time. Models for most compounds showed larger E values in SJD, a result previously noticed by the occurrence data at main WWTPs in the basin. Erythromycin presented k values of −0.15 h^{-1} in both sites being the compound more efficiently removed from the water column. In addition, the proposed model approach proved to be consistent (i.e. E and k values estimated for every compound at both locations studied (ABR and SJD) were similar) and thus qualified as potentially useful for management purposes at basin or water-body scale.

5. Several PhACs (an updated list of 76 relevant compounds out of the 96 studied in this thesis) belonging to the most consumed therapeutic groups were measured in SW and sediments from four major Iberian river basins: Llobregat, Ebro, Júcar and Guadalquivir. The spatial occurrence of PhACs was determined in SW and sediments collected in two extensive sampling campaigns performed in consecutive years along the four catchments (77 locations in total). The use of several statistical methods performed in R (e.g. ANOVA analyses followed by TukeyHSD pairwise comparisons, Non-metric Multidimensional Scaling (NMDS), permanova analyses, ANOVA based on permutation and linear mixed effect models (LME models)) enabled to unveil the general and most important trends of the PhACs studied in the four river basins. Based on the collected data, the specific conclusions from objectives 3 and 4 were:
   (i) PhACs were widespread and pseudo-persistent micropollutants in the Iberian aquatic environment. The concentration of PhACs in SW varied from the low to high ng L^{-1} range; while in sediments they were determined at the low ng g^{-1} level. About the 60% of the compounds studied was present in at least half of the SW and sediment samples analyzed in both sampling campaigns. The 22 and 18% of these compounds were detected in all SW and sediment samples, respectively. Thiabendazole, hydrochlorothiazide and glibenclamide were present in all SW samples; while azithromycin and thiabendazole were the most ubiquitous compounds in sediments.
   (ii) Frequency of detection of PhACs was higher in SW from the Llobregat River, followed by Ebro, Júcar and Guadalquivir.
   (iii) Analgesics/antiinflammatories were the most relevant therapeutic group in SW. Among them, ibuprofen and DCF were of the more concentrated with respective levels in the four river basins as follows: 37 and 31 ng L^{-1} in Llobregat; 37 and 14 ng L^{-1} in Ebro; 3 and 8 ng L^{-1} in Guadalquivir and 2 and 3 in ng L^{-1} Júcar. In river sediments, antibiotics averaged the higher concentrations. These levels were determined in the same order of magnitude in all the catchments (i.e. 43 ng g^{-1} on average). Levels of analgesics/antiinflammatories determined in sediments were close to those reported for antibiotics. To indicate as well as antibiotics, levels of analgesics/antiinflammatories differed slightly among the catchments (19 ng g^{-1} in Llobregat, 15 ng g^{-1} in Ebro, 27 ng g^{-1} in Júcar and 18 ng g^{-1} in Guadalquivir). The gradient of pollution observed in the sewage impacted section studied of the Llobregat River previously was confirmed in the present work for the whole catchment. However, no clear pollution trend was observed for the remaining basins.
   (iv) Significantly positive relationships were found among levels of PhACs and population density and livestock units in both SW and sediment matrices, thus responding to the anthropic pressures in the catchments.

6. The acute toxicity of DCF, SMX and their nitrifying/denitrifying TPs NO_2-DCF, NO-DCF, NO_2-SMX and Des-SMX to *Daphnia magna* and *Vibrio fischeri* was assessed both individually and in mixtures with other compounds of environmental concern (i.e. nonylphenol, diuron, malathion, gliphosate and triclosan). Overall, the associated acute toxicity to D*. magna* and *V. fischeri* of microbial TPs were lower than the corresponding to their parent compounds. However, NO_2-DCF, Des-SMX and NO_2-SMX showed to be slightly more toxic to *V. fischeri* than their precursors, DCF and SMX. Nonetheless, the LOEC values were in all the cases several orders higher than those concentrations found in the environment. These findings confirmed that the studied PhACs and TPs, tested individually, did not showed acute toxicity to the target aquatic organisms. In addition, the potential synergistic or antagonistic effects of DCF, SMX and NO-DCF in binary mixtures of themselves and with other contaminants (i.e. nonylphenol, diuron, malathion, gliphosate and triclosan) were assessed. In general, synergism was the predominant effect, indicating the need to further assess the combination of mixtures. In these cases, although NO-DCF did not showed acute toxicity, at some concentrations it can increase the effects of other toxicants in the same environmental compartments. These findings evidenced the existing lack of knowledge about the combined effects of complex mixtures, in particular considering the presence of TPs.

7. The ecotoxicity of 55 PhACs (out of the 96 compounds studied in this thesis) was assessed in SW of the entire four river basins. TU values were estimated on the basis of acute toxicity to algae, Daphnia and fish species for each location studied. Algae were the most sensitive aquatic organisms towards PhACs, followed by Daphnia and fish. Estimated average ecotoxicological risk to aquatic organisms was most relevant in Llobregat (2.50E − 04), closely followed by Ebro (2.28E − 04) and then Guadalquivir (6.35E−05) and Júcar (3.97E−05). Similar to what was observed for PhACs concentrations, the potential risk of PhACs to aquatic organisms increased downstream at the Llobregat River basin, but no clear trend of increasing concern was observed for the remaining basins. The compounds contributing at least 5% to the total predicted toxicity in the samples were sertraline, erythromycin, losartan and dimetridazole with values of 22, 20, 11 and 6%, respectively, when considering TU based on algae for SW. For TU based on Daphnia there were again four PhACs reaching the 5%- threshold, namely, sertraline (29%), gemfibrozil (12%), loratidine (10%) and fluoxetine (5%). As regards to TU values for fish, gemfibrozil was found to be the PhAC that most contributed to the predicted toxicity of SW samples, 43% on average. Sertraline (11%), loratidine (10) and azithromycin (6) also showed predicted toxicities over 5% of the total TU of the sample. Estimated total toxicity of PhACs, was positively related to population density and livestock units in the upstream sub-basin, thus responding to the anthropic pressures in the catchments.The extensive data presented on the predicted ecotoxicological risk of PhACs to non-target aquatic organisms as well as the computation of their relative contribution to the whole toxicity of the sample, provided valuable information for further prioritization exercices in the risk assessment of Spanish river basins. Overall, sertraline, gemfibrozil and loratidine were regarded as the most relevant PhACs in terms of ecotoxicological risk.

8. The exposure experiments of fluvial biofilms grown in mesocosms to WWe influenced SW of the Llobregat River and the relationships established between the changes observed in biofilms descriptors and the measured PhACs, lead to the following conjectures:

(i) PhACs affected the structure and functioning of fluvial biofilms.
(ii) The effects observed and their relationships with individual PhACs varied among the different microbial communities attached to biofilms.
(iii) Development and functioning of biofilms exposed to PhACs varied under different hydrological conditions.
(iv) The biotic response to the two main stressors studied, namely PhACs pollution and hydrology, varied among the different biofilm compartments examined (photoautotrophs and bacteria).
(v) Differences observed in the PhAC concentrations and on the response of biofilms in changing river water flows indicate the importance of hydrology when studying river conservation in the Mediterranean catchments.
(vi) The flash-flood event reported during among the sampling campaigns of this thesis played a key role on the development of biofilms. Nevertheless the potential negative effects of antibiotics on the bacterial community were steady under different hydrological scenarios.

9. Considering all the studies conducted during this thesis and according to our findings regarding the fate, behaviour and risk of PhACs in the aquatic environment, we propose the following compounds to be included in a future list of priority relevant substances: DCF, SMX, their nitration TPs (i.e. NO_2-DCF and NO_2-SMX), sertraline, gemfibrozil and loratidine.

Chapter 8

Resumen en español

Chapter 8

Resumen en español

8.1. Introducción

8.1.1. Descripción de los fármacos estudiados

Los compuestos farmacológicamente activos (del inglés *pharmaceutically active compounds,* PhACs) son substancias químicas naturales o sintéticas que se aplican para el diagnóstico, tratamiento o prevención de enfermedades. Éstos compuestos están diseñados para desarrollar una determinada actividad biológica y desempeñar propiedades terapéuticas beneficiosas para la salud humana y animal. Estas substancias presentan una amplia variedad de estructuras químicas y propiedades fisicoquímicas.

Los grupos terapéuticos estudiados con mayor frecuencia en el medio ambiente acuático son los antibióticos, y entre ellos los siguientes compuestos: eritromicina, ofloxacina, ciprofloxacina, amoxicilina, sulfametoxazol (SMX), metronidazol y trimetoprima. Los antibióticos son un grupo terapéutico que presenta una amplia variedad de estructuras químicas con la habilidad de inhibir el crecimiento de microorganismos. Estas sustancias se aplican en medicina humana y veterinaria con el propósito de prevenir o tratar infecciones microbianas (Kümmerer, 2009), como por ejemplo el SMX, del grupo de las sulfonamidas. Otros grupos terapéuticos relevantes son los analgésicos y antiinflamatorios, como diclofenaco (DCF), ibuprofeno, naproxeno, ácido acetilsalicilíco y acetaminofén. Los antiinflamatorios no esteroideos (del inglés nonsteroidal antiinflammatory drugs, NSAIDs), son un amplio y heterogéneo grupo terapéutico que se usa principalmente para tratar la inflamación, el alivio del dolor moderado y la reducción de la fiebre, como por ejemplo el DCF. También los β-bloqueantes, como atenolol y metoprolol; los reguladores de lípidos, como gemfibrozilo y bezafibrato; los PhACs de tratamiento psiquiátrico, como carbamazepina, diazepam, fluoxetina o paroxetina (Petrovic et al., 2010).

La figura 1.1 (ver chapter 1) enumera los PhACs de uso humano y animal agrupados según su actividad terapéutica que se estudiaron durante la presente tesis. En la tabla A.1 (ver Annex) se muestran sus correspondientes números CAS, fórmulas moleculares, pesos moleculares y propiedades fisicoquímicas estimadas y experimentales (constante de disociación, pKa; solubilidad en agua, coeficiente de partición octanol-agua, K_{ow}; coeficiente de partición sólido-líquido, K_d y coeficiente de partición de carbono orgánico del suelo, K_{oc}).

Los PhACs constituyen un grupo de compuestos de relevancia medioambiental debido a su intrínseca actividad biológica, pero también debido al continuo incremento de su consumo.

8.1.2. Consumo humano y uso veterinario de PhACs

En la actualidad existen alrededor de 4.000 PhACs que se aplican en medicina humana y veterinaria (Daughton, 2013). El consumo anual de principios activos está estimado en unas 100.000 toneladas (Sadezky et al., 2008) y la cantidad de PhACs aprobada para su consumo en unas 25.000 (Daughton, 2013). De éstos, 9.524 y 9.700 principios activos se han aprobado para su uso humano y humano/veterinario,

respectivamente. Además, se estimó que unas 6.051 toneladas de principios activos fueron destinadas a la producción de PhACs de uso veterinario para animales de consumo en la Unión Europea en 2004, incluyendo 5.393 y 194 toneladas de antibióticos y antiparasitarios, respectivamente (Kools et al., 2008a). Los PhACs NSAIDs, como por ejemplo ibuprofeno y DCF, y los antibióticos, como SMX, se encuentran entre los PhACs con mayores índices de consumo (García et al., 2006; Lázaro et al., 2010; Michael et al., 2014a). El grupo de los antibióticos es particularmente preocupante, ya que se usan en grandes cantidades en ganadería no sólo para finalidades terapéuticas (Kools et al., 2008), sino que también se administra en el ganado para promover el crecimiento (Van Boeckel et al., 2015).

El mercado mundial de PhACs en 2013 se estimó en unos 655.222 millones €. En la figura 1.2 (ver chapter 1) se muestran cifras de expedición de PhACs (como precios de venta en dólares americanos) de catorce países durante el periodo [2004-2012] (OECD, 2014). Entre los mercados mayoritarios, el mercado Norte-Americano (EEUU y Canadá) se ha mantenido entre los mayores mercados muy por encima de Europa y Japón con respectivos porcentajes de ventas del 41.0; 27.4; y 9.7% en 2013 (EFPIA, 2014). En cuanto a España, se ha mantenido en el cuarto puesto entre los cinco mercados punteros Europeos (después de Alemania, Francia, Italia y antes de Reino Unido). Además, España ocupó la octava posición entre los países con mayor Mercado a escala mundial en 2010 (IMS Health, 2011).

Así, debido al elevado e incesante consumo de estas substancias a escala global, los PhACs son actualmente reconocidos como contaminantes ampliamente extendidos en el medio ambiente (Petrovic et al., 2010).

Dados los efectos combinados de la mejora de los estándares de salud en los países en desarrollo y el envejecimiento de las poblaciones en naciones industrializadas, se anticipa un incremento del consumo de PhACs en los próximos años y por último, su riesgo para el medio ambiente (European Environmental Agency, 2010; Van der Aa et al., 2011). Además, dado que también se espera que la producción mundial de carne y el mercado de animales de compañía se incrementen en los próximos años, también se espera que aumente el mercado de PhACs de uso veterinario (Alexandratos and Bruisma, 2012).

La información fiable sobre patrones de consumo de PhACs en el tratamiento de humanos y ganadería es relativamente escasa pero su evaluación indirecta a través de la determinación de concentraciones de PhACs en aguas superficiales impactadas por descargas de efluentes de aguas residuales es una más aproximación más sencilla.

8.1.3. Fuentes y rutas de entrada de los PhACs al medio ambiente acuático

Los PhACs pueden alcanzar los sistemas acuáticos mediante diversas fuentes de emisión (ver figure 1.6 en chapter 1). Éstas pueden ser los residuos industriales, residuos domésticos o ganaderos, las aguas residuales (del inglés *waste water*, WW) urbanas o de hospitales (Daughton 2013).

El consumo veterinario, y en especial para animales de engorde (Van Boeckel et al., 2015), se considera la principal fuente importante de los antibióticos en las aguas superficiales (del inglés *surface waters*, SW). De hecho, el papel de las unidades ganaderas y la actividad ganadera ha sido propuesto como la principal fuente de contaminación de antibióticos en el Río Huangpu (China) (Jiang et al., 2011). En otros

estudios, se identificó la actividad humana como principal fuente de los antibióticos sulfonamidas y otros PhACs presentes en las aguas de los ríos Liaodong Bay y Beiyun (China) (Jia et al., 2011; Dai et al., 2015). Por lo tanto, después de la actividad ganadera, el consumo en la población humana es considerado como la segunda fuente más importante de antibióticos en el medio ambiente. No obstante, en lo que concierne a la literatura disponible, nunca se han llevado a cabo estudios que relacionen de un modo cuantitativo la presencia y el riesgo asociado de los PhACs en el medio ambiente con la población humana y ganadera.

Por lo tanto, la principal ruta de entrada de los PhACs al medio ambiente acuático se produce por vía de la excreción humana/animal además, aunque en menor grado, del vertido directo del PhAC no consumido a través de las aguas residuales domésticas. Generalmente, el metabolismo de los PhACs es incompleto, por lo que un porcentaje del PhAC no alterado llega al medio ambiente acuático a través de la orina y las heces. Este porcentaje, puede variar dependiendo de las propiedades fisicoquímicas y biológicas del PhAC. Por ello, tras el consumo y excreción de los PhACs, éstos entran en el medio ambiente acuático combinados con sus productos de transformación (del inglés *transformation products*, TPs) generados durante el metabolismo humano durante su destino a través del sistema colector de WW y de las estaciones depuradoras de WW (del inglés waste water treatment plants, WWTPs) (Kümmerer et al., 2008: Kunkel and Radke, 2012). Por ejemplo, tras el consumo humano de NSAIDs, entre el 30 y el 90% del PhAC pasa a través del cuerpo humano sin ser metabolizado y finalmente es excretado totalmente inalterado. Tras el consumo de DCF, el metabolismo humano reduce su biodisponibilidad oral hasta en un 50% (Willis et al., 1978; 1980). Durante el metabolismo hepático, la molécula de DCF se hidroxila para dar mayoritariamente 4'-hidroxidiclofenaco (4'-OH-DCF) y en menor proporción 5-hidroxidiclofenaco (5-OH-DCF), así como la glucuronización de su ácido carboxílico produce el 1-O-aciloglucurónido (DCF-gluc) (Kenny et al., 2004). Por lo tanto, el DCF junto con sus metabolitos humanos entra en las WWTPs a través del sistema colector de aguas residuales.

Tras el consumo humano/animal, la principal fuente de entrada de los PhACs, la principal fuente emisora al medio ambiente acuático son las WWTPs. Su presencia en los sistemas acuáticos, se atribuye principalmente a su eliminación parcial en las WWTPs durante el tratamiento de las WW (Gros et al., 2010). En muchos casos, la polaridad combinada con la baja degradabilidad microbiana de los PhACs, resulta en una ineficiente eliminación en las WWTPs que propicia su entrada en el medio ambiente acuático.

8.1.4. Presencia de PhACs y sus TPs en WWTPs y ríos

De los 3,193 PhACs comercialmente disponibles a nivel mundial, sólo 275 substancias se han analizado en el medio ambiente (Howard & Muir, 2011). Los PhACs son detectados en influentes y efluentes de aguas residuales (del inglés *waste water influents* y *effluents*, WWe y WWi, respectivamente) a niveles en el intervalo del µg L^{-1}; mientras que en SW y subterráneas (del inglés ground waters, GW) los niveles son generalmente mucho menores, en el órden del ng L^{-1} (Fent et al., 2006; Celiz et al., 2009; Kassinos et al., 2011; Kunkel and Radke, 2012; Ying et al., 2013, Michael et al., 2014). Las evidencias sobre la presencia de PhACs y sus TPs en el medio ambiente acuático a escala mundial son numerosas (Halling-Sørensen et al., 1998; Daughton and Ternes, 1999; GWRC, 2004; Fent et al., 2006; Sadezky et al., 2008; Mompelat et al., 2009; Pal et al., 2010; Hugues et al., 2012; Ying et al., 2013; Michael et al., 2014; Evgenidou et al., 2015).

Respecto a los niveles de PhACs encontrados en WW, Verlicchi y colaboradores (2012) publicaron un

estudio interesante en el cual se compilaron todas las referencias bibliográficas concernientes a la presencia de 118 PhACs de diversos grupos terapéuticos en WWi y WWe de 264 WWTPs Europeas (ver figure 1.7 en chapter 1). Como se puede observar en la figura 1.7, las diversas familias de PhACs fueron detectadas en Europa en intervalos de concentración diferentes, siendo mayores en las WWi con respecto a las WWe. El grupo terapéutico detectado en mayores concentraciones en WWi fueron los analgésicos y antiinflamatorios, aunque en WWe se detectaron a niveles por debajo de los observados para los reguladores de lípidos y los β-bloqueantes. Es interesante ver como las concentraciones de reguladores de lípidos y β-bloqueantes se detectaron generalmente a mayores niveles en las WWe que las WWi. A su vez, las drogas de tratamiento psiquiátrico se detectaron generalmente en los mismos niveles tanto en WWi como en WWe, lo cual indica su carácter recalcitrante al tratamiento en las WWTPs. Respecto a los compuestos individuales, los más ubicuos fueron ibuprofeno, DCF, naproxeno, ketoprofeno y tramadol. De entre éstos, ibuprofeno y tramadol mostraron las mayores concentraciones absolutas en WWi, mientras que en WWe el compuesto más concentrado fue atenolol. Respecto a los antibióticos, trimetoprima, SMX, eritromicina y ciprofloxacina fueron los más ubicuos, y entre éstos ciprofloxacina fue el antibiótico detectado en mayores concentraciones.

Las aguas superficiales reciben considerables cantidades de contaminantes no regulados, como es el caso de los PhACs (Kemper, 2008; Awad et al., 2014). Hasta el momento, se ha demostrado la presencia de más de 200 PhACs en lagos, ríos y rivieras, por ejemplo en concentraciones hasta un máximo de 6.5 mg L^{-1} para el antibiótico ciprofloxacina (Petrie et al., 2015; Hughes et al., 2012). Incluso en el peor de los casos, han sido detectados en aguas tratadas para el consumo humano (del inglés *drinking water*, DW) (Benotti et al., 2008). La figura 1.8 (ver chapter 1), muestra el mapa de niveles de presencia de PhACs en WWe y SW a escala global que se elaboró a partir de los datos proporcionados en el reciente review de Hugues et al. (2012). En dicho trabajo, se analizaron los datos de niveles de 203 PhACs en WWe i SW en 41 paises alrededor del mundo. La base de datos (Hughues et al., 2012) corresponde a la mediana de las concentraciones de un amplio espectro de PhACs diferentes medidos en 236 estudios llevados a cabo durante el periodo 1998-2010. Estos compuestos se encuentran a escala global en concentraciones que van desde 50 ng L^{-1} a 1.800 µg L^{-1}. Como se puede ver claramente, España y los EE.UU. se encuentran entre los países con mayores niveles de drogas medicinales presentes en sus SW, con concentraciones medias que van desde 4.400 a 9.000 ng L^{-1}. Sin embargo, las naciones en las cuales se han detectado los niveles más altos de PhACS, entre los países examinados en el estudio (Hughes et al., 2012), fueron India, Méjico y Turquía, con concentraciones medias que llegaron a superar los 9.999 ng L^{-1}.

Del total de los medicamentos estudiados, 61 se detectaron con frecuencia tanto en WWe como en SW (Hugues et al., 2012). De estos 61 compuestos, 39, 21, 20 y 3% eran antibióticos, analgésicos, y drogas empleadas para tratamientos cardiovasculares (como agentes β-bloqueantes, diuréticos y bloqueadores de canales de calcio), reguladores lipídicos en la sangre, y antidepresivos (medicamentos psiquiátricos), respectivamente. La frecuencia de detección de PhACS en SW, clasificados por su actividad terapéutica, se comparó entre los ríos receptores de WWe en todo el mundo. Los analgésicos fueron los más ubicuos en Europa, con el 34% de los estudios reportados, mientras que los antibióticos fueron el grupo más frecuentemente detectado en América del Norte y Asia (38% y 42%, respectivamente). Sin embargo, a escala global, los analgésicos resultaron ser la clase terapéutica más ubicua con el 31% de todos los datos reportados y una concentración media de 230 ng L^{-1}, seguidos de los antibióticos (21%, 8,128 ng L^{-1}). Es importante destacar que, en una comparación entre los 10 países más estudiados, los niveles de PhACs en España fueron sustancialmente por encima de la concentración media mundial (171- 441%) para las

principales familias terapéuticas analizadas: analgésicos, PhACs cardiovasculares, reguladores lipídicos y antidepresivos.

En cuanto a los compuestos individuales, carbamazepina, bezafibrato, ácido clofíbrico, ibuprofeno y DCF fueron los PhACs más relevantes en la WWe y SW, entre los 61 más frecuentemente detectados según Hugues et al. (2012). Posteriormente, Ying et al. (2013) compilaron los niveles de 61 PhACs pertenecientes a diferentes clases terapéuticas detectados en SW de ríos de 14 países alrededor del mundo. Entre los antibióticos estudiados, SMX, ciprofloxacina, norfloxacina, ofloxacina y claritromicina fueron los más ubicuos en niveles de hasta varios μg L^{-1}. Las concentraciones determinadas a escala mundial para los analgésicos y antiinflamatorios más frecuentemente detectados (es decir, el ibuprofeno, el DCF, ácido mefenámico, naproxeno, ketoprofeno, ácido salicílico, ácido acetilsalicílico, ácido meclofenámico, ácido tolfenámico, y la indometacina) abarcaron de varios ng L^{-1} a más de varios μg L^{-1}. Varios reguladores lipídicos, tales como ácido clofíbrico, bezafibrato y gemfibrozilo, se observaron también en la mayoría de las SW de todo el mundo. En cuanto a las drogas de tratamiento psiquiátrico, carbamazepina fue el compuesto más frecuentemente detectado en concentraciones que varían desde no detectado a varios μg L^{-1}. El último grupo revisado fue el de los beta-bloqueantes, de los cuales metoprolol, propranolol, y atenolol fueron los más detectados con niveles en el intervalo de no detectado a varios miles de ng L^{-1}.

A diferencia de la extensa literatura sobre la presencia de PhACs en aguas naturales, el número de estudios que evalúan su presencia en los sedimentos es sustancialmente menor. Un buen ejemplo es la revisión realizada por Ying et al. (2013) mencionada anteriormente. Este trabajo, además de SW, también proporciona una compilación de datos de presencia de PhACs en sedimentos de ríos a escala global. Por ejemplo, los antibióticos norfloxacina, ofloxacina y ciprofloxacina fueron detectados con frecuencia en tres ríos chinos en concentraciones de 5.770, 1.290 y 653 ng g^{-1}, respectivamente (Zhou et al., 2011). Por lo contrario, los analgésicos y antiinflamatorios como el ibuprofeno, DCF, y ácido clofíbrico se encuentran raramente en los sedimentos de la región del Mediterránea española y se detectaron en niveles por debajo de los límites de cuantificación del método analítico (Vázquez-Roig et al., 2012). En este estudio, los reguladores lipidícos fenofibrato, ácido clofíbrico; las drogas de tratamiento psiquiátrico como la carbamazepina y diazepam y los β-bloqueantes metoprolol y propranolol fueron algunos de los compuestos detectados con mayor frecuencia y en concentraciones altas. Otros estudios detectaron concentraciones de drogas de tratamiento psiquiátrico en el orden de ng g^{-1} en sedimentos de rivieras estadounidenses (Schultz et al., 2010). Entre ellos, la venlafaxina y fluoxetina fueron los PhACs más relevantes, detectados a niveles de 26 y 19 ng g^{-1}, respectivamente. En la línea del trabajo de Vázquez-Roig et al. (2012), se estudió la distribución de una lista más amplia de PhACs a lo largo de un río español (da Silva et al., 2011). De los 34 compuestos estudiados, las concentraciones más altas que se midieron fueron de paracetamol (222 ng g^{-1}), mevastatina (99 ng g^{-1}) y tilosina A (71 ng g^{-1}). Otros PhACs, tales como eritromicina, ibuprofeno y ranitidina se detectaron en concentraciones máximas de 33, 19 y 25 ng g^{-1}, respectivamente, mientras que la cimetidina y ácido clofíbrico se detectaron a niveles inferiores a 20 ng g^{-1}. Los compuestos restantes se encontraron en concentraciones inferiores a 10 ng g^{-1}.

Aunque la presencia de PhACs en el medio ambiente acuático está bien documentada, la falta de literatura sobre la presencia de TPs de PhACs ha sido evidenciada por Celiz et al. (2009) y Mompelat et al. (2009). Sin embargo, algunos estudios han identificado y detectado niveles de TP en el medio ambiente acuático. Por ejemplo, norfluoxetina, el principal metabolito humano de fluoxetina, se detectó en

concentraciones de entre 4 y 25 ng L^{-1} en WWe (Vanderford et al., 2006); 0.9 y 14 ng L^{-1} en SW; y 0.02 y 3 ng g^{-1} en los sedimentos (Schultz et al., 2010). En este último estudio citado, los niveles de norsertralina, se determinaron en valores que van desde 1.13 a 26.7 ng L^{-1} en SW y desde 0.02 a 10.7 ng g^{-1} en los sedimentos. Del mismo modo, se detectaron cinco metabolitos humanos de la carbamazepina a niveles entre 8.5 y 1571 ng L^{-1} en la WWi y entre 9.3 y 1325 ng L^{-1} en WWe (Miao et al., 2003). Sólo el 10,11-dihidro-10,11 dihydroxycarbamazepine se encontró en SW pero en concentraciones alrededor de 3 veces más altas que la de su compuesto padre. Es importante destacar que los estudios de evaluación de la presencia, destino y comportamiento de los TPs de PhACs en el medio ambiente acuático han aumentado considerablemente en los últimos años (Fatta-Kassinos et al, 2011a; Michael et al, 2014a; Evgenidou et al, 2015). Por ejemplo, se detectaron 13 metabolitos de PhACs pertenecientes a diferentes clases terapéuticas, tales como el 4 'OH-DCF, el glucurónido del oxazepam o el N-acetylsulfametoxazol, en las SW de un río español a niveles entre los 0.96 y los 1.670 ng L^{-1} (López-Serna et al., 2012).

DCF y SMX se detectaron frecuentemente tanto en WWi y WWe Españolas a niveles de 200-1.100 ng L^{-1} (Gros et al., 2010; Díaz-Cruz et al., 2008). A pesar de que la mayoría de los metabolitos humanos del DCF se identificaron hace décadas (Stierlin et al. 1979), su presencia en aguas residuales ha sido descrita recientemente. Respecto al DCF, se demostró que los dos metabolitos hidroxilados 4'-OH-DCF y 5-OH-DCF estaban presentes en aguas residuales de entrada de WWTPs en concentraciones en el intervalo desde 0,06 a 3,0 µg L^{-1} y desde 0,06 a 0,7 µg L^{-1}, respectivamente (Pérez y Barceló, 2008; Langford y Thomas, 2011; Scheurell et al., 2009). El metabolito mayoritario del DCF, 4'-OH-DCF, junto con 5-OH-DCF y la lactama de 4'-OH-DCF (4'-OHD-DCF), se detectaron en concentraciones de 0,71 µg L^{-1}, 0,45 µg L^{-1}, y 0,42 µg L^{-1}, respectivamente mientras que las concentraciones de DCF variaron desde 1,3 a 3,3 µg L^{-1} en muestras de aguas residuales (Stülten et al., 2008). Desde nuestro conocimiento, los metabolitos humanos nunca se han analizado en aguas superficiales. Asimismo, hasta la fecha tampoco existe información cuantitativa sobre la presencia de otros metabolitos humanos del DCF como el 4',5-dihydroxydiclofenac (4',5-diOH-DCF) y el DCF-gluc en WWTPs.

No obstante, el conocimiento acerca del transporte y comportamiento de estos compuestos una vez se han emitido al río a través de los WWe es todavía muy limitado.

8.1.5. Destino y transformación de PhACs y sus TPs en WWTPs

El destino de PhACs en WWTPs se rige por las propiedades fisicoquímicas y biológicas del compuesto (ver tabla A.1 en annex) y el tipo de procesos aplicados durante el tratamiento de WW en la WWTP. Los mecanismos que determinan el comportamiento de los PhACs durante del tratamiento en WWTPs y su posible degradación y/o distribución a lo largo de la WWTP son la adsorción a las partículas y la biodegradación en los lodos activados del tratamiento secundario (Fent et al., 2006). Muy pocos PhACs son volátiles, por lo tanto el proceso de evaporación no es significativo. El destino de PhACs lo largo de las WWTPs se muestra en la figura 1.9 (ver chapter 1).

La **biodegradación** es el mecanismo de eliminación de PhACs más importante durante el tratamiento biológico convencional con lodos activos (del inglés *conventional activated sludge*, CAS) (Joss et al, 2006; Johnson et al, 2008). La biodegradación de PhACs se atribuye principalmente a la actividad co-metabólica

de los microorganismos heterótrofos y autótrofos. Dentro de este grupo, las bacterias y las arqueas que oxidan amonio (AOB y AOA, respectivamente) co-metabolizan una variedad de PhACs mediante enzimas no específicos tales como la Amonio Monooxigenasa (AMO). Estas bacterias nitrificantes pueden aumentar la eficiencia de eliminación de algunos compuestos orgánicos en las WWTPs. Por ejemplo, Vader y colaboradores (2000) demostraron la degradación de etinil estradiol (EE2) en lodos nitrificantes con una alta actividad amoníaco-oxidante. Además pruebas realizadas con cultivos mixtos de nitrificantes sugieren que la enzima AMO podría mediar el co-metabolismo de EE2/amoníaco.

La investigación ha demostrado que muchos productos farmacéuticos no se eliminan completamente durante el tratamiento convencional de aguas residuales, y esto lleva a que su presencia se detecte en WWe, SW y más raramente en GW (Michael et al., 2014a). Como consecuencia de la deficiente eliminación de algunos PhACs durante el tratamiento CAS, se ha sugerido la aplicación de tratamientos terciarios avanzados (del inglés *advanced tertiary treatments*, ATTs) en las WWTPs con el fin de mejorar la calidad química de la WWe (Klavarioti et al, 2009; Ziylan y Ince, 2011) (ver figura 1.9 en chapter 1). Varios ATTs han sido evaluados en los últimos años con el fin de aumentar las tasas de eliminación de PhACs. Estos incluyen el uso de carbón activado en polvo (del inglés *powdered activated carbon*, PAC) y las membranas (por ejemplo, nanofiltración y ósmosis inversa) (Mailler et al, 2015; García et al, 2013), la oxidación química como la cloración o procesos de oxidación avanzada (POAs), (OMS 2011; Oller et al, 2011;.Hey et al, 2012; Lester et al, 2013; Prieto-Rodríguez et al, 2013;. Fatta-Kassinos et al, 2011a; Malato et al, 2014); y humedales artificiales (Matamoros y Bayona, 2013, Verlicchi et al, 2014; Luo et al, 2014) (ver figura 1.9 en chapter 1).

Entre los ATTs, la **oxidación química** y en particular los **procesos avanzados de oxidación** (del inglés *advanced oxidation processes*, AOPs), los cuales son capaces de oxidar y degradar una amplia variedad de contaminantes orgánicos en agua y WW (Ikehata et al., 2006) son los más efectivos (Oller et al., 2011). Estos tratamientos generan reactivos potentes, como los radicales hidroxilo (•OH), que oxidan compuestos recalcitrantes y no biodegradables a su TPs y finalmente convertirlos en dióxido de carbono, vapor de agua y sales inorgánicas (Klavarioti et al., 2009; Ikehata et al., 2006; Antoniadis et al., 2010; Klamerth et al., 2010, Oller et al., 2011).

La mayoría de las WWTPs están generalmente diseñadas para la eliminación eficiente de materia orgánica y nutrientes inorgánicos (por ejemplo carbón biodegradable, nitrógeno y fósforo) que se encuentran presentes en WWi en niveles del orden de los mg L^{-1} a g L^{-1}. Éstas, son WWTPs convencionales que no están equipadas específicamente para la eliminación de microcontaminantes como son los PhACs (Verlicchi et al., 2012). Por este motivo, los sistemas convencionales de tratamiento biológico han demostrado con frecuencia no ser totalmente eficaces, presentando diversos grados de eliminación de PhACs que van desde inferiores al 20% hasta superar el 90% (Chiron et al., 2010; Forrez et al., 2011) (ver tabla 1.2 en chapter 1). Por ejemplo, en WWTPs que aplican CAS como tratamiento secundario biológico, las tasas de eliminación de DCF y SMX varian ampliamente, [7-80]% y [0-98]%, respectivamente (Onessios et al.,2009), por lo que es complicado identificar patrones de comportamiento durante su biotransformación y evaluar su grado. Esta variabilidad en los grados de eliminación de PhACs en WWTPs puede ser debida a parámetros operacionales asó como a factores medio ambientales (Verlicchi et al., 2012). Entre PhACs, las diferencias relevantes que se observan se pueden explicar por la diversas propiedades fisicoquímicas y biológicas que los fármacos presentan (Verlicchi et al., 2012) (ver tabla A.1 en annex). Además, las condiciones climáticas y meteorológicas pueden afectar la eficacia de eliminación de PhACs en WWTPs a través de cambios de

temperatura del agua y dilución por el agua de lluvia, que pueden afectar en último caso el estado biológico de la comunidad microbiana (Fent et al., 2006; Castiglioni et al., 2006; Vieno et al., 2005; 2007; Zhang et al., 2015). El diseño de las WWTPs y factores operacionales como el tiempo de retención de los lodos (del inglés *sludge retention time*, SRT), el tiempo de retención hidráulico (del inglés *hydraulic retention time*, HRT), la temperatura en el reactor biológico y las propiedades de los lodos activados pueden afectar a la eliminación (Suárez et al., 2008; 2012; Alvarino et al., 2014). Las propiedades de la comunidad de los lodos activados, como por ejemplo la actividad de la biomasa (Majewsky et al., 2010) y el potencial nitrificante pueden afectar también a la eliminación (Koh et al., 2009; McAdam et al., 2010). Ambos HRT y SRT gobiernan el tiempo de reacción y la carga (McAdam et al., 2010), y por ende, afectan a la actividad de la biomasa y a su concentración.

Varios estudios han demostrado que los **lodos activados nitrificantes** (del inglés *nitrifying activated sludge, NAS)* tienen la capacidad de degradar microcontaminantes mediante co-metabolimo (Batt et al., 2006; Yi et al., 2007; Forrez et al., 2008; Zhou et al., 2010; Martínez-Hernández et al., 2011). Además, dado que el proceso de nitrificación propicia la eliminación de determinados PhACs, es posible aumentar la eficacia de eliminación mediante el enriquecimiento de los nitrificantes de los lodos activados (Han-Tran et al., 2009).

La biodegradación en el reactor secundario, puede degradar los PhACs y biotransformarlos en TPs (Richardson and Ternes, 2014). Por lo tanto, además de la presencia de metabolitos humanos en WWTPs, la formación microbiana de TPs es el siguiente aspecto a tener en cuenta en el estudio del destino de los PhACs en el sistema acuático. Además, los metabolitos de excreción humana pueden experimentar siguientes transformaciones durante el tratamiento de WW. Por ejemplo, el acetilsulfametoxazol, que es un metabolito del SMX, puede ser transformado de nuevo a su compuesto padre en WWTPs (Gobel et al., 2005). Otro ejemplo de este fenómeno es el observado en el glucurónido de DCF. Se ha especulado que el enlace éster de el conjugado del DCF es inestable hidrolíticamente lo cual podría llevar a la liberación nuevamente de la molécula de DCF durante el tratamiento biológico en la depuradora. Este fenómeno de reconversión de un metabolito a su compuesto padre, explicaría los elevados niveles de DCF en los efluentes de las WWTPs, relativos a las aguas residuales de entrada, que se han observado en algunas ocasiones (Bailey y Dickinson, 2003). Por lo tanto, no sólo el estudio de los procesos de transformación de los PhACs a sus derivados relacionados estructuralmente es interesante, sino que también los son los procesos de reconversión de los TPs a sus compuestos padre.

En comparación con la cantidad de información que trata sobre la distribución de los PhACs en el medio ambiente, bien pocas evidencias se han publicado acerca de las rutas metabólicas que siguen estos compuestos expuestos a comunidades microbiológicas complejas tales como las que se encuentran en los tanques de aereación del tratamiento de lodos activados. Éste es el caso del DCF y SMX, de los cuales su presencia en el medio ambiente acuático se ha estudiado extensamente, pero bien poco se conoce acerca de su destino en las WWTPs, particularmente en lo que respecta a su (co)metabolismo microbiano con bacterias nitrificantes. Uno de los pocos ejemplos descritos en la literatura es el trabajo de Pérez y Barceló (2008), en el cual los nitroso (TP324) y nitro (TP340) derivados del DCF fueron tentativamente identificados mediante el uso de la espectrometría de masas de tiempo de vuelo. Además de la eliminación de la materia orgánica, el proceso más importante que tiene lugar durante el tratamiento con CAS, cuando opera bajo condiciones nitrificantes, es la eliminación de nitrógeno. Este proceso dirigido por la comunidad

microbiana de los lodos, consiste en dos pasos principales que son la nitrificación y la denitrificación. Durante este proceso las bacterias nitrificantes generan especies reactivas de nitrógeno (Chiron et al., 2010) que pueden estar involucradas en la formación de TPs tales como los nitro y nitroso derivados del DCF (TP339 o NO_2-DCF y TP323 o NO-DCF, respectivamente) (Pérez y Barceló, 2008), o en la reacción de nitración del acetaminofén (3-nitro-acetaminofén) (Chiron et al., 2010). Por otra parte, las bacterias denitrificantes son otra fuente potencial de especies reactivas de nitrógeno (Nödler et al., 2012). La formación biótica de especies de nitrógeno derivadas del DCF y el SMX (concretamente NO_2-DCF y 4-NO_2-SMX) se observaron en experimentos a escala laboratorio con microcosmos de agua/sedimento bajo condiciones anóxicas de denitrificación (Nödler et al 2012 y Barbieri et al., 2012). Teniendo en cuenta que la nitrificación y la denitrificación son procesos que también ocurren durante el tratamiento biológico en la WWTP, uno podría conjeturar que la formación de estos productos de transformación del DCF y el SMX también se podría dar en las WWTPs. Por otro lado, el aumento de la concentración de nitrógeno (especies de amonio, NH_4^+-N) se observó con frecuencia en rivieras receptoras de descargas de aguas residuales de salida de WWTPs (Martí et al., 2004). Estas grandes contribuciones de NH_4^+-N desde las WWTPs se relacionaron posteriormente con puntos de elevada actividad microbiana nitrificante que se observaron en rivieras impactadas por el aporte de efluentes de WWTPs (Merseburger et al., 2005).

Por lo general, dependiendo de los grados de eliminación y de transformación de los PhACs y sus TPs durante su destino a lo largo de las WWTPs, es muy probable que una combinación de PhACs y también sus TPs alcancen los sistemas acuáticos de aguas naturales mediante su descarga en las SW a través de las WWe.

8.1.6. Atenuación natural de PhACs en los sistemas colectores de aguas residuales y ríos

De la vasta cantidad de PhACs que son capaces de alcanzar las aguas continentales y debido al amplio abanico de propiedades fisícoquímicas que éstos presentan, se anticipa una distribución de estos compuestos entre los diversos compartimentos acuáticos (por ejemplo entre agua, sedimento y partículas en suspensión) que a menudo resulta compleja de interpretar.

A parte de los procesos de atenuación antropogénicos que los PhACs y TPs experimentan en WWTPs descrito en la sección anterior, estas substancias pueden experimentar procesos de atenuación naturales durante su camino hacia las depuradoras una vez son descargados a las SW colectoras. Cuando los PhACs y TPs alcanzan el sistema colector urbano de WW o los ríos, su destino está sujeto a numerosos factores, incluyendo sus propiedades fisicoquímicas (ver tabla A.1 en annex), factores medioambientales y condiciones climáticas (por ejemplo temperatura del agua, pH y radiación solar) y lo más importante, la presencia y actividad de microorganismos capaces de biodegradarlos (Jelic et al., 2015; Caracciolo et al., 2015). La atenuación natural de los PhACs y TPs se puede dar por varios procesos: (i) la **dilución** en SW; (ii) la **adsorción** en sedimentos y material particulado en suspensión (del inglés *suspended particulate matter*, SPM); (iii) la **biodegradación** biótica; (iv) la **fotodegradación** directa o indirecta; y (v) la **bioacumulación** en biota y su biomagnificación a través de la escala trófica (Mompelat et al., 2009). Cuando llegan a los ríos mediante las WWe, los niveles de PhACs y TPs son atenuados mediante **dilución** en las SW receptoras. Por lo general, la concentración de estos micocontaminantes disminuye al menos un orden de magnitud en el río con respecto a los de WWe (desde elevados ng L^{-1}-µg L^{-1} a bajos ng L^{-1}) (Gros et al., 2010). La **adsorción** de

los PhACs y TPs a sólidos del medio ambiente acuático depende de sus propiedades fisicoquímicas como pK_a, el peso molecular, $\log k_{ow}$, $\log K_{oc}$; y otros muchos parámetros medioambientales como capacidad de intercambio de iones, el contenido de carbono orgánico, la calidad de los sólidos, el pH o la presencia y el tipo de materiales iónicos y coloidales (Delle, 2001). Debido a la naturaleza polar y frecuentemente iónica de los PhACs, su adsorción a los sólidos está gobernada por diversos procesos como la partición hidrofóbica, el intercambio iónico, la adsorción en la superficie, la complejación y los enlaces de hidrógeno (Tolls, 2001; Schwarzenbach et al., 2003). En función del tipo de compuesto y de la heterogeneidad del río, se puede esperar que los fármacos se adsorban en los sedimentos (da Silva et al., 2011; Zhou et al., 2011), SPM (Maskaoui and Zhou, 2010; da Silva et al., 2011) y/o la fase coloidal (Yang et al., 2011). Una vez los PhACs alcanzan los ríos, éstos son transportados a lo largo de la columna de agua y pueden adsorberse al material particulado en suspensión y posteriormente acumularse en los sedimentos. Sin embargo, tras la adsorción estos compuestos pueden ser re-mobilizados y re-suspendidos e incluso desorberse para disolverse de nuevo en la columna de agua. Por ejemplo, se ha demostrado la presencia de algunos PhACs en partículas en suspensión (Matamoros and Bayona 2006) y estudios más recientes han sugerido que la fase coloidal de la columna de agua puede representar un adsorbente de PhACs más potente que las partículas en suspensión y los sedimentos (Maskaoui and Zhou 2010 and Yang et al. 2011).

Además, las concentraciones de PhACs en el medio ambiente acuático pueden atenuarse mediante diversos procesos naturales de degradación. Estudios realizados en microcosmos acuáticos demostraron que la foto-degradación es el proceso más relevante en la atenuación de PhACs en el medio ambiente acuático, siendo la hidrólisis y la degradación microbiana procesos minoritarios (Lam et al. 2004, 2005). No obstante, es probable que la foto-degradación sea menos importante en condiciones de radiación solar reducida debido al alto contenido en materia orgánica disuelta y partículas en suspensión en la columna de agua, por ejemplo.

La **biodegradación** en el medio ambiente acuático está gobernada por los microorganismos asociados a los biofilms en la interfase agua/sedimento o en los sedimentos de base (Radke et al. 2014). La atenuación natural de los PhACs mediante la biodegradación se da un grado que depende del número y el tipo de microorganismos presentes así como de las propiedades fisicoquímicas del compuesto (Fent et al., 2006; Alvarino et al., 2014).

La **fotodegradación** directa o indirecta es el principal mecanismo abiótico por el cual se produce la atenuación de PhACs en el medio ambiente acuático, dado que la mayoría de estos compuestos están diseñados para el consumo oral y por ende son resistentes a la hidrólisis. Mientras que la fotólisis directa se da por la adsorción de la luz solar, en la fotólisis indirecta hay involucradas especies fuertemente oxidantes (por ejemplo radicales hidroxilo y singuletes de oxígeno) generadas de forma natural por fotosintetizadores como nitratos y ácidos húmicos (Andreozzi et al., 2003).

Se ha sugerido que la **bioacumulación** de PhACs en biota está determinada por el transporte activo a través de las membranas biológicas (Daughton et al., 2011). En general, estas substancias son moderadamente lipofílicas y por ende, su potencial de bioacumulación es bajo. No obstante, algunos compuestos como las drogas de tratamiento psiquiátrico han sido detectados en biota acuática (Brooks et al., 2005).

La efectividad de los procesos naturales de atenuación está fuertemente influenciada por las

condiciones climatológicas y meteorológicas como la intensidad de radiación solar y la temperatura o el régimen hidráulico en el río (Vieno et al., 2005).

Además, la efectividad de todos estos procesos está estrechamente sujeta a la variación climatológica estacional, que influye sobre varios factores medioambientales tales como la duración de radiación solar, la temperatura o la precipitación. Se ha observado que los niveles de PhACs pueden disminuir (Kolpin et al. 2000) o bien incrementarse (Boyd et al. 2004) cuando aumenta el caudal del río debido a episodios de lluvia.

8.1.7. Riesgo ecotoxicológico de los PhACs y sus TPs en los organismos acuáticos

Por lo general las concentraciones de PhACs a las que se encuentran en el medio ambiente acuático, del orden de los ng L^{-1}, son de tal nivel que no suponen ningún riesgo toxicológico en humanos (Christensen, 1998). No obstante, los organismos acuáticos son susceptibles de experimentar una exposición continuada a los PhACs a través de las WW a lo largo de toda su vida y por lo tanto sufrir efectos adversos a largo plazo (Oaks et al., 2004; Richard and Hinton, 2008).

Puesto que los PhACs son compuestos intrínsecamente bioactivos y su entrada en el medio ambiente acuático es continua, es de vital importancia entender cuáles podrían ser los efectos en las comunidades biológicas y por ende, en los ecosistemas acuáticos resultantes de la exposición a largo plazo de bajas dosis de estos compuestos. Desafortunadamente, a pesar de de las intensas investigaciones sobre esta problemática ecológica llevadas a cabo en los últimos 15 años, aún quedan amplias lagunas de conocimiento en términos de efectos crónicos en organismos acuáticos no-diana y los efectos en el funcionamiento del ecosistema, así como la pérdida de biodiversidad (Bartelt-Hunt et al., 2011; Hughes et al., 2013).

Varios estudios recientes realizados a escala laboratorio han demostrado que algunos PhACs pueden actuar como disruptores endocrinos siendo sospechosos de causar la feminización en peces; mientras que para los antibióticos, se ha probado que su amplia distribución en el medio ambiente ha llevado al desarrollo de bacterias resistentes a los antibióticos.

A pesar de la pseudo-persistencia del DCF y el SMX en el medio ambiente acuático, es poco probable que éstos PhACs supongan un riesgo para los ecosistemas acuáticos, al menos en términos de toxicidad a corto plazo, ya que sus concentraciones medioambientales son del orden 10^3-10^7 veces menores que los valores de toxicidad aguda (EC_{50}) en organismos acuáticos no-diana que se conocen (Fent et al., 2006). Además, debido a la diversidad estructural que presentan los PhACs, las mezclas complejas de estas substancias pueden exhibir efectos diferentes a los correspondientes a los compuestos por separado (Pomati et al., 2008). En mezclas, los PhACs pueden interactuar entre ellos para derivarse en efectos ecotoxicológicos sinérgicos o antagónicos. Como consecuencia de las miríadas de PhACs presentes en el medio ambiente acuático, es muy probable que se desencadenen efectos crónicos en los organismos acuáticos, incluyendo adición, antagonismo y sinergismo (Daughton and Ternes, 1999; Forrez et al., 2011). Sin embargo, la atribución directa de los efectos toxicológicos en los ecosistemas a los fármacos es un punto difícil de probar y se debe tratar con cautela, dada la concurrencia simultánea de muchos otros compuestos químicos, así como otros estresores medio ambientales (como por ejemplo nutrientes o condiciones hidrológicas).

Se ha aceptado de forma natural que el metabolismo y la transformación de los PhACs lleva a una disminución de su toxicidad asociada. Por ejemplo, los TPs hidroxilados del DCF mostraron actividades farmacológicas menores que el propio DCF o incluso ninguna actividad (Menassé et al., 1978). Sin embargo, en otro trabajo se observó la formación de TPs bioactivos a partir de varios PhACs (incluyendo acetaminofén, carbamazepina y DCF), los cuales se relacionaron con reacciones adversas hepatológicas (Walgren et al., 2005). A pesar de que la toxicidad de los PhACs se conoce ampliamente, la ecotoxicidad que pueden presentar los TPs es un área prácticamente inexplorada (Michael et al., 2014). No obstante, los estudios publicados han demostrado que incluso los metabolitos pueden ejercer efectos perjudiciales en los organismos acuáticos (Celiz et al., 2009; Escher et al., 2011). De igual modo, los TPs generados mediante la transformación microbiana o fotodegradación tanto en los compartimentos medioambientales (como por ejemplo sedimentos o SW) como en sistemas de ingeniería (CAS o AOPs en WWTPs) pueden suponer un riesgo ecotoxiclógico para las especies acuáticas (Escher and Fenner, 2011). Así pues, la desaparición de un PhAC durante el tratamiento en la WWTP, no implica necesariamente la reducción de la toxicidad asociada a la WWe ya que los TPs formados pueden conservar la actividad biológica del compuesto padre (Calza et al., 2006). Por consiguiente, los TPs son considerados como aditivos de concentración en mezclas con sus compuestos padre, de los que también se anticipan potenciales efectos sinérgicos (Escher et al., 2011).

8.1.8 Efectos de la presencia de PhACs en los ecosistemas acuáticos Mediterráneos

Además del impacto que pueden provocar los efluentes de las WWTPs, los ríos pueden sufrir el cambio hidrológico global como resultado del cambio climático, los cambios en el uso de la tierra, el agua y la ingeniería de ríos. Estos impactos se manifiestan en cambios del caudal del agua que desencadenan efectos en la erosión de los suelos, la transferencia y almacenamiento de carbono, nutrientes, contaminantes, aporte de sedimentos a los océanos, biodiversidad continental de los ecosistemas acuáticos, además de la sostenibilidad del desarrollo humano. Por ejemplo, cuando ocurren periodos de sequía, los caudales de los ríos se reducen y por ende su capacidad de dilución de contaminantes, de modo que el riesgo medioambiental asociado a la presencia de estas sustancias se incrementa. En este sentido, son muchos los estudios que han informado sobre el aumento de la frecuencia e intensidad de episodios hidrológicos extremos (New et al. 2001; Huntington 2006; Hirabayashi et al. 2008) en las últimas décadas.

Según las predicciones del Panel Intergubernamental para el Cambio Climático (IPCC) (Christensen et al., 2007), se ha pronosticado que la región Mediterránea experimentará alteraciones severas en el régimen de caudal de sus sistemas acuáticos, no sólo por el descenso de días de precipitación, sino también por el aumento de días de fuertes lluvias. Además, los modelos climáticos regionales pronostican para el sur de Europa una crecida de la frecuencia y duración de las olas de calor y de las precipitaciones severas durante el verano y remarcan que la región Mediterránea será especialmente vulnerable al cambio climático (Sánchez et al. 2004; Giorgi y Lionello, 2008). Debido a ello, se prevén episodios hidrológicos más extremos e impredecibles, como las riadas o las sequías, además de temperaturas más altas y mayor variabilidad de éstas creando, en consecuencia, nuevas condiciones medioambientales en los ecosistemas acuáticos de esta región (Acuña y Tockner, 2010). Además, los ríos Mediterráneos presentan una contaminación severa debida a la elevada presión humana que proviene de las extensivas actividades urbanas, industriales y agricultoras, que afectan a los recursos y al ecosistema. Por estos motivos, los niveles de contaminación en los ríos

Mediterráneos son con frecuencia sustancialmente mayores que en otras cuencas Europeas (Ginebreda et al., 2010). Un buen ejemplo de ello es el río Llobregat (Cataluña, NE España), el cual experimenta condiciones de bajo caudal en condiciones normales (5 m^3s^{-1}) y eventos de picos extraordinarios (máximo registrado de 2.500 m^3s^{-1}) que reajustan periódicamente el sistema (Marcé et al., 2012). Adicionalmente, el río recibe las descargas de aguas residuales de salida de más de 55 WWTPs, y en algunos puntos especialmente durante periodos de sequía, los WWe llegan a representar casi el 100% del total del caudal del río.

Esta situación puede explicar a los elevados niveles de contaminantes orgánicos que se detectan en el río y que aumentan de acuerdo con el creciente número de WWTPs y presión de población desde la cabecera hasta la desembocadura del río en el mar Mediterráno (Céspedes et al. 2005; Huerta-Fontela et al. 2008). Por otra parte, el río Llobregat abastece de agua de consumo humano a la gran ciudad de Barcelona, por lo que abstracción de agua de este río es considerable.

En lo que concierne a la contaminación, como resultado del estatus hidrológico previamente descrito, diversos fenómenos fisicoquímicos pueden desencadenarse a la vez: primero, la ausencia de dilución durante periodos de sequía puede incrementar la concentración de los contaminantes: segundo, y trabajando en la dirección opuesta, caudales bajos aumentan el tiempo de residencia, lo cual facilita los procesos de degradación (Lam et al., 2004); finalmente, las riadas pueden contribuir a la re-mobilización de los contaminantes desde los sedimentos (Petrovic et al., 2011).

La Directiva Marco del Agua (del inglés, *Water Framework Directive*, WFD, 2000/60/CE)) establece las bases para regular los sistemas acuáticos en Europa con el objetivo de conservar, proteger y mejorar la calidad de sus aguas y favorecer su uso sostenible. Bajo esta Directiva, todas las aguas superficiales Europeas están llamadas a alcanzar un buen estado químico y ecológico previsto para el año 2015. La WFD fue más allá del concepto tradicional de calidad de aguas e inició el seguimiento del estado ecológico basándose en las estructuras de las comunidades biológicas mediante el uso de elementos biológicos, hidromorfológicos y fisicoquímicos. Los estados miembros desarrollaron y aplicaron sistemas de medida apropiadas, teniendo en consideración las diversas características biogeográficas de cada región. Mientras que la caracterización por separado de cada elemento ha sido más o menos satisfactoria, el entendimiento de sus respectivas interrelaciones y efectos cruzados es un tema que permanece prácticamente desconocido. Este hecho es particularmente obvio para el caso de los ríos Mediterráneos, los cuales están sujetos a severas sequías y riadas súbitas (Gasith and Resh, 1999). La respuesta del sistema (ecológica y fisicoquímica) a tales variaciones es aún apenas entendida. Como ya se ha descrito previamente, el río Llobregat es un buen ejemplo de un sistema acuático altamente antropizado (Sabater et al., 2012), que experimenta también importantes variaciones de caudal debido a los cambios estacionales que pueden causar alteraciones temporales del caudal base en factores que incluso llegan a superar los 100 (Marcé et al., 2012). Debido a esta relevancia, es de gran interés entender en qué grado la hidrología local afecta la dinámica de los contaminantes y sus efectos potenciales en los organismos acuáticos. Los estudios realizados no van más allá de la aplicación de la WFD mediante el uso de sistemas de medida biológicos basados en macroinvertebrados, diatomeas, macrófitos y peces (Munné et al., 2012a). Sin embargo, la mayoría de estos estudios hacen referencia a respuestas estructurales e ignoran las respuestas funcionales de la comunidad biológica a los contaminantes.

Los biofilms de los ríos son comunidades biológicas complejas compuestas mayoritariamente por

algas, cianobacterias, bacterias, hongos y microfauna que viven sumergidos en un sustrato (Lock, 1993). Las comunidades microbianas adheridas a los biofilms de los ecosistemas de aguas naturales pueden jugar un papel clave en la cadena trófica y en los ciclos biogeoquímicos que tienen lugar en los ecosistemas acuáticos. El ciclo de vida corto de los microorganismos del biofilm y las interacciones tróficas entre la biota (algas, bacterias, hongos, protozoos) permiten la detección de efectos a corto y largo plazo y efectos directos e indirectos en el consorcio de los biofilms (Proia et al., 2012a). Además, en ríos y rivieras, los biofilms son los primeros en interaccionar con substancias disueltas que pueden integrar los efectos de variación de las condiciones en el sistema fluvial durante largos periodos de tiempo. Este comportamiento característico de los biofilms hace que sean útiles descriptores de los efectos microcontaminantes relevantes, como los PhACs, en el ecosistema y por ello, apropiados bioindicadores del estado ecológico de los ríos (Sabater et al., 2007). Varios estudios, tanto a escala laboratorio como de campo, han empleado las comunidades de biofilms fluviales para evaluar los efectos agudos y crónicos de los PhACs (Lawrence et al., 2005; Bonnineau et al., 2010; Lawrence et al., 2012; Rosi-Marshall et al., 2013; Proia et al., 2013; Corcoll et al., 2014).

Por estos motivos sería de máximo interés estudiar la respuesta combinada de la contaminación de PhACs y la estructura y funcionamiento de las microcomunidades biológicas unidas a las biofilms a variaciones del caudal del río.

8.1.9. Legislación y medidas de estudio del riesgo ambiental de PhACs

De acuerdo con la WFD, los PhACs nos están incluidos en la lista de substancias peligrosas prioritarias ni en la de substancias prioritarias (Directivas 2008/105/EC and 2013/39/EU) y por lo tanto, no se dispone de estándares medioambientales de calidad. No obstante, la misma Directiva establece claramente que las substancias descargadas en las cuencas hidrográficas, como es el caso de los PhACs, deberían ser controladas. Además, la Directiva 2013/39/EU reconoce la relevancia de los PhACs para el medio ambiente acuático de la Unión Europea (del inglés *European Union*, EU) (Art. 8c "Specific provisions for pharmaceutical substances) y compromete a la Comisión a desarrollar una aproximación estratégica para 2015 y proponer una serie de medidas específicas para 2017.

De hecho, la Comisión estableció una lista de observación de substancias para la recolección de datos de seguimiento en la UE con los que puedan elaborarse ejercicios de priorización en el futuro (Directiva 2013/39/EU and Decision 2015/495/EU). Es interesante ver como en la citada lista se han incluido 6 PhACs incluyendo el NSAID DCF, las dos hormonas etinil estradiol (EE2) y estradiol (E2); y los antibióticos eritromicina, claritromicina y azitromicina.

La Directiva 2004/27/EC en medicina humana y la Directiva 2004/28/EC en medicina veterinaria, establecieron una evaluación del riesgo medioambiental (del inglés Environmental Risk Assessment, ERA) en el marco de la aprobación de nuevos productos medicinales. De acuerdo con la Directiva 2004/27/EC en PhACs humanos, para todas las nuevas autorizaciones de PhACs, se deben examinar los efectos medioambientales y este seguimiento debe estar incluido en cada aprobación de su aplicación. De acuerdo con los límites d seguridad establecidos por la EU de 0.01 μg L^{-1} (EMA, 2006), sólo los compuestos que excedan estas concentraciones en el medio ambiente deben de estar sujetos a una ERA. Los procedimientos para realizar ERA de PhACs se han desarrollado en base a datos publicados de ecotoxicidad, mayoritariamente mediante unidades de toxicidad (del inglés *toxic units*, TU) o cocientes de riesgo (del inglés *hazard quotients*,

HQ). Los valores de TUs o HQs están asociados al riesgo ecotoxicológico de un determinado compuesto, o de una mezcla de compuestos, para ejercer efectos a corto o largo plazo en organismos no-diana (Gros et al. 2010, Ginebreda et al., 2010; Ginebreda et al., 2014). Los TUs o HQs se definen como la razón entre la concentración medio ambiental medida (del inglés *measured environmental concentration*, MEC) de un determinado compuesto y su valor de toxicidad aguda EC_{50} o LC_{50} (Sprague, 1970) o su toxicidad crónica, normalmente expresada como concentraciones de efectos no observados (del inglés *non-observed effect concentrations,* NOEC) (Castiglioni et al., 2004; Cooper et al., 2008). Los valores de EC_{50}, LC_{50} y NOEC se determinan normalmente empleando tests de ecotoxicidad acuática estándares en dafnias, algas o peces. En el caso que los valores de NOEC o MEC no se han podido determinar, los TUs o HQs se pueden estimar teóricamente. Si no se ha podido estimar la toxicidad crónica, lo cual ocurre frecuentemente para los PhACs, las concentraciones estimadas de efectos no observados (del inglés *non-observed effect concentrations*, PNEC) se pueden extrapolar mediante la división de los valores de EC_{50} or LC_{50} de toxicidad aguda mediante un factor de evaluación (del inglés *assessment factor*, AF) que es normalmente de 1000. De igual modo, si los valores MEC no se han podido determinar, se pueden aplicar las concentraciones medioambientales estimadas (del inglés *predicted environmental concentrations*, PEC). El valor de PEC se estima normalmente en base al porcentaje de penetración en el Mercado, la dosis diaria máxima, el grado de excreción metabólica, la cantidad de WW por habitante, las tasas de eliminación en las WWTPs y el factor de dilución (EMA, 2006; Riva et al., 2015). De acuerdo con los documento de guías de seguridad sobre ERA (EMA, 2006), si los valores de TU o HQ estimados para un compuesto determinado o una mezcla de compuestos están por debajo de la unidad no se espera ningún riesgo ecotoxicológico. No obstante, si los valores estimados igualan o superan la unidad, se anticipa un riesgo medioambiental potencial.

8.1.10. Aplicación de modelos matemáticos para el estudio del destino de los PhACs en el medio ambiente acuático

El uso de modelos matemáticos como herramientas de predicción para interpretar la compleja realidad en un contexto de escasa disponibilidad de información experimental, como en el caso de la evaluación del destino de los compuestos químicos, ha crecido sustancialmente durante las últimas décadas (por ejemplo Beven, 2006). La incorporación de Sistemas de Información Geográfica (del inglés *Geographic Information Systems*, GIS) para la modelización ha mejorado en gran medida sus posibilidades (Pistocchi 2014). Como complemento a las concentraciones medidas en campañas de muestreo, se han desarrollado modelos de calidad del agua para generar predicciones de concentraciones efectivas (del inglés *Predicted Effect Concentration*, PEC) desde fuentes puntuales o difusas de productos químicos en el medio ambiente. De hecho, los estudios de modelización han demostrado que las concentraciones de PhACs en la WWe y en SW se pueden predecir con una exactitud razonable cuando datos realistas sobre las emisiones químicas y de caudal están disponibles (Pistocchi et al., 2010).

El uso de enfoques de flujo simples en el campo de la modelización del destino de los compuestos químicos, ha permitido la evaluación de los patrones de concentración de compuestos derivados de una determinada fuente. Entre estos modelos, el modelo de flujo de pistón (del inglés *Plug-flow,* PF) es a menudo la herramienta elegida para la simulación de la calidad del río (por ejemplo Chapra, 1997). Esta aproximación describe la concentración de una sustancia química a lo largo de la red fluvial aguas abajo de una fuente de

emisión.

Además, estos modelos simplificados, pueden implementarse directamente utilizando análisis GIS, proporcionando distribuciones espaciales de concentraciones químicas estimadas razonablemente realistas, a través de cálculos matemáticos extremadamente simples. Esto se ha demostrado con referencia a la distribución continental de muchas sustancias químicas de relevancia ambiental, aunque su aplicación con PhACs es muy limitada (Pistocchi et al., 2010).

Los modelos de calidad de agua que dependen de los programas de ordenador georreferenciados se están volviendo cada vez más populares. Ejemplos de este tipo de modelos son el GREAT-ER (Geography-referenced Regional Exposure Assessment Tool for European Rivers) (Feijtel et al., 1997) o su equivalentes estadounidenses PhATE (Pharmaceutical Assessment and Transport Evaluation), y LF2000 -WQX (Keller et al 2004; Johnson et al 2007), entre otros (Pistocchi et al 2010). Estos modelos muestran las concentraciones ambientales estimadas de sustancias químicas a lo largo de toda una cuenca fluvial así como perfiles de concentración a escala regional y también puntos calientes de elevada concentración, lo que permite ubicar las fuentes puntuales. Estos enfoques, que integran los procesos que influyen en el destino de PhACs en el medio acuático (por ejemplo, el metabolismo humano, la eliminación en las WWTP, la dilución en las aguas y otros procesos de atenuación natural en los sistemas fluviales receptores), pueden predecir con resolución espacial las concentraciones de compuestos que se liberan a las SW a través de la WWe de WWTPs como principal fuente de emisión (Alder et al., 2010).

La ventaja de este tipo de programas de simulación respecto a modelos más genéricos es el aumento del realismo en la evaluación de la exposición química mediante la incorporación de características espaciales y temporales del medio receptor. Por otra parte, estas metodologías son fáciles de usar y altamente rentables económicamente (Alder et al., 2010).

Uno de los programas de simulación de ordenador georreferenciados más relevantes es el GREAT-ER, un programa informático basado en GIS desarrollado y validado por el Centro Europeo de Ecotoxicología y Toxicología de las Sustancias Químicas (del inglés *European Centre for Ecotoxicology and Toxicology of Chemicals*, ECETOC), como una herramienta exacta de predicción de la exposición a sustancias químicas en el medio ambiente acuático para su uso dentro del esquema de ERA de la EU. El software acopla datos específicos del mercado de sustancias con información relevante de los compuestos en el medio ambiente con el fin de calcular la distribución de los PECs reales de los productos químicos de consumo en SW, tanto para tramos de ríos, así como para cuencas enteras, generando un mapa georreferenciado (Schowanek et al ., 2000). GREAT-ER ya se ha aplicado con éxito y validado para un número de productos de consumo en las cuencas fluviales europeas (Wind et al., 2004).

8.2. Objetivos

En el contexto de la problemática asociada a los PhACs y TPs comentada anteriormente; el objetivo general de esta tesis es el estudio de su destino en WWTPs y en los ríos Ibéricos y la evaluación del riesgo ambiental que estas sustancias pueden suponer para los ecosistemas acuáticos.

Los objetivos específicos son:

1. El desarrollo de un método analítico basado en cromatografía de líquidos acoplada a espectrometría de masas en tándem (LC-MS/MS) para la cuantificación a niveles traza DCF y SMX, sus metabolitos humanos y sus nitro/nitroso productos de transformación, de los cuales su formación en bioreactores a escala laboratorio fue previamente descrita en Pérez y Barceló (2008) y Nödler et al. (2012), con el fin de evaluar si también son detectados en muestras reales de WWTPs.

2. Investigación de la biodegradación de análogos estructurales del DCF

(ácido 2-anilinofenilacético, ácido mefenámico, ácido tolfenámico, ácido meclofenámico y ácido flufenámico) para averiguar sin también son capaces de biotransformarse para generar nitro/nitroso productos de transformación bajo las mismas condiciones experimentales de biodegradación de DCF en reactores nitrificantes a escala laboratorio en las cuales se identificaron sus correspondientes TPs (Pérez y Barceló, 2008) mediante el análisis por espectrometría de masas de alta resolución.

3. Llevar a cabo estudios de seguimiento a gran escala de un total de 96 PhACs en muestras de aguas superficiales y sedimentos muestreadas a lo largo de cuatro importantes cuencas hidrográficas de la Península Ibérica, caracterizadas por una elevada presión antropogénica, identificando los factores clave que afectan su presencia en los ríos.

4. El uso de la quimiometría para la evaluación temporal y especial de la distribución de una selección de 76 PhACs (del total de 96 compuestos estudiados en esta tesis) en aguas superficiales y sedimentos medidos en el punto 3, y la aplicación de un modelo de tipo “plug-flow” (Pistocchi et al., 2010) para estimar la atenuación natural de 14 PhACs a lo largo del curso del curso del Río Llobregat.

5. La evaluación del riesgo ecotoxicológico que los PhACs pueden representar para los ecositemas acuáticos mediante (i) la medida de la toxicidad aguda de PhACs y sus productos de transformación en *Daphnia magna* y *Vibrio fischeri*; (ii) la identificación de los PhACs que contribuyen en mayor grado a la toxicidad total de muestras de agua superficial y (ii) la evaluación del impacto de la variabilidad de los niveles de PhACs y del caudal del río Llobregat en la estructura y funcionamiento de los biofilms.

8.3. Análisis e identificación de DCF, compuestos relacionados y sus TPs.

8.3.1 Determinación simultánea de DCF, sus metabolitos humanos y sus nitro/nitroso productos de transformación en aguas residuales por cromatografía de líquidos acoplada a espectrometría de masas en tándem de quadrupolo/trampa linear de iones.

En la actualidad, existe un creciente interés por el estudio de la presencia, comportamiento y destino de los PhACs en las WWTPs y en el medio ambiente acuático, el cual es ampliamente propiciado por los avances tecnológicos en la instrumentación apropiada para el análisis de estos compuestos orgánicos polares en matrices tan complejas.

Estudios sobre el destino del DCF durante el tratamiento de aguas residuales en WWTPs, fueron el objetivo de Pérez y Barceló (2008), quienes investigaron sobre la biotransformación del DCF en bioreactores

a escala laboratorio que contenían licor mixto tomado del bioreactor secundario de una WWTP municipal. Mediante el uso de varias aproximaciones de la espectrometría de masas de alta resolución, dos productos de transformación del DCF, desconocidos hasta el momento, concretamente el nitroso derivado (TP323 o NO-DCF) y el nitro derivado (TP339 o NO_2-DCF) fueron descritos por primera vez (Pérez y Barceló, 2008). Sin embargo, dado que sus correspondientes patrones estándar puros no estaban disponibles comercialmente, la detección en WWTPs de estos TPs no se pudo llevar a cabo.

Una de las mayores dificultades a la hora de analizar TPs es la necesidad de patrones estándar puros para el desarrollo de los métodos analíticos apropiados y también la cuantificación de las muestras, que no están disponibles comercialmente. Una alternativa razonable para obtener estos compuestos de referencia es mediante su preparación "en-casa" aplicando la síntesis orgánica clásica o la síntesis bioquímica. Con el objetivo de generar metabolitos humanos que no estaban disponibles comercialmente en aquel momento, para el análisis de muestras de aguas residuales, Pérez y Barceló (2008) biosintetizaron 4'-OH-DCF mediante la recombinación del citocromo humano P450.

En base a la problemática medioambiental comentada en la introducción concerniente al DCF y sus TPs, identificados a escala laboratorio, en esta tesis ha sido desarrollado y validado un novedoso modelo analítico multi-residuo, para la determinación simultanea de DCF, cinco de sus metabolitos humanos (4'-OH-DCF, 5-OH-DCF, 4',5-diOH-DCF, 5-OHD-DCFand DCF-gluc) y dos TPs de nitrificación/desnitrificación (NO_2-DCF, NO-DCF) en WWi y WWe con el fin de entender mejor el destino general del DCF. El método de análisis se basó en la determinación del DCF y sus derivados (algunos de ellos sintetizados químicamente) por extracción en fase sólida "off-line" usando como material sorbente un polímero de balance hidrofílico-lipofílico, seguido de CL acoplada QqLIT-MS. La cuantificación se llevó a cabo por el método de calibración de patrón interno, para corregir los posibles efectos de matriz.

La exactitud del método fue generalmente por encima del 40% para aguas residuales de entrada y de salida con una precisión por debajo del 12%. Los límites de detección para a la mayoría de los del compuestos fueron entre el 0.3-2.5 ng L^{-1} y 0.1-3.1 ng L^{-1}, respectivamente.

Con el fin de obtener una herramienta adicional para la identificación y confirmación de los compuestos derivados del DCF estudiados, se llevaron a cabo experimentos de adquisición de información dependiente (IDA), con monitorización por selección de reacción (SRM) y barrido electrónico del ión producto mejorado (EPI) como barrido dependiente.

El DCF y su metabolito humano mayoritario, el 4'-OH-DCF, se detectaron en todas las muestras de WWi en concentraciones de 447-1080 ng L^{-1} y 3.000-6.000 ng L^{-1}, respectivamente; t también de WWe a niveles de 331-1.150 ng L^{-1} y 585-2.610 ng L^{-1}, respectivamente. Por otra parte, 5-OHDCF se detectó solo en una WWi a 417 ng L^{-1} y en cinco de las diez WWe analizadas, en concentraciones en el intervalo de 180 hasta 755 ng L^{-1}. En cambio, 4',5-diOH-DCF se detectó con mayor frecuencia, siendo detectado en seis y siete WWi y WWe, respectivamente, de las 10 WWTPs estudiadas. Las concentraciones determinadas para 4',5-diOH-DCF en WWi fueron entre 255 y 1.028 ng L^{-1}, mientras que en WWe el intervalo fue considerablemente menor de12 a 229 ng L^{-1}. Por el contrario, 5-OHD-DCF se detectó en todas las muestras en concentraciones que no excedieron los 111 ng L^{-1}. En lo que respecta al DCF-gluc, se detectó en seis de las diez WWi mientras que en WWe sólo se detectó en 4 muestras. Ninguno de los TPs del DCF se detectó

en las muestras de WWi analizadas. Por el contrario, NO-DCF se detectó en seis de las 7 WWe analizadas en concentraciones en el intervalo de 4 a 105 ng L^{-1}, mientras que NO_2-DCF sólo se detectó en tres WWe a niveles desde 20 a 29 ng L^{-1}.

8.3.2. Comportamiento de DCF y otros NSAIDs estructuralmente relacionados en NAS de WWTPs.

Con la finalidad de ampliar la investigación de los procesos de nitración y nitrosación del DCF, que se dan en el reactor biológico de las WWTPs, se estudió la biodegradación en condiciones nitrificantes de otros compuestos con estructuras químicas similares al DCF pero que varían en el tipo y número de sustituyentes halogenados. Los compuestos objeto de estudio fueron: el DCF y los ácidos 2-anilinofenilacético, fenámico, mefenámico, flufenámico, tolfenámico y meclofenámico.

Para ello, se llevaron a cabo experimentos de biodegradación de los compuestos de interés en bioreactores nitrificantes a escala laboratorio, preparados a partir de licor mixto obtenido del reactor biológico de una WWTP. Las muestras de biodegradación se analizaron mediante LC-MS/MS usando un espectrómetro de masas híbrido de tipo quadrupolo-trampa orbital (Q-Exactive)-MS para la identificación de TPs. Bajo las mismas condiciones experimentales que se aplicaron cuando se llevó a cabo la biodegradación del DCF (Pérez y Barceló, 2008), la incubación de los NSAIDs análogos estructuralmente al DCF con bacterias procedentes de la WWTP, generó una serie de TPs de nitrosación/nitración. De hecho, todos los compuestos estudiados confirmaron la formación de TPs de nitrosación/nitración identificada mediante mecanismos bióticos. Así, este experimento demostró que el camino de reacción que sigue el DCF bajo condiciones nitrificantes en los lodos activados, no es único para este PhAC.

El análisis de las muestras de biodegradación mediante la espectrometría de masas de alta resolución permitió la identificación y proponer tentativamente las estructuras químicas de los nuevos TPs observados. Se determinaron los perfiles de degradación de todos los compuestos y de sus correspondientes TPs, en los diversos reactores y controles. La evaluación de estos perfiles de degradación proporcionó información valiosa sobre el comportamiento del DCF y otros compuestos con estructuras análogas en los reactores biológicos de las WWTPs. Se observó una cinética de biotransformación del análogo no halogenado del DCF, ácido 2-anilinofenilacético, a su nitro-derivado mayor que la correspondiente a la del DCF. Este comportamiento se explicó por los efectos estéricos que los dos átomos de cloro de la molécula del DCF posiblemente impidan la aproximación enzimática en las reacciones de nitración y nitrosación.

Mediante la adición del isotopo marcado estable $^{15}NH_4$-N en el bioreactor, se observó en el espectro de masas un incremento de +1 Da en la masa del ^{15}N NO_2-DCF marcado isotópicamente, comparado con el mismo TP no marcado NO_2-DCF. De este modo se pudo confirmar la posición del grupo NO_2 en la molécula.

8.4. Estudio de la presencia y modelización de PhACs en WWTPs y ríos Mediterráneos.

8.4.1. PhACs en una sección impactada por aguas residuales de WWTPs de un río Mediterráneo (Río Llobregat, NE España) y su relación con la variación de las condiciones hidrológicas.

En este estudio se determinaron las concentraciones de PhACs en un tramo seleccionado del

río Llobregat y se correlacionaron estos resultados con parámetros hidrológicos del río como el caudal y el carbón orgánico disuelto. Se recabó más información acerca del destino de los PhACs en un tramo severamente impactado por efluentes de WWTPs de un río típicamente Mediterráneo y se quiso estudiar en qué modo éstos se pueden ver afectados por las concentraciones de los PhACs y los principales eventos del cambio climático, concretamente las riadas y las sequías. Además, los niveles determinados de PhACs (C_X para un determinado compuesto X) se relacionaron con los factores hidrológicos como el caudal del río (Q) y el carbono orgánico disuelto (del inglés *dissolved organic carbon*, DOC) y se calcularon las correlaciones respectivas r(C_X/Q) y r(C_X/DOC). Además, se evaluó el impacto de los cambios del caudal y el DOC en la concentración de los PhACs en el río mediante el cálculo de coeficientes relativos de sensibilidad s(C_X/Q) y s(C_X/DOC), respectivamente.

Para ello se tomaron muestras de agua fresca del río dos veces por semana durante un periodo de cinco semanas en tres puntos de muestreo. La sección estudiada del río Llobregat, que se dividió en tres puntos de muestreo localizados entre el tramo superior al tramo inferior en Castellbell i el Vilar, Abrera (ABR) y Sant Joan Despí (SJD), está caracterizada por recibir la descarga de aguas residuales de salida de un elevado número y diversidad de WWTPs, así como del aporte de diversos afluentes y rivieras tributarias.

Se analizó una lista inicial de 66 PhACs considerados relevantes (según su elevado consumo) pertenecientes a diversos grupos terapéuticos mediante LC-MS/MS. Las muestras de aguas superficiales, previamente filtradas a 0,45 µm se pre-concentraron y pre-limpiaron mediante la extracción en fase sólida usando cartuchos de balance hidrofílico-lipofílico, dada la variedad de PhACs analizados. Para el posterior análisis de los compuestos seleccionados para el estudio en los extractos, se usó un método analítico multiresiduo previamente desarrollado y validado por Gros et al (2009) mediante LC-MS/MS (QqLIT) acoplado a una fuente de ionización por electroespray trabajando tanto en modo positivo como negativo. El analizador de masas trabajó en modo de monitorización por selección de ión. Para la separación cromatográfica se usó una columna cromatográfica precedida por una precolumna C18, dada la complejidad de las matrices analizadas.

Se determinaron los niveles de PhACs en aguas superficiales entre los órdenes de ng L^{-1} y µg L^{-1}. Entre los diversos grupos terapéuticos estudiados, los analgésicos y antiinflamatorios fueron las familias de PhACs más detectados en el intervalo de 700-1.700 ng L^{-1} para todos los puntos de muestreo. Del total de 66 PhACs estudiados, todos fueron detectados en más del 85% de las muestras. Las concentraciones máximas detectadas para los compuestos individuales fueron las correspondientes al ibuprofeno, acetaminofén, naproxeno, metoprolol, lorazepam, tetraciclina y SMX; que alcanzaron valores superiores a los 500 ng L^{-1}.

Se observó un incremento de la contaminación de PhACs directamente relacionado con el aumento del número de WWTPs distribuidas a lo largo de la sección estudiada, así como el aumento del volumen de WWe en las WWTPs más importantes localizadas en el tramo bajo del río.

Se observaron correlaciones positivas y negativas entre los compuestos objeto de estudio y las variables hidrológicas como el caudal del río y el carbono orgánico disuelto. La respuesta de los PhACs al caudal del río fue negativa (los valores significativos de r(C_X/Q) fueron desde −0,305 para DCF hasta 0,807 para SMX), básicamente debido a los esperados efectos de dilución. Sólo en pocos casos se observaron relaciones positivas entre las concentraciones de los compuestos detectados y el caudal del río, sugiriendo la relevancia de los diversos fenómenos hidrológicos tales como los efectos de dilución y la re-suspensión de

sedimentos asó como las fuentes de estos contaminantes. La respuesta de los PhACs al DOC fue positiva debido a las asociaciones anticipadas de estas substancias al DOC. Estos resultados sugirieron que la concentración de los PhACs aumenta con el DOC, lo cual significa también que cuando los valores de DOC son elevados, la movilidad de estos compuestos en la fase acuosa aumenta a su vez.

Los análisis de sensibilidad respecto al caudal del río y el carbono orgánico disuelto, mostraron que la mayoría de los PhACs estudiados eran sensibles a estas variables hidrológicas. Los PhACs mostraron valores de $s(C_X/Q)$ en el interval de 0,33 para tetraciclina a −1,43 para lorazepam; mientras que para $s(C_X/DOC)$ los valores variaron desde 0,04 para butalbial hasta 2,40 para ciprofloxacina. En general, la ordenación en la sensibilidad a ambos parámetros hidrológicos fue la misma para todos los compuestos. No obstante, los PhACs mostraron mayor sensibilidad al DOC con respecto al Q, sugiriendo la importancia de la respuesta de estas substancias a la materia orgánica.

8.4.2. Presencia y modelización de PhACs en una sección impactada por aguas residuales de WWTPs de un río Mediterráneo y su dinámica bajo condiciones hidrológicas diferentes.

En este estudio, se continuó siguiendo la presencia de PhACs en un tramo seleccionado del río Llobregat en el curso bajo del río, donde las concentraciones de PhACs presentaron mayores niveles en el estudio anterior. Respecto a la lista anterior de PhACs, se incluyeron 7 PhACs para analizar un total de 73 compuestos. El principal interés fue estudiar la presencia y el destino de estos contaminantes orgánicos bajo condiciones hidrológicas diferentes mediante el muestreo de dos puntos diferenciados del río (ABR y SJD) durante diversas estaciones.

Además se establecieron relaciones cuantitativas entre las concentraciones de PhACs detectadas y el caudal del río bajo condiciones hidrológicas diferentes. Para este fin, se aplicó un modelo sencillo denominado “plug-flow” tal y como propuso Pistocchi et al. (2010), para poder realizar una evaluación cuantitativa sobre (a) la carga de cada PhAC generado por el sistema de aguas residuales aguas abajo desde el punto de control, y (b) la desaparición general observada en los diferentes compuestos a lo largo del río.

El seguimiento de PhACs en aguas superficiales se realizó durante un año, en 4 campañas de muestreo (periodos de 4-5 semanas cada una y 9-13 muestras por campaña) correspondientes a las cuatro estaciones y por tanto, bajo condiciones climáticas y por ende, hidrológicas diferentes.

El protocolo analítico que se empleó para el análisis de las muestras de aguas superficiales fue el mismo que se describió en la sección anterior y que se basó en la extracción en fase sólida “off-line” seguida de la LC- MS/MS (QqLIT) (Gros et al., 2009).

De nuevo, se demostró que los PhACs son microcontaminantes ampliamente extendidos en el río Llobregat. De entre los 73 compuestos analizados, 50 se encontraron presentes en todas las muestras analizadas. Los niveles detectados de PhACs fueron entre los órdenes del ng L^{-1} y del µg L^{-1}. Entre los diversos grupos terapéuticos estudiados, los analgésicos y antiinflamatorios fueron la familia de PhACs más representativa, con frecuencias de detección entre el 67% y el 100% de las muestras y valores medianos superiores a los 350 ng L^{-1} para todos los puntos de muestreo ([200-1.100] ng L^{-1} en ABR y [200-18.000] ng L^{-1} en SJD). Por lo general, las concentraciones individuales fueron elevadas, entre las decenas y los

centenares del orden del ng L^{-1}. Ibuprofeno, acetaminofén y DCF presentaron las mayores concentraciones en el intervalo medio-alto de los ng L^{-1} (100–500 ng L^{-1}).

Se observó un gradiente de concentración a lo largo de la sección del río estudiada. En promedio, las concentraciones menores de PhACs se determinaron en ABR (~2.000 ng L^{-1}), mientras que las máximas se observaron en SJD (~16.800 ng L^{-1}). Esta tendencia se explicó por las crecientes cargas de PhACs desde las WWTPs que aumentan en número río abajo.

En cuanto a la variabilidad temporal de los niveles de PhACs, se detectaron niveles máximos durante periodos fríos y secos correspondientes a las estaciones de otoño e invierno. Las mayores concentraciones totales de PhACs determinadas durante otoño e invierno alcanzaron los 2.000 y 2.500 ng L^{-1} en ABR y 35.000 y 12.000 ng L^{-1} en SJD, respectivamente. En cambio, en primavera y verano, las concentraciones totales medidas sólo llegaron a los 1,400 y 1,200 ng L^{-1} en ABR y niveles similares en ambas estaciones de hasta 3.500 ng L^{-1} en SJD. Los niveles más bajos observados en primavera y verano se explicaron por los efectos de dilución debidos a los episodios de lluvias fuertes, elevadas temperaturas y por ende mayores grados de eliminación de PhACs en WWTPs; procesos de degradación naturales (fotodegradación debida a la radiación solar y/o biodegradación); y la disminución de su consumo humano durante estos periodos.

Se detectaron tres episodios de picos de caudal que proporcionaron información sobre la respuesta de los PhACs estudiados a episodios hidrológicos extremos típicos de un río Mediterráneo como es el río Llobregat. Para evaluar los factores que influyen en la variabilidad de las concentraciones, se seleccionaron 14 PhACs considerados relevantes (de acuerdo con las concentraciones elevadas detectadas) y se aplicó el modelo “plug-flow” para obtener constantes de desaparición “k” de los PhACs seleccionados. El modelo aplicado describió el destino de los PhACs en términos del caudal del río y parámetros específicos del compuesto; la emission del PhAC (E), asociada a la carga promedio liberada río arriba y la constante general de atenuación (k) interpretada como la atenuación del PhAC a lo largo del tiempo. Los modelos aplicados para muchos compuestos mostraron valores mayores de E en SJD, lo cual cabía esperar teniendo en cuenta las mayores concentraciones de PhACs en las principales WWTPs que descargan sus WWe en la cuenca.

La eritromicina mostró valores de k de −0,15 h^{-1} en ambos puntos de muestreo, siendo el compuesto que se eliminó de la columna de agua con la máxima eficiencia. Los siguientes PhACs presentaron valores de k inferiores; ibuprofeno, furosemida, enrofloxacina, enalapril, acetaminofén, DCF o ketoprofeno que fueron entre −0,04 and −0,10 h^{-1} mostrando menor grado de desaparición de la columna de agua que la eritromicina. Sin embargo, otros compuestos mostraron valores de k <0,06, lo cual sugirió el comportamiento conservativo de estos compuestos en la columna de agua.

La aproximación del modelo propuesta, demostró ser consistente (por ejemplo los valores de E y k estimados para cada compuesto en ambas localizaciones estudiadas, ABR and SJD, fueron similares), y por tanto la fiabilidad de los valores calculados de desaparición de estos compuestos en las aguas de los ríos. Además se consideró por tanto potencialmente útil para fines de administración a nivel de cuenca o de todo el sistema acuático.

8.4.3. La concentración y el riesgo de los PhACs en los sistemas acuáticos están relacionados con la densidad de población y las unidades ganaderas en los ríos Ibéricos.

Con el fin de contrastar los datos de contaminación por PhACs en el Río Llobregat con otras cuencas de la península Ibérica, en el presente trabajo se analizó la presencia de PhACs en cuatro cuencas hidrográficas representativas: Llobregat, Ebro, Júcar y Guadalquivir. También se evaluaron las distribuciones espaciales y temporales en las aguas superficiales y los sedimentos de dichas cuencas. Además se evaluó el riesgo ecotoxicológico de la presencia de los PhACs en los sistemas acuáticos puede suponer para los organismos acuáticos (concretamente, algas, Dafnia y peces). Para evaluar la ecotoxicidad de una selección de 55 PhACs (de los 96 compuestos estudiados en esta tesis) en SW de las cuatro cuencas estudiadas, se estimaron valores de TU en base a los datos consultados en la literatura sobre la toxicidad aguda de estos compuestos en algas, dafnias y peces para cada punto de muestreo analizado. Por último, tanto las concentraciones de PhACs determinadas como su riesgo estimado, se correlacionaron con sus principales fuentes de emisión: el consumo humano y animal. Así, se presentó el primer estudio cuantitativo que relaciona la presencia de los PhACs y su ecotoxicidad estimada con la densidad de población humana y las unidades ganaderas. Dado el gran volumen de datos obtenidos, se aplicaron herramientas estadísticas y quimiométricas desarrolladas en R, como por ejemplo análisis ANOVA seguidos de comparaciones; “TukeyHSD pairwise“; escalado multidimensional no métrico, del inglés *Non-metric Multidimensional Scaling*, (NMDS); permanovas, y ANOVA basados en la permutación de modelos lineares de efectos mixtos, del inglés *linear mixed effect models*, (LME models). Estas herramientas facilitaron la interpretación de los resultados y evaluar así las tendencias generales más importantes de los PhACs estudiados en las cuatro cuencas hidrográficas.

Esta vez se preparó una nueva lista de PhACs (76) considerados relevantes para el estudio de acuerdo con su consumo y la literatura consultada sobre su relevancia para el medio ambiente acuático. Se realizaron dos campañas de muestreo en años consecutivos a lo largo de las cuatro cuencas hidrográficas en un total de 77 puntos de muestreo. En ambos muestreos se tomaron muestras puntuales de aguas superficiales (77) y sedimentos (77). Para el análisis cuantitativo de los PhACs objeto de estudio en dichas matrices, se siguieron los protocolos analíticos desarrollados y validados previamente (Gros et al., 2012; Jelic et al., 2009). La extracción de las muestras de aguas superficiales, previamente filtradas a 0,45 µm, se realizó mediante la extracción en fase sólida utilizando un material sorbente de tipo balance hidrofílico-lipofílico dada la variedad de los 76 PhACs analizados. Respecto a los sedimentos, la extracción de los analitos se llevó a cabo mediante la técnica de líquidos presurizados seguida de la etapa de purificación mediante extracción en fase sólida utilizando un material sorbente de tipo balance hidrofílico-lipofílico. El análisis de los extractos se realizó mediante LC-MS/MS (QqLIT) acoplado a una fuente de ionización por electroespray trabajando tanto en modo positivo como negativo. El analizador de masas trabajó en modo de monitorización por selección de reacción. Para la separación cromatográfica se usó una columna cromatográfica precedida por una precolumna C18, dada la complejidad de las matrices analizadas.

Se demostró que los PhACs son microcontaminantes ampliamente distribuidos y pseudo-persistentes en el medio ambiente acuático Ibérico. Se detectaron niveles de PhACs del orden del bajo a alto ng L^{-1} en SW y del nivel de pocos ng g^{-1} en sedimentos. Alrededor del 60% de los compuestos estudiados estaba presente en al menos la mitad de las muestra de SW y sedimentos analizadas en ambas campañas de

muestreo. El 22% y el 18% de compuestos fueron detectados en todas las muestras de SW y sedimentos.

Los PhACs se detectaron con la mayor frecuencia en las SW del río Llobregat, seguidos del Ebro, Júcar y Guadalquivir. Los niveles más elevados de PhACs se detectaron en la cuenca del Llobregat, seguida del Ebro, el Guadalquivir y el Júcar.

El grupo terapéutico detectado con mayor frecuencia y concentración fueron los analgésicos y antiinflamatorios, seguidos de los antibióticos, los diuréticos y los PhACs de tratamiento psiquiátrico. En SW los analgésicos y antiinflamatorios fueron el grupo más relevante. Entre ellos, ibuprofeno y DCF fueron de los más concentrados en las cuatro cuencas hidrográficas presentando los siguientes niveles: 37 y 31 ng L^{-1} en Llobregat; 37 y 14 ng L^{-1} en Ebro; 3 y 8 ng L^{-1} en Guadalquivir y 2 y 3 en ng L^{-1} Júcar. En los sedimentos, los antibióticos mostraron las mayores concentraciones. Estos niveles se detectaron en el mismo orden de magnitud en todas las cuencas (43 ng g^{-1} como promedio). No obstante, los niveles de analgésicos y antiinflamatorios determinados en sedimentos se acercaron a los observados para los antibióticos. Estas concentraciones fueron ligeramente diferentes entre las cuencas (19 ng g^{-1} en Llobregat, 15 ng g^{-1} en Ebro, 27 ng g^{-1} en Júcar y 18 ng g^{-1} en Guadalquivir).

El gradiente de concentración de PhACs que se observó previamente en una sección del río Llobregat impactada por descargas de WWe (Osorio et al., 2012a; 2012b), se confirmó en el presente estudio para toda la cuenca. Sin embargo, para el resto de cuencas no se observó ninguna tendencia clara de contaminación.

Respecto a los compuestos individuales, la hidroclorotiazida y el gemfibrozilo, así como la azitromicina y el ibuprofeno fueron los PhACs más ubicuos y concentrados en SW y sedimentos, respectivamente.

El riesgo ecotoxicológico promedio de los PhACs en los organismos acuáticos fue más relevante en el Llobregat (2.50E − 04), seguida de cerca del Ebro (2.28E − 04) y el Guadalquivir (6.35E−05) y Júcar (3.97E−05). Del mismo modo que se observó para las concentraciones de PhACs, el riesgo potencial de estos compuestos en los organismos acuáticos fue en aumento río debajo de la cuenca del Llobregat, aunque no se pudo observar una clara tendencia en el resto de cuencas. Los compuestos que contribuyeron con un mínimo del 5% a la toxicidad total estimada fueron sertralina, eritromicina, losartan y dimetridazol, con valores del 22, 20, 11 y 6%, repectivamente, considerando TUs en algas para SW. Considerando Tus estimadas para dafnias, se encontraron de nuevo 4 PhACs que alcanzaron el mínimo del 5%: sertralina (29%), gemfibrozilo (12%), loratidina (10%) and fluoxetina (5%). Para Tus estimadas en peces, gemfibrozilo fue el PhAC que más contribuyó a la toxicidad total estimada en SW con un 43% de promedio. Sertralina (11%), loratidina (10%) y azitromicina (6%) también mostraron toxicidades totales estimadas por encima del 5% del total del TU de la muestra. A pesar de que la presencia de los PhACs no supuso ningún riesgo ecotoxicológico estimado para los organismos acuáticos estudiados expuestos a corto plazo, sí que se observaron TUs en el límite de los considerados efectos crónicos. Respecto a los compuestos individuales, sertralina, gemfibrozilo y loratidina fueron identificados como los compuestos más relevantes en términos de riesgo ecotoxicológico y por tanto los que merecen especial atención en futuras evaluaciones de las contaminaciones de PhACs en las cuencas hidrográficas. La extensa cantidad de datos presentada sobre la estimación del riesgo ecotoxicológico de los PhACs en organismos acuáticos no-diana, así como la computación de las contribuciones relativas a la toxicidad total de una determinada muestra, proporcionaron información valiosa para futuros ejercicios de

priorización para la realización de ERA en cuencas hidrográficas Españolas.

Se encontraron relaciones positivas significativas entre la presencia y la toxicidad de los PhACs y la densidad de población humana y las unidades ganaderas., respondiendo así a las presiones antropogénicas en las cuencas.

8.5. Riesgo de los PhACs en los ecosistemas acuáticos.

8.5.1. Investigando la formación y la toxicidad de productos de transformación microbiana de DCF y SMX en WWTPs.

En esta tesis se ha desarrollado y validado un novedoso modelo analítico multi-residuo, basado en SPE-LC-MS/MS offline, para la determinación simultanea de DCF, SMX y cuatro TPs de nitrificación/desnitrificación (NO_2-DCF, NO-DCF, NO_2-SMX and Des-SMX) en WWi, WWe y SW.

El método analítico desarrollado se aplicó para profundizar en el conocimiento del destino de los PhACs frecuentemente detectados en WWTPs y SW, el antiinflamtorio no esteroideo DCF y el antibiótico SMX y la posible formación de sus correspondientes TPs en WWi y de WWe de diversas WWTPs de Cataluña que operan bajo el tratamiento NAS. Para ello, se determinó la presencia de los derivados microbianos del DCF y del SMX en muestras de WW tomadas de diversas WWTPs y SW del río Llobregat que reciben WWe de las mismas WWTPs. También se investigó la relación entre los niveles de las especies de nitrógeno (NO_2^--N and NO_3^--N) involucradas en los procesos de nitrificación y denitrificación y los TPs detectados en las WW.

Además, se contribuyó al conocimiento del riesgo potencial ecotoxicológico que suponen estos TPs del DCF y el SMX para los ecosistemas acuáticos mediante la medida de la toxicidad aguda individual y combinada en *Dafnia magna* y *Vibrio fischeri*. Se amplió y aplicó un método de análisis previamente desarrollado y validado para el DCF y sus TPs (ver sección 8.3.1) para el análisis adicional de SMX y sus TPs, basado en extracción en fase sólida “off-line” seguida de LC-MS/MS (QqLIT), con una fuente de ionización de electroespray trabajando en ambos modos positivo y negativo.

En las WWi se detectaron los TPs NO_2-DCF, NO-DCF y NO_2-SMX con frecuencias del 43%, 14% y 43%, respectivamente. En cambio, en las WWe se detectaron todos los TPs analizados y la frecuencia de detección fue más elevada: NO_2-DCF 71%, NO-DCF 100%, NO_2-SMX 57% y Des-SMX 14%. El DCF y el SMX se detectaron en WW y en SW en respectivas concentraciones de [500-1000] ng L^{-1} y [50-1000] ng L^{-1}, respectivamente. Sus TPs se detectaron por primera vez en ambas WW y SW en concentraciones un orden de magnitud inferiores a las correspondientes a sus compuestos padre. Los TPs del DCF NO_2-DCF y NO-DCF se detectaron en los siguientes intervalos de concentración en WWi [<MQL- 7,1] ng L^{-1} y 8,64 ng L^{-1} (NO-DCF se detectó solo en una muestra de WWi); mientras que para WWe se detectaron en los intervalos [4-4,9] ng L^{-1} y [1.1- 7,8] ng L^{-1}.En cuanto a SMX, los niveles de NO_2-SMX y Des-SMX detectados en WWi, WWe y SW variaron en el intervalo entre 8 y 36 ng L^{-1}. A nuestro entender este es el primer estudio que describe la presencia de TPs de nitrificación/desnitrificacion de DCF i SMX en WWe y SW en ríos impactados por entradas de WWTPs.

Las relaciones que se observaron entre los niveles del NO-DCF, NO_2-DCF, las especies de nitrógeno determinadas en los WWe, el HRT y el SRT de las WWTPs; sugirió tentativamente que los procesos de nitrificación y denitrificación están involucrados en la nitración y nitrosación del DCF durante el tratamiento biológico de las WW. Por lo tanto, sería interesante incluir estos TPs en los estudios de monitoreo ambiental como información complementaria para entender la ocurrencia, el destino y el comportamiento del DCF y SMX en el medio ambiente acuático.

En general, la toxicidad aguda de los TPs en D. *magna* y *V. fischeri* fueron menores que las correspondientes a sus compuestos padre. Sin embargo, NO_2-DCF, Des-SMX y NO_2-SMX se mostraron ligeramente más tóxicos en *V. fischeri* que sus precursores DCF y SMX. No obstante, los valores de LOEC fueron en todos los casos de varios órdenes mayores que las concentraciones encontradas en el medio ambiente acuático. Por lo tanto, los efectos observados en *D. magna* y *V. fischeri* que mostraron tanto DCF y SMX como sus TPs por separado resultaron no ser tóxicos. Sin embargo, se observaron efectos sinérgicos en mezclas binarias de DCF, SMX, NO-DCF y otros compuestos de relevancia medio ambiental (nonilfenol, diurón, malatión, glifosato y triclosan). Estas observaciones indicaron la necesidad de llevar a cabo más evaluaciones sobre los efectos combinados de mezclas de contaminantes. En todos los casos, a pesar de que NO-DCF no mostró toxicidad aguda alguna, en determinadas concentraciones puede aumentar los efectos de otros contaminantes tóxicos presentes en los mismos compartimentos medioambientales. Estos resultados evidenciaron la carencia de conocimiento de los efectos combinados de las mezclas complejas, en especial considerando la presencia de TPs.

8.5.2. La variabilidad hidrológica regula los niveles de PhACs y la respuesta de los biofilms en un río Mediterráneo.

En este trabajo se enfocó el interés en la evaluación del impacto de las fluctuaciones de concentraciones de PhACs y las condiciones variables de caudal del río en la estructura y funcionamiento de los biofilms bénticas fluviales con la finalidad de comprender la influencia relativa de la hidrología y la presencia de PhACs en las comunidades biológicas del río. Para ello, se llevaron experimentos de translocación de biofilms desde un punto del río menos contaminado (ABR) a otro más contaminado (SJD) durante dos periodos caracterizados por condiciones hidrológicas diferentes. Con esto se propuso probar la hipótesis de que los descriptores químicos y biológicos podrían responder a las fluctuaciones de condiciones de caudal, los PhACs influyendo más en condiciones de caudal de base del río que después de un episodio de riada.

Esta conjetura se fundamentó en la elevada sensibilidad que han mostrado los biofilms a los compuestos bioactivos así como a los factores medioambientales. Además, se emplearon biofilms translocados para enfatizar esta sensibilidad. Así pues, en este trabajo se evaluaron los efectos de la fluctuación del caudal y de las concentraciones de PhACs del río y su relación con los parámetros celulares de los biofilms.

Para ello, en dos puntos de muestreo seleccionados en el curso bajo del río Llobregat (ABR y SJD) se tomaron muestras de agua dos veces por semana durante dos campañas de muestreo que duraron 4-5 semanas cada una y durante las que se produjeron condiciones hidrológicas diversas de caudal alto y bajo.

Se determinaron niveles elevados de PhACs que siguieron un gradiente de contaminación entre ABR y SJD. Al margen del régimen hidrológico, los analgésicos y antiinflamatorios y los reguladores de lípidos fueron los grupos terapéuticos más abundantes en ambos puntos de muestreo, presentes en concentraciones totales de hasta 1.000 ng L^{-1} y 550 ng L^{-1} en ABR y SJD, respectivamente. Los antibióticos fluoroquinolonas y las drogas de tratamiento psiquiátrico también se detectaron a altas concentraciones llegando a presentar niveles totales de hasta 500 ng L^{-1}.

El análisis por componentes principales realizado para las concentraciones de PhACs medidas en las dos campañas y en los dos puntos de muestreo, reveló diferencias entre los diversos grupos terapéuticos que dependían del lugar y el periodo de muestreo. Tras un episodio súbito de riada, que ocurrió durante la segunda campaña de muestreo, se diluyeron las concentraciones de los PhACs, pero el promedio de las concentraciones medidas antes de la riada se restableció en dos semanas.

Para la mayoría de los compuestos, las concentraciones de PhACs mostraron una relación inversa con el caudal del río. Se evaluaron los efectos de la presencia de los PhACs de las aguas a las que se encontraban expuestos los biofilms y se relacionaron con las fluctuaciones del caudal.

Las translocaciones de las comunidades de biofilms desde el punto menos contaminado al más contaminado, demostraron un aumento de la mortalidad de las bacterias en los biofilms translocados. Después del episodio de riada, la actividad extracelular de la peptidasa y la concentración de clorofila-a se redujeron significativamente y la tasa de crecimiento de los biofilms fue significativamente más baja. Los experimentos de exposición de biofilms fluviales preparados en mesocosmos a SW influenciadas por WWe del río Llobregat y las relaciones establecidas entre los cambios observados en los descriptores de los y las concentraciones medidas de PhACs, llevaron a varias conjeturas:

(i) Los efectos observados y sus relaciones con los PhACs individuales, variaron a entre las diferentes comunidades microbiológicas adheridas a los biofilms.

(ii) El desarrollo y funcionamiento de los biofilms expuestos a PhACs varió bajo condiciones hidrológicas diferentes.

(iii) La respuesta biótica a los dos principales estresores estudiados, la contaminación de PhACs y la hidrología, varió entre los diferentes compartimentos del biofilm examinados (fotoautrófos y bacterias).

(iv) Las diferencias observadas en las concentraciones de PhACs y en la respuesta de los biofilms

a los cambios de caudal en el río indicaron la importancia de la hidrología cuando se estudia la conservación de los ríos en las cuencas Mediterráneas.

(v) El episodio de riada súbita que se registró a lo largo del estudio, jugó un papel importante en el desarrollo de los biofilms. No obstante, los efectos negativos potenciales de los antibióticos en las comunidades de bacterias se mantuvieron iguales bajo condiciones hidrológicas diferentes.

8.6. Discusión general

En general, en esta tesis se ha mostrado que los PhACs son microcontaminantes ampliamente distribuidos en el medio ambiente acuático y que pueden ser tóxicos para los ecosistemas acuáticos.

8.6.1. Análisis e identificación de los PhACs y sus TPs en WWTPs y SW receptoras

El método desarrollado basado en SPE seguida de LC-(ESI)-MS/MS para el análisis de DCF y SMX, sus principales metabolitos humanos y sus TPs de nitrificación/denitrificación en WW y SW, permitió la determinación de DCF, sus metabolitos humanos 4'-OH-DCF, 5-OH-DCF, 4',5-diOH-DCF, 5-OHD-DCF y DCF-gluc) y sus TPs NO-DCF y NO_2-DCF en WWi y WWe (Osorio et al., 2014b). Los metabolitos humanos se encontraron a elevadas concentraciones en WWi comparadas con las correspondientes a WWe, lo cual se también observaron Larsson et al. (2014) para los principales metabolitos 4'-OH-DCF y 5-OH-DCF. También se observó que DCF no se continúa metabolizando microbiológicamente a estos compuestos durante el tratamiento CAS, o al menos que las posibles transformaciones posteriores de los metabolitos se dan a mayor velocidad con respecto a la formación de los mismos en el reactor biológico. En cuanto a la biotransformación del DCF en WWTPs, los nitroso/nitro TPs se detectaron y cuantificaron por primera vez en la prsente tesis.

Después, se dirigió la atención a los TPs de los PhACs formados surante los procesos de nitrificación/denitrificación que se dan durante el tratamiento NAS en las WWTPs. SMX y sus derivados de nitrificación/denitrificación (NO_2-SMX and Des-SMX) también se detectaron en WW y SW (Osorio et al., *submitted*). El estudio dio nuevos e interesantes descubrimientos: (i) NO_2-SMX y Des-SMX previamente identificados por Nödler et al. (2012) en GW se pudieron determiner en WW y SW; y (ii) los TPs de nitrosación/nitración del DCF se encontraron presentes en SW. Considerando los descubrimientos de Chiron et al. (2010) que investigó sobre la nitrosación del acetaminofén, se formuló la hipótesis que las especies radicales de $NO^{\cdot}$ generadas durante los procesos de nitrificación/denitrificación posiblemente estén implicadas en la formación de los TPs de DCF y SMX. Las relaciones observadas entre los niveles de TPs de nitrificación/denitrificación del DCF y el SMX y algunos parámetros operacionales como HRT y SRT, respaldan la hipótesis inicial sobre la implicación de las especies radicales de $NO^{\cdot}$ en la formación de estos derivados. Además, los niveles de TPs de nitrificación/denitrificación detectados en WWi y SW llevaron a considerar que la biotransformación del DCF y el SMX mediada por la comunidad microbiana de los lodos activados, se puede dar también en el sistema colector de las WW urbanas. De hecho, Jelić et al. (2015) demostraron la transformación microbiana dentro del sistema colector de WW. Por ejemplo, los autores observaron un acusado descenso de las concentraciones (25-60%) de diltiazem, citalopram, claritromicina, bezafibrato y amlodipina durante su

paso a través del sistema. Además, los autores calcularon eliminaciones negativas para SMX (-66±15%) y irbesartán (-58±25%), las cuales se explicaron por la reconversión de los TPs conjugados a sus compuestos padre a lo largo del sistema colector. Otra explicación podría ser la formación de estos TPs mediante el metabolismo de DCF y SMX, su entrada en las WWTPs mediante la excreción humana y su emisión a las SW receptoras debido a su eliminación ineficiente en la WWTP. Por ejemplo, NO_2-SMX se consideró un metabolito minoritario de SMX (Bonvin et al., 2012). Por lo tanto, se deberían llevar a cabo más estudios para esclarecer la fuente de estos derivados.

Con esto en mente, se decidió recabar más información acerca de los mecanismos de biotransformación de compuestos relacionados con el DCF bajo condiciones de tratamiento NAS en WWTPs (Osorio et al. *submitted*). Por lo tanto, se llevaron a cabo experimentos de biodegradación en reactores a escala laboratorio con licor mixto NAS de WWTP. Los compuestos de estudio fueron DCF y otros NSAIDs con estructuras químicas análogas a la del DCF. De estos experimentos se observó que no se formaban TPs en los reactores control abióticos, lo cual sugirió que una reacción biótica es la responsable de la formación de los TPs de nitrosación/nitración. Dado que el crecimiento de las AOB en los reactores estaba favorecido bajo condiciones de elevada concentración de amonio (pH controlado), se conjeturó que la nitrosación/nitración de los TPs se origina con la oxidación de NH_3 por AOB. Con el fin de demostrar esta hipótesis, se adicionó $^{15}NH_4$-N marcado isotópicamente a los reactores con DCF. Como se esperaba, se observó un cambio de masa de +1 Da debido a la incorporación de los grupos ^{15}NO y $^{15}NO_2$ en la molécula de DCF. Respecto a la biodegradación de los NSAIDs relacionados (i.e. ácido meclofenámico, ácido mefenámico, ácido tolfenámico y ácido flufenámico), este estudió documentó la primera evidencia de TPs de nitrosación/nitración de otros PhACs. Los grados de biotransformación de los compuestos padre y sus derivados fueron generalmente bajos, probablemente debido a los impedimentos estéricos o a la poca cantidad de biomasa microbiana. Bastantes estudios evaluaron los efectos de las condiciones de operación en las WWTPs (e.g. SRT, cantidad de AOB, concentración de contaminantes) en los lodos activados y/o las eliminaciones de microcontaminantes en las WWTPs (e.g. Suárez et al., 2010; Fernández-Fontaina et al., 2012). Tras el estudio de Pérez y Barceló (2008), pocos fueron los trabajos que trataron de entender los procesos subyacentes microbianos involucrados en las reacciones de biotransformación que tienen lugar en las WWTPs (Chiron et al., 2010; Helbling et al., 2012; Tran et al., 2012). Las lecciones aprendidas de estos trabajos son que los PhACs se pueden biotransformar mediante el metabolismo de los microbios heterotrófos o el co-metabolismo mediante AOB o AOA; y que AOB están involucradas en la generación de radicales NO radicales durante los procesos de nitrificación y denitrificación. La elucidación completa del mecanismo de reacción del proceso de biotransformación por mediación microbiana de DCF y sus compuestos relacionados a nitro y nitroso TPs, no se consiguó. Por lo tanto, las investigaciones futuras llevadas por el grupo de la Dra. Sandra Pérez deberían enfocarse en: (i) el estudio de los mecanismos de reacción de las especies reactivas de nitrógeno y la incorporación de los grupos NO y NO_2 en la molécula; (ii) la caracterización de la diversidad y actividad de la comunidad microbiana de los lodos activados; y (iii) los efectos de la variación de los parámetros de operación de las WWTPs.

Es interesante ver como los TPs por ellos mismos pueden experimentar siguientes transformaciones. Por ejemplo, Stadler et al. (2012) demostraron la rotura de los glucurónidos y los sulfatos para dar lugar a su compuesto padre. Desafortunadamente, este fenómeno no se pudo confirmar cuando se determinaron

niveles de DCF y su glucurónido en WWi y WWe (Osorio et al., 2014b), porque no se implementaron los requerimientos necesarios en el muestreo para llevar a cabo la evaluación de eliminaciones. Sin embargo, se observó la conversión de los nitro TPs del DCF y el SMX (NO_2-DCF y NO_2-SMX) en sistemas suelo-acuífero bajo condiciones denitrificantes (Barbieri et al., 2012). Este trabajo se llevó a cabo en colaboración con Manuela Barbieri (IDAEA-CSIC), por lo que la publicación correspondiente no se ha incluido en esta tesis. Brevemente, los autores investigaron los mecanismos de eliminación del DCF y SMX en procesos suelo-acuífero que tienen lugar durante la recarga artificial de GW. Se llevaron a cabo experimentos en material de acuífero fortificados con DCF y SMX en concentraciones medioambientales. La formación biótica de nitro derivados de DCF y SMX (los mismos nitro derivados identificados en los reactores con lodos activados estudiados en la presente tesis) que se observó, se relacionó con la presencia de nitritos generados durante la denitrificación. No obstante, no se pudo describir el mecanismo de biotransformación del DCF y SMX ni en qué modo estaban involucrados los nitritos. Como dato de interés, estos nitro-TPs se reconviertieron en sus compuestos padre, lo cual ya se había observado para SMX durante la denitrificación abiótica (Nödler et al., 2012).

En esta tesis también se contribuyó al entendimiento de los caminos de degradación que pueden seguir los PhACs bajo los tratamientos AOP. En el marco de la colaboración científica con Despo Fatta-Kassinos e Irene Michael (Universdad de Chipre) se llevó a cabo la elucidación de TPs de ibuprofeno, DCF y trimetoprima generados durante la aplicación del tratamiento foto-fenton solar, fotocatálisis con TiO_2 mediante UV-A o irradiación solar simulada, sonólisis, y fotocatálisis integrada con irradiación de ultrasonidos (sonofotocatálisis) en varias matrices en experimentos a escala piloto (Michael et al., 2012; Michael et al., 2014). Brevemente, 21, 7 y 10 TPs de trimetorpima, ibuprofeno y DCF respectivamente, fueron identificados y atribuidos al ataque consecutivo de radicales hidroxilo (HO•) junto con la degradación de los compuestos primarios. Se propusieron sus mecanismos de degradación: (i) trimethoprima se transformó mediante hidroxilación, demetilación y reacciones de rotura; (ii) ibuprofeno experimentó principalmente reacciones de decarboxilación, demetilación e hidroxilación; y (iii) la oxidación de DCF se produjo mediante reacciones de oxidación e hidroxilación. La poca mineralización de estos compuestos junto con su elevado grado de transformación y la presencia de algunos TPs hidroxilados recalcitrantes; evidenció que es necesario llevar a cabo más investigaciones antes de implementar AOPs en WWTPs.

Además, otros estudios (Zwiener et al., 2002; Hernández et al., 2011; Moussa et al., 2012) documentaron la elucidación de nuevos TPs con estructuras similares a metabolitos humanos conocidos (1-OH-ibuprofen and carboxy-ibuprofen; 14-OH-clarithromycin; 4'-OH-DCF and 5-OH-DCF, respectivamente). Como dato de interés, NO_2-SMX también se identificó entre los TPs de SMX formados tras la aplicación de un ATT basado en reacciones de radicales sulfato (Ahmed et al., 2012). Estos descubrimientos sugieren que se los diversos procesos que tienen lugar en el medio ambiente acuático pueden estar involucrados en la transformación

de los PhACs.

De las observaciones de esta tesis, se puede señalar que existe una necesidad de incluir TPs en los estudios de seguimiento de los PhACs con el fin de mejorar el entendimiento del destino de estos compuestos en el medio ambiente acuático.

8.6.2. Presencia de PhACs y sus TPs y su destino temporal y espacial a lo largo de las WWTPs y las cuencas hidrográficas Ibéricas

Respecto a los niveles de PhACs documentados para WW y SW (Osorio et al., 2014a; Osorio et al. *submitted*), las concentraciones de DCF fueron similares en su camino desde la entrada a WWTPs, su emisión en las WWe y su llegada a las SW receptoras; mientras que para SMX fueron disminuyendo gradualmente. A pesar de que se ha probado que el tratamiento de WW resulta eficiente para eliminar ciertos PhACs, las concentraciones emitidas de PhACs y sus TPs recalcitrantes son todavía un tema de preocupación para los ecosistemas de aguas naturales receptores.

Con el propósito de ampliar el conocimiento de la presencia de PhACs (hasta 96) en el medio ambiente acuático, se llevaron a cabo estudios de seguimiento a lo largo de cuencas hidrográficas Ibéricas (Osorio et al 2012a,b; 2014a; 2015). Con respecto a la presencia de grupos terapéuticos en SW, los analgésicos y los antiinflamatorios fueron sin duda los más ubicuos y abundantes. En el caso de los sedimentos, su predominancia fue compartida con los antibióticos. Otras familias relevantes en SW fueron los antibióticos, reguladores de lípidos y colesterol y los antihipertensivos; mientras que las drogas de tratamiento psiquiátrico y los diuréticos fueron igualmente ubicuos en ambos SW y sedimentos.

Respecto a los compuestos individuales, aquellos detectados en mayores concentraciones fueron: ibuprofeno, DCF, naproxeno, indometacina, ketoprofeno, acetaminofén, iopromida, carbamazepina, lorazepam, gemfibrozilo, bezafibrato, valsartán, irbesartán, losartán, hidroclorotiazida, furosemida, atenolol, tetraciclina, ofloxacina, tiabendazol y metronidazol. Respecto a los sedimentos, los compuestos en concentraciones más elevadas fueron: sertralina, ketoprofeno, hidroclorotiazida, tetraciclina, codeína, ibuprofeno, claritromicina y azitromicina. Los más ubícuos fueron: hidroclorotiazida, gemfibrozilo, ibuprofeno y azitromizina en SW y sedimentos en concentraciones relativamente similares. Estas observaciones están de acuerdo con aquellas documentadas por Carmona et al. (2014) pero no con las de Löffer et al. (2005), quien observó que el ibuprofeno no mostraba una afinidad significativa por el sedimento debido a sus propiedades físicoquimicas (ver table A.1 en annex). Estas observaciones reflejan lo complicados que pueden resultar los procesos de distribución de los PhACs entre las fases SW-sedimento. Además, esto también revela que la partición de los PhACs en el compartimento acuático no sólo depende de sus propiedades físicoquímicas como la solubilidad, sino que también depende de las condiciones del sistema acuático, concretamente: (i) la físicoquimica, como el pH y la composición del SPM; (ii) la hidrología incluyendo el régimen del caudal del río; y (iii) la morfología, como la topografía de los sedimentos en el fondo del río.

La distribución espacial y temporal observada para los PhACs estudiados a lo largo de las cuencas hidrográficas puede ser la consecuencia de diversos factores o bien una combinación de ellos, concretamente: (i) elevados patrones de consumo humano y animal; (ii) elevados porcentajes de excreción de drogas no metabolizadas; (iii) descarga directa de PhACs al sistema colector de WW, (iv) bajas tasas de eliminación en WWTPs; (v) re-transformación de los TPs al compuesto activo; y (vi) atenuación natural en ríos.

En la presente tesis se evaluaron las relaciones entre los niveles de PhACs determinadas en SW y sedimentos por una parte con la densidad de población y por otra parte con las unidades ganaderas en las cuatro cuencas hidrográficas estudiadas. De modo similar, un estudio reciente llevado a cabo en SW en Taiwan, correlacionó la distribución espacial de los PhACs con las principales fuentes de aporte (por ejemplo, doméstica por el uso humano, antibióticos por el uso animal y en hospitales) en los ríos (Jiang et al., 2015). Sin embargo, la relación entre el perfil de los PhACs y las presiones humana/animal de las áreas estudiadas fueron meramente descriptivas (Jiang et al., 2015), mientras que las relaciones entre los niveles de Pacas y la densidad de población y unidades ganaderas evaluadas en esta tesis fueron cuantitativas y específicas de cada localización (Osorio et al., 2015).

Además, en la presente tesis se demostraron correlaciones positivas significativas entre la densidad de población humana y unidades ganaderas y la concentración de los PhACs detectados en SW y sedimentos (Osorio et al., 2015). Aun así, esta relación no fue proporcional lo cual evidenció que otros factores antropogénicos y naturales pueden influenciar en la variabilidad de los niveles de PhACs en el medio ambiente acuático. Por ejemplo, tras haber documentado un gradiente de concentración de PhACs en el río Llobregat, que se incrementó con el número de WWTPs distribuídas río abajo (Osorio et al., 2012a; 2012b; 2015), se consideraron las WWe como la principal fuente de emisión de PhACs a las SW y la principal causa de este gradiente de contaminación. De hecho, esta asunción se ha confirmado recientemente en regiones altamente urbanizadas de China (Wang et al., 2015). En este estudio, la aplicación de PCA a niveles de 34 PhACs determinados en diversas muestras de río y WWe reveló la contribución de las WWTPs distribuidas a lo largo de los ríos estudiados a la contaminación observada en sus SW.

Estas observaciones, ponen de manifiesto que en las cuencas hidrográficas caracterizadas por una elevada presión antropogénica, como es el caso del río Llobregat, los esperados efectos de atenuación natural de PhACs a lo largo del curso del río pueden ser contrarrestados por la entrada continua de estas substancias a través de las descargas de WWe. Por otra parte, algunos compuestos (por ejemplo, hidroclorotiazida, gemfibrozilo, norfloxacina y DCF) se comportaron de modo opuesto, con concentraciones que disminuyeron río abajo (Osorio et al., 2012b). Además, no fue posible describir ninguna tendencia clara para el comportamiento de los PhACs a lo largo del resto de cuencas estudiadas (Osorio et al., 2015). Estos descubrimientos evidenciaron la complejidad de los factores que afectan el comportamiento variable de los Pacas en el medio ambiente acuático.

Entre éstos, los procesos naturales de biodegradación y fotodegradación pueden jugar un papel clave en la atenuación de los PhACs en el río. Se ha demostrado que la actividad de los microorganismos en la interfaz agua-sedimento de los sedimentos del fondo del río es relevante para la biodegradación de

los PhACs (e.g. Li et al; 2015). Por ejemplo, se estudió el destino de 19 PhACs en experimentos a escala laboratorio reproduciendo la zona hiporhéica (i.e. la región por debajo y a lo largo del sedimento del fondo) (Li et al., 2015). Se document la persistencia de los PhACs entre fácilmente degradable (DT_{50} = 1.8 días (por ejemplo acetaminofén, ibuprofeno) y no degradable (clorotalidona, y fluconazol). Además, los autores identificaron 11 TPs (incluyendo carbamazepina- 10, 11-epóxido, ácido metoprolol) (Li et al., 2015). Los procesos naturales de fotodegradación de PhACs han sido ampliamente investigados (Schulze et al., 2010; Gonçalves et al., 2011; Bonvin et al., 2012; Zonja et al., 2015). En colaboración con Carlos Gonçalves (IAREN-Universidad de Porto), se evaluaron los caminos y grados de fotodegradación de los antivirales oseltamivir ester y oseltamivir carboxilato (Tamiflu) bajo radiación solar artificial y natural (Gonçalves et al., 2011). Se probó que la radiación solar simulada a escala laboratorio es capaz de fotodegradar oseltamivir ester y oseltamivir carboxilato en 15 y 150 días, respectivamente. Sin embargo, los foto-TPs identificados fueron más recalcitrantes a la siguiente fotodegradación que sus compuestos padre. Es interesante mencionar que se demostró la ocurrencia de este proceso natural en el campo, ya que las vidas medias de algunos TPs se pudieron detectar en el río Ebro.

Otro factor clave en la atenuación es el efecto de dilución el cual depende del caudal del río y de los usos antropogénicos del agua. Por ello, uno de los objetivos de esta tesis fue determinar en qué grado los niveles de PhACs están relacionados con el caudal del río e identificar qué compuestos eran más sensibles a los efectos de dilución bajo diferentes regímenes de caudal. Para ello, se evaluaron las correlaciones entre las concentraciones medidas de PhAC y el caudal registrado en el río Llobregat a lo largo de cuatro campañas de muestreo de un mes de duración cada una (9-13 muestras) (Osorio et al., 2012a; 2012b). Los resultados permitieron clasificar los compuestos seleccionados para el estudio (66 y 18, respectivamente de los 96 PhACs estudiados en esta tesis) en tres categorías de acuerdo con la correlación de las concentraciones con el caudal del río: (i) positivamente relacionadas y por tanto no afectadas por los efectos de dilución (sólo acetaminofén); (ii) negativamente relacionadas y por tanto afectadas por los efectos de dilución (por ejemplo SMX); y (ii) positivamente y negativamente relacionadas dependiendo de la localización y por lo tanto relacionados a otros factores adicionales de igual modo (por ejemplo DCF). Además, se clasificaron los PhACs de acuerdo con su sensibilidad al caudal del río, lo cual permitió identificar aquellos compuestos que son más sensibles a los efectos de dilución (enrofloxacina < furosemida < ibuprofeno < fluoxetina < SMX <propilfenazona < eritromicina). Además, se examinó la importancia del DOC como un factor influyente en el comportamiento de los PhACs en los ríos. Para ello, se evaluaron las relaciones entre las concentraciones de los compuestos y el DOC en SW del río Llobregat (Osorio et al., 2012a). Se observaron correlaciones positivas medioambientalmente aceptables entre las concentraciones de PhACs y el DOC para la mayoría de los compuestos estudiados. Además, los PhACs se mostraron más sensibles al DOC, comparados con la sensibilidad al caudal del río observada previamente. Estas observaciones eran en realidad esperadas, ya que la asociación de los solutos al DOC aumenta con el incremento del compuesto en la fase acuosa (Tolls, 2001). No obstante, a pesar de la correlación negativa entre DOC y caudal, diversos PhACs mostraron correlaciones positivas con ambos parámetros. Se conjeturó que el fenómeno de re-suspensión de sedimentos bajo condiciones de régimen de caudal turbulento en el río podría dar explicación a las concentraciones de PhACs en aumento con DOC y caudal del río a la vez. Cuando ocurre este proceso, una cierta fracción de los compuestos adsorbidos en el sedimento se transfiere a la fase acuosa, modificando así la partición agua-sedimento del compuesto. Por lo tanto, los sedimentos y SPM pueden ser una reserva y fuente adicional de

PhACs a las SW durante aumentos estacionales del caudal del río o bajo ciertas condiciones como drenados o eventos de riadas.

En la presente tesis se investigaron los efectos esperados de la variabilidad de las condiciones hidrológicas y las lluvias estacionales en el comportamiento de los PhACs en el río Llobregat. Para ese fin, se monitoreó el río durante cuatro meses correspondientes a las cuatro estaciones del año (Osorio et al., 2012b). En general, los resultados revelaron que los PhACs se encuentran a mayores concentraciones en SW durante periodos fríos y secos correspondientes al otoño e invierno, mientras que las menores concentraciones se dan en periodos lluviosos y templados correspondientes a primavera y verano. Las tendencias estacionales de los niveles de PhACs observadas, se pueden explicar teniendo cuenta todos los factores mencionados anteriormente que afectan la fluctuación de los niveles de PhACs en los ríos. Durante otoño e invierno, las concentraciones de PhACs en SW fueron mayores probablemente debido a: (i) elevado consumo humano/animal; (ii) bajas temperaturas y por ende eliminación reducida en las WWTPs y en el río; (iii) menor dilución debido al caudal más bajo; (iv) menor intensidad de radiación solar y por tanto menor grado de fotodegradación. En un estudio similar llevado a cabo en el río Beiyun (China), se observaron niveles elevados de PhACs al final del invierno-inicio de la primavera (Dai et al., 2015). En este trabajo, las concentraciones del 60% de los PhACs analizados fueron mayors durante la estación seca (marzo), con concentraciones medianas que llegaron a ser 2.6 veces mayores que en otras estaciones. El máximo de concentraciones que se observó en primavera se explicó por dos factores principales: bajo caudal del río (desde noviembre hasta abril es la estación típica de bajos caudales en China) y temperatura del agua baja (por lo que la actividad microbiana y por ende la biodegradación de los PhACs es reducida) (Dai et al., 2015). Otro trabajo documentó los efectos de dilución de los PhAC en WWi de WWTPs urbanas en China después de un episodio de lluvias (Sui et al., 2015). En este estudio se midieron concentraciones de 10 PhACs en WWi que disminuyeron entre 5 y 76% después de las lluvias debido a la dilución de las WWi por el agua de lluvia, la cual se infiltró en el sistema colector de WW. Sin embargo, en las WWTPs localizadas fuera de las zonas urbanas, el aumento del caudal llevó a una disminución de las eficiencias de eliminación de los compuestos (Sui et al., 2015). Por ejemplo el porcentaje de eliminación de trimetoprima y metoprolol se redujo de un 78 y 58% a un 21 y29%, respectivamente, tras el episodio de lluvias. Por el contrario, la influencia de las lluvias no afectó a los niveles de PhACs en WWTPs urbanas, lo cual se explicó por el caudal de WWi prácticamente igual, buenas eficiencias de eliminación, o el uso de sistemas de bypass para bombear a las aguas SW receptoras el exceso de WWi entrante del sistema colector de WW (Sui et al., 2015).

Cabe destacar que durante esta tesis se evaluaron los efectos de un evento súbito de riada, que ocurrió tras un episodio de fuertes lluvias, en los niveles de PhACs en SW del río Llobregat (Osorio et al., 2012b; 2014a). Mientras que se observaron efectos de dilución río arriba, las concentraciones de los PhACs estudiados aumentaron río abajo como consecuencia del evento súbito de riada. A pesar de que las WWe de las WWTPs distribuidas a lo largo del río no fueron analizadas, se atribuyeron estas concentraciones en aumento a la disminución de las eficiencias de eliminación de estas sustancias en las WWTPs tras el evento súbito de riada. Las observaciones de Sui et al. (2015) apoyan nuestras hipótesis. Por otra parte, es posible que los efectos naturales de dilución de las concentraciones de PhACs observadas río arriva tras el episodio de lluvias, no sean contrarrestadas debido al menor número de WWTPs distribuidas en esta sección del río y por tanto un menor aporte de descargas de WWe.

A pesar de los esfuerzos realizados para evaluar la presencia y el comportamiento de los PhACs a lo largo de los ríos y las explicaciones proporcionadas para las tendencias observadas, su estudio teniendo en cuenta todos los factores mencionados previamente aún resulta complicado. Por lo tanto, se aplicó un modelo "plug-flow" con el propósito de facilitar la evaluación del rol de los factores que afectan a la variación de los niveles de PhACs río abajo desde las principales fuentes de emisión (Osorio et al., 2012b). así, se modelaron las concentraciones de 14 PhACs en dos puntos de muestreo del río Llobregat, localizados río abajo de fuentes de emisión que se asumieron como un agregado de descargas de WWe de diversas WWTPs. De acuerdo con el modelo descrito por Pistocchi et al. (2010) se consideraron los siguientes parámetros: (i) la longitud del río desde la fuente emisora hasta el punto de control, el cual fue un valor ponderado incluyendo la distancia a cada WWTP localizada río arriba y la correspondiente descarga de WWe anual; (ii) el caudal del río; (iii) el tiempo de residencia hidráulico desde el punto de emisión hasta el de medición (calculado como un valor ponderado variable considerando la distancia a cada WWTP usando una red del río digitalizada en una plataforma GIS y el correspondiente volumen de WWe anual); y (iv) la constante de atenuación de primer orden (k) del PhAC la cual se asumió que comprendía todos los procesos de atenuación que pueden contribuir (es decir, biodegradación, fotodegradación, dilución y adsorción a sedimentos y SPM).

Es importante señalar que se demostró la validez de la aplicación del modelo, ya que se obtuvieron valores de k similares para un determinado compuesto en ambas localizaciones estudiadas. Además, las emisiones calculadas para los PhACs fueron mayores en el punto localizado río abajo. Estas observaciones eran concordes con los elevados niveles de PhACs detectados que se pueden explicar por la mayor cantidad de WWTPs que descargan sus WWe al río distribuidas a lo largo del curso bajo. Como consecuencia, se propusieron los valores ajustados de k y de emisión para su uso como descriptores razonables de agregados de propiedades de la red de aguas río arriba de puntos de muestreo. Además, los resultados de los modelos ajustados permitieron estimar las tendencias de atenuación de los PhACs. Por ejemplo, furosemida, enrofloxacina, enalapril, acetaminofén, DCF y ketoprofeno mostraron valores de k entre −0.04 y −0.10 h^{-1}. Cabe destacar se identificó la eritromicina como el compuesto que aparentemente se elimina de la columna de agua con mayor rapidez (k≈ −0.15 h^{-1}); mientras que otros compuestos mostraron un comportamiento más conservativo.

Debido a las suposiciones hechas en la construcción de este modelo simple, no se pudo proporcionar una explicación concluyente. Sin embargo, se propusieron posibles razones: tendencias de consumo humano/animal más alta, mayores emisiones de WWTPs debido a tasas de eliminación más bajas; o atenuación menos eficiente en los ríos o incluso efectos re-suspensión de sedimentos. No obstante, el enfoque desarrollado fue considerado como una herramienta fiable para la predicción del destino de PhACs en el medio ambiente acuático. Más recientemente, la atenuación en la corriente de 75 PhACs en 4 segmentos fluviales del río Ebro se evaluó con el fin de evaluar la variabilidad de las tasas de atenuación entre diferentes PhACs así como entre los segmentos de río que difieren en las condiciones ambientales (Acuña et al., 2015). Los autores observaron que la atenuación era muy variable entre PhACs y segmentos de los ríos, pero ninguna de las propiedades físicoquímicas consideradas resultaron ser relevantes en la determinación de las tasas medias de atenuación. Curiosamente, se encontraron con que el log K_{ow} influyó en la variabilidad de las tasas entre los segmentos de los ríos, lo que se explica por su efecto sobre la absorción de los sedimentos y partículas suspendidas, influyendo así en el equilibrio entre los diferentes mecanismos de atenuación

(biotransformación, fototransformación, adsorción y volatilización). La conclusión importante de este estudio fue que todos los procesos de atenuación naturales que PhACs pueden someterse a lo largo de su destino en el curso de los ríos deben ser considerados tan importantes como los efectos de dilución, cuando el objetivo es predecir las concentraciones en los ecosistemas acuáticos.

Teniendo las consideraciones y conclusiones anteriores en mente, en el marco del proyecto SCARCE, la colaboración con Joana Aldekoa y Félix Francés (Universidad Politécnica de Valencia) se dispuso a poner en práctica el modelo GREAT-ER en la cuenca del río Llobregat, con el fin de estudiar el comportamiento de DCF. A tal efecto, las concentraciones de DCF medidas a lo largo de la cuenca del río Llobregat (Osorio et al., 2015) se utilizaron para desarrollar y calibrar el modelo mediante la estimación de la precisión de las concentraciones estimadas de DCF (Aldekoa et al., 2013). El modelo georreferenciado estimó el patrón espacial de la concentración de DCF en toda la red fluvial. Además, el modelo fue capaz de estimar valores de concentración en la mayoría de los puntos de muestreo con un error aceptable (es decir, la raíz mínima de error cuadrático medio obtenido fue de 3,9 ng L^{-1}, mientras que el error promedio para todas las concentraciones medidas fue 28,1 ng L^{-1}), lo que demuestra ser un modelo más preciso que otro desarrollado previamente (Alder et al., 2010). Como se confirma en otros estudios (Whelan et al., 1999; Johnson et al, 2007; Ort et al, 2009), se demostró que el modelo GREAT-ER sería una herramienta útil para simular concentraciones de PhACs en los ríos y por lo tanto comprender mejor su destino a lo largo del curso de agua. Esta aplicación de un enfoque de modelado de estudios ambientales en última instancia, beneficiará a la evaluación de la calidad del agua y la gestión de los recursos hídricos. En vista de los resultados anteriores sobre GREAT-ER, se puede considerar que cuando los datos medidos de concentración de PhACs no están disponibles, los datos estimados podrían ser tan fiables como los datos medidos en la hora de tratar de reducir costes y esfuerzos en los largos y amplios estudios de vigilancia que se llevan a cabo actualmente (Navarro-Ortega et al, 2012; 2015). Por desgracia, la exactitud de los datos estimados se ve afectada por la limitación de datos disponibles e inciertos para la calibración de los modelos, como las variables hidrológicas, las tasas de eliminación y las emisiones de PhACs de WWTPs, las tasas de atenuación natural de PhACs y tendencias de consumo humano / animal. Además, la estimación de datos requiere que sean comparados con los datos medidos para confirmar la validez del método (Celle-Jeanton et al., 2014).

Sin embargo, actualmente no es necesario el análisis de todos los PhACs en el medio ambiente. En lugar de ello, los esfuerzos deberían centrarse en la evaluación de los compuestos ecotoxicológicamente relevantes para el medio ambiente acuático. Con ese objetivo un, tal como se describe para los procedimientos de ERA en la sección 1.9, sería necesario aplicar un criterio de selección en una etapa inicial. Después los PhACs serían valorados con un cierto grado de relevancia, para llegar finalmente a una lista de los que se consideran más importantes de acuerdo con los criterios aplicados de selección o prioridad. La WFD fue pionera en estos enfoques y promulgó las listas de sustancias prioritarias peligrosas, sustancias prioritarias, y más recientemente, la lista de vigilancia de sustancias; que son objeto de revisión permanente y por lo tanto actualización periódicamente. Los ejercicios de priorización también están siendo incluidos en la investigación actual sobre PhACs en el medio acuático (Riva et al, 2015; Daouk et al, 2015). Varios criterios de priorización se pueden considerar para limitar el número de PhACs que se deben estudiar en el medio ambiente acuático, a saber: (i) la probabilidad de su ocurrencia; (ii) el volumen de ventas o de los datos de consumo; (ii) las tasas metabólicas y excreción después del consumo humano / animal; (iii) el destino

en la WWTP; (iv) la persistencia en los sistemas de agua dulce; y (v) el riesgo para la salud humana del medio ambiente (por ejemplo, toxicidad o bioacumulación) (Riva et al, 2015;. Daouk et al, 2015). Se puede considerar que cuanto más amplio sea el criterio de selección, más realista será la lista de PhACs prioritarios en el medio ambiente acuático.

En general, la emisión continua de PhACs de WWTPs generalmente desencadena un aumento de concentraciones aguas abajo en los ríos receptores que puede ser constante bajo diferentes condiciones hidrológicas y que, por tanto, puede tener consecuencias a largo plazo para las comunidades biológicas.

8.6.3. Efectos ecotoxicológicos de PhACs y la hidrología como factores relevantes de estrés de los ecosistemas acuáticos

Preocupados por las consecuencias de la pseudo-persistencia de PhACs en el medio ambiente acuático desde el punto de vista ecológico, esta tesis también ha contribuido al conocimiento de los efectos de PhACs en los ecosistemas acuáticos. La primera publicación incluida en este capítulo (Osorio et al., *submitted*) presentó los resultados de la evaluación de la toxicidad aguda de DCF, SMX y sus derivados de nitrificación/denitrificación derivados en los organismos acuáticos (es decir, *D. magna* y *V. fischeri*). Los valores de LOEC calculados para DCF y SMX revelaron que, en los niveles generales que estos PhACs están presentes en el medio ambiente acuático, no se espera que supongan ningún riesgo ecotoxicológico a las especies acuáticas como *D. magna* y *V. fischeri* después de la exposición a corto plazo . En general, estos resultados estaban de acuerdo con la literatura. Por ejemplo, la toxicidad de DCF y SMX a *D. magna* estaba en el mismo orden que la concentración observada por Cleuvers (2003) y Kim et al. (2007).

Si bien los efectos ecotoxicológicos de PhACs están relativamente bien documentados en comparación con otras sustancias de riesgo emergentes (Farré et al, 2008; Brausch et al, 2013; Vásquez et al, 2014). Hay una brecha sustancial de la información a que se refiere a la amenaza potencial de los TPs de PhACs a los ecosistemas acuáticos. En el pasado, sólo los estudios dispersos incluyeron la evaluación de la toxicidad de los TPs como el realizado por Henschel et al. (1997), que demostró los efectos adversos agudos de metabolitos activos de PhACs (por ejemplo ácido salicílico y ácido clofíbrico) hacia los organismos no diana (por ejemplo, *D. magna*, algas y bacterias). Es importante destacar que la contribución al conocimiento de la ecotoxicidad de los metabolitos y TPs de drogas ha aumentado progresivamente en los últimos años (por ejemplo Rosal et al, 2010; Majewsky et al, 2014; Rubirola et al, 2014). Por ejemplo, entre los PhACs y metabolitos analizados Rosal et al. (2010) informaron de la más alta toxicidad para *V. fischeri* del ácido fenofíbrico (es decir, EC_{50} = 1,7 mg L^{-1}), el metabolito del regulador de lípidos fenofibrato. Del mismo modo, Rubirola et al. (2014) evaluaron el potencial de toxicidad aguda a *V. fischeri* de metoprolol y sus TPs identificados después del tratamiento de lodos activados en reactores a escala de laboratorio. Es importante destacar que los estudios de ecotoxicidad llevados a cabo revelaron que el metabolito O-desmethylmetoprolol exhibió toxicidad aguda más (es decir, EC_{50} = 18 mg L^{-1}) que su compuesto padre (es decir, EC_{50} = 65 mg L^{-1}). De hecho, el principal interés del estudio en la presente tesis era averiguar si los TPs de nitrificación/denitrificación de DCF y SMX eran más tóxicos para especies acuáticas sensibles, o no. La comparación de los valores de toxicidad a *V.*

fischeri determinado para SMX, Des-SMX y NO_2-SMX reveló que los TPs mostraron efectos toxicológicos más altos que su PhAC original (por ejemplo, el valor de EC_{50} para SMX fue >> 100 mg L^{-1}, mientras que para Des-SMX y NO_2-SMX éstos fueron 89,3 y 41, 4 mg L^{-1}). Por otra parte, NO_2-DCF (EC_{50} = 11,7 mg L^{-1}) también mostró mayor toxicidad a *V. fischeri* que DCF (EC_{50} = 22.9 mg L^{-1}). Sin embargo, los LOEC calculados para los TPs eran en varios órdenes superiores a las concentraciones determinadas en WW y SW receptoras. Por ejemplo los LOEC de Des-SMX y NO_2-DCF en *V. fischeri* fueron respectivamente 48,4 y 1,8 mg L^{-1}; mientras que los niveles respectivos determinados de la WWe fueron 11,4 y 3,62 a 4,94 ng L^{-1} y 8,04 hasta 17,7 y <MQL-2,64 ng L^{-1} en SW.

A pesar de que los PhACs estudiados y sus derivados de nitrificación/denitrificación no ejercieron efectos toxicológicos agudos relevantes sobre las especies acuáticas evaluadas, las consecuencias de la exposición a largo plazo a DCF y SMX son todavía limitadas o completamente inexploradas como en el caso de sus TPs de nitrificación/denitrificación. En particular, algunos ejemplos fueron discutidos por Oliveira et al. (2015) que informan sobre el deterioro reproductivo significativo para *D. magna* expuesta a concentraciones DCF desde 29,5 hasta 72 mg L^{-1}. Por otro lado, Sarma et al. (2013) observaron la respuesta frente al rotífero *Plationus patulus y el* cladócero *Moina macrocopa* a niveles de exposición de DCF de 1,56 a 25 mg L^{-1}. Del mismo modo, Lee et al. (2011) informaron de una reducción significativa de la tasa de población *D. magna* expuesta a DCF en concentraciones de 0,93 a 25 mg L^{-1}. Además, se informó de un valor de EC_{50} de 23,8 crónica mg L^{-1}, que era varios órdenes de magnitud más altos que los niveles de DCF detectados en el medio ambiente acuático y por tanto no se esperaron efectos crónicos de DCF en el medio ambiente acuático. No obstante, serían necesarios más estudios que apoyen estas observaciones, así como la inclusión de otros organismos acuáticos no diana (por ejemplo, algas, peces) para llegar a una conclusión definitiva acerca de los posibles efectos a largo plazo de DCF, y asimismo cualquier PhAC, a los ecosistemas acuáticos.

Además, la co-ocurrencia de PhAC junto con sus TPs en muestras ambientales complejas, como WW y SW receptoras, puede dar lugar a efectos aditivos y sinérgicos o antagónicos en los organismos acuáticos no diana (Farré et al, 2008; Ginebreda et al., 2014; Vásquez et al, 2014; Backhaus et al, 2014). Por ejemplo, no se observaron efectos de dosis bajas de PhACs para una mezcla de 10 quinolonas y también para una mezcla de 12 PhACs con diferentes mecanismos de acción (Backhaus et al, 2000a; 2000b). Incluso las mezclas de pocos compuestos a menudo muestran un patrón similar. Una mezcla de fluoxetina y ácido clofíbrico mató a más del 50% de una población de *D. magna* después de una exposición de 6 días, a pesar de que los componentes individuales se encontraban a concentraciones que se habían demostrado no causar efectos significativos (Flaherty y Dodson, 2005). También en mezclas binarias se observó que la trimetoprima desplaza la curva de respuesta a la concentración de SMX y sulfadiazina por un factor de 4 a 5 hacia toxicidades superiores, incluso si está presente solamente en su NOEC (Egucci et al., 2004). De manera similar, se observaron claros efectos sinérgicos en algas para mezclas de flumequina + eritromicina y oxitetraciclina + flumequina por (Christensen et al., 2006). Según estas evidencias, se pueden esperar mayores efectos ecotoxicológicos en la hora de evaluar la toxicidad asociada a mezclas de PhACs, con modos de acción similares o diferentes y con otros contaminantes del medio ambiente acuático también. De hecho, se observaron efectos sinérgicos, y en menor medida antagónicos, durante esta tesis cuando DCF, SMX y NO-DCF se mezclaron con otros compuestos de relevancia ambiental (es decir, nonilfenol, malatión, diurón, glifosato y triclosan) (Osorio et al. *submitted*). Estos resultados están

de acuerdo con las evidencias disponibles que han documentado principalmente efectos sinérgicos de las mezclas binarias de PhACs (Backhaus et al., 2014). Para conocimiento de los autores, los efectos de las interacciones sinérgicas o antagónicas entre PhACs y sus TPs es un campo raramente investigado. Sin embargo, la posible biotransformación de PhACs durante el tratamiento WW ha sido considerado recientemente en los estudios de evaluación ecotoxicológicos de WW tratadas (Hidaka et al., 2012; Michael et al, 2012; 2014b; Czech et al, 2014). Estas investigaciones se han llevado a cabo principalmente para la implementación de ATTS en WWTPs. Por ejemplo, Michael et al. (2012; 2014b) realizaron ensayos de inhibición de biolumiscencia en *V. fischeri* y pruebas de inmovilización sobre *D. magna* para evaluar en qué medida la toxicidad asociada a WW se redujo después de la aplicación de un tratamiento solar AOP. Como se ha descrito anteriormente, los autores llevaron a cabo experimentos de fotodegradación en WWe artificial fortificada con PhACs individuales (es decir, ibuprofeno, DCF y trimetoprima) en reactores a escala laboratorio. Se evaluaron el perfil de toxicidad de cada medicamento en WW durante la aplicación del tratamiento avanzado y atribuyeron los diversos efectos observados para los TPs que se generaron en las diferentes etapas del proceso. Los autores concluyeron que los TP intermedio generados durante la oxidación de trimetoprims no mostraron ningún efecto tóxico a *V. fischeri.* En cuanto a DCF e ibuprofeno, demostraron la capacidad de tratamiento sonophotocatalysis para reducir la toxicidad inicial de estos PhACs hacia *D. magna* con un rendimiento de 20% y 40% de inmovilización, respectivamente, al final del tratamiento. Es importante destacar que un estudio reciente sobre los efectos ecotoxicológicos de PhACs en el medio acuático ha abarcado la contribución potencial de los recursos naturales de foto-TPs a toda la toxicidad de una mezcla medioambiental dada (Wang et al., 2014). Por ejemplo, a pesar de que la fotodegradación solar ha sido considerada como un proceso de atenuación natural significativa de PhACs disminuyendo así el riesgo ecológico, Wang et al. (2014) identificaron por primera vez el aumento de la toxicidad a *V fischeri* de una mezcla irradiada de 27 PhACs (por ejemplo SMX, ofloxacina, trimetoprima, ibuprofeno y DCF) en SW en concentraciones ambientales. Curiosamente, ya que la foto-transformación de los compuestos incluidos en la mezcla se había estudiado previamente (Chowdhury et al, 2011; Jiao et al, 2008; Li et al, 2011; Lin et al, 2013; Trovó et al., 2009; Wang y Lin, 2012), se atribuyeró la mayor toxicidad del SW irradiado a los efectos sinérgicos de los foto-TPs de PhACs generados. Estos resultados revelaron la falta de comprensión de las implicaciones ambientales de la transformación natural de PhACs y el riesgo que plantea la posterior formación de TPs para el ecosistema acuático.

Con todo, a pesar de que ciertos PhACs y sus TPs, puede que no representen una amenaza ecotoxicológica para las especies acuáticas expuestas a corto plazo, su contribución a la toxicidad total de mezclas ambientales complejas deben ser evaluados para mejorar la evaluación del riesgo asociado a estos compuestos. Por otra parte, las posibles interacciones (es decir, los efectos sinérgicos y antagónicos) entre PhACs, otros contaminantes de preocupación ambiental y sus TPs que podrían ocurrir en los sistemas acuáticos se deben evaluar para dar a conocer su magnitud e importancia biológica en los ecosistemas acuáticos. Por lo tanto, la comprensión del destino de mezclas de compuestos farmacéuticos y sus efectos crónicos en organismos acuáticos es una investigación difícil, así como un tema de preocupación para los organismos de gestión del agua.

Siguiendo con la exploración de los efectos ecotoxicológicos sobre organismos no diana asociados a la presencia de mezclas complejas de PhACs en el medio ambiente acuático, se realizó en la presente tesis

una evaluación del riesgo asociado a 55 PhACs a lo largo de cuatro cuencas de los ríos ibéricos (Osorio et al., 2015, en sección 4.4). Con ese objetivo, el uso del modelo de concentración, además de mezclas de sustancias (Ginebreda et al., 2014) se combinó con datos de toxicidad para calcular las TUs de PhACs presentes en cada muestra recogida a lo largo del curso de agua. Los valores individuales de TU estimados para los PhACs seleccionados no revelaron riesgos agudos significativos en los organismos acuáticos objeto de ensayo. Sin embargo, se pueden esperar efectos ecotoxicológicos crónicos potenciales sobre las algas en dos puntos calientes de contaminación de PhACs identificados en las cuencas del Llobregat y Ebro (es decir LLO7 y ZAD, respectivamente). En el ámbito específico de la región, el Llobregat y el Ebro se caracterizaron como con mayor riesgo ecotoxicológico, seguidos de Júcar y Guadalquivir. Se identificaron los puntos calientes de riesgo ecotoxicológico en cada cuenca (por ejemplo LLO7 de Llobregat; ZAD en Ebro; GUA6 en Guadalquivir, y JUC7 en el Júcar). Por otro lado, se determinaron también a lo largo de toda la cuenca los lugares menos contaminados, y por ende en menor riesgo (por ejemplo CAB5 en el Júcar; LLO2 de Llobregat; ESE en Ebro, y GUA1 en Guadalquivir). Ya se ha mencionado la necesidad de establecer prioridades de PhACs. Por esa razón, se calculó la contribución relativa de las diferentes sustancias a la toxicidad total en la muestra en los lugares estudiados con el fin de enumerar los principales PhACs en las cuencas de la Península Ibérica. Además, teniendo en cuenta que la contribución relativa de cada PhAC a la ecotoxicidad puede variar de acuerdo a su toxicidad individual y la concentración, se identificaron los compuestos que estaban contribuyendo más a la toxicidad total del agua en cada sitio. La sertralina, eritromicina, losartán, dimetridazol, loratadina y fluoxetina fueron los compuestos identificados para contribuir al menos el 5% a la toxicidad total de la muestra de SW. Entre ellas, la sertralina, gemfibrozilo y loratadina fueron considerados como los compuestos más relevantes, y por lo tanto los PhACs para los cuales se debería concentrar el mayor interés si la contaminación de los sistemas acuáticos por PhACs necesita ser controlada.

La caracterización de PhACs por su potencial riesgo ecotoxicológico para los organismos acuáticos no diana se ha realizado en numerosos estudios (por ejemplo, Hernando et al, 2006; Ginebreda et al, 2010; Gros et al, 2010; Damásio et al, 2011; Pereira et al, 2015; Johnson et al, 2015; Kuzmanovic et al, 2015; de Castro-Català et al, 2015b). Es importante destacar que, Hernando et al. (2006) aplicó por primera vez una primera aproximación, para caracterizar el riesgo ambiental para PhACs (es decir, antibióticos, analgésicos y antiinflamatorios, reguladores de lípidos, β-bloqueantes, antiepilépticos y hormonas esteroides) detectados con mayor frecuencia en la WWe, SW y sedimentos a escala global (Hernando et al., 2006). Con ese objetivo, se utilizaron datos de presencia recogidos de la literatura para calcular HQs basados en datos de toxicidad aguda en los organismos acuáticos (bacterias, algas e invertebrados). Se sospechaba del alto riesgo que la presencia de los siguientes medicamentos en las WWe pueden inducir: antibióticos (eritromicina), antiinflamatorios (ibuprofeno, naproxeno, DCF, ketoprofeno), agentes reguladores de lípidos (gemfibrocilo, ácido clofíbrico), β-bloqueantes (propranolol, metoprolol) y antiepilépticos (carbamazepina). También se sospechaba de un elevado riesgo en SW de antiinflamatorios (ibuprofeno, naproxeno, DCF, ketoprofeno) y antiepilépticos (carbamazepina). Las concentraciones conocidas en los sedimentos de estos residuos de medicamentos no eran sospechosas de inducir riesgo. Más recientemente, se llevó a cabo un ERA de once PhACs en WWi y WWe de WWTPs Portuguesas mediante el cálculo de HQs en diferentes organismos acuáticos (es decir, algas, dafnias y peces) (Pereira et al., 2015). De acuerdo a sus valores estimados de HQ (> 1), se estimaó que los siguientes compuestos podían suponer efectos adversos para los organismos acuáticos: ciprofloxacina, bezafibrato, gemfibrozil, simvastatina y DCF. Un estudio similar

llevado a cabo a mayor escala, tuvo como objetivo determinar el potencial riesgo ecotoxicológico asociado a los antibióticos (por ejemplo, ciprofloxacina, SMX, trimetoprima y eritromicina) presentes en ríos europeos (Johnson et al., 2015). Los niveles de antibióticos se estimaron a partir de datos disponibles revisados de las tasas de consumo nacionales, excreción y tasas de eliminación tras el tratamiento de WW. Como ambas concentraciones medidas y estimadas en WWe estaban por debajo de los niveles documentados de efectos para los organismos acuáticos más sensibles, no se esperaba una toxicidad directa en los ríos. Sin embargo, las concentraciones medidas y estimadas de los ríos observados para la ciprofloxacina y eritromicina eran más cercanas a los niveles de efectos en la biota acuática (2 órdenes de magnitud inferior), seguido por SMX (3 órdenes de magnitud inferiores). En vista de estos resultados, ciprofloxacina y eritromicina fueron considerados por los autores como los PhACs de interés para los ecosistemas acuáticos.

En cuanto al estudio incluido en esta tesis (Osorio et al., 2015), ninguno de los valores totales de TUs calculados en cada localización para las algas, Daphnia y peces, superó el valor unitario, por lo tanto, de acuerdo con los umbrales estándar (Malaj et al., 2,014), no se observó riesgo agudo asociado a PhACs. Sin embargo, aunque sólo para LLO7 y ZAD, los correspondientes valores totales de TUs de algas se estimaron por encima de ~ 1E - 03 en ambas campañas de muestreo, lo que evidencia los potenciales efectos ecotoxicológicos a largo plazo sobre estos productores primarios (Malaj et al., 2014). Aunque la probabilidad de efectos de toxicidad aguda de PhACs fue considerada como de menor importancia en todos estos estudios, su contribución a la toxicidad total de la mezcla, así como sus posibles efectos a largo plazo necesitan más estudios. Por lo tanto, otras consideraciones de riesgo deben mantenerse en la caracterización de PhACs relativos a los efectos crónicos. Estos resultados destacan la importancia de realizar este tipo de estudios de seguimiento y ERA para apoyar futuras medidas de priorización por las autoridades del agua.

Un elemento crucial para cualquier estudio que se esfuerza por analizar el impacto de las mezclas de PhACs en el campo, es desentrañar los vínculos causales entre los compuestos presentes en un compartimento determinado del medio ambiente acuático (por ejemplo, la columna de agua o los sedimentos) y los efectos ecotoxicológicos observados. Este reto ha sido abordado mediante el uso de métodos basados en correlación, empleando experimentos de translocación y estudios avanzados de análisis químicos. Por ejemplo, Muñoz et al. (2009) investigaron la correlación entre la presencia de 21 PhACs y la estructura de la comunidad bentónica. Los autores observaron efectos adversos sobre la diversidad de diatomeas en uno de los lugares contaminados y una correlación significativa entre la biodiversidad global de diatomeas y las concentraciones de PhACs. Sin embargo, esta correlación se encontró entre los niveles de indometacina, propranolol, atenolol e ibuprofeno y la abundancia y biomasa de varios invertebrados bentónicos (Chironomus y Tubifex). En cambio, Ginebreda et al. (2010) basó su evaluación del riesgo de una mezcla en la adición de índices de riesgo, siguiendo el modelo de la concentración, y encontró una buena correlación entre la biodiversidad de invertebrados y la suma de los cocientes de riesgo para dafnias.

Curiosamente, de Castro-Català et al. (2015b) realizaron una evaluación de riesgos de los sedimentos recogidos de diversos lugares de las mismas cuencas. A diferencia del estudio presentado en esta tesis, se evaluó la toxicidad de la muestra sobre la que proporciona una estimación más realista y adecuada de

los riesgos ecotoxicológicos en los sitios estudiados. Los autores calcularon los valores de TU de las aguas intersticiales (*V. fischeri, Pseudokirchneriella subcapitata* y *D. magna*) y pruebas de exposición de todo el sedimento (V. fischeri, Chironomus riparius) y evaluaron la composición de la comunidad de invertebrados (análisis multivariante) para detectar respuestas a corto y largo plazo de los organismos. La combinación de los diferentes enfoques les permitió detectar los efectos ecotoxicológicos en los organismos e identificar los principales contribuyentes a la toxicidad de estos ríos multi-estresados. Por otra parte, se correlacionaron los efectos adversos observados en organismos acuáticos con una gran cantidad de productos químicos medidos en los lugares evaluados (por ejemplo, metales, PhACs, pesticidas). Los puntos calientes de riesgo de toxicidad se identificaron en tres sitios (los sitios aguas abajo del Llobregat (LLO5) y el Júcar (JUC5), y el sitio más aguas arriba del Ebro (EBR1)). Además, los insecticidas organofosforados y metales fueron identificados como los principales contribuyentes responsables de esta toxicidad, particularmente en los sedimentos. Estos sitios fueron diferentes de los encontrados en esta tesis (Osorio et al., 2015), lo que evidencia la importancia de otros contaminantes presentes en la mezcla que podrían contribuir a la toxicidad global del sedimento. Respecto PhACs, sólo los sedimentos del río Guadalquivir, que presentan una alta toxicidad para la exposición de *V. fischeri* a corto plazo, estaban relacionados con los antibióticos. Sin embargo, su contribución a la toxicidad de todo el sedimento fue compartida con metales (es decir, Cu, Ni y Hg).

La presencia de otros contaminantes de interés emergente, con diferentes modos de acción que PhACs, en matrices ambientales complejas, y por lo tanto su contribución relativa a la toxicidad de la mezcla completa; ya se discutió en la publicación se presenta en esta tesis (Osorio et al., 2015). En dos estudios contemporáneos (Kuzmanovic et al 2015; De Castro-Català et al, 2015b), el ERA realizado para la caracterización de los contaminantes de interés ambiental (incluyendo PhACs) sobre SW de las mismas cuencas ibéricas, demostraron que PhACs no eran el grupo más relevante de contaminantes que contribuyen a toda la toxicidad de matrices SW. Diez compuestos que pertenecen a los grupos de insecticidas organofosforados y compuestos de disrupción endocrina alquilfenólicos (EDCs) fueron identificados como los principales contribuyentes de la toxicidad para la biota acuática (es decir, las algas, *D. magna* y peces) en SW (Kuzmanovic et al. 2015). Sin embargo, la sertralina, la eritromicina y losartán fueron identificados entre los principales contribuyentes a toda la toxicidad de la muestra SW para las algas; mientras que la sertralina era relevante para *D. magna* y gemfibrozilo para los peces. Por otra parte, de Castro-Català et al. (2015a) encontró que PhACs y EDCs en SW de los mismos ríos son las familias químicas más probablemente relacionadas con las respuestas de los invertebrados bentónicos. En cambio, en el siguiente estudio de los mismos autores (de Castro-Català et al. (2015b), los metales y algunos insecticidas organofosforados fueron los principales contribuyentes a la toxicidad de los sedimentos.

Además, dentro de esta tesis también se relacionó cuantitativamente la ecotoxicidad estimada de PhACs para los organismos acuáticos (es decir, algas, dafnias y peces) con la presión de la población y la agricultura humana animal (Osorio et al., 2015). Relaciones significativamente positivas entre TUs de PhACs en SW y la densidad de población y unidades ganaderas fueron empíricamente demostradas por primera vez. TUs para Daphnia y peces mostraron una respuesta más fuerte, al aumento de densidad de la población y unidades ganaderas con las TUs de algas. Sin embargo, las TUs eran más altas para las algas y más bajas para los peces, con las de dafnias mostrando valores en el medio, lo que sugiere que la

toxicidad de PhACs perjudicaría el conjunto de los productores primarios más que otra biota. Por lo tanto, los efectos sobre los procesos del ecosistema en el que las algas son importantes, ya que los productores primarios, no cambiará mucho con de densidad de población o unidades ganaderas; mientras que se espera que otros organismos en las redes alimentarias principales (es decir, invertebrados y peces) se vean más afectados con el aumento de los niveles de PhACs en los ecosistemas acuáticos evaluados. Estas inconsistencias observadas revelaron la necesidad de ampliar la caracterización de riesgo de PhACs a toda la cadena alimentaria de la biota acuática, cubriendo la gama de sensibilidades diferentes a los compuestos con diferentes modos de acción. Los análisis de las respuestas a nivel de la comunidad a los compuestos individuales y sus mezclas proporcionan una impresión más realista de la gama potencial de los efectos de los contaminantes y con mayor relevancia ecológica. En este sentido, las comunidades microbianas son excelentes sustitutos para el ecosistema en su conjunto, los efectos que implican todo el flujo de los niveles, el carbono y la energía trófica y los impactos sobre los ciclos biogeoquímicos vitales para la salud general y función del ecosistema y la biodiversidad que revela (Lawrence et al., 2012) .

Cabe destacar que durante esta tesis se contribuyó a la investigación sobre los efectos de PhACs detectados en el río Llobregat en biofilms fluviales (Proia et al., 2013a) y más específicamente a los efectos de los antibióticos en las comunidades bacterianas adjuntas (Proia et al., 2013b). Los biofilms se cultivaron en condiciones controladas en mesocosmos que contenían SW del río de tres localizaciones siguiendo un gradiente de contaminación. Después de la colonización, los biofilms fueron trasladados a aguas de diferentes grados de contaminación y se midieron las diferentes respuestas. La translocación del sitio menos contaminado al más contaminado fue la más efectiva. El análisis multivariante reveló que los analgésicos y antiinflamatorios afectaban significativamente las respuestas de los biofilms En particular, el ibuprofeno y acetaminofén se asociaron con efectos negativos sobre la fotosíntesis, y con la disminución de la relación de algas verdes/cianobacterias. Dado que estos descriptores están relacionados principalmente con los organismos autótrofos, las observaciones sugieren que el ibuprofeno y el acetaminofén podrían afectar a la estructura y función de la comunidad autótrofa del biofilm (es decir algas verdes, cianobacterias y diatomeas). Por otro lado, DCF no mostró ninguna relación con los autótrofos del biofilm. En cambio, el análisis estadístico reveló la asociación de DCF a la actividad de fosfatasa. Dado que el enzima fosfatasa en los biofilms se produce principalmente por bacterias, se sugirió que el DCF podría afectar a esta comunidad heterotrófica en biofilms tanto de forma directa o indirecta. Estos resultados están de acuerdo con los observados por Lawrence et al. (2012). Las comunidades bacterianas de los biofilms que crecieron en las SW de los diferentes sitios, difirieron notablemente en su estructura, pero no tanto en términos funcionales. Curiosamente, la abundancia de actinobacteria aumentó después de la translocación al sitio más contaminado y este efecto se asoció a las concentraciones más altas de antibióticos presentes en el agua. Es importante destacar que las especies de este grupo de bacterias son productores naturales de antibióticos (por ejemplo, género *Streptomyces* producen estreptomicina) siendo, por lo tanto intrínsecamente resistentes a ellos (D'Costa et al, 2011). Además, los biofilms mostraron aumento de la mortalidad bacteriana, lo cual se asoció a la presencia de antibióticos en el agua. De hecho, se observó una correlación positiva significativa entre la concentración de tetraciclina y la proporción de bacterias muertas en los biofilms translocados a las SW más contaminadas. Además de los efectos sobre la estructura, también la actividad bacteriana fue afectada después de la translocación. De hecho, se observó que la capacidad de metabolismo de las bacterias heterótrofas disminuyó. En general, se observó una correlación significativa entre las

concentraciones de los antibióticos y las respuestas de biofilm. Estos resultados no fueron totalmente en la línea de los reportados por Yergueau et al. (2012). En cambio, los autores observaron ligeros cambios en la estructura de la comunidad bacteriana; mientras que los biofilms muostraron una variedad de cambios funcionales después de una exposición a corto plazo a las concentraciones ambientales de eritromicina, SMX, sulfametazina y gemfibrozilo. De acuerdo con las observaciones de la colaboración relaizada en esta tesis, también se observaron efectos en autótrofos (es decir, disminución de las cianobacterias y la actividad fotosintética) después de la exposición a la eritromicina y SMX. Además, se observaron de varios cambios en los descriptores relacionados con los biofilms de absorción de nutrientes, tales como hidratos de carbono, nitrógeno y fósforo. Sin embargo, la lista de PhACs incluida en los estudios citados (Lawrence et al., 2012, Yergueau et al., 2012) fue sustancialmente más corta que la analizada en los estudios realizados en esta tesis, lo cual limita la comparación de los resultados.

Teniendo en cuenta la variación observada de concentración PhACs en SW, debido a las fluctuaciones en el caudal del río (Osorio et al, 2012a; 2012b; 2014a), se llevaron a cabo nuevas investigaciones encaminadas a evaluar cómo esta modulación afectada la situación biológica del río. Con ese fin, se evaluaron los efectos de los cambios de caudal en la concentración de PhACs y su relación con las respuestas de los biofilms fluviales. Este fue el tercer trabajo realizado dentro de la colaboración científica con Lorenzo Proia (Universidad de Girona), para más detalles, véase el chapter 5 (sección 5.2). Valiosamente, se realizaron experimentos de translocación de las comunidades del biofilm de un un lugar menos contaminado a otro más contaminado del río en mesocosmos asentados en el campo. Curiosamente, el evento de riada súbita relevante que se produjo al comienzo de la segunda campaña de estudio, lo que corresponde a las fases iniciales de colonización de biofilms, tuvo consecuencias importantes en su estructura y funcionamiento. Después del episodio de riada súbita, se observó que la tasa de crecimiento de biofilms se redujo significativamente indicando la disminución de las tasas de acumulación y recuperación posterior lenta. Curiosamente, la clorofila-a, un descriptor relacionado con la comunidad autótrofa, disminuyó significativamente en biofilms translocados a los sitios más contaminados durante la primera campaña, pero no cambió en la segunda. De acuerdo con las correlaciones negativas significativas entre la clorofila-a y algunos grupos terapéuticos, se sugirió que la disminución de la clorofila-a en el sitio más contaminado podría ser una consecuencia de los niveles más altos de PhACs. Por otro lado, la clorofila-a no respondió en absoluto a la translocación durante la segunda campaña de muestreo. Dado que los PhACs se diluyeron sustancialmente debido al evento de riada repentina, se sugirió que sus posibles efectos sobre las biopelículas podrían haber sido contrarrestados. Independientemente de las condiciones hidrológicas, los resultados mostraron un aumento de la mortalidad de bacterias en los biofilms trasladados al lugar más contaminado. Además, se observó una correlación negativa significativa entre los antibióticos macrólidos y la cantidad de bacterias vivas. Estos hallazgos apoyan la hipótesis del aumento de la mortalidad de bacterias en los biofilms fluviales como consecuencia directa del aumento de las concentraciones de antibióticos en SW. Por lo tanto, el compartimento bacteriano de biofilms ha demostrado mantenerse igualmente sensible a los antibióticos a pesar de los efectos de dilución asociados a condiciones de inundación.

Un estudio similar evaluó los efectos a largo plazo de una mezcla de PhACs en concentraciones ambientales combinados con la intermitencia del caudal de los ríos en los arroyos artificiales de interior (Corcoll et al., 2015). Los resultados de este trabajo fueron considerados como complementarios a las

conclusiones de esta tesis, ya que los autores investigaron sobre los efectos de la concentración PhACs posteriores durante los períodos de sequía, un fenómeno que se había demostrado anteriormente (Osorio et al., 2012b). De acuerdo con las conclusiones de esta tesis, observaron que los biofilms se vieron afectados negativamente por PhACs, tales como cambios en la estructura de la comunidad bacteriana o el metabolismo de las algas verdes y heterótrofos. Además, la intermitencia de caudal también moduló estos efectos en biofilms. Por ejemplo, la comunidad de algas se hizo más sensible a la exposición a corto plazo de PhACs durante la intermitencia del agua, lo que indicó efectos acumulativos entre los dos factores de estrés estudiados. Estos efectos están de acuerdo con los observados durante esta tesis después del evento de inundación repentina para el caso particular de la clorofila-a, un descriptor relacionado con el metabolismo de las algas. Los efectos observados de PhACs sobre la estructura y función de los biofilms, llevó a conjeturar que la presencia de estos microcontaminantes en los ríos podría causar alteraciones importantes en el funcionamiento del ecosistema fluvial. De hecho, las comunidades de biofilms del río son productores primarios netos generalmente muy eficaces en la transformación de la materia orgánica (Romaní et al., 2004), y son transductores de energía a niveles tróficos superiores (Lamberti, 1996). Además, juegan un papel clave en la absorción de nutrientes y la remineralización (House, 2003; Von Schiller et al, 2007), siendo por lo tanto relevantes para los procesos de auto-depuración que se producen en los ríos (Pusch et al., 1998). Sin embargo, la co-ocurrencia de muchos otros contaminantes traza, no considerados en este estudio, pero detectados en el río Llobregat (por ejemplo Köck-Schulmeyer et al., 2012), también puede interferir con los resultados observados. La dirección de las respuestas de los biofilms se explicó por los efectos directos e indirectos de los factores ambientales y la contaminación química sobre la estructura y función de la comunidad.

Por lo tanto, podría decirse que no se encontró una causalidad concluyente entre los PhACs estudiados y los efectos observados. Por otra parte, estos resultados manifiestan que el potencial riesgo ambiental de PhACs en los ecosistemas fluviales está sujeto al régimen de caudal del río, y es particularmente sensible a los acontecimientos de riadas súbitas o de intermitencia del caudal. En general, la interpretación de las complejas interacciones entre múltiples factores de estrés de ecosistemas acuáticos, como la carga continua de PhACs o la alta variabilidad del régimen hidrológico y sus efectos combinados sobre biofilms; resultó ser una tarea complicada. Las interacciones entre los diferentes factores de estrés podrían ser principalmente no aditivas (es decir, sinergias o antagonismos) que sugieren que múltiples factores estresantes pueden interactuar con más frecuencia para generar “sorpresas ecológicas” en lugar de efectos aditivos simples (Darling y Cote, 2008). Determinar qué factores estresantes específicos interactúan para generar estos

efectos en los ecosistemas acuáticos y la prevalencia y magnitud de estas interacciones sigue siendo un reto para la comunidad científica. En consecuencia, el análisis, la cuantificación y la predicción de las respuestas a múltiples factores de estrés a nivel comunitario deben ser los principales objetivos para el trabajo futuro en la evaluación ecotoxicológica del medio acuático.

Las conclusiones de esta tesis, junto con las observaciones de otros autores mencionados anteriormente discutidas, han evidenciado la importancia de integrar diversas disciplinas, concretamente la química, toxicología y ecotoxicología para la evaluación apropiada del destino y riesgo de los PhACs en el medio ambiente acuático.

8.7. Conclusiones

(i) El método analítico desarrollado basado en extracción en fase sólida "off-line" seguido de LC-MS/MS permitió la detección simultánea de ocho compuestos. El método proporcionó límites de detección en el orden de pocos ng L^{-1} y una buena precisión para aguas residuales, proporcionando un protocolo fiable y robusto para el análisis de rutina de un compuesto tan ubicuo como es el DCF, sus principales metabolitos humanos y sus TPs de nitración/nitrosación microbiana en aguas residuales. La aplicación del método en las aguas residuales de entrada y de salida de las WWTPs demostró la presencia de los metabolitos y TPs del DCF en tales matrices en el orden del ng L^{-1}. Por lo que respecta a la literatura disponible, ésta es la primera evidencia de la presencia de TPs de nitración/nitrosación microbiana del DCF en WWTPs. Estos resultados corroboran los estudios previos de los coautores en los cuales se observó la transformación del DCF en nito/nitroso compuestos en reactores a escala laboratorio a través de reacciones biológicas. El conocimiento de la presencia de metabolites y TPs en el medio ambiente acuático es todavía limitado y serían necesarios más estudios para evaluar el destino global de los PhACs en las WWTPs.

(ii) Se descubrieron TPs de nitración/nitrosación microbiana de antiinflamatorios no esteroideos relacionados con el DCF en reactores con licor mixto de WWTPs. Estos resultados corroboran las hipótesis formuladas previamente acerca de la transformación del DCF mediante reacciones biológicas para generar estos TPs en reactores a escala laboratorio, por tanto demostrando que este camino de reacción no es solo único para el DCF. Además de la elucidación y la determinación de metabolitos y TPs desconocidos, la evaluación de la toxicidad de estos compuestos seria así mismo relevante para definir los efectos toxicológicos potenciales en los ecosistemas acuáticos.

(iii) Los valores elevados de PhACs detectados (el intervalo de concentraciones totales fue de LOQ-1.500 ng L^{-1}) y su ubicuidad en las muestras analizadas se explican por la elevada tasa de consumo de estos PhACs, así como por su resistencia a la biodegradación en WWTPs convencionales que aplican tratamiento secundario biológico. La información acerca de las relaciones entre los niveles

de concentración de PhACs en los ríos y las condiciones hidrológicas es aún escasa y por tanto sería necesario poner mayor interés en este tema. Los PhACs pueden adsorberse a los sólidos en suspensión, la fase coloidal y los sedimentos y por tanto la adsorción es uno de los procesos claves que controlan en el transporte y destino de estos compuestos en los sistemas acuáticos. Por este motivo, sería de especial interés determinar los coeficientes de partición de los PhACs entre las diversas fases sólida y acuosa de la columna de agua del río, así como los factores involucrados en sus mecanismos de distribución. Los resultados sugieren interacciones complejas entre las fuentes de contaminación, el transporte y la degradación de los PhACs en un río tan dinámico como es el río Llobregat. Otros factores medioambientales se deberían también tener en cuenta, como la foto-degradación y la biodegradación, los cuales pueden afectar al destino de los PhACs en las aguas superficiales.

(iv) La presencia de PhACs en ríos Mediterráneos como el río Llobregat está sujeta a una fuerte variación estacional. Este hecho se puede explicar parcialmente en términos de cambios extremos de caudal característicos de la hidrología Mediterránea. Por ejemplo, durante la estación de lluvias se produjeron picos de caudal que sobrepasaron hasta dos órdenes de magnitud el caudal de base del río, lo cual afectó directamente a la concentración de los PhACs. Sin embargo, varios fenómenos concurrentes (con el tiempo operando en sentido opuesto) como la re-suspensión, la adsorción, la degradación, la variabilidad del tiempo de residencia hidráulico del río, o la eficiencia de las WWTPs pueden dar lugar a un patrón complejo, el cual sólo puede ser desenmarañado a través de una modelización apropiada. En este contexto, se trató de desarrollar un modelo de tipo “plug-flow”, que permitió explicar las variaciones observadas en la carga de los compuestos analizados en las dos localizaciones del río estudiadas en términos del caudal y de dos parámetros característicos de cada compuesto, concretamente un parámetro asociado a la carga promedio descargada aguas arriba y otro interpretable como la constante general de desaparición durante el tiempo de circulación. Los resultados obtenidos para los dos puntos de muestreo estudiados, mostraron consistencia y por tanto el método se propuso para ser aplicado en la gestión de las cuencas o en tramos concretos de sistemas acuáticos.

(v) Se demostró la ubicuidad de los PhACs en aguas superficiales y sedimentos de los ríos Ibéricos, a pesar de que algunos puntos de muestreo presentaron concentraciones extraordinariamente elevadas, lo cual indicó que se debería poner especial atención en estas localizaciones concretas. Tanto las concentraciones medianas de PhACs como sus ecotoxicidades estimadas, mostraron relaciones positivas con la densidad de población humana así como con las unidades ganaderas, respondiendo así a la presiones antropogénicas en las cuencas hidrográficas. A pesar de que la contribución de los PhACs a la toxicidad total estimada en aguas superficiales es dependiente del lugar específico analizado, cinco compuestos (eritromicina, gemfibrozilo, loratidina, losartan y sertralina) fueron los responsables de más del 50% de las unidades de toxicidad calculadas para algas, dafnias y peces y por lo tanto estos compuestos deberian ser especialmente considerados cuando se aborda el tema de la contaminación de PhACs en las aguas superficiales. Los resultados de este estudio resaltaron que las aguas superficiales pueden recibir cantidades ingentes de PhACs que pueden interferir en la organización natural de los organismos acuáticos y afectar a los procesos ecosistémicos y, así, a los servicios.

(vi) De acuerdo con la literatura consultada, se mostró por vez primera la evidencia de la presencia de TPs del DCF y SMX en aguas residuales así como en aguas superficiales impactadas por efluentes de WWTPs. La detección de los derivados del DCF y SMX en aguas residuales de entrada a las WWTPs

sugirió que los procesos de transformación microbiana pueden ocurrir a lo largo del sistema colector de aguas residuales. Las relaciones de los niveles de NO-DCF y NO_2-DCF con las especies de nitrógeno (NO_2^--N and NO_3^--N) sugirieron tentativamente la biotransformación del DCF en NO-DCF y NO_2-DCF y del SMX en NO_2-SMX y Des-SMX; como consecuencia de los procesos de nitrificación y denitrificación que tienen lugar en los lodos activados. Según los trabajos publicados, también se evaluó por vez primera la toxicidad aguda en *D. magnia* y *V. fischeri* de los productos de transformación microbiana del DCF y el SMX de modo individual así como en mezclas binarias con otros contaminantes relevantes del medio ambiente acuático (nonilfenol, diurón, malatión, glifosato y triclosan). Por lo general, la toxicidad aguda en *D. magnia* y *V. fischeri* de los TPs fue inferior a la correspondiente a sus productos padre. No obstante, el sinergismo observado para NO-DCF en mezclas, demostró que estos derivados no deberían ser descartados en la evaluación de muestras tan complejas como son las aguas residuales o las aguas superficiales receptoras de efluentes de WWTPs. Debido a este fenómeno, los productos de transformación microbiana del DCF y el SMX deberían incluirse en los estudios medioambientales de estos PhACs como complemento para el entendimiento de la presencia, destino y comportamiento del DCF y el SMX en el medio ambiente acuático.

(vii) Las diferencias observadas en las concentraciones de PhACs y la respuesta de los biofilms a las fluctuaciones del caudal del río, puntualizó la importancia que las variaciones hidrológicas tienen en el estado químico y ecológico de los ríos Mediterráneos. El estudio reveló que las diferencias entre los biofilms desarrolladas bajo condiciones de caudal diferentes, eran más relevantes que las diferencias observadas entre los puntos de muestreo. Esta diferencia fue más marcada por la aplicación de la translocación de comunidades de biofilms entre puntos de muestreo, siguiendo un gradiente de polución. Esta evidencia sugiere que el episodio de riada ocurrido, representó un papel fundamental en el desarrollo de las comunidades biológicas en el Río Llobregat. No obstante, este estudio también reveló que algunos efectos potencialmente negativos de ciertos grupos de PhACs, como los antibióticos, en los organismos acuáticos, como las bacterias, se pueden mantener inalterables bajo diversas situaciones hidrológicas. En base a estos resultados, se concluye que para esclarecer los efectos de la combinación de los cambios en la hidrología y en la contaminación en los ecosistemas acuáticos, se requiere la unificación de esfuerzos en la química analítica, la hidrología y la ecología.

References

References

Acuña V, Tockner K. “The effects of alterations in temperature and flow regime on organic carbon dynamics in Mediterranean river networks” *Global and Planetary Change* 16 (2010): 2638–2650.

Acuña V, von Schiller D, García-Galán MJ, Rodríguez-Mozaz S, Corominas L, Petrovic M, Poch M, Barceló D, Sabater S. "Occurrence and in-stream attenuation of wastewater-derived pharmaceuticals in Iberian rivers." *Science of the Total Environment* 503 (2015): 133-141.

Agüera A, Martínez Bueno MJ, Fernández-Alba AR. "New trends in the analytical determination of emerging contaminants and their transformation products in environmental waters." *Environmental Science and Pollution Research* 20, no. 6 (2013): 3496-3515.

Ahmed MM, Barbati S, Doumenq P, Chiron S. "Sulfate radical anion oxidation of diclofenac and sulfamethoxazole for water decontamination." *Chemical Engineering Journal* 197 (2012): 440-447.

Al Aukidy M, Verlicchi P, Voulvoulis N. “A framework for the assessment of the environmental risk posed by pharmaceuticals originating from hospital effluents” *Science of the Total Environment* 493 (2014) 54–64.

Aldekoa J, Medici C, Osorio V, Pérez S, Marcé R, Barceló D, Francés F. "Modelling the emerging pollutant diclofenac with the GREAT-ER model: Application to the Llobregat River Basin." *Journal of Hazardous Materials* 263 (2013): 207-213.

Alder, AC, Schaffner C, Majewsky M, Klasmeier J, Fenner K. "Fate of β-blocker human pharmaceuticals in surface water: Comparison of measured and simulated concentrations in the Glatt Valley Watershed, Switzerland." *Water research* 44, no. 3 (2010): 936-948.

Alexandratos N, Bruinsma J. "World agriculture towards 2030/2050: the 2012 revision." (2012) ESA Work. Pap 3.

Alvarino T, Suarez S, Lema J M, Omil F. "Understanding the removal mechanisms of PPCPs and the influence of main technological parameters in anaerobic UASB and aerobic CAS reactors." *Journal of hazardous materials* 278 (2014): 506-513.

Anderson PD, D'Aco VJ, Shanahan P, Chapra SC, Buzby ME, Cunningham VL, Duplessie BM, Hayes EP , Mastrocco FJ, Parke NJ, Rader JC, Samuelian JH, Schwab BW. "Screening analysis of human pharmaceutical compounds in US surface waters." *Environmental science & technology* 38, no. 3 (2004): 838-849.

Andreozzi R, Raffaele M, and Nicklas P. "Pharmaceuticals in STP effluents and their solar photodegradation in aquatic environment." *Chemosphere* 50, no. 10 (2003): 1319-1330.

Antoniadis A, V. Takavakoglou, G. Zalidis, E. Darakas, I. Poulios. "Municipal wastewater treatment by sequential combination of photocatalytic oxidation with constructed wetlands." *Catalysis today* 151, no. 1 (2010): 114-118.

Aristi I, Schiller D, Arroita M, Barceló D, Ponsatí L, García-Galán MJ, Sabater S, Elosegi A, Acuña V. "Mixed effects of effluents from a wastewater treatment plant on river ecosystem metabolism: subsidy or stress?" *Freshwater Biology* 60, no. 7 (2015): 1398-1410.

Awad YM, Sung-Chul K, El-Azeem A, Samy AM, Kye-Hoon K, Kwon-Rae K, Kangjoo K, Jeon C, Soo S, Yong L, Ok S. “Veterinary antibiotics contamination in water, sediment, and soil near a swine manure composting facility” *Environ. Earth Sci.* 71, no. 3 (2014): 1433–1440.

Backhaus T, Altenburger R, Boedeker W, Faust M, Scholze M, Grimme LH. “Predictability of the toxicity of a multiple mixture of dissimilarly acting chemicals to Vibrio fischeri.” *Environ. Toxicol. Chem.* 19 (2000a): 2348–2356. (doi:10.1002/etc.5620190927)

Backhaus T, Faust M. "Predictive environmental risk assessment of chemical mixtures: a conceptual framework." *Environmental science & technology* 46, no. 5 (2012): 2564-2573.

Backhaus T, Scholze M, Grimme LH. "The single substance and mixture toxicity of quinolones to the bioluminescent bacterium Vibrio fischeri." *Aquatic Toxicology* 49, no. 1 (2000b): 49-61.

Backhaus T. "Medicines, shaken and stirred: a critical review on the ecotoxicology of pharmaceutical mixtures." Philosophical Transactions of the Royal Society of London B: Biological Sciences 369.1656 (2014): 20130585.

Bailey MJ, Dickinson RG. "Acyl glucuronide reactivity in perspective: biological consequences." Chemico-biological interactions 145.2 (2003): 117-137.

Banjac Z, Ginebreda A, Kuzmanovic M, Marcé R, Nadal M,. Riera JM, Barceló D. "Emission factor estimation of ca. 160 emerging organic microcontaminants by inverse modeling in a Mediterranean river basin (Llobregat, NE Spain)." *Science of The Total Environment* 520 (2015): 241-252.

Barbieri M, Carrera J, Ayora C, Sanchez-Vila X, Licha T, Nödler K, Osorio V, Pérez S, Köck-Schulmeyer M, López de Alda M, Barceló D. "Formation of diclofenac and sulfamethoxazole reversible transformation products in aquifer material under

denitrifying conditions: batch experiments." *Science of the Total Environment* 426 (2012): 256-263.

Bartels P, von Tümpling W. "Solar radiation influence on the decomposition process of diclofenac in surface waters." *Science of the total environment* 374, no. 1 (2007): 143-155.

Bartelt-Hunt S, Snow DD, Damon-Powell T, Miesbach D, "Occurrence of steroid hormones and antibiotics in shallow groundwater impacted by livestock waste control facilities" *J. Contam. Hydrol.* 123 (2011): 94–103.

Batt AL, Kim S, Aga DS. "Enhanced biodegradation of iopromide and trimethoprim in nitrifying activated sludge." *Environmental science & technology* 40, no. 23 (2006): 7367-7373.

Bendz D, Paxeus NA, Ginn TR, Loge FJ. "Occurrence and fate of pharmaceutically active compounds in the environment, case study: Hoje River in Sweden." *Journal of Hazardous Materials* 122 (2005): 195–204.

Benotti MJ, Trenholm RA, Vanderford BJ, Holady JC, Stanford BD, Snyder SA. "Pharmaceuticals and endocrine disrupting compounds in US drinking water." *Environmental Science & Technology* 43, no. 3 (2008): 597-603.

Besse JP, Garric J. "Human pharmaceuticals in surface waters: implementation of a prioritization methodology and application to the French situation." *Toxicology letters* 176, no. 2 (2008): 104-123.

Beven, K. "A manifesto for the equifinality thesis." *Journal of hydrology* 320, no. 1 (2006): 18-36.

Bock E, and Michael Wagner. "Oxidation of inorganic nitrogen compounds as an energy source." In The prokaryotes, pp. 457-495. Springer New York, 2006.

Boix C, Ibáñez M, Zamora T, Sancho JV, Niessen WMA, Hernández F. "Identification of new omeprazole metabolites in wastewaters and surface waters" *Science of the Total Environment* 468–469 (2014) 706–714

Boleda M, Élida RM, Alechaga E, Moyano E, Galceran MT, Ventura F. "Survey of the occurrence of pharmaceuticals in Spanish finished drinking waters" *Environmental Scicience and Pollutution Research* (2014) 21:10917–10939.

Bonnineau C, Guasch H, Proia L, Ricart M, Geiszinger A, Romaní AM, Sabater S. "Fluvial biofilms: a pertinent tool to assess β-blockers toxicity." *Aquatic toxicology* 96, no. 3 (2010): 225-233.

Bonvin F, Omlin J, Rutler R, Schweizer WB, Alaimo PJ, Strathmann TJ, McNeill K, Kohn T. "Direct photolysis of human metabolites of the antibiotic sulfamethoxazole: evidence for abiotic back-transformation." *Environmental science & technology* 47, no. 13 (2012): 6746-6755.

Borg MA. “National cultural dimensions as drivers of inappropriate ambulatory care consumption of antibiotics in Europe and their relevance to awareness campaigns”*J. Antimicrob. Chemother.* 67 (2012) 763–767.

Boxall ABA, Keller VDJ, Straub JO, Monteiro SC, Fussell R, Williams RJ. "Exploiting monitoring data in environmental exposure modelling and risk assessment of pharmaceuticals." *Environment international* 73 (2014): 176-185.

Boxall ABA, Kolpin DW, Halling-Sørensen B, Tolls J. "Peer reviewed: are veterinary medicines causing environmental risks?" *Environmental science & technology* 37, no. 15 (2003): 286A-294A.

Boyd, RG, Palmeri MJ, Zhang S, and Grimm AD. “PhACs and personal care products (PPCPs) and endocrine disrupting chemicals (EDCs) in stormwater canals and bayou St. John in New Orleans, Louisiana, USA” *Science of the Total Environment* 333 (2004): 137– 148.

Brandmayr C, Kerber H, Winker M, Schramm E. "Impact assessment of emission management strategies of the pharmaceuticals Metformin and Metoprolol to the aquatic environment using Bayesian networks." *Science of The Total Environment* 532 (2015): 605-616.

Brausch JM, Connors KA, Brooks BW, Rand GM. "Human pharmaceuticals in the aquatic environment: a review of recent toxicological studies and considerations for toxicity testing." In *Reviews of Environmental Contamination and Toxicology* Volume 218, pp. 1-99. Springer US, 2012.

Brooks BW, Chambliss CK, Stanley JK, Ramirez A, Banks KE, Johnson RD, and Lewis RJ. "Determination of select antidepressants in fish from an effluent-dominated stream." Environmental *Toxicology and Chemistry* 24, no. 2 (2005): 464-469.

Brooks BW, Foran CM, Richards SM, Weston J, Turner PK, Stanley JK, Solomon KR, Slattery M, La Point TW. "Aquatic ecotoxicology of fluoxetine." *Toxicology letters* 142, no. 3 (2003): 169-183.

Buesing N, Gessner MO. “Benthic bacterial and fungal productivity and carbon turnover in a freshwater marsh” *Appl. Environ. Microbiol*. 72 (2006) 596–605.

Buser HR, Poiger T, Müller MD. "Occurrence and environmental behavior of the chiral pharmaceutical drug ibuprofen in surface waters and in wastewater.” *Environmental Science & Technology* 33, no.15 (1999): 2529–2535.

Buser HR, Poiger T, Müller MD. "Occurrence and fate of the pharmaceutical drug diclofenac in surface waters: rapid photodegradation in a lake." *Environmental Science & Technology* 32, no. 22 (1998): 3449-3456.

Calza P, Sakkas VA, Medana C, Baiocchi C, Dimou A, Pelizzetti E, Albanis T. "Photocatalytic degradation study of diclofenac over aqueous TiO 2 suspensions." *Applied Catalysis B: Environmental* 67, no. 3 (2006): 197-205.

Caracciolo AB, Topp E, Grenni P. "Pharmaceuticals in the environment: Biodegradation and effects on natural microbial communities. A review." *Journal of pharmaceutical and biomedical analysis* 106 (2015): 25-36.

Carballa M, Omil F, Lema JM, Liompart M, Garcia-Jares C, Rodriguez I, Gómez M, Ternes T. "Behavior of pharmaceuticals, cosmetics and hormones in a sewage treatment plant." *Water Research* 38 (2004): 2918–26.

Carmona E, Andreu V, Picó V. "Occurrence of acidic pharmaceuticals and personal care products in Turia River Basin: from waste to drinking water." *Science of the Total Environment* 484 (2014): 53-63.

Carucci A, Cappai G, Piredda M. "Biodegradability and toxicity of pharmaceuticals in biological wastewater treatment plants." *Journal of Environmental Science and Health Part A* 41, no. 9 (2006): 1831-1842.

Castiglioni S, Bagnati R, Fanelli R, Pomati F, Calamari D, Zuccato E. "Removal of pharmaceuticals in sewage treatment plants in Italy." *Environmental Science & Technology* 40, no. 1 (2006): 357-363.

Castiglioni S, Fanelli R, Calamari D, Bagnati R, Zuccato E. "Methodological approaches for studying pharmaceuticals in the environment by comparing predicted and measured concentrations in River Po, Italy" *Regul Toxicol Pharmacol* 39 no.1 (2004): 25–32.

Celiz MD, Tso J, Aga DS. "Pharmaceutical metabolites in the environment: analytical challenges and ecological risks." *Environmental Toxicology and Chemistry* 28, no. 12 (2009): 2473-2484.

Celle-Jeanton H, Schemberg D, Mohammed N, Huneau F, Bertrand G, Lavastre V, Le Coustumer P. "Evaluation of pharmaceuticals in surface water: Reliability of PECs compared to MECs." *Environment International* 73 (2014): 10-21.

Céspedes, R, Lacorte S, Raldúa D, Ginebreda A, Barceló D, Piña B. "Distribution of endocrine disruptors in the Llobregat River basin (Catalonia, NE Spain)" *Chem* 61, no. 11 (2005): 1710–1719.

Challis JK, Hanson ML, Friesen KJ, Wong CS. "A critical assessment of the photodegradation of pharmaceuticals in aquatic environments: defining our current understanding and identifying knowledge gaps." *Environmental Science: Processes & Impacts* 16, no. 4 (2014): 672-696.

Chapra S. "Surface water-quality modeling" (1997) New York: McGraw-Hill Int. Ed.

Chiron S, Gomez E, Fenet H. "Nitration processes of acetaminophen in nitrifying activated sludge." *Environmental science & technology* 44, no. 1 (2010): 284-289.

Chitescu CL, Kaklamanos G, Nicolau AI, aStolker AAM. “High sensitive multiresidue analysis of pharmaceuticals and antifungals in surfacewater using U-HPLC-Q-Exactive Orbitrap HRMS. Application to the Danube river basin on the Romanian territory” *Science of the Total Environment* 532 (2015) 501–511.

Chow, L, Waldron L, Gillings MR. Potential impacts of aquatic pollutants: sub-clinical antibiotic concentrations induce genome changes and promote antibiotic resistance. *Front. Microbiol*. 6 (2015) http://dx.doi.org/10.3389/fmicb.2015.00803.

Chowdhury RR, Charpentier PA, Ray MB. “Photodegradation of 17bestradiol in aquatic solution under solar irradiation: kinetics and influencing water parameters.” *J. Photochem. Photobiol*. A Chem. 219 (2011) 67-75.

Christensen AM, Ingerslev F, Baun A. "Ecotoxicity of mixtures of antibiotics used in aquacultures." *Environmental Toxicology and Chemistry* 25, no. 8 (2006): 2208-2215.

Christensen FM. "Pharmaceuticals in the environment—a human risk?" *Regulatory Toxicology and Pharmacology* 28, no. 3 (1998): 212-221.

Christensen JH, Hewitson B, Busuioc A, Chen A, Gao X, Held R, Jones R, Kolli RK, Kwon WK, Laprise R, Magana Rueda V, Mearns L, Menendez CG, Räisänen J, Rinke A, Sarr A, Whetton P, Arritt R, Benestad R, Beniston M, Bromwich D, Caya D, Comiso J, de Elia R, Dethloff K. (2007): “Regional climate projections, Climate Change”: The Physical Science Basis. Contribution of Working group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, University Press, Cambridge, (2007) Chapter 11, ISBN: 978-0-521-88009-1.

Clara M, Kreuzinger N, Strenn B, Gans O, Kroiss H. "The solids retention time—a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants." *Water research* 39, no. 1 (2005): 97-106.

Clara M, Strenn B, Ausserleiter M, Kreuzinger N. “Comparison of the behaviour of selected micropollutants in a membrane bioreactor and a conventional wastewater treatment plant.” *Water Science & Technology* 50, no. 5 (2004): 29-36.

Cleuvers M. "Aquatic ecotoxicity of pharmaceuticals including the assessment of combination effects." *Toxicology letters* 142, no. 3 (2003): 185-194.

COMMISSION IMPLEMENTING DECISION (EU) 2015/495 of 20 March 2015 establishing a watch list of substances for Union-wide monitoring in the field of water policy pursuant to Directive 2008/105/EC of the European Parliament and of the Council.

Comninellis C, Kapalka A, Malato S, Parsons SA, Poulios I, Mantzavinos D. "Advanced oxidation processes for water treatment: advances and trends for R&D." *Journal of Chemical Technology and Biotechnology* 83, no. 6 (2008): 769-776.

Cooper ER, Siewicki TC, Phillips K. “Preliminary risk assessment database and risk ranking of pharmaceuticals in the environment” *Science of the Total Environment* 398 no 1-3 (2008): 26–33.

Corcoll N, Acuña V, Barceló D, Casellas M, Guasch H, Huerta B, Petrovic M, Ponsatí L, Rodríguez-Mozaz S, Sabater S. "Pollution-induced community tolerance to non-steroidal anti-inflammatory drugs (NSAIDs) in fluvial biofilm communities affected by WWTP effluents." *Chemosphere* 112 (2014): 185-193.

Corcoll N, Casellas M, Huerta B, Guasch H, Acuña V, Rodríguez-Mozaz S, Serra-Compte A, Barceló D, Sabater S. "Effects of flow intermittency and pharmaceutical exposure on the structure and metabolism of stream biofilms." *Science of the Total Environment* 503 (2015): 159-170.

Corcoran J, Winter MJ, Tyler CR. "Pharmaceuticals in the aquatic environment: a critical review of the evidence for health effects in fish." *Critical reviews in toxicology* 40, no. 4 (2010): 287-304.

Czech, Bożena, Izabela Jośko, and Patryk Oleszczuk. "Ecotoxicological evaluation of selected pharmaceuticals to Vibrio fischeri and Daphnia magna before and after photooxidation process." *Ecotoxicology and environmental safety* 104 (2014): 247-253.

D’Costa VM, King CE, Kalan L, Morar M, Sung WWL, Schwarz C, Froese D, Zazula G, Calmels F, Debruyne R, Golding GB, Wright GD. "Antibiotic resistance is ancient." *Nature* 477, no. 7365 (2011): 457-461.

da Silva BF, Jelic A, López-Serna R, Mozeto AA, Petrovic M, Barceló D. "Occurrence and distribution of pharmaceuticals in surface water, suspended solids and sediments of the Ebro river basin, Spain." *Chemosphere* 85, no. 8 (2011): 1331-1339.

Dai G, Wang B, Huang J, Dong R, Deng S, Yu G. "Occurrence and source apportionment of pharmaceuticals and personal care products in the Beiyun River of Beijing, China." *Chemosphere* 119 (2015): 1033-1039.

Damásio J, Barceló D, Brix D, Postigo C, Gros M, Petrovic M, Sabater S, Guasch H, de Alda M, Barata C. “Are pharmaceuticals more harmful than other pollutants to aquatic invertebrate species: a hypothesis tested using multi-biomarker and multispecies responses in field collected and transplanted organisms” *Chemosphere* 85, no.10 (2011): 1548–1554.

Daouk S, Chèvre N, Vernaz N, Bonnabry N, Dayer P, Daali Y, Fleury-Souverain S. "Prioritization methodology for the monitoring of active pharmaceutical ingredients in hospital effluents." *Journal of environmental management* 160 (2015): 324-332.

Darling, ES, Côté IM. "Quantifying the evidence for ecological synergies." *Ecology letters* 11, no. 12 (2008): 1278-1286.

Darwano H, Vo Duy S, Sauve S. "A New Protocol for the Analysis of Pharmaceuticals, Pesticides, and Hormones in Sediments and Suspended Particulate Matter From Rivers and Municipal Wastewaters" *Arch Environ Contam Toxicol* 66(2014): 582–593

Daughton CG, Brooks BW. "Active pharmaceutical ingredients and aquatic organisms." *Environmental contaminants in biota: interpreting tissue concentrations* 2 (2011).

Daughton CG, Ruhoy IS. "Environmental footprint of pharmaceuticals: the significance of factors beyond direct excretion to sewers." *Environmental toxicology and chemistry* 28, no. 12 (2009): 2495-2521.

Daughton CG, Ternes TA. "Pharmaceuticals and personal care products in the environment: agents of subtle change?" *Environmental health perspectives* 107, no. Suppl 6 (1999): 907.

Daughton CG. "Pharmaceuticals in the environment: sources and their management." In: *Analysis, removal, effects and risk of pharmaceuticals in the water cycle—occurrence and transformation in the environment*. pp. 37-69. Elsevier ,2013.

Daughton G, Ruhoy IS. "Lower-dose prescribing: minimizing "side effects" of pharmaceuticals on society and the environment." *Science of the Total Environment* 443 (2013): 324-337.

de Almeida CAA, Oliveira M, Mallmann CA, Martins AF. "Determination of the psychoactive drugs carbamazepine and diazepam in hospital effluent and identification of their metabolites". *Environmental Science and Pollution Research* (2015) doi:10.1007/s11356-015-4948-y

de Castro-Català N, Kuzmanovic M, Roig N, Sierra J, Ginebreda A, Barceló D, Pérez S, Petrovic M, Picó Y, Scumacher M, Muñoz I. "Ecotoxicity of sediments in rivers: Invertebrate community, toxicity bioassays and the toxic unit approach as complementary assessment tools." *Science of The Total Environment* (2015b).

De Castro-Català N, Muñoz I, Armendáriz L, Campos B, Barceló D, López-Doval J, Pérez S, Petrovic M, Picó Y, Riera JL. "Invertebrate community responses to emerging water pollutants in Iberian river basins." *Science of the Total Environment* 503 (2015a): 142-150.

de Gusseme B, Vanhaecke L, Verstraete W, Boon N. "Degradation of acetaminophen by Delftia tsuruhatensis and Pseudomonas aeruginosa in a membrane bioreactor." *Water research* 45, no. 4 (2011): 1829-1837.

de Jongh CM, Kooij PJF, Voogt P, ter Laak TL. "Screening and human health risk assessment of pharmaceuticals and their transformation products in Dutch surface waters and drinking water." *Science of the Total Environment* 427 (2012): 70-77.

Delle Site A. "Factors affecting sorption of organic compounds in natural sorbent/water systems and sorption coefficients for selected pollutants. A review." *Journal of Physical and Chemical Reference Data* 30, no. 1 (2001): 187-439.

Di Giulio RT, Hinton DE, eds. *The toxicology of fishes*. Crc Press, 2008.

Díaz-Cruz MS, García-Galán, MJ, Barceló D. "Highly sensitive simultaneous determination of sulfonamide antibiotics and one metabolite in environmental waters by liquid chromatography-quadrupole linear ion trap-mass spectrometry" *Journal of Chromatography A* 1193 (2008): 50–59.

Dietrich S, Ploessl F, Bracher F, Laforsch C. "Single and combined toxicity of pharmaceuticals at environmentally relevant concentrations in Daphnia magna–A multigenerational study." *Chemosphere* 79, no. 1 (2010): 60-66.

Donnachie RL, AC Johnson AC, Sumpter JP. "A rational approach to selecting and ranking some pharmaceuticals of concern for the aquatic environment and their relative importance compared to other chemicals." *Environmental Toxicology and Chemistry* (2015).

EFPIA. "The Pharmaceutical Industry in figures" (2014) – European Federation of Pharmaceutical Industries and Associations (EFPIA)

Eguchi K, Nagase H, Ozawa M, Endoh YS, Goto K, Hirata K, Miyamoto K, Yoshimura H. (2004). Evaluation of antimicrobial agents for veterinary use in the ecotoxicity test using microalgae. Chemosphere, 57(11), 1733-1738.

EMA. "Committee for medicinal product for veterinary use (CVMP)" (2008) Doc. Ref. EMEA/CVMP/ERA/418282/2005-Rev.1 Consultation

EMA. "Guideline on Environmental Impact Assessment for Veterinary Medicinal Products in Support of the VICH Guidelines GL 6 and GL 38. (Draft Veterinary TGD)." (2006) Committee for Medicinal Products for Veterinary Use (CVMP) p. 59.

Emnet P, Gaw S, Northcott G, Storey B, Graham L. "Personal care products and steroid hormones in the Antarctic coastal environment associated with two Antarctic research stations, McMurdo Station and Scott Base." *Environmental research* 136 (2015): 331-342.

Enick OV, Moore MM. "Assessing the assessments: pharmaceuticals in the environment." *Environmental Impact Assessment Review* 27, no. 8 (2007): 707-729.

EPA U.S. Office of Compliance Sector Notebook Project: Profile of the Pharmaceutical Manufacturing Industry; EPA/310-R-97-005 (1997) http://www.epa.gov/Compliance/resources/publications/assistance/sectors/notebooks/pharmapt1.pdf; Washington, DC.

Erguder TH, Boon N, Wittebolle L, Marzorati M, Verstraete W. "Environmental factors shaping the ecological niches of ammonia-oxidizing archaea." *FEMS Microbiology Reviews* 33, no. 5 (2009): 855-869.

Escher BI, Bramaz N, Lienert J, Neuwoehner J, Straub JO. "Mixture toxicity of the antiviral drug Tamiflu®(oseltamivir ethylester) and its active metabolite oseltamivir acid." *Aquatic toxicology* 96, no. 3 (2010): 194-202.

Escher BI, Fenner K. "Recent advances in environmental risk assessment of transformation products." *Environmental science & technology* 45, no. 9 (2011): 3835-3847.

European Council Directive 2013/39/EU of the European parliament and of the council of 12 August 2013 amending Directives 2000/60/EC and 2008/105/EC as regards priority substances in the field of water policy.

European Council. “Directive 2004/27/EC requiring Member States to “ensure that appropriate collection systems are in place for human medicinal products that are unused or have expired” (Article 127b).” (2004) Official Journal of the European Communities.

European Council**.** “Directive 2004/28/EC of the European Parliament and of the Council of 31 March 2004 amending Directive 2001/82/EC on the Community Code relating to Veterinary Medicinal Products” (2004). Official Journal of the European Communities.

European Council. “Directive 2008/105/EC of the European Parliament and of the Council of 16 December 2008 on environmental quality standards in the field of water policy, amending and subsequently repealing Council Directives 82/176/ EEC, 83/513/EEC, 84/156/EEC, 84/491/EEC, 86/280/EEC and amending Directive 2000/60/EC of the European Parliament and of the Council.” Official Journal of the European Communities L 348 (2008), 0084.

European Environment Agency. “Technical report No. 1/2010: Pharmaceuticals in the Environment”. (2010) EEA: Copenhagen.

Evgenidou EN, Konstantinou IK, Lambropoulou DA. “Occurrence and removal of transformation products of PPCPs and illicit drugs in wastewaters: A review” *Science of the Total Environment* 505 (2015): 905–926

Farré M, Pérez S, Kantiani L, Barceló D. "Fate and toxicity of emerging pollutants, their metabolites and transformation products in the aquatic environment." *TrAC Trends in Analytical Chemistry* 27, no. 11 (2008): 991-1007.

Fatta-Kassinos D, Meric S, Nikolaou A. "Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research." *Analytical and bioanalytical chemistry* 399, no. 1 (2011b): 251-275.

Fatta-Kassinos D, Vasquez MI, Kümmerer K. "Transformation products of pharmaceuticals in surface waters and wastewater formed during photolysis and advanced oxidation processes–degradation, elucidation of byproducts and assessment of their biological potency." *Chemosphere* 85, no. 5 (2011a): 693-709.

Feijtel T, Boeije G, Matthies M, Young A, Morris G, Gandolfi C, Hansen B, Fox K, Matthijs E, Koch V, Schroder R, Cassani G, Schowanek D, Rosenblom J, Holt M. "Development of a geography-referenced regional exposure assessment tool for European rivers—GREAT-ER." *Journal of hazardous materials* 61, no. 1 (1998): 59-65.

Fent K, Weston AA, Caminada D. "Ecotoxicology of human pharmaceuticals." *Aquatic toxicology* 76, no. 2 (2006): 122-159.

Fernández-Fontaina E, Omil F, Lema JM, Carballa M. "Influence of nitrifying conditions on the biodegradation and sorption of emerging micropollutants." *Water Research* 46, no. 16 (2012): 5434-5444.

Ferrando-Climent L, Collado N, Buttiglieri G, Gros M, Rodriguez-Roda I, Rodriguez-Mozaz S, Barceló D. "Comprehensive study of ibuprofen and its metabolites in activated sludge batch experiments and aquatic environment." *Science of the total environment* 438 (2012): 404-413.

Ferrari B, Mons R, Vollat B, Fraysse B, Paxēaus N, Lo Giudice R, Pollio A, Garric J. "Environmental risk assessment of six human pharmaceuticals: are the current environmental risk assessment procedures sufficient for the protection of the aquatic environment?" *Environmental toxicology and chemistry* 23, no. 5 (2004): 1344-1354.

Ferrer I, Thurman EM. "Analysis of pharmaceuticals in drinking water, groundwater, surface water, and wastewater." *Comprehensive Analytical Chemistry* 62 (2013): 91-128.

Ferrer I, Thurman EM. "Identification of a new antidepressant and its glucuronide metabolite in water samples using liquid chromatography/quadrupole time-of-flight mass spectrometry." *Analytical chemistry* 82, no. 19 (2010): 8161-8168.

Filby AL, Neuparth T, Thorpe KL, Owen R, Galloway TS, Tyler CR. "Health impacts of estrogens in the environment, considering complex mixture effects." *Environmental Health Perspectives* (2007): 1704-1710.

Flaherty CM, Dodson SI. “Effects of pharmaceuticals on Daphnia survival, growth, and reproduction”. *Chemosphere* 61 (2005): 200–7.

Fono LJ, Kolodziej EP, Sedlak DL. "Attenuation of wastewater-derived contaminants in an effluent-dominated river." *Environmental science & technology* 40, no. 23 (2006): 7257-7262.

Forrez I, Carballa M, Boon N, Verstraete W. "Biological removal of 17α-ethinylestradiol (EE2) in an aerated nitrifying fixed bed reactor during ammonium starvation." *Journal of chemical technology and biotechnology* 84, no. 1 (2009): 119-125.

Forrez, I, Boon N, Verstraete W, Carballa M. "Biodegradation of micropollutants and prospects for water and wastewater biotreatment." *Comprehensive biotechnology* 6 (2011): 485-494.

Furhacker M. "The water framework directive-can we reach the target?" (2008).

García del Pozo J, De Abajo FJ, Sanz E, De las Cuevas C. “Use of benzodiazepines in Spain (1992–2006)” (2006). http://www.aemps.gob.es/medicamentosUsoHumano/observatorio/docs/ansioliticos_hipnoticos.pdf.

García del Pozo J, De Abajo FJ. “Evolution of the use of anti-inflammatory non-steroidal in Spain from 1990 until 2003.” Prim Care 36(8) (2005). http://www.aemps.gob.es/medicamentosUsoHumano/observatorio/docs/AINE.pdf.

García del Pozo J. “Study of antiulcer utilization in Spain (2000–2008).” *Inf Ter Sist Nac Salud* 33 (2009): 49–54.

García N, Moreno J, Cartmell E, Rodriguez-Roda I, Judd S. "The application of microfiltration-reverse osmosis/nanofiltration to trace organics removal for municipal wastewater reuse." *Environmental technology* 34, no. 24 (2013): 3183-3189.

Garcia-Armisen T, Vercammen K, Passerat J, Triest D, Servais P, Cornelis P. "Antimicrobial resistance of heterotrophic bacteria in sewage-contaminated rivers" *Water Research*. 45 (2011): 788-796.

García-Galán MJ, Díaz-Cruz MS, Barceló D. “Occurrence of sulfonamide residues along the Ebro River basin. Removal in wastewater treatment plants and environmental impact assessment.” *Environment International* 37, no. 2 (2011): 462-473.

García-Galán MJ, González Blanco S, López Roldán R, Díaz-Cruz S, Barceló D. "Ecotoxicity evaluation and removal of sulfonamides and their acetylated metabolites during conventional wastewater treatment." *Science of the Total Environment* 437 (2012): 403-412.

Garrison AW, Pope JD, Allen JR. “GC/MS Analysis of organic compounds in domestic wastewater” In *Identification and analysis of organic pollutants in water* pp. 517-566 Ann Arbor Science, Michigan, 1976.

Gasith A, Resh VH. “Streams in Mediterranean climate regions: abiotic influences and biotic responses to predictable seasonal events” *Annual Review of Ecology, Evolution and Systematics* 30 (1999): 51–81.

Ghosh S, Ramsden SJ, LaPara TM. "The role of anaerobic digestion in controlling the release of tetracycline resistance genes and class 1 integrons from municipal wastewater treatment plants." *Applied Microbiology and Biotechnology* 84, no. 4 (2009): 791-796.

Giger W, Alder AC, Golet EM, Kohler HPE, McArdell CS, Molnar E, Siegrist H, Suter MJF. "Occurrence and fate of antibiotics as trace contaminants in wastewaters, sewage sludges, and surface waters." *CHIMIA International Journal for Chemistry* 57, no. 9 (2003): 485-491.

Ginebreda A, Kuzmanovic M, Guasch H, López de Alda M, López-Doval JC, Muñoz I, Ricart M, Romaní AM, Sabater S, Barceló D. "Assessment of multi-chemical pollution in aquatic ecosystems using toxic units: compound prioritization, mixture characterization and relationships with biological descriptors." *Science of the Total Environment* 468 (2014): 715-723.

Ginebreda A, Muñoz I, López de Alda M, Brix R, López-Doval J, Barceló D. "Environmental risk assessment of pharmaceuticals in rivers: relationships between hazard indexes and aquatic macroinvertebrate diversity indexes in the Llobregat River (NE Spain)." *Environment International* 36, no. 2 (2010): 153-162.

Giorgi F, Lionello P. “Climate change projections for the Mediterranean region” *Global and Planetary Change* 63 (2008): 90–104

Göbel A, Thomsen A, McArdell CS, Joss A, Giger W. "Occurrence and sorption behavior of sulfonamides, macrolides, and trimethoprim in activated sludge treatment." *Environmental science & technology* 39, no. 11 (2005): 3981-3989.

Göbel A, Thomsen A, McArdell CS, Joss A, Giger W. Occurrence and sorption behaviour of sulfonamides, macrolides, and trimethoprim in activated sludge treatment. *Environmental science & technology* 39, no. 11 (2005): 3981-3989.

Golet EM, Strehler A, Alder AC, Giger W. "Determination of fluoroquinolone antibacterial agents in sewage sludge and sludge-treated soil using accelerated solvent extraction followed by solid-phase extraction." *Analytical Chemistry* 74, no. 21 (2002s): 5455-5462.

Gómez-Gutiérrez AI, Jover E, Bodineau L, Albaigés J, Bayona JM. "Organic contaminant loads into the Western Mediterranean Sea: estimate of Ebro River inputs." *Chemosphere* 65, no. 2 (2006): 224-236.

Gonçalves C, Perez S, Osorio V, Petrovic M, Alpendurada MF, Barceló D. "Photofate of oseltamivir (Tamiflu) and oseltamivir carboxylate under natural and simulated solar irradiation: kinetics, identification of the transformation products, and environmental occurrence." *Environmental science & technology* 45, no. 10 (2011): 4307-4314.

Grenni P, Patrolecco L, Ademollo N, Tolomei A, Caracciolo A. "Degradation of gemfibrozil and naproxen in a river water ecosystem." *Microchemical Journal* 107 (2013): 158-164.

Gros M, Petrović M, Barceló D. "Wastewater treatment plants as a pathway for aquatic contamination by pharmaceuticals in the Ebro river basin (northeast Spain)." *Environmental Toxicology and Chemistry* 26, no. 8 (2007): 1553-1562.

Gros M, Petrović M, Ginebreda A, Barceló D. "Removal of pharmaceuticals during wastewater treatment and environmental risk assessment using hazard indexes." *Environment international* 36, no. 1 (2010): 15-26.

Gulkowska A, Leung HW, So MK, Taniyasu S, Yamashita N, Yeung LWY, Richardson BJ, Lei AP, Giesy JP, Lam PKS. "Removal of antibiotics from wastewater by sewage treatment facilities in Hong Kong and Shenzhen, China." *Water Research* 42, no. 1 (2008): 395-403.

GWRC. *Pharmaceuticals and Personal Care Products in the Water Cycle: An International Review*. Global Water Research Coalition, 2004.

Haddad T, Kümmerer K. “Characterization of photo-transformation products of the antibiotic drugCiprofloxacin with liquid chromatography–tandem mass spectrometry in combination with accurate mass determination using an LTQ-Orbitrap” *Chemosphere* 115 (2014) 40–46.

Halling-Sørensen B, Nors Nielsen S, Lanzky PF, Ingerslev F, Holten Lützhøft HC, Jørgensen SE. "Occurrence, fate and effects of pharmaceutical substances in the environment-A review." *Chemosphere* 36, no. 2 (1998): 357-393.

HAZCHEM, a mathematical model for use in risk assessment of substances. ECETOC Special Report, No. 8 (1994).

Heberer T. "Occurrence, fate, and removal of pharmaceutical residues in the aquatic environment: a review of recent research data." *Toxicology Letters* 131, no. 1 (2002): 5-17.

Helbling DE, Johnson DR, Honti M, Fenner K. "Micropollutant biotransformation kinetics associate with WWTP process parameters and microbial community

characteristics." *Environmental science & technology* 46, no. 19 (2012): 10579-10588.

Henschel K-P, WenzelA, Diedrich M, Fliedner A. "Environmental hazard assessment of pharmaceuticals." *Regulatory Toxicology and Pharmacology* 25, no. 3 (1997): 220-225.

Hernández F, Ibáñez M, Gracia-Lor E, Sancho JV. "Retrospective LC-QTOF-MS analysis searching for pharmaceutical metabolites in urban wastewater." *Journal of Separation Science* 34, no. 24 (2011): 3517-3526.

Hernando MD, Mezcua M, Fernández-Alba AR, Barceló D. "Environmental risk assessment of pharmaceutical residues in wastewater effluents, surface waters and sediments." *Talanta* 69, no. 2 (2006): 334-342.

Hey G, Ledin A, Jansen JLC, Andersen HR. “Removal of pharmaceuticals in biologically treated wastewater by chlorine dioxide or peracetic acid.” *Environmental technology* 33, no. 9 (2012): 1041-1047.

Hirabayashi Y, Kanae S, Emori S, Oki T, Kimoto M. “Global projections of changing risks of floods and droughts in a changing climate.” *Hydrological Sciences Journal* 53, no. 4 (2008): 754-772.

Holčapek M, Kolářová L, Nobilis M. "High-performance liquid chromatography–tandem mass spectrometry in the identification and determination of phase I and phase II drug metabolites." *Analytical and bioanalytical chemistry* 391, no. 1 (2008): 59-78.

House WA. “Geochemical cycling of phosphorus in rivers”. *Applied Geochemistry* 18 (2003): 739–748.

Huerta B, Jakimska A, Gros M, Rodríguez-Mozaz S, Barceló D. "Analysis of multi-class pharmaceuticals in fish tissues by ultra-high-performance liquid chromatography tandem mass spectrometry." *Journal of Chromatography A* 1288 (2013): 63-72.

Huerta-Fontela M, Galcerán MT, Ventura F. “Stimulatory drugs of abuse in surface waters and their removal in a conventional drinking water treatment plant” *Environmental Science & Technology* 42, no. 18 (2008): 6809–6816.

Huggett DB, Brooks BW, Peterson B, Foran CM, Schlenk D. "Toxicity of select beta adrenergic receptor-blocking pharmaceuticals (B-blockers) on aquatic organisms." *Archives of Environmental Contamination and Toxicology* 43, no. 2 (2002): 229-235.

Hughes SR, Kay P, Brown LE. "Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems." *Environmental science & technology* 47, no. 2 (2012): 661-677.

Hughes SR, Kay P, Brown LE. “Global synthesis and critical evaluation of pharmaceutical data sets collected from river systems” *Environmental Science & Technology* 47 no. 2 (2013): 661–677.

Huntington TG. “Evidence for intensification of the global watercycle: review and synthesis” *Journal of Hydrology* 319 (2006): 83–95.

Huntscha S, Singer HP, McArdell CS, Frank CE, Hollender J. "Multiresidue analysis of 88 polar organic micropollutants in ground, surface and wastewater using online mixed-bed multilayer solid-phase extraction coupled to high performance liquid chromatography–tandem mass spectrometry." *Journal of Chromatography A* 1268 (2012): 74-83.

Ikehata K, Naghashkar NJ, El-Din MG. "Degradation of aqueous pharmaceuticals by ozonation and advanced oxidation processes: a review." *Ozone: Science and Engineering* 28, no. 6 (2006): 353-414.

IMS “Health Market Prognosis 2014 Global Outlook for Medicines Through 2018.” Report by the IMS Institute for Healthcare Informatics. (2014) http://www.imshealth.com

IMS. “Health Market Prognosis” (2011) http://www.imshealth.com

Isidori M, Nardelli A, Pascarella L, Rubino M, Parrella A. "Toxic and genotoxic impact of fibrates and their photoproducts on non-target organisms." *Environment International* 33, no. 5 (2007): 635-641.

Jasper JT, Sedlak DL. "Phototransformation of wastewater-derived trace organic contaminants in open-water unit process treatment wetlands." *Environmental science & technology* 47, no. 19 (2013): 10781-10790.

Jelić A, Gros M, Ginebreda A, Cespedes-Sánchez R, Ventura F, Petrovic M, Barcelo D. "Occurrence, partition and removal of pharmaceuticals in sewage water and sludge during wastewater treatment." *Water Research* 45, no. 3 (2011): 1165-1176.

Jelić A, Rodríguez-Mozaz S, Barceló D, Gutiérrez O. “Impact of in-sewer transformation on 43 pharmaceuticals in a pressurized sewer under anaerobic conditions.” *Water Research* 68 (2015):98-108

Jia A, Hu J, Wu X, Peng H, Wu S, Dong Z. “Occurrence and source apportionment of sulfonamides and their metabolites in Liaodong Bay and the adjacent Liao River basin, North China” *Environmental Toxicology and Chemistry* 30, no. 6 (2011): 1252–1260.

Jiang JJ, Lee CL, Brimblecombe P, Vydrova L, Fang MD. "Source contributions and mass loadings for chemicals of emerging concern: Chemometric application of pharmaco-signature in different aquatic systems." *Environmental Pollution* (2015) doi:10.1016/j.envpol.2015.06.039.

Jiang L, Hu X, Yin D, Zhang H, Yu Z. “Occurrence, distribution and seasonal variation of antibiotics in the Huangpu River, Shanghai, China” *Chemosphere* 82 (2011): 822–828.

Jiao S, Zheng S, Yin D, Wang L, Chen L. "Aqueous photolysis of tetracycline and toxicity of photolytic products to luminescent bacteria." *Chemosphere* 73, no. 3 (2008): 377-382.

Johnson AC, Keller V, Dumont E, Sumpter JP. "Assessing the concentrations and risks of toxicity from the antibiotics ciprofloxacin, sulfamethoxazole, trimethoprim and erythromycin in European rivers." *Science of the Total Environment* 511 (2015): 747-755.

Johnson AC, Keller V, Williams RJ, Young A. "A practical demonstration in modelling diclofenac and propranolol river water concentrations using a GIS hydrology model in a rural UK catchment." *Environmental Pollution* 146, no. 1 (2007): 155-165.

Johnson AC, Ternes T, Williams RJ, Sumpter JP. "Assessing the concentrations of polar organic microcontaminants from point sources in the aquatic environment: measure or model?" *Environmental science & technology* 42, no. 15 (2008): 5390-5399.

Jones OAH, Voulvoulis N, Lester JN. "Potential ecological and human health risks associated with the presence of pharmaceutically active compounds in the aquatic environment." *Critical Reviews in Toxicology* 34, no. 4 (2004): 335-350.

Jones OAH, Voulvoulis N, Lester JN. "The occurrence and removal of selected pharmaceutical compounds in a sewage treatment works utilizing activated sludge treatment." *Environmental Pollution* 145, no. 3 (2007): 738-744.

Joss A, Zabczynski S, Göbel A, Hoffmann B, Löffler D, McArdell CS, Ternes TA, Thomsen A, Siegrist H. "Biological degradation of pharmaceuticals in municipal wastewater treatment: proposing a classification scheme." *Water Research* 40, no. 8 (2006): 1686-1696.

Kang S, Kang SY, Kanaly RA, Lee E, Lim Y, Hur HG. "Rapid oxidation of ring methyl groups is the primary mechanism of biotransformation of gemfibrozil by the fungus Cunninghamella elegans." *Archives of Microbiology* 191, no. 6 (2009): 509-517.

Keller VDJ, Young AR. "Development of the Integrated Water Resources and Water Quality Modelling System." Science Report P2-248/SR. Environment Agency. Bristol, UK." (2004).

Kemper N. "Veterinary antibiotics in the aquatic and terrestrial environment" *Ecological Indicators* 8 (2008): 1–13.

Kim I, Yamashita N, Tanaka H. "Performance of UV and UV/H 2 O 2 processes for the removal of pharmaceuticals detected in secondary effluent of a sewage treatment plant in Japan." *Journal of Hazardous Materials* 166, no. 2 (2009): 1134-1140.

Kim Y, Choi K, Jung J, Park S, Kim PG, Park J. "Aquatic toxicity of acetaminophen, carbamazepine, cimetidine, diltiazem and six major sulfonamides, and their potential ecological risks in Korea." *Environment International* 33, no. 3 (2007): 370-375.

Kimura K, Hara H, Watanabe Y. “Elimination of selected acidic pharmaceuticals from municipal wastewater by an activated sludge system and membrane bioreactors.” *Environmental Science & Technology* 41 (2007): 3708–3714.

Klamerth N, Malato S, Maldonado MI, Aguera A, Fernández-Alba AR. "Application of photo-fenton as a tertiary treatment of emerging contaminants in municipal wastewater." *Environmental Science & Technology* 44, no. 5 (2010): 1792-1798.

Klavarioti M, Mantzavinos D, Kassinos D. "Removal of residual pharmaceuticals from aqueous systems by advanced oxidation processes." *Environment International* 35, no. 2 (2009): 402-417.

Köck-Schulmeyer M, Ginebreda A, González S, Luis Cortina J, López de Alda M, Barceló D. "Analysis of the occurrence and risk assessment of polar pesticides in the Llobregat River Basin (NE Spain)." *Chemosphere* 86, no. 1 (2012): 8-16.

Koh YKK, Tze YC, Boobis AR, Scrimshaw MD, Bagnall JP, Soares A, Pollard S, Cartmell E, Lester JN. "Influence of operating parameters on the biodegradation of steroid estrogens and nonylphenolic compounds during biological wastewater treatment processes." *Environmental Science & Technology* 43, no. 17 (2009): 6646-6654.

Kolpin DW, Furlong ET, Meyer MT, Thruman EM, Zaugg SD, Barber LB, and Buxton H. “PhACs, hormones and other wastewater contaminants in U.S. streams” *Environmental Science & Technology* 32 (2000): 2498–2506.

Kools SAE, Boxall A, Moltmann JF, Bryning G, Koschorreck J, Knacker T. "A ranking of European veterinary medicines based on environmental risks." *Integrated environmental assessment and management* 4, no. 4 (2008b): 399-408.

Kools SAE, Moltmann JF, Knacker T. "Estimating the use of veterinary medicines in the European Union." *Regulatory Toxicology and Pharmacology* 50, no. 1 (2008a): 59-65.

Koops HP, Pommerening-Röser A. "Distribution and ecophysiology of the nitrifying bacteria emphasizing cultured species." *FEMS Microbiology Ecology* 37, no. 1 (2001): 1-9.

Kosjek T, Perko S, Zupanc M, Hren MZ, Dragičević TL, Žigon D, Kompare B, Heath E. "Environmental occurrence, fate and transformation of benzodiazepines in water treatment." *Water Research* 46, no. 2 (2012): 355-368.

Kosma CI, Lambropoulou DA, Albanis TA. “Comprehensive study of the antidiabetic drugmetformin and its transformation product guanylurea in Greek wastewaters” *Water Research* 7 0 (2015) 436-448.

Kostich MS, Batt AL, Lazorchak JM. “Concentrations of prioritized pharmaceuticals in effluents from 50 large wastewater treatment plants in the US and implications for risk estimation” *Environmental Pollution* 184 (2014): 354-359.

Kreuzinger N, Clara M, Strenn B, Kroiss H. "Relevance of the sludge retention time (SRT) as design criteria for wastewater treatment plants for the removal of endocrine disruptors and pharmaceuticals from wastewater." *Water Science & Technology* 50, no. 5 (2004): 149-156.

Kumar S, Samuel K, Subramanian R, Braun MP, Stearns RA, Chiu SHL, Evans DC, Baillie TA. "Extrapolation of diclofenac clearance from in vitro microsomal metabolism data: role of acyl glucuronidation and sequential oxidative metabolism of the acyl glucuronide." *Journal of Pharmacology and Experimental Therapeutics* 303, no. 3 (2002): 969-978.

Kümmerer K, ed. *Pharmaceuticals in the environment: sources, fate, effects and risks*. Springer Science & Business Media, 2008.

Kümmerer K. "Pharmaceuticals in the environment." *Annual Review of Environment and Resources* 35, no. 1 (2010): 57-75.

Kümmerer K. "The presence of pharmaceuticals in the environment due to human use–present knowledge and future challenges." *Journal of environmental management* 90, no. 8 (2009): 2354-2366.

Kunkel U, Radke M. "Fate of pharmaceuticals in rivers: Deriving a benchmark dataset at favorable attenuation conditions." *Water Research* 46, no. 17 (2012): 5551-5565.

Kuzmanović M, Ginebreda A, Petrović M, Barceló D. "Risk assessment based prioritization of 200 organic micropollutants in 4 Iberian rivers." *Science of The Total Environment* 503 (2015): 289-299.

Lam MW, Young CJ, Brain RA, Johnson DJ, Hanson MA, Wilson CJ,Richards SM, Solomon KR, Mabury SA. “Aquatic persistence of eight PhACs in a microcosm study” *Environmental Toxicology and Chemistry* 23 (2004): 1431–1440.

Lam MW, Young CJ, Mabury SA. “Aqueous photochemical reaction kinetics and transformations of fluoxetine” *Environmental Science & Technology* 39 (2005): 513–522.

Lamberti GA. “The role of periphyton in benthic food webs”. In *Algal Ecology: Freshwater Benthic Ecosystems*. pp. 533–572. Academic Press, San Diego, 1996

Langford K, Thomas KV. "Input of selected human pharmaceutical metabolites into the Norwegian aquatic environment." *Journal of Environmental Monitoring* 13.2 (2011): 416-421.

Lara-Martín PA, Renfro A, Cochran K, Brownawell B. "Geochronologies of Pharmaceuticals in a Sewage-Impacted Estuarine Urban Setting (Jamaica Bay, New York)." *Environmental Science & Technology* 49, no. 10 (2015): 5948-5955.

Larsson E, al-Hamimi S, Jönsson JÅ. "Behaviour of nonsteroidal anti-inflammatory drugs and eight of their metabolites during wastewater treatment studied by hollow fibre liquid phase microextraction and liquid chromatography mass spectrometry." *Science of the total environment* 485 (2014): 300-308.

Larsson, DGJ. "Pollution from drug manufacturing: review and perspectives." *Philosophical Transactions of the Royal Society of London B: Biological Sciences* 369, no. 1656 (2015): 20130571.

Latch DE, Stender BL, Packer JL, Arnold WA, McNeill K. "Photochemical fate of pharmaceuticals in the environment: cimetidine and ranitidine." *Environmental Science & Technology* 37, no. 15 (2003): 3342-3350.

Laville N, Aıt-Aıssa S, Gomez E, Casellas C, Porcher JM. "Effects of human pharmaceuticals on cytotoxicity, EROD activity and ROS production in fish hepatocytes." *Toxicology* 196, no. 1 (2004): 41-55.

Lawrence JR, Swerhone GDW, Wassenaar LI, Neu TR. "Effects of selected pharmaceuticals on riverine biofilm communities." *Canadian journal of microbiology* 51, no. 8 (2005): 655-669.

Lawrence JR, Zhu B, Swerhone GDW, Roy J, Tumber V, Waiser MJ, Topp E, Korber DR. "Molecular and microscopic assessment of the effects of caffeine, acetaminophen, diclofenac, and their mixtures on river biofilm communities." *Environmental Toxicology and Chemistry* 31, no. 3 (2012): 508-517.

Lázaro E, Montero D. “Use of antibiotics in Spain”. Ministry of Health, Social Policy and Equality. Spanish Medicines and Medical Devices Agency. Directorate-General for Pharmacy and Healthcare Products (2010). http://www.aemps.gob.es/medicamentosUsoHumano/observatorio/docs/antibioticos.pdf.

Lazarus RS, Rattner BA, Brooks BW, Du B, McGowan PC, Blazer VS, Ottinger MA. "Exposure and food web transfer of pharmaceuticals in ospreys (Pandion haliaetus): Predictive model and empirical data." *Integrated environmental assessment and management* 11, no. 1 (2015): 118-129.

Lee J, Ji K, Kho YL, Kim P, Choi K. "Chronic exposure to diclofenac on two freshwater cladocerans and Japanese medaka." *Ecotoxicology and environmental safety* 74, no. 5 (2011): 1216-1225.

Lester Y, Mamane H, Zucker I, Avisar D. "Treating wastewater from a pharmaceutical formulation facility by biological process and ozone." *Water Research* 47, no. 13 (2013): 4349-4356.

Li B, Zhang T. "Mass flows and removal of antibiotics in two municipal wastewater treatment plants." *Chemosphere* 83, no. 9 (2011): 1284-1289.

Li W, Shi Y, Lihong G, Liu J, Cai Y. "Occurrence of antibiotics in water, sediments, aquatic plants, and animals from Baiyangdian Lake in North China." *Chemosphere* 89, no. 11 (2012): 1307-1315.

Li Y, Niu J, Wang W. "Photolysis of enrofloxacin in aqueous systems under simulated sunlight irradiation: kinetics, mechanism and toxicity of photolysis products." *Chemosphere* 85, no. 5 (2011): 892-897.

Li Z, Maierb MP, Radkea M. "Screening for pharmaceutical transformation products formed in riversediment by combining ultrahigh performance liquidchromatography/high resolution mass spectrometry with a rapid data-processing method" *Analytica Chimica Acta* 810 (2014): 61– 70.

Li Z, Sobek A, Radke M. "Flume experiments to investigate the environmental fate of pharmaceuticals and their transformation products in streams." *Environmental science & technology* (2015).

Lin AYC, Wang XH, Lee WN. "Phototransformation determines the fate of 5-fluorouracil and cyclophosphamide in natural surface waters." *Environmental Science & Technology* 47, no. 9 (2013): 4104-4112.

Lindberg R, Wennberg P, Johansson M, Tysklind M, Andersson B. "Screening of human antibiotic substances and determination of weekly mass flows in five sewage treatments plants in Sweden." *Environmental Science & Technology* 39, no. 10 (2005): 3421-3429.

Lindqvist N, Tuhkanen T, Kronberg L. "Occurrence of acidic pharmaceuticals in raw and treated sewage and in receiving waters." *Water Research* 39 (2005): 2219–28.

Lock MA. "Attached microbial communities in rivers." In *Aquatic microbiology: an ecological approach*. pp. 113-138 Blackwell, Oxford, 1993.

Löffler D, Römbke J, Meller M, Ternes TA. " Environmental fate of pharmaceuticals in water/sediment systems." *Environmental Science & Technology* 39, no. 14 (2005): 5209- 5218.

López-Serna R, Petrović M, Barceló D. "Direct analysis of pharmaceuticals, their metabolites and transformation products in environmental waters using on-line

TurboFlow™ chromatography–liquid chromatography–tandem mass spectrometry." *Journal of Chromatography A* 1252 (2012): 115-129.

Luo Y, Guoa W, Ngo HH, Nghiemb LD, Hai FI, Zhang J, Liang S, Wang XC. “A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment” *Science of the Total Environment* 473–474 (2014): 619–641.

Lupo A, Conye S, Berendonk TU. “Origin and evolution of antibiotic resistance: the common mechanisms of emergence and spread in waterbodies” *Frontiers in Microbiology* 3 (2012) 19.

Machado TC, Pizzolato TM, Arenzon A, Segalin J, Lansarin MA. “Photocatalytic degradation of rosuvastatin: Analytical studies and toxicity evaluations” *Science of the Total Environment* 502 (2015): 571–577.

Mailler R, Gasperi J, Coquet Y, Deshayes S, Zedek S, Cren-Olivé C, Cartiser N., Eudesc V, Bressya A, Cauposa E, Moillerona R, Chebboe G, Rocherf V. "Study of a large scale powdered activated carbon pilot: Removals of a wide range of emerging and priority micropollutants from wastewater treatment plant effluents." *Water Research* 72 (2015): 315-330.

Majewsky M, Gallé T, Zwank L, Fischer K. "Influence of microbial activity on polar xenobiotic degradation in activated sludge systems." *Water science and technology* 62, no. 3 (2010): 701.

Majewsky M, Wagner D, Delay M, Bräse S, Yargeau V, Horn H. "Antibacterial Activity of Sulfamethoxazole Transformation Products (TPs): General Relevance for Sulfonamide TPs Modified at the para Position." *Chemical research in toxicology* 27, no. 10 (2014): 1821-1828.

Malaj E, Peter C, Grote M, Kühne R, Mondy CP, Usseglio-Polatera P, Brack W, Schäfer RB. “Organic chemicals jeopardize the health of freshwater ecosystems on the continental scale.” *Proceedings of the National Academy of Sciences* 111, no. 26 (2014): 9549-9554.

Malato S, Fernández-Ibáñez P, Oller I, Prieto-Rodriguez L, Miralles-Cuevas S, Cabrera-Reina A. "Approaches to Water and Wastewater Treatment for Removal of Emerging Contaminants: Ongoing Research and Recommendations for Future Work." In *Transformation Products of Emerging Contaminants in the Environment: Analysis, Processes, Occurrence, Effects and Risks* pp. 161-178, 2014.

Marcé R, Honey-Rosés J, Manzano A, Moragas L, Catllar B, Sabater S. “The Llobregat River Basin: a paradigm of impaired rivers under climate change threats.” In: *The Llobregat: the story of a polluted Mediterranean river*. pp. 1–26 Hdb. Env. Chem.Berlin Heidelberg: Springer-Verlag, 2012.

Martí E, Aumatell J, Gode J, Poch M, Sabater F. “Nutrient retention efficiency in streams receiving inputs from wastewater treatment plants.” *Journal of Environmental Quality* 33 (2004): 285-293.

Martín B. “Health care and pharmacological aspects of depression in Spain in the field of primary and specialized care: current situation and evolution in recent years.” Rev Psiq Fac Med Barna 32, no. 3 (2005): 143–7.

Martínez-Hernández S, Texier AC, Cuervo-López FM, Gómez J. "2-Chlorophenol consumption and its effect on the nitrifying sludge." *Journal of hazardous materials* 185, no. 2 (2011): 1592-1595.

Maskaoui K, Zhou JL. "Colloids as a sink for certain pharmaceuticals in the aquatic environment." *Environmental science and pollution research* 17, no. 4 (2010): 898-907.

Matamoros V, Bayona JM. "Removal of Pharmaceutical Compounds from Wastewater and Surface Water by Natural Treatments." In *Analysis, Removal, Effects and Risk of Pharmaceuticals in the Water Cycle: Occurrence and Transformation in the Environment* 62. pp. 409-, 2013.

Matamoros V, Bayona JM. “Elimination of pharmaceuticals and personal care products in subsurface flow constructed wetlands.” *Environmental science & technology* 40, no. 18 (2006): 5811-5816.

Maurer M, Escher BI, Richle P, Schaffner C, Alder AC. “Elimination of β-blockers in sewage treatment plants.” *Water Research* 41, no. 7 (2007): 1614-1622.

McAdam EJ, Bagnall JP, Koh YKK, Chiu TY, Pollard S, Scrimshaw MD, Lester JN, Cartmell E. "Removal of steroid estrogens in carbonaceous and nitrifying activated sludge processes." *Chemosphere* 81, no. 1 (2010): 1-6.

Menassé R, Hedwall PR, Kraetz J, Pericin C, Riesterer L, Sallmann A, Ziel R, Jaques R. "Pharmacological properties of diclofenac sodium and its metabolites." *Scandinavian Journal of Rheumatology* 7, no. S22 (1978): 5-16.

Merseburger G, Martí E, Sabater F. “Net changes in nutrient concentrations below a point source input in two streams draining catchments with contrasting land uses.” *Science of the Total Environment* 347 (2005): 217-229.

Metcalfe CD, Chu S, Judt C, Li H, Oakes KD, Servos MR, Andrews DM. "Antidepressants and their metabolites in municipal wastewater, and downstream exposure in an urban watershed." *Environmental Toxicology and Chemistry* 29, no. 1 (2010): 79-89.

Metcalfe CD, Miao XS, Koenig BG, Struger J. "Distribution of acidic and neutral drugs in surface waters near sewage treatment plants in the lower Great Lakes, Canada." *Environmental Toxicology and Chemistry* 22, no. 12 (2003): 2881-2889.

Metzger JW. "Drugs in municipal landfills and landfill leachates." In *Pharmaceuticals in the Environment*, pp. 133-137. Springer Berlin Heidelberg, 2004.

Miao XS, Metcalfe CD. "Determination of carbamazepine and its metabolites in aqueous samples using liquid chromatography-electrospray tandem mass spectrometry." *Analytical Chemistry* 75, no. 15 (2003): 3731-3738.

Michael I, Achilleos A, Lambropoulou D, Osorio Torrens V, Pérez S, Petrović M, Barceló D, Fatta-Kassinos D. "Proposed transformation pathway and evolution profile of diclofenac and ibuprofen transformation products during (sono) photocatalysis." *Applied Catalysis B: Environmental* 147 (2014b): 1015-1027.

Michael I, Hapeshi E, Osorio V, Perez S, Petrovic M, Zapata A, Malato S, Barceló D, Fatta-Kassinos D. "Solar photocatalytic treatment of trimethoprim in four environmental matrices at a pilot scale: Transformation products and ecotoxicity evaluation." *Science of the Total Environment* 430 (2012): 167-173.

Michael I, Vasquez MI, Hapeshi E, Haddad T, Baginska E, Kümmerer K, Fatta-Kassinos D. "Metabolites and transformation products of pharmaceuticals in the aquatic environment as contaminants of emerging concern." In *Advanced Mass Spectrometry-based techniques for the identification and structure elucidation of transformation products of emerging contaminants.* pp. 413-459. Wiley, 2014a.

Mompelat S, Le Bot B, Thomas O. "Occurrence and fate of pharmaceutical products and by-products, from resource to drinking water." *Environment International* 35, no. 5 (2009): 803-814.

Monteiro SC, Boxall ABA. "Occurrence and fate of human pharmaceuticals in the environment" In *Reviews of environmental contamination and toxicology*. pp. 53-154. Springer New York, 2010.

Munné A, Tirapu L, Solà C, Olivella L, Vilanova M, Ginebreda A, Prat N. "Comparing chemical and ecological status in Catalan Rivers. Analysis of river quality status following the Water Framework Directive" In: *Emerging and priority pollutants in rivers: bringing science into river management plans.* pp. 243–265. Hdb. Env. Chem.Berlin Heidelberg: Springer-Verlag, 2012.

Muñoz I, López-Doval JC, Ricart M, Villagrasa M, Brix R, Geiszinger A, Ginebreda A, Guasch H, José López de Alda M., Romaní AM, Sabater S, Barceló D. "Bridging levels of pharmaceuticals in river water with biological community structure in the Llobregat river basin (northeast Spain)." *Environmental Toxicology and Chemistry* 28, no. 12 (2009): 2706-2714.

Murdoch RW, Hay AG. "Formation of catechols via removal of acid side chains from ibuprofen and related aromatic acids." *Applied and Environmental Microbiology* 71, no. 10 (2005): 6121-6125.

Muto CA “Why are antibiotic-resistant nosocomial infections spiraling out of control” *Infection Control & Hospital Epidemiology* 26 (2005): 10–12.

Nakada N, Tanishima T, Shinohara H, Kiri K, Takada H. “Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment.” *Water Research* 40, no. 17 (2006): 3297-3303.

Nakamura Y, Yamamoto H, Sekizawa J, Kondo T, Hirai N, Tatarazako N. "The effects of pH on fluoxetine in Japanese medaka (Oryzias latipes): acute toxicity in fish larvae and bioaccumulation in juvenile fish." *Chemosphere* 70, no. 5 (2008): 865-873.

Nałęcz-Jawecki G, Wojcik T, Sawicki J. "Evaluation of in vitro biotransformation of propranolol with HPLC, MS/MS, and two bioassays." *Environmental Toxicology* 23, no. 1 (2008): 52-58.

National Office of Animal Health. “Facts and figures about the UK animal medicines industry” www.noah.co.uk/focus/facts_figures.htm.

Navarro-Ortega A, Acuña V, Batalla RJ, Blasco J, Conde C, Elorza FJ, Elosegi A et al. "Assessing and forecasting the impacts of global change on Mediterranean rivers. The SCARCE Consolider project on Iberian basins." *Environmental Science and Pollution Research* 19, no. 4 (2012): 918-933.

Navarro-Ortega A, Acuña V, Bellin A, Burek P, Cassiani G, Choukr-Allah R, Dolédec S et al. "Managing the effects of multiple stressors on aquatic ecosystems under water scarcity. The GLOBAQUA project." *Science of the Total Environment* 503 (2015): 3-9.

Nebot C, Falcon R, Boyd KG, Gibb SW. "Introduction of human pharmaceuticals from wastewater treatment plants into the aquatic environment: a rural perspective." *Environmental Science and Pollution Research* (2015): 1-10.

Neuwoehner J, Fenner K, Beate I. Escher. "Physiological modes of action of fluoxetine and its human metabolites in algae." *Environmental Science & Technology* 43, no. 17 (2009): 6830-6837.

New M, Todd M, Hulme M, Jones P. “Precipitation measurements and trends in the twentieth century.” *International Journal of Climatology* 21, no. 15 (2001): 1889-1922.

Nödler K, Licha T, Barbieri M, Pérez S. "Evidence for the microbially mediated abiotic formation of reversible and non-reversible sulfamethoxazole transformation products during denitrification." *Water Research* 46, no. 7 (2012): 2131-2139.

Oaks JL, Gilbert M, Virani MZ, Watson RT, Meteyer CU, Rideout BA, Shivaprasad HL, Ahmed S, Chaudhry MJI, Arshad M, Mahmood S, Ali A, Khan AA. "Diclofenac

residues as the cause of vulture population decline in Pakistan." *Nature* 427, no. 6975 (2004): 630-633.

Oaks JL, Watson RT. "South Asian vultures in crisis: Environmental contamination with a pharmaceutical." In *Wildlife Ecotoxicology*, pp. 413-441. Springer New York, 2011.

OECD "Pharmaceutical expenditure per capita", *Health: Key Tables from OECD*. (2014) *http://dx.doi.org/10.1787/pharmexpcap-table-2014-1-en.*

Oliveira LLD, Antunes SC, Gonçalves F, Rocha O, Nunes B. "Evaluation of ecotoxicological effects of drugs on Daphnia magna using different enzymatic biomarkers." *Ecotoxicology and Environmental Safety* 119 (2015): 123-131.

Oller I, Malato S, Sánchez-Pérez JA. "Combination of advanced oxidation processes and biological treatments for wastewater decontamination—a review." *Science of the Total Environment* 409, no. 20 (2011): 4141-4166.

Onesios KM, Yu JT, Bouwer EJ. “Biodegradation and removal of pharmaceuticals and personal care products in treatment systems: A review” *Biodegradation* 20 (2009): 441-466.

Oppenheimer J, Stephenson R, Burbano A, Liu L. “Characterizing the passage of personal care products through wastewater treatment processes” *Water Environment Research* 79, no. 13 (2007): 2564-2577.

Ort C, Hollender J, Schaerer M, Siegrist H. "Model-based evaluation of reduction strategies for micropollutants from wastewater treatment plants in complex river networks." *Environmental Science & Technology* 43, no. 9 (2009): 3214-3220.

Ortiz SdG, Pinto GP, Encina PG, Mata RI. "Consumption and occurrence of pharmaceutical and personal care products in the aquatic environment in Spain." *Science of the total environment* 444 (2013): 451-465.

Osorio V, Imbert-Bouchard M, Zonja B, Abad JL, Pérez S, Barceló D. "Simultaneous determination of diclofenac, its human metabolites and microbial nitration/nitrosation transformation products in wastewaters by liquid chromatography/quadrupole-linear ion trap mass spectrometry." *Journal of Chromatography A* 1347 (2014b): 63-71.

Osorio V, Larrañaga A, Aceña J, Pérez S, Barceló D. “Concentration and risk of pharmaceuticals in freshwater systems are related to the population density and the livestock units in Iberian Rivers.” *Science of The Total Environment* (2015) doi:10.1016/j.scitotenv.2015.06.143.

Osorio V, Marcé R, Pérez S, Ginebreda A, Cortina JL, Barceló D. "Occurrence and modeling of pharmaceuticals on a sewage-impacted Mediterranean river and their dynamics under different hydrological conditions." *Science of the Total Environment* 440 (2012b): 3-13.

Osorio V, Pérez S, Ginebreda A, Barceló D. "Pharmaceuticals on a sewage impacted section of a Mediterranean River (Llobregat River, NE Spain) and their relationship with hydrological conditions." *Environmental Science and Pollution Research* 19, no. 4 (2012a): 1013-1025.

Osorio V, Proia L, Ricart M, Pérez S, Ginebreda A, Cortina JL, Sabater S, Barceló D. "Hydrological variation modulates pharmaceutical levels and biofilm responses in a Mediterranean river." *Science of the Total Environment* 472 (2014a): 1052-1061.

Oulton RL, Kohn T, Cwiertny DM. "Pharmaceuticals and personal care products in effluent matrices: a survey of transformation and removal during wastewater treatment and implications for wastewater management." *Journal of Environmental Monitoring* 12, no. 11 (2010): 1956-1978.

Pal A, Gin KYH, Lin AYC, Reinhard M. "Impacts of emerging organic contaminants on freshwater resources: review of recent occurrences, sources, fate and effects." *Science of the Total Environment* 408, no. 24 (2010): 6062-6069.

Parkinson A, Ogilvie BW, Paris BL, Hensley TN, Loewen GJ. “Human biotransformation.” In: *Biotransformation and Metabolite Elucidation of Xenobiotics: Characterization and Identification*. John Wiley & Sons, 2010.

Parsons S. *Advanced oxidation processes for water and wastewater treatment*. IWA publishing, 2004.

Paterson G, Metcalfe CD. "Uptake and depuration of the anti-depressant fluoxetine by the Japanese medaka (Oryzias latipes)." *Chemosphere* 74, no. 1 (2008): 125-130.

Patrolecco L, Capri S, Ademollo N. “Occurrence of selected pharmaceuticals in the principal sewage treatment plants in Rome (Italy) and in the receiving surface waters” *Environ Sci Pollut Res* 22 (2015):5864‑5876.

Peng X, Wang Z, Kuang W, Tan J, Li K. “A preliminary study on the occurrence and behavior of sulfonamides, ofloxacin and chloramphenicol antimicrobials in wastewater of two sewage treatment plants in Guangzhou, China.” *Science of the Total Environment* 371, no. 1 (2006): 314-322.

Pereira AMPT, Silva LJG, Meisel LM, Lino CM, Pena A. "Environmental impact of pharmaceuticals from Portuguese wastewaters: geographical and seasonal occurrence, removal and risk assessment." *Environmental Research* 136 (2015): 108-119.

Pérez S, Barceló D. “First evidence for occurrence of hydroxylated human metabolites of diclofenac and aceclofenac in wastewater using QqLIT-MS and QqTOF-MS.” *Analitical Chemistry* 80 (2008): 8135-8145.

Pérez S, Eichhorn P, Aga DS. "Evaluating the biodegradability of sulfamethazine, sulfamethoxazole, sulfathiazole, and trimethoprim at different stages of sewage treatment." *Environmental Toxicology and Chemistry* 24, no. 6 (2005): 1361-1367.

Pérez S, Eichhorn P, Celiz MD, Aga DS. "Structural characterization of metabolites of the X-ray contrast agent iopromide in activated sludge using ion trap mass spectrometry." *Analytical chemistry* 78, no. 6 (2006): 1866-1874.

Petrie B, Barden R, Kasprzyk-Hordern B. "A review on emerging contaminants in wastewaters and the environment: Current knowledge, understudied areas and recommendations for future monitoring." *Water Research* 72 (2015): 3-27.

Petrovic M, Ginebreda A, Acuña V, Batalla RJ, Elosegi A, Guasch H, López de Alda M, Marcé R, Muñoz I, Navarro-Ortega A, Navarro E, Vericat D, Sabater S, Barceló D "Combined scenarios of chemical and ecological quality under water scarcity in Mediterranean rivers" TrAC 30, no.8 (2011): 1268–78.

Petrovic M, Postigo C, de Alda ML, Ginebreda A, Gros M, Radjenovic J, Barceló D. "Occurrence and fate of pharmaceuticals and illicit drugs under water scarcity" In *Water scarcity in the Mediterranean: perspectives under global change* pp.197-229 Hdb Env Chem 8, 2010.

Petrović M, Škrbić B, Živančev J, Ferrando-Climent L, Barceló D. "Determination of 81 pharmaceutical drugs by high performance liquid chromatography coupled to mass spectrometry with hybrid triple quadrupole–linear ion trap in different types of water in Serbia" *Science of the Total Environment* 468–469 (2014): 415–428.

Pistocchi A, Marinov D, Pontes S, Gawlik BM. "Continental scale inverse modeling of common organic water contaminants in European rivers." *Environmental Pollution* 162 (2012): 159-167.

Pistocchi A, Sarigiannis DA, Vizcaino P. "Spatially explicit multimedia fate models for pollutants in Europe: State of the art and perspectives" *Science of the Total Environment* 408 (2010): 3817–30.

Pistocchi A. *GIS based chemical fate modeling: Principles and applications*. John Wiley & Sons, 2014.

Pomati F, Orlandi C, Clerici M, Luciani F, Zuccato E. "Effects and interactions in an environmentally relevant mixture of pharmaceuticals." *Toxicological Sciences* 102, no. 1 (2008): 129-137.

Prieto-Rodríguez L, Oller I, Klamerth N, Agüera A, Rodríguez EM, Malato S. "Application of solar AOPs and ozonation for elimination of micropollutants in municipal wastewater treatment plant effluents." *Water research* 47, no. 4 (2013): 1521-1528.

Proia L, Cassió F, Pascoal C, Tlili A, Romaní AM. "The use of attached microbial communities to assess ecological risks of pollutants in river ecosystems: the role of heterotrophs." In *Emerging and Priority Pollutants in Rivers*, pp. 55-83. Springer Berlin Heidelberg, 2012.

Proia L, Lupini G, Osorio V, Pérez S, Barceló D, Schwartz T, Amalfitano S, Fazi S, Romaní AM, Sabater S. "Response of biofilm bacterial communities to antibiotic pollutants in a Mediterranean river." *Chemosphere* 92, no. 9 (2013a): 1126-1135.

Proia L, Osorio V, Soley S, Köck-Schulmeyer M, Pérez S, Barceló D, Romaní AM, Sabater S. "Effects of pesticides and pharmaceuticals on biofilms in a highly impacted river." *Environmental Pollution* 178 (2013b): 220-228.

Proia L, Osorio V. "The Effect of PhACs on Biological Communities in Rivers: Field Studies." In: *Comprehensive Analytical Chemistry. Analysis, Removal, Effects and Risk of Pharmaceuticals in the Water Cycle - Occurrence and Transformation in the Environment*, 62, pp. 649 - 670. Elsevier, 2013.

Pusch M, Fiebig D, Brettar I, Eisenmann H, Ellis BK, Kaplan LA, Lock MA, Naegeli, MW, Traunspurger W. “The role of micro-organisms in the ecological connectivity of running waters.” *Freshwater Biology* 40 (1998): 453–495.

Quintana JB, Weiss S, Reemtsma T. “Pathways and metabolites of microbial degradation of selected acidic pharmaceutical and their occurrence in municipal wastewater treated by a membrane bioreactor.” *Water Research* 39 (2005): 2654–2664.

Radjenovic J, Petrovic M, Barceló D. “Analysis of pharmaceuticals in wastewater and removal using a membrane bioreactor.” *Analytical and bioanalytical chemistry* 387, no. 4 (2007): 1365-1377.

Radjenovic J, Petrovic M, Barceló D. “Fate and distribution of pharmaceuticals in wastewater and sewage sludge of the conventional activated sludge (CAS) and advanced membrane bioreactor(MBR) treatment.” *Water Research* 43 (2009): 831–841.

Radke M, Maier MP. "Lessons learned from water/sediment-testing of pharmaceuticals." Water Research 55 (2014): 63-73.

Radović T, Grujić S, Petković A, Dimkić M, Laušević M. “Determination of pharmaceuticals and pesticides in river sediments and corresponding surface and ground water in the Danube River and tributaries in Serbia” *Environmental Monitoring and Assessment* 187 (2015): 4092.

Richards NL, Cook G, Simpson V, Hall S, Harrison N, Scott KS. "Qualitative detection of the NSAIDs diclofenac and ibuprofen in the hair of Eurasian otters (Lutra lutra)

occupying UK waterways with GC–MS." *European journal of wildlife research* 57, no. 5 (2011): 1107-1114.

Richardson ML, Bowron JM. "The fate of pharmaceutical chemicals in the aquatic environment." *Journal of Pharmacy and Pharmacology* 37, no. 1 (1985): 1-12.

Richardson SD, Ternes TA. "Water analysis: emerging contaminants and current issues." *Analytical Chemistry* 86, no. 6 (2014): 2813-2848.

Riva F, Zuccato E, Castiglioni S. "Prioritization and analysis of pharmaceuticals for human use contaminating the aquatic ecosystem in Italy." *Journal of Pharmaceutical and Biomedical Analysis* 106 (2015): 71-78.

Roberts PH, Thomas KV. "The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment." *Science of the Total Environment* 356, no. 1 (2006): 143-153.

Robinson PF, Liu QT, Riddle AM, Murray-Smith R. "Modeling the impact of direct phototransformation on predicted environmental concentrations (PECs) of propranolol hydrochloride in UK and US rivers." *Chemosphere* 66, no. 4 (2007): 757-766.

Rodriguez-Caballero A, Pijuan M. "N_2O and NO emissions from a partial nitrification sequencing batch reactor: exploring dynamics, sources and minimization mechanisms." *Water Research* 47, no. 9 (2013): 3131-3140.

Romaní AM, Giorgi A, Acuña V, Sabater S. “The influence of substratum type and nutrient supply on biofilm organic matter utilization in streams.” *Limnology and Oceanography* 49 (2004): 1713-1721.

Roose-Amsaleg C, Laverman AM. “Do antibiotics have environmental side-effects? Impact of synthetic antibiotics on biogeochemical processes” *Environmental Science and Pollution Research* (2015) doi: 10.1007/s11356-015-4943-3

Rosal R, Rodea-Palomares I, Boltes K, Fernández-Piñas F, Leganés F, Gonzalo S, Petre A. “Ecotoxicity assessment of lipid regulators in water and biologically treated wastewater using three aquatic organisms.” *Environmental Science and Pollution Research* 17, no. 1 (2010): 135-144.

Rosi-Marshall EJ, Kincaid DW, Bechtold HA, Royer TV, Rojas M, Kelly JJ. "Pharmaceuticals suppress algal growth and microbial respiration and alter bacterial communities in stream biofilms." *Ecological Applications* 23, no. 3 (2013): 583-593.

Rúa-Gómez PC, Püttmann W. "Occurrence and removal of lidocaine, tramadol, venlafaxine, and their metabolites in German wastewater treatment plants." *Environmental Science and Pollution Research* 19, no. 3 (2012): 689-699.

Rubirola A, Llorca M, Rodriguez-Mozaz S, Casas N, Rodriguez-Roda I, Barceló D, Buttiglieri G. "Characterization of metoprolol biodegradation and its transformation

products generated in activated sludge batch experiments and in full scale WWTPs." *Water Research* 63 (2014): 21-32.

Ruel M, Esperanza M, Choubert J, Valor I, Budzinski H, Coquery M. "On-site evaluation of the efficiency of conventional and advanced secondary processes for the removal of 60 organic micropollutants." *Water Science & Technology* 62, no. 12 (2010): 2970–2978.

Sabater S, Ginebreda A, Barceló D. "The Llobregat. The story of a polluted Mediterranean river" Hdb. Env. Chem. Berlin Heidelberg: Springer-Verlag; 2012.

Sabater S, Guasch H, Ricart M, Romaní A, Vidal G, Klünder C, Schmitt-Jansen M. "Monitoring the effect of chemicals on biological communities. The biofilm as an interface." *Analytical and Bioanalytical Chemistry* 387, no. 4 (2007): 1425-1434.

Sadezky A, Löffler D, Ternes T. "Proposal of an environmental indicator and classification system of pharmaceutical product residues for environmental management." *Projet Européen KNAPPE, Deliverable D12* (2008). http://www.knappe-eu.org/

Sahar E, Ernst M, Godehardt M, Hein A, Herr J, Melin T, et al. Comparison of two treatments for the removal of selected organic micropollutants and bulk organic matter: conventional activated sludge followed by ultrafiltration versus membrane bioreactor. *Water Science and Technology* 63, no. 4 (2011): 733.

Salgot M, Huertas E, Weber S, Dott W, Hollender J. "Wastewater reuse and risk: definition of key objectives." *Desalination* 187, no. 1 (2006): 29-40.

Sánchez EC, Gallardo MA, Gaertner A. "Future climate extreme events in the Mediterranean simulated by a regional climate model: a first approach" *Global and Planetary Change* 44 (2004): 163–180.

Sanderson H, Brain RA, Johnson DJ, Wilson CJ, Solomon KR. "Toxicity classification and evaluation of four pharmaceuticals classes: antibiotics, antineoplastics, cardiovascular, and sex hormones." *Toxicology* 203, no. 1 (2004): 27-40.

Santos J, Aparicio I, Callejón M, Alonso E. "Occurrence of pharmaceutically active compounds during 1-year period in wastewaters from four wastewater treatment plants in Seville (Spain)" *Journal of Hazardous Materials* 164 (2009):1509–16.

Santos JL, Aparicio I, Alonso E. "Occurrence and risk assessment of pharmaceutically active compounds in wastewater treatment plants. A case study: Seville city (Spain)." *Environment International* 33 (2007): 596–601.

Sarma SSS, González-Pérez BK, Moreno-Gutiérrez RM, Nandini S.. "Effect of paracetamol and diclofenac on population growth of Plationus patulus and Moina macrocopa." *Journal of Environmental Biology* 35 (2013): 119-126.

Scheurell M, Franke S, Shah RM, Hühnerfuss H. “Occurrence of diclofenac and its metabolites in surface water and effluent samples from Karachi, Pakistan.” *Chemosphere* 77, no.6 (2009): 870-876.

Schmitt-Jansen M, Bartels P, Adler N, Altenburger R. "Phytotoxicity assessment of diclofenac and its phototransformation products." *Analytical and bioanalytical chemistry* 387, no. 4 (2007): 1389-1396.

Schowanek D, Webb S. "Examples of exposure assessment simulation for pharmaceuticals in river basins with the GREAT-ER 1.0 system." In *Proceedings KVIV Seminar ‘Pharmaceuticals in the Environment’March*, 9 pp. 2000, 2000.

Schultz MM, Furlong ET, Kolpin DW, Werner SL, Schoenfuss HL, Barber LB, Blazer VS, Norris DO, Vajda AM. "Antidepressant pharmaceuticals in two US effluent-impacted streams: occurrence and fate in water and sediment, and selective uptake in fish neural tissue." *Environmental Science & Technology* 44, no. 6 (2010): 1918-1925.

Schulze T, Weiss S, Schymanski E, von der Ohe PC, Schmitt-Jansen M, Altenburger R, Streck G, Brack W. "Identification of a phytotoxic photo-transformation product of diclofenac using effect-directed analysis." *Environmental Pollution* 158, no. 5 (2010): 1461-1466.

Schuster A, Hädrich C, Kümmerer K. "Flows of active pharmaceutical ingredients originating from health care practices on a local, regional, and nationwide level in Germany—is hospital effluent treatment an effective approach for risk reduction?." *Water, Air, & Soil Pollution: Focus* 8, no. 5-6 (2008): 457-471.

Schwaiger J, Ferling H, Mallow U, Wintermayr H, Negele RD. "Toxic effects of the non-steroidal anti-inflammatory drug diclofenac: Part I: histopathological alterations and bioaccumulation in rainbow trout." *Aquatic Toxicology* 68, no. 2 (2004): 141-150.

Schwarzenbach RP, Gschwend PM, Imboden D. “Environmental Organic Chemistry” 2nd edition, John Wiley & Sons, Inc., Hoboken, New Jersey, USA, 2003

Seehusen DA, Edwards J. "Patient practices and beliefs concerning disposal of medications." *The Journal of the American Board of Family Medicine* 19, no. 6 (2006): 542-547.

Segalin J, Sirtori C, Jank L, Lima MF, Livotto PR, Machado TC, Pizzolato TM. “Identification of transformation products of rosuvastatin in water during ZnO photocatalytic degradation through the use of associated LC–QTOF–MS to computational chemistry” *Journal of Hazardous Materials* 299 (2015): 78-85.

Simpson VR, Tomlinson AJ, Molenaar FM, Lawson B, Rogers KD. "Renal calculi in wild Eurasian otters (Lutra lutra) in England." *The Veterinary record* 169, no. 2 (2011): 49-49.

Singer H, Jaus S, Hanke I, Lück A, Hollender J, Alder AC. “Determination of biocides and pesticides by on-line solid phase extraction coupled with mass spectrometry and their behaviour in wastewater and surface water” *Environmental Pollution* 158 (2010): 3054–64.

SNS, Sistema Nacional de Salud. Ministerio de Sanidad, Servicios Sociales e Igualdad. Informe anual del Sistema Nacional de Salud, 2011 (2012) www.msssi.gob.es

Sprague JB. "Measurement of pollutant toxicity to fish. II. Utilizing and applying bioassay results." *Water Research* 4, no. 1 (1970): 3-32.

Stadler LB, Ernstoff AS, Aga DS, Love NG. "Micropollutant fate in wastewater treatment: redefining “removal”.” *Environmental Science & Technology* 46, no. 19 (2012): 10485-10486.

Stierlin H, Faigle JW, Sallmann A, Kung W, Richter WJ, Kriemler H-P, Alt KO, Winkler T. "Biotransformation of diclofenac sodium (Voltaren®) in animals and in man: I. Isolation and identification of principal metabolites." *Xenobiotica* 9, no. 10 (1979a): 601-610.

Stierlin H, Faigle JW. "Biotransformation of Diclofenac Sodium (Voltaren®) in Animals and in Man.: II. Quantitative determination of the unchanged drug and principal phenolic metabolites, in urine and bile." *Xenobiotica* 9, no. 10 (1979b): 611-621.

Streeter HW, Phelps EB. *A study of the pollution and natural purification of the Ohio River*. US Department of Health, Education, & Welfare, 1958.

Strenn B, Clara M, Gans O, Kreuzinger N. “Carbamazepine, diclofenac, ibuprofen and bezafibrate—investigations on the behaviour of selected pharmaceuticals during wastewater treatment.” *Water Science and Technology* 50 (2004): 269–76.

Stülten D, Zühlke S, Lamshöft M, Spiteller M.. “Occurrence of diclofenac and selected metabolites in sewage effluents.” *Science of the Total Environment* 405, no. 1 (2008): 310-316.

Stumpf M, Ternes TA, Wilken RD, Rodrigues S, Baumann W. "Polar drug residues in sewage and natural waters in the state of Rio de Janeiro, Brazil." *Science of the Total Environment* 225, no. 1 (1999): 135-141.

Suárez S, Carballa M, Omil F, Lema JM. "How are pharmaceutical and personal care products (PPCPs) removed from urban wastewaters?" *Reviews in Environmental Science and Bio/Technology* 7, no. 2 (2008): 125-138.

Suárez S, Lema JM, Omil F, “Removal of Pharmaceutical and Personal Care Products (PPCPs) under nitrifying and denitrifying conditions.” *Water Research* 44 (2010): 3214-3224.

Suárez S, Ramil M, Omil F, Lema JM. “Removal of pharmaceutically active compounds in nitrifying–denitrifying plants.” *Water Science & Technology* 52, no. 8 (2005): 9-14.

Sui Q, Wang D, Zhao W, Huang J, Yu G, Cao X, Qiu Z, Lu S. “Pharmaceuticals and consumer products in four wastewater treatment plants in urban and suburb areas of Shanghai” *Environmental Science and Pollution Research* 22, no. 8 (2015): 6086-6094.

Sumpter JP, Johnson AC. "10th Anniversary perspective: reflections on endocrine disruption in the aquatic environment: from known knowns to unknown unknowns (and many things in between)." *Journal of Environmental Monitoring* 10, no. 12 (2008): 1476-1485.

Tauxe-Wuersch A, De Alencastro LF, Grandjean D, Tarradellas J. “Occurrence of several acidic drugs in sewage treatment plants in Switzerland and risk assessment.” *Water Research* 39 (2005): 1761–72.

Ternes T, Joss A. “Human Pharmaceuticals, Hormones and Fragrances: The Challenge of Micropollutants in Urban Water Management” (2007). London: IWA Publishing.

Ternes TA, Bonerz M, Herrmann N, Teiser B, Andersen HR. "Irrigation of treated wastewater in Braunschweig, Germany: an option to remove pharmaceuticals and musk fragrances." *Chemosphere* 66, no. 5 (2007): 894-904.

Ternes TA, Joss A, Siegrist H. "Peer reviewed: scrutinizing pharmaceuticals and personal care products in wastewater treatment." *Environmental Science & Technology* 38, no. 20 (2004): 392A-399A.

Ternes TA. "Occurrence of drugs in German sewage treatment plants and rivers." *Water Research* 32, no. 11 (1998): 3245-3260.

Terzić S, Senta I, Ahel M, Gros M, Petrović M, Barcelo D, Müller J, Knepper T, Martí I, Ventura F, Jovančić P, Jabučar D. “Occurrence and fate of emerging wastewater contaminants in Western Balkan Region” *Science of the Total Environment* 399 (2008): 66–77.

Thomas PM, Foster GD. “Tracking acidic pharmaceutical, caffeine, and triclosan through the wastewater treatment process.” *Environmental Technology and Chemistry* 24 (2005): 25–30.

Tolls J. "Sorption of veterinary pharmaceuticals in soils: a review." *Environmental Science & Technology* 35, no. 17 (2001): 3397-3406.

Tran NH, Urase T, Kusakabe O. "The characteristics of enriched nitrifier culture in the degradation of selected pharmaceutically active compounds." *Journal of Hazardous Materials* 171, no. 1 (2009): 1051-1057.

Tran NH, Urase T, Ngo HH, Hu J, Ong SL. "Insight into metabolic and cometabolic activities of autotrophic and heterotrophic microorganisms in the biodegradation of emerging trace organic contaminants." *Bioresource Technology* 146 (2013): 721-731.

Triebskorn R, Casper H, Heyd A, Eikemper R, Köhler H-R, Schwaiger J. "Toxic effects of the non-steroidal anti-inflammatory drug diclofenac: Part II. Cytological effects in liver, kidney, gills and intestine of rainbow trout (Oncorhynchus mykiss)." *Aquatic Toxicology* 68, no. 2 (2004): 151-166.

Trovó AG, Nogueira RFP, Agüera A, Sirtori C, Fernández-Alba AR. "Photodegradation of sulfamethoxazole in various aqueous media: persistence, toxicity and photoproducts assessment." *Chemosphere* 77, no. 10 (2009): 1292-1298.

Urase T, Kikuta T. "Separate estimation of adsorption and degradation of pharmaceutical substances and estrogens in the activated sludge process." *Water Research* 39, no. 7 (2005): 1289-1300.

Vader JS, Van Ginkel CG, Sperling FMGM, De Jong J, De Boer W, De Graaf JS, van der Most M, Stokman PGW. “Degradation of ethinyl estradiol by nitrifying activated sludge.” *Chemosphere* 41 no. 8 (2000): 1239-1243.

Van Boeckel TP, Brower C, Gilbert M, Grenfell BT, Levin SA, Robinson TP, Teillant A, Laxminarayan R. “Global trends in antimicrobial use in food animals” *Proceedings of the National Academy of Sciences* 112, no. 18 (2015): 5649-5654.

van der Aa NGFM, Kommer G, van Montfoort J, Versteegh J. "Demographic projections of future pharmaceutical consumption in the Netherlands." *Water Science & Technology* 63, no. 4 (2011): 825-831.

Van der Hoeven N. "Current issues in statistics and models for ecotoxicological risk assessment." *Acta Biotheoretica* 52, no. 3 (2004): 201-217.

van Zelm R, Huijbregts MAJ, van de Meent D. "Transformation products in the life cycle impact assessment of chemicals." *Environmental science & technology* 44, no. 3 (2010): 1004-1009.

Vanderford BJ, Snyder SA. "Analysis of pharmaceuticals in water by isotope dilution liquid chromatography/tandem mass spectrometry." *Environmental Science & Technology* 40, no. 23 (2006): 7312-7320.

Vasquez MI, Lambrianides A, Schneider M, Kümmerer K, Fatta-Kassinos D. “Environmental side effects of pharmaceutical cocktails: What we know and what we should know.” *Journal of Hazardous Materials* 279, (2014): 169-189.

Vasskog T, Berger U, Samuelsen PJ, Kallenborn R, Jensen E. "Selective serotonin reuptake inhibitors in sewage influents and effluents from Tromsø, Norway." *Journal of Chromatography A* 1115, no. 1 (2006): 187-195.

Vázquez-Roig P, Andreu V, Onghena M, Blasco C, Picó Y. "Assessment of the occurrence and distribution of pharmaceuticals in a Mediterranean wetland (L'Albufera, Valencia, Spain) by LC-MS/MS." *Analytical and bioanalytical chemistry* 400, no. 5 (2011): 1287-1301.

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.

Verlicchi P, Zambello E. "How efficient are constructed wetlands in removing pharmaceuticals from untreated and treated urban wastewaters? A review" *Science of the Total Environment* 470–471 (2014): 1281–1306

Vermeire TG, Jager DT, Bussian B, Devillers J, Den Haan K, Hansen B, Lundberg I, Niessen H, Robertson S, Tyle H, van der Zandt PTJ. "European union system for the evaluation of substances (EUSES). Principles and structure." *Chemosphere* 34, no. 8 (1997): 1823-1836.

Vieno N, Tuhkanen T, Kronberg L. "Elimination of pharmaceuticals in sewage treatment plants in Finland." *Water Research* 41, no. 5 (2007): 1001-1012.

Vieno NM, Tuhkanen T, Kronberg L. "Seasonal variation in the occurrence of pharmaceuticals in effluents from a sewage treatment plant and in the recipient water." *Environmental Science & Technology* 39, no. 21 (2005): 8220-8226.

Von Schiller D, Martí E, Riera JL, Sabater F. "Effects of nutrients and light on periphyton biomass and nitrogen uptake in Mediterranean streams with contrasting land uses" *Freshwater Biology* 52 (2007): 891–906.

Walgren JL, Mitchell MD, Thompson DC. "Role of metabolism in drug-induced idiosyncratic hepatotoxicity." *Critical reviews in toxicology* 35, no. 4 (2005): 325-361.

Walsh TR, Weeks J, Livermore DM, Toleman MA. "Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study" *The Lancet Infectous Diseases* 11(2011): 355–362.

Wang J, Gardinali PR. "Identification of phase II pharmaceutical metabolites in reclaimed water using high resolution benchtop Orbitrap mass spectrometry" *Chemosphere* 107 (2014): 65–73.

Wang XH, Lin AYC. "Phototransformation of cephalosporin antibiotics in an aqueous environment results in higher toxicity." *Environmental Science & Technology* 46, no. 22 (2012): 12417-12426.

Wang Z, Zhang XH, Huang Y, Wang H. "Comprehensive evaluation of pharmaceuticals and personal care products (PPCPs) in typical highly urbanized regions across China." *Environmental Pollution* 204 (2015): 223-232.

Water Framework Directive (Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy) European Parliament and Council Article 175(1) OJL; 2000. p. 1–73. (22 December).

Watkinson AJ, Murby EJ, Costanzo SD. “Removal of antibiotics in conventional and advanced wastewater treatment: implications for environmental discharge and wastewater recycling.” *Water research* 41, no. 18 (2007): 4164-4176.

Whelan MJ, Gandolfi C, Bischetti GB. “A simple stochastic model of point source solute transport in rivers based on gauging station data with implications for sampling requirements” *Water Research* 33 (1999): 3171-3181

WHO. “Pharmaceuticals in Drinking-water”. Health and Environment Water, Sanitation, Hygiene and Health. WHO Press, World Health Organization (2011)

Willis JV, Kendall MJ, Flinn RM, Thornhill DP, Welling PG. “The pharmacokinetics of diclofenac sodium following intravenous and oral administration.” *European journal of clinical pharmacology* 16, no. 6 (1979): 405-410.

Wilson BA, Smith VH, deNoyelles F, Larive CK. "Effects of three pharmaceutical and personal care products on natural freshwater algal assemblages." *Environmental Science & Technology* 37, no. 9 (2003): 1713-1719.

Wind T, Werner U, Jacob M, Hauk A. "Environmental concentrations of boron, LAS, EDTA, NTA and Triclosan simulated with GREAT-ER in the river Itter." *Chemosphere* 54, no. 8 (2004): 1145-1154.

Xu W, Zhang G, Li X, Zou S, Li P, Hu Z, Li J. "Occurrence and elimination of antibiotics at four sewage treatment plants in the Pearl River Delta (PRD), South China." *Water Research* 41, no. 19 (2007): 4526-4534.

Yang Y, Fu J, Peng H, Hou L, Liu M, Zhou JL. "Occurrence and phase distribution of selected pharmaceuticals in the Yangtze Estuary and its coastal zone." *Journal of Hazardous Materials* 190, no. 1 (2011): 588-596.

Yasojima M, Nakada N, Komori K, Suzuki Y, Tanaka H. "Occurrence of levofloxacin, clarithromycin and azithromycin in wastewater treatment plant in Japan." *Water Science & Technology* 53, no. 11 (2006): 227-233.

Yergeau E, Sanschagrin S, Waiser MJ, Lawrence JR, Greer CW. “Sub-inhibitory concentrations of different pharmaceutical products affect the meta-transcriptome of river biofilm communities cultivated in rotating annular reactors.” *Environmental Microbiology Reports* 4, no. 3 (2012): 350-359.

Yi T, Harper WF. "The link between nitrification and biotransformation of 17α-ethinylestradiol." *Environmental Science & Technology* 41, no. 12 (2007): 4311-4316.

Ying GG, Zhao JL, Zhou LJ, Liu S. “Fate and Occurrence of Pharmaceuticals in the Aquatic Environment (Surface Water and Sediment)” In *Analysis, Removal, Effects and Risk of Pharmaceuticals in the Water Cycle: Occurrence and Transformation in the Environment* 62, 453, 2013.

Yu JT, Bouwer EJ, Coelhan M. “Occurrence and biodegradability studies of selected pharmaceuticals and personal care products in sewage effluent.” *Agricultural Water Management* 86 (2006): 72–80.

Zhang H, Du M, Jiang H, Zhang D, Lin L, Ye H, Zhang X. “Occurrence, seasonal variation and removal efficiency of antibiotics and their metabolites in wastewater treatment plants, Jiulongjiang River Basin, South China” *Environmental Science: Processes & Impacts* 17, no.1 (2015): 225-234.

Zhou H; Wu C, Huang X, Mijun G, Xianghua W, Hiroshi T, Hiroaki T. “Occurrence of selected pharmaceuticals and caffeine in sewage treatment plants and receiving Rivers in Beijing, China” *Water Environment Research* 82 (2010): 2239–48.

Zhou LJ, Ying GG, Zhao JL, Yang JF, Wang L, Yang B, Liu S. "Trends in the occurrence of human and veterinary antibiotics in the sediments of the Yellow River, Hai River and Liao River in northern China." *Environmental Pollution* 159, no. 7 (2011): 1877-1885.

Zhou X, Oleszkiewicz JA. "Biodegradation of oestrogens in nitrifying activated sludge." *Environmental technology* 31, no. 11 (2010): 1263-1269.

Ziylan A., Ince NH. "The occurrence and fate of anti-inflammatory and analgesic pharmaceuticals in sewage and fresh water: treatability by conventional and non-conventional processes." *Journal of Hazardous Materials* 187, no. 1 (2011): 24-36.

Zonja B, Delgado A, Pérez S, Barceló D. "LC-HRMS suspect screening for detection-based prioritization of iodinated contrast media photodegradates in surface waters." *Environmental Science & Technology* 49, no. 6 (2015): 3464-3472.

Zorita S, Martensson L, Mathiasson L. “Occurrence and removal of pharmaceuticals in municipal sewage treatment system in the south of Sweden.” *Science of the total environment* 407, no. 8 (2009): 2760-2770.

Zwiener C, Seeger S, Glauner T, Frimmel F. "Metabolites from the biodegradation of pharmaceutical residues of ibuprofen in biofilm reactors and batch experiments." *Analytical and bioanalytical chemistry* 372, no. 4 (2002): 569-575.

Annex

Table A.1. Physicochemical properties of PhACs, classified by their therapeutic activity, studied in this thesis

	CAS Number	Molecular formula	Average mass (Da)	pKa[a]	Water Solubility[b] (mgL^{-1} at 25 deg C) (exp)	Log K_{ow}[b] (exp)	Log K_{ow}[b] (est)	log D_{ow}[a] (pH=6)	log D_{ow}[a] (pH=8)	Log K_d	Log K_{oc}[b] (est)
Analgesics and antinflamatories											
Ketoprofen	22071-15-4	$C_{16}H_{14}O_3$	254.281	3.78	51	3.12	3.00	1.35	-0.08	1.2[d]	2.459
Naproxen	22204-53-1	$C_{14}H_{14}O_3$	230.259	4,15	15.9	3.18	3.10	1.18	-0.46	1.1[d]	2.543
Ibuprofen	15687-27-1	$C_{13}H_{18}O_2$	206.281	4.85	21	3.97	3.79	2.65	0.79	0.9[d]	2.596
Indomethacine	53-86-1	$C_{19}H_{16}ClNO_4$	357.788	3.80	0.94	4.27	4.23	1.13	-0.25	-	3.366
Diclofenac	15307-86-5	$C_{14}H_{11}Cl_2NO_2$	296.149	4.00	2.37	4.51	4.02	1.97	0.45	1.2[d]	2.921
Mefenamic acid	61-68-7	$C_{15}H_{15}NO_2$	241.285	3.89	20	5.12	5.28	2.94	1.50	2.6[d]	2.664
Acetaminophen	103-90-2	$C_8H_9NO_2$	151.163	9.46	1.40E+04	0.46	0.27	1.09	1.07	3.06[d]	1.790
Propyphenazone	479-92-5	$C_{14}H_{18}N_2O$	230.305	0.47	3.00E+06	1.94	2.05	2.61	2.61	-	3.060
Phenazone	1698-60-8	$C_{11}H_{12}N_2O$	188.226	0.09	5.19E+04	0.38	0.59	1.61	1.61	-	2.349
Phenylbutazone	50-33-9	$C_{19}H_{20}N_2O_2$	308.374	5.13	47.5	3.16	3.52	3.32	2.15	-	4.199
Codeine	76-57-3	$C_{18}H_{21}NO_3$	299.364	9.19	9000	1.19	1.28	-1.84	0.05	-	3.115
Piroxicam	36322-90-4	$C_{15}H_{13}N_3O_4S$	331.346	3.79/4.76/ 11.73	23	3.06	2.58	-0.11	-0.56	-	2.177
Meloxicam	71125-39-8	$C_{14}H_{13}N_3O_4S_2$	351.401	0.47/4.47/ 10.64	7.152[c]	3.43	3.50	0.24	-0.07	-	2.120
Tenoxicam	59804-37-4	$C_{13}H_{11}N_3O_4S_2$	337.374	4.78/7.55/ 13.63	66.13[c]	-	2.40	0.71	0.17	-	1.911
Lipid regulators and cholesterol lowering stating drugs											
Clofibric acid	882-09-7	$C_{10}H_{11}ClO_3$	214.645	3.37	582.5[c]	2.57	2.84	0	-1.04	-	1.640
Gemfibrozil	25812-30-0	$C_{15}H_{22}O_3$	250.333	4.42	4.694	-	4.77	2.74	1.00	1.28[d]	2.656
Bezafibrate	41859-67-0	$C_{19}H_{20}ClNO_4$	361.819	3.83/14.57	1.224[c]	-	4.25	1.36	-0.04	-	3.166
Fenofibrate	49562-28-9	$C_{20}H_{21}ClO_4$	360.831	n.i.	0.1957[c]	-	5.19	4.60	4.60	-	3.638
Atorvastatin	134523-00-5	$C_{33}H_{35}FN_2O_5$	558.640	4.33/13.88/ 14.90/15.58	300	-	5.00[a]	3.32	1.62	-	-
Mevastatin	73573-88-3	$C_{23}H_{34}O_5$	390.513	14.91	4.801[c]	3.95	4.32	3.14	3.14	-	3.603
Fluvastatin	93957-54-1	$C_{24}H_{26}FNO_4$	411.466	4.56/14.64/ 15.44	0.4681[c]	-	4.85	2.40	0.61	-	3.423

Psychiatric drugs											
Fluoxetine	54910-89-3	$C_{17}H_{18}F_3NO$	309.326	9.80	38.35[c]	4.05	4.65	0.80	2.39	0.7[d]	5.317
Paroxetine	61869-08-7	$C_{19}H_{20}FNO_3$	329.365	9.77	537.1[c]	-	2.57	-0.13	1.47	-	2.635
Diazepam	439-14-5	$C_{16}H_{13}ClN_2O$	284.740	2.92	50	2.82	2.70	3.01	3.01	1.3[d]	4.050
Lorazepam	846-49-1	$C_{15}H_{10}Cl_2N_2O_2$	321.158	10.61/12.46	3.666[c]	-	3.98	3.49	3.49	-	3.300
Carbamazepine	298-46-4	$C_{15}H_{12}N_2O$	236.269	15.96	17.66[c]	2.45	2.25	3.22	3.22	0.1[d]	3.588
Sertraline	79559-97-0	$C_{17}H_{17}Cl_2N$	306.230	9.56	3.517[c]	-	5.21	1.72	3.43	-	5.534
Citalopram	59729-32-7	$C_{20}H_{21}FN_2O$	324.392	9.78	31.09[c]	-	3.74	0.66	1.77	-	4.404
Venlafaxine	99300-78-4	$C_{17}H_{27}NO_2$	277.402	8.91/14.42	266.7[c]	-	3.28	-0.60	1.30	-	3.166
Olanzapine	132539-06-1	$C_{17}H_{20}N_4S$	312.432	4.01/7.24 13.17	53.33[c]	-	2.56	1.82	3.02	-	4.792
Trazodone	25332-39-2	$C_{19}H_{22}ClN_5O$	371.864	7.09	8.239[c]	-	3.21	3.66	4.73	-	4.685
Alprazolam	28981-97-7	$C_{17}H_{13}ClN_4$	308.765	1.79/5.08	13.1[c]	2.12	3.87	2.74	2.79	-	6.333
Histamine H_1 and H_2 receptor antagonists											
Famotidine	76824-35-6	$C_8H_{15}N_7O_2S_3$	337.445	1.74/8.38/ 9.29	1000	-0.65	-0.64	-2.49	-1.60	-	4.274
Ranitidine	66357-35-5	$C_{13}H_{22}N_4O_3S$	314.404	0.47/7.80	2.466e+004[c]	0.27	0.29	-0.91	0.67	-	4.443
Cimetidine	51481-61-9	$C_{10}H_{16}N_6S$	252.339	4.53/6.53/ 14.16	7426[c]	-	0.57	-0.53	0.07	-	2.963
Loratidine	79794-75-5	$C_{22}H_{23}ClN_2O_2$	382.883	4.33	0.01099[c]	5.20	5.66	4.47	4.48		6.352
β-Blocking agents											
Atenolol	29122-68-7	$C_{14}H_{22}N_2O_3$	266.336	9.67/14.08/ 15.95	685.2[c]	0.16	-0.03	-3.07	-1.41	-0.68[d]	2.171
Sotalol	3930-20-9	$C_{12}H_{20}N_2O_3S$	272.364	9.43/10.07/ 14.10	5513[c]	0.24	0.37	-3.15	-1.47	-	1.580
Metoprolol	37350-58-6	$C_{15}H_{25}NO_3$	267.364	9.67/14.09	4777[c]	1.88	1.69	-1.85	-0.18	-	1.794
Propanolol	525-66-6	$C_{16}H_{21}NO_2$	259.343	9.67/14.09	228[c]	3.48	2.60	-0.84	0.83	2.6[d]	3.086
Timolol	26839-75-8	$C_{13}H_{24}N_4O_3S$	316.420	9.76/14.08	2741[c]	1.83	1.75	-2.21	-0.60	-	1.000
Betaxolol	63659-18-7	$C_{18}H_{29}NO_3$	307.428	9.67/14.09	450.7[c]	2.81	2.98	-1.14	0.53	-	2.601
Nadolol	42200-33-9	$C_{17}H_{27}NO_4$	309.401	9.76/13.59 14.22/15.23	2.24e+004[c]	0.81	1.17	-3.01	-1.39	-	2.149
Carazolol	57775-29-8	$C_{18}H_{22}N_2O_2$	298.379	9.67/14.03/ 15.00	8.524[c]	3.59	2.66	-0.64	1.02		3.874
Barbiturates											
Butalbital	77-26-9	$C_{11}H_{16}N_2O_3$	224.256	7.48/11.15	1700	-	1.87	1.63	1.02	-	2.024
Pentobarbital	76-74-4	$C_{11}H_{18}N_2O_3$	226.272	7.48/11.15	679	2.10	2.00	1.85	1.24	-	2.058
Phenobarbital	50-06-6	$C_{12}H_{12}N_2O_3$	232.235	7.14/10.80	1110	1.47	1.33	1.53	0.66	-	2.390

Diuretics											
Hydrochlorothiazide	58-93-5	$C_7H_8ClN_3O_4S_2$	297.739	5.00	0.011	-0.07	-0.10	-0.16	-0.19	1.8[d]	1.901
Furosemide	54-31-9	$C_{12}H_{11}ClN_2O_5S$	330.744	4.25/9.83	73.1	2.03	2.32	-0.09	-1.76	-	2.275
Torasemide	56211-40-6	$C_{16}H_{20}N_4O_3S$	348.420	4.20/5.92	136.6[c]	-	1.95	1.50	0.72	-	3.895
Antihypertensives											
Amlodipine	111470-99-6	$C_{20}H_{25}ClN_2O_5$	408.876	9.45	75.32[c]	3.00	2.07	-1.89	-0.21	-	3.253
Losartan	124750-99-8	$C_{22}H_{23}ClN_6O$	422.911	4.12/7.40/ 14.27	0.8223[c]	-	4.01	5.33	4.66	-	5.959
Irbesartan	138402-11-6	$C_{25}H_{28}N_6O$	428.529	4.12/7.40	0.05991[c]	-	5.31	5.71	5.08	-	7.911
Valsartan	137862-53-4	$C_{24}H_{29}N_5O_3$	435.519	4.37/7.40	1.406[c]	-	3.65	3.98	1.62	-	6.011
Enalapril	75847-73-3	$C_{20}H_{28}N_2O_5$	376.447	3.67/5.20	34.88[c]	-	2.45	-0.25	-1.49	-	3.133
Antiplatelet agent											
Clopidogrel	135046-48-9	$C_{16}H_{16}ClNO_2S$	321.822	4.57	50.78[c]	-	3.82	3.79	3.80	-	4.367
X-ray contrast agent											
Iopromide	73334-07-3	$C_{18}H_{24}I_3N_3O_8$	791.112	11.09/12.17/ 13.69/14.27/ 15.38/15.96	>60[a]	-	-0.72[a]	-0.72	-0.72	1[d]	-
Antihelmintics											
Albendazole	54965-21-8	$C_{12}H_{15}N_3O_2S$	265.331	4.21/9.68/ 13.95	40.76[c]	-	3.14	3.46	3.46	-	3.272
Thiabendazole	148-79-8	$C_{10}H_7N_3S$	201.248	4.08/10.28	50	2.47	2.00	2.19	2.19	-	3.345
Levamisole	16595-80-5	$C_{11}H_{12}N_2S$	204.291	6.98	1116[c]	1.84	2.87	1.85	2.82		3.937
Cancer treatment drugs											
Tamoxifen	10540-29-1	$C_{26}H_{29}NO$	371.515	8.76	0.1936[c]	-	6.30	4.01	5.91	-	6.885
Syntethic glucocorticoid											
Dexamethasone	50-02-2	$C_{22}H_{29}FO_5$	392.461	12.42/13.48/ 14.09	66.5	1.94	1.72	1.75	1.75	-	1.982
Sedation and muscle relaxation drugs											
Xylazine	23076-35-9	$C_{12}H_{16}N_2S$	220.334	6.94	4.776[c]	-	4.52	2.80	3.75	-	3.588
Tranquilizer											
Azaperone	1649-18-9	$C_{19}H_{22}FN_3O$	327.396	5.21/7.16	131[c]	3.30	3.23	1.89	3.08		4.210

Antibiotics											
Erythromycin	114-07-8	$C_{37}H_{67}NO_{13}$	733.927	8.38/12.45/ 12.92/13.35/ 13.98/14.61	>60[a]	-	1.22[a]	-1.15	0.69	2.2[d]	-
Metronidazole	443-48-1	$C_6H_9N_3O_3$	171.154	2.57/5.42	9500	-0.02	-0.00	-0.57	-0.57	-	1.000
Azithromycin	83905-01-5	$C_{38}H_{72}N_2O_{12}$	748.984	8.74	7.09	-	0.80[a]	-5.47	-1.73	2.5-2.7[d]	-
Roxithromycin	80214-83-1	$C_{41}H_{76}N_2O_{15}$	837.047	2.29/9.08/ 12.45/12.93/ 13.36/14.00/ 14.64	>60[a]	-	1.95[a]	-1.06	0.83	2.2-2.7[d] 2.3-2.6[d]	-
Clarithromycin	81103-11-9	$C_{38}H_{69}NO_{13}$	747.953	8.38/12.46/ 12.94/13.41/ 14.48	>60[a]	-	1.80[a]	-0.57	1.26	2.5-2.6[d]	-
Tylosin A	1401-79-0	$C_{46}H_{77}NO_{17}$	916.100	7.20/12.45/ 12.95/13.43/ 14.39/14.97	>60[a]	-	0.74[a]	-0.48	0.68	-	-
Josamycin	16846-24-15	$C_{42}H_{69}NO_{15}$	827.995	7.90/12.71/ 13.82/15.94	>60[a]	-	1.70[a]	-0.20	1.44	-	-
Spiramycin	8025-81-8	$C_{43}H_{74}N_2O_{14}$	843.053	8.54/9.35/ 12.94/13.85/ 14.73	>60[a]	-	2.14[a]	-3.62	0.14	-	-
Tilmicosin	10850-54-0	$C_{46}H_{80}N_2O_{13}$	869.133	7.93/9.58/ 12.55/13.14/ 13.75/14.67	>60[a]	-	3.23[a]	-2.09	1.38	-	-
Ofloxacin	82419-36-1	$C_{18}H_{20}FN_3O_4$	361.367	5.45/6.20	6.762e+005[c]	-	-2.00	0.61	-0.89	4.2[d]	1.648
Ciprofloxacin	85731-33-1	$C_{17}H_{18}FN_3O_3$	331.341	5.76/8.68	1.148e+004[c]	0.28	-0.00	-1.26	-1.16	4.3[d]	1.550
Enoxacin	74011-58-8	$C_{30}H_{40}F_2N_8O_9$	694.684	0.99/5.50/ 8.59	-	-	-0.98[a]	-	-	-	-
Danofloxacin	112398-08-0	$C_{19}H_{20}FN_3O_3$	357.379	5.65/6.73	5818[c]	-	0.44	0.70	-0.36	-	1.892
Enrofloxacin	93106-60-6	$C_{19}H_{22}FN_3O_3$	359.395	5.69/6.68	3397[c]	-	0.70	1.12	0.03	4.5[d]	1.922
Tetracycline	60-54-8	$C_{22}H_{24}N_2O_8$	444.435	2.92/7.61/ 8.19/8.76/ 12.07/14.11/ 15.77	231	-1.30	-1.33	-5.27	-5.92	3.9[d]	1.760
Doxycycline	564-25-0	$C_{22}H_{24}N_2O_8$	444.435	2.93/7.46/ 8.08/8.75/ 11.99/14.59/ 15.70/15.82	630	-0.02	-1.36	-4.76	-5.51	-	1.810
Sulfamethoxazole	723-46-6	$C_{10}H_{11}N_3O_3S$	253.278	0.25/1.97/ 6.16	610	0.89	0.48	0.84	-0.03	2.1-2.7[d] 2.3-2.6[d]	3.185
Sulfadiazine	68-35-9	$C_{10}H_{10}N_4O_2S$	250.277	2.01/6.99	77	-0.09	-0.34	0.33	-0.44	-	2.276
Trimethoprim	738-70-5	$C_{14}H_{18}N_4O_3$	290.318	7.16	400	0.91	0.73	0.09	0.99	2.2-2.6[d] 2.3[d]	2.957
Chloramphenicol	56-75-7	$C_{11}H_{12}Cl_2N_2O_5$	323.129	10.39/13.55/ 15.09	2500	1.14	0.92	0.57	0.57	-	1.000
Nifuroxazide	965-52-6	$C_{12}H_9N_3O_5$	275.217	8.33/13.04	1416[c]	-	1.49	1.80	1.64	-	3.874

Antibiotics											
Flumequine	42835-25-6	$C_{14}H_{12}FNO_3$	261.248	6.00	308.4[c]	-	2.60	1.96	0.26	-	1.853
Dimetridazole	551-92-8	$C_5H_7N_3O_2$	141.128	3.13	9.203e+005[c]	-	-1.23	0.18	0.18	-	1.341
Ronidazole	7681-76-7	$C_6H_8N_4O_4$	200.152	1.02/14.19	2900	-0.97	-0.38	-0.52	-0.52	-	1.469
Cefalexin	15686-71-2	$C_{16}H_{17}N_3O_4S$	347.389	3.45/7.23/ 11.91/12.72	1789[c]	0.65	0.40	-3.11	-3.69	-	2.822
Calcium channel blockers											
Diltiazem	42399-41-7	$C_{22}H_{26}N_2O_4S$	414.518	8.18/12.86	12.3[c]	2.70	2.79	0.51	2.28	-	3.978
Verapamil	152-11-4	$C_{27}H_{38}N_2O_4$	454.602	9.68	4.471[c]	3.79	4.80	1.11	2.87	-	6.666
Ashtma treatment drugs											
Salbutamol	18559-94-9	$C_{13}H_{21}NO_3$	239.311	9.40/10.12/ 14.18/15.15	1,43E+04[c]	-	0.64	-2.72	-1.06	-	1.501
Prostatic hyperplasia treatment drugs											
Tamsulosin	106463-17-6	$C_{20}H_{28}N_2O_5S$	408.512	9.28/9.33	215.9[c]	-	2.47	-0.93	0.80	-	5.285
Anticoagulant											
Warfarin	81-81-2	$C_{19}H_{16}O_4$	308.328	5.56	17	2.70	2.23	1.63	0.65	-	2.436
Antidiabetic											
Glibenclamide	10238-21-8	$C_{23}H_{28}ClN_3O_5S$	494.004	4.32/13.72	4	-	4.79	2.39	2.29	2.4[d]	4.402

(a) Predicted ChemAxon (https://www.chemaxon.com/products/marvin/marvinsketch/); **(b)** Predicted EPI Suite (www.chemspider.com); **(c)** estimated water solubility from log $K_{ow;}$ **(d)** from Verlicchi et al. 2012.