Elsevier
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, UK
Radarweg 29, PO Box 211, 1000 AE Amsterdam, The Netherlands

British Library Cataloguing in Publication Data
A catalog record for this book is available from the British Library

Library of Congress Cataloging-in-Publication Data
A catalogue record for this book is available from the Library of Congress

ISBN: 978-0-444-62657-8
ISSN: 0166-526X

Printed and bound in Poland

13 14 15 16 17 10 9 8 7 6 5 4 3 2 1

Contributors to Volume 62

Jaume Aceña, Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

Mustafa Al Aukidy, Department of Engineering, and Terra & AcquaTech Technopole of the University of Ferrara, Ferrara, Italy

Sandra Babić, Department of Analytical Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Zagreb, Croatia

Patrick J. Babin, Maladies Rares: Génétique et Métabolisme (MRGM), University of Bordeaux, Talence, France

Ruth Babington, Nb4D–Nanobiotechnology for Diagnostics, IQAC-CSIC, Barcelona, and Centro de Investigación Biomédica en Red en Bioingeniería Biomateriales y Nanomedicina (CIBER-BBN), Zaragoza, Spain

Jose Luis Balcázar, Catalan Institute for Water Research (ICRA), Girona, Spain

Zoran Banjac, Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

Carlos Barata, Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Spain

Damia Barcelo, IDAEA-CSIC, Department of Environmental Chemistry, Barcelona, and Catalan Institute for Water Research (ICRA), Girona, Spain

Josep M. Bayona, Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

Magnus Breitholtz, Department of Applied Environmental Science, Stockholm University, Stockholm, Sweden

Marta Broto, Nb4D–Nanobiotechnology for Diagnostics, IQAC-CSIC, Barcelona, and Centro de Investigación Biomédica en Red en Bioingeniería Biomateriales y Nanomedicina (CIBER-BBN), Zaragoza, Spain

Bruno Campos, Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Spain

Christian G. Daughton, Environmental Chemistry Branch, National Exposure Research Laboratory, U.S. Environmental Protection Agency, Las Vegas, Nevada, USA

Silvia Díaz-Cruz, Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

Davor Dolar, Department of Physical Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Zagreb, Croatia

Peter Eichhorn, Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

Despo Fatta-Kassinos, NIREAS, International Water Research Centre, University of Cyprus, Nicosia, Cyprus

Imma Ferrer, Center for Environmental Mass Spectrometry, Department of Environmental Engineering, University of Colorado, Boulder, Colorado, USA

Zacharias Frontistis, NIREAS, International Water Research Centre, University of Cyprus, Nicosia, Cyprus

Roger Galve, Nb4D–Nanobiotechnology for Diagnostics, IQAC-CSIC, Barcelona, and Centro de Investigación Biomédica en Red en Bioingeniería Biomateriales y Nanomedicina (CIBER-BBN), Zaragoza, Spain

María Jesús García-Galán, Catalan Institute for Water Research (ICRA), Girona, Spain

Natàlia García-Reyero, Institute for Genomics, Biocomputing & Biotechnology, Mississippi State University, Starkville, Mississippi, USA

Marlene Ågerstrand, Department of Applied Environmental Science, Stockholm University, Stockholm, Sweden

Antoni Ginebreda, Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

Belinda Huerta, Catalan Institute for Water Research (ICRA), Girona, Spain

Krešimir Košutić, Department of Physical Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Zagreb, Croatia

Maja Kuzmanovic, Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

Juan M. Lema, Department of Chemical Engineering, School of Engineering, University of Santiago de Compostela, Santiago de Compostela, Spain

Shan Liu, State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, China

Sung Kyu Maeng, Department of Civil and Environmental Engineering, Sejong University, Seoul, Republic of Korea

Maria-Pilar Marco, Nb4D–Nanobiotechnology for Diagnostics, IQAC-CSIC, Barcelona, and Centro de Investigación Biomédica en Red en Bioingeniería Biomateriales y Nanomedicina (CIBER-BBN), Zaragoza, Spain

Elisabet Marti, Catalan Institute for Water Research (ICRA), Girona, Spain

Víctor Matamoros, Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

Irene Michael, NIREAS, International Water Research Centre, University of Cyprus, Nicosia, Cyprus

Dragana Mutavdžić Pavlović, Department of Analytical Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Zagreb, Croatia

Francisco Omil, Department of Chemical Engineering, School of Engineering, University of Santiago de Compostela, Santiago de Compostela, Spain

Victoria Osorio, Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

Salvador Petanas, Nb4D–Nanobiotechnology for Diagnostics, IQAC-CSIC, Barcelona, and Centro de Investigación Biomédica en Red en Bioingeniería Biomateriales y Nanomedicina (CIBER-BBN), Zaragoza, Spain

Mira Petrovic, Catalan Institute for Water Research (ICRA), Girona, and ICREA, Catalan Institute for Research and Advanced Studies, Barcelona, Spain

Benjamin Piña, Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Spain

Sandra Pérez, Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

Lorenzo Proia, Catalan Institute for Water Research (ICRA), Girona, Spain

Demetrio Raldua, Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA, CSIC), Barcelona, Spain, and Maladies Rares: Génétique et Métabolisme (MRGM), University of Bordeaux, Talence, France

Ruben Reif, Department of Chemical Engineering, School of Engineering, University of Santiago de Compostela, Santiago de Compostela, Spain

Sara Rodríguez-Mozaz, Catalan Institute for Water Research (ICRA), Girona, Spain

Christina Rudén, Department of Applied Environmental Science, Stockholm University, Stockholm, Sweden

Carlos N.A. Salinas Rodriguez, Department of Civil and Environmental Engineering, Sejong University, Seoul, Republic of Korea

Saroj K. Sharma, UNESCO-IHE Institute for Water Education, Delft, The Netherlands

E. Michael Thurman, Center for Environmental Mass Spectrometry, Department of Environmental Engineering, University of Colorado, Boulder, Colorado, USA

Enrique Valera, Nb4D–Nanobiotechnology for Diagnostics, IQAC-CSIC, Barcelona, and Centro de Investigación Biomédica en Red en Bioingeniería Biomateriales y Nanomedicina (CIBER-BBN), Zaragoza, Spain

Paola Verlicchi, Center for Environmental Mass Spectrometry Department of Engineering, and Terra & AcquaTech Technopole of the University of Ferrara, Ferrara, Italy

Guang-Guo Ying, State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, China

Elena Zambello, Department of Engineering, and Terra & AcquaTech Technopole of the University of Ferrara, Ferrara, Italy

Jian-Liang Zhao, State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, China

Li-Jun Zhou, State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, China

Bozo Zonja, Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

Series Editor's Preface

Sometime in 2007, I wrote that, as series editor of Comprehensive Analytical Chemistry, I have certain duties. The first is to be able to acquire new titles for this successful series in the field of analytical chemistry. The second is that I should also bring in titles from my own field of expertise. In this respect, in 2003, I was coeditor of Volume 40 of the series *Analysis and Fate of Surfactants in the Aquatic Environment*, together with my two old friends, Thomas Knepper and Pim de Voogt. In 2007, and 10 volumes later, Volume 50 was published, again with me as coeditor together with my colleague Mira Petrovic. This was for the first edition of the present book *Analysis, Fate and Removal of Pharmaceuticals in the Water Cycle*. Now, in 2013, I am again coeditor of the second edition now in your hands, with a slightly modified title including the effects and risks, and we also have another colleague as coeditor, Sandra Peréz.

In the European Union, around 3000 different pharmaceutically active compounds are used in human medicine. Most modern drugs are small organic compounds, which are moderately water-soluble but still lipophilic, which allows them to be bioavailable and biologically active. They are designed to have specific pharmacological and physiological effects at low doses and thus are inherently potent, often with unintended outcomes for wildlife. Their consumption has increased over the years and will continue to increase due to the expanding population, general aging, increase of per capita consumption, expanding potential markets, and new target age groups.

After being administrated, pharmaceuticals are excreted via the liver and/or kidneys as a mixture of parent compounds and metabolites that are usually more polar and hydrophilic than the original drugs. Thus, after their usage for the intended purpose, a large fraction of these substances is discharged into wastewater, unchanged or in the form of degradation products, which are often not eliminated in conventional wastewater treatment plants. Depending on the efficiency of the treatment and chemical nature of these compounds, pharmaceuticals can reach surface and groundwaters. The need for research on the pathways of exposure, bioavailability, and risk assessment and risk management has been identified by a large number of scientists working in this field.

Pharmaceuticals commonly occur in treated sewage effluents, in surface waters, and in soil, sediments, sludge, biota, and tap water. Although the levels are generally low, there is rising concern about their potential long-term impacts on both humans and aquatic organisms, the latter being continuously

exposed to these compounds. These levels are capable of inducing acute effects in humans, that is, even though they are far below the recommended prescription dose, they have been found to affect aquatic ecosystems. Antibiotics and estrogens are among the many pharmaceuticals suspected of persisting in the environment due either to their resistance to natural biodegradation or to their continuous release.

Pharmaceuticals in the aquatic environment have been a topic of interest in conferences and in the literature for the last 20 years. One of the reasons for the increasing concern on pharmaceuticals has certainly been the improvement in analytical techniques. The use of various forms of liquid chromatography–tandem mass spectrometry includes exact mass measurement methods. It is possible to detect and confirm low levels of common pharmaceutical residues and their metabolites in water, solid, and biota samples. The fate of pharmaceuticals during sewage treatment is a key issue since wastewater treatment processes represent point source pollution of human pharmaceuticals. Investigation into removal technologies is also of high interest to the scientific community and the most common technologies being applied are included in the book. Finally, the growing occurrence of human and veterinary pharmaceuticals in the environment is driving toxicological studies and publications on ecological and risk assessment, including antibiotic resistance prioritization of the most harmful compounds with toxicity to different types of aquatic organisms, mainly daphnia, fish, and algae.

All the abovementioned topics have been included in the present book, which contains 21 chapters written by worldwide experts in the field, not only mainly from Europe and the United States but also from China. Analytical and environmental scientists will find a comprehensive view on the problems associated with the emerging and pseudopersistent problem of pharmaceutical residues in the environment. The book is addressed to a broad audience, from experts in the field to newcomers who will benefit from taking time out to familiarize themselves with its content.

Finally, I would like to thank all the authors, many of them friends and colleagues, for their efforts in compiling the literature references and writing their book chapters. I am especially thankful to my coworkers and colleagues in the department, Mira Petrovic and Sandra Pérez, for their efforts and time spent communicating with the different contributors of this comprehensive book on pharmaceuticals in the water cycle.

Damia Barcelo
Barcelona, August 2013

Preface

Pharmaceuticals are a diverse group of chemicals used in veterinary medicine, agricultural practices, human health, and cosmetic care. Many are highly bioactive, most are water soluble, and all (when present in the environment) occur usually at no more than trace concentrations.

Pharmaceuticals are a class of new, so-called "emerging" contaminants that have raised great concern in the last years. Human and veterinary drugs are continuously being released in the environment mainly as a result of the manufacturing processes, the disposal of unused or expired products, and the excreta. (i) They are referred to as "pseudo" persistent contaminants (i.e., high transformation/removal rates are compensated by their continuous introduction into environment), (ii) they are developed with the intention of exerting a desired biological effect, (iii) they often are moderately lipophilic to be able to cross membranes, and (iv) they are used by man in rather large quantities (i.e., similar to those of many pesticides).

The continuous introduction of pharmaceuticals and their bioactive metabolites into the environment may lead to a high long-term concentrations and promote continual, but unnoticed, adverse effects on aquatic and terrestrial organisms. The analytical methodology for the determination of trace pharmaceuticals in complex environmental matrices is still evolving and the number of methods described in the literature has grown considerably. Moreover, future introduction of selected pharmaceutical compounds on the regulatory lists (e.g., diclofenac) of the EU WFD and others such as carbamazepine (antiepileptic) and chloramphenicol (antibiotic) that are on the US EPA Contaminant Candidate List (CCL) as drinking water contaminants raise the interest for practical analytical methods and their applications in routine analysis. Attention has been paid during the last few years to develop a better understanding of the toxicology issues including low-dose multi-generational exposure to multiple chemical stressors and how human and ecological risks might be affected by these chemical cocktails.

The main objectives of this book is to provide the reader with a well-founded overview of the state of the art of the analytical methods for trace determination of pharmaceuticals in the environmental samples, and to give a review of the fate and occurrence of pharmaceuticals in the water cycle (elimination in wastewater and drinking water treatment), including latest developments in the treatment technologies, such as membrane bioreactors, advance oxidation, and natural attenuation processes. To reach these objectives, the book includes a concise and critical compilation of the information

published in the last years regarding the occurrence, analysis, and fate of pharmaceuticals in the environment. Following the first edition of this book in 2007, this book will extend the scope focusing on transformation products and including chapters on methods for elucidation of transformation pathways, transformation occurring in wastewater treatment processes, and transformations in the environment.

The book is structured with five parts:

The *first part* deals with the general introduction divided into two subchapters, the first one giving an overview of drug discovery and development in the pharmaceutical industry from the stage of compound design to clinical trials and marketing authorization. The second introduces the problem of pharmaceuticals as environmental contaminants.

The *second part* of the book is devoted to the analysis of pharmaceuticals and consists of five sub-chapters dealing with modern analytical techniques for the analysis of pharmaceuticals in the environment. It starts with discussion of needs for prioritization in selecting target compounds for chemical analysis and risk assessment. The following three chapters are devoted to highly sophisticated and established hyphenated mass spectrometric methods such as LC–MS and LC–MS–MS, and GC–MS used for target and nontarget analysis of aqueous samples (wastewater, surface, ground, and drinking water), solid matrices (soil, sediment, and sludge), and biota. In addition, sample preparation methods are thoroughly evaluated for all groups of pharmaceuticals including their major metabolites. Finally, one sub-chapter also addresses the application of bioassays and biosensors for the analysis of pharmaceuticals in the environment.

The *third part* deals with the removal of pharmaceuticals in wastewater and drinking water treatment, including also discussion of removal mechanisms. Of the treatment techniques discussed, not only conventional wastewater treatment (activated sludge) is evaluated, but also advanced treatment technologies such as biotic and abiotic membrane technologies, advanced oxidation processes, as well as natural treatments (constructed wetlands, bank filtration).

The *fourth part* gives an overview on occurrence data and fate in the aquatic and terrestrial environment, as well as an overview of evaluation of biotic and abiotic transformations in the environment through different analytical approaches.

Finally, the *fifth part* deals with the effect and risk assessment of pharmaceuticals. It will include chapters on field studies conducted to assess ecotoxicity, effects on biological communities, effects on microbial resistance, and finally evaluation on environmental risk assessment of pharmaceuticals.

The last chapter will summarize the current state of the art in the field and outline future trends and research needs.

Overall the present book is certainly timely since the interest and the developments in the analysis, fate, and removal of pharmaceuticals from the

environment have grown considerably during the last few years. This book will be of interest for a broader audience of analytical chemists and environmental scientists already working in the field of pharmaceuticals in the water cycle or newcomers who want to learn more about this emerging contamination problem.

Finally, we would like to thank all the contributing authors of this book for their time and efforts in preparing their chapters. Without their cooperation and engagement, this volume would certainly not have been possible.

Mira Petrovic, Damia Barcelo, and Sandra Pérez
Girona, July 2013

Chapter 1

General Introduction on Pharmaceuticals

Peter Eichhorn
Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

Chapter Outline

1 INTRODUCTION

The key objective of this introductory chapter is to provide basic knowledge to environmental scientists being involved in studying occurrence, fate, and effects of pharmaceuticals with respect to aspects pertaining to modern drug discovery and drug development. As these researchers commonly concentrate their efforts on understanding those processes that take place once a human

Comprehensive Analytical Chemistry, Vol. 62. http://dx.doi.org/10.1016/B978-0-444-62657-8.00001-X

drug, including metabolites, is discharged into the sewer, that is, anything that happens down the drain, this chapter provides a brief overview of the pharmaceutical industry. It addresses its economic importance and illustrates the fierce competition between major players, discusses strategies leading toward the discovery of new drugs, and summarizes the phases of drug development from the first clinical assay up to New Drug Application (United States) or Marketing Authorization Application (European Union) for obtaining marketing approval. Section 4 provides a concise picture on fundamental terms used in this highly interdisciplinary field and describes the role of physicochemical properties as the key determinants for absorption, distribution, metabolism, and excretion (ADME) of drugs.[a] While the distribution of a pharmaceutically active compound (API) between systemic circulation and periphery organs and tissues is of minor importance to what fraction eventually may enter wastewater streams, the other three components are of particular relevance as they directly affect the extent to which drugs, along with their biotransformation products, find entry into sewer systems. The role of drug metabolism in drug discovery is highlighted and the most common drug-metabolizing enzymes are portrayed. In the last part of this introduction, the position of major pharmaceutical companies on the presence of pharmaceuticals in the environment and their view on possible adverse effects on aquatic organisms and human health is reviewed.

2 THE PHARMACEUTICAL INDUSTRY

2.1 How Big Is Big Pharma?

The private pharmaceutical sector is undoubtedly the driving force in researching, developing, and marketing of innovative medicines for the prevention and treatment of pathological conditions and disorders. In the global pharmaceutical market, being worth more than 800 billion $US, the United States is the single largest market accounting for 37% of sales followed by Europe (28%) and Japan (12%) [1]. With the exception of TEVA as an Israel-based company, all other corporations in the top 20 are headquartered in these three geographic regions (Table 1). In their mission to improving health and quality of life of patients, the pharmaceutical industry puts major efforts in offering efficacious and safe quality drugs to patients who suffer from widespread, chronic diseases. Taking into account the ranking of causes of death in industrialized countries (cardiovascular diseases (24%), cancers (23%), chronic lower respiratory diseases (5.7%), and CNS diseases (5.1%) [2]), the incentives for targeting common diseases while rather neglecting

[a]As environmentally relevant pharmaceuticals fall into the category of small-molecule drugs, biologicals (naturally occurring or modified polypeptides, protein, DNA, or RNA products) are beyond the scope of this chapter and not further discussed.

TABLE 1 Sales Figures of Top 20 Global Pharmaceutical Companies in 2006–2011, in Billion $US (Total Audited Markets)

		2011	2010	2009	2008	2007	2006
Global Market	**Rank**	*855*	*795*	*754*	*727*	*670*	*608*
Pfizer	1	56.4	56.8	58.6	60.6	62.2	61.7
Novartis	2	51.6	46.9	41.9	39.5	36.9	33.6
Merck & Co.	3	40.1	37.5	38.0	38.5	38.4	35.1
Sanofi	4	39.5	38.5	38.2	39.0	36.4	33.4
AstraZeneca	5	37.0	35.9	34.7	32.7	30.0	27.4
Roche	6	34.9	33.0	32.6	30.1	27.0	23.0
GlaxoSmithKline	7	34.5	34.0	35.4	36.9	37.5	36.0
Johnson & Johnson	8	27.7	27.7	27.4	30.2	29.5	28.0
Abbott	9	25.9	24.3	23.3	22.7	20.3	18.6
Teva	10	23.9	24.5	21.8	20.8	18.2	16.3
Lilly	11	23.7	22.1	20.3	19.0	17.1	15.1
Takeda	12	17.8	16.8	18.1	18.1	16.9	15.5
Bristol-Myers Squibb	13	16.4	15.0	14.1	13.5	12.0	11.3
Bayer	14	16.4	15.7	15.6	15.7	13.9	12.2
Amgen	15	16.3	15.6	15.1	15.4	16.0	16.0
Böhringer Ingelheim	16	16.2	14.6	15.2	14.0	12.5	11.3
Novo Nordisk	17	11.2	9.73	8.60	7.94	6.73	5.76
Daiichi Sankyo	18	10.4	9.75	8.71	8.07	7.11	6.70
Otsuka	19	10.0	8.74	7.88	6.46	5.30	4.65
Mylan	20	8.98	8.02	6.89	6.12	5.96	0.51

Source: IMS Health Midas, December 2011; $US: sales and rank are in $US with quarterly exchange rates; sales cover direct and indirect pharmaceutical channel wholesalers and manufacturers. The figures in the preceding text include prescription and certain over the counter data and represent manufacturer prices.

disorders occurring at very low frequency are, unfortunately, all too obvious. With profit-driven businesses dominating the landscape, the priorities in the pharmaceutical industry are defined according to economic considerations. Whether this is ethically justifiable or not [3]—in the developing world tuberculosis and malaria are among the five most common causes of death—statistics confirms this preferences.

Among all other major industries, the pharmaceutical sector stands out with respect to the percentage of R&D expenditure on net sales [4]. With more than 15% of sales being reinvested to feed the R&D pipeline, it is far ahead of software and computer services (9.6%), technology hardware and equipment (7.8%), electronic and electrical equipment (4.2%), automobile industry (4.1%), the chemical industry (3.1%), or let alone oil and gas producers (0.4%). Unlike many other industries enjoying short innovation cycles with rapid returns on investment, the R&D processes in the pharmaceutical business are characterized by rather long-term investments into projects with intrinsically uncertain outcomes. The chances of unexpected failure at any point during the lengthy drug discovery and drug development are considerable (see Section 3), and even achieving the milestone of marketing authorization does by no means guarantee the economic success of a novel drug product during its patent lifetime (see Section 2.2). In absolute terms, the R&D spending for developing a single drug rose from an estimated 300 million $US in the year 1991 to 800 million $US at the turn of the century to 1.3 billion $US in 2005 [5]. In the United States alone, the pharmaceutical industry increased their spending from 23 to 55 billion $US over the period from 1999 to 2010, while productivity has at best remained flat staggering at 15–25 new drugs launched each year [6]. That these soaring costs have not translated into any statistically significant increase in the number of drug approvals is the topic of intense discussions and ongoing debates among the major players [7,8]. In response to the dropping productivity per dollar invested, the pressure to successfully place a product on the market is therefore higher than ever before.

2.2 Intellectual Property: Time Is Precious

Patents, as a property right granted by a sovereign state to the inventor of a novel, nonobvious, and useful invention, are at the heart of the R&D process [9,10]. The owner of a patent has the right to exclude competitors from making, using, offering for sale, or selling the invention for a period of 20 years from the filing of the patent application. The aim is to maximize the profit accruing to the inventor and those who have supplied the capital necessary to research and develop the product. Of those 20 years of patent protection, more than half the time is devoured by the R&D efforts as the patent is filed during the early stages of drug discovery. Once a new drug product with an outstanding benefit/risk ratio or a unique mode of action (first-in-class) has been launched on the market, it is only a question of time that competitor products with similar or improved properties become available to prescribers and patients [11]. By cautiously navigating around the competitor's patent space, follow-on drugs can share a great deal of structural features with the original compound up to the point where they are literally just a few atoms away [12]. One of the most striking examples is the phoshodiesterase

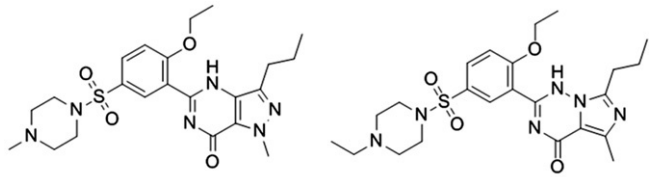

FIGURE 1 Chemical structures of first-in-class sildenafil (Viagra™, Pfizer) and follow-on vardenafil (Levitra™, Bayer). The dates indicate US registration date.

type 5 (PDE-5) inhibitor vardenafil (Levitra™), which differs from sildenafil (Viagra™) by the position of a single nitrogen atom and substitution of a methyl by an ethyl group (Figure 1). What may be considered a not particularly innovative approach of the inventor, it certainly was a smart one.[b]

How a single top-line product can contribute to the revenues of a pharmaceutical company is illustrated in Table 2, which compiles the global sales of drug best sellers for the period of 2007–2011. These blockbusters, that is, drugs generating >1 billion $US annually, can make up a substantial share in the overall revenues. In 2011, Bristol-Myers Squibb's platelet aggregation inhibitor clopidogrel (Plavix™) as the second best-selling product on the list accounted for a 57% of its global sales (see Table 1). Shareholders will unquestionably greet this success with standing ovations but the day of patent expiration is irrevocably approaching. Like carrion feeders awaiting the ailing animal to perish, generic drug manufacturers with bioequivalence certificates in their pockets are in the starting blocks to flood the market with cheap copies of the drug product on the day after a patent expires. The foreseeable yet abrupt decline in sales figures is shown in Figure 2 for three blockbusters beyond the time period of Table 2. The patent of Pfizer's cash cow Lipitor™, containing the blood cholesterol-lowering atorvastatin, expired in the United States in November 2011. Within half a year, sales had dropped by more than threefold.

3 DRUG DISCOVERY AND DRUG DEVELOPMENT

3.1 Discovery: Screening Thousands of Compounds

The starting point for drug discovery programs is the realization that for a given medical condition or disease, no suitable medicines are available to patients. It is this unmet clinical need that motivates pharmaceutical companies to embark on this lengthy and cost-intensive venture [14]. First-in-class drugs with novel mechanisms of action aiming at unproven targets have an

[b]The structurally very dissimilar tadalafil (Cialis™, Eli Lilly), reaching the market as the third PDE-5 inhibitor, achieved blockbuster status and outperformed vardenafil in part because of its considerably longer plasma half-life [13]. This resulted in duration of effectiveness of as long as 24–36 h and the drug was nicknamed "weekend pill."

TABLE 2 Evolution of Global Sales of Top 20 Products in 2007–2011, in Billion $US (Total Audited Markets)

Rank	Brand	Active Pharmaceutical Ingredient	Company	Therapeutic Class	2011	2010	2009	2008	2007
1	Lipitor	Atorvastatin	Pfizer	Lipid regulator	12.5	12.7	13.3	13.7	13.4
2	Plavix	Clopidogrel	Bristol-Myers Squibb	Platelet aggregation inhibitor	9.3	8.8	9.1	8.7	7.3
3	Seretide	Fluticasone/salmeterol	GlaxoSmithKline	Respiratory agents	8.7	8.6	8.2	7.8	7.2
4	Crestor	Rosuvastatin	AstraZeneca	Lipid regulator	8.0	6.8	5.4	4.0	3.0
5	Nexium	Esomeprazole	AstraZeneca	Antiulcerant	7.9	8.4	8.2	7.8	7.1
6	Seroquel	Quetiapine	AstraZeneca	Antipsychotic	7.6	6.8	6.0	5.4	4.6
7	Humira	Adalimumab[a]	Abbott	Autoimmune agents	7.3	6.0	5.1	4.0	2.7
8	Enbrel	Etanercept[b]	Pfizer	Autoimmune agents	6.8	6.2	5.9	5.5	5.0
9	Remicade	Infliximab[a]	Janssen	Autoimmune agents	6.8	6.1	5.5	4.9	4.2
10	Abilify	Aripiprazole	Otsuka	Antipsychotic	6.3	5.4	4.7	3.6	2.7
11	Singulair	Montelukast	Merck	Respiratory agents	6.1	5.5	5.0	4.6	4.4
12	Zyprexa	Olanzapine	Lilly	Antipsychotic	5.7	5.7	5.4	5.1	5.0
13	Mabthera	Rituximab[a]	Roche	Oncologics	5.7	5.1	4.6	4.4	3.7
14	Lantus	Insulin glargine[b]	Sanofi-Aventis	Antidiabetics	5.5	4.7	4.0	3.4	2.7
15	Avastin	Bevacizumab[a]	Genentech	Oncologics	5.4	5.6	5.0	4.0	2.8

16	Herceptin	Trastuzumab[a]	Roche	Oncologics	4.8	4.3	3.8	3.7	3.2
17	Cymbalta	Duloxetine	Lilly	Antipsychotic	4.7	3.9	3.4	2.8	2.2
18	Spiriva	Tiotropium	Böhringer Ingelheim	Respiratory agents	4.7	4.0	3.5	3.1	2.5
19	Neulasta	Pegfilgrastim[b]	Amgen	Immunomodulator	4.2	3.8	3.7	3.7	3.5
20	Glivec	Imatinib	Novartis	Oncologics	4.1	3.8	3.4	3.3	2.7

APIs not marked with "[a]" and "[b]" are small-molecule drugs.
[a]*Monoclonal antibody.*
[b]*Other protein drugs (etanercept is a fusion protein produced by recombinant DNA; insulin glargine is a long-acting basal insulin analogue; pegfilgrastim is a PEGylated form of the recombinant human granulocyte colony-stimulating factor (GCSF) analogue filgrastim).*
Source: IMS Health MIDAS, December 2011; $US: sales and rank are in $US with quarterly exchange rates.

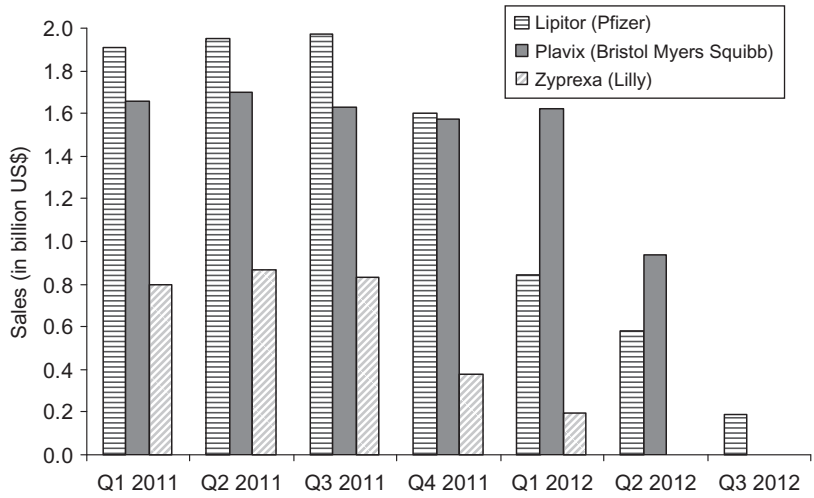

FIGURE 2 Effect of patent expiration on US quarterly sales figures of three of the top 20 (2011) drugs (see Table 2). (Lipitor: November 2011; Plavix: May 2012; Zyprexa: October 2011). Missing columns in Q2 and Q3 of 2012 indicate that data were not available.

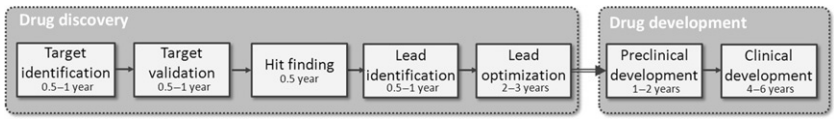

FIGURE 3 Phases and approximate timelines of drug discovery and development.

inherently higher risk of failure than so-called follow-on drugs that take advantage of preclinical or even clinical validation of the target. For a follow-on drug to be competitive, that is, economically successful, climbing on the bandwagon always implies to strive for launching the best-in-class.

It is often at academic institutions that the knowledge on the understanding of biochemical pathways as the sequence of chemical reactions in a biological organism is generated. If there is sufficient scientific evidence that inhibition or activation of such signaling cascades might be exploited to modulate a disease state, a new discovery program is born (Figure 3). Once a promising target has been identified, it needs to be validated by applying a combination of *in vitro* tools and animal models [15,16]. During the next phase, high-throughput screening (HTS) campaigns are commonly run in order to identify molecules from large libraries (>100,000 different compounds of highly diverse chemical structures) that display specific activity at the macromolecular target. In addition to these biological assays that are typically performed in 384- or 1536-well plate format, inexpensive computational screens can aid in designing virtual compounds provided that the X-ray structure of the target protein is known. Positive HTS hits are then further characterized with respect

to potency (determination of IC_{50} values), their activity in functional assays (cell or tissue-based), and the ability to modify their target affinity by structural changes of the molecule (exploring structure–activity relationships). At the end of this hit-finding phase, a small number of compounds with the most promising profile are selected for further optimization relying on classical organic synthesis. The subsequent lead identification phase begins by defining a screening cascade that consists of a broad panel of assays designed to discard compounds with suboptimal properties as early as possible while advancing interesting candidates to the next level. Being aware of time pressure and budget constraints, the fundamental notion of pharmaceutical researchers in drug discovery is as follows: fail early, fail cheap. In fact, it was not until the 1990s that the necessity to optimize compound properties far beyond the essential requirements of potency, activity, and selectivity was recognized. An instructive comparison of trends in clinical attrition rates between 1991 and 2000 indicated that the primary factor causing drug failure in 1991 was unacceptable pharmacokinetics (PK) profile in humans [17]. A stunning 40% of compounds failed in the clinics due to PK and bioavailability issues. By the year 2000, this number had dropped to $<10\%$ as consequence of adopting several preclinical screens to address ADME issues. Nowadays, these are fully implemented at strategic positions in screening cascades; microsomal stability (see Section 5.1), membrane permeability, and cytochrome P450 (CYP450) inhibition constitute decisive filters at early stages, while animal PK, *in vivo* tissue distribution, characterization of metabolites, and identification of excretion routes (see Section 4.1) are investigated for more advanced molecules.

The lead identification phase concludes with picking lead compounds from distinct chemical series deemed to have the largest potential to undergo further optimization. Besides tuning of ADME properties, compounds are tested in depth for efficacy in mechanistic animal models being predictive of human disease and are subject to early safety and toxicity studies in preclinical species. In view of the wide scope of the screening cascade addressing all aspects from potency to drug–drug interactions to potential hepatotoxicity upon repeated dosing to animals, drug discovery is unquestionably a very challenging undertaking, and identifying a suitable candidate molecule (and subsequently a backup compound) for development is by no means guaranteed. If no compound meeting the criteria defined in the target candidate profile is obtained within a reasonable timeframe, program cancellation as the last resort becomes inevitable.

3.2 Development: Is This the Right Compound?

After candidate selection, the preclinical phase of the drug development is initiated (Figure 3). One of the key objectives is the generation of enough information that supports the safety of the investigational drug since the

compilation of a comprehensive data package is required to eventually convince regulators and clinical ethical committees to grant permission for starting the first-in-man studies. Toxicology studies in animal species are performed to determine the effects of organ function and to identify target organ for toxicity. By establishing dose–response curves of toxic effects in animal testing, maximum admissible doses are defined for the first dosing in humans on the basis of no observed adverse effect levels and safety factors [18]. In addition, a comprehensive panel of *in vitro* studies is run to assess potential immunotoxicity, mutagenicity, and genotoxicity. Safety pharmacology studies are carried out to investigate the potential undesirable pharmacodynamic (PD) effects of the drug substance on physiological functions. The battery of tests evaluates effects on cardiovascular function, respiratory function, and behavior of the central nervous system [19]. Pharmaceutical development is another important component that deals with converting the active ingredient (drug substance) into a dosage form (drug product). Formulations suitable for the administration route need to be developed; mechanical, physical, and chemical characterizations of the drug product are performed including the development of analytical methodologies [20].

After successful completion of the preclinical development, the sponsor submits an application (clinical trial application) to the regulatory agency, which contains information related to PD, PK, safety pharmacology, toxicology, and the estimation on the first dose in humans, in order to obtain authorization for initiating clinical trials. If authorization is granted, the investigational drug (product) can be tested for the very first time in humans. Depending on the number and type of subjects enrolled, the endpoints measured, and the geographic scope, clinical trials can be divided into three major phases [21]:

- Clinical phase I

  The major objective of phase I studies is to assess safety and tolerability of a new drug. It usually consists of administering single, ascending doses to determine the maximally tolerated dose while closely monitoring any side effects. The studies involve some 10–20 individuals, usually healthy volunteers, although for certain indications such as cancer or HIV, individuals suffering from the disease are treated. Frequent sampling of blood in conjunction with collection of excrements (urine and feces) allows to analyze the PK profiles, to identify the metabolic routes, and to determine the relevance of excretion of unchanged drug as compared to that of metabolites. Giving the compound by intravenous administration allows to assess the absolute bioavailability for routes other than direct injection or infusion into the bloodstream. Phase I studies are completed within less than a year and are conducted in an uncontrolled and unblinded manner.

- Clinical phase II

  In phase II studies, the major goal is to evaluate the efficacy and to establish dose–response curves. By recruiting about 200–300 patients with the target disease (their selection is based on well-defined entry criteria), the efficacious dose is determined. Depending on availability of standard comparators, the efficacy can either be benchmarked against an existing agent with an identical mode of action or one approved for the same indication, or it can be compared with the effect of a placebo. In this series of studies where patients receive the drug in a chronic dosing regimen over a period of several weeks, any observations on side effects or adverse events are thoroughly reported. In addition, in phase II studies, the potential of any drug–drug interactions is assessed and common risks for specific populations (e.g., the elderly or patients with renal impairment) are identified. The total duration of the phase II studies is up to 2 years.
- Clinical phase III

  Upon obtaining satisfactory outcomes in the phase II studies, the efficacy is now to be confirmed in a large patient population comprising several hundreds to thousands of patients with the target disease from geographically diverse background. A typical phase III study is a multicenter, randomized, double-blinded, placebo-controlled trial in which the patients are divided into treatment and control groups in a random manner and neither the overseeing physician nor the patient knows whether the administered formulation contains the API or not (placebo). Individual studies for chronic treatment with the drug under development can extend over a year, while the entire series of phase III trial commonly has a duration of 2–4 years. In the end, the overall risk–benefit ratio has to be evaluated.

Despite the tremendous amount of information gathered during the six to eight years of discovery and preclinical development for the single compound under investigation, the road toward achieving the next milestone is paved with uncertainties, and all too often with unexpected, and above all insurmountable, obstacles that make to abandon the entire project. Khanna analyzed the productivity trend in clinical trials for the years 2009–2010 [22]. In phase I, the rate of success was 70% for all molecules having been approved as new investigational drugs but a mere 17.5% survived phase II studies. Conducting phase III studies further reduced the number of molecules to 8.5%. That means that the success rate of the most expensive phase of the overall R&D efforts, accounting for about one-third of the overall costs, amounted to a mere 50%. Eventually, only 6% achieved the level of new drug application. Regarding the reasons for attrition during clinical phase II trials, Arrowsmith reported for the period of 2008–2010 that out of 87 drugs (including new drugs and new indications for existing drugs), 51% failed due to insufficient efficacy, while 29% were discarded for strategic reasons [23].

For 19% of drugs, safety concerns were the reason for failure. On the other hand, issues associated with poor PK or bioavailability accounted for only 1% of failure. Considering phase III and submission failures for 83 drugs analyzed in the period of 2007–2010, insufficient efficacy was by far the major reason for failure (66%), while safety issues, including unfavorable risk–benefit evaluation, accounted for 21% of failure [24].

3.3 Regulatory Review and Beyond

The last hurdle to market access is the thorough drug review process in which regulatory agencies assess safety, quality, and efficacy of new medicines. The evaluation of the application by the responsible authorities (Food and Drug Administration (FDA) in the United States; European Medicines Agency (EMA) in the European Union; and Ministry of Health, Labour and Welfare in Japan) comprises four main stages: receipt of dossier, scientific assessment, sponsor response, and issuing of authorization (or rejection in case of unsuccessful application) [25]. For novel therapeutics, the median total review time for small-molecule drugs submitted between 2001 and 2010 was 314 days reviewed by the FDA and 366 days by the EMA [26]. In case of a positive decision of the regulatory agency, the approved drug is ready to be manufactured, distributed, and launched on the market(s) and thereby enters into the so-called phase IV where continuous postmarketing surveillance (pharmacovigilance) is initiated. Under uncontrolled and observational conditions, patient safety is monitored and any (unexpected) adverse events are reported back to the marketing authorization holder within a context of an epidemiological focus. Furthermore, the observations may help identify additional indications of the drug for future approval. As the patient population usually exceeds by far the size of that enrolled in clinical phase III studies and duration of treatment may be much longer, adverse drug reactions that were not observed in clinical trials may surface. Depending on the severity of the events and the level of evidence for causal relationship with drug treatment, the approved product may either be excluded for treatment of certain subpopulations or, in the worst case, the market authorization may be revoked. Of a total of 548 new chemical entities that had been approved by the FDA between 1975 and 1999, 45 drugs acquired so-called black box warnings (alerts that appear on the package insert to indicate that the drug carries a significant risk of serious adverse effects) whereas 16 were withdrawn from the market [27]. In such instances, the economic damage to the company and the negative impact on the perception by prescribers and patients may be considerable.

4 PHARMACOKINETICS AND PHARMACODYNAMICS

In order to exert its desired pharmacological effect, a drug has to travel from the site of administration (or application) to the target site where the binding

to the receptor stimulates a biological response. Depending on the affinity of the drug to the biochemical target, a certain local concentration of the ligand is required. Whereas the study of these concentration-dependent effects of a drug to the body is referred to as PD, the processes describing how the human body handles a drug are termed pharmacokinetics (PK).

4.1 ADME: A Journey Through the Body

The schematic in Figure 4 depicts the key processes a drug is subject to after oral dosing as the most frequently used route of drug administration [28–30]. It illustrates that on its way to the site of action (in this case located in a peripheral compartment), a combination of physical and particularly biological events governs its fate in the organism. When taken as a solid pharmaceutical formulation (tablet and capsule), the API has to be released by disintegration and can then dissolve in the fluids of the gastrointestinal tract (GIT). Given the large pH difference between stomach and intestine, the solubility of acidic and basic drugs can greatly vary in these two sections of the GIT. Although drug absorption through the gastric mucosa is possible, its small surface area of only about 0.053 m^2 limits the importance of this pathway. In contrast, the surface area of the small intestine of about 250 m^2 in conjunction with a transit time of 2–4 h makes intestinal absorption the major route of entry into the bloodstream [31]. As only compound being present in the dissolved phase is available for crossing the brush-border membrane of the enterocytes, the undissolved fraction of the drug is subject to fecal excretion in unaltered form and thus becomes a sewage-borne pollutant.

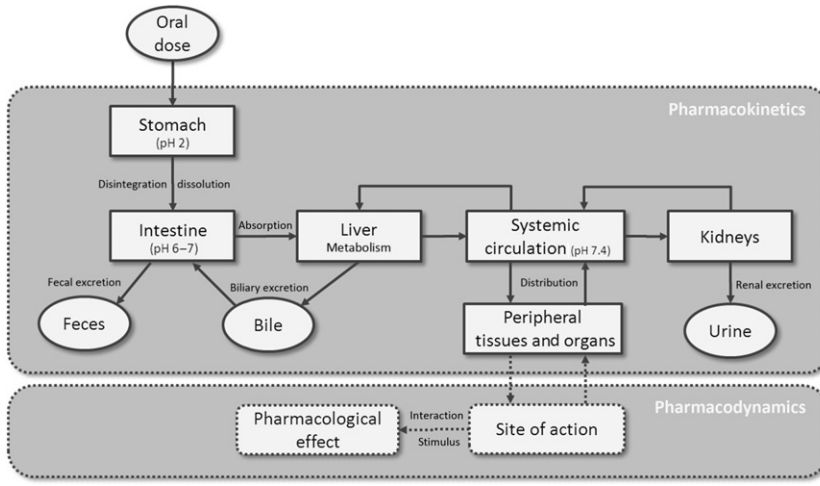

FIGURE 4 Schematic of pharmacokinetics of orally dosed drug and interface with pharmacodynamics.

Although active transport across the intestinal epithelium applies to some compounds, passive diffusion is the determining factor for most drugs, that is, the concentration gradient is the driving force for entry into the enterocytes [32]. The extent and rate of intestinal uptake of the soluble fraction depends on the physicochemical properties of the compound. The intrinsic permeability is a composite of molecular size, charge state at intestinal pH, and lipophilicity (expressed as the logarithm of the *n*-octanol–water partition coefficient, log *P*). As cell membranes are composed of phospholipid bilayers in which negatively charged phosphate groups are exposed to the lumen and cytosol, respectively, while the lipophilic tails are directed toward the inside of the membrane, the diffusion rate correlates positively with log *P*. Simply put, the compound has to be "soluble" in the lipophilic environment of the cell membrane (too high log *P*, however, is counterproductive as the molecule remains inside the lipid membrane). This circumstance implies that an ionic character of a drug molecule is unfavorable for passive diffusion and therefore compounds being uncharged at intestinal pH generally exhibit higher permeation rates than charged ones. Once the drug has reached capillaries irrigating the gut wall, the bloodstream takes it through the portal vein to the liver as the point of entry into systemic circulation. The liver represents the second important barrier before drug distribution in the organism can take place, and its unique function in protecting the body from potentially hazardous substances is reflected by the physiological phenomenon that it is the only organ receiving venous blood in addition to direct supply of oxygenated blood through the hepatic artery (adding up to about 25% of cardiac output). Upon uptake into hepatocytes through the sinusoidal membrane, a high affinity of the drug to metabolizing enzymes can already substantially reduce the amount of drug eventually available for distribution. Biotransformation during the first passage through the liver is termed first-pass effect and, for obvious reasons, is an undesirable process. The fraction surviving the attack of hepatic enzymes is then distributed between the central and peripheral compartments of the cardiovascular system. According to Figure 4 in which the drug target, for example, an enzyme, receptor, or ion channel is proposed to reside in a specific organ, the drug reaches the site of action and ultimately interacts with macromolecules to trigger the pharmacological response (carbamazepine as a frequently detected environmental contaminant, e.g., acts by blocking neuronal sodium channels in the brain and therefore limits repetitive firing of action potentials).

Besides the liberation of the API from the formulated product, it is the complex interplay of the four processes of ADME that defines and governs the overall fate. Since the human body recognizes drugs as foreign substances lacking an apparent physiological benefit, defense mechanism has been put in place during the evolution of mankind in order to eliminate them from the organism. While the aforementioned first-pass effect stands at the frontline of defense, the efficiency of metabolic reactions in conjunction with excretory

processes do ultimately limit the lifetime inside the organism. The function of the former is to convert drugs into more polar, water-soluble metabolites with improved susceptibility for excretion. With the liver being the major metabolizing organ, metabolites either can directly be secreted across the canalicular membrane of the hepatocytes into bile that, following intermediate storage in the gall bladder, is drained through the bile duct into the duodenum or can diffuse back into the bloodstream (at first glance, it may seem contradictory that more polar compounds formed in an intracellular space such as the liver cells are more amenable to excretion—membrane permeability is negatively correlated with polarity—but active transporter protein embedded in the cell membrane are capable of performing this task even against concentration gradients). Traveling further down to the distal parts of the small intestine and on to the large intestine, the ultimate fate is excretion with fecal matters. The other important route of excretion of drug metabolites is accomplished by the kidneys where glomerular filtration and active tubular secretion help eliminate biotransformation products. Apart from excretion of metabolites, certain drugs may also be excreted in unchanged form. Irrespective of the route of elimination, however, the drug will show up along with its metabolites in raw sewage at the inlet of sewage treatment plants (this, of course, assumes that sanitary wastes are collected in the first place, which probably does not apply to many less developed countries).

4.2 Reducing the Release of Bioactive Drugs into the Environment: Not as Easy as It Appears

Commissioning an environmental scientist with devising strategies to minimizing the environmental input of orally dosed drugs based on the simplified scheme outlined in Figure 4, the logical answers would likely include these PD-related aspects:

- Enhancing affinity of the drug toward the macromolecular target
- Maximizing effect concentrations at the site of action
- Perhaps achieving a sustained stimulus for a long-lasting effect even after drug concentrations in the effect compartment have started to decline

All this is addressed during the biological screening process in drug discovery (see Section 3.1) where optimization of potency and activity at the target is a primary goal. As far as the PK side of the story is concerned, possible solutions would include

- fast liberation of the API and rapid and complete dissolution in the GIT fluids,
- quantitative absorption through the gut wall to avoid any excretion of intact drug without appearing in systemic circulation,
- reduction of hepatic first pass to maximize oral bioavailability,

- minimization of affinity to drug-metabolizing enzymes in the liver for sustained circulating blood levels,
- avoidance of direct excretion of intact parent drug into bile or urine (perhaps the most relevant aspect for reducing the release of bioactive pharmaceutical compounds into the environment).

Medicinal chemists, scientists running biological screening assays, and ADME experts will undoubtedly endorse the recommendations but the daily routine in drug discovery tells that all those processes and pathways are interwoven in a highly complex way that can only be successfully addressed by carefully balancing out compound properties. To illustrate the challenges faced in designing small-molecule drugs with suitable ADME properties, one needs to take into account that the physicochemical properties may work in opposite directions on the various parameters determining the overall fate of drug molecules in the human body. But in the first place, it is the drug binding to the target that defines which molecular entities are ligands with high affinity. The task of developing quantitative structure–activity relationships is definitely not a trivial one. Since most biochemical targets are proteins involved in signal transduction cascades, for their physiological role to be modulated in a planned manner, the drug molecule has to establish specific interactions at the molecular level with amino acids constituting its structure [33]. Through a combination of polar (hydrogen bonding) and hydrophobic interactions (van der Waals), the drug binds tightly to the protein, competitively replaces its natural ligand from the binding site, and eventually impairs or enhances the normal signaling function. Let us imagine a highly potent ligand for a given protein target has been identified by conducting *in vitro* binding assays. It now just has to make it from the oral cavity to the receptor (e.g., orally taken selegiline inhibits the mitochondrial enzyme monoamine oxidase B in the brain and thus prevents neurotransmitters such as dopamine from being broken down [34]).

Uncoupling the PD requirements from the PK properties (ignoring the latter has been shown to be highly detrimental for a successful drug development (see Section 3.1)), a high compound solubility would be considered desirable in terms of achieving the maximum soluble fraction in the intestinal lumen. However, the incorporation of polar functional groups, in particular ionizable moieties that act as charge carriers (e.g., carboxylic acids and basic aliphatic amines), leads to a drop in membrane permeability that compromises the movement of drug molecules across the multiple membrane barriers encountered on its way to the site of action [35]. Therefore, a hydrophilic–lipophilic balance needs to be found in order to accommodate good solubility (at this point, the dose plays an important role) and acceptable membrane permeability in the same molecular structure. At the same time, too high a lipophilic character ($\log P$) makes the compound more

susceptible to CYP450-mediated metabolic reactions (see Section 5.1) in the liver and thus results in faster drug clearance. Although not explicitly depicted in Figure 4, plasma protein binding (PPB) [36,37] is a key determinant in the distribution of a drug between central and peripheral compartments (it also influences hepatic and renal clearance). Almost all drugs bind to some extent to abundant plasma proteins with neutral and acidic drugs showing preference for serum albumin while basic compounds preferably attach to α_1-acid glycoprotein. As a general rule, the PPB of a drug increases with increasing lipophilicity and is most prominent for acidic compounds that strongly bind through electrostatic interactions to protonated amino acid residues in albumin. The extent of PPB in turn affects the distribution behavior insofar as only the free drug fraction in blood plasma can diffuse across membranes. Finally, the affinity of a drug to tissue (components) is again largely influenced by charge and lipophilicity. Due to electrostatic interactions with negatively charged phospholipids forming the membrane bilayer, basic drugs exhibit a pronounced affinity for tissues, whereas acidic drug molecules are rather repulsed and thus tissue–blood concentration ratios are fairly low. Another aspect to be considered is molecular size (and shape): in general terms, solubility decreases with increasing molecular weight (MW), so does membrane permeability.

In conclusion, this brief survey of ADME principles has illustrated that rational drug design requires a multifactorial optimization [38]. Modulation of the physicochemical properties has a direct consequence not only on the interaction with the drug target itself but also on many of the processes governing the disposition in the organism. As far as drug elimination, that is, the combined effects of metabolism and excretion, is concerned, the clearing organs of the human body—first and foremost the liver and kidney—are actively pursuing to eliminate the exogenous substance, regardless of the pathway. Although it may be of concern to the environmental scientist whether a drug is subject to excretion in unaltered form or extensively metabolized in the liver to inactive biotransformation products, to a sophisticated system such as the human body, this difference does not matter: clearance is its ultimate mission.

4.3 Physicochemical Space: What Do Drugs Look Like?

The previous section has illustrated how changes of physicochemical properties of small-molecule drugs modulate their ADME profile (see Section 5.1). Beside the fact that medicinal chemists consciously design molecules with drug-like properties and through reiterative processes optimize their PK and PD characteristics, most marketed (oral) drugs eventually fall within a certain range of molecular properties. The most frequently used parameters used to describe the structural features that affect the physicochemical properties are

MW, lipophilicity (log P), number of hydrogen bond donors (OH, NH groups), and hydrogen bond acceptors (O, N). The landmark paper by Lipinski et al. [39,40] examined the impact of these four parameters of a total of 2245 orally dosed drugs with respect to solubility and permeability as crucial requirements for acceptable oral bioavailability (see previous section). Their comprehensive analysis revealed that about 90% of molecules complied with what later became to be known as the rule of five (RO5): MW $\leq$500, log P $\leq$5, H-bond donors $\leq$5, and H-bond acceptors $\leq$10. Compounds outside that range were less likely to exhibit satisfactory bioavailability; nonetheless, there do exist clearly successful drugs beyond the space defined by the RO5, such as macrolide antibiotics. Following the revealing findings of Lipinski's computational approach, trends between therapeutics classes were compared, the evolution over time was examined, and drugs with parenteral routes of administration were included [41–43]. By adding further, readily accessible molecular properties describing structural flexibility (number of rotatable bonds), rigidity (ring count), and the solvent-exposed surface area covered by polar atoms (so-called polar surface area), valuable insights into the determinants of physicochemical properties have been gained. The data presented in Table 3 nicely illustrate what structural properties define different drug classes based on their administration route. The values for oral drugs in the lower half of the table are in close agreement with the RO5. Injectable drugs, in turn, reside in a different range of molecular properties characterized by lower lipophilicity (high aqueous solubility is required) and fewer constraints regarding MW.

5 DRUG METABOLISM

With the growing interest in including human metabolites of pharmaceuticals in environmental monitoring surveys in order to generate a more comprehensive picture of the fate, a concise overview of the major human drug-metabolizing enzymes and pathways is presented here. Metabolic reactions can broadly be classified into phase I reactions (hydrolysis, oxidation, and reduction) and phase II reactions (conjugation) [44,45] (Figure 5). Although in some instances, a combination of both classes is required to convert the drug into a readily excretable species, modification of the chemical structure by a single enzyme may be sufficient to generate a metabolite that is subject to rapid excretion. As indicated earlier, hepatic metabolism represents the most prominent pathway with its inherent possibility of direct secretion of the metabolite into bile (it may return to systemic blood by passive diffusion or active transport across the sinusoidal membrane), but other organs such as the kidneys and lungs may also play a role in affording metabolic inactivation. It is worth noting that hepatic drug clearance commonly displays large interindividual differences that are determined by genetics, sex, and age but are also influenced by disease state [46].

TABLE 3 Molecular Properties of Marketed Drugs

Route	MW	clogP	O + N	OH + NH	H-Bond Acceptors	H-Bond Donors	Rings	Rotatable Bonds	PSA (in Å^2)
	Mean values								
Oral (1193)	343.7	2.3	5.5	1.8	3.2	1.8	2.6	5.4	78
Absorbent (116)	392.3	1.6	6.5	3.0	3.6	3.0	2.5	7.9	101
Injectable (308)	558.2	0.6	11.3	4.7	6.2	4.7	3.2	12.7	144
Topical (112)	368.5	2.9	5.0	1.9	3.2	1.8	2.9	5.3	75
	10–90% percentiles								
Oral (1193)	200–475	−0.8–5.2	2–9	0–3	1–6	0–3	1–4	1–10	22–134
Absorbent (116)	172–666	−2.3–4.8	2–14	0–7	1–7	0–7	0–4	2–16	20–219
Injectable (308)	196–1085	−3.3–4.9	3–23	0–11	1–11	0–11	1–6	2–27	28–311
Topical (112)	188–495	−0.6–6.0	2–8	0–3	0–5	0–3	1–5	1–9	21–114

The class "absorbent" refers to dosage forms in which the drug is anticipated to absorb through membranes (ophthalmic, otic, nasal, inhalation, vaginal, or rectal). "Injectable" denotes drugs for intramuscular, intravenous, or subcutaneous administration.
Adapted from Ref. [42].

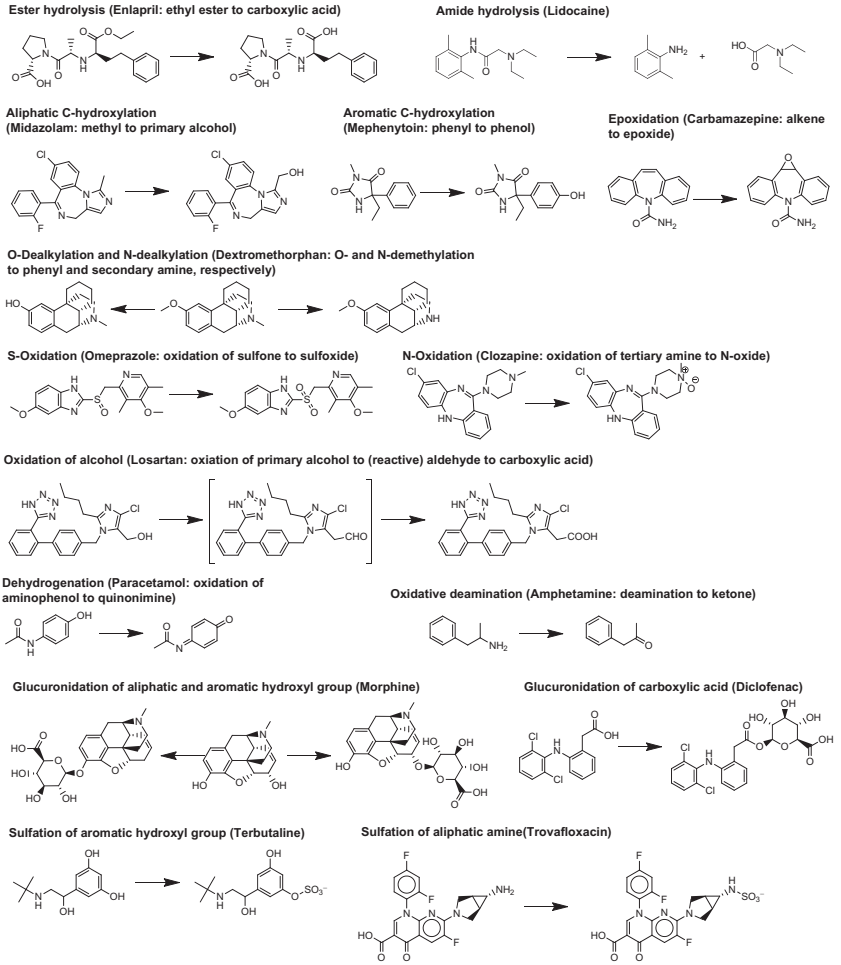

FIGURE 5 Examples of relevant phase I and phase II biotransformations.

5.1 Phase I and Phase II Reactions: Whoever Is Faster

Hydrolytic reactions mediated by widely distributed esterases (also present in blood plasma itself) afford the cleavage of ester and amide bonds thereby releasing two far more polar molecular entities [47]. In view of the general ease of hydrolyzing esters, the presence of ester bonds in small-molecule drugs is uncommon as the bond *per se* is highly prone to hydrolysis. The exception to this rule is so-called prodrugs (e.g., enalapril, oseltamivir, and adefovir dipivoxil) that are specifically designed to produce active drugs upon hydrolysis [48,49]. Applying this simple synthetic strategy allows to convert drugs with suboptimal properties in terms of membrane permeability or intestinal solubility into molecules with greatly improved ADME profiles.

With respect to oxidative reactions, the monooxygenase CYP450 is by far the most prominent drug-metabolizing enzyme being responsible for metabolism of 70–80% of all marketed drugs [50,51]. Of the more than 50 CYP families identified to date in humans, metabolizing thousands of endogenous and exogenous compounds, those isoforms involved in oxidizing drug molecules belong mainly to the families CYP1, CYP2, and CYP3 (order of importance: CYP3A4 > CYP2C9 ~ CYP2C19 > CYP2D6 ~ CYP1A2 > CYP1A1 ~ CYP2B6 ~ CYP2E1). These membrane-bounded enzymes, residing in the endoplasmic reticulum (ER) of cells, catalyze the NADPH-dependent oxidation of structurally diverse substrates. The selectivity of the various isoforms ranked earlier depends on structural features of the substrate including size, charge, lipophilicity, and shape [52]. CYP450 accomplishes C-oxidations (aliphatic hydroxylation and aromatic hydroxylation), alkene epoxidations, heteroatom dealkylations (cleavages of C–N, C–O, and C–S bond[c]), and oxidations of alcohol and aldehydes (Figure 5). Furthermore, they are capable of oxidizing aromatic amines to the corresponding hydroxylamines, while N-oxygenations of tertiary amines and N-heterocycles give rise to N-oxides. Heteroatom oxidation of thioether-bearing drugs can produce the corresponding sulfoxide and sulfone. The catalytic repertoire of the versatile CYP450 enzyme system also includes dehydrogenation reactions and oxidative deaminations. The second most important enzyme catalyzing oxidations of drugs is flavin-containing monooxygenase, which, unlike CYP, exclusively oxidizes nucleophilic heteroatom-containing substrates [53]. Regardless the involved enzymes, the large majority of these reactions enhance the polarity of the substrate and frequently imply partial or complete loss of pharmacological activity (as mentioned earlier, the interactions with the macromolecular target are sensitive to structural modifications such as the incorporation of an oxygen atom).

This fundamental principle holds true even more in case of phase II reactions in which a larger moiety is catalytically transferred from the cofactor to the substrate and covalently bound to an existing functional group with nucleophilic character. The two most relevant transferases are soluble cytosolic sulfotransferases (SULT), conjugating hydroxyl and amino groups with sulfonate [54], and ER-membrane-embedded UDP-glucuronosyltransferases (UGT) that link glucuronic acid to hydroxyl, thiol, amino, and carboxylic acid groups present in the substrate [55]. As SULTs are high-affinity but low-capacity enzymes, sulfate conjugates are quantitatively less important in humans than glucuronides. In addition, depletion of the cofactor 3'-phosphoadenosine-5'-phosphosulfate (PAPS) may become the limiting factor for extensive sulfation. A decisive feature of many sulfate and glucuronide conjugates is the

[c]By comparing substrate and metabolite structures of dealkylation reactions (Figure 5), it may not be immediately obvious where the oxidation has taken place. In fact, it is the carbon atom in the leaving group that has been oxidized to a carbonyl function.

susceptibility of the formed bond to enzymatic hydrolysis. In particular, sulfate esters originating from the sulfation of alcohols or phenols and acyl glucuronides (esters) formed by conjugation of carboxylic acids can be easily cleaved to release the free drug molecule. When such labile conjugates undergo biliary excretion and are subsequently discharged into the small intestine, microbial sulfatases and glucuronidases produced by gut microflora can liberate the unconjugated product. From a PK perspective, this may give rise to a phenomenon called enterohepatic recirculation in which reabsorption of the parent drug through the intestinal epithelium enables reentering the blood flow in the portal vein (see Figure 4) [56]. From a mass balance point of view, which focuses on determining the fraction of the administered dose exiting the human body intact, enzymatic breakdown of drug conjugates translates into excretion of bioactive compound and consequently adds to the environmental burden. In this context, the question of the relevance and impact of conjugate cleavage in biological sewage treatment by mixed microbial communities has been brought up [57,58]. The liberation of the active drug has been speculated to be associated with the observation that for certain compounds, such as diclofenac [59], the levels measured in treated effluents exceed those in corresponding raw sewage samples.

5.2 Metabolism Versus Direct Excretion: A Delicate Balance

In their quest for discovering suitable candidates to be progressed to the development stage, scientists working in drug discovery teams do probably not share the concerns—if they are aware of the current environmental discussion at all—related to the extent the parent drug may appear in altered form or as readily cleavable conjugate, in feces and urine. In the first place, the criteria for selecting appropriate drug candidates include *in vitro* potency, *in vivo* efficacy in animal models, promising ADME profile in preclinical species, and absence of relevant safety issues. It is not until conducting the first-in-man studies (see Section 3.2) that quantitative data on elimination pathways become available.

In most oral drug discovery programs, metabolic clearance is required to be low as this increases the likelihood of achieving high exposure (see Figure 4) and ultimately ensures the necessary compound levels in the effect compartment. During lead identification and lead optimization (see Figure 3), a set of *in vitro* and *in vivo* tools are employed to assess the metabolic fate in quantitative terms (kinetic measurements) and from a qualitative perspective (metabolite identification). Incubations with subcellular liver fractions prepared from preclinical species and human donors allow to address both aspects in samples obtained in the same experiment. Given the importance of CYP450 in the biotransformation of drugs, microsomal incubations are commonly the starting point in metabolic screening [60]. Based on mass spectrometry-assisted structure elucidation of the metabolites, the medicinal

chemist then attempts to eliminate or deactivate soft metabolic spots in the molecule. To circumvent, for example, parahydroxylation on aromatic rings (see Section 5.1), the hydrogen atom on the paraposition of the phenyl can be substituted with fluorine [61].

To assess the potential for glucuronidation, liver microsomal incubations supplemented with the cofactor UDPGA are performed and the formation of glucuronides is confirmed by mass spectrometric analysis based on their diagnostic mass shift. For compounds having advanced in the optimization process, incubations in hepatocyte suspensions allow to investigate the role of different metabolic pathways since these the cells contain the full complement of drug-metabolizing enzymes [62]. Among the preferred substrates of UGT are compounds bearing either phenolic hydroxyl groups or carboxylic acids. Whenever drug molecules contain carboxylic acids—in some cases their presence may be required for achieving strong binding to the macromolecular target through electrostatic interactions [63]—there is a good chance that glucuronidation takes place in humans. Prominent examples include the nonsteroidal anti-inflammatories diclofenac, naproxen, ketoprofen, and indomethacin [64]. Although from an environmental perspective, oxidative metabolism appears advantageous in terms of generating products with largely reduced biological activity, synthetic strategies rather aim to reduce the relevance of this pathway. Regardless the balance of phase I and phase II metabolism, or combinations thereof, the extent of direct renal or biliary excretion in humans is difficult to predict based on the outcomes of excretion studies in preclinical species [65,66]. Prior to selecting drug candidates for development, mass balance studies are conducted in animals by collecting urine and feces (or preferably of bile if bile duct ligation is technically feasible) following intravenous bolus administration. But due to interspecies differences in metabolic enzyme activity and expression of drug transporter proteins [67], the findings do not necessarily translate to the behavior in humans. Therefore, it is not until the first clinical studies that the presence of intact drugs, or readily cleavable conjugates, can be confirmed and quantified.

6 PHARMACEUTICALS IN THE ENVIRONMENT: THE MANUFACTURERS' VIEW

Given the scientific interest in occurrence, fate, and effects of pharmaceuticals in the environment, and in particular the public concern about the potential hazards posed to human health by the consumption of drinking water containing traces of common drugs, pharmaceutical companies have become aware of the situation and the need to take initiatives to better understand any environmental and human health impact. Table 4 compiles key information posted on the websites of a number of selected Big Pharma representatives as regards the presence of pharmaceuticals in the environment. To begin with, they stress that their positive detection is closely linked to the improvements of analytical

TABLE 4 Summary of Position Papers of Major Pharmaceutical Companies Regarding the Presence and Effects of Human Pharmaceuticals in the Environment

	Bristol-Myers Squibb [68]	GlaxoSmithKline [69]	Lilly [70]	Novartis [71]	Pfizer [72]	Roche [73]	Sanofi [74]
Reason for detectability	Improved analytical methods	Improvements in analytical capabilities	Modern advances in chemical measurement techniques		Advances in analytical technology	Ever-increasing sensitivity of analytical methods	Improvement in analytical methods since the mid-1970s
Occurrence in the environment	At extremely low levels (concentrations in the ppb or ppt range)	At extremely low levels	At concentrations usually in the range of ppt or lower		At trace levels (tiny amounts)	In low (ppb) to extremely low (ppt) concentrations	In very low concentrations (ng/L or µg/L)
Major route of entry into environment	Patient use and excretion of unmetabolized materials	Residues of the pharmaceutical or its breakdown products (i.e., metabolites) may be excreted as part of normal biological processes	Pharmaceutical active ingredients are routinely broken down or eliminated by the body	Pharmaceuticals entering the aquatic environment are an inevitable consequence of business activity and of science-based healthcare treatment	Prescribed and normal patient use and excretion (accounting for over 90% of the detected concentrations)	Patient use (increasing rate of widely metabolized and readily degradable biopharmaceuticals in company is a welcome development)	After pharmaceuticals are absorbed or administered, they are partly excreted by patients either in the same form or as metabolites

Minor route of entry into environment	Unused medicines discarded by consumers	Unused products or via pharmaceutical manufacturing discharges			Improper disposal of unused medicines and normal manufacturing discharges	Manufacturing process and from improper disposal of unused medicines	Effluent from drug production plants and discharge resulting from the inappropriate disposal of unused medicines
Risk to human health and environment	Studies conducted to date indicate it is highly unlikely the quantities of pharmaceuticals detected in the environment would be harmful to human health	Unlikely to affect human health at the levels detected (according to WHO's 2011 Technical Report on Pharmaceuticals in Drinking Water) Some potential for impact on aquatic life Carries out state-of-the-art environment testing on all their pharmaceuticals and use these data in risk assessments to evaluate potential for harm to human health and the	Information published to date shows the extremely low concentrations in surface waters are very unlikely to be harmful to human health or have short-term impacts on aquatic organisms The potential for subtle and long-term effects on aquatic organisms is still being studied by the scientific community	Believes that the levels of APIs found in the environment do not present a health risk for humans, as they are below the doses approved as safe by medicinal regulatory agencies according to current knowledge	Based on current observations it is very unlikely that exposure to very low levels of pharmaceuticals in drinking water would result in appreciable adverse effects on human health There are no reported adverse human health effects attributed to drugs in the aquatic environment; recent studies including those from the WHO	Environment quantities are in general far below the level at which they have been shown to have a therapeutic or adverse effect in humans Even a lifetime of consuming drinking water containing these trace concentrations of APIs would not correspond to one single daily therapeutic dose of the respective pharmaceuticals	Risk to human health appears low in light of small concentrations based on current information Environment risks are a genuine concern, particularly for certain classes of pharmaceutical products such as hormonal substances, cytotoxic drugs, and antibiotics

Continued

TABLE 4 Summary of Position Papers of Major Pharmaceutical Companies Regarding the Presence and Effects of Human Pharmaceuticals in the Environment—Cont'd

Bristol-Myers Squibb [68]	GlaxoSmithKline [69]	Lilly [70]	Novartis [71]	Pfizer [72]	Roche [73]	Sanofi [74]
	environment. Results of these assessments indicate no adverse impact to public health or the environment from post-patient releases of GlaxoSmithKline (GSK) pharmaceuticals to the environment Comparisons of measured concentrations with predicted no effect concentration (PNECs) for humans find that the levels of pharmaceuticals present in the environment are too low to pose any acute or chronic risk to people			conclude that trace amounts of pharmaceuticals measured in water should not be of concern to human health even if consumed for many years Data currently fail to show any connection between the concentration of pharmaceuticals detected in the aquatic environment and acute environment effects (exception:	Potential long-term effects of low concentrations and the potential combination effects need to be investigated further To date, studies concur that the low levels do not cause short-term impact to aquatic life Further studies are needed to evaluate the potential effects associated with long-term exposure of aquatic organisms	

	However, questions about the potential for chronic effects on aquatic life for multiple compounds or certain classes of compounds have been raised		certain hormones) Some studies suggest that in specific situations, chronic environment exposure of certain species (e.g., fish) to select classes of pharmaceuticals (e.g., hormones) may be linked with environment effects Further studies are needed to determine any environment effects arising from chronic exposure	There are indications that certain hormones (in particular sex hormones) and other substances exhibiting hormone-like activity may have detrimental long-term effects on aquatic populations

Continued

TABLE 4 Summary of Position Papers of Major Pharmaceutical Companies Regarding the Presence and Effects of Human Pharmaceuticals in the Environment—Cont'd

	Bristol-Myers Squibb [68]	GlaxoSmithKline [69]	Lilly [70]	Novartis [71]	Pfizer [72]	Roche [73]	Sanofi [74]
Actions	Collects an extensive amount of ecotoxicological information about own compounds to support environmental assessments required as part of a New Drug Application (NDA)	Performs environmental risk assessment (ERA) to meet current regulatory requirements and internal global environment, health, and safety standards for all new pharmaceutical and consumer healthcare products before they are launched	Tests and assesses own medicines for potential effects on the environment to meet current regulatory requirements and internal standards before new medicines are launched Regularly updates testing protocols for new and existing pharmaceuticals as knowledge and testing methods improve	Researches the potential impacts of newly developed medicines on human and environment health already at an early stage in their R&D process, and, where necessary, develops tailored strategies to minimize that impact	Since implementation of the EU ERA Guidelines (2006), a comprehensive ERA data package (chronic effects, fate, and physical–chemical properties) has been developed on most New Chemical Entity (NCEs) registered in the past 5 years	Carries out investigations and supports or contributes to research programs to better understand the human and environment health impacts of pharmaceuticals in the environment (PIE) and to promote appropriate approaches to wastewater treatment Investigates new APIs for biodegradability and initial ecotoxicity during their development Develops ERA based on chronic environment effects and advanced	ERA is currently required as part of the marketing authorization application dossier for any new pharmaceutical launched on the market in the EU, United States, and some other countries Is committed to improving their knowledge about the potential environment impact, if any, of own products already on the market (ERA for several marketed drugs on voluntary basis)

			environment fate data and is required by regulations Investigates older APIs, normally at a simpler scale, in order to assess their environment risks (not a regulatory requirement)	In total some 30 of own major products have been analyzed → no significant environment risk at the expected environment concentration
Manufacturing processes and waste disposal	Designs clean and efficient pharmaceutical manufacturing processes that do not have an adverse impact on the environment Treats wastewater from manufacturing facilities efficiently before being discharged to the environment	Strives to minimize discharges of APIs in their wastewater Incinerates, whenever possible, pharmaceutical waste from their operations	Manufacturing processes and facilities are designed and operated to ensure that, as far as practicable, the APIs are not discharged into the wastewater All aqueous manufacturing emissions are treated in wastewater treatment plant (WWTPs), where a	Points out that some recent publications suggest that the emissions from manufacturing may be significant at a local level and may have an environment impact

Continued

TABLE 4 Summary of Position Papers of Major Pharmaceutical Companies Regarding the Presence and Effects of Human Pharmaceuticals in the Environment—Cont'd

	Bristol-Myers Squibb [68]	GlaxoSmithKline [69]	Lilly [70]	Novartis [71]	Pfizer [72]	Roche [73]	Sanofi [74]
	Treatment is provided by company owned and operated on-site infrastructure or off-site municipal WWTP, or a combination of both					significant part of this waste is degradable and thus readily removed via biological mechanisms If required by risk assessments, facilities pretreat wastewater using additional technologies prior to discharge	
Collaborations	Works closely with regulatory and environment agencies such as the US FDA, US EPA, and the USGS to ensure the potential impact of pharmaceuticals on the aquatic	Continues to work with industry groups and regulators to develop the science and methodologies to continually evaluate our products and management practices	Continues to collaborate with regulatory, academic, and research organizations to identify new data needs on the transport, fate, and effects of pharmaceuticals	Supports research initiatives that advance society's understanding about the environment fate and effects of pharmaceuticals	Pfizer works directly with and in partnership with other member companies on trade associations (e.g., PhRMA, EFPIA) to ensure relevant science	Has provided during the past decade financial support and technical assistance for academic research programs and investigations into the presence, effects, and risks of PIE	Participates in the voluntary environment classification system initiated by the Swedish Association of the Pharmaceutical Industry (LIF)

	environment and on human health is understood and minimized		in the environment Has published articles and made presentations to drinking water and wastewater forums on the topic of pharmaceuticals in the environment for many years	Actively supports academia, regulators, and other stakeholders in developing more efficient risk assessment practices for pharmaceuticals in the environment	is understood and where necessary, further advanced to best ensure these activities do not pose risk to human health and the aquatic environment		Acquires essential information through collaborative projects within the pharmaceutical industry in Europe and the United States, through membership in trade groups such as PhRMA, EFPIA, LEEM, and LIF. The aim of these projects is to assess the potential impact of pharmaceuticals in the environment, including for human health
Information sharing practices	Safety data sheets available on website		Summary results from environment fate and effect studies are available in		Effective management and communication of risk based on sound science	Has published several in-depth ERA of important older own APIs in scientific literature	

Continued

TABLE 4 Summary of Position Papers of Major Pharmaceutical Companies Regarding the Presence and Effects of Human Pharmaceuticals in the Environment—Cont'd

	Bristol-Myers Squibb [68]	GlaxoSmithKline [69]	Lilly [70]	Novartis [71]	Pfizer [72]	Roche [73]	Sanofi [74]
		Publishes environment data, assessments, and related topics in the scientific literature Works with regulators to ensure that relevant precautions are included on labels and in information to patients	product safety data sheets and are routinely updated		should ensure a well-informed public, regulatory community and industry. Through this approach, stakeholders should be better assured that controls are protective of human health and the environment	Makes available to the public its safety data sheets, which contain relevant environment data on their APIs	
Disposal methods of unused and returned medication		Encourages proper and safe disposal by patients and supports the use of approved voluntary "take-back" programs in the	Support efforts to educate the public in the United States about the proper drug disposal			Has established financial incentives to ensure that unused or outdated products are returned by retailers	Supports take-back programs for unused medicines where applicable and available

communities and countries where they are available Endorses the Federal Guidelines on the Proper Disposal of Prescription Pharmaceuticals developed by the White House Office of National Drug Control Policy and supports the SMARxT Disposal initiative	methods for unused medicines through trade association sponsorship of the SMARxT DISPOSAL™ website	and others in the supply chain Requires any returned or waste pharmaceutical product to be incinerated rather than disposed of in landfills Participates in pharmaceutical take-back programs in the EU and supports the use of existing local take-back programs in the United States and elsewhere, as well as the implementation of take-back programs on national levels	

sensitivity afforded by modern instrumentation, which has allowed to measure levels ranging from "tiny amounts" to "extremely low concentrations" (it is true that typical therapeutic blood levels by far exceed environmental concentrations). There is general agreement that excretion of drugs (and metabolites) is a logical consequence of patient use and constitutes the major source of entry into the environment, whereas discharges of unused medicines and effluents from manufacturing facilities are secondary. The exposure of humans through intake of contaminated drinking water is considered very minor and deemed to pose no risk of therapeutic or adverse effects. The position papers recognize the need for conducting further studies to assess long-term effects in aquatic organisms caused by chronic exposure. In this respect, drug classes of concern that are specifically mentioned include hormones, antibiotics, and cytotoxic compounds. From a regulatory perspective, all seven corporations portrayed in Table 4 point out that they conduct mandatory environmental risk assessments as integral component of the marketing authorization application of new drugs [75–78]. In addition, they all assert to participate in various collaborations with industry groups, governmental agencies, and academic research groups committed to improve the knowledge about the environmental impact of pharmaceuticals.

The state of the science in analysis, removal, effects, and risk is the topic of this book in which researchers from academia and independent research institutions provide their views on drug residues in the environment.

REFERENCES

[1] IMS Health Market Prognosis, London, United Kingdom, 2012.
[2] D.L. Hoyert, J. Xu, Natl. Vital Stat. Rep. 61 (2012) 1.
[3] H. Brody, Med. Care 50 (2012) 910.
[4] European Commission, The 2010 EU Industrial R&D Investment Scoreboard, Joint Research Centre, Seville, Spain, 2010.
[5] N.A. Meanwell, Chem. Res. Toxicol. 24 (2011) 1420.
[6] Future Pharma, Five Strategies to Accelerate the Transformation of the Pharmaceutical Industry by 2020, London, United Kingdom, 2011.
[7] J.W. Scannell, A. Blanckley, H. Boldon, B. Warrington, Nat. Rev. Drug Discov. 11 (2012) 191.
[8] E.F. Schmid, D.A. Smith, Drug Discov. Today 10 (2005) 1031.
[9] M.A. Bader, O. Gassmann, N. Ziegler, F. Ruether, Drug Discov. Today 17 (2012) 281.
[10] C.S. Hemphill, B.N. Sampat, J. Heal. Econ. 31 (2012) 327.
[11] J.A. Dimasi, L.B. Faden, Nat. Rev. Drug Discov. 10 (2011) 23.
[12] F. Giordanetto, J. Boström, C. Tyrchan, Drug Discov. Today 16 (2011) 722.
[13] P.J. Wright, Int. J. Clin. Pract. 60 (2006) 967.
[14] J.P. Hughes, S.S. Rees, S.B. Kalindjian, K.L. Philpott, Br. J. Pharmacol. 162 (2011) 1239.
[15] E.H. Kerns, J. Pharm. Sci. 90 (2001) 1838.
[16] E.H. Kerns, L. Di, Drug Discov. Today 8 (2003) 316.
[17] I. Kola, J. Landis, Nat. Rev. Drug Discov. 3 (2004) 711.

[18] B.G. Reigner, K. Blesch, Eur. J. Clin. Pharmacol. 57 (2002) 835.
[19] L. Ewart, D.J. Gallacher, G. Gintant, J.M. Guillon, D. Leishman, P. Levesque, N. McMahon, L. Mylecraine, M. Sanders, W. Suter, R. Wallis, J.P. Valentin, J. Pharmacol. Toxicol. Methods 66 (2012) 66.
[20] P. Narayan, Curr. Protoc. Pharmacol. (Suppl. 55) (2011) 7.3.1–7.3.29.
[21] C.A. Umscheid, D.J. Margolis, C.E. Grossman, Postgrad. Med. 123 (2011) 194.
[22] I. Khanna, Drug Discov. Today 17 (2012) 1088.
[23] J. Arrowsmith, Nat. Rev. Drug Discov. 10 (2011) 328.
[24] J. Arrowsmith, Nat. Rev. Drug Discov. 10 (2011) 87.
[25] M. Hirako, N. McAuslane, S. Salek, C. Anderson, S. Walker, Drug Inf. J. 41 (2007) 291.
[26] N.S. Downing, J.A. Aminawung, N.D. Shah, J.B. Braunstein, H.M. Krumholz, J.S. Ross, N. Engl. J. Med. 366 (2012) 2284.
[27] K.E. Lasser, P.D. Allen, S.J. Woolhandler, D.U. Himmelstein, S.M. Wolfe, D.H. Bor, JAMA 287 (2002) 2215.
[28] M.S. Benedetti, R. Whomsley, I. Poggesi, W. Cawello, F.X. Mathy, M.L. Delporte, P. Papeleu, J.B. Watelet, Drug Metab. Rev. 41 (2009) 344.
[29] S. Chillistone, J. Hardman, Anaesth. Intens. Care Med. 9 (2008) 167.
[30] B. Testa, P. Crivori, M. Reist, P.A. Carrupt, Perspect. Drug Discov. Des. 19 (2000) 179.
[31] J.M. deSesso, A.L. Williams, Chapter 21: Contrasting the gastrointestinal tracts of mammals: factors that influence absorption, in: Annual Reports in Medicinal Chemistry, vol. 43, Elsevier, 2008, p. 353.
[32] S. Hurst, C.M. Loi, J. Brodfuehrer, A. El-Kattan, Expert Opin. Drug Metab. Toxicol. 3 (2007) 469.
[33] C. Bissantz, B. Kuhn, M. Stahl, J. Med. Chem. 53 (2010) 5061.
[34] M.S. Song, D. Matveychuk, E.M. MacKenzie, M. Duchcherer, D.D. Mousseau, G.B. Baker, Prog. Neuropsychopharmacol. Biol. Psychiatry 44 (2013) 118.
[35] D.F. Veber, S.R. Johnson, H.Y. Cheng, B.R. Smith, K.W. Ward, K.D. Kopple, J. Med. Chem. 45 (2002) 2615.
[36] R. Mehvar, Am. J. Pharm. Educ. 69 (2005) 1.
[37] S. Schmidt, D. Gonzalez, H. Derendorf, J. Pharm. Sci. 99 (2010) 1107.
[38] M.P. Gleeson, J. Med. Chem. 51 (2008) 817.
[39] C.A. Lipinski, J. Pharmacol. Toxicol. Methods 44 (2000) 235.
[40] C.A. Lipinski, Drug Discov. Today Technol. 1 (2004) 337.
[41] J.R. Proudfoot, Bioorg. Med. Chem. Lett. 15 (2005) 1087.
[42] M. Vieth, M.G. Siegel, R.E. Higgs, I.A. Watson, D.H. Robertson, K.A. Savin, G.L. Durst, P.A. Hipskind, J. Med. Chem. 47 (2004) 224.
[43] M.C. Wenlock, R.P. Austin, P. Barton, A.M. Davis, P.D. Leeson, J. Med. Chem. 46 (2003) 1250.
[44] M. Pirmohamed, Medicine 36 (2008) 355.
[45] B. Testa, Chem. Biodivers. 6 (2009) 2055.
[46] A. Rostami-Hodjegan, G.T. Tucker, Nat. Rev. Drug Discov. 6 (2007) 140.
[47] F.G. Bahar, K. Ohura, T. Ogihara, T. Imai, J. Pharm. Sci. 101 (2012) 3979.
[48] J. Rautio, H. Kumpulainen, T. Heimbach, R. Oliyai, D. Oh, T. Järvinen, J. Savolainen, Nat. Rev. Drug Discov. 7 (2008) 255.
[49] V.J. Stella, K.W. Nti-Addae, Adv. Drug Deliv. Rev. 59 (2007) 677.
[50] F.P. Guengerich, Chem. Res. Toxicol. 14 (2001) 611.
[51] F.P. Guengerich, AAPS J. 8 (2006) E105.

[52] D.F. Lewis, M. Dickins, Drug Discov. Today 7 (2002) 918.
[53] J.R. Cashman, Expert Opin. Drug Metab. Toxicol. 4 (2008) 1507.
[54] N. Gamage, A. Barnett, N. Hempel, R.G. Duggleby, K.F. Windmill, J.L. Martin, M.E. McManus, Toxicol. Sci. 90 (2006) 5.
[55] M.B. Fisher, M.F. Paine, T.J. Strelevitz, S.A. Wrighton, Drug Metab. Rev. 33 (2001) 273.
[56] M.S. Roberts, B.M. Magnusson, F.J. Burczynski, M. Weiss, Clin. Pharmacokinet. 41 (2002) 751.
[57] H.J. Lee, E. Lee, S.H. Yoon, H.R. Chang, K. Kim, J.H. Kwon, Chemosphere 87 (2012) 969.
[58] V. Kumar, A.C. Johnson, N. Nakada, N. Yamashita, H. Tanaka, J. Hazard. Mater. 227–228 (2012) 49.
[59] H.B. Lee, T.E. Peart, M.L. Svoboda, J. Chromatogr. A 1094 (2005) 122.
[60] N. Plant, Drug Discov. Today 9 (2004) 328.
[61] M.B. Fisher, K.R. Henne, J. Boer, Curr. Opin. Drug Discov. Dev. 9 (2006) 101.
[62] N.J. Hewitt, M.J.G. Lechón, J.B. Houston, D. Hallifax, H.S. Brown, P. Maurel, J.G. Kenna, L. Gustavsson, C. Lohmann, C. Skonberg, A. Guillouzo, G. Tuschl, A.P. Li, E. Lecluyse, G.M.M. Groothuis, J.G. Hengstler, Drug Metabol. Rev. 39 (2007) 159.
[63] C.J. Hawkey, Best Pract. Res. Clin. Gastroenterol. 15 (2001) 801.
[64] M.J. Bailey, R.G. Dickinson, Chem. Biol. Interact. 145 (2003) 117.
[65] Y. Lai, Expert Opin. Drug Metab. Toxicol. 5 (2009) 1175.
[66] I. Mahmood, Life Sci. 63 (1998) 2365.
[67] R. Masereeuw, F. Russel, Drug Discov. Today Technol. 1 (2004) 357.
[68] Bristol-Myers Squibb, Wastewater Effluents. http://www.bms.com/sustainability/environmental_performance/Pages/wastewater_effluents.aspx, (accessed 17.04.13).
[69] GlaxoSmithKline, Pharmaceuticals in the Environment. www.gsk.com/policies/GSK-on-pharmaceuticals-in-environment.pdf, (accessed 17.04.13).
[70] Lilly, Lilly's Position on Pharmaceuticals in the Environment. http://www.lilly.com/Documents/Pharmaceuticals%20in%20the%20Environment%20Position%20Statement.pdf, (accessed 17.04.13).
[71] Novartis, Pharmaceuticals in the Environment. http://www.novartis.com/downloads/corporate-responsibility/resources/positions/pharmaceuticals-environment.pdf, (accessed 17.04.13).
[72] Pfizer, Pharmaceuticals in the Environment (PIE). http://www.pfizer.com/files/responsibility/protecting_environment/WWP_Purple_Paper-Pharmaceuticals_in_the_Environment.pdf, (accessed 17.04.13).
[73] Roche, Roche Position on Pharmaceuticals in the Environment (PIE). www.roche.com/pharmaceuticals_in_the_environment.pdf, (accessed 17.04.13).
[74] Sanofi, Pharmaceuticals in the Environment. http://csrreporting.sanofi.com/web/planet/pharmaceuticals_environnement, (accessed 17.04.13).
[75] European Medicines Agency, Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use, Committee for Medicinal Products for Human Use (CHMP), London, United Kingdom, 2006.
[76] Food and Drug Administration, Environmental Assessment of Human Drug and Biologics Applications, Center for Drug Evaluation and Research, Rockville, MD, 1998.
[77] K. Webber, P. Spindler, Drug Inf. J. 41 (2007) 155.
[78] European Medicines Agency, Questions and Answers on 'Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use, Committee for Medicinal Products for Human Use (CHMP), London, United Kingdom, 2006.

Chapter 2

Pharmaceuticals in the Environment: Sources and Their Management

Christian G. Daughton
Environmental Chemistry Branch, National Exposure Research Laboratory, U.S. Environmental Protection Agency, Las Vegas, Nevada, USA

Chapter Outline

Comprehensive Analytical Chemistry, Vol. 62. http://dx.doi.org/10.1016/B978-0-444-62657-8.00002-1

1 INTRODUCTION

Many issues and concerns surround the presence in the environment of anthropogenic chemical contaminants. Those involving pharmaceuticals can be extremely complex—made even more so by a vast network of interacting variables, coupled with numerous unknowns spanning human actions, activities, and behaviors and environmental processes (Figure 1). A comprehensive understanding of the overall problem requires an integrated study of the spectrum of drugs that establish a presence in the environment, in what quantities and by what routes they enter the environment (loadings and resulting concentrations), their transport and fate across an array of environmental compartments, their ecotoxicity (the ramifications regarding exposures for microorganisms, animals, and plants), their human toxicity (ramifications for human exposure to drug residues via contaminated foods and water), unknowns involving exposure to multiple stressors at extremely low concentrations, and the countless driving forces that facilitate or prevent their entry to the environment.

A continuum of source–exposure–effects threads its way through these interwoven dimensions (see Figure 8.1 in [1]; standalone illustrated poster available at http://www.epa.gov/esd/bios/daughton/exposure-continuum.pdf). To reduce the odds of adverse or untoward effects for the environment or humans, this continuum can be actively short-circuited in key places. This is the role played by various mitigation strategies such as engineered schemes for removing drug contaminants from wastewater, solid waste, and drinking water. Other than specific programs such as consumer take-backs designed to collect unused, unwanted medications [2], these downstream (control) strategies are largely incidental since they are designed primarily for improving indirect measures of chemical contamination (such as chemical or biological oxygen demand). During these processes (such as sewage treatment), the residues of drugs (and many other synthetic chemicals) are coincidentally removed to various degrees; moreover, these mitigation measures involve costly infrastructure and are resource-intensive. More targeted and efficient strategies for minimizing the entry of drug residues to the environment involve upstream (preventative) approaches using more sustainable countermeasures centered on pollution prevention and sustainable design.

Central to the study of the source–exposure–effects continuum and for guiding the development of sustainable approaches for reducing the entry of drug residues to the environment is a comprehensive understanding of their

FIGURE 1 The origins and sources of active pharmaceutical ingredients (APIs) in the environment; partly adapted from Ref. [128]; original illustration available [129]. Red denotes possible exposure pathways for humans and wildlife. Orange diamonds denote pollution prevention opportunities for reducing the entry of APIs to the environment. API, active pharmaceutical ingredient; CAFO, confined animal feeding operation; LTCF, long-term care facility; OTC, over the counter; RCRA, Resource Conservation and Recovery Act (of 1976). Note that also available is an analogous illustration that covers the use of illicit drugs as well as the illicit use of legal pharmaceuticals [22]. Illustration available at http://www.epa.gov/esd/bios/daughton/illicit-drug-lifecycle.pdf.

sources and origins. This can reveal which specific active pharmaceutical ingredients (APIs) enter the environment, in what quantities and spatiotemporal distributions, and where, why, and how they gain entry. Understanding the sources and origins of APIs is important because it allows assessment of the ways in which sources can be prevented or minimized and ways in which the connections between sources and the environment can be reduced. A better understanding can also serve as an initial guide or filter for selecting those APIs that should be targeted for environmental monitoring—after prioritization on the basis of potential for ecotoxicity (e.g., [3,4]) or human toxicity [5]. This knowledge can also be used to influence or guide the prescribing habits of physicians and purchasing habits of consumers, inform new legislation, or reveal data gaps (e.g., those APIs that have received insufficient attention). Important to keep in mind is that the presence in the environment of a particular API may result from multiple sources: excretion, bathing, disposal, and manufacture, among others. Apportioning occurrence data back to sources (e.g., [6]) is not frequently done, especially when the contributions from each source can be episodic, sporadic, continual, or diurnal—with variability imposed by human activity patterns (e.g., bathroom usage), time of day (dosing schedules), day of week (lifestyle or enhancement drugs, or recreational use), season (cold and flu medications, e.g., [7]), and weather (temperature, precipitation, and sunlight, all of which can impact the efficiency of sewage treatment, natural transformation processes, or cause raw sewage overflow events, e.g., [8]).

A comprehensive examination of the sources and origins of drugs in the environment was covered in the first edition of this book [9]. Provided here is an attempt to expand and update that original chapter, with minimal repetition of original materials. Some other, more recent overviews of API sources are also available [10–15].

2 WHAT DO WE MEAN WITH THE TERM "DRUG"? A VERNACULAR OF TERMINOLOGY

Discussion of any of the many dimensions of the overarching issue involving drugs as environmental contaminants requires an understanding of the basic terminology pertinent to drug products and their active ingredients. A shared understanding of definitions for basic, widely used terms is required especially for communicating across disciplines—and the topic of drugs in the environment attracts specialists across an extremely broad spectrum of disciplines, including analytical and environmental chemistry, environmental and human toxicology, veterinary sciences, animal husbandry, aquatic and marine sciences, entomology, agricultural and plant sciences, hydrology, pharmacology, pharmacy science and practice, medical science and practice, healthcare practice, nursing, civil and sanitary engineering, risk assessment and communication, policy making, legislating, forensics, risk communication, and even

social psychology. There are indeed many dimensions to this topic—a topic often intensified by the fact that pharmaceuticals can possess profound life-enhancing or life-saving abilities as well as extreme toxicity that can lead to tragic injury or deaths of humans, pets, and wildlife.

A clearer appreciation for the importance of terminology can be gained from the experiences of the National Institutes of Health (NIH) Chemical Genomics Center (NCGC). The NCGC launched a major program to screen small-molecule drugs—those substances that had already been market-approved—for previously unrecognized biological activity [16]. The objective was to identify candidate substances for potential "repurposing" (already approved drugs possessing new or extended therapeutic indications that had previously been unrecognized or unknown). Repurposing is believed to hold potential as a cost-effective way to address rare or neglected diseases. It also represents an additional way in which certain approved drugs may gain increased usage and therefore display higher potential for entry to the environment. This pioneering effort by the NCGC was to involve systematic screening of *all known approved drugs* using a wide array of several hundred high-throughput biological assays.

An initial objective for the NCGC was to assemble a definitive, comprehensive physical collection of all known active ingredients used in approved drugs. The project immediately faced complications posed by the challenge of not just assembling such a physical collection, but moreover in the unforeseen difficulty in identifying the substances that such a definitive list should actually contain; the difficulty was amplified in that the list would need to be one that exclusively comprised unique (nonredundant) chemicals. The NCGC's efforts resulted in what is now called the NCGC Pharmaceutical Collection (NPC). While still evolving, the NPC is probably the most definitive and comprehensive physical collection of drug ingredients that have been registered or approved (worldwide) for use in either humans or animals.

The information gathered for the NPC concerning the world's inventory of approved drugs is extremely useful to the topic of drugs as environmental contaminants because it presents the most complete and accurate picture to date of the universe of bioactive chemicals currently being used in healthcare. It can therefore reveal the entire gamut of pharmacological substances having the potential to gain entry to the environment. Prior to the NPC project, the numerous estimates of the number and identities of drugs in use varied wildly—a problem caused by poorly curated databases, by confusion over chemical terminology, and even (as we will see) by an inaccurate understanding of what is meant by the term "drug."

A most unanticipated obstacle faced at the outset in creating the NPC was the realization that the term "drug" had no definitive definition. Likewise, unambiguous definitions for other terms used in the vernacular of what constitutes a "drug" (such as API) were also lacking. Any discussion of the many aspects of "drugs" as environmental contaminants therefore warrants some

attention to the salient terminology—especially so that we have a common understanding of how and when many of the terms are used rather loosely and sometimes interchangeably. The definitions are critical to answer fundamental questions such as "How many drugs are there?"

The NCGC was surprised to discover that a definitive list of approved drugs simply did not exist—even from drug regulatory agencies. This was partly a result of the lack of a common understanding of what constitutes a "drug." As a result, even the supposedly "authoritative" lists maintained by regulatory agencies were found to be incomplete, they comprised replicate entries (often as a result of confusion over multiple names for the same name drug), and they were often outdated (many drugs were no longer marketed, for any number of reasons including hazard-based market withdrawal). One of the outcomes from the lack of a definitive list has been the inability of anyone to offer a confident estimate of the total number of distinct "drugs"—where each is based on a unique chemical. So the universe of distinct chemicals that could potentially contaminate the environment as a result of healthcare practices had essentially been unknown. This may partly be the reason for an overwrought focus on seemingly simpler lists compiled by healthcare informatics companies, such as the 100 or 200 most commonly prescribed drugs (e.g., [17]). Important to recognize is that these lists can also be inaccurate. Even worse is that these truncated lists tend to impart their own biases as a result of sometimes ignoring over-the-counter drugs and by overlooking numerous other commonly used drugs. This problem, which may serve to actively bias the research surrounding drugs in the environment, is later discussed in Section 8.

At the risk of oversimplifying, the following will try to briefly provide a more accurate picture of the total number of "drugs" currently approved or under evaluation. With respect to most discussions involving the entry of pharmaceuticals to the environment, the most commonly used terms—"drug" and "active pharmaceutical ingredient" (API)—are usually being used in reference to what is called the molecular or chemical entity (ME or CE). The ME represents the most fundamental aspect of a drug—its unique chemical structure (encompassing steric conformation—such as enantiomers and polymorphs) that ultimately affords interaction with targeted (as well as unintended) biological receptors. When formulated into a particular drug, the constituent ME may be present in various "extended" chemical iterations such as esters (e.g., as used for certain prodrugs), salts, chelates, complexes, clathrates, solvates, or hydrates. While these different forms of an ME may exhibit different pharmacokinetic properties, they do not display significantly different ultimate interaction with receptors. These different iterations of an ME are referred to as the API. This means that there can be multiple APIs based on the same ME, but each of these APIs shares the basic biological activity of the parent ME.

Next in the hierarchy of terminology is "drug," which is manufactured to contain one or more APIs, along with formulation ingredients (which are

often euphemistically referred to as "inert" or "inactive" ingredients). It is the "drug" that receives market approval (e.g., by the FDA in the United States). Multiple or numerous trade/brand names and adopted generic (nonadvertised) names can eventually receive approval when based on the same API or unique combination of APIs (a "combination" drug), and the drug may be approved for prescription only (℞ or Rx), for availability over the counter (OTC), or (in certain countries) for availability behind the counter (BTC—which sometimes serves as a transition for an eventual Rx-to-OTC switch). More frequently than not, newly approved drugs are based upon an API (or combination of APIs) that has already been incorporated in prior drugs. Only what is known as newly approved molecular entities (NMEs) represent structurally unique APIs appearing for the first time in any drug. NMEs therefore represent chemicals having the potential to widely contaminate the environment for the first time (as opposed to sporadic contamination during more limited use in research laboratories and clinical trials). In contrast, for drugs withdrawn from market (which usually occurs during postmarket or post-approval—"phase 4"—clinical trials), should they represent the only source for a particular ME, this marks an instance where environmental levels of an ME may begin a downward trajectory.

The overarching term in the hierarchy relevant to drugs is "drug product." A drug product is the ultimate packaged form of a drug readied for commerce and intended for ultimate end use. Drug products for a given API can contain a range of dose strengths, dose forms (e.g., tablet, capsule, and lotion), or variety of dose shapes, colors, and flavors; facilitate different routes of administration (e.g., oral, intravenous, transdermal, subcutaneous, ocular, and insufflation); and offer a spectrum of packaging/container designs or integration with a delivery device such as a dispenser. Worldwide, there can literally be hundreds of different drug products that contain the exact same API (and ME).

For the sake of environmental considerations, the universe of drugs is not limited to those that have been approved for market or for investigational use (e.g., clinical trials)—for humans, agriculture, or animals. Veterinary drugs are sometimes repurposed for human use. Certain substances registered primarily for use as pesticides have also been approved for medical use—examples being insecticides and anthelmintics repurposed for systemic or topical use against head lice, such as pyrethroids, ivermectin, malathion, lindane, and spinosad; some of the more persistent pesticides (such as lindane), however, have experienced local bans on use [18]. Pharmaceuticals can also become repurposed as pesticides—affording another route by which APIs enter the environment; one example is the use of dead mice laced with acetaminophen as bait to control invasive brown tree snakes in Guam [19]. Acutely toxic levels of certain APIs can also be inadvertently introduced to the environment, such as via tainted carcasses; two examples are the fatal poisoning of vultures by cattle carcasses containing residues of NSAIDs such as

diclofenac (e.g., [20]) and the fatal poisonings of eagles and other scavengers by consuming improperly disposed carcasses from animals euthanized with pentobarbital (e.g., [21]).

The universe also includes substances that have been explicitly or implicitly banned as illicit, such as the controlled substances maintained by the US DEA under Schedule I (see discussion in [22]). Furthermore, some approved drugs are ultimately withdrawn from the market (often because of severe adverse events) but sometimes only in certain countries. Therefore, a drug withdrawn by one country may turn up in environmental monitoring surveys in other countries or in the same country as a result of casual or illegal importation or from continued use of existing consumer stockpiles.

Finally, while "drugs" can comprise a wide spectrum of basic chemical structures, functional groups, structural motifs, and molecular sizes and shapes (represented by general categories such as biologics, antibodies, nanoparticles, diagnostic agents, and radiopharmaceuticals), it is generally the "small-molecule" substances (<800 Da), which are amenable to oral administration, and various diagnostics (the iodinated contrast imaging agents being one example) that have attracted nearly all of the attention from environmental scientists. Separate perspectives are available on biologics [23] and nanoparticles [24–27]. Various other classes of potential contaminants related to healthcare and medical research also exist. One example is the synthetic plasmid antibiotic resistance genes, which have been shown to be unintentionally released from lab settings and enter the environment via waste discharge. These synthetic plasmids may confer antibiotic resistance in the wild and in humans [28].

With this vernacular, it should be clearer that when the discussion involves trace environmental contamination resulting from the use of drugs (usually residues released primarily via urine and feces and secondarily via sweat, bathing, and direct dermal transfer [29] or from disposal of leftover or unwanted drugs to sewers or trash), it is invariably the residual ME (or API) that is being measured or referenced. When contamination or waste relates directly to the actual physical commodity (such as undissolved pills or packaging), only then are the terms drug or drug product technically appropriate.

3 HOW LARGE IS THE UNIVERSE OF DRUG ENTITIES AND WHY SHOULD IT MATTER?

With this brief summary of terminology as background, the NPC study [16] arrived at the following tallies (as of 2011) for the numbers captured by the hierarchy of drug terms; these are summarized in Table 1. The key number for purposes of discussions involving environmental contamination is that 2356 unique MEs are approved for human use by the FDA and 3936 MEs are approved for human use when including major markets worldwide.

TABLE 1 Number of Entities in Each of the Four Major Hierarchical Terminologies Involving Human and Veterinary Drugs[a]

Terminology Hierarchy	Number of Market-Approved by FDA	Number of Market-Approved Worldwide[b]	Number Approved for Investigational Use[c]
Drug product	>100,000	Unknown	
Drug	>10,000	>25,000	
API[d]	5206 (human use) 5445 (human/vet use)[e]	9524 (human use) 9700 (human/vet use)	*4935*
ME[f] or CE	2356 (human use) 2508 (human/vet use)	3936 (human use) *4034* (human/vet use)	
		8969 total approved/ investigational (human/vet)[g]	

[a] *Data adapted from Ref. [16].*
[b] *Worldwide: only includes the major markets—the United States, the United Kingdom, Canada, EMA, and Japan.*
[c] *Investigational use includes ME approved for clinical trials and experimental use.*
[d] *API: active pharmaceutical ingredient—structurally unique.*
[e] *Human/vet use: combined unique entities from both human use and veterinary use.*
[f] *ME: molecular entity (or chemical entity, CE)—structurally unique.*
[g] *Sum of two italicized numbers.*

Inclusion of veterinary drugs only increases these totals of unique MEs an additional 6%: a combined 2508 human and veterinary drugs approved by the FDA and 4034 approved worldwide. An additional 4935 unique MEs are cataloged in various major databases for experimental use but not yet approved for market.

The total number of APIs (combined human and veterinary use) resulting from these MEs are 5445 approved by the FDA and 9700 approved worldwide. The MEs are incorporated into over 10,000 drugs and over 100,000 drug products approved by the FDA and into over 25,000 drugs approved worldwide (no estimate on the worldwide number of drug products); see Table 1. Important to note with regard to the worldwide distribution of APIs in the environment is that APIs do not necessarily gain approval uniformly across countries.

These numbers mean that a combined 8969 unique human and veterinary MEs hold the potential for entering the environment worldwide. Undoubtedly, these would display a very broad range of loadings, with many exhibiting extremely low rates—either because they are infrequently used or because they are highly potent and therefore manufactured and used in extremely

small amounts; yearly production rates across APIs span a range of 6 or more orders of magnitude (from grams to tons). Clearly, the mass loadings entering waterways (e.g., via treated and raw sewage) would be dominated by those drugs prescribed in the highest quantities (on the basis of their constituent ME masses) but modulated by their pharmacokinetics; note, however, that overall loadings or ultimate concentrations are but one variable in determining ultimate toxicological significance, as potency and the complexities of simultaneous exposures to multiple APIs (as well as other unrelated toxicants) also play major roles. The escalation in design and manufacturing of the so-called highly potent APIs (HPAPIs; see [30]) could pose considerable challenges for their environmental monitoring as a result of their undoubtedly exceedingly low levels in any environmental compartment.

Now that we understand the world of drugs (or pharmaceuticals) as comprising thousands of unique chemical entities (MEs or APIs) and many tens of thousands of commercially formulated drug products, it is not surprising that they exhibit a broad range of physicochemical and physiological properties. Each API can be assigned to one or several therapeutic groups, such as those implemented in the tiered Anatomic Therapeutic Chemical classification system or the analogous system for veterinary medicines (e.g., see [31], Table 5 therein). Some APIs are promiscuous in their ability to interact with a wide range of receptors—both the intended target and unintended targets—often leading to adverse effects but also sometimes revealing new potential therapeutic uses, which is one of the objectives of the NCGC program.

Of possible importance (but yet to be examined) are the very low individual potential loadings that could emanate from the thousands of investigational APIs used in clinical and laboratory studies; investigational use often extends beyond formal clinical trials (i.e., expanded access or compassionate use), but such use is comparatively small. Environmental studies on investigational drugs are just beginning to appear, but they have focused on the future potential for persistence in the environment once they are introduced to the market rather than on entry to the environment during investigational use (e.g., [32]). Nonetheless, certain unapproved substances currently sold as illicit drugs but which have future potential as approved drugs have been identified as environmental contaminants. One example comprises the many synthetic (but unapproved) analogs of the approved phosphodiesterase type-5 (PDE-5) inhibitors (e.g., sildenafil, vardenafil, and tadalafil, used primarily in treating erectile dysfunction) [33–37].

The annual rate at which new molecular entities (NMEs; or new chemical entities, NCEs) gain FDA market approval in the United States is extremely low compared with the total number of extant MEs. For example, the rates were unusually high in 2012, 2011, and 2009, where the FDA approved only 39, 35, and 37 NMEs, respectively; these numbers include small molecules and biologics. These were the highest rates since 1996, where 53 were approved [38,39]; furthermore, significant numbers of these NMEs may be

for orphan, neglected, or rare diseases, where their overall usage may represent minuscule quantities compared with mainstream drugs. An important facet of NMEs with respect to their potential for environmental impacts is that no routine mechanism seems to be in use that prompts efforts for examining their presence in the environment upon their market introduction.

The value of these drug terminology statistics is in lending accurate perspective when discussing the various issues surrounding the environment and drugs. For example, it is the ME (or API) that is the target of environmental monitoring and ecotoxicology studies or the chemical entity that we wish to remove by engineered treatment of sewage and water. It is the drug (and drug product) that is the target of pollution prevention programs (such as alteration in prescribing practices) or consumer take-backs of leftover drugs; statistics for drugs and drug products are used to assess diversion, abuse, and poisonings. Moreover, an accurate understanding of sales and consumption of drug products is essential for predicting the quantities of MEs (or APIs) that can enter various environmental compartments or for better understanding solid waste treatment or disposal (such as incineration or landfill). To illustrate the importance of correct use of this terminology, for many discussions involving data on "drug waste" (such as the mass of "drugs" collected in consumer take-backs), it is often unclear if the data pertain to the physical drug products (including their packaging), to the drugs themselves (such as pills or capsules), or to the constituent ME or API. While use of these terms interchangeably is common, it should always be made clear (as one example) whether the term *drug* is intended as vernacular shorthand for API (or ME) or rather refers to the formulated drug.

4 UNDERSTANDING THE SOURCES FROM WHERE MEDICATIONS CAN BECOME ENVIRONMENTAL CONTAMINANTS

Drug residues in the environment are perhaps the most recognizable indicators of how everyday human actions, activities, and behaviors can directly impact the environment. Their usually low but measurable levels in the environment often reflect the collective, continual contributions of seemingly minuscule quantities from very large numbers of individual, disconnected point sources. A key aspect of the continual introduction of APIs to waterways via sewage is that detectable levels can persist indefinitely even when environmental half-lives are short. Nonpersistent chemicals such as most APIs can exhibit a perpetual presence by way of continual replenishment—a phenomenon that was termed "pseudopersistence" in 2002 [40].

The diversity and scope of chemicals involved is extremely broad and made further complicated by a wide array of factors that drive their entry to the environment from an extraordinarily complex network of sources. The basics of this intricate network are captured in the stylized illustration in

Figure 1; note that also available is an analogous network illustration that covers the use of illicit drugs (and the illicit use of legal pharmaceuticals) [22]. Medications pervade most societies, posing an extraordinarily wide spectrum of countless diffuse and point sources [31]. Understanding this network is critical to managing the many risks posed by medications in society—risks that range from overt, acute poisonings of humans [41] and wildlife (e.g., [20]) to subtle perturbations such as altered wildlife behavior (e.g., [42–47]).

Potential sources for API entry to the environment are spun off at many different points along the trajectory of a drug product's life cycle. These sources are created intentionally (as a result of the designed and prudent use of a medication) and unintentionally (from imprudent storage or disposal or from irrational use). The focus in this chapter concerns the points between manufacture and final use of the commercial product.

Each source point can feed into another potential source or serve as a shortcut to direct entry to the environment. Depending on the perspective, secondary sources are created during myriad other points of the life cycle of an API; two examples among many are the occurrence of residual APIs in commercial biosolids created from human sewage (e.g., [48]) and the creation of parent APIs from the hydrolysis of excreted glucuronide conjugates [29]. Understanding and characterizing these sources could inform the design of the most effective risk management strategies to prioritize where effort and resources should be expended to reduce the entry to the environment of those APIs posing the greatest risk and to cost-effectively reduce their overall environmental loadings. Among numerous possible examples, compare the potential contributions to environmental loadings for a particular API when directly disposed to sewers with its discharge via urinary excretion. If this API is extensively metabolized to nonconjugates, disposal has the potential to contribute significantly compared with excretion. But if the API is extensively excreted unchanged, disposal might be a minor contributor. Likewise, the source contributions from excretion might be insignificant for an API that is intended solely for topical use (e.g., perhaps because of systemic toxicity), where bathing would serve as the major source. These factors are discussed in Daughton and Ruhoy [29].

5 FACTORS THAT OBSCURE OR CONFOUND THE ORIGIN OF APIs OR SOURCE APPORTIONMENT

Many factors can confuse the process of tracing drugs back to their sources. Some serve to obscure their presence in the environment. Others complicate deducing from where an API originated. Four examples involve reversible metabolic conjugates, natural products, prodrugs, and the disconnects in the place and time and where drugs are sold and actually used for their intended purpose.

5.1 Stealth or "Hidden" Secondary Sources of APIs: Metabolic Conjugates and Back-Transformations

Reversible metabolic conjugates (i.e., glucuronides) may serve as "hidden" reservoirs (secondary sources) for many APIs after discharge to sewage or the environment [29,49]. Deconjugation that does not occur in the gut prior to excretion can take place later in sewage or in the ambient environment via hydrolysis mediated by exogenous bacteria or abiotic processes. Reconversion of these conjugates back to the parent API is often the cause of higher levels of an API measured in treated sewage effluent than in the raw influent. Some APIs can be recreated in the environment not only just from hydrolysis of reversible conjugates but also by abiotic processes such as photolysis of metabolites. One example is the back-transformation of sulfamethoxazole by the photolysis of one of its metabolites, 4-nitroso-sulfamethoxazole [50].

5.2 Natural Products Versus Semisynthetics

Another factor that can confound apportionment of an API to its source is that some APIs can originate from both anthropogenic and natural sources. One example is 17β-estradiol, an endogenous hormone excreted not only just by humans and other mammals but also by fish. It is also an API formulated in various systemic and transdermal estrogen replacement drugs. Another, more widespread example includes the antibiotics, many thousands of which have been isolated from native microorganisms (and represent but a minute fraction of those that exist in nature but have yet to be discovered). Some of these are also manufactured as "semisynthetics" (i.e., manufactured analogs produced via fermentation) for use in medications (e.g., see [51]); a prototypical example is penicillin G (benzylpenicillin). Other of many possible examples of natural products (from fungi, bacteria, plants, and animals) that have semisynthetic, unaltered counterparts include artemisinin, bleomycin, cyclosporine, cocaine, colchicine, digitalis, epibatidine, lovastatin, morphine, paclitaxel, quinine, streptomycin, testosterone, and tubocurarine. Many others are derived from natural products—the so-called second-generation natural products [52].

5.3 Prodrugs

A prodrug is a chemical structure that serves as a precursor for an intended drug. A prodrug incorporates an active molecular entity within another molecular structure. The prodrug itself is often biologically inactive but may also possess biological activity—serving as a drug itself. The molecular entity is subsequently released from the prodrug upon metabolic or physicochemical processes, such as hydrolysis. Prodrugs are usually designed to improve

bioavailability; the conversion process (release of the molecular entity) can take place intra- or extracellularly.

It is important to recognize that drug design and pharmacokinetics can play a confounding role in tracing sources. For example, some APIs can originate from multiple other APIs—as metabolites. This occurs by design via prodrugs, which comprise a significant percentage of all marketed drugs. Roughly 15% of the top 100 small-molecule drugs (ranked by sales) in 2009 were prodrugs, and roughly 10% of all approved small-molecule drugs may be prodrugs [53]. Among the "promoiety" prodrugs, the active metabolite is released (detached) from the generally inactive promoiety. Often, however, the released metabolite is marketed as an API itself; both the prodrug and the released API serve as drugs on their own (drug–prodrug pairs). APIs from these particular prodrug–drugs therefore have at least two separate sources; for some APIs (5-fluorouracil is one example), numerous market-approved prodrug forms may be available. This can be important in assessments of environmental impact. For example, models used for calculating predicted environmental concentrations (PECs) for these prodrug APIs must account for two or more separate drugs as contributory sources. Prodrugs may also have intrinsic biological activity of their own—in addition to the active metabolite (e.g., [54]).

Since prodrugs are a rapidly growing area of drug development, the number of potential sources of drugs that may need to be examined for specific APIs will escalate accordingly. Surprisingly, the issue of prodrugs is rarely mentioned in the environmental literature. Some of the numerous examples of drug–prodrug connections (in contrast to API/prodrug connections) include meprobamate/carisoprodol, prednisolone/prednisone, beclomethasone/beclomethasone dipropionate, 17α-ethynylestradiol/mestranol, phenobarbital/primidone, dextroamphetamine/lisdexamfetamine, acyclovir/valaciclovir, 5-fluorouracil/capecitabine, enalaprilate/enalapril, morphine/codeine, and canrenone (which can be formed from both spironolactone and canrenoate). In the environmental science literature, such pairs are rarely mentioned together in the same article. Two exceptions involve isolated articles that discuss combined API contributions from phenobarbital and primidone [55] and from 5-fluorouracil and capecitabine [56]. In other instances, individual APIs that could have been released from prodrugs might sometimes be mentioned, but their interconnection via metabolic conversion is not noted (e.g., for prednisolone/prednisone [57]). One common exception is morphine and codeine, which are widely recognized as being metabolically linked, probably because of the extensive research done on dose reconstruction for the purposes of gauging community-wide illicit drug use [22].

5.4 Spatiotemporal Disconnects: Medication Stockpiling and Transient Populations

At the same time, sufficient knowledge of sources can inform decisions regarding which APIs should be targeted for environmental monitoring and

the actual locations to monitor. Coupled with knowledge of human and animal pharmacokinetics, environmental transformation processes, and toxicity for real-world exposure routes and levels, models could be formulated to predict the risk associated with individual APIs. Furthermore, since such an approach would facilitate the unbiased selection of APIs to target for monitoring, it would greatly help to reduce the incidence of bias that derives from the insidious Matthew effect (see Section 8). By factoring in demographics (e.g., to account for changes in consumption as a function of population and age distribution) and the market introduction of drugs based on NMEs, models could be used to anticipate new trends in API emissions. Note that a major obstacle with determining per capita usage of APIs is posed by the many limitations and complexities surrounding accurate estimation of local population size [58].

One variable in the use of medications plays a central but underappreciated role in creating considerable unknown in models and also in dictating the types of subsequent exposures that may occur. This variable is the delay in the time from when a medication is purchased or prescription filled and when it is finally used as intended (if ever). This means, for example, that even when real-time, local sales data are available, they cannot necessarily be used reliably as a proxy for real-time medication usage. The two major causes for delay are dispensing of long-term maintenance medications in large quantities sufficient for many months of treatment coupled with poor patient compliance. This results in considerable delay in ultimate use and also in the accumulation of leftover or unwanted medications; large, perpetual stockpiles of unused medications can accumulate when automatic refills are allowed to continue unabated—a phenomenon with several different causes, one of which derives from patient behavior and another from deaths of patients [41].

Patient behavior is a factor that strongly determines the fate of medications. It is also a factor that is particularly refractory to modeling. Adherence or compliance to prescribed medication regimens dictates if, how, and when a drug is consumed or instead relegated to unused stockpiles that must later be disposed or otherwise risk being diverted for other uses. This behavior dictates the frequency, duration, and extent of usage, which in turn determines the extent to which an API enters sewerage (as a result of excretion, bathing, or disposal). While these factors are complex, they all have potential for modification and control in order to reduce an API's entry to the environment (see [59], Table 4 therein: "Major variables involving dose and its administration that can be optimized to reduce excretion as well as the incidence of ADRs and leftovers").

Perhaps the major limitation to quantifying the scope (types, amounts, and locations) of API sources is access to real-time, geographic usage data. In some countries, such as the United States, comprehensive commercial informatics services compile detailed data on prescription sales/dispensing and demographics, but access is fee-based and the costs usually preclude utility

for modeling purposes. Assessing the flow of APIs through commerce is problematic. The quantity of an individual API that enters commerce via prescription could be estimated from the commercial informatics from total sales volume or total prescriptions written (on a local basis but only for retail prescription drugs, which does not account for institutional drugs), but unknown is the percentage that is ultimately used versus that which is indefinitely stored or disposed. The temporal delay between time of dispensing and time of ultimate use can extend to years. There are also disparities in spatial disconnects between the location of prescription sale and the geographic locale where the drug is ultimately used (due to population mobility); many drugs experience ultimate use in countries where they were not originally prescribed, and many prescription drugs are also widely purchased illegally. Additional problems in assessing actual API use are shown by Greenblatt [60].

Another factor that may serve to bias sales data is that counterfeit or fraudulent drugs are a growing problem worldwide but especially so for low- and middle-income countries [61]. Counterfeit drugs have been falsified in one or more ways. Their relevance to the issue of APIs as environmental contaminants is that they introduce an unknown degree of uncertainty for models that rely on API sales data—as an unknown percentage of drugs that are purchased OTC or Rx do not contain the amount or types of active ingredients claimed on the label. Instead, they can contain (i) incorrect doses (ranging from insufficient or excessive) of the declared (legitimate) API, (ii) fluctuating doses of the declared API (within and between batches), (iii) solely "inactive ingredients" (absence of any active ingredient), (iv) undeclared (but approved) APIs, or (v) unapproved or unregistered APIs (or other nonpharmaceutical bioactive ingredient). When they contain registered APIs, these APIs may be legitimate (reclaimed from original manufacturers packaging) or they may be counterfeit themselves (and contain substandard API or unapproved impurities). Thousands of nonaccredited Internet pharmacies serve not only as a conduit just for counterfeit drugs but also as an illegal source of unknown magnitude for bona fide prescription medications (including controlled substances) and drugs that have been removed from the market [62–64].

6 SOURCE AS A VARIABLE INFLUENCING EXPOSURE AND ENTRY TO THE ENVIRONMENT

The spatial and temporal distributions of APIs in society comprise myriad fixed and transient locations [31]; a rhetorical question that lends perspective to the ubiquity of medications is "where are medications *not* commonly stored or used in society?" These locations all serve as potential sources that feed the routes of API transfer to the environment. The primary routes of transfer include excretion (via urine, feces, and sweat), bathing (e.g., from sweat and topical drugs), and disposal (to sewers and trash); these routes are modulated by the route of drug administration, as dictated by pharmacokinetics.

Secondary routes of transfer include dispersion to air and the direct transfer to the anthropogenic, made environment (including the physical surfaces that we touch in the course of our daily activities)—where residues of many drugs have the potential to be transferred by direct dermal contact. This includes drugs that are applied dermally (often at much higher levels than systemic drugs) and those that are excreted partly via sweat [29].

Subsequent routes of unintended exposure include levels ranging from trace residues (e.g., in contaminated finished drinking water) to complete dose forms (e.g., incidental or accidental but otherwise avoidable ingestion of medications that are imprudently stored). These exposures often reflect the routes of transfer, but sometimes, sources translate immediately and directly to exposures. Exposure routes include ingestion (e.g., via residues that make their way to ambient waters and foods), inhalation (e.g., airborne dust from medicated animal feed [65] or residues suspended in the air such as from smoking [66]), and dermal (e.g., contact with contaminated ambient waters, sediments, or physical surfaces [29]). Drug residues in fin- and shellfish destined for human consumption have both incidental origins (uptake of ambient API residues) and intentional origins (use of unapproved drugs or levels that exceed regulated tolerances in aquaculture and mariculture [67–72]).

Exposure levels can range from acute (resulting from comparatively high episodic levels) to chronic (resulting from sustained trace levels). Exposures can occur from residues in both the ambient environment and the immediate made environment. Exposures can result from multiple APIs or APIs in conjunction with other types of anthropogenic or naturally occurring toxicants. Exposures to trace residues are often unintended, undetectable, unavoidable, and unrecognized at the time; this aspect of exposure is a particular concern for pregnant women, infants, and aquatic systems. Exposures to whole-dose forms (e.g., the medication itself) can occur from accidental ingestion or dermal contact (especially a concern for children and the elderly; see [41]); wildlife scavengers can also ingest whole-dose forms that have been discarded in trash. Whole-dose exposures also manifest in purposeful but imprudent human consumption, such as drugs diverted from their prescribed or intended use (resulting in abuse and poisonings); this is a concern especially for medications that are improperly stored or imprudently disposed. Finally, unknown exposures may occur from the occupational preparation or use of highly toxic chemotherapeutics or from inadvertent exposure to patients undergoing chemotherapeutic treatment—by way of contact with high levels excreted via sweat, urine, or feces and which can later contaminate other materials such as laundry [41].

7 KEY QUESTIONS RELATED TO SOURCE AND THE LIFE CYCLE OF DRUGS

Of the top 20 critical questions surrounding APIs in the environment—as set forth in the consensus developed by Boxall et al. [73]—the subject of sources

is prominently featured in question #9: "What are the environmental exposure pathways for organisms (including humans) to PPCPs in the environment, and are any of these overlooked in current risk assessment approaches?" This includes "Review of potential pathways of release of PPCPs to the environment at different stages of the product life cycle for different regions of the world; analysis of existing risk assessment frameworks against this information; refinement of frameworks to include ignored exposure pathways where appropriate."

Lacking a comprehensive understanding of the numerous potential API sources that permeate society, progress will be limited in addressing key questions that are important for managing, mitigating, or preventing exposure risks. Better knowledge of source contributions is necessary to facilitate the apportionment of discrete API sources to overall environmental loadings and for better understanding spatiotemporal distributions. Important questions include the following:

How much of an API's environmental loading results from

- disposal versus excretion or bathing (reducing the contribution of APIs to the environment by disposal to sewers has served as a major justification in implementing nationwide collection programs for leftover drugs; this assumption, however, had never been based on any scientific assessment—see [41]);
- disposal to sewers versus disposal via landfilled trash;
- disposal to land of sewage biosolids (or compost) versus direct discharge from STPs;
- discharge from treated sewage versus raw sewage, which maximizes the potential for APIs to enter the environment (the incidence of raw sewage discharge is very high worldwide [74]);
- CAFOs, agricultural, and veterinary practice [75,76] versus human use;
- semisynthetic forms versus naturally occurring production (e.g., pertinent to some antibiotics and steroids);
- waste discharge from manufacture [77] or formulation [78,79] versus therapeutic end use;
- hospitals, nursing homes, long-term care facilities, and hospices versus consumers (types and quantities of a limited set of drugs—many of greater potency—might vary greatly; hospitals use many APIs not generally prescribed to patients on an outpatient basis) (e.g., [80–87]);
- release to sewers during bathing (APIs designed for topical use—with minimal systemic use) [29];
- delivery devices (many devices, such as medicated transdermal patches, retain significant quantities of highly concentrated API residuals; some of these must be disposed by flushing to sewers because of acute toxicity and abuse potential); for example, sewer disposal of one used patch for certain medications can introduce the mass of an API equivalent to that resulting from combined excretion from thousands of oral doses [29].

Likewise, important questions relevant to sources and immediate exposure include the comparative significance of

- morbidity and mortality (including childhood fatalities) resulting from acute human poisonings from ingestion of imprudently disposed or stored medications [41] versus chronic risks posed by incidental exposures to trace residues in the environment; one medicated transdermal patch for certain drugs can contain multiple lethal doses for a child;
- human exposure via API residues occurring in foods (crops that take up APIs from treated sewage streams or fin-/shellfish exposed to sewage effluents) versus finished drinking water;
- human exposure via food and drinking water versus dermal contact (incidental "bystander" exposure) [29];
- the entire spectrum and quantities of APIs involved in unintended exposures (simultaneous and sequential), a topic that poses toxicological concerns regarding dose addition and dose interaction;
- the percentage of unwanted, unused drugs that are indefinitely stockpiled versus disposed (relevant to concerns regarding diversion);
- the percentage of a particular drug (or drugs overall) that goes unused (and later require disposal) versus the percentage that is consumed as intended or diverted to unintended use (recreational use, self-treatment, or abuse);
- incidence of acute poisonings (humans, pets, and wildlife) resulting directly from drugs that are imprudently disposed or improperly stored; common OTC medications can be very toxic to pets [88];
- effectiveness of consumer take-backs in directing drugs away from disposal to trash or sewers (at least one US study revealed, e.g., that they are not effective [89]).

The ramifications of sources are critical to understanding the full life cycle of drugs, as shown from an assessment of Cook et al. [90] and an ensuing critique [91,92].

8 THE "MATTHEW EFFECT": A MAJOR POTENTIAL OBSTACLE TO A COMPREHENSIVE UNDERSTANDING OF DRUGS AS ENVIRONMENTAL CONTAMINANTS

Historically, the spectrum of chemical stressors considered in risk assessments has been narrowly restricted to regulate priority and legacy pollutants and associated conventional chemicals—high-volume commercial products or those unintentionally produced as ubiquitous by-products from industrial processes. These, however, have comprised few chemicals compared with the tens of thousands in commercial use. These select, targeted chemicals most likely also represent but a very small subset of the unknown numbers of yet-to-be-identified xenobiotics (both anthropogenic and naturally occurring) that play ongoing roles in the totality of biological exposure. Many studies

over the years have demonstrated the limited extent to which the chemical composition of environmental samples is understood. A recent example of the extent to which the totality of exposure is unknown—even in widely studied matrices—is provided by the chemical characterization of the hydrocarbons released from the Deepwater Horizon oil spill, where only 50% of the total oil was amenable to characterization, with the remainder being dominated by oxygenated hydrocarbons not amenable to the analytical techniques employed [93]. One outcome was that the fate of the oil could not be fully accounted for.

This situation can be viewed as an "iceberg" effect, where escalating numbers of potential chemical stressors may be present at ever-lower, undetectable concentrations in various environmental matrices (see Figure 2). Indeed, studies emerging just in the last few years are beginning to present evidence that the chemicals remaining unidentified in a given sample may hold the potential for the predominant share of total biological stress (e.g., [94]).

This same biased, iceberg perspective may also apply to the study of APIs as environmental contaminants—where only a select few have attracted most of the attention from the universe of roughly 9000 unique NMEs in use worldwide. This tendency of comparatively few, select chemicals to occupy the attention of the many disciplines involved in risk assessment has been noted over the last decade, even in light of the new perspective on environmental

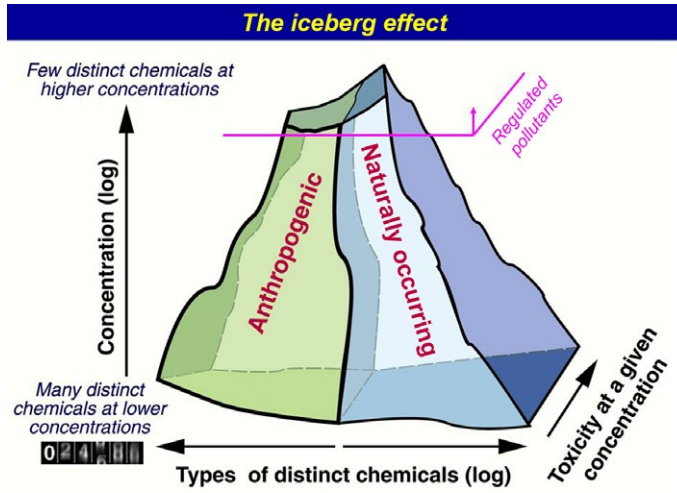

FIGURE 2 The iceberg effect: escalating numbers of chemical stressors at ever-lower concentrations in environmental matrices. Chemicals targeted for monitoring may represent a very small percentage of the totality of anthropogenic and naturally occurring stressors to which biological systems are exposed. *Illustration reproduced from Daughton [130].*

contamination afforded by the so-called "emerging" contaminants, which was largely catalyzed with interest in pharmaceuticals [95,96].

A potential disparity in data from environmental monitoring continues to grow as a result of the comparatively few chemical stressors that are targeted in environmental monitoring. This phenomenon has been deemed as a manifestation of a self-fulfilling selection bias referred to as the "Matthew effect"—where the prominence of those few chemicals targeted for investigation is dictated largely by the attention already devoted to them in the past. The Matthew effect as a psychosocial phenomenon was first articulated by Robert Merton in his well-known work of 1968 [97] and since used by Grandjean et al. in 2011 [98] to explain the biased path followed by many of the incremental and repetitive findings of environmental science.

The critical importance of exposure assessment and its role as the weak link in both ecological and human health risk assessment is made clear most recently in the European Commission's report "New Challenges for Risk Assessment" [99]. Notably, however, this report (like all prior evaluations of the risk assessment paradigm) perpetuates an extremely limited and biased view of the chemical space occupied by chemical stressors. The report does not entertain the question as to whether the universe of stressors is sufficiently known, nor does it recognize the potential for bias and data disparity created by ignoring large numbers of chemicals. The same oversight is evident in the recent NAS report "Environmental Decisions in the Face of Uncertainty" [100], where the IOM was requested by the US EPA to provide guidance on managing risk in the face of uncertainty. The possibility of bias and uncertainly (as introduced by the Matthew effect) is not alluded to in this report either.

While some of the potential ramifications of the Matthew effect in environmental science have been discussed by Grandjean et al. [98], evidence of its playing a possible role in introducing bias has never been actively sought. After all, establishing an absence of published data for a particular subject is clearly an onerous task demanding rigorous and time-consuming examination of as much of the published literature as possible. This would usually be perceived as a thankless endeavor—trying to establish that something has not been reported—and explains why the Matthew effect (if indeed an active phenomenon) could escape notice. Perhaps the only published example of the Matthew effect in play involves a recent 2013 study that examined the potential impact of medication prescribing practices on environmental contamination by APIs [59]. Data are presented ([59], Table S1 therein) showing a select group of APIs that are prescribed frequently and whether there is evidence that they also occur in the environment as contaminants. Among the 53 frequently prescribed APIs subject of the evaluation, minimal evidence existed in the published literature for whether roughly a dozen had ever been the targets of environmental monitoring or whether they had even been investigated as contaminants in the environment (an absence of data as opposed to

data of absence). This absence of environmental occurrence data for 22% of a sampling of 53 commonly used medications indicates the possibility of a substantially greater incidence of absence of data for the much larger universe of APIs in use today.

It should be noted, however, that some of these APIs may have been actively excluded from consideration for monitoring because of a low predicted potential to enter the environment as indicated by models based on pharmacokinetics or because a suitable analytical method was lacking (one with a sufficient limit of detection). But this overlooks the possibility that an otherwise poorly excreted API may instead enter the environment as a result of disposal or bathing rather than excretion [29]. This failure to target some of the more commonly prescribed drugs points to a possible bias in how drugs are selected for targeted monitoring.

Another example comes from an examination of the presence of 203 APIs in monitoring studies conducted in 41 countries [101]. The study revealed that most of the monitoring effort was devoted to just 14 (7%) of the 203 APIs.

A comprehensive understanding of the APIs that have the highest potential to enter the environment would be useful for preventing overwrought attention on a select few APIs simply because they have been the focus of prior studies. The factors responsible for driving the entry of APIs to the environment have been discussed in detail in Daughton and Ruhoy [29]. This knowledge would allow better targeting of research across the entire spectrum of APIs.

After several decades of published works on the occurrence of APIs in the environment, no centralized, publically accessible, standardized database yet exists that compiles any type of environmental occurrence data—whether positive data, negative data (data of absence), or verified absence of data. Now with the comprehensive database of NPC extant APIs, it would indeed be useful to crosswalk this universe of known APIs with those that have been identified in a wide variety of published environmental monitoring projects—from which a subset of APIs that have yet to be targeted for monitoring (those with an absence of data) could then be derived.

It is surprising that lists of the most widely used drugs (e.g., most frequently prescribed) are not periodically evaluated for those APIs that have not yet been detected in the environment. Have they been actively ignored or simply overlooked—casualties of the Matthew effect? APIs lacking occurrence data should be further investigated to determine the cause of the absence of data.

Given that the NMEs in use worldwide roughly total almost 9000, a major objective should be the development of a filter that selects a subset with the greatest chance of entry to the environment (a complex function of pharmacokinetics and human activities and behaviors) coupled with inherent hazard (a complex function of pharmacodynamics and potency). Hazard is particularly problematic in that it may manifest in a variety of ways, including

(i) adverse effects in humans as well as in nontarget species; (ii) predictable acute, extreme toxicity (e.g., single-dose lethality in humans [29,41]); (iii) unpredictable acute toxicity in nontarget species (e.g., renal toxicity from many NSAIDs for certain vultures (e.g., [20])); and (iv) unpredictable subtle effects from chronic low-level exposures (e.g., alteration of behaviors in aquatic organisms (e.g., [42,102–104])). The difficulties faced by these aspects are yet further amplified by the complexities in understanding real-world exposures, which can entail long-term chronic exposure to multiple APIs (which may or may not share the same mechanisms of action), at individual levels that may be substantially lower than currently established no-effect levels.

9 SUSTAINABILITY, STEWARDSHIP, AND POLLUTION PREVENTION FOR MINIMIZING THE ENVIRONMENTAL IMPACT OF APIs

A considerable body of published literature exists on many of the aspects of pharmaceuticals as environmental contaminants [105]. This literature addresses the many facets of fate and transport, environmental monitoring, biological effects, and engineered treatment of wastewater, drinking water, and solid (medical) waste. Notably absent, however, has been concerted discussion regarding potential solutions to the overall problem—how to design a sustainable system of pharmaceutical use that maximizes therapeutic utility and minimizes the environmental footprint.

Given the countless complexities associated with society's relationship with pharmaceuticals and the widespread occurrence of APIs in the environment (the full magnitude and scope of which is still emerging), it should not be surprising that the many approaches currently relied upon for mitigating or controlling the entry of APIs to the environment or for reducing the potential for exposure may not be sufficiently effective. These control measures usually involve considerable infrastructure and resources. They range from conventional engineering approaches (engineered treatment of industrial wastewaters, sewage, or drinking water) to waste diversion measures, such as consumer take-back programs for collection and centralized destruction (often involving incineration) of leftover medications—thereby averting disposal to sewers or landfills.

Unwanted leftover medications have been recognized as a public hazard and as a challenge to waste management since the 1960s [106–108], where a primary focus was on how to remove them from households—a forerunner to today's organized collection programs. Despite the costs associated with these conventional downstream control approaches and the many questions as to whether they are sufficiently effective, little consideration has been paid to applying the principles of sustainability and pollution prevention—to reduce or avoid the need to control waste by reducing or eliminating it to

begin with. The current conventional approaches (downstream end-of-pipe control) essentially address symptoms rather than the causes of the overall problem. Addressing the causes requires upstream solutions guided by sustainability and pollution prevention.

Few actions have been considered for tackling the problem at its main points of origination: prescribing and dispensing (and the influence of requirements imposed by health insurers) coupled with ultimate usage by the consumer or patient. A major impediment has been the persistent tacit assumption that the standards of care promulgated in the healthcare industry regarding the use of medication cannot be modified without jeopardizing the quality of healthcare. But this long-standing assumption may be fallacious.

A series of articles spanning 2003–2013 [41,59,109–113] presents a framework for the sustainable use of pharmaceuticals that makes this assumption moot. These articles assert that the current paradigm for the use of pharmaceuticals in medical care is not sustainable. As an alternative, they present a framework for designing a healthcare system that employs the sustainable use of pharmaceuticals, with emphasis on pollution prevention. This series of articles argues for directly linking environmental concerns with the practice of healthcare—under an umbrella system of the *Green Pharmacy* guided by *pharmEcovigilance*. PharmEcovigilance is a holistic version of conventional pharmacovigilance that ties the environment and the individual together as a single, integral patient—emphasizing the need to treat the patient and the environment as an interconnected whole. A key message is that drugs have afterlives that extend far past their intended medical uses.

The objective of the Green Pharmacy and pharmEcovigilance framework is to guide prudent prescribing, dispensing, and end use of medications in order to minimize the entry of drug residues to the environment from excretion and also to reduce the incidence of leftover medications that later require disposal or result in indefinite stockpiling in the home (sometimes extending to decades); stockpiling, in turn, breeds unlimited opportunities for uses never intended—many leading to failed or compromised therapies (e.g., via self-medication), diversion, abuse, and unintended poisonings. Drugs tend to not experience a routine or predictable path from manufacture to end use. Countless intervening factors (many involving human behavior) can block their intended consumption and lead to other problems. The eventual usage rates for many drugs can be extremely low, a problem greatly exacerbated when prescriptions are continually refilled but never used. A key to success is that the very same measures designed for incorporating sustainability and pollution prevention into the practice of prescribing could also have collateral benefits in dramatically improving therapeutic outcomes, reducing some of the major costs associated with healthcare (leftover drugs are often an overt symptom of numerous inefficiencies, imprudence, and irrationality in the conduct and administration of healthcare), and reducing the incidence of drug diversion and unintended drug poisonings (a major problem in the United

States and a key priority for the White House Office of National Drug Control Policy—ONDCP).

Poisonings from intended and unintended ingestion or other types of exposure (including dermal contact and inhalation) to certain drugs are a very real concern. Drugs with extreme acute toxicity pose demonstrated risks for morbidity and mortality, especially for children [29,41]. These highly potent drugs pose challenges in developing prudent, efficient, and protective strategies for disposing of leftover medications, as well as for their safe storage. One perspective on the range of drugs that pose particular hazards can be obtained by examining the US FDA's list of "Approved Risk Evaluation and Mitigation Strategies (REMS)" [114]. REMS are required for certain drugs to ensure that their benefits outweigh their known, significant risks. This is often achieved by limiting the subpopulation targeted for therapy, determined, for example, by screening prospective patients for various risk factors (some of which are genetic markers) or by considerations of outright toxicity. Many of these REMS drugs (as well as certain non-REMS drugs) need to be stored and disposed with utmost care.

This body of work posits that any of the numerous actions, behaviors, and customs involved with the prescribing and dispensing of drugs can be altered to (1) reduce the incidence of leftover medications (and thereby lessen the need for disposal—which is usually done by flushing to sewers, discarding in trash, or collecting by infrastructure-intensive consumer take-back programs—while at the same time reducing drug diversion, abuse, and unintended poisonings caused by directed leftovers) and (2) reduce the quantities of unmetabolized residues excreted or washed into sewers by bathing [29]. The second point is one that had essentially been discounted as infeasible (purportedly because it would compromise medical care), but one that actually offers the greatest potential for minimizing the environmental burden of pharmaceutical ingredients. This can be done in large part by implementing lower-dose, off-label prescribing [59].

Some of the summary materials contained within this core group of papers that might prove useful in pollution prevention are the following. Daughton and Ruhoy ([109], Table 1 therein) provides a summary of failures in healthcare that lead to the accumulation and imprudent disposal of leftover medications. Box 2 in Daughton and Ruhoy [109] lists the many factors that influence the consumption of medications (which, in turn, impacts excretion of APIs) and lead to the accumulation of unused medications (thereby leading to the need for disposal). The first examination of how adjustment in dose could be a viable means of reducing excretion of APIs is presented in Daughton and Ruhoy [59], where a network illustration (Figure 1 therein; also available at http://www.epa.gov/esd/bios/daughton/how-prescribing-impacts-the-environment-(13Nov12).pdf) shows the interactions within the prescriber–patient relationship network that lead to leftover drugs specifically from the failure to consider adjustment of doses to levels lower than the

"standard" (on-label) dose. Daughton and Ruhoy ([59], Table 2 therein) lists the many advantages of prudent prescribing (especially lower doses) in reducing adverse outcomes for patients, public safety, and the environment. Daughton and Ruhoy ([59], Table 4 therein) lists the major variables involving dose and its administration that can be optimized to reduce excretion as well as the incidence of adverse drug reactions and leftovers.

Finally, a large collection of nearly 2000 articles has been compiled that covers the numerous aspects of drug stewardship, sustainable use of medications, and issues relevant to the disposal and diversion of leftover, unwanted drugs, and acute poisonings in humans and wildlife from improperly disposed medications [115].

Physicians and other healthcare professionals currently have no resource for quickly learning about Green Pharmacy and pharmEcovigilance and how they could benefit medical care. Since environmental pollution by pharmaceuticals is ubiquitous, this far-reaching topic could be incorporated in medical core curricula and in continuing education. There are innate connections between the natural environment and human health. This topic makes this dramatically evident and provides an ideal context within which healthcare professionals can learn about the natural environment, how their actions can directly affect the environment, and how corrective actions could improve healthcare.

With respect to downstream pollution control measures for reducing the entry of leftover medications to the environment, several recent developments in the United States are worth noting.

Landmark federal legislation—the "Secure and Responsible Drug Disposal Act of 2010"—was signed into law on 12 October 2010 [116]. This act is partly designed to allow the Drug Enforcement Administration (DEA) to rectify the problems imposed on consumer drug collection take-back programs by long-standing, stringent requirements designed to ensure the secure handling of controlled substances [117].

Attention in the United States is beginning to be directed toward pharmaceutical manufacturers, with EPR (extended producer responsibility) as a preferred means of dealing with postconsumer drug waste [118]. The first attempt at legislating EPR in the United States for postconsumer drug waste was made by Alameda County, California, in September 2012 [119] but faced immediate opposition by the pharmaceutical industry [120]. Legislation introduced in the State of Rhode Island would address (for the first time in the United States) possible actions for preventing entry to the environment from excretion of highly toxic chemotherapeutics [121]. Ontario and British Columbia, Canada, have also been targeting EPR [122,123].

Finally, interest in developing new approaches for consumers to deactivate unwanted medications to facilitate a safer means of in-home disposal is shown by a solicitation from the NIH and the Centers for Disease Control and Prevention [124].

Additional developments will undoubtedly emerge as innovative approaches for minimizing leftover drug waste or for dealing more effectively with drug waste. One example that shows the large range of possibilities is one that proposes to add value to drug waste by reclaiming APIs for use as corrosion inhibitors (e.g., [125–127]).

10 FINAL THOUGHT: TREATING THE PATIENT AND THE ENVIRONMENT TOGETHER AS ONE

The pervasive use of pharmaceuticals worldwide, coupled with the growing risks and attendant problems surrounding leftover, stockpiled, and disposed medications throughout society, indicates that the current paradigm for the use of medications in medical and self-care may not be sustainable. A sustainable system would incorporate designs for prudent, efficient, and safe use of pharmaceuticals. Such a system would serve not just to reduce or eliminate the entry of APIs to the environment. By striving to protect the environment, collateral benefits for sustainable use could be significant. Therapeutic outcomes could be improved, medical care costs could decline, and the incidence of drug diversion and unintended drug poisonings (which are recognized as major problems at least in the United States) could be greatly lessened. Leftover medications—later requiring disposal—are a direct measure of wasted healthcare resources. Even the excretion of a certain portion of APIs—some of which later survive in the environment—is a direct measure of nonoptimal practices used in dispensing and prescribing [59]. The many benefits that could derive from designing a sustainable system for pharmaceutical use that directly links environmental concerns with the practice of healthcare—treating the patient and the environment as an integral whole—are substantial. A comprehensive understanding of the ubiquitous occurrence and distribution of drugs throughout society provides the basis for mitigating the seemingly countless sources from which they later gain entry to both the natural and human-made environments.

A sustainable system of medication usage can only be sought by optimizing the many variables at work in the intersection between human and ecological health. The ultimate question is "What types (APIs) and quantities (doses and durations) of medications are necessary to maintain, improve, or protect human health and the well-being of society, while also ensuring a sustainable environment?" Perhaps, the practice of prescribing and the ultimate consumption of medications may serve as an integrative measure of societal and ecological health and well-being. A perfectly optimized system of healthcare might be one that would not generate any leftover medications and also result in minimal excretion of API residues [110].

US EPA Notice: The US Environmental Protection Agency through its Office of Research and Development funded and managed the research described here. It has been subjected to agency's administrative review and approved for publication.

REFERENCES

[1] C.G. Daughton, B.W. Brooks, in: W.N. Beyer, J. Meador (Eds.), Environmental Contaminants in Biota: Interpreting Tissue Concentrations, CRC Press, Taylor and Francis, Boca Raton, FL, 2011, p. 287.
[2] S.T. Glassmeyer, E.K. Hinchey, S.E. Boehme, et al., Environ. Int. 35 (2009) 566.
[3] A.C. Capleton, C. Courage, P. Rumsby, et al., Toxicol. Lett. 163 (2006) 213.
[4] V. Roos, L. Gunnarsson, J. Fick, et al., Sci. Total Environ. 421–422 (2012) 102.
[5] C.G. Daughton, in: R.U. Halden (Ed.), Contaminants of Emerging Concern in the Environment: Ecological and Human Health Considerations, American Chemical Society, Washington, DC, 2010, p. 9.
[6] H. Chang, Y. Wan, J. Hu, Environ. Sci. Technol. 43 (2009) 7691.
[7] T. Azuma, N. Nakada, N. Yamashita, et al., Environ. Sci. Technol. 46 (2012) 12873.
[8] Y. Valcárcel, S. Alonso, J. Rodríguez-Gil, et al., Environ. Sci. Pollut. Res. 20 (2013) 1396.
[9] C.G. Daughton, in: M. Petrović, D. Barceló (Eds.), Analysis, Fate and Removal of Pharmaceuticals in the Water Cycle, Elsevier Science, Amsterdam, 2007, p. 1.
[10] D.J. Lapworth, N. Baran, M.E. Stuart, et al., Environ. Pollut. 163 (2012) 287.
[11] F. Heeb, H. Singer, B. Pernet-Coudrier, et al., Environ. Sci. Technol. 46 (2012) 8680.
[12] D. Fatta-Kassinos, S. Meric, A. Nikolaou, Anal. Bioanal. Chem. 399 (2011) 251.
[13] K.V. Thomas, K.H. Langford, in: K. Kümmerer, M. Hempel (Eds.), Green and Sustainable Pharmacy, Springer-Verlag, Berlin, Heidelberg, 2010, p. 211.
[14] M. Petrovic, J. Radjenovic, C. Postigo, et al., in: D. Barceló, M. Petrović (Eds.), The Handbook of Environmental Chemistry, Emerging Contaminants from Industrial and Municipal Waste: Occurrence, Analysis and Effects, Springer-Verlag, Berlin, Heidelberg, 2008, p. 1.
[15] K. Kümmerer, A. Schuster, in: K. Kümmerer (Ed.), Pharmaceuticals in the Environment – Sources, Fate, Effects and Risks, Springer, Berlin, Heidelberg, 2008, p. 43.
[16] R. Huang, N. Southall, Y. Wang, et al., Sci. Transl. Med. 3 (2011) 1.
[17] M. Bartholow, Top 200 Drugs of 2011: An Analysis of the Top 200 Drugs by Prescriptions Dispensed and Total Sales Reveals Key Trends in Today's Pharmaceutical Market, Intellisphere, LLC, Plainsboro, NJ, 2012; available: http://www.pharmacytimes.com/publications/issue/2012/July2012/Top-200-Drugs-of-2011.
[18] E.H. Humphreys, S. Janssen, A. Heil, et al., Environ. Health Perspect. 116 (2008) 297.
[19] J.J. Johnston, P.J. Savarie, T.M. Primus, et al., Environ. Sci. Technol. 36 (2002) 3827.
[20] J.L. Oaks, R.T. Watson, in: J.E. Elliott, C.A. Bishop, C.A. Morrissey (Eds.), Wildlife Ecotoxicology, Springer, New York, NY, 2011, p. 413.
[21] K.M. Langelier, in: P.T. Redig, J.E. Cooper, J.D. Remple, D.B. Hunter (Eds.), Raptor Biomedicine, University of Minnesota Press, Minneapolis, MN, 1993, p. 231.
[22] C.G. Daughton, Rev. Environ. Contam. Toxicol. 210 (2011) 59.
[23] T.C. Kühler, M. Andersson, G. Carlin, et al., Drug Saf. 32 (2009) 995.
[24] P. Hoet, B. Legiest, J. Geys, et al., Drug Saf. 32 (2009) 625.
[25] B. Jovanović, D. Palić, Aquat. Toxicol. 118–119 (2012) 141.
[26] J.A. Hernandez-Viezcas, H. Castillo-Michel, J.C. Andrews, et al., ACS Nano 7 (2013) 1415.
[27] I. Mahapatra, J. Clark, P.J. Dobson, et al., Environ. Sci. Process. Impacts 15 (2013) 123.
[28] J. Chen, M. Jin, Z.-G. Qiu, et al., Environ. Sci. Technol. 46 (2012) 13448.
[29] C.G. Daughton, I.S. Ruhoy, Environ. Toxicol. Chem. 28 (2009) 2495.

[30] D. Bormett, High-Potency APIs: Containment and Handling Issues, PharmTech.com (Pharmaceutical Technology), 2008 (1 September); available: http://www.pharmtech.com/pharmtech/Ingredients/High-Potency-APIs-Containment-and-Handling-Issues/ArticleStandard/Article/detail/548824.
[31] I.S. Ruhoy, C.G. Daughton, Environ. Int. 34 (2008) 1157.
[32] S.J. Lim, P. Fox, Sci. Total Environ. 444 (2013) 417.
[33] W.T. Poon, Y.H. Lam, C.K. Lai, et al., Hong Kong Med. J. 13 (2007) 359.
[34] B.J. Venhuis, D. de Kaste, Forensic Sci. Int. 182 (2008) e23.
[35] B.J. Venhuis, D.M. Barends, M.E. Zwaagstra, et al., Recent Developments in Counterfeits and Imitations of Viagra, Cialis and Levitra: A 2005–2006 Update, RIVM (Netherlands National Institute for Public Health and the Environment), Bilthoven, The Netherlands, 2007; available: http://rivm.openrepository.com/rivm/bitstream/10029/16459/1/370030001.pdf.
[36] B.J. Venhuis, M.E. Zwaagstra, J.D.J. van den Berg, et al., Illicit Erectile Dysfunction Products in The Netherlands: A Decade of Trends and a 2007–2010 Product Update, National Institute for Public Health and the Environment, Ministry of Health, Welfare and Sport, RIVM Report 370030003/2010, 75 pp., Bilthoven, The Netherlands, 2010; available: http://www.rivm.nl/Bibliotheek/Wetenschappelijk/Rapporten/2011/september/Illicit_erectile_dysfunction_products_in_the_Netherlands_A_decade_of_trends_and_a_2007_2010_product_update.
[37] B.J. Venhuis, D. de Kaste, J. Pharm. Biomed. Anal. 69 (2012) 196.
[38] L.M. Jarvis, Chem. Eng. News 91 (2013) 15.
[39] M. McCoy, Chem. Eng. News 89 (2011) 6.
[40] C.G. Daughton, Lancet 360 (2002) 1035.
[41] C.G. Daughton, Drugs and the Environment: Stewardship & Sustainability, National Exposure Research Laboratory, Environmental Sciences Division, US EPA, NERL-LV-ESD 10/081, EPA/600/R-10/106, 196 pp., Las Vegas, NV, 2010; available: http://www.epa.gov/nerlesd1/bios/daughton/APM200-2010.pdf.
[42] T. Brodin, J. Fick, M. Jonsson, et al., Science 339 (2013) 814.
[43] D.E. Conners, E.R. Rogers, K.A. Armbrust, et al., Environ. Toxicol. Chem. 28 (2009) 2671.
[44] K.M. Gaworecki, S.J. Klaine, Aquat. Toxicol. 88 (2008) 207.
[45] J.A. Mennigen, E.A. Harris, J.P. Chang, et al., Regul. Pept. 155 (2009) 99.
[46] D.M. Oggier, C.J. Weisbrod, A.M. Stoller, et al., Environ. Sci. Technol. 44 (2010) 7685.
[47] D. Pascoe, W. Karntanut, C.T. Müller, Chemosphere 51 (2003) 521.
[48] B.P. Chari, R.U. Halden, Water Res. 46 (2012) 4814.
[49] C.G. Daughton, T.A. Ternes, Environ. Health Perspect. 107 (1999) 907.
[50] F. Bonvin, J.V. Omlin, R. Rutler, et al., Environ. Sci. Technol. 47 (2012) 6746–6755, http://dx.doi.org/10.1021/es303777k.
[51] J. Clardy, M.A. Fischbach, C.T. Walsh, Nat. Biotechnol. 24 (2006) 1541.
[52] D.J. Newman, G.M. Cragg, J. Nat. Prod. 70 (2007) 461.
[53] K.M. Huttunen, H. Raunio, J. Rautio, Pharmacol. Rev. 63 (2011) 750.
[54] A. Qandil, Int. J. Mol. Sci. 13 (2012) 17244.
[55] U. Hass, U. Duennbier, G. Massmann, Water Res. 46 (2012) 6013.
[56] J.O. Straub, Integr. Environ. Assess. Manag. 6 (2010) 540.
[57] M. Schriks, J.A. van Leerdam, S.C. van der Linden, et al., Environ. Sci. Technol. 44 (2010) 4766.
[58] C.G. Daughton, Sci. Total Environ. 414 (2012) 6.

[59] C.G. Daughton, I.S. Ruhoy, Sci. Total Environ. 443 (2013) 324.
[60] D.J. Greenblatt, J.S. Harmatz, R.I. Shader, J. Clin. Psychopharmacol. 31 (2011) 1.
[61] G.J. Buckley, L.O. Gostin (Eds.), Countering the Problem of Falsified and Substandard Drugs, Committee on Understanding the Global Public Health Implications of Substandard, Falsified, and Counterfeit Medical Products, Board on Global Health, Institute of Medicine, National Academy of Sciences, Washington, D.C., 2013.
[62] USFDA, FDA Takes Action Against Thousands of Illegal Internet Pharmacies: Agency Participates in International Operation Pangea V to Protect Consumers from Potentially Dangerous, Unapproved Drugs, US Food and Drug Administration, Silver Spring, MD, 2012; available: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm322492.htm.
[63] CASA, in: A CASA White Paper, The National Center on Addiction and Substance Abuse (CASA) at Columbia University, New York, NY, 2008, p. 23.
[64] D.E. deKieffer, J. Pharm. Pract. 19 (2006) 171.
[65] G. Hamscher, J. Hartung, in: K. Kümmerer (Ed.), Pharmaceuticals in the Environment – Sources, Fate, Effects and Risks, Springer, Berlin, Heidelberg, 2008, p. 95.
[66] M. Viana, C. Postigo, X. Querol, et al., Environ. Pollut. 159 (2011) 1241.
[67] S.B. Turnipseed, S.B. Clark, J.M. Storey, et al., J. Agric. Food Chem. 60 (2012) 4430.
[68] M.C. Bermúdez-Almada, A. Espinosa-Plascencia, in: E.D. Carvalho, G. Silva David, R.J. da Silva (Eds.), Health and Environment in Aquaculture, InTech, Rijeka, Croatia, 2012, p. 199.
[69] D.C. Love, S. Rodman, R. Neff, et al., Environ. Sci. Technol. 45 (2011) 7232.
[70] L. Du, W. Liu, Agron. Sustain. Dev. 32 (2012) 309.
[71] T. Heberer, in: A. Pruden, L.S. Shore (Eds.), Hormones and Pharmaceuticals Generated by Concentrated Animal Feeding Operations, Springer, New York, NY, 2009, p. 95.
[72] T. Heberer, in: P.L. Keen, M.H.M.M. Montforts (Eds.), Antimicrobial Resistance in the Environment, John Wiley & Sons, Hoboken, NJ, 2012, p. 337.
[73] A.B.A. Boxall, M.A. Rudd, B.W. Brooks, et al., Environ. Health Perspect. 120 (2012) 1221.
[74] R. Baum, J. Luh, J. Bartram, Environ. Sci. Technol. 47 (2013) 1994.
[75] K.-R. Kim, G. Owens, S.-I. Kwon, et al., Water Air Soil Pollut. 214 (2011) 163.
[76] K.L.D. Henderson, J.R. Coats, in: K.L. Henderson, J.R. Coats (Eds.), Veterinary Pharmaceuticals in the Environment, American Chemical Society, Washington, DC, 2009, p. 3.
[77] D.G.J. Larsson, C. de Pedro, N. Paxeus, J. Hazard. Mater. 148 (2007) 751.
[78] P. Phillips, S. Smith, D. Kolpin, et al., Toxicol. Lett. 211 (Suppl.) (2012) S16.
[79] P.J. Phillips, S.G. Smith, D.W. Kolpin, et al., Environ. Sci. Technol. 44 (2010) 4910.
[80] B.I. Escher, R. Baumgartner, M. Koller, et al., Water Res. 45 (2011) 75.
[81] K.S. Le Corre, C. Ort, D. Kateley, et al., Environ. Int. 45 (2012) 99.
[82] P.M. Nagarnaik, A.L. Batt, B. Boulanger, Water Environ. Res. 84 (2012) 339.
[83] C. Ort, M.G. Lawrence, J. Reungoat, et al., Water Res. 44 (2010) 605.
[84] A. Schuster, C. Hädrich, K. Kümmerer, Water Air Soil Pollut. 8 (2008) 457.
[85] P. Verlicchi, M. Al Aukidy, A. Galletti, et al., Sci. Total Environ. 430 (2012) 109.
[86] D. Weissbrodt, L. Kovalova, C. Ort, et al., Environ. Sci. Technol. 43 (2009) 4810.
[87] P. Verlicchi, A. Galletti, M. Petrovic, et al., in: D. Barceló (Ed.), Emerging Organic Contaminants and Human Health, Springer-Verlag, Berlin/Heidelberg, 2012, p. 139.
[88] ASPCA, Prescription Meds Top ASPCA's List of Harmful Pet Toxins for 4th Straight Year, American Society for the Prevention and Cruelty to Animals (ASPCA), New York, NY, 2012; available: http://www.aspca.org/Pressroom/press-releases/020812.

[89] University of Wisconsin Extension, Product Stewardship Institute, Inc, Wisconsin Household Pharmaceutical Waste Collection: Challenges and Opportunities, Wisconsin Department of Natural Resources, 68 pp., 2012; available: http://dnr.wi.gov/topic/HealthWaste/documents/2012HouseholdPharmStudy.pdf.
[90] S.M. Cook, B.J. VanDuinen, N.G. Love, et al., Environ. Sci. Technol. 46 (2012) 5535.
[91] C.G. Daughton, Environ. Sci. Technol. 46 (2012) 8519.
[92] S.M. Cook, N.G. Love, S.J. Skerlos, Environ. Sci. Technol. 46 (2012) 8521.
[93] C. Aeppli, C.A. Carmichael, R.K. Nelson, et al., Environ. Sci. Technol. 46 (2012) 8799.
[94] B.I. Escher, C. van Daele, M. Dutt, et al., Environ. Sci. Technol. 47 (2013) 7002–7011, http://dx.doi.org/10.1021/es304793h.
[95] C.G. Daughton, J. Am. Soc. Mass Spectrom. 12 (2001) 1067.
[96] C.G. Daughton, Environ. Impact Assess. Rev. 24 (2004) 711.
[97] R.K. Merton, Science 159 (1968) 56.
[98] P. Grandjean, M.L. Eriksen, O. Ellegaard, et al., Environ. Health 10 (2011) 96.
[99] SCENIHR, SCCS, SCHER, Addressing the New Challenges for Risk Assessment, Scientific Committee on Emerging and Newly Identified Health Risks (SCENIHR), Scientific Committee on Consumer Safety (SCCS), and Scientific Committee on Health and Environmental Risks (SCHER), 154 pp., European Commission; available: http://ec.europa.eu/health/scientific_committees/emerging/docs/scenihr_o_037.pdf.
[100] IOM, Environmental Decisions in the Face of Uncertainty, Committee on Decision Making Under Uncertainty, Board on Population Health and Public Health Practice, Institute of Medicine, National Academies, National Academies Press, 260 pp., 2013.
[101] S.R. Hughes, P. Kay, L.E. Brown, Environ. Sci. Technol. 47 (2013) 661.
[102] P. Fong, N. Molnar, Bull. Environ. Contam. Toxicol. 81 (2008) 535.
[103] M.M. Schultz, M.M. Painter, S.E. Bartell, et al., Aquat. Toxicol. 104 (2011) 38.
[104] M.A. Thomas, R.D. Klaper, PLoS One 7 (2012) e32917.
[105] C.G. Daughton, M.S.T. Scuderi, Pharmaceuticals and Personal Care Products (PPCPs): Relevant Literature, U.S. Environmental Protection Agency, Las Vegas, NV (a comprehensive bibliographic database of literature references; first implemented 19 February 2008), 2013; available: http://www.epa.gov/ppcp/lit.html.
[106] W.A. Nicholson, Br. Med. J. 3 (1967) 730.
[107] C.L. Baltimore, J. Meyer, Pediatrics 42 (1968) 312.
[108] H. Matthew, Br. Med. J. 2 (1966) 788.
[109] C.G. Daughton, I.S. Ruhoy, Expert Rev. Clin. Pharmacol. 4 (2011) 211.
[110] C.G. Daughton, I.S. Ruhoy, in: K. Kümmerer, M. Hempel (Eds.), Green and Sustainable Pharmacy, Springer-Verlag, Berlin, Heidelberg, 2010, p. 77.
[111] C.G. Daughton, I.S. Ruhoy, Drug Saf. 31 (2008) 1069.
[112] C.G. Daughton, Environ. Health Perspect. 111 (2003) 757.
[113] C.G. Daughton, Environ. Health Perspect. 111 (2003) 775.
[114] USFDA, Approved Risk Evaluation and Mitigation Strategies (REMS), U.S. Food and Drug Administration, Silver Spring, MD, Silver Spring, MD, 2013; available: http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm111350.htm.
[115] C.G. Daughton, M.S.T. Scuderi, Drug Disposal and Environmental Stewardship: Relevant Literature, U.S. Environmental Protection Agency, Las Vegas, NV (a comprehensive bibliographic database of literature references; first implemented 19 February 2008), 2013; available: http://www.epa.gov/ppcp/lit.html.

[116] A. Klobuchar, C. Grassley, S. Brown, S. 3397: Secure and Responsible Drug Disposal Act of 2010: To Amend the Controlled Substances Act to Provide for Take-Back Disposal of Controlled Substances in Certain Instances, and for Other Purposes, Senate and the House of Representatives of the United States of America in Congress, 111th US Congress (2009–2010), 2010; available: http://www.govtrack.us/congress/bill.xpd?bill=s111-3397.

[117] DEA, Disposal of Controlled Substances (Proposed Rule to Implement the Secure and Responsible Drug Disposal Act of 2010), US Department of Justice, Drug Enforcement Administration, 1117-AB18, Washington, DC, 2011; available: http://www.reginfo.gov/public/do/eAgendaViewRule?pubId=201110&RIN=1117-AB18.

[118] PSI, Protecting Our Health and the Environment: The Need for Sustainably Financed Drug Take-Back Programs, Product Stewardship Institute, Inc. (PSI), Boston, MA, 2012; available: http://fyi.uwex.edu/pharma/files/2012/10/Pharmaceuticals-White-Paper-on-EPR_Final.pdf.

[119] Alameda County, Safe Drug Disposal, Alameda County Environmental Health, Alameda County, CA, 2013; available: https://www.acgov.org/aceh/safedisposal/index.htm; https://www.acgov.org/aceh/documents/SafeMed_FINAL.pdf.

[120] E. Warmerdam, No You Don't, Drug Makers Tell County, Courthouse News Service, Oakland, CA, 2012; available: http://www.courthousenews.com/2012/12/10/52963.htm.

[121] R.T. Szyba, Bill Calls for Safe Disposal of Potentially Toxic Human Waste, Legislative Press & Information Bureau, Rhode Island State House, Providence, RI, 2012; available: http://webserver.rilin.state.ri.us/News/pr1.asp?prid=8183.

[122] Ministry of the Environment, Extended Producer Responsibility Regulation for the Collection of Post Consumer Waste Pharmaceuticals and Sharps (including syringes) to be made under the Environmental Protection Act, Government of Ontario, Toronto, Ontario, Canada, 2012; available: http://www.ebr.gov.on.ca/ERS-WEB-External/displaynoticecontent.do?noticeId=MTE3MDM4&statusId=MTc1MTQ3&language=en.

[123] F. Bula, B.C. Moving Quickly Toward Producer-Pays Recycling System, The Globe and Mail, Toronto, Ontario, Canada, 2012; available: http://www.theglobeandmail.com/news/british-columbia/bc-moving-quickly-toward-producer-pays-recycling-system/article4517083/.

[124] US Department of Health and Human Services, Solicitation of the National Institutes of Health and the Centers for Disease Control and Prevention for Small Business Innovation Research Contract Proposals: Products for At-Home Deactivation of Psychoactive Prescription Medicines, US Department of Health and Human Services, OMB No. 0990-0115 PHS 2013-1, pp. 94–96, 2012; available: http://grants.nih.gov/grants/funding/SBIRContract/PHS2013-1.pdf.

[125] N. Vaszilcsin, V. Ordodi, A. Borza, Int. J. Pharm. 431 (2012) 241.

[126] G. Gece, Corros. Sci. 53 (2011) 3873.

[127] M. Sobhi, M. Abdallah, K. Khairou, Monatsh. Chem. – Chem. Monthly 143 (2012) 1379.

[128] C.G. Daughton, in: K. Heggenhougen, S. Quah (Eds.), International Encyclopedia of Public Health, Academic Press, Oxford, 2008, p. 66.

[129] C.G. Daughton, How Prescribing Impacts the Environment. One-Size-Fits-All Drug Dosing: Risks to Health and the Environment & Increased Health Care Costs, U.S. EPA, Las Vegas, NV; illustrated poster, 13 November 2012 [note: this illustration was published in: Daughton, C.G. and Ruhoy, I.S. "Lower-Dose Prescribing: Minimizing 'Side Effects' of Pharmaceuticals on Society and the Environment" Science of the Total Environment 2013, 443:324–337; 10.1016/j.scitotenv.2012.10.092]; available: http://www.epa.gov/esd/bios/daughton/how-prescribing-impacts-the-environment-(13Nov12).pdf.

[130] C.G. Daughton, Pharmaceuticals in the Environment: Overview of Significance, Concerns, and Solutions, Keynote Presentation at First Integral Virtual Symposium sponsored by the American Chemical Society [ACS] Division of Environmental Chemistry and the U.S. EPA, ACS 228th National Meeting in Philadelphia, PA, 2004; available: http://www.epa.gov/esd/bios/daughton/acs-25aug2004.htm; http://www.epa.gov/esd/bios/daughton/daughton-ACS2004.ppt.

Chapter 3

Prioritization: Selection of Environmentally Occurring Pharmaceuticals to Be Monitored

Maja Kuzmanovic*, Zoran Banjac*, Antoni Ginebreda*, Mira Petrovic†,‡ and Damia Barcelo*,†

*Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

†Catalan Institute for Water Research (ICRA), Scientific and Technological Park of the University of Girona, Girona, Spain

‡ICREA, Passeig Lluis Companys, Barcelona, Spain

Chapter Outline

1 INTRODUCTION

Continuous contamination of the environment with diverse groups of chemical compounds and their adverse effects on both ecosystem and human health is one of the most relevant environmental issues of today. According to European Inventory of Existing Commercial Chemical Substances (EINECS), in European Union (EU), there are more than 100,000 registered chemicals of which 70,000 are in daily use. Moreover, since analytic

Comprehensive Analytical Chemistry, Vol. 62. http://dx.doi.org/10.1016/B978-0-444-62657-8.00003-3

techniques continue to improve, the number and frequency of detections of still unregulated, emerging contaminants are increasing [1]. Additionally, chemical compounds that have been previously detected in the environment but whose potential adverse effects on human health and environment are only now being noted are also considered emerging contaminants. Although many of those compounds are present at low concentrations in the environment, their effects on ecosystems are still unknown, especially because they occur in complex chemical mixtures [2]. Some of those compounds are continuously introduced into the environment; therefore, regardless of their persistence in given conditions, they are permanently present (pseudo-persistent), which might lead to unexpected chronic effects of affected species [3]. Considering the high number of chemical compounds entering the environment on a daily basis and their potential adverse effects on ecosystem and human health, while having in mind budget limitation and time restrictions, there is a definite need to develop prioritization schemes for risk assessment, regulation, and management.

Pharmaceuticals are a large group of chemicals that are in daily use in terms of human medicine and veterinary use. This group of anthropogenic chemicals is among the ones with the largest input into the environment. After consumption of pharmaceuticals, their active substances undergo metabolic processes in the organism. Many transformation products and some percentage of unmetabolized compounds are excreted from the body and discharged into the sewage system where further biotic and abiotic transformation processes may also take place, giving rise to additional transformation products. As a whole, parent compounds and the associated transformation products (TPs) may enter the environment since conventional wastewater treatment plants are not efficient enough for their removal [4]. On the other hand, they might reach natural systems by improper disposal of sewage or unused medicines as well. Furthermore, pharmaceuticals can enter environmental systems from sludge that is used as fertilizer, manure that comes from veterinary medicine-treated animals, or directly into water from use in aquaculture [5]. The detection of pharmaceuticals in environmental samples has been reported worldwide [6–9]. Even though pharmaceuticals are the groups of chemicals designed to affect specific receptors in human or animal organisms, their environmental effects are still not examined enough. Occurrence of pharmaceuticals in freshwater systems is most commonly in orders of nanograms to micrograms per liter. Whereas acute ecotoxicity effects at those levels of concentrations are not very probable [5], chronic effects can be more likely expected. Moreover, toxicity in real systems can be influenced by additive and synergistic effects of constituents of mixture [10]. Several examples of toxic effects of pharmaceuticals on aquatic species have been examined [11,12]. However, more adverse effects of pharmaceuticals in natural ecosystems can be expected, such as endocrine disruption, genotoxicity, and development of antibiotic-resistant pathogenic bacteria.

This chapter will give the overview of (a) general principles of prioritization, (b) pharmaceutical risk assessment, and (c) existing prioritization schemes for prioritization of pharmaceuticals.

2 GENERAL PRINCIPLES FOR PRIORITIZATION OF CHEMICAL COMPOUNDS

Increasing number of manufactured chemical compounds in volumes of few hundred million tons that are being emitted into the environment each year [13] represent a big challenge in risk assessment and management. Some of environmentally occurring chemicals might have notable adverse effects. However, not all of them pose the threat to ecosystem and human health, certainly not in the same extent. The knowledge of hazardous properties of majority of these chemicals is still unknown and data for assessment of their risk are required. Still, because of the huge number of chemicals released and present into the environment, it is not possible to conduct environmental monitoring for all of them. This has led to development of chemical compound prioritization schemes for risk management and regulation purposes.

2.1 EU Existing Legislation

2.1.1 Registration, Evaluation and Authorization of Chemicals

Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) is the European Community regulation on chemicals [14], which is in power since 2007. It is based on the idea that chemical industry itself should ensure that the chemicals it produces and puts on the market do not adversely affect human health or the environment. It requires early identification of the intrinsic properties of chemical substances by placing the responsibility of supporting that information on the industry. The aim of REACH is to achieve better protection of environmental and human health in the EU but without obstructing development and competitiveness of chemical industry. Industry is obliged to have certain knowledge of the properties of its substances and to provide information of the chemicals, which are then registered in a central database run by the European Chemicals Agency (ECHA). The database is open to public and provides hazard information. Authorities should ensure that manufacturers are fulfilling their obligations and taking action on substances of very high concern. REACH was established in order to fulfill the knowledge gaps on the vast number of chemicals in use and to ensure that industry is able to provide risk and hazard assessment of the substances to finally implement the risk management measures to protect humans and the environment. However, it should be stressed that pharmaceuticals are not included in the domain of the REACH regulation.

2.1.2 Water Framework Directive

Considering environmental perspective with the aquatic environment focus, the big upturn was made by establishing Water Framework Directive (WFD), which aims to achieve good ecological and good chemical status of European surface waters by the year 2015. WFD identifies a list of 33 priority substances [15] and 8 other hazardous substances regulated by previous legislation that pose a significant risk to the EU aquatic environment. The lists of priority and hazardous substances include contaminants that have been recognized as dangerous especially for the human health and are regulated mainly on the basis of persistence, bioaccumulation, and toxicity (PBT) properties. Water bodies must meet the Environmental Quality Standards (EQS) [15] for these substances, that is, to keep the levels of concentrations of these compounds below the EQS to successfully achieve water quality requirements. The list of priority substances is reviewed and updated every 4 years. In last update proposal [15] of the European Commission, 15 new substances were added. As far as pharmaceuticals are concerned, until now, only the anti-inflammatory diclofenac has been included in the last update proposal [15].

Furthermore, EU member states are obliged to identify pollutants of regional or local importance and provide EQS, monitoring schemes and regulatory measures for them.

2.2 Prioritization of Chemical Compounds

Prioritization of chemical compounds may be done for several purposes and with different focuses, that is, ranking for identifying data gaps or data gathering and organizing, ranking for further testing (to select the compounds of highest concern and to focus testing efforts), ranking for risk assessment, and, finally, ranking for decision making and legislation establishment. According to their importance as aquatic contaminants, many prioritization schemes have been developed [16]. Some of the most representative are summarized in Table 1.

In general terms, the majority of prioritization schemes follow the same order sequence (Figure 1). First step involves the preselection of the chemicals to be prioritized. The preselection of chemicals may be done according to existing legislation and monitoring data or by identification of sources and pressures [22]. Afterward, it is followed by the exposure and hazard estimation. The occurrence and hazard data quantification might be done in different ways. Exposure can be determined by experimental measurements of concentration, that is, measured environmental data (MEC), or can be estimated by different models that use the information about the chemical's production quantity, frequency of its release to the environment, and predictions of its persistence and mobility in the environment giving predicted environmental data (PEC) [16]. Effect assessments in environmental risk assessment

TABLE 1 Prioritization Schemes with Focus on Aquatic Environment

Preselected Compounds	Criteria	Results	References
78 Compounds of "high concern"	PBT properties estimated exposure levels	Chlorpyrifos, ametryn, dichlofluanid, prometryn, chlorothalonil, cyanzine, trifluralin, atrazine	[17]
100 Pharmaceuticals, personal care products, and endocrine disruptors	Occurrence treatment in water treatment plants Ecological effects, health effects	Mestranol, bisphenol A, AHTN, TDIP, estrone, tri(2-butoxyethyl) phosphate, celestolide, ethylhexyl methoxycinnamate, musk xylene, musk ambrette, bezafibrate, propylparaben, linuron, HHCB, atorvastatin, lindane, 17β-estradiol	[18]
250 Compounds (WFD, relevant substances for river Rhine, measured in Swiss waters)	Potential occurrence in the water phase	Pentachlorophenol, Perfluorooctanoic acid (PFOA), Perfluorooctanesulfonic acid (PFOS), azithromycin, ofloxacin, clarithromycin, erythromycin roxithromycin, fluconazole, diatrizoate, pentachlorobenzene	[19]
500 Classical (WFD) and emerging organic contaminants	Frequency and extent of exceedance of PNEC (predicted no-effect concentration)	Diazinon, azoxystrobin, terbuthylazine, heptachlor, endosulfan I, 4,4′DDD, diuron, DEHP, Irgarol, 2,4′-DDD, alachlor, pyrene, endosulfan II, PCB-180, 4,4′-DDE, heptachlor epoxide B	[20]
Chemicals of Japanese Pollutant Release and Transfer Register (PRTR)	Human health environmental effects	Dichlorvos, arsenic, cobalt and beryllium compounds, disulfoton, fenitrothion, parathion, diazinon, antimony compounds, chlorpyrifos-methyl	[21]

Adapted from Ref. [16].

(ERA) most commonly include acute or chronic toxicity of chemicals, which can be determined by *in vivo* toxicity tests for standard test species representative of different trophic levels (algae, *Daphnia magna*, and fish), combined with bioaccumulation and persistence potential of substances. By *in vivo* tests, concentration of the chemical that provokes certain harmful effect or lethality of test species is being measured. The most common is the use of EC50 or LC50 (50% effect concentration or 50% lethal concentration, respectively)

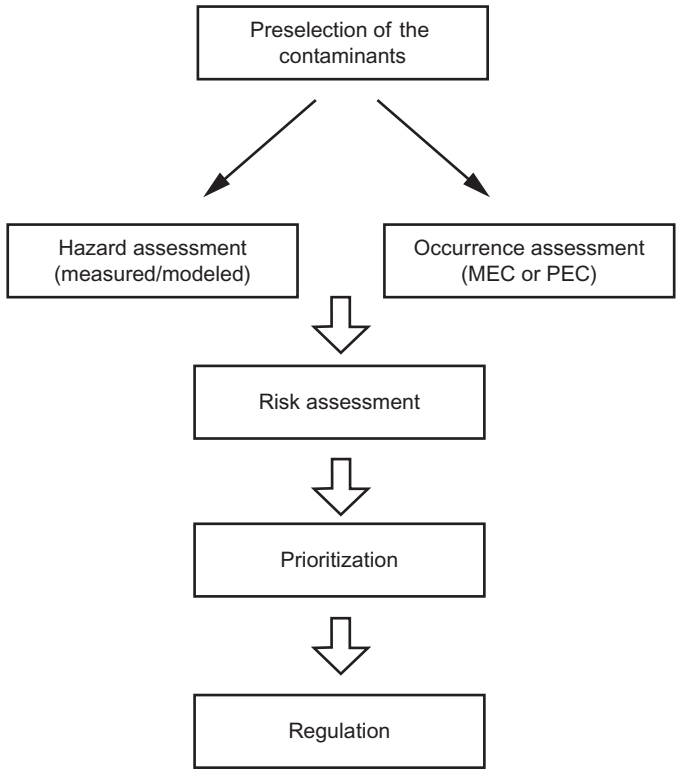

FIGURE 1 General prioritization scheme for legislation purposes. Preselection of candidates for prioritization might be done according to existing legislation, production volume, usage data, etc. Occurrence data might be measured (MEC—measured environmental concentration) or predicted by models (PEC—predicted environmental concentration).

as the indicator of acute toxicity of the chemical. Acute toxicity tests provide information of chemical concentration, which, after short-term exposure to test species, provokes targeted endpoint effect (mortality, immobility, growth stagnation, etc.). On the other hand, chronic toxicity gives information concerning the dose of a chemical compound to which an organism is exposed for longer time (or even its whole lifetime) and is provoking certain sublethal effect.

Chronic toxicity data seem to be quite scarce and sometimes can be derived from LC50 or EC50 by applying certain assessment factor (AF). Another approach is estimation of toxicity by QSAR (quantitative structure–activity relationship) models [23], sometimes referred as *in silico* methods. Most commonly used tool for environmental toxicity assessment is EPA's ECOSAR^{TM} tool. The structure–activity relationships (SARs) in the ECOSAR^{TM} are used for aquatic toxicity prediction based on the similarity

of structures to chemicals for which the aquatic toxicity-measured data exist. Toxicity estimations are based on mathematical relationships between the Kow values and the corresponding measured toxicity. Since 1981, the US EPA has used SARs to predict the aquatic toxicity of new industrial chemicals in the absence of test data [24] and several authors [25–27]. ECOSAR™ predicts toxicity for three general types of chemicals, that is, neutral organics, organic chemicals with excess toxicity, and surfactant-active chemicals. Neutral organics are chemicals that are nonreactive and provoke effect of narcosis, which is referred as baseline toxicity. Organic chemicals with excess toxicity represent the group of chemicals that have reactive functional groups and due to that have different toxicological mode of action. Surface-active compounds have hydrophobic and hydrophilic part of their molecule. In ECOSAR™, they are grouped on the basis of the total charge of the molecules (anionic, cationic, neutral, and amphoteric).

Besides risk of toxic effects, substances with persistency and bioaccumulation potential pose an additional risk to the environment because they can remain present in the environment for a long time or they can be easily accumulated in biota. To provide integrated information of risk, indexes or scoring systems for integration of information of possible adverse properties of chemicals can be used [17,18,28].

The last step for prioritization includes procedure or models for calculating the comparable risk of chemicals and final ranking or grouping the chemicals according to their risk.

3 PHARMACEUTICALS: ENVIRONMENTAL RISK ASSESSMENT

Occurrence and potential risk of pharmaceuticals in the environment become an issue of increased concern over the years [10]. This is especially due to worldwide detections of those products in the environment [6–9]. Pharmaceuticals are in general less persistent than other known persistent organic pollutants, but because of their everyday use and continuous release into the environment, they are constantly present, that is, so-called pseudo-persistent [3]. For some pharmaceuticals, adverse effects are already noted, for example, synthetic sex hormones [29]. For some, because their low levels of detection in the environment and reasonably low acute toxicity, acute effects are not expected. On the other hand, pharmaceuticals are compounds designed to affect biological receptors even in small quantities and therefore they need special attention [30]. Moreover, chronic environmental effects are still unknown for the majority of these compounds.

The regulation of pharmaceutical products in Europe started in 1965 by implementation of European Economic Community's directive [31]. In 1993, assessment of pharmaceutical product risk toward the environment

was introduced by European Economic Community [32]. Later in that year, the European Medicines Evaluation Agency (EMEA) was created and subsequent guidelines for the ERA of pharmaceutical products were issued [32]. In the current EMEA regulations, a threshold safety value of 10 ng/l is set, and compounds whose PEC exceed this quantity have to be subjected to toxicity tests and can, therefore, be considered as potential candidates to be included in monitoring programs [33]. A draft of the guideline was published in 2001, and the final document came into force in December 2006. The latter EMEA guideline describes the stepwise tiered procedure for estimation of potential risks of pharmaceutical products to the environment. However, whatever the impact the pharmaceutical product has on the environment, this will not be a criterion for prevention of its marketing, since the benefit of pharmaceutical to patients is considered priority. If it is likely that certain pharmaceutical product poses risk for the environment, precautionary and mitigation safety measures must be taken [33]. The guideline is focused on the possible environmental risks associated with the use of the pharmaceutical under concern, although possible ways of entering into the environment arising from disposal and manufacture are not considered. The general principles of the approach are presented in Figure 2.

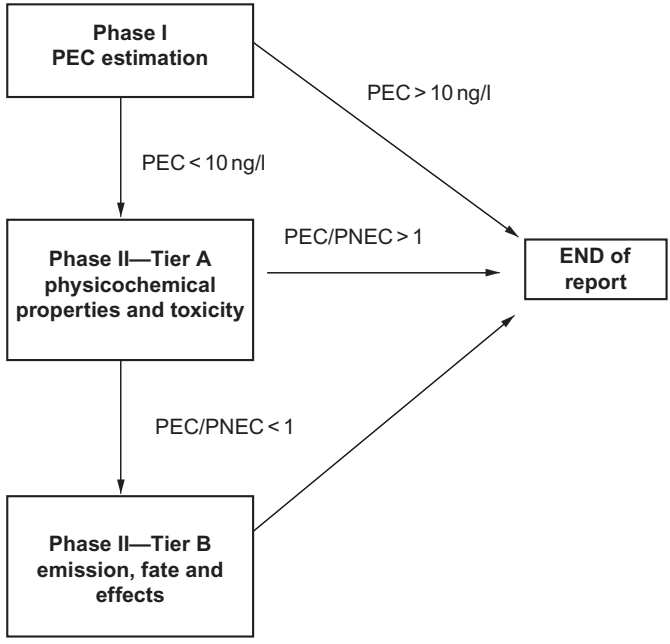

FIGURE 2 Scheme for tiered environmental risk assessment as proposed by EMEA guideline.

3.1 Phase I

First phase includes estimation of exposure by calculation of the PEC. It is restricted to the aquatic compartment and is calculated according to some general information of pharmaceutical as release information and environmental fate. The calculations are made according to Equation (1):

$$\mathrm{PEC\,(surface\,water)} = \frac{\mathrm{DOSEai} \times \mathrm{Fpen}}{\mathrm{WASTEWinhab} \times \mathrm{DILUTION}} \tag{1}$$

Where, DOSEai is maximum daily dose consumed per inhabitant, Fpen is market penetration factor of the active ingredient of pharmaceutical product, WASTEWinhab is volume of wastewater generated per inhabitant, and DILUTION is dilution of wastewater effluent in recipient surface waters. If the estimated PEC of pharmaceutical product does not exceed the threshold value of 10 ng/l, no further risk assessment is necessary. Exceptions are the compounds with high endocrine disruption potential or very lipophilic properties that may have adverse effects even below the threshold value. Regardless of their PEC values, second-tier assessment must be performed for them. Additionally, in case the log Kow values of the compound are equal or higher than 4.5, PBT assessment is required.

3.2 Phase II

Second phase involves tier A and tier B. In tier A, base set data of physicochemical properties and on the fate of a substance in the environment are determined. This includes degradation, transformation in aquatic environment, adsorption–desorption properties, and organic carbon–water partition coefficient (Koc).

Besides, toxicity data for three standard test species (algae, *Daphnia* sp., and fish) are required. Acute toxicity data for selected common pharmaceuticals either experimental or calculated using ECOSAR are given in Tables 2 and 3 as an example. Risk assessment is conducted for surface water, groundwater, and microorganisms in water, and if one or more result show indication of risk, further assessment is necessary leading to tier B. PEC value from the first tier is compared with the respective predicted no-effect concentrations (PNECs). PNEC values are obtained from derivation of acute toxicity data (EC50 or LC50) by applying AF of 1000 or by applying AF of 10 to no observed effect concentration (NOEC) as represented by Equation (2):

$$\mathrm{PNEC_{water}} = \frac{\text{lowest acute EC50/LC50}}{1000}, \quad \mathrm{PNEC_{water}} = \frac{\mathrm{NOEC}}{10} \tag{2}$$

If indication of risk is presented by one or more of the resulting risk quotients, that is, they exceed 1 ($\mathrm{HQ} > 1$), in case of surface water, more data need to be provided for specific risk assessment in tier B. If PEC/PNEC ratios

TABLE 2 Acute Toxicity Data for Some Common Pharmaceuticals Obtained Using ECOSAR

	ECOSAR Data [24]		
Pharmaceutical	*EC50 Algae (mg/l)*	*EC50* Daphnia *sp. (mg/l)*	*LC50 Fish (mg/l)*
Amoxicillin	**3316**	**350**	**366**
Acetaminophen	2549	42	1
Clarithromycin	**2.08**	**3.31**	**17.36**
Clofibric acid	192	293	53
Carbamazepine	70	111	101
Cimetidine	40	35	571
Diclofenac	2911	5057	532
Erythromycin	4.3	7.8	61
Gemfibrozil	6.7	4.9	11
Ibuprofen	26	38	5
Naproxen	34	15	22
Ofloxacin	**2444**	**1786**	**19352**
Roxithromycin	4	6	50
Sulfamethoxazole	51	4.5	890

Numbers in bold were not taken from Ref. [24], but estimated by authors using ECOSAR.

in tier A show the value higher than 1, additional data of the compound are required. PEC and PNEC values are refined in this step by implementing more data of emission, fate, and effects of tested compound. Even if the risk of the compound is proven, still, refusal for marketing authorization is not an outcome due to precedence for patient benefit. However, some risk mitigation measures are introduced considering mainly proper disposal suggestions to the consumers by labeling the product.

Risk may be generally defined as the combination (i.e., product) of a probability of occurrence of a certain event by its hazard effects [16]:

$$\text{Risk} = \text{Occurrence} \times \text{Effects}$$

Different existing risk assessment approaches have been developed in order to identify and rank compounds of environmental concern for both regulatory and monitoring purposes. Whereas most of all the existing schemes share the basic underlying risk assessment paradigm, they differ on how risk,

TABLE 3 Acute Toxicity Data for Most Representative Pharmaceuticals

Pharmaceutical	EC50 Algae (mg/l)	EC50 Invertebrates (mg/l)	LC50 Fish (mg/l)	References
Amoxicillin	0.004	–	–	[34]
Acetaminophen	105	300	900	[35]
Clarithromycin	0.09	25.72	280	[36–38]
Carbamazepine	74	14	35	[39–41]
Cimetidine	–	271	–	[41]
Diclofenac	16	22	–	[40]
Erythromycin	0.02	15	900	[35]
Gemfibrozil	4	10	0.9	[42]
Ibuprofen	342.2	101	110	[35,39]
Naproxen	626	166	600	[35,39]
Ofloxacin	1.5	30	10	[35]
Roxithromycin	–	7	50	[43,44]
Sulfamethoxazole	0.027	>100	563	[40,43]

occurrence, and effects are defined and hence quantified. While hazard is usually represented by intrinsic properties of compounds and includes PBT estimations, since not all hazardous compounds are present in all geographic areas, estimations of risk are performed by adding the corresponding exposure data to hazard information. Exposure data can be expressed in the form of MEC or PEC, which can be indirectly predicted on the basis of, for example, the data of annual production, sales rate, and number of prescriptions. Toxicity data of chemicals can as well be measured *in vivo* or be predicted by the so-called *in silico* models. All the schemes commented in the succeeding text use different types of PBT and/or exposure methods to rank and prioritize pharmaceutical compounds and systematic comparison of those approaches is convenient to make improvements in pharmaceutical risk assessment and prioritization to be further used on the previously described tiered process. The following section is a summary of some of the most relevant proposed pharmaceutical prioritization methods.

The topic has been recently reviewed by Ross et al., and the classification given in the succeeding text is largely based on this comprehensive work.

4 SUMMARY OF SOME RELEVANT PRIORITIZATION METHODS: EXPOSURE-, HAZARD-, AND RISK-BASED SCHEMES

Reliable prioritization schemes must be clearly defined, based on validated data, and widely applicable to as much compounds as possible. Furthermore, adequate prioritization methods to be used in tiered procedures rely mostly on the generation of a minimum number of false-negatives. False-positives are less crucial since they are considered in the next tier step, while false-negatives are omitted.

Main drawbacks are usually the lack of data (i.e., ecotoxicity data). On the other hand, compounds whose biological target is different than prokaryotes are often excluded from the ranking (i.e., antibiotics and antiviral) and those whose purpose is other than pharmaceuticals (e.g., caffeine and nicotine) [45].

In the succeeding text, we briefly examine 12 existing prioritization methods. They may be classified as exposure-, hazard-, or risk-based depending on the data they use, which are summarized in Table 4. On the other hand, their more relevant features are reported on Table 5.

Finally, the 20 top-ranked compounds as issued from some of the methods considered are shown in Table 6.

5 CONCLUSIONS

Due to the large number of chemical compounds entering the natural environment on a daily basis, it is necessary to expand the knowledge on their possible adverse effects on ecosystem and human health. Pharmaceuticals are the group of compounds that are in common use and their input in the environment is constant. Regardless, their long-term adverse effects are still largely unknown. Due to their low concentrations in the environment, acute effects of most of the pharmaceuticals are not likely expected; however, chronic toxicity and other possible adverse effects should not be excluded. The existing legislation in EU gives the guidelines for ERA for pharmaceuticals for her member states. However, to perform more accurate risk assessment, it is necessary to obtain more information about toxicity of those compounds, to develop models based on real test data, and possibly to link the observed concentrations and risk expected to real ecosystem status. To identify pharmaceuticals of possible environmental concern, many prioritization schemes have been developed. In general, only few among the vast number of pharmaceuticals are identified as possible pollutants. However, it should be stressed that the risk of pharmaceuticals is strongly dependent not only on their hazard, fate, and transport patterns but also on the site-specific sociogeographic situation and consequential usage. This should be taken into account when prioritization works are done.

Finally, the relevance of prioritization schemes for public management purposes should be recognized since they provide the necessary scientific background to properly allocate monitoring efforts under the always increasing budgetary constraints.

TABLE 4 Classes of Data Included in Some Selected Prioritization Methods

Data	Method 1	Method 2	Method 3	Method 4	Method 5	Method 6	Method 7	Method 8	Method 9	Method 10	Method 11	Method 12
Sales statistics (kg)	√	√	√	–	–	–	–	–	–	–	–	–
Prescriptions	–	–	–	–	–	–	–	–	–	–	–	–
Production	–	–	–	–	–	–	–	–	–	–	–	–
Potency (pharma)	√	–	–	–	–	√	–	–	–	√	–	–
Effect data	–	–	√	√	√	–	√	–	–	√	√	√
Water concentration	–	–	–	√	√	–	–	–	–	–	–	–
Log *P*	√	—	—	—	√	√	√	√	√	√	√	–
Modeling	–	–	–	–	–	–	–	√	–	√	√	–
Persistency/ fate	–	–	–	–	√	–	√	–	–	–	–	–
WWTP removal	–	–	–	–	–	–	–	–	–	–	√	–
Model type	Risk	Exposure	Risk	Risk	Risk	Hazard	Hazard	Hazard	Hazard	Risk	Risk	Hazard

Adapted from Ref. [45].

TABLE 5 Main Characteristics of Selected Prioritization Methods

Method #	Remarks	References
1	*FPM fish-plasma model* Comparison between human plasma concentration for a determined pharmaceutical and fish-plasma steady-state concentration	[46]
2	*Sales annual* Based on sales statistics. Used often for regulatory purposes. In this study, data taken from Sweden (2009)	[47]
3	*PEC/PNEC* Ranking based on the risk quotient RQ ratio "predicted environmental concentration/predicted no-effect concentration." It is the most classical and general approach to ERA. RQ≥1 indicates environmental concern	[48]
4	*MEC/PNEC* Idem as the previous but based on "measured environmental concentration" (MEC) rather than PEC	[49]
5	*"Aquatic environment" ranking* Focused on risk to the aquatic environment. Based on surface water concentration, half-life, and fish and crustacean toxicity	[50]
6	*Critical environment concentration* (*CEC*) Hazard measure similar to FPM but independent of exposure value	[51]
7	*PBT* (*persistence–bioaccumulation–toxicity*) Classical effect-based approach widely used in ERA	[52]
8	*QSAR* Pharmaceuticals are ranked according to their predicted aquatic toxicity, removal by WWTP, bioaccumulation potential, and number of compounds included in each therapeutic class (as surrogate of volumes produced) Cornerstone method is the prediction of aquatic ecotoxicities using QSAR (EPIWIN software from EPA) and specifically ECOSAR for predicting fish, *Daphnia*, and algae chronic toxicities from Kow About 3000 substances belonging to 51 classes are assessed. Different ranks of the different classes (rather than single compounds) according to the various criteria are issued	[53]

TABLE 5 Main Characteristics of Selected Prioritization Methods—Cont'd

Method #	Remarks	References
9	*Log P* (*log Kow*) Octanol–water partition coefficient can be experimentally measured or estimated by SAR. In this method log octanol-water partition coefficients were calculated using EPI Suite™ KOWWIN software.	[54]
10	*Extended EMEA* (*2-tier*)(*France*) Method essentially inspired on the EMEA guidelines (2-tier procedure). Exposure is predicted (PEC) based on consumption/sales and excretion. Effects considered include ecotoxicology, pharmacological factors (mode of action, enzyme modulation, and adverse effects, such as carcinogenicity), and log *P*. If a compound shows a potential for any of listed adverse effects it is considered as a priority supstance. Expert judgment is also included as part of the process The method has been applied to the French case. 120 Pharmaceuticals and 30 active metabolites belonging to blood-lipid-lowering agents, analgesics, anxiolytics, antidepressants, nonsteroidal anti-inflammatory drugs, antihypertensives, antipsychotics, antibacterial, anticonvulsants, and corticoids are assessed, giving rise to a list of 40 priority parent compounds and 15 metabolites	[55]
11	*Toxic load* (*TL*) Quantitative risk-based ranking approach, combining mass load (estimated through number of prescriptions and/or annual production), human metabolism (elimination), WWTP removal, and multiple toxic endpoints (human, mouse, and aquatic toxicity). This allows estimating a toxic load ratio TL = mass loading/toxicity threshold Different priority lists are issued based on the different criteria The 200 most prescribed drugs are assessed using this method	[56]
12	*EOCRank* Ranking system for pharmaceuticals in stream/source water based on: occurrence, treatment, ecological effect, and human health effects. The following properties were considered in the ranking: prevalence, frequency of detection, removal, bioaccumulation, ecotoxicity, pregnancy effects, and health effects (carcinogenicity, mutagenicity, impairment of fertility, central nervous system acting, endocrine effects, immunotoxicity, and developmental effects)	[18]

TABLE 6 Ranked Lists of Top 20 Compounds for Some Selected Methods

	Method 1	Method 2	Method 3	Method 4	Method 5	Method 6	Method 7	Method 8	Method 9
1	Orlistat	Acetylsalicylic acid	Ethinyl estradiol	Propranolol	Fluoxetine	Iloprost	Acitretin	Permethrin	Mivacurium bromide
2	Fluphenazine	Paracetamol	Atovaquone	Ethinyl estradiol	Ibuprofen	Ethinyl estradiol	Aprepitant	Loperamide	Atracurium
3	Montelukast	Metformin	Sertraline	Estradiol	Paracetamol	Estradiol	Atovaquone	Biperiden	Montelukast
4	Loratadine	Ibuprofen	Estradiol	Naproxen	Estradiol	Loratadine	Beclometasone	Clomifene	Fulvestrant
5	Simvastatin	Acetylcysteine	Mycophenolate mofetil	Fluvoxamine	Diclofenac	Clemastine	Betamethasone	Amiodarone	Fluphenazine
6	Fulvestrant	Glucosamine	Propranolol	Sertraline	Carvedilol	Azelastine	Bromocriptine	Haloperidol	Amiodarone
7	Telmisartan	Levodopa	Acetylsalicylic acid	Felodipine	Propranolol	Buprenorphine	Carvedilol	Itraconazole	Lumefantrine
8	Estradiol	Metoprolol	Naproxen	Fluoxetine	Gemfibrozil	Misoprostol	Citalopram	Bromhexine	Cetylpyridinium
9	Felodipine	Naproxen	Felodipine	Ketoconazole	Naproxen	Etonogestrel	Clemastine	Stiripentol	Verteporfin
10	Amiodarone	Mesalazine	Ketoconazole	Amlodipine	Diazepam	Medroxyprogesterone	Clobetasol	Pentamidine isethionate	Telmisartan
11	Sertraline	Cholestyramine	Paracetamol	Citalopram	Paroxetine	Estriol	Clozapine	Acitretin	Orlistat
12	Verapamil	Sulfasalazine	Amitriptyline	Bromhexine	Amitriptyline	Flupentixol	Cyproterone	Dinoprostone	Bexarotene
13	Irbesartan	Valproic acid	Fluoxetine	Furosemide	Carbamazepine	Meclozine	Dasatinib	Meloxicam	Permethrin
14	Dextropropoxyphene	Gabapentin	Dipyridamole	Budesonide	Risperidone	Felodipine	Docetaxel	Desogestrel	Paricalcitol

15	Meclozine	Carbamazepine	Chlorprothixene	Metoprolol	Codeine	Terbinafine	Estradiol	Oxybuprocaine	Acitretin
16	Clomipramine	Tramadol	Bromhexine	Carbamazepine	Phenobarbital	Simvastatin	Ethinyl estradiol	Amylmetacresol	Lercanidipine
17	Duloxetine	Furosemide	Entacapone	Carvedilol	Fosinopril	Haloperidol	Felodipine	Estriol	Cinacalcet
18	Levomepromazine	Diclofenac	Fulvestrant	Mirtazapine	Fenofibrate	Loperamide	Isradipine	Felodipine	Clomifene
19	Atorvastatin	Atenolol	Galantamine	Loratadine	Furosemide	Levomepromazine	Ketoconazole	Pizotifen	Toremifene
20	Estriol	Allopurinol	Propofol	Tamoxifen	Atenolol	Pizotifen	Ketotifen	Tamoxifen	Tafluprost

Rank	**Method 10(a)**	**Method 10(b)**	**Method 11(a)**	**Method 11(b)**	**Method 11(c)**	**Method 11(d)**	**Method 12**
1	Allopurinol	Salicylic acid	Acetaminophen	Potassium chloride	Metformin HCl	Levothyroxine sodium	Musk moskene
2	Amiodarone	Fenofibric acid	Hydrocodone bitartrate	Acetaminophen	Polyethylene glycol	Ranitidine HCl	Octocrylene
3	Amoxicillin	Perindoprilat	Hydrochlorothiazide	Metformin HCl	Amoxicillin trihydrate	Clopidogrel bisulfate	Desulfinyl fipronil
4	Amphotericin B	Ramiprilat	Lisinopril	Ranitidine HCl	Cephalexin	Fluticasone propionate	Demeclocycline
5	Atenolol	Demethyltramadol	Levothyroxine sodium	Gabapentin	Ranitidine HCl	Furosemide	Celestolide
6	Bezafibrate	Hydroxy-ibuprofen	Simvastatin	Amoxicillin trihydrate	Trimethoprim	Montelukast sodium	Ethylhexyl methoxycinnamate
7	Buflomedil	Carboxy-ibuprofen	Amoxicillin	Ibuprofen	Furosemide	Trimethoprim	Musk xylene
8	Carbamazepine	Acetyl sulfamethoxazole	Metoprolol succinate	Cephalexin	Levothyroxine sodium	Atenolol	Musk ambrette

TABLE 6 Ranked Lists of Top 20 Compounds for Some Selected Methods—Cont'd

Rank	Method 10(a)	Method 10(b)	Method 11(a)	Method 11(b)	Method 11(c)	Method 11(d)	Method 12
9	Ceftriaxone	14-OH-clarithromycin	Amlodipine besylate	Methocarbamol	Fluticasone propionate	Tramadol HC1	Bezafibrate
10	Ciprofloxacin	Norfluoxetine	Metformin HCl	Divalproex sodium	Gabapentin	Simvastatin	Propylparaben
11	Clarithromycin	OH-metronidazole	Ethinyl estradiol	Polyethylene glycol	Atenolol	Hydrochlorothiazide	Ethylparaben
12	Cyamemazine	B-Hydroxy-acid metabolite	Azithromycin	Levothyroxine sodium	Hydrochlorothiazide	Acetaminophen	Methyl parathion
13	Diclofenac	2-OH-atorvastatin	Albuterol sulfate	Metoprolol succinate	Ciprofloxacin HCl	Metformin HCl	Methylparaben
14	Diosmin	4-OH-atorvastatin	Oxycodone HCl	Trimethoprim	Acetaminophen	Bupropion HC1	Norfluoxetine
15	Doxycycline	–	Alprazolam	Furosemide	Clopidogrel bisulfate	Olmesartan	Equilenin
16	Fluoxetine	–	Atorvastatin calcium	Sulfamethoxazole	Levetiracetam	Sulfamethoxazole	17α-Estradiol
17	Fosfomycin	–	Fluticasone propionate	Ciprofloxacin HCL	Levofloxacin	Pioglitazone	Equilin
18	Fosfomycin	–	Atenolol	Omeprazole	Sulfamethoxazole	Levetiracetam	Clofibric acid
19	Furosemide	–	Omeprazole	Guaifenesin	Fexofenadine HCl	Risperidone	Musk ketone
20	Ibuprofen	–	Zolpidem tartrate	Carisoprodol	Valacyclovir HCl	Citalopram HBr	Sulfamethoxine

Method 1: FPM fish-plasma model; Method 2: Sales annual; Method 3: PEC/PNEC; Method 4: MEC/PNEC; Method 5: "Aquatic environment" ranking; Method 6: Critical environmental concentration (CEC); Method 7: PBT (persistence–bioaccumulation–toxicity); Method 8: QSAR; Method 9: log *P* (the same as method 1); Method 10(a): Extended EMEA (2-tier)(France), parent compounds; Method 10(b): Extended EMEA (2-tier)(France), active metabolites; Method 11(a): Toxic load (TL), number of prescriptions; Method 11(b): Toxic load (TL), production kg/year; Method 11(c): Toxic load (TL), lad; Method 11(d): Toxic load (TL), all endpoints; Method 12: EOCRank, overall.
Adapted from Ref. [45].

REFERENCES

[1] K.E. Murray, S.M. Thomas, A.A. Bodour, Environ. Pollut. 158 (2010) 3462.
[2] R.P. Schwarzenbach, B.I. Escher, K. Fenner, T.B. Hofstetter, C.A. Johnson, U. von Gunten, B. Wehrli, Science 313 (2006) 1072.
[3] C.G. Daughton, T.A. Ternes, Environ. Heal. Perspect. 107 (1999) 907.
[4] J. Radjenovic, M. Petrovic, D. Barcelo, Trends Anal. Chem. 28 (2009) 562.
[5] C. Carlsson, A.-K. Johansson, G. Alvan, K. Bergman, T. Kuhler, Sci. Total. Environ. 364 (2006) 67.
[6] M.J. Hilton, K.V. Thomas, J. Chromatogr. A 1015 (2003) 129.
[7] R. Andreozzi, V. Caprio, C. Ciniglia, M. De Champdore, R. Lo Giudice, R. Marotta, E. Zuccato, Environ. Sci. Technol. 38 (2004) 6832.
[8] D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, H.T. Buxton, Environ. Sci. Technol. 36 (2002) 4007.
[9] T. Heberer, Toxicol. Lett. 131 (2002) 5.
[10] K. Fent, A.A. Weston, D. Caminada, Aquat. Toxicol. 76 (2006) 122.
[11] G. Nentwig, Arch. Environ. Contam. Toxicol. 52 (2007) 163.
[12] S. Schnell, N.C. Bols, C. Barata, C. Porte, Aquat. Toxicol. 93 (2009) 244.
[13] W. Mucke, K. Voigt, H. Schulze, Toxicol. Environ. Chem. 13 (1986) 129.
[14] E.C. Agency, European Community Agency, 2006/1907/EC, 2006.
[15] E. Commission, European Commission, 2008/105/EC, 2008.
[16] D. Guillen, A. Ginebreda, M. Farre, R.M. Darbra, M. Petrovic, M. Gros, D. Barcelo, Sci. Total. Environ. 440 (2012) 236.
[17] K. Daginnus, S. Gottardo, A. Paya-Perez, P. Whitehouse, H. Wilkinson, J.-M. Zaldivar, Int. J. Environ. Res. Public Health 435 (2011) 435–455.
[18] A. Kumar, I. Xagoraraki, Sci. Total. Environ. 408 (2010) 5972.
[19] C.W. Goetz, C. Stamm, K. Fenner, H. Singer, M. Schaerer, J. Hollender, Environ. Sci. Pollut. Res. 17 (2010) 341.
[20] P.C. von der Ohe, V. Dulio, J. Slobodnik, E. De Deckere, R. Kuehne, R.-U. Ebert, A. Ginebreda, W. De Cooman, G. Schueuermann, W. Brack, Sci. Total. Environ. 409 (2011) 2064.
[21] D. Lerche, S.Y. Matsuzaki, P.B. Sorensen, L. Carlsen, O.J. Nielsen, Chemosphere 55 (2004) 1005.
[22] M. Petrovic, A. Ginebreda, V. Acuna, R.J. Batalla, A. Elosegi, H. Guasch, M. Lopez de Alda, R. Marce, I. Munoz, A. Navarro-Ortega, E. Navarro, D. Vericat, S. Sabater, D. Barcelo, Trends Anal. Chem. 30 (2011) 1269.
[23] H.J. Pedersen, P. Kudsk, A. Helweg, Pestic. Sci. 44 (1995) 131.
[24] H. Sanderson, D.J. Johnson, C.J. Wilson, R.A. Brain, K.R. Solomon, Toxicol. Lett. 144 (2003) 383.
[25] H. Qin, J. Chen, Y. Wang, B. Wang, X. Li, F. Li, Y. Wang, Chin. Sci. Bull. 54 (2009) 628.
[26] H. Kusic, B. Rasulev, D. Leszczynska, J. Leszczynski, N. Koprivanac, Chemosphere 75 (2009) 1128.
[27] Z. Dashtbozorgi, H. Golmohammadi, Eur. J. Med. Chem. 45 (2010) 2182.
[28] M. Nadal, V. Kumar, M. Schuhmacher, J.L. Domingo, Chemosphere 64 (2006) 1526.
[29] J.P. Nash, D.E. Kime, L.T.M. Van der Ven, P.W. Wester, F. Brion, G. Maack, P. Stahlschmidt-Allner, C.R. Tyler, Environ. Heal. Perspect. 112 (2004) 1725.
[30] G.T. Ankley, B.W. Brooks, D.B. Huggett, J.P. Sumpter, Environ. Sci. Technol. 41 (2007) 8211.

[31] E.E. Community, European Economic Community, 65/65/EEC, 1965.
[32] E.E. Community, European Economic Community, 93/39/EEC, 1993.
[33] J.O. Straub, Toxicol. Lett. 135 (2002) 229.
[34] B. Halling-Sørensen, Chemosphere 40 (2000) 731.
[35] C. Boillot, C. Bazin, F. Tissot-Guerraz, J. Droguet, M. Perraud, J.C. Cetre, D. Trepo, Y. Perrodin, Sci. Total. Environ. 403 (2008) 113.
[36] G. Huschek, P.D. Hansen, H.H. Maurer, D. Krengel, A. Kayser, Environ. Toxicol. 19 (2004) 226.
[37] M. Isidori, M. Lavorgna, A. Nardelli, L. Pascarella, A. Parrella, Sci. Total. Environ. 346 (2005) 87.
[38] M. Grung, T. Kallqvist, S. Sakshaug, S. Skurtveit, K.V. Thomas, Ecotoxicol. Environ. Saf. 71 (2008) 328.
[39] M. Cleuvers, Ecotoxicol. Environ. Saf. 59 (2004) 309.
[40] B. Ferrari, R. Mons, B. Vollat, B. Fraysse, N. Paxeus, R. Lo Giudice, A. Pollio, J. Garric, Environ. Toxicol. Chem. 23 (2004) 1344.
[41] S.D. Kim, J. Cho, I.S. Kim, B.J. Vanderford, S.A. Snyder, Water Res. 41 (2007) 1013.
[42] M.D. Hernando, A. Aguera, A.R. Fernandez-Alba, Anal. Bioanal. Chem. 387 (2007) 1269.
[43] K. Choi, Y. Kim, J. Jung, M.-H. Kim, C.-S. Kim, N.-H. Kim, J. Park, Environ. Toxicol. Chem. 27 (2008) 711.
[44] Y.-J. Lee, S.-E. Lee, D.S. Lee, Y.-H. Kim, Environ. Toxicol. Pharmacol. 26 (2008) 216.
[45] V. Roos, L. Gunnarsson, J. Fick, D.G.J. Larsson, C. Ruden, Sci. Total. Environ. 422 (2012) 102.
[46] D.B. Huggett, J.C. Cook, J.F. Ericson, R.T. Williams, Hum. Ecol. Risk Assess. 9 (2003) 1789.
[47] Apotekens Service. http://www.apotekensservice.se/Nyheter-och-Press/Presskontakt/Lakemedelsstatistik/, 2010 (accessed on November 2).
[48] B. Mattson, Drug Inf. J. 91 (2007) 41.
[49] C. Wahlberg, B. Bjorlenius, N. Paxeus, Water Sci. Technol. 63 (2006) 1772.
[50] E.R. Cooper, T.C. Siewicki, K. Phillips, Sci. Total. Environ. 398 (2008) 26.
[51] J. Fick, R.H. Lindberg, J. Parkkonen, B. Arvidsson, M. Tysklind, D.G.J. Larsson, Environ. Sci. Technol. 44 (2010) 2661.
[52] A.K. Wennmalm, B. Gunnarsson, Drug Inf. J. 39 (2005) 291.
[53] H. Sanderson, D.J. Johnson, T. Reitsma, R.A. Brain, C.J. Wilson, K.R. Solomon, Regul. Toxicol. Pharmacol. 39 (2003) 158.
[54] US EPA, KOWWIN v 1.68, in Estimation Programs Interface Suite™ for Microsoft Windows, v 4.10, 2011. United States Environmental Protection Agency, Washington, DC.
[55] J.-P. Besse, J. Garric, Toxicol. Lett. 176 (2008) 104.
[56] Z. Dong, D.B. Senn, R.E. Moran, J.P. Shine, Regul. Toxicol. Pharmacol. 65 (2013) 60.

Chapter 4

Analysis of Pharmaceuticals in Drinking Water, Groundwater, Surface Water, and Wastewater

Imma Ferrer and E. Michael Thurman

Center for Environmental Mass Spectrometry, Department of Environmental Engineering, University of Colorado, Boulder, Colorado, USA

Chapter Outline

Comprehensive Analytical Chemistry, Vol. 62. http://dx.doi.org/10.1016/B978-0-444-62657-8.00004-5

1 INTRODUCTION: PHARMACEUTICALS IN WATERS

Pharmaceuticals found in water samples, due to human activities (via direct or indirect sources), are by far the most extensive range of emerging contaminants reported to date. In the late 1990s, reports and studies began to appear on the detections of pharmaceuticals and personal care products in drinking water sources, including both groundwater and surface water [1,2]. It was quickly realized that discharges from domestic wastewater treatment plants (WWTPs) were a major source for these contaminants. Initially, there was not as much concern as there is today because concentrations that could reach drinking water were below many laboratory analysis detection limits, or it was believed that dilution of the contaminants, combined with water treatment, would remove them from our water supplies. However, the level of concern increased due to the detection of three classes of pharmaceuticals: endocrine disruptors, antibiotics, and antidepressants.

Endocrine-disrupting compounds (EDCs) in raw water supplies were discovered at extremely low levels downstream of WWTPs. The concentrations were at the low sub-ng/L levels. Several key studies showed that fish were being impacted by these low levels of hormones [3,4]. In particular, male fish were being feminized by the female hormone, 17-beta-estradiol. This work culminated in a US Geological Survey study in 2002 that showed the presence of hormones in many US streams [2].

The presence of pharmaceuticals in drinking water sources has now become an important water quality issue, as evidenced by the 2008 Associated Press's report on pharmaceuticals in drinking water of the United States, which states, "A vast array of pharmaceuticals — including antibiotics, anticonvulsants, mood stabilizers and sex hormones — have been found in the drinking water supplies of at least 41 million Americans" [5].

Research has not determined the human health effects of exposure to concentrations of pharmaceuticals in drinking water. However, federal research has demonstrated the potential impact to human health from exposure to some pharmaceuticals found in drinking water, such as antibiotics and those that interfere with the functioning and development of hormones in humans [6]. Little is known about the potential interactive effects (such as synergistic or antagonistic toxicity) that may occur from complex mixtures of these compounds in the environment, but some studies have shown that toxicity increases when other compounds are present [7].

Recent research also shows that the traditional toxicological approach used to determine the doses at which compounds become toxic is inadequate for some compounds, in particular, for endocrine disruptors [8]. This study demonstrated that non-monotonic responses and low-dose effects are remarkably common in studies of natural hormones and EDCs. Epidemiological studies show that environmental exposure to EDCs is associated with human diseases and disabilities. When non-monotonic dose–response curves occur, the effects of low doses cannot be predicted by the effects observed at high doses. Thus,

fundamental changes in chemical testing and safety determination are needed to protect human health. The rapidly growing body of research demonstrates the importance to water professionals of understanding the occurrence, fate, and potential environmental and human health effects of emerging contaminants in general and for their source waters in particular.

It has already been more than a decade since pharmaceutical compounds were widely reported in several water bodies of the United States [2]. This paper is still the most cited paper in the history of pharmaceuticals in water samples. The results of this reconnaissance by the US Geological Survey showed that 80% of all surface water had detectable concentrations of pharmaceutical compounds. Approximately 82 compounds were detected including steroids, antibiotics, analgesics, heart medications, and other compounds. Since then, hundreds of papers have been published on the detection and identification of pharmaceuticals in diverse types of water samples. In the last 10 years, pharmaceuticals have been extensively detected in surface water in Europe [9–11] and in the United States [12–15]. Thus, the identification of pharmaceuticals in water samples has been the focus of many water agencies and water treatment facilities around the world.

In general, there is a trend in the literature to only report and measure already known and published emerging contaminants. Only a few studies have reported newly identified and discovered new pharmaceutical compounds and their degradation products [16–18]. It is important to mention that, sometimes, degradation products or metabolites exceed concentrations of parent compounds, becoming then more environmentally relevant than the starting active ingredients. The advent of new methodologies (more sensitive and selective) has controlled the concentrations and type of analytes reported in the environment. However, no consensus about which of the pharmaceuticals to report has been achieved from any of the regulatory agencies. Specifically, in the United States, the Environmental Protection Agency (EPA) has guided and released new regulations [19] in order to narrow the contaminant candidate list (CCL3) to possible toxic emerging compounds of interest. Most recently, a new candidate list called "The Third Unregulated Contaminant Monitoring Rule (UCMR 3)" from EPA was launched in May 2012 [20]. The Unregulated Contaminant Monitoring Rule (UCMR) provides EPA and other interested parties with scientifically valid data on the occurrence of contaminants in drinking water. These data serve as a primary source of occurrence and exposure information that the agency uses to develop regulatory decisions. The UCMR 3 monitoring will take place from 2013 to 2015 and includes monitoring for 28 chemicals and two viruses. Regulatory water agencies will be required to report concentrations for these contaminants in the near future. No pharmaceuticals are included in this recent list, only hormones. But in the meantime, a trend to detect as many compounds as possible in environmental water sources has become the main challenge.

This chapter gives an overview of the different analytic techniques used in LC/MS for the detection of pharmaceutical compounds in water samples, with a specific focus on tandem mass spectrometry and time-of-flight (TOF)

techniques, and the applications that have recently generated in the environmental field. This manuscript gives several examples of pharmaceutical analysis that exemplify the unique features of these techniques for the identification of target and non-target or unknown compounds.

2 ANALYTICAL TECHNIQUES

Emerging contaminants are a growing concern to human health and the environment, particularly in drinking water supplies. The laboratory analyses can be costly and there is currently no clear standard list of constituents as analytical methods continue to develop. Due to their polarity, the majority of the pharmaceuticals identified in environmental samples have been detected using liquid chromatography/mass spectrometry (LC/MS). This is the technique most commonly used for the identification and quantitation of pharmaceuticals in water samples [21]. Among diverse LC/MS techniques commonly used for the routine monitoring and quantitation of pharmaceuticals in water samples, the preferred one is tandem mass spectrometry (LC/MS–MS), using either collision cells or linear traps, to obtain information on fragment ions.

However, it is worth mentioning that the advent of TOF techniques applied to environmental analyses has also begun in the last few years [22]. Applications range from routine analytical methods that analyze a few target compounds to more extensive methods that include a variety of analytes, including also non-target and unknown identification. Due to the high complexity of some environmental samples (i.e., wastewater, sludge samples, and soil samples), high-resolution techniques with additional structural information on fragment ions are needed and this has made these techniques become more and more popular. These techniques provide a high degree of confidence for identification of target analytes and aid in the structural elucidation of degradation products and unknown compounds, which are also usually present in environmental samples. Furthermore, the possibility of creating universal accurate mass databases with TOF analyses for sets of compounds has broadened the range of applications as well, going from target to non-target identification, as we will see in the next sections.

2.1 Solid Phase Extraction

Because detections at low concentrations (usually at the ng/L) have to be achieved, a priori preconcentration step is necessary for water samples in order to isolate the analytes of interest. The main challenge is to perform a simultaneous extraction of groups of analytes with widely diverse polarities. There is no doubt that the most effective and used preconcentration technique is based on solid phase extraction (SPE). In this sense, a water sample is extracted by a solid media (i.e., C18 or polymeric sorbents), which traps the analytes of interest. Following a solvent elution, analytes are desorbed from SPE cartridges and collected into tubes. Evaporation

of solvent to almost dryness concentrates the analytes and allows them to be analyzed by regular LC/MS techniques.

Several authors have reported the use of SPE for a wide variety of pharmaceuticals in water samples [11–18]. Usually, a single-step extraction procedure is performed for a rather small number of compounds. However, there are also multiple extraction procedures reported for a wide range of compounds, as that outlined by the original EPA method 1694 [23]. This method classifies target analytes in two or more groups according to their physical–chemical properties, and extractions are performed under different conditions. Another option consists of the combination of two different SPE sorbents in series. Without doubt, Oasis HLB cartridges are preferred for the extraction of pharmaceutical compounds from water samples since they allow the extraction of acidic, neutral, and basic compounds at neutral pH. Silica based C_{18} is another sorbent usually employed for these types of extractions, although in this case, sample pH adjustment prior to extraction is generally required depending on the nature of the compounds. There is a nice review by Gross et al. [21] that reviews different SPE approaches for the extraction of several groups of pharmaceuticals in water samples.

Our group recently reported the optimization of a single SPE procedure for a large group of pharmaceuticals [15]. The optimization was performed with the aim of reaching acceptable recoveries for wide variety of compounds in a single extraction step. For recovery studies, environmental water samples were spiked with a known amount of pharmaceuticals and processed through the cartridges. Areas obtained after chromatographic analyses were then compared to the areas corresponding to the analyses of blank matrixes of the same type spiked directly with the same amount of pharmaceutical compounds. In general, acceptable recoveries were obtained for the majority of compounds, which was in agreement with previous methods. Comparison at neutral pH and at acidic and alkaline conditions was also tested. Recoveries were not better, in general, after pH adjustment due to the incompatibility of the compounds and hydrolysis reactions of several analytes, which was especially true for the penicillin family that is highly susceptible to hydrolysis, as commented earlier. Tetracyclines were not recovered under the conditions used here; they involve addition of a complexing agent, such as EDTA, which requires a separated SPE method [24].

Initial recovery experiments were carried out in spiked deionized water, surface water, drinking water, and wastewater. Each matrix presents a different set of circumstances that must be addressed. Deionized water, because of the low ionic strength, often gave the highest recoveries but do not reflect real water samples. Likewise, drinking water, which contains adjuvants or treatment substances such as alum, organic coagulants, metal ions, and chlorine, gave varying results. Finally, wastewater samples have higher concentrations of suspended solids that also may affect recovery of pharmaceuticals. In general, recoveries from wastewater were between 10% and 15% lower than reagent water samples, probably due to strong matrix effects and competition of interferents for

specific sites in the sorbent. After testing these various matrices, we determined that surface water gave the most reproducible recoveries by SPE and the recovery experiments reported in the previous work were carried out with this matrix. The absolute recoveries of the pharmaceuticals varied from 10% to 123% and they were similar to the ones reported by other works [11,23]. Usually, the use of labeled standards is a necessity for good recovery data and quantitation in order to account for potential losses during the extraction process.

2.2 Triple Quadrupole Mass Spectrometry Analysis (LC/MS–MS)

To develop a triple quadrupole tandem mass spectrometry method, one needs to first generate multiple reaction monitoring (MRM) transitions for each compound. An optimized MRM transition includes a precursor ion, a product ion, and an optimized collision energy. The first step consists in selecting a proper precursor ion, which usually consists of the protonated or deprotonated molecule. The second step is to generate product ions at different collision energies and then choosing a couple of fragments. Each pair of precursor and a fragment ion is considered a transition. According to EU identification criteria [25], it is enough to achieve identification of a certain compound using two MRM transitions and their relative ion abundance ratio, provided the retention time matches. This application of identification criteria is essential to ensure the unequivocal identification of target analytes in environmental samples. Usually, the transition with the higher abundance is used for quantitation, while the other transition is used as a confirmatory one. The instrument is then set up to monitor as many transitions as possible for a wide range of pharmaceutical compounds. Some instruments require the use of retention time windows for a multianalyte approach, whereas other instruments will schedule the different transitions by using time-dependent algorithms.

As a generality, LC/MS–MS is more focused to target analysis where the analyst is looking at a specific group of analytes; some may vary from few analytes within a family (3–4) to large multiresidue methods (>100). Thus, LC/MS–MS using linear traps and triple quadrupoles seems to be the preferred method for routine analysis of pharmaceutical compounds in environmental samples. Overall, hundreds of papers have been published reporting findings of pharmaceuticals in nontreated and treated waters using these types of methodologies [26–44]. However, in spite of the numerous papers reported for analysis of pharmaceuticals, no analytical methodology seems to be the preferred one as a standardized methodology for these types of compounds. Each analyst chooses the specific methodology that is more adequate for the analysis of certain families of pharmaceuticals and each method is optimized for the detection of trace amounts of these compounds in water samples. However, some generalities can be made regarding the analysis of pharmaceuticals by tandem mass spectrometry techniques, as discussed next.

Because pharmaceutical compounds contain chemical groups with amino, carboxylic, and keto moieties, they are easily ionized under electrospray (ESI)

conditions. Most of the pharmaceuticals ionize well under positive ion ionization [15] due to the existence of nitrogen atoms in their chemical structures. However, some groups, such as the anti-inflammatory/analgesic drugs (i.e., ibuprofen, naproxen, and gemfibrozil), are easily ionized under negative ion conditions due to their carboxylic group moieties in their structures.

Most of the pharmaceuticals fragment well under tandem mass spectrometric conditions, yielding two or more product ions. However, there are a few cases when some compounds only yield one fragment ion. In these cases, either the signal of an isotope such as S or Cl could be used as a secondary transition or retention time has to be taken into account. It is the view of many authors that confirmation of positive identifications in real samples requires the additional second MRM transition and the evaluation of ion ratios between the two monitored transitions as compared to a reference standard [15,25]. Confirmation of the identity of target analytes in real samples is usually based on ion ratio statistics for the transitions monitored. Thus, the confirmation criteria using tandem mass spectrometry cover a range of maximum permitted tolerances according to relative ion intensity, expressed as a percentage of the intensity of the most intense transition.

Another issue that has usually been discussed in detail is the existence of matrix effects, which can cause an underestimation or overestimation of detected concentrations of pharmaceuticals in water samples. Matrix effects are common in surface and wastewater samples due to the presence of natural organic matter in such samples [13]. Matrix effects typically mean suppression; however, they also mean matrix interferences that are present in the sample and, hence, they have an effect on the ionization and/or detection of the compounds. In some cases, the elimination of sample preconcentration prior to analysis minimizes suppression or enhancement effects from interfering matrix components during analysis. Direct analysis of aqueous samples permits reducing the amount of matrix going into the system, thus decreasing the matrix effects. Other approaches used consist of reducing sample volume extraction (from 1 L to hundreds of mL of sample extracted) or performing extra cleanup steps.

Finally, limits of detection (LODs) achieved by tandem mass spectrometric techniques have seen a huge improvement in the last few years. Newer and more sensitive systems with innovative ionization sources have been recently developed by several instrument companies. This has allowed decreasing LODs for pharmaceuticals to even an order of magnitude in many cases, thus permitting the identification of very low levels of these types of compounds in environmental waters. Similarly, quantitative performance in terms of dynamic range, linear response, and reproducibility generally covers three orders of magnitude, thus making LC/MS–MS systems great tools for the quantitation of pharmaceutical compounds in water samples.

As a general rule, LC/MS–MS analyses, using triple quadrupoles or linear traps, can target hundreds of compounds in one single run once the methodology has been optimized for each individual compound [21]. Often, these types

of methodologies will require a lot of initial work for the optimization of the best fragmentor voltages and collision energies for each analyte. Once the method is optimized, low sensitivity levels can be achieved by monitoring the characteristic transitions for each compound. However, sensitivity can become an issue when targeting a large number of compounds, as well. Another issue is that targeted LC/MS–MS methods usually do not take into account potential metabolites or degradation products that may be also present in the samples. These are some of the reasons why TOF techniques that operate in full spectrum have gained terrain on the identification of pharmaceutical compounds [22].

2.3 Quadrupole Time-of-Flight Mass Spectrometry Analysis (Q-TOF-MS)

LC/MS employing accurate mass measurements has been proven as a successful technique for quantitative analysis of target compounds and rapid qualitative analysis of "unknown" environmental mixtures. One of the main reasons that TOF has become so popular in the last few years is the fact that accurate mass measurements are specific and universal for any kind of analyte and do not depend on the type, brand, or specific instrumentation used. The degree of fragmentation may vary depending on the instrument, but the specific accurate mass value and/or accurate isotope information will be consistent for a given analyte, no matter what type of ionization, collision-induced dissociation, and MS–MS fragmentation are used. Accurate mass determination allows obtaining unique information for a given molecule, plus additional information from isotopic patterns, mass defect, and specific fragment ions [22].

Recently, LC/TOF-MS has been used for the unequivocal confirmation of contaminants (including pharmaceuticals, pesticides, and surfactants) in a variety of samples, such as water and sediments [26] by accurate mass measurement of protonated molecules. Similarly, several authors have reported accurate mass confirmation of pharmaceuticals in surface and wastewater samples [45–49] and sediment and sludge [50] using TOF techniques. Detection of drugs in urine has also been one of the topics that have been widely covered by LC/TOF-MS techniques [51–54]. In many of these studies, TOF techniques were successfully used for the unequivocal identification of degradation products and unknown compounds [16,55]. It is worth mentioning also several applications of TOF mass analysis for the identification and confirmation of metabolites or degradation products of pesticides and pharmaceuticals in environmental samples [56–60].

In the last few years, major improvements such as sensitivity, mass accuracy, and resolving power have been achieved with LC/MS instruments, mainly driven by competition between instrument companies. This improvement on resolving power benefits analyses involving complex environmental

matrices, by separating isobaric interferences from the contaminant signals of interest. The improved resolution also facilitates the measurement of accurate masses within 3 ppm, which is accepted for the verification of elemental compositions. Elemental compositions of contaminants and their fragment ions clearly constitute higher-order identifications than those afforded by nominal mass measurements.

Sometimes, a single stage time-of-flight mass analyzer (TOF/MS) generates valuable information by imparting enough energy into the $[M+H]^+$ ions in the source region to cause fragmentation [22]. Some of these fragments generated by single-stage mass spectrometry can be used for the elucidation of fragmentation pathways and/or identification of target compounds. But specific MS–MS accurate mass measurements of fragment ions become particularly important in the structure elucidation of non-targets and unknowns. In this sense, the Q-TOF-MS–MS is unique among TOF instruments in its ability to give accurate mass measurements (1–2 millimass units) of the fragment ions that are ejected from the collision chamber. This is very useful when trying to elucidate the identity of unknown or non-target compounds; the more fragment accurate mass information one can get from TOF mass techniques, the better understanding for the structural elucidation of a certain compound. The same reasoning applies to the elucidation of possible degradation products or metabolites. When knowing what the starting compound is, the information about fragment ions and their accurate masses will play an important role in deciphering the chemical structure of the metabolite or degradation product.

Most published methods only include information on the exact mass of the protonated or deprotonated molecule; a few report just one fragment ion per compound. To our knowledge, no studies include also accurate mass information of more than one fragment ion obtained by MS–MS for a large number of compounds (>80). Only recently, an extensive accurate mass library was developed and commercialized by Broecker et al. [61] for more than 2500 compounds. Another study by our group compiled information on 100 pharmaceutical compounds including detailed data on fragment ions obtained by a Q-TOF-MS instrument [62]. We also included a total of 16 different metabolites for the most environmentally relevant pharmaceuticals. Accurate mass information for each compound was obtained and compiled, as it is shown in Table 1.

Another important tool that has made TOF one of the key methodologies for identification of compounds is the existence of accurate mass databases, as published extensively. An individual scientist can apply these universal databases to each specific problem and then often get a correct identification on the analyte of interest [63–65]. Other tools that are available with TOF instrumentation, and will be discussed in this chapter, include the use of molecular features, accurate mass filters, and isotopic mass defect and the use of mass profiling to distinguish between control samples and positive samples. Examples will be given for each one of these accurate mass tools.

TABLE 1 LC/Q-TOF-MS Exact Masses for the Protonated and Deprotonated Molecules of 100 Pharmaceuticals and Their Main Fragment Ions

Compound	Ret. Time (min)	Elemental Composition[a]	$[M+H]^+$	Frag. Ion 1	Frag. Ion 2	Frag. Ion 3	Frag. Ion 4
1,7-Dimethylxanthine	6.3	$C_7H_8N_4O_2$	181.0720	163.0614	124.0505		
10,11-Dihydroxy-carbamazepine	12.9	$C_{15}H_{14}N_2O_3$	271.1077	253.0972	236.0706	210.0913	180.0808
10-Hydroxy-carbamazepine	13.9	$C_{15}H_{14}N_2O_2$	255.1128	237.1022	194.0964		
Acetaminophen	6.5	$C_8H_9NO_2$	152.0706	134.0600	110.0600	93.0335	
Albuterol	4.0	$C_{13}H_{21}NO_3$	240.1594	222.1489	166.0863	148.0757	
Ampicillin	10.4	$C_{16}H_{19}N_3O_4S$	350.1169	192.0478	160.0427	106.0651	
Atenolol	4.2	$C_{14}H_{22}N_2O_3$	267.1703	190.0863	225.1234	145.0648	
Azithromycin	12.2	$C_{38}H_{72}N_2O_{12}$	749.5158	591.4215	375.2615	158.1176	
Bupropion	13.6	$C_{13}H_{18}ClNO$	240.1150	184.0524	166.0418	131.0730	
Caffeine	9.8	$C_8H_{10}N_4O_2$	195.0877	138.0662	110.0713	123.0427	
Carbamazepine	17.2	$C_{15}H_{12}N_2O$	237.1022	194.0964	179.0730		
Cefotaxime	12.9	$C_{16}H_{17}N_5O_7S_2$	456.0642	396.0431	368.0482	324.0583	
Cetirizine	16.3	$C_{21}H_{25}ClN_2O_3$	389.1626	201.0466	166.0777		
Cimetidine	4.3	$C_{10}H_{16}N_6S$	253.1230	159.0699	117.0481	95.0604	
Ciprofloxacin	11.0	$C_{17}H_{18}FN_3O_3$	332.1405	314.1299	288.1507	231.0564	
Citalopram	15.2	$C_{20}H_{21}FN_2O$	325.1711	262.1027	109.0448		
Clarithromycin	16.0	$C_{38}H_{69}NO_{13}$	748.4842	590.3899	158.1176		

Clofibric acid[b]	20.3	$C_{10}H_{11}ClO_3$	213.0324	126.9956	85.0295		
Clonidine	7.7	$C_9H_9Cl_2N_3$	230.0246	212.9981	44.0495		
Cloxacillin	19.7	$C_{19}H_{18}ClN_3O_5S$	436.0728	277.0374	178.0054	160.0427	
Codeine	7.0	$C_{18}H_{21}NO_3$	300.1594	243.1016	215.1067	199.0754	165.0699
Cotinine	3.2	$C_{10}H_{12}N_2O$	177.1022	146.0600	98.0600	80.0495	
Dehydronifedipine	20.5	$C_{17}H_{16}N_2O_6$	345.1081	284.0917	268.0968		
Demethyldextrorphan	12.0	$C_{16}H_{21}NO$	244.1696	201.1274	199.1117		
Des-venlafaxine	11.3	$C_{16}H_{25}NO_2$	264.1958	246.1852	201.1274	58.0651	
Dextromethorphan	14.6	$C_{18}H_{25}NO$	272.2009	213.1274	171.0804	147.0804	
Dextrorphan	12.1	$C_{17}H_{23}NO$	258.1852	201.1274	199.1117	159.0804	
Diazepam	20.7	$C_{16}H_{13}ClN_2O$	285.0789	193.0897	154.0418		
Diclofenac	23.0	$C_{14}H_{11}Cl_2NO_2$	296.0240	250.0185	215.0496		
Digoxigenin	13.4	$C_{23}H_{34}O_5$	391.2479	373.2373	355.2268	337.2162	
Digoxin	15.2	$C_{41}H_{64}O_{14}$	781.4369	651.3739	521.3109	391.2479	
Dihydrocarbamazepine	17.1	$C_{15}H_{14}N_2O$	239.1179	222.0913	194.0964	180.0808	
Diltiazem	15.2	$C_{22}H_{26}N_2O_4S$	415.1686	370.1108	178.0321	150.0372	
Diphenhydramine	15.1	$C_{17}H_{21}NO$	256.1696	167.0855	152.0621		
Enrofloxacin	11.5	$C_{19}H_{22}FN_3O_3$	360.1718	342.1612	316.1820	245.1085	

Continued

TABLE 1 LC/Q-TOF-MS Exact Masses for the Protonated and Deprotonated Molecules of 100 Pharmaceuticals and Their Main Fragment Ions—Cont'd

Compound	Ret. Time (min)	Elemental Composition	$[M+H]^+$	Frag. Ion 1	Frag. Ion 2	Frag. Ion 3	Frag. Ion 4
Erythro-hydrobupropion	13.7	$C_{13}H_{20}ClNO$	242.1306	186.0680	168.0575		
Erythromycin	14.6	$C_{37}H_{67}NO_{13}$	734.4685	576.3742	558.3637	158.1176	
Erythromycin anhydrate	15.6	$C_{37}H_{65}NO_{12}$	716.4580	558.3637	158.1176		
Flumequine	18.1	$C_{14}H_{12}FNO_3$	262.0874	244.0768	202.0299	174.0350	
Fluoxetine	16.9	$C_{17}H_{18}F_3NO$	310.1413	148.1121	117.0699	91.0542	
Fluvoxamine	16.1	$C_{15}H_{21}F_3N_2O_2$	319.1628	258.1100	200.0682	71.0491	
Furosemide[b]	12.0	$C_{12}H_{11}ClN_2O_5S$	329.0004	285.0106	204.9839	126.0111	
Gabapentin	6.5	$C_9H_{17}NO_2$	172.1332	154.1226	137.0961	67.0542	
Gemfibrozil[b]	25.0	$C_{15}H_{22}O_3$	249.1496	121.0659			
Guaifenesin	12.7	$C_{10}H_{14}O_4$	199.0965	163.0754	151.0754	135.0804	125.0597
Hydrocodone	10.1	$C_{18}H_{21}NO_3$	300.1594	243.1016	199.0754	171.0804	
Hydroxy-bupropion	12.3	$C_{13}H_{18}ClNO_2$	256.1099	238.0993	166.0418		
Ibuprofen[b]	23.6	$C_{13}H_{18}O_2$	205.1234	161.1336			
Iopromide	4.4	$C_{18}H_{24}I_3N_3O_8$	791.8770	773.8665	572.7784		
Ketoprofen	19.0	$C_{16}H_{14}O_3$	255.1016	209.0961	105.0335	77.0386	
Ketorolac	19.7	$C_{15}H_{13}NO_3$	256.0968	105.0335	77.0386		
Lamotrigine	12.1	$C_9H_7Cl_2N_5$	256.0151	210.9824	166.0292	58.0400	
Lincomycin	8.7	$C_{18}H_{34}N_2O_6S$	407.2210	359.2177	317.2071	126.1277	

Lomefloxacin	11.2	$C_{17}H_{19}F_2N_3O_3$	352.1467	334.1362	308.1569	265.1147	
Mefenamic acid	24.6	$C_{15}H_{15}NO_2$	242.1176	224.1070			
Meprobamate	14.7	$C_9H_{18}N_2O_4$	219.1339	158.1175	97.1012	55.0542	
Metformin	2.3	$C_4H_{11}N_5$	130.1087	113.0822	88.0869	71.0604	
Methadone	16.6	$C_{21}H_{27}NO$	310.2165	265.1587	105.0335		
Metoprolol	12.1	$C_{15}H_{25}NO_3$	268.1907	116.1070	56.0495		
Metoprolol acid	9.4	$C_{14}H_{21}NO_4$	268.1543	165.0546	145.0648	56.0495	
Miconazole	19.2	$C_{18}H_{14}Cl_4N_2O$	414.9933	227.0137	158.9763		
2-*N*-Glucuronide lamotrigine	8.4	$C_{15}H_{15}Cl_2N_5O_6$	432.0472	256.0151			
Naproxen[b]	20.9	$C_{14}H_{14}O_3$	229.0870	185.0972	170.0737	169.0659	
Norcitalopram	15.3	$C_{19}H_{19}FN_2O$	311.1554	262.1027	109.0448		
Nordiazepam	18.5	$C_{15}H_{11}ClN_2O$	271.0633	243.0684	208.0995	165.0214	140.0262
Norfloxacin	10.7	$C_{16}H_{18}FN_3O_3$	320.1405	302.1299	276.1507	233.1085	
Norfluoxetine	16.7	$C_{16}H_{16}F_3NO$	296.1257	134.0964			
Ofloxacin	10.7	$C_{18}H_{20}FN_3O_4$	362.1511	344.1405	318.1612	261.1034	
Oxacillin	19.2	$C_{19}H_{19}N_3O_5S$	402.1118	243.0764	160.0427	144.0444	
Oxolinic acid	15.4	$C_{13}H_{11}NO_5$	262.0710	244.0604	216.0291	160.0393	
Oxcarbazepine	15.8	$C_{15}H_{12}N_2O_2$	253.0972	236.0706	210.0913	208.0757	180.0808

Continued

TABLE 1 LC/Q-TOF-MS Exact Masses for the Protonated and Deprotonated Molecules of 100 Pharmaceuticals and Their Main Fragment Ions—Cont'd

Compound	Ret. Time (min)	Elemental Composition	$[M+H]^+$	Frag. Ion 1	Frag. Ion 2	Frag. Ion 3	Frag. Ion 4
Oxycodone	9.4	$C_{18}H_{21}NO_4$	316.1543	298.1438	256.1332	241.1097	
Paroxetine	15.9	$C_{19}H_{20}FNO_3$	330.1500	192.1183	70.0651		
Penicillin G	16.9	$C_{16}H_{18}N_2O_4S$	335.1060	160.0427	176.0706	114.0372	
Penicillin V	17.9	$C_{16}H_{18}N_2O_5S$	351.1009	160.0427	192.0655	114.0372	
Phenytoin	17.1	$C_{15}H_{12}N_2O_2$	253.0972	225.1022	182.0964	104.0495	
Primidone	12.7	$C_{12}H_{14}N_2O_2$	219.1128	162.0913	119.0855	91.0542	
Propranolol	14.4	$C_{16}H_{21}NO_2$	260.1645	242.1539	218.1176	183.0804	
Ranitidine	4.6	$C_{13}H_{22}N_4O_3S$	315.1485	270.0907	224.0978	176.0488	130.0559
Roxithromycin	16.2	$C_{41}H_{76}N_2O_{15}$	837.5318	679.4376	158.1176		
Sarafloxacin	12.4	$C_{20}H_{17}F_2N_3O_3$	386.1311	368.1205	342.1412	299.0990	
Sertraline	16.9	$C_{17}H_{17}Cl_2N$	306.0811	275.0389	158.9763	129.0699	
Simvastatin	26.9	$C_{25}H_{38}O_5$	419.2792	285.1849	243.1743	225.1638	199.1481
Sulfachloropyridazine	15.6	$C_{10}H_9ClN_4O_2S$	285.0208	156.0114	108.0444	92.0495	130.0167
Sulfadiazine	9.9	$C_{10}H_{10}N_4O_2S$	251.0597	156.0114	108.0444	92.0495	
Sulfadimethoxine	16.5	$C_{12}H_{14}N_4O_4S$	311.0809	156.0114	156.0768	108.0444	92.0495
Sulfamerazine	11.5	$C_{11}H_{12}N_4O_2S$	265.0754	156.0114	110.0713	108.0444	92.0495

Sulfamethazine	12.4	$C_{12}H_{14}N_4O_2S$	279.0910	186.0332	156.0114	108.0444	124.0869
Sulfamethizole	12.7	$C_9H_{10}N_4O_2S_2$	271.0318	156.0114	108.0444	92.0495	
Sulfamethoxazole	15.3	$C_{10}H_{11}N_3O_3S$	254.0594	156.0114	108.0444	92.0495	
Sulfanilamide	4.6	$C_6H_8N_2O_2S$	173.0379	156.0114	108.0444	92.0495	
Sulfathiazole	10.6	$C_9H_9N_3O_2S_2$	256.0209	156.0114	108.0444	92.0495	
Thiabendazole	8.8	$C_{10}H_7N_3S$	202.0433	175.0324	131.0604	92.0495	
Tramadol	11.1	$C_{16}H_{25}NO_2$	264.1958	246.1852	58.0651		
Triclocarban	25.6	$C_{13}H_9Cl_3N_2O$	314.9853	161.9872	128.0262	127.0183	
Trimethoprim	10.4	$C_{14}H_{18}N_4O_3$	291.1452	261.0982	230.1162	123.0665	
Tylosin	15.0	$C_{46}H_{77}NO_{17}$	916.5264	772.4478	174.1125		
Venlafaxine	13.5	$C_{17}H_{27}NO_2$	278.2115	260.2009	215.1430	58.0651	
Virginiamycin	18.4	$C_{28}H_{35}N_3O_7$	526.2548	508.2442	355.1288	109.1012	
Warfarin	21.4	$C_{19}H_{16}O_4$	309.1121	251.0703	163.0390	121.0284	

All exact masses have been theoretically corrected.
[a]*Elemental compositions correspond to neutral molecules.*
[b]*Compounds detected in negative ion mode* $[M-H]^-$.

3 TARGET ANALYSIS OF PHARMACEUTICALS: LOW-LEVEL DETECTION AND CASE STUDIES

3.1 Establishment of a Selective Target List for Low-Level Work

The development of a multiresidue method for the analysis of a large group of pharmaceuticals was carried out by our group [15]. The implementation for this method consisted of the analysis of 70 analytes and 18 labeled internal standards, which are a mixture of pharmaceuticals and personal care products that are currently analyzed by LC/MS–MS. In our work, we addressed some of the analytic issues encountered, such as degradation of some compounds in solvent mixtures and assignment of a second transition for MRM transitions for additional mass spectrometry quality assurance. The main goal of this work was to show the usefulness of this method for generic screening and monitoring of pharmaceuticals in water and wastewater. This method was applied initially to the analysis of several drinking water, surface water, and wastewater samples from several locations in Colorado, United States. Surprisingly, only 8 out of the 70 compounds were consistently found in environmental water samples: caffeine, carbamazepine, clarithromycin, diltiazem, diphenhydramine, erythromycin, sulfamethoxazole, and trimethoprim, which were confirmed with two MRM transitions. Concentrations ranged from 5 to 1200 ng/L. These samples are representative of several inputs of wastewater contamination. One drinking water sample was also analyzed and gave a positive hit for carbamazepine, a common antiepileptic and antidepressant prescribed drug.

Since then, we have refined our target methods using triple quadrupole mass spectrometry for a subset of 20 compounds that are regularly found in surface and wastewater samples. The pharmaceuticals chosen for LC/MS–MS analysis met three criteria. First, the compounds posed health concerns that merited their monitoring. Second, the compounds are not removed through wastewater treatment processes, since WWTPs were hypothesized as the major source of pharmaceuticals in surface water samples. This means that the compounds should not be degraded rapidly by bacteria nor adsorbed to the sediments and sludge of the WWTP. Third, the compounds should be measurable and accurately detectable by modern mass spectrometry techniques at the trace levels that have been reported in the environment [2]. A few of the compounds on the list were included to determine the impacts of recreational activities and septic systems. For example, the presence of caffeine, and sucralose, while a by-product in WWTP effluent, could also indicate human impacts from recreational activities. Sucralose is an artificial sweetener and its presence in water not only occurs in WWTP effluent but also may be a result of people directly discarding portions of their artificially sweetened drinks into the water. Similarly, the presence of caffeine in water could be the result of discarding unfinished caffeinated beverages directly into the water.

Table 2 shows a list of the analytes selected for low-level detection, including the LODs for surface water samples. Most of the compounds included in this list do not have any aquatic life or drinking water quality standards associated with them. In fact, many of the drugs and pharmaceuticals are normally consumed at levels that are many orders of magnitude higher than the part-per-trillion levels detected in water. However, waters flowing through our environment and being used as drinking water supplies may be of higher concern, particularly since the potential interactive effects (such as synergistic or antagonistic toxicity) that may occur from complex mixtures of these compounds in the environment are unknown. Additionally, some compounds, particularly the endocrine disruptors, can produce harmful effects even at very low concentrations [66].

3.2 Case Study: Analysis of Pharmaceuticals in WWTP Effluents

In Colorado, Northern Water, a public agency created in 1937, provides water for agricultural, municipal, domestic, and industrial uses to an eight-county service area with a population of about 830,000. Northern Water and the US Bureau of Reclamation operate the Colorado-Big Thompson (C-BT) Project, which collects water on the West Slope and delivers it to northeastern Colorado through a 13 mile tunnel beneath Rocky Mountain National Park. The C-BT Project annually delivers an average of 213,000 acre feet of water to northeastern Colorado. Water is provided to many cities and several smaller communities, rural and domestic water districts, and local industries.

As explained previously, emerging contaminants are a growing concern to human health and the environment, particularly in drinking water supplies. Our lab was involved in the analysis of pharmaceuticals for ~130 water samples collected over a 3-year time period from November 2008 until November 2011. The samples were analyzed for the selected pharmaceuticals as mentioned in the earlier section. The monitoring program included 21 sites throughout the C-BT Project and South Platte River tributaries. The program also evolved to include more sample events during the year.

In order to better understand contributions from WWTPs, a baseline of compounds was established by collecting and analyzing samples from various effluents. This analysis was not conducted to pinpoint the source of contaminants to a specific WWTP, as there were several in the study area, but rather to pinpoint what compounds were unique to the study area. The effluents sampled were considered to be representative of WWTP discharges in the area and were later used to help identify which contaminants to look for at the sampling sites downstream of WWTPs.

The WWTP effluent samples collected were analyzed using the low-level LC/MS–MS method described in Section 3.1. Eighteen of the 20 compounds on the low-level list were detected, many with concentrations in the 1000s and 100s of ng/L range, which is typical of WWTP effluents [2]. Table 3 shows

TABLE 2 MRM Transitions and MS Operating Parameters Selected for the Analysis of the Selected Group of Pharmaceutical Compounds

Compound	Fragmentor Voltage	MRM Transitions (*m*/*z*)	Collision Energy (eV)	LOD (ng/L)
Acetaminophen	90	152 > 110	15	5
		152 > 65	35	
$^{13}C_2$-^{15}N-acetaminophen	*90*	*155 > 111*	*15*	
		155 > 93	*25*	
Atenolol	110	267 > 190	15	5
		267 > 145	20	
Bisphenol A	120	227 > 212	15	20
		227 > 133	25	
Bupropion	80	240 > 184	5	1
		240 > 166	10	
Caffeine	110	195 > 138	15	10
		195 > 110	25	
$^{13}C_3$-caffeine	*110*	*198 > 140*	*15*	
		198 > 112	*25*	
Carbamazepine	120	237 > 194	15	2

		237 > 179	35	
Carbamazepine-d_{10}	*120*	*247 > 204*	*15*	
		247 > 202	*35*	
Clarithromycin	110	748.5 > 590	15	2
		748.5 > 158	25	
Cotinine	90	177 > 98	25	5
		177 > 80	25	
Cotinine-d_3	*90*	*180 > 80*	*25*	
		180 > 101	*25*	
Diltiazem	130	415 > 178	25	5
		415 > 150	25	
Diphenhydramine	70	256 > 167	15	5
		256 > 152	35	
Erythromycin	90	734.5 > 576	15	10
		734.5 > 158	35	
$^{13}C_2$-erythromycin	*90*	*736.5 > 160*	*25*	
		736.5 > 578	*15*	
Gemfibrozil	70	249 > 121	5	5
Gemfibrozil-d_6	*70*	*255 > 121*	*5*	

Continued

TABLE 2 MRM Transitions and MS Operating Parameters Selected for the Analysis of the Selected Group of Pharmaceutical Compounds—Cont'd

Compound	Fragmentor Voltage	MRM Transitions (*m*/*z*)	Collision Energy (eV)	LOD (ng/L)
Lamotrigine	120	256 > 211	25	5
		258 > 213	25	
Metoprolol	110	268 > 116	15	1
		268 > 56	30	
Propranolol	120	260 > 116	15	1
		260 > 56	30	
Sucralose	110	419 > 221	15	15
		419 > 239	15	
Sulfamethoxazole	80	254 > 156	10	5
		254 > 92	30	
$^{13}C_6$-sulfamethoxazole	*110*	*260 > 162*	*15*	
		260 > 98	*25*	
Triclosan	70	287 > 35	5	20
		289 > 37	5	
$^{13}C_{12}$-triclosan	*75*	*299 > 35*	*5*	

Trimethoprim	110	291 > 230	20	5
		291 > 261	25	
$^{13}C_3$-trimethoprim	*110*	*294 > 233*	*20*	
		294 > 264	*25*	
Venlafaxine	90	278 > 260	5	1
		278 > 58	15	

The labeled standards are shown in italics.

TABLE 3 Concentrations (in ng/L) for Pharmaceuticals Found in Two Representative Wastewater Effluent Samples in Northern Colorado in 2010

Compound	WWTP Eff-1	WWTP Eff-2
Acetaminophen	<5	<5
Atenolol	1515	160
Bisphenol A	<20	<20
Bupropion	756	74.6
Caffeine	336	393
Carbamazepine	368	114
Clarithromycin	2877	68.3
Cotinine	48.0	49.3
Diltiazem	494	<5
Diphenhydramine	2000	5.6
Erythromycin	793	139
Gemfibrozil	2881	370
Lamotrigine	456	266
Metoprolol	2535	9.9
Propranolol	286	<1
Sucralose	45,100	29,096
Sulfamethoxazole	1261	133
Triclosan	856	777
Trimethoprim	1531	15.3
Venlafaxine	547	129

the concentrations for these compounds from two wastewater effluents. The "<" values listed on this table indicate that the compound was not detected above the reporting limit; the value is set equal to the reporting limit. It is clear from these preliminary data that the selection of the targeted list was successful, as 90% of the compounds selected for monitoring were found in these samples.

3.3 Case Study: Analysis of Pharmaceuticals in Surface Waters

Now that a baseline was established for the type of compounds detected, a more specific monitoring program was carried out in the same area. Most of

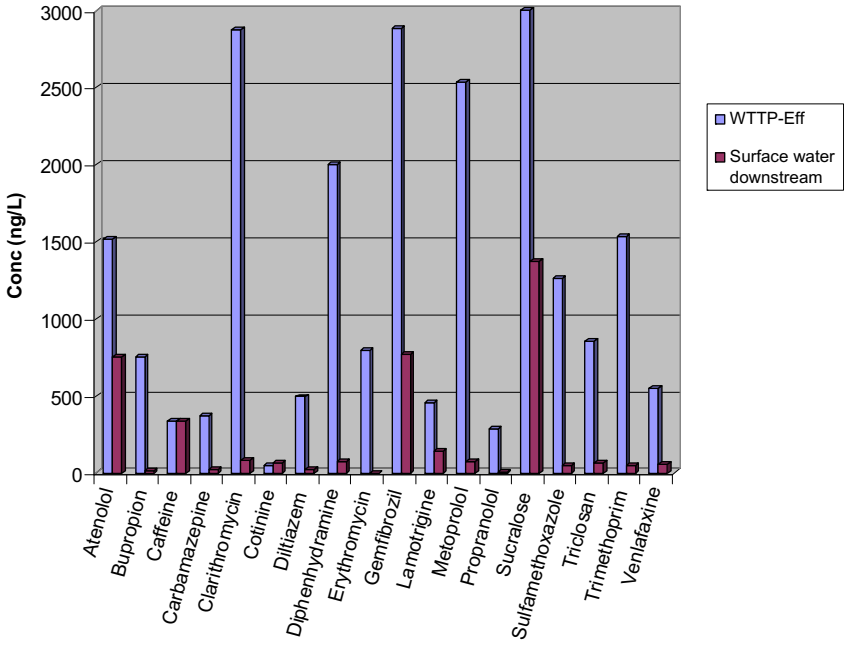

FIGURE 1 Concentrations for the pharmaceuticals found in a wastewater treatment plant (WWTP) effluent and 1 mile downstream of the WWTP. Graph has been rescaled for sucralose due to higher concentration for this compound (45,100 μg/L).

the sampling sites for surface water were located downstream of one or more WWTPs. Samples downstream (~1 mile) of the WWTPs were collected and analyzed using the same method in order to assess the impact of these effluents on surface water. Concentrations of the compounds present at the WWTP site were considerably lower at the downstream site due to dilution, but the low-level analysis was sensitive enough to capture any compound that would be of concern at other sampling sites further downstream (see Figure 1). As it can be seen in this figure, the concentrations for most of the compounds decrease by a factor of at least two, with most of them decreasing by a factor of 10, except for two compounds: caffeine and cotinine. These two compounds were present at a similar concentration in the downstream site compared to the WWTP site.

These results showed that effluent from WWTPs is the probable source of many of the pharmaceuticals found in the downstream surface waters. In many cases, the sampling sites influenced by the WWTPs may not show a strong correlation to the WWTP effluent due to the large distances between the WWTP and the sampling site and the presence of significant diluting flows. As expected, the influence of WWTP effluent is more apparent at the sites closest to points of discharge and decreases as the water moves through the system.

Most of these discharges are significantly diluted as they are mixed with reservoir water and the influence is insignificant at downstream sampling sites.

This study constituted a baseline study for pharmaceuticals in wastewater, surface water, and drinking water sources and represents an important first step in monitoring these compounds in the environment in this specific area. It is important to reach as low of a concentration level as possible, while still maintaining quality control and accuracy, to develop baseline information for future studies and an excellent long-term monitoring program.

It is important to keep in mind the meaning of low-level pharmaceuticals when looking at the results presented in this study. Two things have happened over the past decade. First, the instruments and methods of isolation for pharmaceuticals have been improved so that it is possible to monitor these compounds at the nanogram per liter concentration level compared to a microgram per liter concentration level. This low level of detection means that there will be some detections of the more commonly used drugs and to a lesser extent the endocrine disruptor compounds (such as bisphenol A and triclosan), as they are more difficult to measure at the nanogram per liter level. Secondly, the biological importance of these low-level detections is poorly studied at this time. Generally, biological study lags behind the analytical methodology; thus, one should exercise caution in interpreting the low-level detections. When the concentrations of the pharmaceuticals are detected at microgram per liter levels, there is more concern, since these are the levels that have been studied in the recent past for biological effects in aquatic life [66].

4 NON-TARGET ANALYSIS OF PHARMACEUTICALS

Up to now, we have seen examples of analysis for the most commonly used technique for detection of pharmaceutical compounds, which is tandem mass spectrometry using LC/MS–MS. However, as mentioned in Section 2, TOF mass techniques have become quite popular in the last few years, thus allowing to detect, quantify, and discover new metabolites and degradation products of pharmaceutical compounds. In the next two sections, some examples will be given for the major findings, using the diverse tools that LC/TOF-MS techniques offer.

4.1 Discovery of New Metabolites by TOF Techniques

After analyzing a large number of samples, we have come up with some findings (new compounds detected and new metabolites) that were worth mentioning here and this is the reason that these compounds were included in previous data sets [62]. Identities of compounds were based on retention time and accurate mass of the protonated/deprotonated molecules and their fragment ions. MS–MS acquisition was performed on those cases where a new

compound or metabolite was discovered. For example, a new finding was the anticonvulsant (also used as antidepressant) lamotrigine and its N2-glucuronide found in wastewater, surface water, and even groundwater samples [16]. To date, no other environmental reports of this pharmaceutical and/or metabolite were reported in the literature. This compound is frequently detected in water samples (as shown in the earlier section) and at high concentrations, suggesting that it is replacing the "older" anticonvulsant drugs (carbamazepine, citalopram, and fluoxetine) prescribed for human intake. Other findings include metabolites of already well-known drugs such as bupropion, carbamazepine, and venlafaxine, to mention a few. These are important findings as the metabolite concentrations often exceed the parent compound concentration. Figure 2 shows an example of a common detected drug (metoprolol) and its newly identified acid metabolite in a surface water sample. The MS–MS experiments at 30V revealed the most important fragments of this metabolite (as shown in the inset spectrum). This finding shows that it is possible to fully identify a new metabolite without the need of a standard. Figure 3 shows the complete pathway fragmentation for this compound and shows how the accurate masses obtained in the MS–MS experiment (shown in Figure 2) match very closely with the calculated exact masses.

4.2 Accurate Mass Tools for Identification of Pharmaceuticals and Metabolites

4.2.1 Molecular Features

For many years, the use of reverse-search methods for gas chromatography/mass spectrometry (GC/MS) has made it possible to search large National Institute of Standards and Testing (NIST) pesticide libraries in minutes [67]. Unfortunately, similar reverse-search methods have not been available for LC/MS for two reasons. First, the single quadrupole and triple quadrupole mass spectrometers do not operate in full scan mode for pesticide screening because of a lack of sensitivity [68]. Secondly, although libraries for LC/MS three-dimensional ion trap have been made, they have not been popular due to difficulties in reproducibility of fragmentation and the need for authentic standard analysis for each instrument [69–71]. So, the only approach that uses full spectrum information is liquid chromatography/time-of-flight mass spectrometry (LC/TOF-MS), which is both sensitive and accurate [72], but uses only the accurate mass of the $[M+H]^+$ ion. The combination of accurate mass and sensitivity is needed for screening compounds by their molecular formula.

The molecular feature extraction (MFE) software compiles accurate mass ions, excludes background noise, and plots extracted ion chromatograms of the most intense peaks found in a chromatogram. So a molecular feature is defined as a discrete molecular entity defined by combination of retention

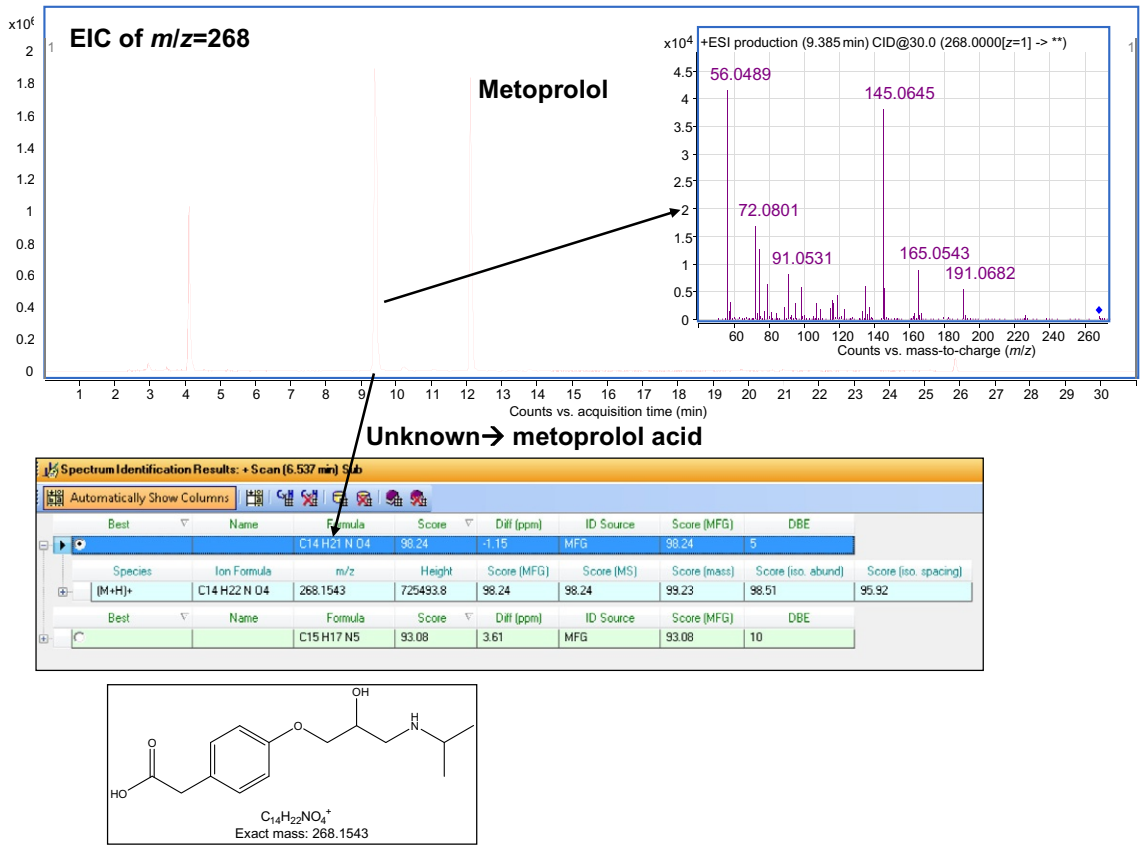

FIGURE 2 Extracted ion chromatogram for *m/z* 268 corresponding to metoprolol and its acid metabolite in a surface water sample from the Platte River near Denver (CO). MS–MS of the identified metabolite is also shown in the inset.

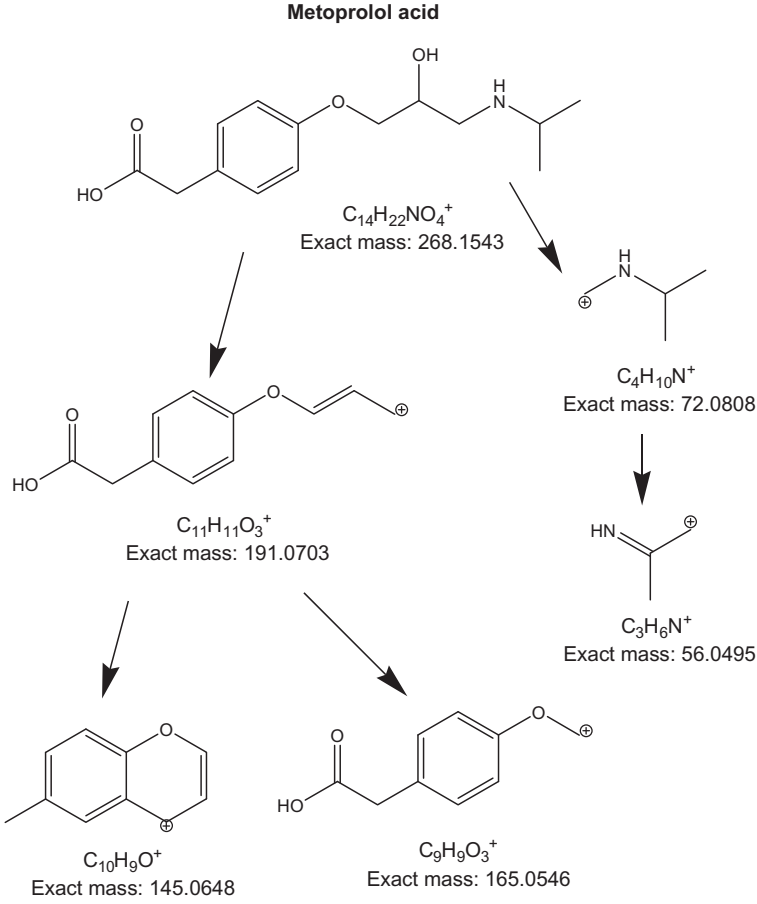

FIGURE 3 Fragmentation pathway for metoprolol acid metabolite.

time, mass, and response in an LC/MS analysis. In general, MFE operates on raw mass spectral data generating lists of chemically qualified molecular features (while background is removed, interferences are resolved, and isotopic clusters and molecular adducts are recognized). The screening criteria usually consist of ±5 ppm accurate mass window, ±0.2 min retention time window, and a minimum of 10000 counts (signal to noise of ~10:1) for moderately complex samples. The ions are grouped by entities that include common adducts (sodium, ammonia, etc.) and isotope clusters.

Usually, by following this approach, a total of 2000–9000 features are found in a chromatogram of a water sample. One can generate as many molecular formulas as wanted and from there one can try to elucidate the chemical structure. But, the most common approach is to compare the data obtained to a known database to try to match as many compounds as possible. This

approach is explained in Section 4.2.2. Strengths of the MFE include rapid screening of hundreds of compounds at sensitive levels compared to a manual approach and the simplicity of use of the library for any accurate mass spectrometer instrumentation capable of routinely measuring sub 5 ppm mass accuracy.

4.2.2 Accurate Mass Databases

The pioneer efforts to search data using an accurate mass database were made by several authors, such as Thurman et al. [73], Bodeldijk et al. [74], and Laks et al. [75]. For example, Thurman et al. [73] used an approach of TOF, ion trap, and the Merck Index database to identify pesticides in food and also degradation products, without the initial use of primary standards. Bobeldijk et al. also used the Merck Index, the NIST library, and their own database to screen water pollutants [74]. The methods in these examples rely on manually searching the databases, compound by compound. Recently, several papers have extended this approach and have been published [54,75] that use mass accuracy of 30 ppm and database analysis to identify ~600 drugs in blood and urine without the use of primary standards, using only the protonated molecule.

In spite of the progress that has been made, the ability to do true library analysis is still a problem to be solved for LC/MS and for rapid analysis of environmental samples. The problems to be overcome include reproducible spectra and ion ratios, routine programs for rapid screening of samples rather than manual checking of data, and some estimate of the probability of the correct identification. Variation in fragmentation intensity is not critical with the use of accurate mass since the accurate mass of the fragment ion gives its molecular formula. In fact, accurate mass measurements are specific and universal for every target analyte regardless the instrumentation used. Usually, unambiguous identification is accomplished by means of accurate mass measurements from (de)protonated molecules, fragment ions, and isotope intensity/signature matching. Thus, the accurate mass database approach is a screening tool and it is powerful and fast because only the molecular formula is needed.

The approach most commonly used is called “reversed database search” in which a total ion chromatogram is searched for ions included in the specified database. Databases usually contain information of the monoisotopic exact mass of the MH^+, at least one product ion, and retention time of the compound. This automatic screening method requires a thorough full optimization of the accurate-mass window used and retention time (always optional) tolerances, which play an important role on the selectivity, accuracy, and successfulness of the whole procedure. In this way, and by running a commercial database, we verified the presence of one of the metabolites of dextromethorphan, also known as dextrorphan, in a surface water sample impacted by a wastewater source.

Figure 4 depicts an excerpt of the automated generated report of a database search for a surface water sample analyzed by LC/Q-TOF-MS. The analyte identified was dextrorphan, a phase I metabolite of the cough suppressant medication that contains dextromethorphan. It is important to note the high score obtained for this particular hit. This score is a combination of mass accuracy, isotope intensity, and isotope matching. Also, as shown in the figure, a good mass accuracy (with an error below 2 ppm) was obtained for this identification, thus confirming the presence of dextrorphan in the sample. Again, in this case, no standard had been analyzed by this instrument when this finding was made, so a pure standard was purchased, analyzed, and verified this positive identification in a water sample. This shows again the power of TOF techniques for the discovery of new metabolites in environmental samples.

4.2.3 Accurate Mass Filters and Isotopic Mass Defect

Chlorine appears in many pesticides and pharmaceutical products that are important to environmental analysis. Because chlorine contains two isotopes, Cl^{35} and Cl^{37}, there is a distinctive A+2 isotope pattern that is generated by a single chlorine atom in a molecule. Furthermore, there is an isotopic mass defect that occurs with chlorine-37 that makes the identification of chlorine in a molecule relatively easy [76]. More than one chlorine atom in a molecule generates an A+2 and A+4 isotopic pattern, which is characteristic and commonly shown in all mass spectrometry books as a key to compound identification of chlorinated compounds [77]. In this sense, a chlorine mass filter was developed by our group [78]. The chlorine mass filter is used to screen both LC/TOF-MS and LC/QTOF-MS data files in order to discover compounds that contain chlorine. The chlorine filter uses MassHunter software to generate formula of chlorine-containing compounds.

An example is given for a wastewater sample. The initial identification of lamotrigine, a nonreported antidepressant pharmaceutical to date, in water samples was accomplished using the mass defect filter that looked for chlorinated analytes in the extract of a wastewater sample after LC/TOF-MS analysis in MS-only mode. The mass defect filter essentially looks at the accurate mass of the monoisotopic mass of an analyte and the A+2 isotopic mass. Both the intensity and the accurate mass are used to detect chlorinated compounds using the mass defect filter. In the case of lamotrigine, the chlorine filter detected a peak at 13.7 min with a mass of m/z 256.0153 and an A+2 isotope with a mass of m/z 258.0122 and an intensity of 66% (see Figure 5). The mass defect filter showed that the A+2 peak had a relative isotopic mass defect of −0.0030 u, indicating a chlorinated compound with two chlorine atoms [76]. The second step after the mass defect filter was to determine the molecular formula of the unknown chlorinated compound. The best fit for the ion formula was $C_9H_8Cl_2N_5$ with a match of 99 out of 100 based on MassHunter software, which evaluates the accurate mass of the A ion, the

Sample Type	Sample	P1-C2
Instrument Name	Instrument 1	
Acq Method	Multiresidue_Pestticide s.m	1/20/2010 12:12:15 PM
IRM Calibration Status	Success	Forensic_search_1.m
Comment		

Compound Table

Label	Tgt Name	Tgt Score	Mass Error (ppm)	Tgt Formula	Obs. RT	Ref. Mass	Obs. Mass
Cpd 313: 5-Butyl-5-phenyl-hydantoin	5-Butyl-5-phenyl-hydantoin	82.57	−1.47	$C_{13}H_{16}N_2O_2$	13.428	232.1212	232.1208
Cpd 317: Oxazidione	Oxazidione	47.19	−1.12	$C_{20}H_{19}NO_3$	13.475	321.1365	321.1361
Cpd 316: Pyriproxifen	Pyriproxifen	47.19	−1.12	$C_{20}H_{19}NO_3$	13.475	321.1365	321.1361
Cpd 320: Dextrorphan (Levorphanol)	Dextrorphan (Levorphanol)	96.73	1.59	$C_{17}H_{23}NO$	13.487	257.178	257.1784
Cpd 319: Pirandamine	Pirandamine	96.73	1.59	$C_{17}H_{23}NO$	13.487	257.178	257.1784
Cpd 318: Racemorphan	Racemorphan	96.73	1.59	$C_{17}H_{23}NO$	13.487	257.178	257.1784
Cpd 321: Vinblastin	Vinblastin	68.85	−0.57	$C_{46}H_{58}N_4O_9$	13.546	810.4204	810.4199
Cpd 323: Chlormadinone	Chlormadinone	46.35	1.83	$C_{21}H_{27}ClO_3$	13.569	362.1649	362.1655
Cpd 322: Cismadinone	Cismadinone	46.35	1.83	$C_{21}H_{27}ClO_3$	13.569	362.1649	362.1655
Cpd 326: Pipratecol	Pipratecol	47.2	−1.08	$C_{19}H_{24}N_2O_4$	13.581	344.1736	344.1732
Cpd 325: Formoterol	Formoterol	47.2	−1.08	$C_{19}H_{24}N_2O_4$	13.581	344.1736	344.1732
Cpd 324: Tolamolol		47.2	−1.08	$C_{19}H_{24}N_2O_4$	13.581	344.1736	344.1732

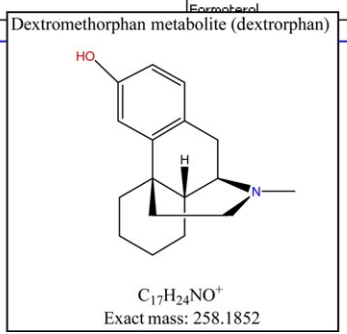

FIGURE 4 Example of a generated automated report with MassHunter using a forensic database.

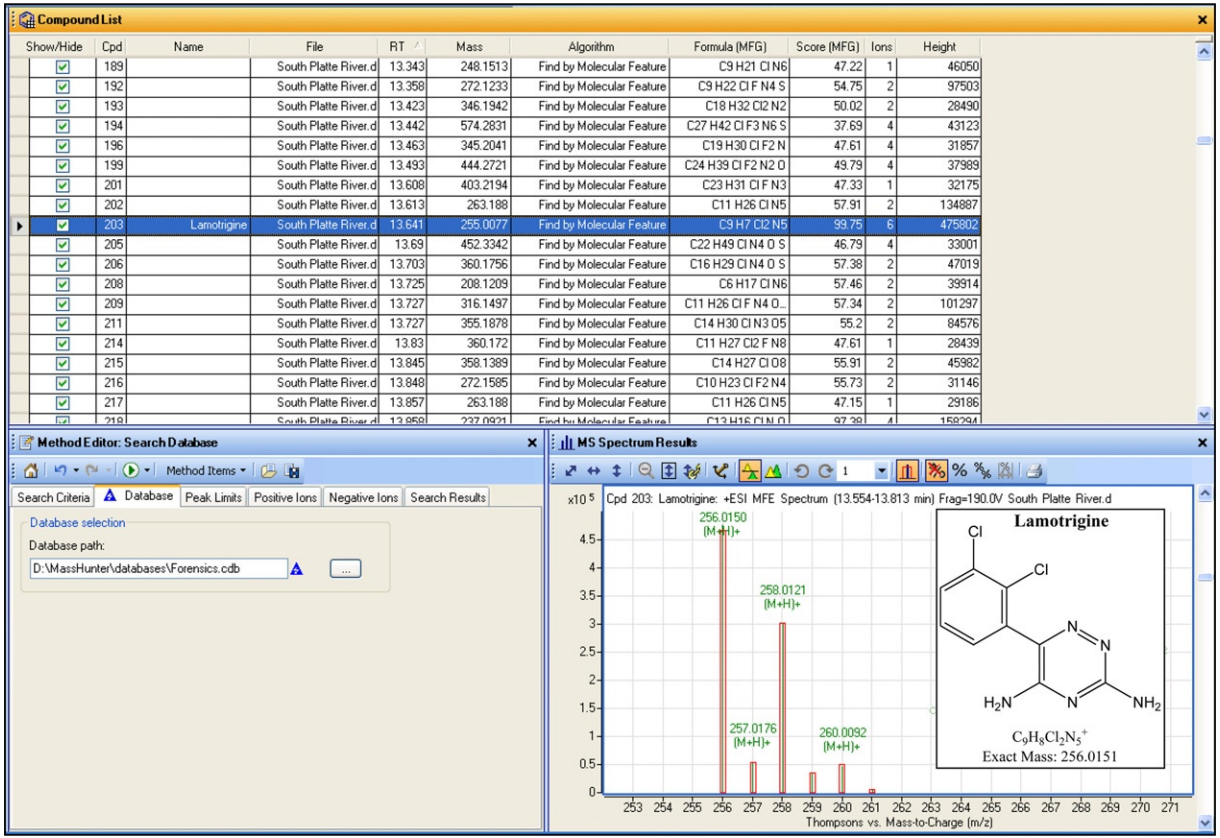

FIGURE 5 Results for a chlorine mass filter used for identification of chlorinated species. In the inset, the accurate mass spectrum for lamotrigine is shown.

isotope intensity matching, and isotope spacing (also called the isotopic mass defect) or accurate mass of the isotopes. The neutral formula, $C_9H_7Cl_2N_5$, was then run through a forensic database for a formula match and gave lamotrigine as its only formula. When the formula was put through a much larger database, ChemSpider, the match was for 65 compounds; however, there were only 13 patented structures and only 1 compound was listed in Wikipedia-available article and that was lamotrigine. A quick read showed that this compound is the number three most used bipolar medication in the United States at this time; thus, it was given the most likelihood of a correct identification. Later on, a standard was purchased and the identification was verified [16].

The combination of mass accuracy, database matching, and identifying a fragment ion shows the power of using the chlorine mass filter to find and identify trace chlorinated substituents in water samples impacted by wastewater. This approach works really well for complex water matrices by identifying specific chlorinated compounds, which in turn could be potential metabolites from known target analytes.

4.2.4 Accurate Mass Profiling

Urine metabolic profiling combined with LC/QTOF-MS was used to find and identify the metabolites of dextromethorphan, a common over-the-counter (OTC) cough suppressant [17]. Chromatograms of both blank urine and urine taken 4 h after ingestion of dextromethorphan were compared using Mass Profiler software. The software first analyzes all groups of ions (known as features) in the chromatogram of both samples and compiles this into a database. Three replicates of each sample are taken and averaged. Next, the software compares the two samples looking for features that are unique to the dextromethorphan urine (Figure 6). The comparison resulted in 27 features (in red or dark gray) that were unique to this sample and 136 individual ions. Ions at the same retention time, for example, 15.7 min, are usually the same fragment ions of a

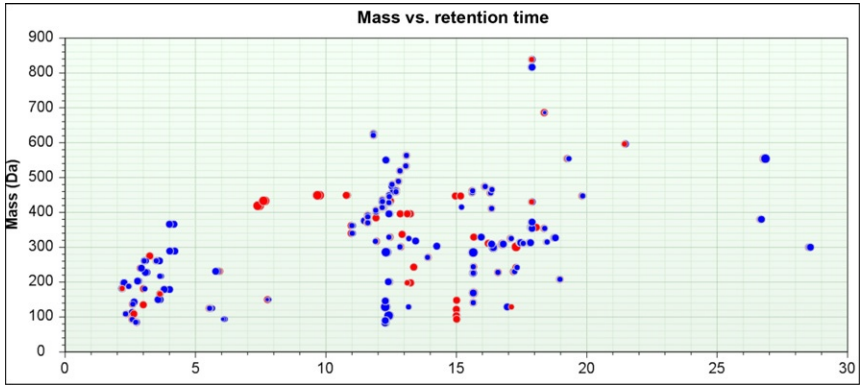

FIGURE 6 Mass profiler plot of a urine sample 4 h after taking a 10mg dose of dextromethorphan.

feature. Using this approach, seven new glucuronide metabolites were identified, as well as dextrorphan and *N*-demethyldextrorphan. Four of these compounds are reported in the pharmaceutical literature [79–82]. The metabolites are dextrorphan and *N*-demethyldextrorphan and glucuronides of each of these two compounds. The calculated exact masses for each of these compounds were extracted from the total ion chromatogram of the positive urine sample and compared to the measured masses. The measured masses for the protonated molecule of each compound varied from 0.1 to 0.3 mmu, which is 1 ppm mass accuracy or less for all targeted compounds. The rest of the metabolites had never been reported in the literature before.

4.2.5 Metabolic Analogy

An interesting approach to identify metabolites in water samples is the use of a metabolic analogy. Diagnostic ions, which are chemical structures that are common of a specific class of compounds, can be used to detect chemically related compounds in a sample. This approach was used by Writer et al. [83] to detect a series of known metabolites of carbamazepine and new metabolites for bupropion in wastewater samples. The extracted ion chromatograms for bupropion and its metabolites, erythro-hydrobupropion, threo-hydrobupropion, and hydroxy-bupropion, are shown in Figure 7A. The erythro and the threo metabolites are isomers and almost coelute in a chromatographic run of 30 min. This figure shows how sometimes metabolites are more important and abundant than the parent compounds. Figure 7B shows the corresponding mass spectrum for each metabolite.

5 CONCLUSIONS

Pharmaceuticals are ubiquitous in many water sources, including surface water, groundwater, and wastewater. Two main methodologies involving LC/MS are commonly used for the detection of low level of pharmaceuticals in water samples. A tandem mass spectrometry approach (LC/MS–MS) is usually applied for the detection of a group of target compounds. On the other hand, TOF-MS analyses using LC/Q-TOF-MS proved to be very successful for the discovery and identification of new pharmaceuticals and related metabolites. Several tools using accurate mass analysis, such as molecular features, database searching, chlorine filters, and metabolic profiling, were highly useful in the identification of several pharmaceutical metabolites. Wastewater treatment plants were identified as the major sources for pharmaceutical occurrence in surface water, and analyses of downstream and upstream samples allowed comparing the presence and degradation or dissipation of selected analytes. Analytical techniques using tandem mass spectrometry for target analysis and TOF for non-target and screening purposes are complementary and can be applied successfully for the identification of pharmaceutical compounds in water samples.

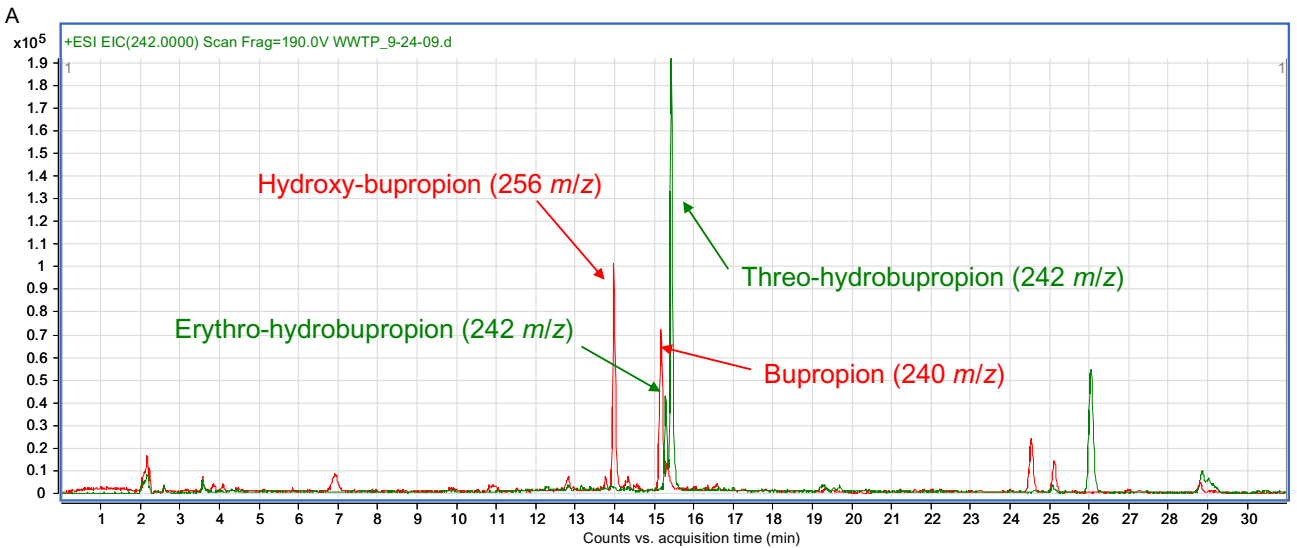

FIGURE 7 (A) Overlaid extracted ion chromatograms for a wastewater effluent sample showing diagnostic ions for bupropion and associated metabolites and

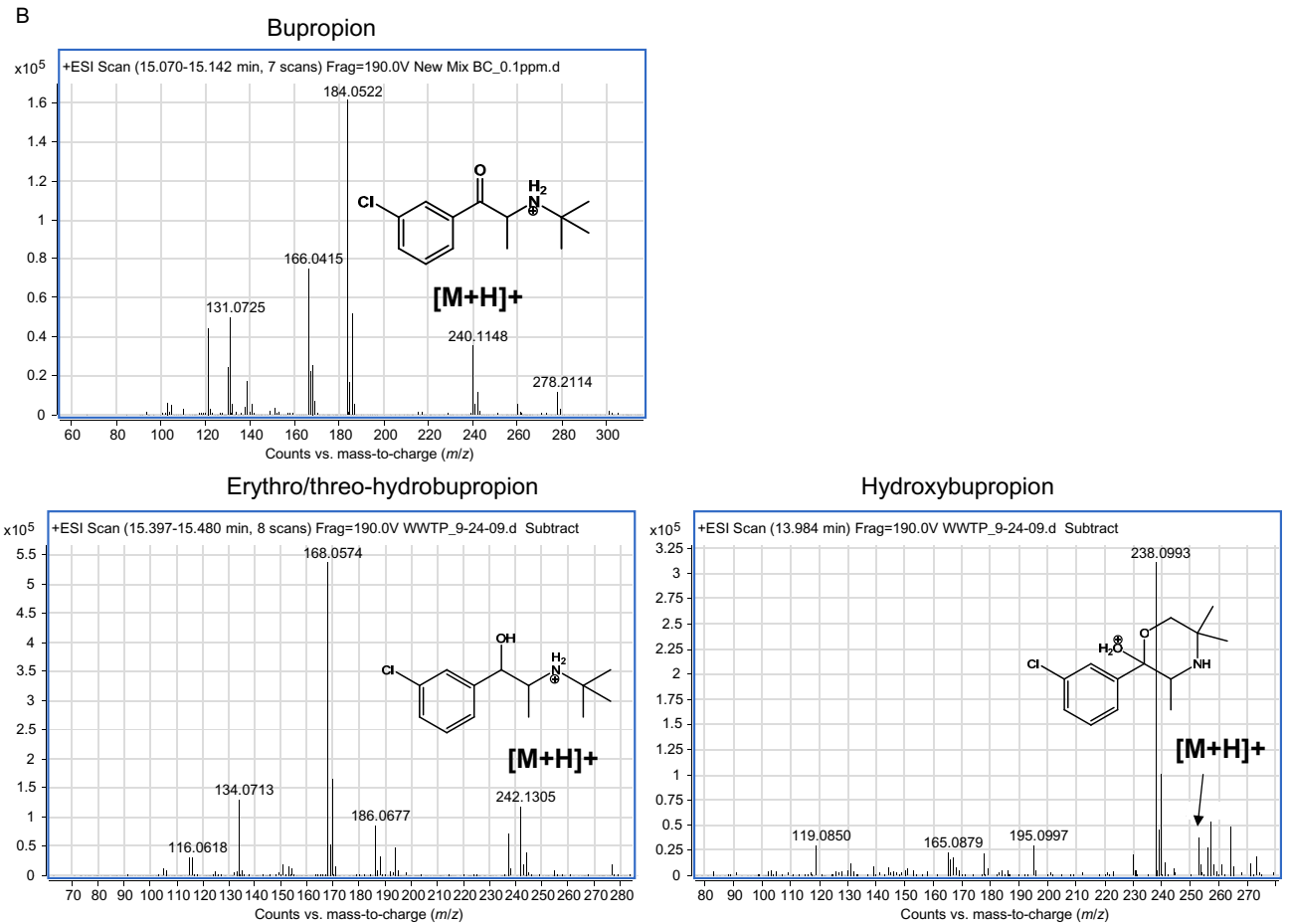

FIGURE 7—Cont'd (B) corresponding mass spectra.

REFERENCES

[1] O. Jones, N. Voulvoulis, J. Lester, Crit. Rev. Environ. Sci. Technol. 35 (2005) 401–427.
[2] D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, H.T. Buxton, Environ. Sci. Technol. 36 (2002) 1202–1211.
[3] J. Sumpter, S. Jobling, Environ. Health Perspect. 103 (1995) 173–178.
[4] S. Jobling, M. Nolan, C. Tyler, G. Brighty, J. Sumpter, Environ. Sci. Technol. 32 (1998) 2498–2506.
[5] J. Donn, M. Mendoza, J. Pritchard, AP: Drugs Found in Drinking Water. Retrieved 2012, from USA Today. http://www.usatoday.com/news/nation/2008-03-10-drugs-tap-water_N.htm, 2008.
[6] U.S. Government Accountability Office. Environmental Health Action Needed to Sustain Agencies' Collaboration on Pharmaceuticals in Drinking Water, 2011.
[7] T.D. Colborn, D. Dumanoski, Our Stolen Future: Are We Threatening Our Fertility, Intelligence, and Survival? A Scientific Detective Story, Dutton, New York, 1996.
[8] F.S. Vom Saal, Toxicol. Sci. 115 (2010) 612–613.
[9] C.G. Daughton, T.A. Ternes, Water Res. 32 (1998) 3245–3260.
[10] R. Hirsch, T. Ternes, K. Haberer, K.L. Kratz, Sci. Total Environ. 225 (1999) 109–118.
[11] M. Petrovic, M. Gros, D. Barceló, J. Chromatogr. A 1124 (2006) 68–81.
[12] S.A. Snyder, P. Westerhoff, Y. Yoon, D.L. Sedlak, Environ. Eng. Sci. 20 (2003) 449–469.
[13] B.J. Vanderford, R.A. Pearson, D.J. Rexing, S.A. Snyder, Anal. Chem. 75 (2003) 6265–6274.
[14] M.J. Benotti, R.A. Trenholm, B.J. Vanderford, J.C. Holady, B.D. Stanford, S.A. Snyder, Environ. Sci. Technol. 43 (2009) 597–603.
[15] I. Ferrer, E.M. Thurman, J. Chromatogr. A 1217 (2010) 5674–5686.
[16] I. Ferrer, E.M. Thurman, Anal. Chem. 82 (2010) 8161–8168.
[17] E.M. Thurman, I. Ferrer, J. Chromatogr. A 1259 (2012) 158–166.
[18] A. Jelic, C. Cruz-Morato, E. Marco-Urrae, M. Sarra, S. Perez, T. Vicent, M. Petrovic, D. Barceló, Water Res. 46 (2012) 955–964.
[19] http://water.epa.gov/scitech/drinkingwater/dws/ccl/ccl3.cfm.
[20] http://water.epa.gov/lawsregs/rulesregs/sdwa/ucmr/ucmr3/index.cfm.
[21] M. Gros, M. Petrovic, D. Barceló, Anal. Bioanal. Chem. 386 (2006) 941–952.
[22] I. Ferrer, E.M. Thurman (Eds.), Liquid Chromatography Time-Of-Flight Mass Spectrometry: Principles, Tools and Applications for Accurate Mass Analysis, John Wiley and Sons, Inc., New York, 2009.
[23] EPA Method 1694: Pharmaceuticals and Personal Care Products in Water, Soil, Sediment, and Biosolids by HPLC/MS/MS, December 2007, EPA-821-R-08-002.
[24] M.E. Lindsey, M. Meyer, E.M. Thurman, Anal. Chem. 73 (2001) 4640.
[25] F. Andre, K.K.G. de Wasch, H.F. de Brabander, S.R. Impens, L.A.M. Stolker, L. van Ginkel, R.W. Stephany, R. Schilt, D. Courtheyn, Y. Bonnaire, P. Furst, P. Gowik, G. Kennedy, T. Kuhn, J.P. Moretain, M. Sauer, Trends Anal. Chem. 20 (2001) 435–445.
[26] M. Petrovic, M. Farre, M.L. de Alda, S. Perez, C. Postigo, M. Kock, J. Radjenovic, M. Gros, D. Barceló, J. Chromatogr. A 1217 (2010) 4004–4017.
[27] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Anal. Bioanal. Chem. 391 (2008) 1293.
[28] L. Bijlsma, J.V. Sancho, E. Pitarch, M. Ibánez, F. Hernández, J. Chromatogr. A 1216 (2009) 3078.
[29] M.R. Boleda, M.T. Galcerán, F. Ventura, J. Chromatogr. A 1175 (2007) 38.
[30] J. Bones, K.V. Thomas, B. Paull, J. Environ. Monit. 9 (2007) 701.

[31] S. Castiglioni, E. Zuccato, E. Crisci, C. Chiabrando, R. Fanelly, R. Bagnati, Anal. Chem. 78 (2006) 8421.
[32] A. Gheorghe, A. Van Nuijs, B. Pecceu, L. Bervoets, P.G. Jorens, R. Blust, H. Neels, A. Covaci, Anal. Bioanal. Chem. 391 (2008) 1309.
[33] M. Huerta-Fontela, M.T. Galcerán, F. Ventura, Anal. Chem. 79 (2007) 3821.
[34] D. Hummel, D. Löffler, G. Fink, T.A. Ternes, Environ. Sci. Technol. 40 (2006) 7321.
[35] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, J. Chromatogr. A 1161 (2007) 132.
[36] B. Loganathan, M. Phillips, H. Mowery, T.L. Jones-Lepp, Chemosphere 75 (2009) 70.
[37] C. Postigo, M.J. Lopez De Alda, D. Barceló, Anal. Chem. 80 (2008) 3123.
[38] E. Zuccato, S. Castiglioni, R. Bagnati, C. Chiabrando, P. Grassi, R. Fanelli, Water Res. 42 (2008) 961.
[39] A.C. Chiaia, C. Banta-Green, J. Field, Environ. Sci. Technol. 42 (2008) 8841.
[40] F. Busetti, K.L. Linge, A. Heitz, J. Chromatogr. A 1216 (2009) 5807.
[41] J.Y. Pailler, A. Krein, L. Pfister, L. Hoffmann, C. Guignard, Sci. Total Environ. 407 (2009) 4736.
[42] M. Gros, M. Petrovic, D. Barceló, Anal. Chem. 81 (2009) 898.
[43] S. Grujic, T. Vasiljevic, M. Lausevic, J. Chromatogr. A 1216 (2009) 4989.
[44] C. Wu, A.L. Spongberg, J.D. Witter, Int. J. Environ. Anal. Chem. 88 (2008) 1033.
[45] M. Farré, M. Gros, B. Hernandez, M. Petrovic, P. Hancock, D. Barceló, Rapid Commun. Mass Sp. 22 (2008) 41–51.
[46] J. Radjenovic, M. Petrovic, D. Barceló, Trends Anal. Chem. 26 (2007) 1132–1144.
[47] M. Ibáñez, J.V. Sancho, D. McMillan, R. Rao, F. Hernández, Trends Anal. Chem. 27 (2008) 481–489.
[48] M.J. Gómez, O. Malato, I. Ferrer, A. Agüera, A.R. Fernández-Alba, J. Environ. Monitor. 9 (2007) 718–729.
[49] A.A.M. Stolker, W. Niesing, E.A. Hogendoorn, J.F.M. Versteegh, R. Fuchs, U.A.Th. Brinkman, Anal. Bioanal. Chem. 378 (2004) 955–963.
[50] I. Ferrer, C.E. Heine, E.M. Thurman, Anal. Chem. 76 (2004) 1437–1444.
[51] A. Kaufmann, P. Butcher, K. Maden, M. Widmer, Anal. Chim. Acta 586 (2007) 13–21.
[52] I. Ojanperä, A. Pelander, S. Laks, M. Gergov, E. Vuori, M. Witt, J. Anal. Toxicol. 29 (2005) 34–40.
[53] S. Ojanperä, A. Pelander, M. Pelzing, I. Krebs, E. Vuori, I. Ojanperä, Rapid Commun. Mass Sp. 20 (2006) 1161–1167.
[54] A. Pelander, I. Ojanperä, S. Laks, I. Rasanen, E. Vuori, Anal. Chem. 75 (2003) 5710–5718.
[55] M. Ibáñez, J.V. Sancho, O.J. Pozo, W.M.A. Niessen, F. Hernández, Rapid Commun. Mass Sp. 19 (2005) 169–178.
[56] A. Aguera, L.A.P. Estrada, I. Ferrer, E.M. Thurman, S. Malato, A.R. Fernandez-Alba, J. Mass Sp. 40 (2005) 908–915.
[57] I. Ferrer, M. Mezcua, M.J. Gomez, E.M. Thurman, A. Aguera, M.D. Hernando, A. R. Fernandez-Alba, Rapid Commun. Mass Sp. 18 (2004) 443–450.
[58] D.A. Lambropoulou, M.D. Hemando, I.K. Konstantinou, E.M. Thurman, I. Ferrer, T.A. Albanis, A.R. Fernandez-Alba, J. Chromatogr. A 1183 (2008) 38–48.
[59] M. Mezcua, I. Ferrer, M.D. Hernando, A.R. Fernandez-Alba, Food Addit. Contam. 23 (2006) 1242–1251.
[60] S. Perez, P. Eichhorn, D. Barceló, D.S. Aga, Anal. Chem. 79 (2007) 8293–8300.
[61] S. Broecker, S. Herre, B. Wüst, J.A. Zweigenbaum, F. Pragst, Anal. Bioanal. Chem. 400 (2011) 101–117.

[62] I. Ferrer, E.M. Thurman, J. Chromatogr. A 1259 (2012) 148–157.
[63] I. Ferrer, A.R. Fernández-Alba, J.A. Zweigenbaum, E.M. Thurman, Rapid Commun. Mass Sp. 20 (2006) 3659–3668.
[64] E.M. Thurman, I. Ferrer, O. Malato, A.R. Fernández-Alba, Food Addit. Contam. 23 (2006) 1169–1178.
[65] A. Polettini, R. Gottardo, J.P. Pascali, F. Tagliaro, Anal. Chem. 80 (2008) 3050–3057.
[66] B. Park, K. Kidd, Environ. Toxicol. Chem. 24 (2005) 2027–2036.
[67] Agilent Technologies Application Note for GC/MS Screening of Pesticides, 2005.
[68] I. Ferrer, E.M. Thurman, Trends Anal. Chem. 22 (2003) 750–756.
[69] C. Baumann, M.A. Cintora, M. Eichler, E. Lifante, M. Cooke, A. Przyborowska, J.M. Halket, Rapid Commun. Mass Sp. 14 (2000) 349–356.
[70] J.L. Josephs, M. Sanders, Rapid Commun. Mass Sp. 18 (2004) 743–759.
[71] M. Gergov, W. Weinmann, J. Meriluoto, J. Uusitalo, I. Ojanpera, Rapid Commun. Mass Sp. 18 (2004) 1039–1046.
[72] I. Ferrer, E.M. Thurman, Anal. Chem. 77 (2005) 3394–3400.
[73] E.M. Thurman, I. Ferrer, A.R. Fernández-Alba, J. Chromatogr. A 1067 (2005) 127–134.
[74] I. Bobeldijk, J.P.C. Vissers, G. Kearney, H. Major, J.A. van Leerdam, J. Chromatogr. A 929 (2001) 63–74.
[75] S. Laks, A. Pelander, E. Vuori, E. Ali-Toppa, E. Sippola, I. Ojanpera, Anal. Chem. 76 (2004) 7375–7379.
[76] E.M. Thurman, I. Ferrer, Anal. Bioanal. Chem. 397 (2010) 2807–2816.
[77] R.M. Smith, Understanding Mass Spectra, John Wiley & Sons, Inc., New York, 2004, 290 pp.
[78] Agilent Technologies Application Note: Using a Chlorine Filter for Accurate-Mass Data Analysis of Environmental Samples.
[79] T.H. Eichhold, L.J. Greenfield, S.H. Hoke II., K.R. Wehmeyer, J. Mass Sp. 32 (1997) 1205–1211.
[80] U. Lutz, N. Bittner, R.W. Lutz, W.K. Lutz, J. Chromatogr. B 871 (2008) 349–356.
[81] S. Lin, C.H. Chen, H.O. Ho, H.H. Chen, M.T. Sheu, J. Chromatogr. B 859 (2007) 141–146.
[82] R. Kikura-Hanajiri, M. Kawamura, A. Miyajima, M. Sunouchi, Y. Goda, Anal. Bioanal. Chem. 400 (2011) 165–174.
[83] J.H. Writer, I. Ferrer, L.B. Barber, E.M. Thurman, Sci. Total Environ., 461–462 (2013) 519–527.

Chapter 5

Analysis of PhACs in Solid Environmental Samples (Soil, Sediment, and Sludge)

Sandra Babić and Dragana Mutavdžić Pavlović
Department of Analytical Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Zagreb, Croatia

Chapter Outline

1 INTRODUCTION

Pharmaceutically active compounds (PhACs) have an important role in treatment and prevention of diseases in both humans and animals. After the administration, they are excreted from the body as a parent compound and/or as their metabolite and released into influents of municipal wastewater

Comprehensive Analytical Chemistry, Vol. 62. http://dx.doi.org/10.1016/B978-0-444-62657-8.00005-7

treatment plants (WWTPs). They are only partially eliminated in such plants, which brings to their release into the environment via effluents or sewage sludge. The degree of removal and biodegradation of PhACs during wastewater treatment (WWT) varies considerably, depending on their physicochemical properties. In terms of soluble PhACs, transfer to sludge is only of minor concern. Strongly hydrophobic (lipophilic) and not readily biodegradable compounds are retained in the sludge. Most pharmaceuticals used for human treatment are not biodegradable [1]. Furthermore, PhACs used in veterinary medicine end up, after excretion by animals, in manure. Treated sewage sludge is often disposed of on agricultural land because of its fertilization value similar to that of manure from farms. Since veterinary drugs tend to end up in manure, either of the earlier-mentioned products have a strong potential to introduce PhACs and contaminate soil, surface, and groundwater [1–4]. In addition, surface water receiving effluents from WWTPs contaminated by PhACs may lead to contamination of sediment, another solid matrix that requires monitoring of pharmaceuticals. Also, effluents from WWTPs are used for facilitation of the ecological flow in wetlands and for irrigation of farm areas thus introducing PhACs in the soil compartment [5]. As a result, pharmaceuticals are found in different environmental compartments (surface water [6], sediment [7], and soil [8]).

Therefore, analysis of solid environmental matrices is of importance in assessing pharmaceuticals' fate and behavior in the environment. Sampling is the first activity in sample analysis, often underestimated, but significantly contributes to the overall uncertainty of the final results. Since environmental matrices, especially solid samples, are very heterogeneous, their heterogeneity is the main source of this uncertainty. Therefore, obtaining representative samples is one of the most important aspects of monitoring campaigns and it ensures valid test results [9]. Furthermore, the four dimensionality (time being the fourth dimension) of solid environmental samples should be regarded during the preparation of sampling protocols. The final step in analysis of solid environmental samples is usually based on chromatographic techniques (liquid chromatography (LC) or gas chromatography (GC)) hyphenated to mass spectrometry (MS). The major difference, in comparison with water sample analysis, is associated with the tedious sample preparation step.

2 TRANSPORT AND FATE OF PhACs IN SOLID MATRICES

Sediments originate from processes of weathering and erosion of minerals and soils and are transported down the river to the coast where they are discharged to seas and oceans. In lowland areas where the river flow rate declines, transported sediments settle along the riverbanks and beds through the sedimentation process. Sediments are, like the water, a highly dynamic part of river systems.

Emission of anthropogenic substances to the surface water as a result of different human activities can cause rapid deterioration of the sediment quality. Depending on pollutant properties, these substances could accumulate in sediments and reflect the history of the pollution in a respective river basin [10,11]. After entering into water, PhACs are distributed to water phases, suspending particles and sediments depending on their physicochemical properties [12]. Water-soluble compounds are presented in the water phase in dissolved forms, while the hydrophobic ones are absorbed by particles in water. With particle dropping, PhACs enter into the sediment. The water temperature, salinity, and pH value could impact adsorption and desorption processes, while the water flow velocity, particle size and shape, and the river bed morphology could affect the particle dropping [10,12]. Therefore, transport of PhACs could be very different in different river systems. In water where stream is rushing, PhACs may travel long distances. On the contrary, in stable water with little disturbance, PhACs are easily sedimentated not far away from the place where they enter the aquatic environment [12]. Particles dropped into the sediment may be suspended into the water again due to various water disturbances, which leads to the second water pollution. Consequently, organic pollutants may be transferred a long way from the discharge point through the repeated sedimentation-suspending process [10,12]. Because of the sediment ability to transport and accumulate contaminants and release this historical contamination, contaminated sediments remain potential sources of adverse effects on the surface and groundwater.

Sewage sludge is, besides the effluent, the end product of WWTPs and could be used for land application as a nutrient source (supplying nitrogen and phosphate) or soil conditioner (improving the organic matter content, water-holding capacity, or structure) [10]. However, lipophilic and not readily biodegradable compounds are retained in the sludge where they are accumulated. Consequently, sludge is a potential source of substances such as heavy metals and organic compounds that are harmful to humans and animals and entail potential adverse effects on the environment [1,9]. After application of sewage sludge or manure contaminated with PhACs to soil, these compounds could reach deeper soil layers or the groundwater through the runoff and leaching, which depends on several factors: washout of soil particles with precipitation (soil erosion), the proportion of soluble organic substances, and the solubility of compounds [13].

Besides the physical transport of PhACs in the sediment and soil, PhACs are subjected to several other processes that can lead to their elimination in the environment and consequently to a loss of their pharmacological activity. These processes refer to adsorption/desorption, degradation by chemical reactions (abiotic degradation)—including photolysis, hydrolysis, and oxidation—and biotic elimination through bioaccumulation and microbial degradation [14,15]. Photodegradation is only likely to occur in the top layer of the soil surface and after plowing of agricultural fields when sorbed PhACs are

exposed to sunlight. Despite the fact that the degradation rate in soil is lower than in aqueous solutions, photochemistry could be a significant degradation path for otherwise chemically resistant PhACs (such as fluoroquinolones (FQs)) [16].

With respect to highly water-soluble pharmaceuticals, adsorption is not a significant elimination process. These pharmaceuticals are mobile and tend to leach through the soil into the groundwater. Unlike highly water-soluble pharmaceuticals, hydrophobic compounds strongly sorb to solid matrices and tend to accumulate. The sorption of pharmaceuticals to the soil or sediment includes different mechanisms. The most important ones are sorption to organic matter, surface adsorption to mineral content, ion exchange, complexation with metal ions (Ca^{2+}, Mg^{2+}, Fe^{3+}, or Al^{3+}), and H-bonding [15]. Depending on a species, interactions with soil can occur through electrostatic attraction, surface bridging, hydrogen bonding, or hydrophobic interactions [17]. Apart from surface adsorption, diffusion into porous soil particles also contributes to PhACs elimination. Compounds adsorbed to the surface of the particles possess exchanging properties that constitute a reversible adsorption part, whereas compounds entering to the interior of the particles form an irreversible adsorption part [12].

The degree of sorption mainly depends on PhACs physicochemical properties (K_d, K_{OC}, K_{OW}, and pK_a), the type of solid matrices (content of organic matter and soil minerals), and environmental conditions (pH and temperature). The distribution of chemicals between the solid and water phase is described by means of a soil–water partition coefficient K_d defined as a ratio of the chemical concentration in the sorbent and in the water at equilibrium. As far as hydrophobic compounds are concerned, K_d varies depending on the organic carbon content, and hence, application of the organic carbon-normalized partition coefficient (K_{OC}) approach is recommended for prediction of the environmental fate [14,15]. Furthermore, K_{OC} values are easily derived from the octanol–water partition coefficient (K_{OW}), which describes chemical lipophilic or hydrophobic properties [18]. The degree of sorption for different PhACs varies to a great extent. These variations could not be explained only by variation in the soil organic content. The sorption and consequently accumulation of antibiotics in solid matrices are firmly governed by the ionization property of numerous PhACs with pK_a values within an environmentally significant pH range [19]. The K_{OW} coefficients of ionizing compounds change considerably in the pH range around the acid dissociation constant (K_a). As a consequence of the PhACs' ionization ability, they are present in the environment as negative, neutral, zwitterionic, and positively charged species with a different tendency of sorption to solid matrices [17]. For example, the adsorption coefficients (K_d) of sulfonamides increase as the soil pH decreases due to the ionization of amphoteric sulfonamides [20]. At pH values 8–9, acidic pharmaceuticals (such as ibuprofen (IBF) and diclofenac) appear as anionic species while basic pharmaceuticals (such as sulfamethazine) are positively charged.

Therefore, it is expected that the sorption would be weak due to electrostatic repulsion from the charged functional groups in the sludge and sediment [5]. Except by the pH value, PhACs' sorption onto solid matrices is influenced by ionic strength as shown for tetracyclines (TCs) since they form reversible complexes with multivalent cations [20]. TCs have three pK_a values: they always possess a local charge and are zwitterionic at an environmentally relevant pH. As a consequence thereof, TCs may interact with cationic and anionic sites in soil. Despite their hydrophilic property (polar structure) and potential biodegradability, they are widely detected in solid matrices. This could be explained by the fact that TCs complexate with divalent metal ions (e.g., magnesium, calcium, and ferric ion) and therefore accumulate in the sediment or in solid fraction during WWT [5,19,21]. Three major mechanisms are proposed for TCs' sorption [20]: complexation with divalent cations, ion exchange, and hydrogen bridging from acidic groups of humic acids to polar groups of the TCs. The most prescribed FQs worldwide, ciprofloxacin and ofloxacin, are frequently detected in solid environmental matrices. FQs have two pK_a values and exist, within an environmentally relevant pH range, mostly as zwitterions that favor their hydrophobicity [5]. High FQ concentrations in solid samples could be related to their high potential to chelate with cations and to bind to solid matrices [22].

3 OCCURRENCE OF PhACs IN SOLID SAMPLES

The discussed sources and fate of PhACs result in detectible concentrations in soil, sediment, and sludge. The number of papers dealing with investigation of PhACs occurrence, fate, and behavior in solid environmental matrices has gone up in the last year, but such papers are still less numerous in relation to those dealing with aqueous matrices. When investigating the fate of PhACs in WWTP, most studies focus only on the aqueous phase (influent and effluent) although screening of sewage sludge can show that PhACs are persistent in this matrix. This is probably due to demanding efforts and the tedious sample preparation step in the analysis of this complex matrix. Anyhow, sewage sludge is the most widely investigated solid sample. The amounts of PhACs found in solid environmental samples are different and range from those below the limit of detection (LOD) to several milligrams/kilograms (Table 1).

Recent investigation of sludge samples from three conventional WWTPs in Spain has revealed accumulation of 21 PhACs out of 43 analyzed ones at concentrations up to 100 μg/kg [62]. The most abundant PhACs included diclofenac, bezafibrate, carbamazepine, hydrochlorothiazide, furosemide, atorvastatin, and clarithromycin, while beta blockers, beta agonist, and histamine H2-receptor antagonists were found at very low concentrations. The investigation has shown that PhACs accumulated in sludge samples belong to different therapeutic classes covering a wide range of physicochemical properties.

TABLE 1 Sample Preparation and Quantification Methods for Pharmaceutical Determination in Solid Environmental Samples

Compounds	Matrix	Sample Preparation	Clean-up	Analysis	Recovery (%)	LOD (μg/kg)	LOQ (μg/kg)	Detected Level (μg/kg)	References
17 PhACs	Sediment Soils	PLE	SPE (SAX-HLB)	LC–ESI–MS/MS	71–119	0.2–6.8 0.1–5.3		≤15.1 ≤8.4	[5]
18 PhAc	Soil Sediment Sewage sludge	MAE	SPE (HLB)	GC–MS	92–101 91–101 91–100	0.8–4.7		9–460 8.5–360 30–2300	[8]
8 Qs 9 SAs 5 MAs	Sediment	PLE	SPE (HLB)	LC–ESI–MS/MS	71.5–132.2 96.8–132.3 63.4–100.5	0.2–0.5 0.02–0.3 0.1–0.3		65.5–1166 <LOD–8.48 0.58–304	[22]
14 SAs	Soil	Shaking	SPE (SAX-HLB)	UHPLC–ESI–MS/MS	3.2–188.2	0.010–0.343		0.034–0.663 (SMZ)	[23]
Antibiotics	Sediment	Shaking	SPE (HLB)	LC–ESI–MS/MS	<30–128.4		0.3–3.6	1.2–32.8	[24]
Ivermectin	Marine sediment	Shaking	SPE (C8)	HPLC–FLD	78.5–87.3	0.5	0.93	1.4–6.8	[25]
8 Acidic drugs Ivermectin 7 Antibiotics	Sediment	USE	SPE (MCX) SPE (Lichrolut EN) SPE (Lichrolut EN and C18)	LC–APCI–MS/MS LC–ESI–MS/MS	56–206 31–41 22–82		0.4–8 0.4 3–20		[26]
11 Acidic PhACs 8 Neutral PhACs	Sludge	USE	SPE (MCX) SPE (C18)	LC–ESI–MS/MS	43–76 25–78		20–50 ng/g	0.20–0.45 (diclofenac)	[27]
7 PhACs	Sludge	USE	SPE (Strata X)	LC–ESI–MS/MS	31–83		0.5–51	3.6–778	[28]
SMZ CTC TYL	Soil	USE	SPE (SAX-HLB)	LC–ESI–MS/MS	38–73		3–5	10.4 (SMZ) 55–87 (CTC) <LOQ (TYL)	[29]

11 Veterinary antibiotics	Soil	USE	SPE (C18)	HPLC–ESI–MS/MS	61–89		0.49–25		[30]
TCs FQs SAs	Soil	USE	SPE (SAX-HLB)	HPLC–DAD HPLC–FLD (for FQs)	60–86 46–55 69–101			10–25 20–50 (ENR) ≤400	[31]
5 FQs	Soil	USE	SPE (MIP)	HPLC–UV (C18) HPLC–UV (MIP)	75.2–85.3 87.9–103.5	40–70 190–350			[32]
4 Qs 5 FQs	Soil	USE	–	HPLC–UV	82.5–104.3	40–80	150–250		[33]
SAs MAs TMP Chloramphenicol	Sewage sludge Sediment	USE	SPE (HLB)	LC–ESI–MS/MS	74.7–111.8		2.2–66.9	6.8–125.6	[34]
7 Acidic PhACs	Sediment	USE	SPE (MIP)	LC–MS/MS	77.4–90.6	4–10		6.6–17.9	[35]
25 Antibiotics	Sediment Sludge	USE + vortex	SPE (SAX-HLB)	RRLC–ESI–MS/MS	<10–343 <10–235		0.64–6.67 1.5–28.6	3.41–127 1.45–5800	[36]
5 Acidic PhACs	Soil	USE	SPE (C18)	GC–MS	99.5–118.3		0.2–1.2	0.55–9.08	[37]
SCP OTC TYL	Soil	USE	SPE (SAX-HLB)	LC–UV–FLD (FLD for SCP)	68–85 27–75 47–105	18 18 40			[38]
66 PhACs	Sewage sludge	Combination PLE and USE	SPE (HLB)	LC–MS/MS UHPLC–MS/MS	40–130			3–8680 ng/g	[39]
SAs MAs TMP	Sewage sludge	PLE	SPE (HLB)	LC–ESI–MS/MS	79–106 91–142 78		3–41	≤197	[40]
MAs IPA TIA	Soil	PLE	SPE (diol)	LC–APCI–MS/MS	38–118	0.2–1.6	0.6–5.3	0.7 (TIA)	[41]
11 Antimicrobials	Sludge	PLE	SPE (HLB)	LC–ESI–MS/MS	1–104	0.001–0.27	0.005–0.59	0.07–0.23 (SDX)	[42]

Continued

TABLE 1 Sample Preparation and Quantification Methods for Pharmaceutical Determination in Solid Environmental Samples—Cont'd

Compounds	Matrix	Sample Preparation	Clean-up	Analysis	Recovery (%)	LOD (μg/kg)	LOQ (μg/kg)	Detected Level (μg/kg)	References
TCs SAs Others	Biosolid	PLE	SPE (HLB)	LC–ESI–MS/MS	49–68 64–95 77–88	0.6–146	1.9–488	2.6–743.6	[43]
17 PhACs	Soil Sediment	PLE	SPE (SAX-HLB)	LC–ESI–MS/MS	34–105	0.1–6.8	0.25–23	MDL-35.62	[44]
7 Avermectins	Sediment Soil	PLE	SPE (HLB)	LC–APCI–MS/MS	63–88 63–80		0.5–2.5	<LOD	[45]
Toltrazuril Toltrazuril sulfoxide Toltrazuril sulfone	Soil	PLE	SPE (C18)	LC–ESI–MS/MS	77–110		0.01–0.03	≤0.335	[46]
5 SAs	Soil	PLE	–	LC–ESI–MS/MS	41–93	5–15		≤530	[47]
43 PhACs	Sewage sludge Sediment	PLE	SPE (HLB)	LC–ESI–(Q-LIT)–MS^2	38.2–215 33.2–206	0.01–8.84 0.01–3.20	0.05–29.4 0.02–10.7	0.2–126 –	[48]
10 PhACs	Sewage sludge	PLE	SPE (HLB)	HPLC–ESI–MS	54–95	2–8	20–100	1300–4000	[49]
3 FQs 2 TCs 2 SAs	Sewage sludge	PLE	SPE (HLB) SPE (SCX)	LC–ESI–MS	26–95 3–96		0.1–160		[50]
TCs SDZ MAs	Soil	PLE	SPE (SAX-HLB)	LC–ESI–MS/MS	50–80 50–80 60–100	0.6–5.6 0.9–2.9 0.4–5.5	1.1–12.8 1.2–6.4 1.2–11.0	0.6–15.5 (CTC) 1.8–57.4 (TYL)	[51]

Acidic PhACs Carbamazepine	Irrigated soils	PLE	SPE (HLB)	GC–MS	62–102 75–118	0.1–2.0 0.5		<1 5.14–6.48	[52]
11 PhACs	Soil	PLE	SPE (Strata X)	UHPLC–Orbitrap-MS	34–100			>50 (OTC)	[53]
4 Avermectins	Soil	SFE Shaking	– Column (Florisil)	LC–ESI–MS/MS	82.5–96.2 56.4–118.6	1.5 0.3	5 1		[54]
4 NSAIDs	Sewage sludge	SHWE	HF–LPME	LC–ESI–MS	38.9–90.3	0.4–3.7	1.5–12.2	7.7–588	[55]
4 Acidic PhACs	Sewage sludge	MAE	SPE (HLB) DME–SPE (HLB)	GC–MS	80–101 83–106		100–540 15–22	10–150	[56]
4 Acidic PhACs	Sediment	MAE	DME–SPE (HLB)	GC–MS	95–103		2–6	2–38	[57]
8 PhACs	Sediment	MAME	SPE (HLB)	HPLC–UV–DAD	6–114	4–167	12–556		[58]
12 PhACs	Sediment	MSPD	–	LC–ESI–MS/MS	37.1–115	0.125–500	0.5–5000	–	[59]
Ibuprofen Hydroxy-IBP Carboxy-IBP	Soils	QuEChERS	–	LC–FLD	82.2–101		≤22.4	46.1 (carboxy-IBP)	[60]
4 NSAIDs	Sewage sludge	HF–LPME	–	LC–ESI–MS	53–62			29–138	[61]

CTC, chlortetracycline; DAD, diode-array detection; ENR, enrofloxacin; FLD, fluorescence detection; FQs, fluoroquinolones; IPAs, ionophore antibiotics; MAs, macrolides; NSAIDs, nonsteroidal anti-inflammatory drugs; OTC, oxytetracycline; RRLC, rapid resolution liquid chromatography; SAs, sulfonamides; SCP, sulfachloropyridazine; SDX, sulfadimethoxine; SDZ, sulfadiazine; SMT, sulfamethazine; SMZ, sulfamethazine; Qs, quinolones; TCs, tetracyclines; TIA, tiamulin; TMP, trimethoprim; TYL, tylosin.

Comprehensive investigation [63] of 110 sewage sludge samples from the United States and 72 target pharmaceuticals and personal care products (PPCPs) has shown that next to disinfectants (triclocarban and triclosan), antibiotics were the most abundant PPCPs. Among antibiotics, FQs, more precisely ciprofloxacin and ofloxacin, appeared to be the most abundant ones, with medium concentrations of 6.8 ± 2.3 mg/kg and 5.4 ± 1.9 mg/kg dry weight (dw), respectively. TCs (4-epitetracycline, tetracycline, minocycline, and doxycycline) were found with mean concentrations around 1–2 mg/kg dw, while the macrolide antibiotic azithromycin was found at 0.8 ± 0.2 mg/kg dw. Azithromycin has also been found in sludge from Switzerland and Germany, at concentration up to 0.16 mg/kg dw [40]. Since azithromycin is a frequently prescribed antibiotic, fairly hydrophobic, and not readily biodegrade, it is expected to be detected at higher concentrations. A relatively low-determined amount could be attributed to incomplete azithromycin extraction from solid samples resulting in low recovery (only 12% [63]).

Sulfonamides have also been found in different activated sewage sludge samples from Germany and Switzerland, with concentrations within the range of 24–197 μg/kg for sulfapyridine and 18–113 μg/kg for sulfamethoxazole [40]. Unlike the German and Swiss samples, sewage sludge samples taken in WWTPs in the south of Catalonia have disclosed that the amount of sulfonamides were below the method limit of quantification (LOQ) [49]. The only pharmaceuticals quantified were tylosin (TYL) and roxithromycin, with the highest value of 4.0 mg/kg dw for TYL and 1.8 mg/kg dw for roxithromycin.

Several studies have indicated that irrigation of soil using wastewater effluents (reclaimed water) [52,64] or spreading treated sewage sludge onto soil [65,66] could introduce these micropollutants into the environment. Similarly, fertilization of agricultural land with manure contaminated with PhACs could contaminate the soil and groundwater and be uptaken by vegetables. Once they enter the soil, PhACs' behavior is very different depending on substance properties, soil type, and environmental conditions. It was reported that carbamazepine and diclofenac can be classified as slow mobile compound in soil rich in soluble organic matter (SOM), while in SOM-poor soil, their mobility increases significantly due to poor sorption [67]. In another study, it was shown that naproxen and trimethoprim showed moderate to strong sorption, while the sorption of diclofenac, IBF, and sulfamethoxazole was negligible [68].

Hu et al. [4] have investigated the occurrence and seasonal changes and migration of TCs, sulfonamides, and quinolones from manure to soil and from soil to vegetables and groundwater. They have observed seasonal changes in antibiotic concentrations with a significantly lower concentration in summer than that in winter in all the investigated matrices. The highest observed concentration in soil referred to TCs (2683 μg/kg for oxytetracycline (OTC)). As it is expected, concentrations of water-soluble antibiotics in groundwater are higher in comparison with low water-soluble TCs, which have high concentrations in soil. Ciprofloxacin was found in most groundwater samples but not

detected in vegetable samples indicating its high mobility from soil to groundwater.

Recent investigation on the occurrence of 17 PhACs with a great variety of polarities and pK_a values in water, soil, and sediment from Mediterranean wetland has revealed their presence in all the investigated matrices. As much as 94% of the sediments and 80% of the agricultural land samples were polluted; the most abundant PhACs were carbamazepine in the sediment and acetaminophen in the soil samples. The concentrations in the sediment reached 15.1 ng/g (acetaminophen) and 8.4 ng/g (norfloxacin) in the soil. Also, diffusion of codeine and FQs to deeper soil horizons was observed [5].

A study of sorption of acidic pharmaceuticals to sediment [57] has shown that the concentration of pharmaceuticals sorbed to the sediment is dependent on pharmaceutical concentration in the water phase (higher in winter) and linearly dependent on total organic carbon content in the solid phase. Naproxen and diclofenac were quantified in sediment from the Danube River and the figures fit within the range of 2–20 and 5–38 ng/g, respectively, while the concentrations of IBF and ketoprofen were below their LOQ values.

Hu et al. [7] have investigated natural accumulation and attenuation of antibiotics in river sediment by long-term field and modeling studies. The concentrations of 12 investigated antibiotics from seven different antimicrobial groups in sediment samples ranged from 97 μg/kg (trimethoprim) to 12.4 mg/kg (rifampicin). They measured antibiotic concentration in the sediments at 1 month, 1 year, and 2 years after the dredging and observed that the antibiotic concentration increased significantly, confirming that sediment accumulates antibiotics and could act as a sink of antibiotic contamination in river water.

Li et al. [22] have investigated the occurrence and distribution of sulfonamides, quinolones, and macrolides in water, sediment, and biota samples. The most abundant antibiotics in the sediment samples were quinolones (up to 1140 μg/kg dw for norfloxacin) and macrolides (up to 302 μg/kg dw for roxithromycin), while sulfonamides were prominent in the water and accounted for only 1.06% of the total antibiotics present in the sediment samples.

Results from different studies have revealed that PhACs are found in solid environmental matrices throughout the world. FQs and TCs seem to be the most abundant ones due to their wide consumption and strong sorption to solid samples. Since pharmaceuticals are excreted as a parent compound and/or metabolite, metabolites should also be included in monitoring of solid samples. To date, only few papers have focused on determination of pharmaceuticals and their metabolites in solid environmental samples [46,60].

4 SAMPLE PREPARATION

Solid samples are complex in their nature and the extraction of pharmaceuticals therefrom has proved to be more challenging than the extraction from aqueous samples [69]. This is the case due to heterogeneous characteristics

of soil, sediments, and sludge [70] that tend to suffer from several interferences that deeply affect the extraction and the separation steps of the analysis. These types of samples are completely different from the homogeneous nature of water or liquid samples, which are easier to handle than solid ones [15]. Furthermore, the target analytes often exist at very low concentrations in these samples, which is why it is essential to carry out effective sample preparation procedure.

Extraction of pharmaceuticals from solid samples is a process in which analytes desorb from the sample matrix and then dissolve into the solvent. The extraction efficiency of the applied extraction method is affected by three interrelated factors: analyte solubility, mass transfer, and matrix effects. A highly soluble analyte can be "unextractable" due to being locked in the matrix pores or being strongly bound to its surface. Mass transfer is dependent on the diffusion coefficient and on the particle size and structure of the matrix, and it is enhance by high pressure, high temperature, low solvent viscosity, and small particle size [71].

Pretreatment of the sample is needed to assure good contact between the solvent and the matrix in the extraction process. The pretreatment usually comprises three different steps and usually depends of sample type. The first step is drying of solid samples. The existence of water in the sample can be eliminated using air-drying, heating, or lyophilization. The temperature applied to different analytes is critical, particularly if the analytes are thermolabile and degrade when they are heated. However, if samples are lyophilized, the analytes are neither evaporated nor degraded and the drying time is shorter. After drying, homogenization by grinding and sieving follows [72].

Solvent selection is probably the most important step in the development of an extraction method. The analyte should have a high solubility in the extraction solvent so as to ensure efficient desorption of analytes from solid ones. Many pharmaceutical compounds, such as antibiotics, are relatively hydrophobic and have relatively low water solubility, making it necessary to use organic solvents for extraction. However, even when pharmaceuticals have high water solubility, they also have high K_d coefficients that could complicate the desorption of analytes. Solvent modifiers (acids, bases, etc.) are sometimes added to extraction solvents to increase the solubility of target analytes in the extraction solvents and to improve extraction efficiencies. Some compounds such as TCs, as mentioned in Section 2, are known to form complexes with di- and trivalent cations in the clay minerals or with hydroxyl groups at the surface of solid particles. Accordingly, complexing agents such as ethylenediaminetetraacetic acid (EDTA) are often added to the extraction buffer to improve the extraction recovery [73].

Various methods have been applied to the extraction of pharmaceuticals from the solid matrices. These include initial slurrying of samples into an aqueous matrix followed by liquid–liquid extraction using various organic solvents and more advanced extraction techniques (Figure 1) such as

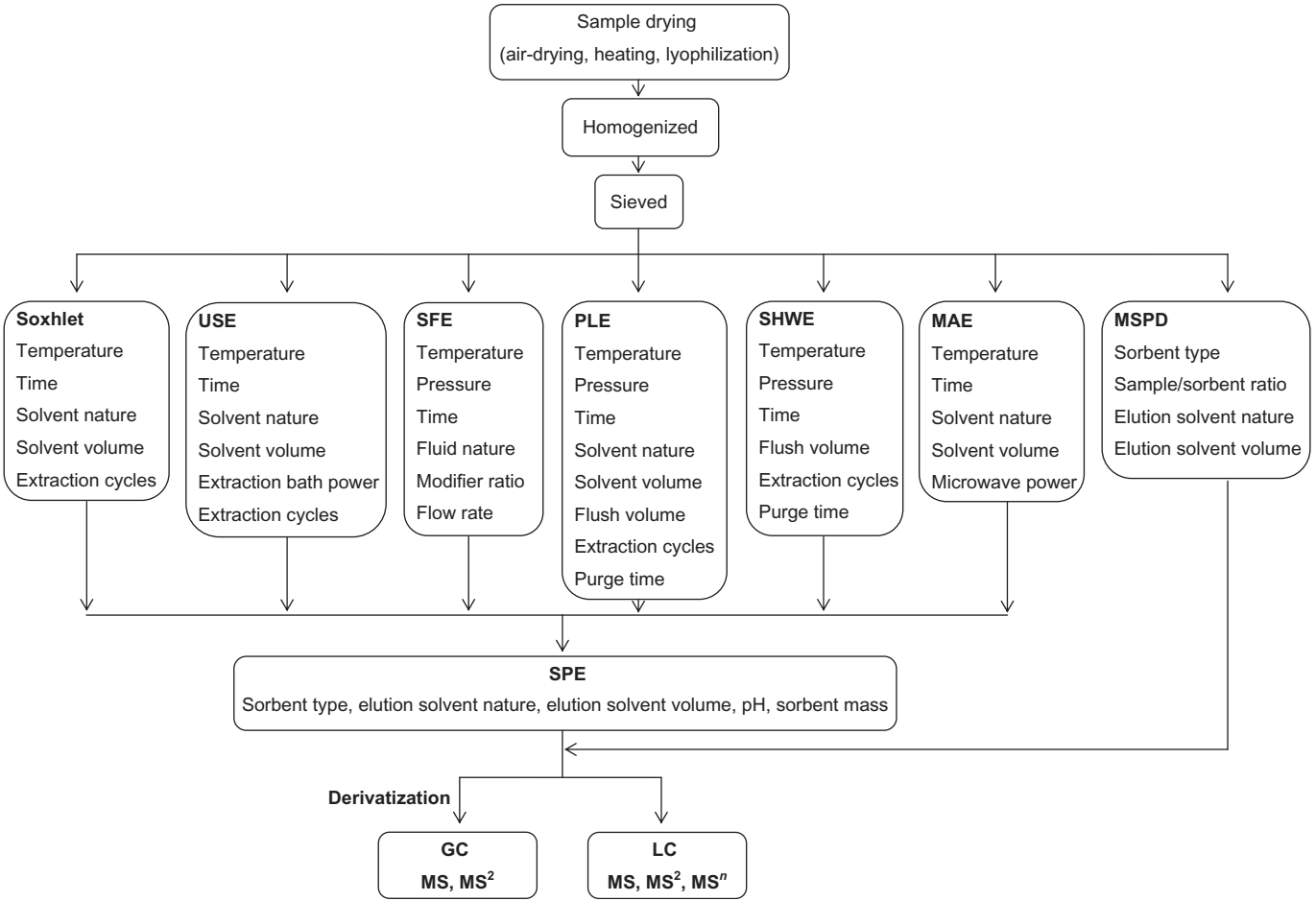

FIGURE 1 Steps in the analytic procedure for determining pharmaceuticals in solid samples. *Adapted from Ref. [72].*

pressurized liquid extraction (PLE), superheated water extraction (SHWE), ultrasonic solvent extraction (USE), supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), and matrix solid-phase dispersion (MSPD), which have been employed in order to improve the extraction efficiency. Sample extracts obtained from solid matrices usually contain interfering coextracts, which dictate an additional cleanup before final analysis [74]. Solid-phase extraction (SPE) has been preferred in most cases for the fact that it is fast, requires low volume of organic solvent, presents low contamination risk, and can be used online [15].

4.1 Soxhlet and Soxtec

Solvent extraction is one of the earliest solid sample preparation methods with the widest application, which was followed by the development of modern extraction methods [71,75]. The method removes and separates compounds of interest not only from insoluble high-molecular-weight fractions but also from other compounds that could interfere with subsequent steps of the analytic process as well [75]. Despite the multitude of modern extraction methods, Soxhlet is still the standard method for the extraction of semivolatile and nonvolatile organics from solid samples [71,76].

The Soxhlet extraction method has its advantages, one of them being that the sample is repeatedly brought into contact with fresh portions of extractant, which facilitates the displacement of the transfer equilibrium [75]. In this way, the sample is extracted with cooled, condensed solvents, but the extraction procedure is slow and can take between 6 and 48 h, which is one of the major drawbacks of Soxhlet extraction in comparison with other methods. The relatively large volume of the extract is another issue, which is why a solvent evaporation step is usually necessary for the concentration of analytes prior to extract cleanup and analysis [71]. The Soxhlet method is limited by the extractant, the disposal of which represents a source of environmental concerns [75].

Although Soxhlet is time-consuming and labor-intensive and requires the use of large volumes of organic solvents, it has thus far been applied in organic compound extraction from solid matrices due to its high extraction efficiency. Despite the disadvantages mentioned in the preceding text, the method has seen numerous applications in the analysis of organic compounds in sewage sludge (polycyclic aromatic hydrocarbons (PAHs), polybrominated diphenyl ethers (PBDEs), polychlorinated biphenyls (PCBs), nonylphenol ethoxylates (NPEOs), etc.). Terzić and Ahel [77] applied the Soxhlet extraction method to the air-dried sediment sample to extract nine pharmaceuticals using the Soxhlet apparatus and dichloromethane (200 mL) and methanol (200 mL) as solvents in two separate cycles.

The idea of the Soxhlet extraction method automation was initially based on the necessary savings in time and extractant, which are substantial. The automated Soxhlet extraction apparatus (Soxtec commercially) combines

reflux boiling and the Soxhlet extraction method (whereby both are assisted by electric heating) to perform two extraction steps (boiling and rinsing), followed by extractant recovery. The move from one step to the next is achieved by switching a lever [75]. In 1994, Soxtec was approved by the Environmental Protection Agency as a standard method [78]. Extraction using Soxtec is faster than the traditional Soxhlet extraction method owing to the contact between the solvent and the sample, which is more vigorous, and to the more rapid mass transfer in a high-temperature boiling solvent [71,78].

4.2 Ultrasonic Solvent Extraction

As an alternative to Soxhlet extraction, ultrasonic energy has been widely applied for the leaching of organic and inorganic compounds from solid samples [78]. In this extraction method, ultrasonic vibration ensures a close contact between the sample and the solvent. USE is relatively quick, but the extraction efficiency is not so high as efficiencies reached with other methods. In addition, it has been noticed that ultrasonic irradiation may lead to decomposition of some compounds with organic phosphor. Since it is a quick method, it is important to follow the specific operating conditions strictly. In terms of samples with an anticipated lower concentration of target analytes, the extraction procedure needs to be conducted two or more times, each time with the fresh solvents. The extracts from the different extractions are then combined. For high concentrations of analytes in samples (over 20 ppm), a single extraction may be suitable. Following extraction, the extract is filtered or centrifuged, with a certain form of cleanup generally required prior to the analysis [71]. The USE method is more favorable owing to the widely available necessary equipment and the extraction that can be carried out by using a reasonably small volume of the solvent (typically 0.1–2 g sample treated with 5–25 mL of solvent) within the extraction time of 10–30 min [78,79], which is extremely reduced in comparison with the classical Soxhlet extraction. A wide range of analytes have been examined using the USE, including the application to pharmaceutical compounds found in the literature [4,26–28,30–32,34,35,37,38]. Even though the solvents applied during USE are similar to those used in the Soxhlet method, the addition of complexing agents to the extraction solvent may be necessary for compounds such as TCs, which form strong complexes with multivalent metal ions [79]. For example, in the studies by Kay et al. [21], the extraction of OTC from soil was carried out by USE using a solvent mixture composed of EDTA, citric acid, sodium phosphate, and methanol. However, the extraction recovery for the applied OTC with this method was only 38.1%. Another complexing agent applied aside from EDTA was the McIlvaine buffer. In combination with methanol and EDTA at pH 7, it was selected as the extractant solution for OTC, TYL, and sulfachloropyridazine (SCP) in the soil. In the paper, Blackwell et al. [38] combined ultrasonic agitation and vortex mixing, allowing for recoveries in the range of 68–85% for SCP, 21–75% for OTC, and 47–105%

for TYL. The recoveries for all three compounds were lower in the soils with higher clay and organic carbon values and especially with OTC and TYL, which have relatively high sorption coefficients in soils. In most of the references, the temperature of the ultrasound bath is not controlled. However, Turiel et al. [32] applied 45 °C as the temperature of the ultrasonic bath for the extraction of five FQs from soil samples. In another paper [33], the same authors optimized different temperatures (ranging from room temperature to 60 °C) and different extraction times (from 10 to 60 min), extraction volumes (from 4 to 12 mL), and concentration of $Mg(NO_3)_2$ in the extraction solution (from 20% to 50%, w/v). Based on the results, they applied the room temperature of ultrasonic bath for the extraction of four quinolones and five FQs from soil samples, also.

4.3 Supercritical Fluid Extraction

SFE was introduced as an alternative extraction method with the advantages of reduced solvent consumption and extraction time compared with the classical extraction methods [78]. Supercritical fluids are defined as fluids at a certain temperature and pressure, which are above their critical value. The single state of the fluid that exists within the supercritical area possesses both gas- and liquid-like properties. Due to this, they are unique solvents for the fact that their solvent effectiveness can be controlled by small changes in pressure and by temperature. Several gases or liquids, such as CO_2, N_2O, $CHClF_2$, ethane, propane, ethylene, and benzene, may be applied as solvent in SFE. CO_2 is the main supercritical solvent (critical conditions = 30.9 °C and 73.8 bar) widely used due to the fact that it is available in high purity, inert, and cheap, has low surface tension and viscosity with high diffusivity, and is environment friendly and generally recognized as safe. Furthermore, CO_2 is gaseous at room temperature and pressure that makes the analyte recovery very simple, especially for the reason that the ability of SFE using CO_2 could be operated at low temperatures using a nonoxidant medium. This property of CO_2 as a supercritical fluid allows the extraction of thermally labile or easily oxidized compounds. In addition, in the supercritical state, CO_2 has a polarity comparable to liquid pentane; therefore, this gas at supercritical condition is suitable for lipophilic compounds. However, the major drawback of the gas lies in its lack of polarity, yielding lesser results of the extraction of polar compounds [80]. Also, when the solutes bind strongly to the matrix, the solvent strength of CO_2 is often inadequate to break the solute–matrix bond. Supercritical solvents such as N_2O and $CHClF_2$ are more efficient in extracting polar compounds, but their routine use is uncommon due to environmental concerns. The extraction efficiency of polar compounds using CO_2 can be improved by adding small quantities (1–10%) of polar organic solvents, such as methanol, referred to as modifiers [71]. Modifiers can also reduce the analyte–matrix interactions, improving their extraction efficiency. Aside from cosolvents, surfactant may also be added to supercritical CO_2, which in turn can boost its extraction

efficiency, especially for several hazardous organic compounds [80]. SFE is quick (10–60 min) and uses minimum amounts of solvents (5–10 mL) per sample. In addition to this, the SFE extract does not require additional filtration since the extraction cell does have frits [71].

Park et al. [54] developed a multiresidue analytic method for the determination of avermectins in soil samples using SFE. Extractions were performed at 80 °C and at a pressure of 300 kg/cm^2 for 40 min of extraction time and with 30% of modifier ratio. The obtained recoveries ranged from 82.5% to 96.2% with relative standard deviation values between 2.1% and 7.9%, thereby showing that SFE is a very reproducible method. In general, the earlier-mentioned method proved to be an efficient sample preparation method for the determination of organic compounds in solid samples [81], such as PAHs and PCBs. With regard to the previously mentioned method, the application of the method for the determination of pharmaceuticals in the environment will hopefully be more common in the future.

4.4 Pressurized Liquid Extraction

PLE is also known as pressurized fluid extraction, enhanced solvent extraction, high-pressure solvent extraction, or accelerated solvent extraction [72]. PLE has become a well-established method and has proven its advantages in the determination of pharmaceuticals in solid samples due to high extraction efficiency within a short period, low solvent consumption, and the possibility of automation. It uses conventional solvents at an elevated temperature (100–180 °C) and pressure (1500–2000 psi), which are below the critical point of the solvent. Sample amounts typically range between 0.5 and 5 g and are often mixed with an inert material to increase the exposure surface area of the sample. For this purpose, the commonly used materials include sand, aluminum oxide, diatomaceous earth, or Hydromatrix as commercially available material [79].

SFE is matrix-dependent and often requires the addition of organic modifiers. PLE was developed in order to overcome these limitations. It was expected that conventional solvents would be less efficient than supercritical fluids, which have higher diffusion coefficient and lower viscosity. In many cases, extraction was faster and more complete with organic solvents at an elevated temperature and pressure than with SFE [71]. PLE has been applied to a wide range of target analytes and to PhACs since both polar and nonpolar extraction solvents or solvent mixtures may be used. Many applications of PLE and a wide variety of solvents can therefore also be found in the literature [78]. In many cases, solvent composition was proven to have a considerable impact on the extraction efficiency of pharmaceuticals present in solid environmental samples, which is why the choice of the extraction solvent is one of the most critical parameters [44,45]. Various types of polar solvents such as acetone, methanol, water, and buffer solution mixtures are commonly applied. Many of these mixtures combine water with other solvents, such as in

the case of acetonitrile/water mixture (7:3, v/v) [43], methanol/water (1:1, v/v) [40,45,49], or methanol/water (1:2, v/v) [48]. When water is used as an extraction solvent or as part of the extraction mixture, pH is also controlled in the analysis of analytes with acid–base properties, as it was the case with the extraction of 66 PPCPs from sewage sludge, which included sulfonamides, TCs, FQs, macrolides, and other antibiotics and pharmaceuticals in general [39]. Strong acids (e.g., hydrochloric or nitric acid) are not to be used for the pH adjustment seeing as how they oxidize the steel components of the extraction cell [78]. Other solvent mixtures not containing water, such as acetone/methanol (1:1, v/v) [46] or acetone/hexane/acetic acid (50:50:2, v/v/v) [52], may also be found in the literature.

Aside from the choice of the extraction solvent, many parameters affect the PLE extraction efficiency. Temperature is a major parameter. Elevated temperatures in PLE lower the surface tension and the viscosity of the extraction solvent, thereby allowing for a better penetration into the interstitial spaces of the sample matrix. The increase in the temperature in turn significantly decreases the dielectric constant of the water, so that organic solvents may be used in smaller amounts or even omitted. However, an overly high temperature may cause compound degradation, coextraction of unwanted soil–matrix components, or decrease in method selectivity due to a more efficient extraction of interfering matrix components [21,48]. In case of the extraction temperature, the range found in the literature is between room temperatures of 40 and 200 °C. One of the lowest temperatures mentioned (40 °C) was used for the extraction of seven avermectins from sediments and soil samples [45], whereas the highest extraction temperature was used for the extraction of five sulfonamides from soils [47]. The most commonly used temperature for the extraction of pharmaceuticals from environmental samples was 100 °C [39,40,43,48,82]. Extractions of the veterinary pharmaceuticals from soil samples were performed at room temperature due to the fact that the TCs are converted to their epi- or anhydro form when heated [51]. The static extraction period is most commonly 5 min [39,40,42,45,47–49,52]. As regards the extraction cycles or the number of times fresh solvents get into the cell and are in contact with the sample [72], the studied range is between one and five cycles, although two [22,40,41,52] or three [39,43–45,48,51] cycles are most frequently used. However, the increase in the number of extraction cycles in turn increases the dilution of analytes, which is not advisable.

In the literature, PLE is also described as a good extraction tool for pharmaceuticals in soil [40,41,51,83] and/or for other substances in environmental matrices [84,85].

4.5 Superheated Water Extraction

SHWE, also called hot water extraction, pressurized (hot) water extraction, high-temperature water extraction, subcritical water extraction, or hot liquid

water extraction, is an emerging method based on the use of water as an extraction solvent at temperatures between 100 and 374 °C (critical point of water, 374 °C and 22 MPa), at a high enough pressure to keep it in the liquid state [86]. SHWE is similar to PLE but uses water as an extraction solvent. Water is nontoxic and inexpensive and could become the solvent of choice in extraction procedures [87]. From a practical point of view, the main advantage of SHWE over PLE is that—since pressure has little effect—only one variable, that is, the temperature, needs to be optimized. This helps simplify the optimization of procedures. On the other hand, water is much too polar to be used for the extraction of non- and moderately polar organic compounds at room temperature. However, owing to polarity, water can be easily modified by changing the temperature [88]. The method looks to have a wider range of applications than PLE or SFE with CO_2, where the available polarity range is narrower and polar compounds cannot easily be included [86]. Water is thus able to extract low-polar compounds at higher temperatures and polar compounds at suitably lower temperatures. The equipment required is relatively simple and by passes the need for high pressures required in SFE. Further advantages are reflected in its linkage to other chromatographic systems and in the fact that, unlike CO_2, there are no issues regarding cooling and condensation [89].

One disadvantage of SHWE is that the extract is a relatively dilute aqueous solution, which has raised concerns about the solubility of analytes and the potential for precipitation and sample loss by readsorption onto the original matrix. However, owing to the fact that the extract solution is a clean matrix, sample handling and concentration is much easier than from the original sample material [89]. The SHWE extract is easily cleaned up and concentrated in comparison with the PLE extract, which always contains a considerable amount of organic solvent [55].

Even though there are a few examples of SHWE of organic compounds in the literature, so far, only one report of the applications of SHWE for pharmaceutical analysis has been found [55]. Saleh et al. used SHWE for the analysis of nonsteroidal anti-inflammatory drugs (NSAIDs), ketoprofen, naproxen, diclofenac, and IBF [55]; temperature, number of cycles, flush volume, and pH of water as the extraction solvent were optimized until extraction pressure was fixed. Three extraction temperatures were explored: 80, 100, and 120 °C. The highest extraction temperatures examined (100 and 120 °C) yielded the highest extraction efficiencies; ketoprofen and naproxen were extracted fully at 100 °C, whereas maximum amounts of diclofenac and IBF were extracted at 120 °C. However, 100 °C was selected as the optimal extraction temperature since the extraction recoveries were slightly higher at 120 °C. Water at three different pH conditions (acidic, neutral, and basic) was studied as well. Ketoprofen and naproxen were quantitatively extracted at both neutral and basic conditions, while basic pH was necessary for the exhaustive extraction of diclofenac and IBF. This may be explained by the acidic characteristics

of the target analytes, which are more soluble in water at basic pH. As regards the number of cycles, the best recoveries were obtained in extractions with five cycles, whereas the maximum recoveries were achieved at 60% flush volume. Flush volume (flush %) defines the amount of solvent necessary to flush through the cell following the static heated step expressed as a percentage of the cell volume.

Even though SHWE is very similar to PLE, the latter offers more possibilities of overcoming the issues that may occur, for example, when using different solvents. It appears to be the key to further development of PLE by finding new solvents [88].

Nonetheless, SHWE does suffer from two disadvantages: a low extraction efficiency and impassability for thermally instable composition. Both of the issues may be solved simply by modifying the water with organic solvents or surfactants, which reduces the extraction temperature and improve extraction efficiencies. In conclusion, SHWE requires further research before it can become more widely applicable [88].

4.6 Microwave-Assisted Extraction

MAE uses microwave energy to heat the sample–solvent mixture [78]. Microwave energy is a nonionizing radiation that causes molecular motion by migration of ions and rotation of dipoles. The effect of microwave energy is strongly dependent on the nature of the solvent and the matrix [87]. Solvents used in the Soxhlet extraction cannot readily be applied to microwave extraction because some of them do not absorb microwaves [71], although the use of solvent mixtures with and without dipoles provides a variety of potential solvent mixtures [78]. MAE—although an earlier method—is similar to PLE, but with short extraction times and low solvent consumption, which help reduce the overall energy input and costs [8,87]. The advantage of PLE-based methods (including SHWE) over MAE lies in the fact that no additional filtration step is required, which is an additional benefit when considering automation and/or online coupling of the extraction and separation–detection parts of the system [86]. In comparison with USE, MAE is usually more robust, but USE is sometimes faster and more simple [90]. The efficiency of MAE may be affected by factors such as the selected solvent, temperature, extraction time, matrix effects, and water contents [71].

Water as an extraction solvent has been successfully applied in the extraction of acidic drugs from sewage sludge [56] and sediment [57]. In both of the earlier-mentioned cases, the obtained recoveries were in the range of 80–105%, depending on the efficiency of the cleanup procedure.

However, the application of water as the extraction liquid has its disadvantages. The fats and oils along with the detergents also present in sewage sludge result in a hard disrupting colloidal solution [56]. A novel use of the MAE is seen as combined with micellar media as extractants (MAME) (Figure 2), which has

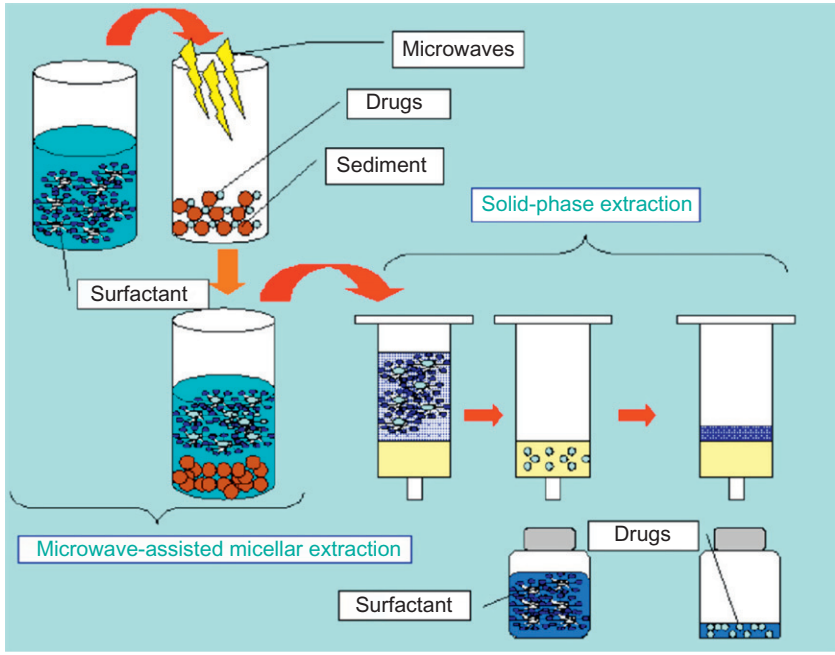

FIGURE 2 MAME–SPE procedure schematic. *Reproduced with permission from Ref. [58].*

previously been used to extract various compound types from environmental samples [58]. This chapter describes the performance of MAME methodology using a nonionic surfactant, polyoxyethylene 10 lauryl ether, as the extractant for the preconcentration of eight pharmaceuticals from several soil samples. The proposed method is faster and the pharmaceuticals can be extracted more selectively and more quickly. The obtained recoveries were in the range of over 80% for most of the target compounds, which is similar to or better than with conventional extraction processes such as the Soxhlet extraction.

Water as a polar substance may be heated using microwave irradiation and it can often improve analyte recovery, but so far, no reports on this have been found for pharmaceuticals. There are a few papers on other organic compounds (PAH, pesticides, etc.) as well, but in some cases, no improvement of extraction efficiency was observed when samples were humidified [78].

According to the literature, the extraction times used in MAE for pharmaceuticals are within 6 min [8,58] and 30 min [56].

4.7 Matrix Solid-Phase Dispersion

MSPD is a method that allows simultaneous extraction and the cleanup of analytes from solid samples, owing to which it can also be used as an alternative technique to classical extraction methods. The major advantages of

MSPD compared to other extraction methods such as PLE or Soxhlet extraction are reflected in its simple usage, low cost, and, in some cases, reduced extraction time. The method allows for the organic contaminants to be extracted more selectively and more quickly with similar or better recoveries than with conventional extraction processes [59].

MSPD has unique features as a sample extraction method. The use of mild extraction conditions (room temperature and atmospheric pressure) together with a suitable combination of dispersant sorbent and elution solvent normally provides acceptable recoveries [78]. The performance of MSPD is mainly affected by the column packing technique and the elution procedure. Particularly, analyzed samples (solid or semisolid) are blended with a suitable adsorbent that is commonly a silica-based material, sand, Florisil, or alumina [88] to form a homogenous packing material. After successful packing, the sample/adsorbent column is eluted using a stepwise solvent program similar to SPE [91]. So far, it has been used mainly for the extraction of organic environmental compounds from food and biological matrices [92]; however, current findings suggest that the method has not been applied to the extraction of pharmaceuticals from soil, sediment, and sludge samples with only one exception [59].

Two of the most important parameters affecting MSPD are the type of sorbent and the solvent polarity. In this procedure, the choice of a suitable adsorbent is vital since the chosen adsorbent is used not only as an adsorption separation material but also as a blending solid support to disrupt and disperse the sample [91]. Mutavdžić Pavlović et al. [59] used C18 sorbent instead of Florisil since it allowed for cleaner extracts to be obtained although they did try to use a combination of the previously mentioned during the MSPD optimization procedure.

The properties of the elution solvent areas are equally important as the choice of the sorbent since target analytes need to be efficiently desorbed and the remaining matrix components need to be retained in the column [91,93]. In this context, the elution profile is also an important factor in the MSPD procedure seeing as how it also has two functions: the separation, wherein the profile appears as a general mobile phase, and the dissolution/extraction of target compounds [91]. Based on different physicochemical properties of examined pharmaceuticals from sediment samples, Mutavdžić Pavlović et al. [59] had to use several 5% acid solutions such as $H_2C_2O_4$, HAc, HCl, and H_3PO_4 in combination with acetonitrile as the extractant solvents. Acetonitrile (5% of $H_2C_2O_4 = 6{:}4$, v/v) was selected as the optimal extraction solvent for the examined pharmaceuticals from the sediment. The earlier-mentioned extraction solvent produced good recoveries (over 80%) and better peak shape of compounds.

4.8 Other Methods

In 2003, Anastassiades et al. [94] introduced the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for the analysis of pesticide residues in

fruits and vegetables. The QuEChERS method was designed to help produce extracts that are directly applicable to both GC and HPLC analysis. The method involves the initial extraction of a well-homogenized sample by shaking with acetonitrile in a centrifuge tube, salt-out partitioning of water with salts including $MgSO_4$, which removes a significant amount of polar matrix components, and cleanup using dispersive SPE (DSPE), in which common matrix components are retained by the sorbent and the analytes that remain in the extract [95]. The QuEChERS method has several advantages over most traditional extraction methods: high recoveries, rapidity, simplicity, reliability and robustness, low costs, low solvent consumption, practically no need for glassware, very accurate results, and no need for chlorinated solvents. Furthermore, a single person can perform the method without much training or technical skill and the method covers a very broad analyte spectrum (from very polar to basic) [60,95]. Although the QuEChERS method has mainly been used for the determination of pesticides, other compounds such as more than 40 pharmaceuticals [96] or veterinary drugs [97,98] have also been determined in several matrices (blood, milk, and animal tissue).

Bragança et al. applied the QuEChERS method for the determination of IBF and its metabolites [60] in soil samples (Figure 3). For that purpose, several parameters were examined in order to optimize the performance of the extraction method, such as the ratio of sample mass per extraction solvent volume, the extraction solvent, the QuEChERS composition, the extraction time, the extraction process, and the addition of ceramic parts with the aim of preventing agglomeration. The best approach to the QuEChERS extraction involved using 3 mL of purified water (with or without adjusted pH) and 7 mL of acidified acetonitrile (1% acetic acid) and 4 min of extraction time. To improve the obtained results, the extraction mixture, once the homogenization using vortex mixing finished, was placed in an ultrasonic bath for an additional 4 min. The obtained recoveries of the fortified samples ranged from 79.5% to 101% with 3% of relative standard deviations for all matrix–compound combinations. Current findings suggest that this is the first analytic report in which QuEChERS was applied to the simultaneous determination of IBF and its two major metabolites (hydroxy-IBF and carboxy-IBF) in soil samples.

The literature does provide a few examples where the analysis of the sludge is not carried out after a solid–liquid extraction, but the sample is diluted in water and then submitted to extraction techniques that are applied to liquid samples [78]. For example, an alternative to the extraction of organic microcontaminants in water, which is commonly used, is the hollow fiber liquid-phase microextraction (HF-LPME). In addition to this, HF-LPME, as compared to other methods, minimizes organic solvent consumption, gives an efficient cleanup and selectivity, needs short analysis time, and has a low cost. There are two different modes of HF-LPME: two-phase and three-phase HF-LPME. Sagristà et al. [61] developed a three-phase hollow fiber liquid-HF-LPME method for the direct determination of four NSAIDs in sewage

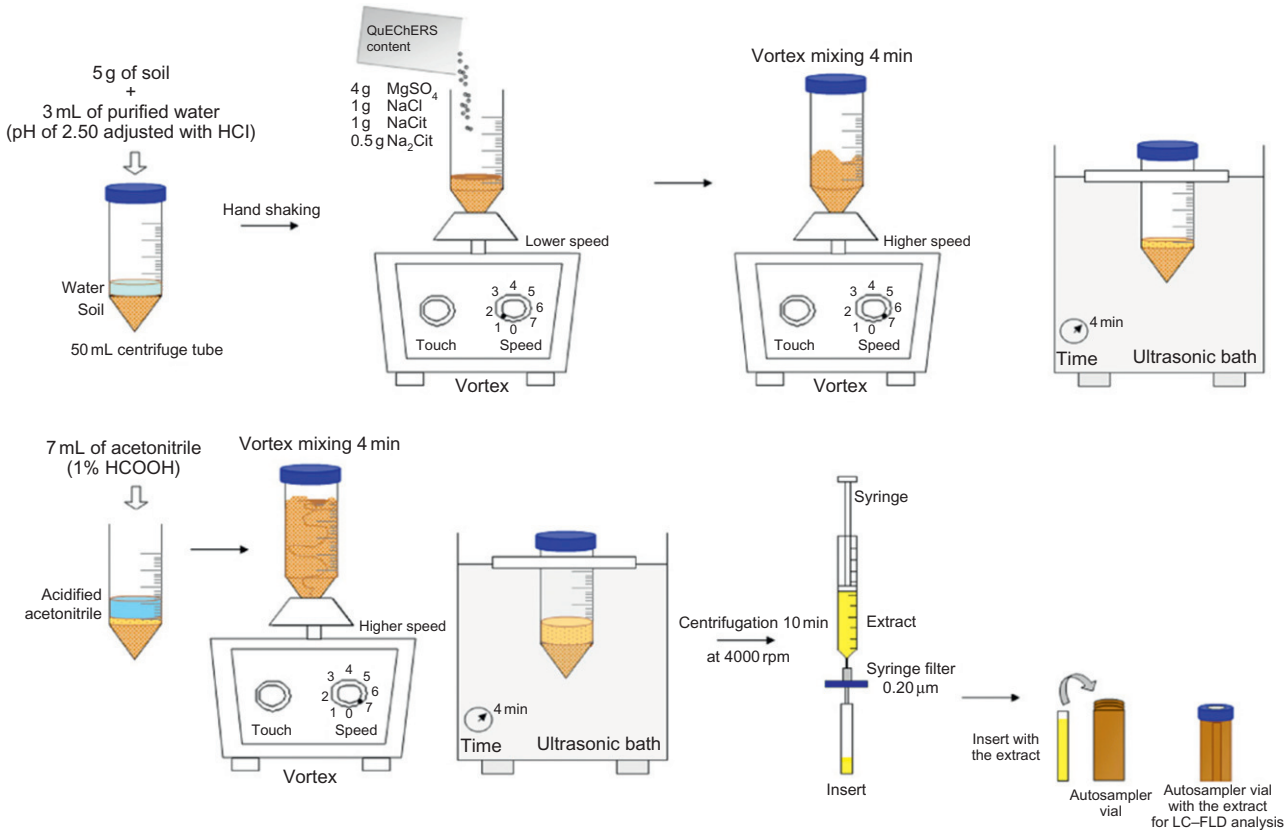

FIGURE 3 Schematic of the QuEChERS procedure for the extraction of IBF and its major metabolites from soils. *Reproduced with permission from Ref. [60].*

sludge. In this case, slurry of the sewage sludge was carried out in water and stirred overnight before extraction. The drugs were extracted from nonspiked and spiked slurry samples with different amounts of sludge into an organic phase and then back-extracted into an aqueous phase held in the lumen of the hollow fiber. The acceptor phase comprised 0.1 mol/L of ammonium carbonate at pH 9 and di-*n*-hexyl ether as the organic solvent. In sludge samples, repeatability and interday precision were tested with relative standard deviation values between 10–18% and 7–15%, respectively.

5 SAMPLE CLEANUP AND CONCENTRATION

Natural organic matters, such as humic and fulvic acids, present in environmental samples are coextracted with the analytes and often complicate analytic detection [73]. Most of the mentioned extraction methods used for the preconcentration of pharmaceuticals from solid samples are not selective, which is why cleanup procedures are a necessary step in analytic methodology. When performed, the cleanup of extracts is usually carried out using SPE [15].

5.1 Solid-Phase Extraction

The most commonly used cleanup method in environmental analysis is the SPE, which allows large sample volumes to be concentrated and purified in one step. The main purpose of SPE is the removal of matrix components such as salts and some organic matter while concentrating analytes. SPE has replaced many conventional liquid–liquid extraction methods due to the advantages gained by minimizing solvent consumption, the increased selectivity by way of choosing both the stationary phase and the elution solvent, and the ability to automate extraction [73]. Reverse-phase SPEs (HLB, C8, C18, etc.), normal-phase SPEs (alumina, diol, Florisil, silica, etc.), or ion-exchange mode SPEs (strong anion-exchange (SAX), mixed-mode cation-exchange (MCX), etc.) have been applied as a disk, column, or cartridge format. Among the previously mentioned, it is the reverse-phase SPE that has been widely applied in solid samples [78]. In general, the copolymer-based sorbent Oasis HLB (Waters, MA, USA) has been the preferred SPE cartridge and has been applied the most [8,22,34,39,40,42,43,45,48–50,52,56,58] owing to their more rugged extraction efficiency, improved recovery for both polar and nonpolar compounds, and greater capacity than reverse-phase silica-based sorbents [99]. Aside from the polymeric SPE cartridge, the addition of a SAX cartridge has also been used in tandem with the HLB cartridge [5,23,29,31,36,38,44,51]. The combination of the SAX and HLB cartridges was chosen since it provided the most satisfactory recoveries from solid samples. For example, the diverse physicochemical properties of the sulfonamides complicate the SPE purification step and require careful selection of the SPE column and conditions, usually by way of adjusting pH value. The combination of the

mentioned cartridges allows the sulfonamides to pass through the SAX column and the unwanted organic materials to be retained. The HLB column, located below the SAX, retained the desired sulfonamides, which could then be eluted with an appropriate solvent [23]. The use of an SAX cartridge facilitates the removal of anionic humic substances that are present in soil extracts, leading to much cleaner samples for analysis. Further sorbents include LiChrolut EN, LiChrolut C18, Strata C18, Oasis MCX, and Strata-X. Strata-X (Phenomenex, CA, USA) serves as an alternative to Oasis HLB, which was also based on an organic copolymer with both hydrophilic and lipophilic functional groups. Some authors observed more reproducible recoveries with the aforementioned and an increased tolerance to a higher ionic strength and the organic solvent in the sample, relative to using Oasis HLB [21].

Aside from the earlier-mentioned commercial and commonly applied sorbents, one promising technique that has been recently applied in order to address the issues occurring during preparation of environmental samples is molecular imprinting. This technique involves using the analyte as a template molecule and creating specific interaction sites within a polymeric solid. The selectivity of the sites depends on the interactions between the template and the monomer used to develop the imprint [73]. However, over the last few years, they have seen an increase in the application as selective sorbents in molecularly imprinted SPEs. SPEs with a molecularly imprinted polymer (MIP) as sorbent have some advantages such as affinity, selectivity, stability, simplicity of their preparation, and the possibility of adaptation to different applications. MIPs have so far been applied mainly for the extraction of pharmaceuticals from biological samples [100–103] and environmental samples [32,35].

Even though the main interest in improving the sorbents for SPE lies in the field of polymers, other materials, such as multiwalled carbon nanotubes (MWCNTs), have also been examined as SPE materials for polar compounds. Among the varied application fields, Cai et al. [104] used MWCNTs as a sorbent in SPE for extracting a group of endocrine disruptors from aqueous samples. In comparative studies, MWCNTs were more effective than or as effective as C18 or styrene–divinylbenzene-based sorbents [105]. Although the mentioned studies claimed that MWCNTs were promising materials in SPE fields, further work should be done with a wider range of polar compounds to confirm that MWCNTs are suitable sorbents for extracting polar pollutants in SPE [106]. However, the complexity of their synthesis and the need for conducting stability and method performance studies have slowed down the development of these applications. More reports are expected in the near future and especially for those dealing with the analysis of real samples, since they represent the most important separation applications at the moment [107].

5.2 Selective PLE

The process known as selective PLE (SPLE) involves PLE combined with *in situ* (in-cell) cleanup of the extract by packing the sample dispersed in an

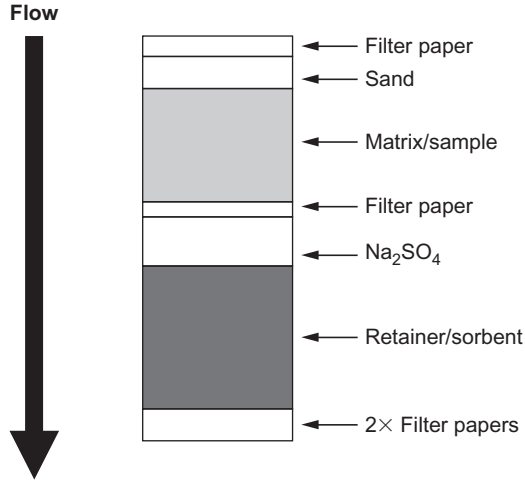

FIGURE 4 Packing of the extraction cell. *Reproduced with permission from Ref. [108].*

adsorbent, such as modified silica, Florisil, or alumina (Figure 4). The use of a SPLE technique significantly reduces the need for exhaustive post-cleanup procedures and allows the automation of cleanup steps. In recent years, SPLE has been developed for the analysis of organic pollutants such as PAHs and PCBs and many other compounds present in environmental [78] and food samples [109] including the estrogenic compounds [108] in soil.

However, current findings suggest that SPLE has not yet been applied in the determination of pharmaceuticals in solid environmental samples; however, a wider application may be expected in the near future.

5.3 Other Cleanup Techniques

Saleh et al. [55] recently used HF-LPME for both the preconcentration and cleanup of some NSAIDs following SHWE from sewage sludge. Ammonium carbonate buffer (0.1 M) was used as the acceptor phase since it is a volatile buffer suitable for electrospray ionization (ESI)-MS and provides a suitable pH (9.5) for analytes to become deprotonated and trapped in the acceptor phase. The hollow fibers were immersed into organic solvent (di-*n*-hexyl ether) for 15 s and in ultrapure water for 10 s to wash away the extra organic solvent from the surface of the fiber. For the study of the effect of the donor-phase pH on the extraction efficiencies of target drugs, different pH values in the range of 1–6 were tested. The extractions were performed at 600 rpm for 90 min. The results showed that by decreasing pH from 6 to 4, the extraction efficiencies of NSAIDs increased, remained constant up to pH 2, and increased slightly at pH lower than 2. Because of that, pH 1.5 was chosen as the optimum value for the rest of the experiments. The cleanup method decreased the matrix effect and produced relatively high enrichment factors

in the extract of the sewage sludge. Matrix effects and enrichment factors in the range of 9–15% and 947–1213, respectively, were obtained. The HF-LPME extraction time was 120 min. This method is a very good alternative since considerable matrix effects for NSAIDs have been reported after cleanup using SPE [48].

DSPE is also one of the relatively new purification and extraction procedures. DSPE and MSPD are similar in some respects but differ in the addition of the sorbent: with DSPE, it is added to an aliquot of the extract rather than to the original sample as it is the case with MSPD. The high cost of the sorbent limits the sample size that can be used in MSPD. This may lead to concerns regarding sample representation and homogeneity; however, DSPE relies on the extraction process to provide a homogenous aliquot from an original sample of any size with only a small amount of sorbent used [110]. Dobor et al. [56] used a modified DSPE (namely, DME) for the precleaning of the decantated extract of NSAIDs obtained by MAE. For that purpose, 0.5 g of neutral alumina as the sorbent and 0.25 g $Al_2(SO_4)_3$ as the electrolyte were added to the water extract and shaken for 10 min. Following that, the mixture was centrifuged for 10 min to separate the liquid and the colloidal fraction of the sludge particles adsorbed on the surface of alumina. The recleaned extract was applied to the Oasis HLB cartridge. The results obtained using the DME + SPE procedure in comparison with simple SPE have shown that the preparation of a new sample is the better option. The developed cleanup method considerably decreased the matrix effect in the sewage sludge extract, which had lower LOQ values of DME + SPE (15–22 ng/g) compared with LOQ of SPE (100–540 ng/g). Nonetheless, the study has shown that the dispersive matrix extraction method may be recommended for sample preparation in case of a high matrix effect, if the extractant is a water or water–water miscible solvent, if the target analytes have polar character, and if the sample is disposed of to produce colloidal solution.

6 QUANTITATIVE ANALYTIC DETERMINATION

PhACs are present in environmental solid samples where their concentration ranges from low milligrams/kilograms to micrograms/kilograms (Table 1). Therefore, advanced separation and detection techniques are required for sensitive and accurate detection of these low concentrations. Most analytic methods reported for determination of PhACs in environmental samples are developed with the aim to analyze aqueous matrices (surface water and wastewater). The same methods are used for PhACs detection in solid environmental samples. The difference refers to the tedious sample preparation step of those matrices (sediment, soil, and sewage sludge). Solid samples represent analytically very challenging matrices because of their high heterogeneity. Moreover, sewage sludge contains numerous components, potential interfering compounds in analysis of target PhACs such as lipids and other naturally

occurring materials, and materials that may be added to sewage during the processing (e.g., surfactants, ferric chloride, polymeric colloids, or lime). So it is of high importance to remove them from the sample using appropriate sample preparation and cleanup procedures [111].

Modern analytic methods used for separation and detection of PhACs in solid environmental samples mainly rely on application of chromatographic techniques (GC or LC) hyphenated to MS, although diode array and fluorescent detectors were used. Since most pharmaceuticals are relatively nonvolatile and some are highly polar compounds containing ionizable functional groups (carboxylic or amino), a derivatization step is required before GC analysis. For this purpose, various derivatization agents are utilized. This way, an additional step is introduced into the analytic procedure, which could influence the accuracy of the method due to a loss of analytes, introduction of unwanted contaminants, or incomplete reaction. Furthermore, many PhACs like TCs are thermolabile [15]. Therefore, high-performance LC is used more frequently than GC. Detailed and comprehensive reviews on application of GC–MS and LC–MS methods for determination of PhACs in solid environmental matrices (soil, sediment, and sludge) have been reported [15,21,111,112].

6.1 GC Methods

Despite the fact that usage of LC–MS is dominant in most environmental analyses, GC–MS is still utilized in many environmental laboratories as a cost-effective technique suitable for routine analysis. In comparison with LC–MS, GC–MS has the advantage of being less submissive to matrix effects, particularly in complex solid environmental matrices or wastewater, which makes it still attractive for PhAC analysis in environmental samples [52,73]. Moreover, using an available standard electron impact (EI)-MS database, full-scan GC–MS can be used for identifying nontarget PhACs and their environmental transformation products [73]. The high selectivity of the method could be provided by applying tandem MS, while high sensitivity could be obtained by a large volume injection [73]. DB5- or HP5-MS columns (30 m × 0.25 mm i.d., 0.25 μm film thickness) are usually used for the GC separation of PhACs. Helium is used as a carrier gas at a flow rate of 1–1.2 mL/min. Samples (1–2 μL) are injected in the GC using split/splitless mode. The column temperature is usually programmed to vary within the range of 50–300 °C. EI ionization at temperatures of 200–250 °C and with the ionization energy of 70 eV is a standard ionization technique for GC–MS analysis of PhACs [8,37,52]. The identification is conducted in the full-scan mode, while the quantification is performed by acquiring compound-specific molecular ions and/or fragment ions in a selected ion monitoring mode [73]. Aside from certain neutral drugs, most pharmaceuticals are polar, nonvolatile, and thermally labile compounds that are unsuitable for GC separation. The derivatization of hydroxyl and carboxyl groups prior

to GC–MS or GC–MS/MS analysis of pharmaceuticals has thus become a necessary step. This is a definite advantage of LC-based MS/MS analysis, seeing as how no derivatization is required to achieve a good separation. Derivatization is usually performed by using organic reactions (e.g., methylation, silylation, and acetylation) once analytes have been extracted and cleaned from the sample matrix [74].

6.2 LC Methods

Nowadays, determination of PhACs in solid environmental samples is dominated by the coupling of the LC separation technique to a sensitive and specific detection system. Although diode-array detection [31–33,38,58] and fluorescence detection (FLD) [25,31,38,60] are used, the majority of determinations in multiresidue analysis refer to LC in combination with mass spectrometers.

FLD is useful for FQ detection; however, when multiresidue analysis has to be performed, an additional derivatization step is required due to the lack of fluorophores in the PhACs other than FQs [25,38]. Nevertheless, FLD without derivatization has been applied to detect IBF and their metabolites, hydroxy-IBF and carboxy-IBF, in soil samples [60].

Reverse-phase (C18) analytic column is most commonly used for the separation of PhACs. Instead of these classical nonselective sorbents, application of MIPs as a stationary phase enables separation of analytes from interfering compounds and analysis without a previous cleanup step. Turiel et al. [32] have synthesized MIPs using ciprofloxacin as a template and applied them as an analytic column in FQ analysis in soil samples. However, the application of MIP analytic columns is scarce probably due to the fact that they are tailor-made materials intended to be used for a specific analyte or closely related compounds. Regarding environmental analysis, multiresidue methods are preferred in order to determine a larger number of pollutants in a single run, while highly selective stationary phases such as MIPs allow determination of PhACs belonging to the same structural group.

Recently, ultra-high-performance LC (UHPLC) has been applied to the analysis of PhACs in solid environmental samples [23,39,53,77]. UHPLC has the sensitivity two to three times greater than HPLC due to the usage of columns packed with particles <2 μm resulting in better chromatographic resolution, increased peak capacity, and reduced run time. The increased efficiency of this type of column leads to shorter analysis runs, narrower peaks, improved separations, and reduced peak overlaps [73,99], which then led to better quality of the mass spectra. Despite the advantages of UHPLC, only a few papers have reported their application in analysis of solid environmental samples (e.g., sediment [77], soil and manure [23], and sewage sludge [39]).

The composition of the mobile phase is an important factor for obtaining good chromatographic separation, reproducible retention times, satisfactory

peak shapes, and good ionization efficiencies. Typically, mixtures of acetonitrile–water or methanol–water at different pH values have been used as mobile phases for the LC separation under gradient elution. In an attempt to improve the ionization of analytes and the sensitivity of MS detection, the mobile phase is usually modified with volatile additives (e.g., formic acid, acetic acid, and ammonium acetate or formate). Nonvolatile additives such as oxalic acid should be avoided when ESI is used. The same applies to higher viscosity eluents that can produce higher back pressure [69].

ESI and atmospheric-pressure chemical ionization (APCI) are the most widely used atmospheric-pressure ionization techniques. Development of APCI expands the range of low-polarity and low-mass compounds amenable for LC–MS analysis [99]. Nevertheless, ESI, with an exception of Schlüsener et al. [41] and Löffler and Ternes [26] who have used APCI, is the ionization technique preferred by most authors since it is excellent for both polar and nonpolar compounds and for compounds with poor thermal stability [113].

Although LC–MS is used for determination of PhACs in complex matrices like solid environmental samples, it still requires efficient separation of the analytes from the interferences. Single quadrupole MS methods produce low fragmentation, and pseudomolecular ions $[M+H]^+$ or $[M-H]^-$ are obtained, which are collected in positive and/or negative ion modes [99,112]. Determination of NSAIDs [55,61], sulfonamides, macrolides, FQs, and TCs [49,50] in sewage sludge using single quadrupole MS has been reported.

In order to overcome the drawback of single quadrupole MS methods of possible cofragmentation of matrix components other than target analytes, LC–MS^2 is preferred for analysis of complex matrices. By using LC–MS^2, it is possible to distinguish individual compounds having the same molecular mass by different fragments obtained after the induced collision with an inert gas [99], thus avoiding cofragmentation of analytes and interferences [111–113]. Therefore, using LC–MS^2, complete separation may not always be necessary. However, good separation may considerably reduce matrix effects, which may suppress or enhance the analyte signal. MS^2 offers increased sensitivity and selectivity, particularly in analysis of complex matrices, so the use of tandem MS is preferred in the analysis of solid environmental samples.

The analyzers used mostly as LC detectors include quadrupole (Q), ion trap (IT), and time of flight (TOF) alone or in different combinations. Triple quadrupole (QqQ) is the most widely used tandem mass spectrometer and can be applied for determination of parent PhACs and their known metabolites. Hybrid mass spectrometers that have been developed by combining two different principles of MS analyzers into one single instrument enable more information on the sample, whereas the analysis run time is significantly reduced. Among them, quadrupole–TOF–MS and quadrupole–linear IT (Q-LIT) are established as powerful tools for target analysis of environmental contaminants [99].

IT analyzers have an ability to perform multiple stages of fragmentation in time (MS^n) and to trap the product ions resulting in high sensitivity and full-scan mass spectra. Application of this kind of MS analyzers in environmental analysis may help to infer the degradation pathways and identification of the unknown substances [99,112]. Hybrid Q-LIT mass spectrometers combine the specificity and robustness of QqQ analyzers and the full-scan tandem MS sensitivity of IT analyzers resulting in an increase of the instrumental dynamic range. This kind of hybrid MS instruments enables true positive analysis of target compounds in complex samples with higher confidence [73,99]. Although the application of hybrid mass spectrometers in analysis of solid environmental samples is scarce, Jelić et al. [48] have reported use of a hybrid instrument consisting of a Q-LIT in the analysis of PhACs in sewage sludge samples. These instruments enable powerful scan combinations leading to rapid identification and confirmation of target analytes. Excellent sensitivity has been obtained with LOD lower than 1 ng/g for most of the compounds. LC-Q-LIT has also been applied in the investigation on the occurrence and distribution of PhACs in the surface water, suspended soil, and sediments of the Ebro River basin (Spain) [114].

TOF coupled with LC is an alternative detection method for identification of unknown residues in complex environmental samples. Due to the high-resolution capability of the method, an accurate mass can be obtained for both the precursor and product ions in full-scan spectra. This method can be used for the screening and qualitative and quantitative analysis of pharmaceuticals in complex matrices [73,99,112]. The high-power resolving technique of the TOF–MS method removes the interference signal, making it easier to identify the nontarget compounds in complex environmental samples. However, comparing it to quadrupole instruments, LC–TOF–MS has a significantly lower effective linear dynamic range, which is one of the most important drawbacks of the utilization of LC–TOF–MS in quantitative analysis [113]. Terzić and Ahel [77] have shown that UHPLCs coupled to Q-TOF–MS have a high capability for identification of nontarget contaminants in complex environmental matrices such as freshwater sediment (Figure 5).

A new approach to multiresidue analysis entails application of the high resolution and reliable mass accuracy of Orbitrap MS systems. Orbitrap represents an alternative to Q-TOF instruments for identification of PhAC transformation products or screening over a wide mass range. The first and so far the only application of Orbitrap MS for PhACs analysis in solid environmental samples has been reported by Chitescu et al. [53]. The application involves trace analysis of pharmaceuticals and fungicides in soil and plant samples.

6.3 Matrix Effect

Complete elimination of interferences is usually not possible in analysis of complex environmental matrices despite tedious sample preparation and

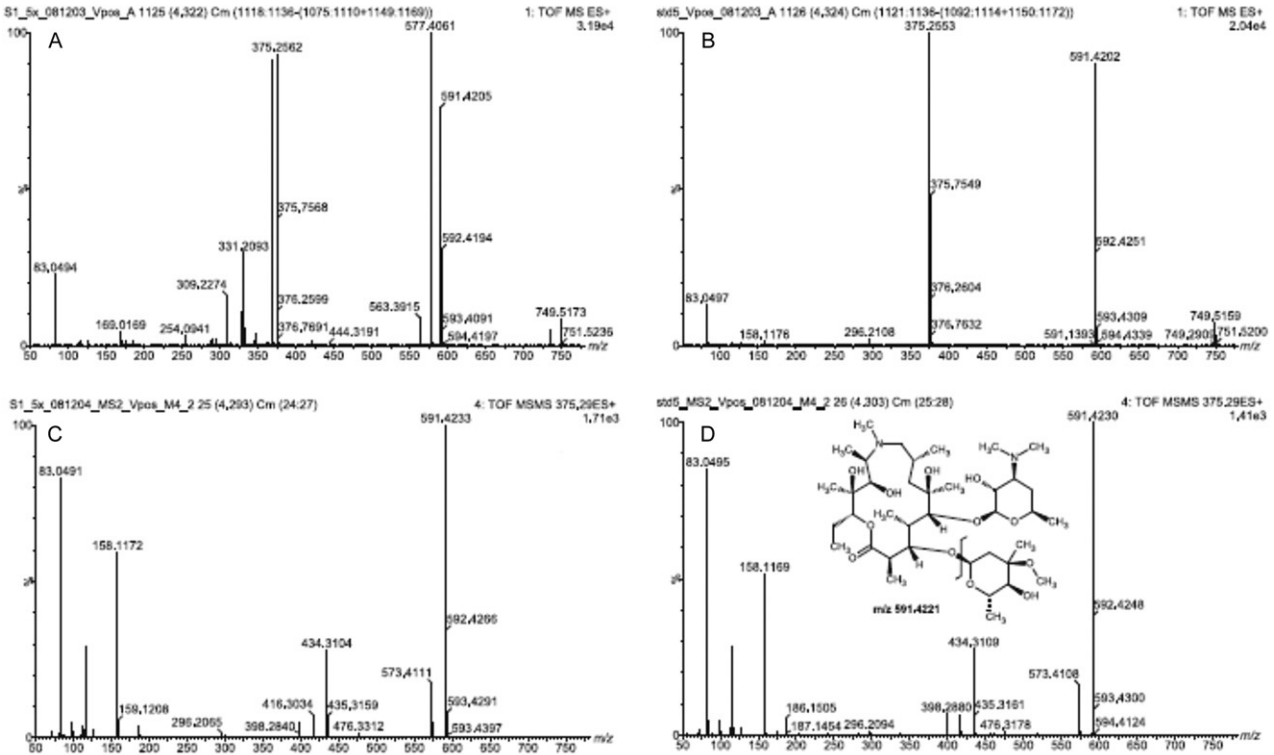

FIGURE 5 Identification of azithromycin in the sediment extract from the Gorjak Creek using UHPLC–Q-TOF–MS with electrospray ionization in positive polarity mode: (A) TOF mass spectrum of the peak at 4.3 min; (B) TOF mass spectrum of azithromycin standard; (C) product ion spectrum of precursor ion *m/z* 375 in sediment sample; (D) product ion spectrum of azithromycin standard using ion *m/z* 375 $[M+2H]^{2+}$ as a precursor. *Reproduced with permission from Ref. [77].*

cleanup steps. These coeluting undetected matrix components that have similar ions in the MS experiment have caused the matrix effect and affected the data quality [73]. The consequence of the matrix effect is primarily suppression or enhancement of the ion intensity of the target analyte. Furthermore, poor accuracy and repeatability and problems with linearity and quantification (overestimation of the analyte concentration due to signal enhancement or a false negative result due to signal suppression) may arise [57]. The matrix effect depends on the sample matrix, specific analyte, or ionization mode, and it has been observed in the analysis by both GC–MS and LC–MS [115]. EI sources used in the GC–MS analysis are much less sensitive to ion suppression and ion enhancement than ESI or APCI. The reason lies in the fact that ionization occurs in the gas phase where the pressure is low and a smaller amount of sample is injected [116]. Comparing ESI and APCI, it has been reported that APCI is less matrix-dependent than ESI. Wick et al. [115] have compared ESI and APCI in the positive and negative ionization mode, in the multiresidue analysis of biocides, UV filters and benzothiazoles in aqueous matrices, and activated sludge by LC–tandem MS. They have observed that ESI exhibited strong ion suppression for most target analytes, while APCI was generally less susceptible to ion suppression but partially leads to ion enhancement.

Since the majority of the application of LC–MS analysis of PhACs uses ESI as an ionization technique, it is essential to evaluate the matrix effect when developing analytic methods for environmental analysis [73].

Several strategies are proposed to overcome the problems resulting from the matrix effect. The most effective one is exhaustive sample preparation and cleanup and it removes interference substances, but it is time-consuming and may result in a loss of target analytes.

Saleh et al. [55] have shown that appropriate sample preparation and cleanup procedures may reduce the matrix effect. They observed that superheated water used as an extraction solvent instead of superheated organic solvents and HF-LPME as a cleanup procedure decreased the matrix effect for determination of NSADs in sewage sludge.

Another strategy to reduce the matrix effect is improvement of chromatographic separation, thus avoiding coelution with matrix components. A third approach is a serial dilution of the final extract, so less matrix components are injected into analytic system. Still, in most cases, it is not possible to completely eliminate the matrix effect. Therefore, several approaches are proposed to overcome problems associated with the matrix effect in the final determination. It could be compensated using an appropriate calibration model with standards in the matrix. It is proposed to use matrix-matched calibration standards to establish a calibration curve. However, an uncontaminated sample matrix must be available for this approach. Another approach refers to use of the standard addition method in which calibration standards are added to the sample to evaluate the calibration curve. This approach is

tedious and time-consuming, thus inappropriate for monitoring campaigns when a large number of samples must be analyzed [73,116]. An effective approach is application of an internal standard (structurally similar unlabeled compound or isotopically labeled standard) that can compensate for the matrix effect. However, poor availability, high costs of internal standards, and the fact that the matrix effect depends on the retention time and that more than one internal standard may be needed make this approach less desirable [73,117]. Despite these, application of isotopically labeled standards is a popular method for compensating the matrix effects in the determination of PhACs in environmental samples. In order to evaluate the extent of the observed matrix effect, many researchers have used the simple method first proposed by Matuszewski et al. [118].

7 CONCLUSION

The data on PhACs occurrence evidence their presence in solid environmental samples in the average concentration of micrograms/kilograms. Therefore, it is of high importance to understand their behavior in soil, sediment, and sewage sludge.

Sample preparation and cleanup procedures play a fundamental role in developing an analytic methodology for such complex environmental samples. Techniques that provide for a fast and simple preparation procedure use a small amount of solvents and samples and enable automatization are favorable. Conventional sample preparation techniques such as Soxhlet and USE are still applied for extraction of PhACs from solid environmental samples despite their disadvantages of being time-consuming, large consumption of sample and organic solvents, and, consequently, generation of large quantities of waste. During the last decade, new sample preparation techniques have been developed in an attempt to endeavor these disadvantages. These techniques include MAE, SHWE, PLE, SFE, and MSPD. Among them, PLE is the most applied technique for solid environmental samples.

Although UV detection and FLD are still used in analysis of solid environmental samples, advanced LC–MS^2 techniques that enable multiresidue analysis of a wide range of structurally different PhACs are utilized in the majority of methods. The application of more sophisticated MS instruments such as IT, TOF, and Orbitrap that may help in identification of nontarget contaminants in complex environmental matrices is still scarce.

Investigations of PhAC occurrence in solid environmental matrices are curious and less numerous in comparison with those dealing with aqueous environmental samples. Therefore, further research should be conducted to collect additional data on the occurrence of PhACs and their metabolites in solid environmental samples necessary for evaluation of their behavior in the environment, determination of degradation pathways, and identification of degradation products that will cater for realistic risk assessments.

ACKNOWLEDGMENT

This work has been supported by the Croatian Ministry of Science, Education and Sports project: 125-1253008-1350 Advanced analytic methods for pharmaceuticals determination in the environment.

REFERENCES

[1] Pollutants in urban waste water and sewage sludge, Final report, prepared by ICON IC Consultants LTD, London, UK, 2001.
[2] D.R. Lapen, E. Topp, C.D. Metcalfe, H. Li, M. Edwards, N. Gottschall, P. Bolton, W. Curnoe, M. Payne, A. Beck, Sci. Total. Environ. 399 (1997) 50–65.
[3] R.H. Lindberg, P. Wennberg, M.I. Johansson, M. Tysklind, B.A.V. Andersson, Environ. Sci. Technol. 39 (2005) 3421–3429.
[4] X. Hu, Q. Zhou, Y. Luo, Environ. Pollut. 158 (2010) 2992–2998.
[5] P. Vazquez-Roig, V. Andreu, C. Blasco, Y. Picó, Sci. Total. Environ. 440 (2012) 24–32.
[6] D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, H.T. Buxton, Environ. Sci. Technol. 36 (2002) 1202–1211.
[7] X. Hu, K. He, Q. Zhou, J. Hazard. Mater. 225–226 (2012) 91–98.
[8] A. Azzouz, E. Ballesteros, Sci. Total. Environ. 419 (2012) 208–215.
[9] D. Lambkin, S. Nortcliff, T. White, Trends Anal. Chem. 23 (2004) 704–715.
[10] W. Salomons, J. Brils (Eds.), Contaminated Sediments in European River Basins, European Sediment Research Network, 2004, pp. 13–36. www.SedNet.org, (January 2013).
[11] U. Förstner, Trends Anal. Chem. 23 (2004) 217–236.
[12] H. Qiu, J. Geogr. Geol. 3 (2011) 239–246.
[13] Organic contaminants in sewage sludge for agricultural use, Project coordination: European Commission, Joint Research Centre, Institute for Environment and Sustainability, Soil and Waste Unit, H. Langenkamp, P. Part (2001), http://ec.europa.eu/environment/waste/sludge/pdf/organics_in_sludge.pdf (January 2013).
[14] G.L. Northcott, K.C. Jones, Environ. Pollut. 108 (2000) 19–43.
[15] M.S. Díaz-Cruz, M.J. López de Alda, D. Barceló, Trends Anal. Chem. 22 (2003) 340–351.
[16] M. Sturini, A. Speltini, F. Maraschi, A. Profumo, L. Pretali, E. Fasani, A. Albini, Chemosphere 86 (2012) 130–137.
[17] K. Kummerer (Ed.), Pharmaceuticals in the Environment, Springer, Berlin, 2008.
[18] G. Jacobsen, I. Berglind, Aquaculture 70 (1988) 365–370.
[19] M. Hörsing, A. Ledin, R. Grabic, J. Fick, M. Tysklind, J. Cour Jansen, H.R. Andersen, Water Res. 45 (2011) 4470–4482.
[20] S. Thiele-Bruhn, J. Plant Nutr. Soil Sci. 166 (2003) 145–167.
[21] S. O'Connor, D.S. Aga, Trends Anal. Chem. 26 (2007) 456–465.
[22] W. Li, Y. Shi, L. Gao, J. Liu, Y. Cai, Chemosphere 89 (2012) 1307–1315.
[23] W.L. Shelver, H. Hakk, G.L. Larsen, T.M. DeSutter, F.X.M. Casey, J. Chromatogr. A 1217 (2010) 1273–1282.
[24] S.-C. Kim, K. Carlson, Anal. Bioanal. Chem. 387 (2007) 1301–1315.
[25] A. Cannavan, R. Coyne, D.G. Kennedy, P. Smith, Aquaculture 182 (2000) 229–240.
[26] D. Löffler, T.A. Ternes, J. Chromatogr. A 1021 (2003) 133–144.
[27] T.A. Ternes, M. Bonerz, N. Herrmann, D. Löffler, E. Keller, B. Bagó Lacida, A.C. Alder, J. Chromatogr. A 1067 (2005) 213–223.

[28] W. Chenxi, A.L. Spongberg, J.D. Witter, Chemosphere 73 (2008) 511–518.
[29] M.-O. Aust, F. Godlinski, G.R. Travis, X. Hao, T.A. McAllister, P. Leinweber, S. Thiele-Bruhn, Environ. Pollut. 156 (2008) 1243–1251.
[30] E. Martínez-Carballo, C. González-Barreiro, S. Scharf, O. Gans, Environ. Pollut. 148 (2007) 570–579.
[31] A. Karcı, I.A. Balcıoğlu, Sci. Total. Environ. 407 (2009) 4652–4664.
[32] E. Turiel, A. Martín-Esteban, J.L. Tadeo, J. Chromatogr. A 1172 (2007) 97–104.
[33] E. Turiel, A. Martín-Esteban, J.L. Tadeo, Anal. Chim. Acta 562 (2006) 30–35.
[34] C.-M. Tang, Q.-X. Huang, Y.-Y. Yu, X.-Z. Peng, Chin. J. Anal. Chem. 37 (2009) 1119–1124.
[35] Y.-P. Duan, C.-M. Dai, Y.-L. Zhang, L. Chen, Anal. Chim. Acta 758 (2013) 93–100.
[36] L.-J. Zhou, G.-G. Ying, S. Liu, J.-L. Zhao, F. Chen, R.-Q. Zhang, F.-Q. Peng, Q.-Q. Zhang, J. Chromatogr. A 1244 (2012) 123–138.
[37] J. Xu, L. Wu, W. Chen, A.C. Chang, J. Chromatogr. A 1202 (2008) 189–195.
[38] P.A. Blackwell, H.-C. Holten Lützhøft, H.-P. Ma, B. Halling-Sørensen, A.B.A. Boxall, P. Kay, Talanta 64 (2004) 1058–1064.
[39] T. Okuda, N. Yamashita, H. Tanaka, H. Matsukawa, K. Tanabe, Environ. Int. 35 (2009) 815–820.
[40] A. Göbel, A. Thomsen, C.S. McArdell, A.C. Alder, W. Giger, N. Theiß, D. Löffler, T.A. Ternes, J. Chromatogr. A 1085 (2005) 179–189.
[41] M.P. Schlüsener, M. Spiteller, K. Bester, J. Chromatogr. A 1003 (2003) 21–28.
[42] M.S. Díaz-Cruz, M.J. López de Alda, D. Barceló, J. Chromatogr. A 1130 (2006) 72–82.
[43] Y. Ding, W. Zhang, C. Gu, I. Xagoraraki, H. Li, J. Chromatogr. A 1218 (2011) 10–16.
[44] P. Vazquez-Roig, R. Segarra, C. Blasco, V. Andreu, Y. Picó, J. Chromatogr. A 1217 (2010) 2471–2483.
[45] K.A. Krogh, E. Björklund, D. Loeffler, G. Fink, B. Halling-Sørensen, T.A. Ternes, J. Chromatogr. A 1211 (2008) 60–69.
[46] J. Olsen, E. Björklund, K.A. Krogh, M. Hansen, Anal. Chim. Acta 755 (2012) 69–76.
[47] K. Stoob, H.P. Singer, S. Stettler, N. Hartmann, S.R. Mueller, C.H. Stamm, J. Chromatogr. A 1128 (2006) 1–9.
[48] A. Jelić, M. Petrović, D. Barceló, Talanta 80 (2009) 363–371.
[49] A. Nieto, F. Borrull, R.M. Marcé, E. Pocurull, J. Chromatogr. A 1174 (2007) 125–131.
[50] M. Lillenberg, S. Yurchenko, K. Kipper, K. Herodes, V. Pihl, K. Sepp, R. Lõhmus, L. Nei, J. Chromatogr. A 1216 (2009) 5949–5954.
[51] A.M. Jacobsen, B. Halling-Sørensen, F. Ingerslev, S. Honoré Hansen, J. Chromatogr. A 1038 (2004) 157–170.
[52] J.C. Durán-Alvarez, E. Becerril-Bravo, V. Silva Castro, B. Jiménez, R. Gibson, Talanta 78 (2009) 1159–1166.
[53] C.L. Chitescu, E. Oosterink, J. de Jong, A.A.M. Stolker, Talanta 88 (2012) 653–662.
[54] J.-H. Park, J.-H. Choi, A.M.A. El-Aty, J.-S. Park, B.M. Kim, T.-W. Na, K.H. Park, A. Yang, M.M. Rahman, J.-H. Shim, J. Sep. Sci. 36 (2013) 148–155.
[55] A. Saleh, E. Larsson, Y. Yamini, J.Å. Jönsson, J. Chromatogr. A 1218 (2011) 1331–1339.
[56] J. Dobor, M. Varga, J. Yao, H. Chen, G. Palkó, G. Záray, Microchem. J. 94 (2010) 36–41.
[57] M. Varga, J. Dobor, A. Helenkár, L. Jurecska, J. Yao, G. Záray, Microchem. J. 95 (2010) 353–358.
[58] R. Cueva-Mestanza, Z. Sosa-Ferrera, M.E. Torres-Padrón, J.J. Santana-Rodríguez, J. Chromatogr. B 863 (2008) 150–157.

[59] D. Mutavdžić Pavlović, T. Pinušić, M. Periša, S. Babić, J. Chromatogr. A 1258 (2012) 1–15.
[60] I. Bragança, A. Plácido, P. Paíga, V.F. Domingues, C. Delerue-Matos, Sci. Total. Environ. 433 (2012) 281–289.
[61] E. Sagristà, E. Larsson, M. Ezoddin, M. Hidalgo, V. Salvadó, J.Å. Jönsson, J. Chromatogr. A 1217 (2010) 6153–6158.
[62] A. Jelic, M. Gros, A. Ginebreda, R. Cespedes-Sánchez, F. Ventura, M. Petrovic, D. Barceló, Water Res. 45 (2011) 1165–1176.
[63] K. McClellan, R.U. Halden, Water Res. 44 (2010) 658–668.
[64] C.A. Kinney, E.T. Furlong, S.L. Werner, J.D. Cahill, Environ. Toxicol. Chem. 25 (2006) 317–326.
[65] G.J.H.P. Gielen, M.R. van den Heuvel, P.-W. Clinton, L.G. Greenfield, Chemosphere 74 (2009) 537–542.
[66] G. Carbonell, J. Pro, N. Gómez, M.M. Babín, C. Fernández, E. Alonso, J.V. Tarazona, Ecotoxicol. Environ. Saf. 72 (2009) 1309–1319.
[67] B. Chefetz, T. Mualem, J. Ben-Ari, Chemosphere 73 (2008) 1335–1343.
[68] K. Lin, J. Gan, Chemosphere 83 (2011) 240–246.
[69] S.-C. Kim, K. Carlson, In: M. Petrović, D. Barceló (Eds.), Analysis, Fate and Removal of Pharmaceuticls in the Water Cycle, Elsevier, Oxford, 2007, pp. 95–131.
[70] D. Barceló, Trends Anal. Chem. 23 (2004) 677–679.
[71] D. Kou, S. Mitra, In: S. Mitra (Ed.), Sample Preparation Techniques in Analytical Chemistry, Wiley-Interscience, New Jersey, 2003, pp. 139–182.
[72] C. Klein, S. O´Connor, J. Locke, D. Aga, In: D.S. Aga (Ed.), Fate of Pharmaceuticals in the Environment and in Water Treatment Systems, CRC Press, Boca Raton, 2008.
[73] C. Hao, X. Zhao, P. Yang, Trends Anal. Chem. 26 (2007) 569–580.
[74] A. Nieto, F. Borrull, E. Pocurull, R.M. Marce, Trends Anal. Chem. 29 (2010) 752–764.
[75] M.D. Luque de Castro, F. Priego-Capote, J. Chromatogr. A 1217 (2010) 2383–2389.
[76] http://www.epa.gov/osw/hazard/testmethods/sw846/pdfs/3540c.pdf (January 2013).
[77] S. Terzić, M. Ahel, Environ. Pollut. 159 (2011) 557–566.
[78] O. Zuloaga, P. Navarro, E. Bizkarguenaga, A. Iparraguirre, A. Vallejo, M. Olivares, A. Prieto, Anal. Chim. Acta 736 (2012) 7–29.
[79] W.W. Buchberger, J. Chromatogr. A 1218 (2011) 603–618.
[80] J. Sunarso, S. Ismadji, J. Hazard. Mater. 161 (2009) 1–20.
[81] M. Rezaee, Y. Yamini, M. Moradi, A. Saleh, M. Faraji, M.H. Naeeni, J. Supercrit. Fluids 55 (2010) 161–168.
[82] È.A.M. Gilroy, V.K. Balakrishnan, K.R. Solomon, E. Sverko, P.K. Sibley, Chemosphere 86 (2012) 578–584.
[83] Y. Zielezny, Analysis of Veterinary Pharmaceuticals in Soil and Their Impact on Microbial Populations, PhD Dissertation, Bonn, 2008.
[84] T. Christian, R.J. Schneider, H.A. Färber, D. Skutlarek, M.T. Meyer, H.E. Goldbach, Acta Hydrochim. Hydrobiol. 31 (2003) 36–44.
[85] J.L. Luque-García, M.D. Luque de Castro, J. Chromatogr. A 1010 (2003) 129–140.
[86] L. Ramos, E.M. Kristenson, U.A.Th. Brinkman, J. Chromatogr. A 975 (2002) 3–29.
[87] B. Albero, C. Sánchez-Brunete, E. Miguel, R.A. Pérez, J.L. Tadeo, J. Chromatogr. A 1248 (2012) 9–17.
[88] Y. Chen, Z. Guo, X. Wang, C. Qiu, J. Chromatogr. A 1184 (2008) 191–219.
[89] R.M. Smith, J. Chromatogr. A 975 (2002) 31–46.
[90] J.L. Luque-García, M.D. Luque de Castro, Trends Anal. Chem. 22 (2003) 41–47.

[91] H.B. Xiao, M. Krucker, K. Albert, X.M. Liang, J. Chromatogr. A 1032 (2004) 117–124.
[92] C. Sánchez-Brunete, E. Miguel, J.L. Tadeo, J. Chromatogr. A 1148 (2007) 219–227.
[93] M.T. Pena, M.C. Casais, M.C. Mejuto, R. Cela, Anal. Chim. Acta 626 (2008) 155–165.
[94] M. Anastassiades, S.J. Lehotay, D. Štajnbaher, F.J. Schenck, J. AOAC Int. 86 (2003) 412–431.
[95] M. Anastassiades, E. Scherbaum, In: A.R. Fernández-Alba (Ed.), Chromatographic-Mass Spectrometric Food Analysis for Trace Determination of Pesticide Residue, Elsevier, Amsterdam, 2005, pp. 122–123.
[96] F. Plössl, M. Giera, F. Bracher, J. Chromatogr. A 1135 (2006) 19–26.
[97] M.M. Aguilera-Luiz, J.L. Martínez Vidal, R. Romero-González, A. Garrido Frenich, J. Chromatogr. A 1205 (2008) 10–16.
[98] G. Stubbings, T. Bigwood, Anal. Chim. Acta 637 (2009) 68–78.
[99] M.S. Díaz-Cruz, D. Barceló, Trends Anal. Chem. 26 (2007) 637–646.
[100] L. Chen, J. Liu, Q. Zeng, H.A. Yu, H. Zhang, L. Ding, J. Chromatogr. A 1216 (2009) 3710–3719.
[101] E. Caro, R.M. Marcé, P.A.G. Cormack, D.C. Sherrington, F. Borrull, Anal. Chim. Acta 552 (2005) 81–86.
[102] A. Beltran, R.M. Marcé, P.A.G. Cormack, F. Borrull, J. Chromatogr. A 1216 (2009) 2248–2253.
[103] B. Rezaei, M.T. Jafari, R. Khademi, Talanta 79 (2009) 669–675.
[104] Y.Q. Cai, G.B. Jiang, J.F. Liu, Q.X. Zhou, Anal. Chem. 75 (2003) 2517–2521.
[105] Y.Q. Cai, G.B. Jiang, J.F. Liu, Q.X. Zhou, Anal. Chim. Acta 494 (2003) 149–156.
[106] N. Fontanals, R.M. Marcé, F. Borrull, Trends Anal. Chem. 24 (2005) 394–406.
[107] A.V. Herrera-Herrera, M.Á. González-Curbelo, J. Hernández-Borges, M.Á. Rodríguez-Delgado, Anal. Chim. Acta 734 (2012) 1–30.
[108] Z. Zhang, S.M. Rhind, C. Kerr, M. Osprey, C.E. Kyle, Anal. Chim. Acta 685 (2011) 29–35.
[109] S. Sporring, E. Björklund, J. Chromatogr. A 1040 (2004) 155–161.
[110] A. Posyniak, J. Zmudzki, K. Mitrowska, J. Chromatogr. A 1087 (2005) 259–264.
[111] M.S. Díaz-Cruz, M.J. García-Galán, P. Guerra, A. Jelic, C. Postigo, E. Eljarrat, M. Farré, M.J. López de Alda, M. Petrovic, D. Barceló, Trends Anal. Chem. 28 (2009) 1263–1275.
[112] S.-C. Kim, K. Carlson, Trends Anal. Chem. 24 (2005) 635–644.
[113] M.S. Díaz-Cruz, D. Barceló, Trends Anal. Chem. 24 (2005) 645–657.
[114] B. Ferreira da Silva, A. Jelic, R. López-Serna, A.A. Mozeto, M. Petrovic, D. Barceló, Chemosphere 85 (2011) 1331–1339.
[115] A. Wick, G. Fink, T.A. Ternes, J. Chromatogr. A 1217 (2010) 2088–2103.
[116] M. Caban, N. Migowska, P. Stepnowski, M. Kwiatkowski, J. Kumirska, J. Chromatogr. A 1258 (2012) 117–127.
[117] A. Kot-Wasik, J. Dębska, J. Namieśnik, Trends Anal. Chem. 26 (2007) 557–568.
[118] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 70 (1998) 882–889.

Chapter 6

Analysis of Pharmaceutical Compounds in Biota

Belinda Huerta*, Sara Rodríguez-Mozaz* and Damia Barcelo*,†
*Catalan Institute for Water Research (ICRA), Girona, Spain
†Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

Chapter Outline

1 INTRODUCTION

Pharmaceutical residues have been detected in the aquatic environment for a number of years at low concentrations (nanograms per liter to micrograms per liter) in wastewater treatment plant (WWTP) effluents, surface water, and groundwater [1–4]. Despite the usually low levels and tendency to swiftly degrade, they are found in the natural ecosystems: their introduction rates in the aquatic environment exceed their degradation rate, and thus, they are considered pseudopersistent contaminants [5].

A major route of entrance of these substances in the environment is a consequence of their intended purpose. After the administration of a pharmaceutical, the unchanged parent compound and, in many cases, bioactive and inactive metabolites are excreted and released into the municipal sewage systems. Many of them pass through WWTPs unchanged or as their transformation products [6], as conventional WWTPs are not specifically designed to remove these compounds. Wastewater effluents are usually discharged to surface water or used for irrigation. Solid effluents, that is, sludge, containing pharmaceutical residues can result in leaching into the soil or lead to runoff

Comprehensive Analytical Chemistry, Vol. 62. http://dx.doi.org/10.1016/B978-0-444-62657-8.00006-9

to receiving waters [7]. Veterinary drug usage for treating domestic animals, livestock, and aquaculture is another route of entrance, whether through direct deposition on land via feces or through runoff to receiving waters. Agriculture and aquaculture also require large quantities of specific pharmaceutical compounds, such as antibiotics and hormones for growth promotion, therapeutic treatment, or disease prevention [8].

One of the main concerns related to the presence of pharmaceutical compounds in the environment is that they are biologically active, resulting in unexpected effects in nontarget aquatic organisms [9–11]. Little is still known about the long-term effects of exposure to low levels of these emerging contaminants on wildlife and, ultimately, on humans [9] and the potential for effects when organisms are exposed to multiple like-acting drugs.

There are a few examples of unintended side effects, such as the feminization of male fish attributed to the estrogen derivate ethinyl estradiol in combination with other hormones and the toxicity of the anti-inflammatory drug diclofenac, which caused the death of millions of vultures in Asia [12–14]. A recent study showed that environmentally relevant concentrations of oxazepam, a benzodiazepine drug, can affect fish behavioral traits, such as boldness, activity, and sociality [15]. These traits are considered ecologically and evolutionarily important and are used to predict how individuals respond to environmental changes [15]. These studies only refer to one or a few compounds when, as a matter of fact, organisms are exposed to hundreds of pharmaceuticals at the same time, so the full environmental impact of these compounds and the possible additive or synergistic effects are still unknown.

2 SAMPLE PREPARATION

Literature specifically focusing on sample preparation for the analysis of pharmaceuticals in solid samples, including food and biological matrices, has been recently published [16–20]. This section reviews the main extraction and cleanup procedures and the most used detection techniques applied to the determination of pharmaceuticals in aquatic wildlife (see Figure 1). Table 1 summarizes the main steps and experimental conditions reported in the literature for the analysis of pharmaceuticals in biota samples.

2.1 Sample Extraction

For the analysis of environmental samples, the target analytes must be previously isolated and concentrated from the sample matrix. Sample preparation is particularly critical when biota samples are involved due to the higher complexity of these matrices, especially rich in undesirable components that could interfere with the analysis (lipids, proteins, and pigments), and the low concentration of analytes.

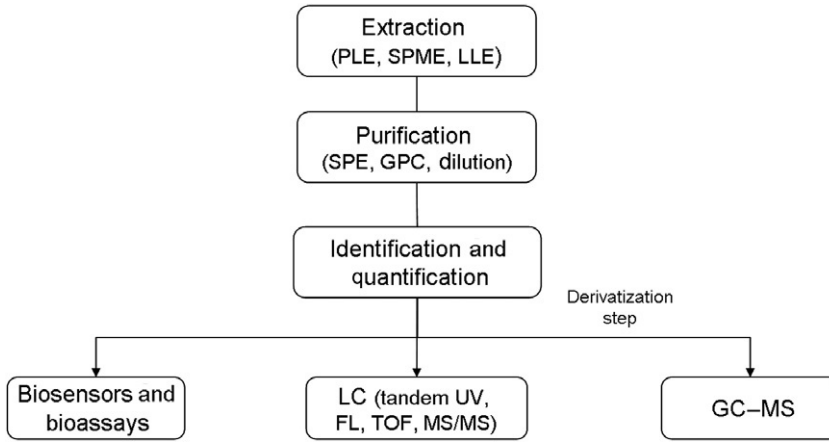

FIGURE 1 Basic analytic steps in methodologies for determination of pharmaceuticals in biota.

Considering the aforementioned problems, along with the widely varying physicochemical properties (e.g., polarity, solubility, and stability) between groups of pharmaceuticals, one of the main challenges for the development of a good analytic method is to obtain efficient extraction for the target compounds [17,20]. Even though the current trend is to develop multiresidue methods for the analysis of pharmaceuticals in environmental matrices [60,61], the development of methods for multiclass pharmaceutical determination (three or more therapeutic families) in biota is difficult, as it requires a compromise in the selection of the experimental conditions, which usually are not the best conditions for all the analytes studied. The more differences there are between compounds, the greater the difficulty in finding a single extraction procedure for all analytes with acceptable recoveries [20]. Probably for this reason, few multiresidue methods have been developed for the screening of biota (see Table 1) [54–59]. Consequently, most of the methods reported so far focus on single compounds or a family of compounds (psychiatric drugs, antibiotics, etc.). All things considered, extensive and lengthy sample preparation is essential to solve these issues that might be a hindrance during the method optimization [16].

There are two main types of biota samples: liquid samples (such as bile or plasma) and solid samples (muscle, liver, etc.), which require very different analytic approaches. In the case of nonsolid biological matrices, the main objective is to preconcentrate the analytes. This usually means a dilution step, to reduce matrix interferences, followed by a preconcentration through the use of solid-phase extraction (SPE) cartridges. The most frequently chosen sorbent for SPE cartridges is Oasis HLB or mixed-mode cation-exchange (MCX) (specific for basic compounds) sorbent or Strata-X, as they have shown an efficient performance during the extraction of a wide range of pharmaceuticals [22–24,43,55]. An alternative technique for the analysis of

TABLE 1 Analytic Methodologies for the Determination of Pharmaceutical Compounds in Biota

Family	Compounds	Matrix	Extraction	Cleanup	Detection	LOD (ng/g)	Rec. (%)	References
Analgesics and anti-inflammatories	Diclofenac	Fish gills, kidney, liver, muscle	–	SPE EXtrelut NT 20 + deriv. TMSH	GC–MS	10	–	[21]
		Fish bile	Dilution	Strata-X-AW	UPLC–TOF/MS	–	–	[22]
	Diclofenac and metabolites	Fish bile	Dilution	SPE Oasis HLB	LC–MS/TOF	–	–	[23]
	Diclofenac, ibuprofen, naproxen	Fish bile, plasma	Centrifugation + dilution	SPE Oasis HLB	LC–MS/MS	0.9–20[a]	92–105	[24]
	Ibuprofen	Fish plasma, gills, kidney, liver, muscle	Homogenization	SPE ResPrep™ Florisil + deriv. BF3/MeOH	GC–MS	14[a]	90–104	[25]
		Mussel digestive gland tissue, homogenate	Microwave-assisted micellar extraction (MAME)	SPE Oasis HLB	LC–MS/MS	–	–	[26]
	Ibuprofen metabolites [b]	Fish plasma	Homogenization	SPE ResPrep™ Florisil + deriv. BF3/MeOH	LC–MS	–	90–104	[25]
	Ibuprofen, ketoprofen, naproxen	Mussel homogenate, gills	MAME	SPE Oasis HLB	HPLC–UV	0.2	85–119	[27]

Antibiotics	Aminoglycosides	Fish muscle	Centrifugation	SPE Oasis MCX	UPLC–MS/MS	2–25	70–82	[28]
	Amphenicols, penicillin, sulfonamides, tetracyclines	Fish and mussel muscle	Enzymatic-microwave-assisted extraction	Centrifugation	HPLC-DAD/FLD	2–16	70–100	[29]
	Flumequine	Fish muscle	Sonication	Centrifugation	HPLC–FL	1	63	[30]
	Fluoroquinolones, quinolones	Fish homogenate	Centrifugation	Wash with hexane	Optical SPR biosensor	0.3		[31]
	Flumequine, oxolinic acid	Bryophyte homogenate	Extraction with NaOH + wash with chloroform		HPLC–FL	5	–	[32]
	Macrolides	Fish muscle	PLE	–	LC–MS	18–51	66–91	[33]
	Oxytetracycline	Crustacean hemolymph and hepatopancreas	Protein denaturalization + buffered extraction	SPE Sep-Pak C_{18}	HPLC–UV	40[a]	96–108	[34]
		Bryophyte homogenate	Extraction with acetone and McIlvaine buffer	SPE Bond Elut C_{18}	HPLC–UV	30		[32]
	Quinolones	Crustacean homogenate	Centrifugation	Liquid–liquid extraction	HPLC–FL	6.9	68	[35]
β-blockers	Carvedilol	Crustacean and insect homogenate	PLE	–	Scintillation (LSC)			[36]
	Propranolol	Fish plasma	Addition of NaOH + vortexing	Centrifugation	LC–MS		98–103	[37]

Continued

TABLE 1 Analytic Methodologies for the Determination of Pharmaceutical Compounds in Biota—Cont'd

Family	Compounds	Matrix	Extraction	Cleanup	Detection	LOD (ng/g)	Rec. (%)	References
Hormones	17α-Ethinylestradiol	Fish homogenate	PLE	GPC + solvent wash + deriv. PFBCl	GC–MS	0.7	74–94	[38]
		Crustacean and insect homogenate	Sonication	Centrifugation	LC–MS/MS	3.7–6.5	95–98	[39]
	17β-Estradiol	Fish muscle	Centrifugation	SPE + deriv. BSTFA	GC–MS	0.003	95–98	[40]
	17α-Ethinyl estradiol, 17β-estradiol	Biofilm	Centrifugation + ultrasonication	Filtration + deriv. MSTFA	GC–MS	–	–	[41]
		Mussel homogenate	Ultrasonication + filtration	SPE Florisil	LC–MS/MS	0.3–5.0	48–55	[42]
Lipid regulators	Atorvastatin	Crustacean and insect homogenate	Sonication	Centrifugation	LC–MS/MS	0.3–1.3	61–108	[39]
	Bezafibrate	Mussel digestive gland tissue, gills, homogenate	MAME	SPE Oasis HLB	LC–MS/MS	–	–	[26]
		Mussel homogenate	MAME	SPE Oasis HLB	HPLC–UV	0.2	85–119	[27]
		Fish plasma	Centrifugation	SPE Oasis HLB	LC–MS/MS	–	89 ± 6	[43]
	Simvastatin	Fish liver	Shaking with solvent	SPE Oasis HLB	LC–MS/MS	7.9	80 ± 7	[44]

Psychiatric drugs	Carbamazepine	Fish bile, plasma	Centrifugation	SPE Oasis HLB	LC–MS/MS	0.9–100[a]	92–105	[24]
		Mussel digestive gland, gills, homogenate	MAME	SPE Oasis HLB	LC–MS/MS	–	–	[26]
		Mussel homogenate	MAME	SPE Oasis HLB	HPLC–UV	0.2	85–119	[27]
		Algae, Cnidarian, Crustacean homogenate	Liquid–liquid extraction	SPE anhydrous sodium sulfate	LC–MS/MS	0.2	93 ± 4	[45]
		Fish brain, liver, muscle, plasma	Centrifugation		LC–MS/MS		95–110	[46]
		Crustacean and insect homogenate	Sonication	Centrifugation	LC–MS/MS	0.8–1.1	118–129	[39]
	Carbamazepine, diazepam	Fish muscle	PLE	GPC + silica gel + deriv. MSTFA	GC–MS	3.7–18	88–97	[47]
		Fish liver	Centrifugation	SPE Oasis HLB	LC–MS/MS	8.2	84 ± 4	[44]
	Carbamazepine, diazepam, fluoxetine	Crustacean and insect homogenate	Extraction with hydrogen peroxide		Scintillation (LSC)	–	–	[36]

Continued

TABLE 1 Analytic Methodologies for the Determination of Pharmaceutical Compounds in Biota—Cont'd

Family	Compounds	Matrix	Extraction	Cleanup	Detection	LOD (ng/g)	Rec. (%)	References
	Bupropion, citalopram, fluoxetine, paroxetine, sertraline, venlafaxine[b]	Fish muscle	PLE + protein denaturalization with HCl	SPE Oasis® MCX	LC–APCI-MS/MS	0.5	5–111	[48]
	Bupropion, citalopram, duloxetine, fluoxetine, fluvoxamine, paroxetine, sertraline, venlafaxine[b]	Fish brain	Sonication	Centrifugation	LC–MS/MS	0.015	77–97	[49]
	Fluoxetine	Mussel homogenate	Bed shaker		LC–MS/MS	0.05	51–94	[50]
	Fluoxetine, sertraline[b]	Fish brain, liver, muscle	Rotary extraction	SPE bond elute + deriv. PFPA	GC–MS	0.01	49–107	[51]
	Fluoxetine, paroxetine[b]	Fish muscle	PLE + protein denaturalization with HCl	SPE Oasis® MCX	LC–APCI-MS/MS	0.02–0.07	86–99	[52]
	Oxazepam	Fish muscle	BeadBeater	Centrifugation	LC–MS/MS	0.5	100 ± 12	[15]
Barbiturates	Barbital, secobarbital	Crustacean homogenate, fish muscle	Ultrasonic extraction + wash with hexane	SPE Oasis HLB	LC–MS/MS	0.25	75–85	[53]

	Therapeutic Family	Matrix	Extraction	Cleanup	Detection	LOD (ng/g)	Rec. (%)	References
Multiresidue methods[c]	Analgesics and anti-inflammatories, antibiotics, β-blockers, lipid regulators, psychiatric drugs, anticoagulants, anti-acid reflux drugs, antihistamines	Fish muscle	Rotary extractor	Centrifugation	LC–MS/MS	0.01–3.14	31–97	[54]
	Analgesics and anti-inflammatories, β-blockers, lipid regulators, psychiatric drugs	Fish bile	SPE Oasis HLB/MCX		LC–MS/MS	1.1–77[a]	74–136	[55]
	Analgesics and anti-inflammatories, lipid regulators, psychiatric drugs	Fish bile, muscle	Solid-phase microextraction (SPME)		LC–MS/MS			[56,57]
	Lipid regulators, psychiatric drugs, antihistamines, calcium channel blockers	Fish liver, muscle	Rotary extractor	Centrifugation	LC–MS/MS	0.04–9.6	91–142	[58]
	Analgesics and anti-inflammatories, β-blockers, psychiatric drugs, antihelminthics, antiplatelet agents, diuretics, anti-asthma drugs	Fish homogenate, liver, muscle	PLE	GPC	UPLC–MS/MS	0.01–0.98	27–92	[59]

[a] *ng/mL for bile and plasma analysis.*
[b] *Method includes pharmaceutical metabolites.*
[c] *Three or more therapeutic families.*

propranolol in plasma was based on the dilution of the samples with 100 µl of 0.1% (w/v) NaOH and centrifugation and obtained recoveries in the range 98–103% [37]. Nallani et al. [25] applied a simple centrifugation step with acetone followed by a derivatization step for the analysis of ibuprofen metabolites in blood samples. Togunde et al. [56] developed an analytic method based on solid-phase microextraction (SPME) to investigate the uptake and the bioconcentration of pharmaceuticals in fish bile. Polydimethylsiloxane and C_{18} fiber coatings were inserted in the bile samples previously deconjugated and set for equilibrium extraction at under 1200 rpm continual vortex agitation. Recovery efficiency was not very high (5–65%), but the extraction strategy was solvent-free and integrated sampling and sample preparation into a single step.

Extraction of pharmaceuticals from solid matrices usually requires more extensive procedures, such as ultrasonication [30,53], microwave-assisted micellar extraction (MAME) [26,27], pressurized liquid extraction (PLE) [47,52,59], centrifugation with solvent [24,31,40,43,46], and rotary extraction [51,54,58].

Ultrasonication has been used for the extraction of several pharmaceutical classes, such as antibiotics, analgesics, antidepressants, and hormones using different solvents, such as methanol/acetone [62], acetonitrile [30,49], and hexane/acetone (70:30, v/v) [42]. For instance, Schultz et al. [49] added formic acid and acetonitrile to fish brain samples and homogenized with an ultrasonic tissue disruptor for the analysis of antidepressants. Vannini et al. [62] developed a method for the analysis of 13 compounds, which included antibiotics, anti-inflammatories, lipid regulators, diuretic, β-blockers, psychiatric drugs, and other therapeutic families, in algal homogenates consisting of two ultrasonication steps (10 min each) with a mixture of methanol/2% NH_4 solution in methanol/acetone (1:1:1, v/v), followed by a centrifugation step. Wang et al. [53] described an extraction method for barbital residues in fish tissue and shrimp muscle based on ultrasonication extraction with 0.1% acetic acid (v/v) in acetonitrile and hexane wash for extract purification, with recoveries between 75% and 85%. Two sonication extraction steps with hexane–acetone (70:30, v/v) as extractant were applied for the analysis of 17α-ethinylestradiol and 17β-estradiol in mussel samples, with recoveries around 50% [42].

Ramirez et al. [54] evaluated the efficiency of ten solvents (i.e., dichloromethane, hexane, methanol, and acetonitrile), differing in pH or polarity for the extraction of 24 pharmaceuticals from fish muscle. In this study, moderate-polarity solvents were found to be most effective at removing target analytes from fish muscle, whereas aqueous solvents resulted in relatively poor extraction efficiency. As most of the target analytes were basic, tested pH (2.4–6) had little effect on recovery. This method applied a simple homogenization with the selected solvent and a centrifugation step for removal of macromolecules as the extraction procedure.

A less common technique is, for example, the use of micellar systems for the extraction of the target analytes, where a micellar surfactant is used as the extractant, reducing solvent consumption and production of residues. This is the case of MAME, which Cueva-Mestanza et al. [27] applied to the detection of six pharmaceuticals in mussels with recoveries higher than 80%. Fernandez-Torres et al. [29] applied a similar technique, enzymatic-microwave-assisted extraction (MAE), using an enzymatic digestion for the extraction of 11 antibiotics in fish tissue and mussel samples. A comparison between classical MAE and enzymatic-assisted MAE was performed in order to improve extraction efficiencies, and recoveries of almost all analytes increased at low irradiation powers when enzymatic-assisted MAE was applied.

The study of bioaccumulation and tissue distribution of contaminants in living organisms has also benefited from advances in techniques like SPME, which has been applied to samples for the analysis of several therapeutic classes of pharmaceuticals *in vivo* in fish. The procedure consists in inserting a fiber in the fish muscle to adsorb the target analytes. After a short exposure, the fiber is extracted from the organism and desorbed through agitation in an organic solvent. In the majority of the cases, results were comparable with those obtained by a solid–liquid extraction to determine the extractable concentrations of target analytes in fish muscle [57,63,64].

A spreading trend is the use of PLE for the determination of contaminants in solid samples. This technique involves extraction with conventional solvents at high pressure (100–140 bar) and temperatures (80–180 °C), without reaching their critical point, to increase the extraction of pollutants from solid samples [65,66]. An important point to consider is that, although this extraction process may be more efficient than the procedures mentioned earlier, it may also extract more matrix components. Extraction solvent and temperature are considered the critical parameters during optimization of this technique. Huerta et al. [59] evaluated the effect of various solvents at different temperatures for the extraction efficiency. Tested temperatures were in the range 50–90 °C, as the thermal stability of the compounds due to the relatively high extraction temperatures was essential. At 50 °C, methanol provided the highest recoveries when compared to other solvents (acetonitrile), solvent mixtures (methanol/water, 1:1; methanol/acetonitrile, 1:1), or solvent with additive (acidified methanol). The rest of the parameters did not significantly improve the recoveries. Berrada et al. [33] extracted macrolide antibiotics in fish muscle, even though these compounds are especially temperature-sensitive, with recoveries over 77% and no further purification preanalysis. Chu and Metcalfe [52] described a procedure for sample preparation by PLE testing three solvents: methyl *tert*-butyl ether, ethyl acetate, and methanol. With the optimized method, which involved a T^{a} 100 °C and methanol as extraction solvent, recoveries greater than 85% were obtained for paroxetine, fluoxetine, and norfluoxetine in fish tissue.

2.2 Sample Purification

Direct analysis after extraction is not always possible for the quantitative determinations of pharmaceuticals in biota samples without further purification to clean the extracts, as they may contain matrix coeluents, which could spoil or hinder an accurate detection.

The great majority of the cleanup procedures is based on SPE, as it is relatively swift, requires small quantities of solvent, and can adsorb compounds with very different physicochemical properties. Florisil, alumina, or silica gel columns have been used especially in the case of fatty samples [32,34,42,51]. In the method developed by Nallani et al. [25], use of ResPrep^{TM} Florisil SPE cartridges was the cleanup method chosen for the analysis of ibuprofen and metabolites in different fish tissues. More often, polymeric sorbents mixed with polymeric and cation-exchange sorbents are applied to the analysis of pharmaceuticals in environmental matrices. SPE Oasis HLB cartridges have been frequently applied to preconcentrate the target analytes and reduce the presence of coeluents, as they can extract acidic, neutral, and basic compounds with high efficiencies [27,53]. Vannini et al. [62] used SPE cleanup with Oasis HLB and MCX and reversed-phase cartridges for the analysis of 13 compounds in algal homogenates. Chu and Metcalfe [52] described a procedure for sample preparation by PLE, followed by cleanup on a mixed-mode SPE cartridge (Oasis MCX).

Removal of lipids from the biota extract is a crucial step, as the fat content often constitutes between 5% and 50% of the samples [59]. Gel-permeation chromatography (also known as size exclusion chromatography) has the advantage of good separation of large molecules from the small molecules with a minimal volume of eluate, and the column usually can be used over several months with no detriment on cleanup capacity [66]. This technique has been used in tandem with other cleanup strategies, such as SPE, liquid–liquid extraction with hexane to eliminate nonpolar matrix residues, and freezing the sample extracts in acetonitrile to precipitate remaining lipids such as cholesterol [38,47]. Chu and Metcalfe [52] tried gel permeation chromatography (GPC) cleanup with Bio-Beads S-X3 to remove lipids from the samples, yet they settled for SPE to remove the coextractives from the PLE extract, as the needed solvent volume was large and another cleanup step was still needed to make the sample suitable for analysis. Huerta et al. [59] tested GPC with an EnviroPrep (Agilent) column compared to SPE with Florisil and Oasis HLB cartridges. GPC purification step was selected as cleanup method, as it provided satisfactory results for most of the target compounds and reduced considerably interferences during analysis, and not further steps were needed.

3 SAMPLE SEPARATION AND DETECTION

Analytic techniques to detect pharmaceuticals at trace quantities in environmental matrices have advanced significantly in the last few years. Most of

the analytic determination methods for pharmaceuticals in biota matrices found in the literature are based on liquid chromatography (LC) [46,50,54–56,58,59] or gas chromatography (GC) [21,25,38,40,47,51] in combination with mass spectrometry (MS) or mass spectrophotometry detection, but alternative techniques have also been described and applied, for instance, immunoassays [31].

3.1 Bioassays and Biosensors

Several biosensors and immunoassays have recently been developed for selected pharmaceuticals in biological matrices [67]. Albeit these biological techniques have been sparingly used for the analysis of pharmaceuticals in wildlife, their high sensitivity, simplicity, and cost-effectiveness make them a good screening technique for different therapeutic classes [68]. Various screening methods to detect antibiotics based on immunoassays have been developed for their application to fish samples, such as time-resolved fluoroimmunoassay and enzyme-linked immunosorbent assay [69]. The most commonly used biological element for the detection of veterinary drug residues is the antibody/antigen affinity pair, which is frequently used as an immunochemical method [68,70]. Huet et al. [31] developed an optical immunosensor, based on the surface plasmon resonance principle, as a screening test for 13 antibiotics at concentrations below the established maximum residue levels. This method was applied to different biological matrices, including fish samples.

3.2 Gas Chromatography

Gas chromatography–tandem mass spectrometry (GC–MS) has been mostly limited to compounds that are volatile enough to be transferred into the gas phase or that can be derivatized to volatile species without difficulty, which is a minority in the case of pharmaceuticals, that is, synthetic hormones [38,40]. This derivatization step may be an arduous process in complex sample matrices like biota, although it has the advantage of being less susceptible to the matrix effects than other techniques.

GC has been adeptly applied for the detection of fluoxetine, sertraline, and metabolites in several fish tissues, including some as fatty samples such as the liver, with excellent limit of detection (LOD) of 0.01 ng/g [51], and carbamazepine and diazepam in fish muscle with LOD of less than 4 ng/g [47], as well as some anti-inflammatories [21,25].

3.3 Liquid Chromatography

LC has grown to be a fundamental separation method for the determination of polar and thermolabile compounds. This separation technique has the

advantage over GC of avoiding the derivatization step previously mentioned, but it can be seriously affected by matrix effects derived from interfering compounds extracted from the biological samples, particularly when it is in combination with MS detection [71].

Most analysis of pharmaceuticals from biota samples has been conducted in reversed-phase columns, such as C8 and C18 [23,24,26,46,48,54,58]. Acetonitrile and methanol [39,43,52] have often been used in mobile phases for chromatographic separation of pharmaceuticals, often accompanied by modifiers, such as formic acid [15,39], ammonium acetate [24,54,59], or ammonium hydroxide [55] to stabilize the pH and obtain a better peak shape and reproducibility and to increase the ionization efficiency when an MS is used as detection method.

LC in tandem with spectrophotometric detection, such as diode-array detection, UV absorbance detection, and fluorescence detection, has been used for the detection of some pharmaceutical classes in aquatic organisms. Studies to determine quinolones in fish, crustaceans, and bryophytes (aquatic plants) were performed by means of high-performance LC with fluorescence detection, with LOD between 1 and 7 ng/g [30,32,35,72]. Another study reported the application of high-performance LC with UV absorbance detection for anti-inflammatories, lipid regulators, and psychiatric drugs in mussels, with LOD below 0.2 ng/g and recoveries between 85% and 119% [27]. These applications are, however, limited to those compounds with a specific physicochemical characteristic, such as the presence of chromophores or fluorescent properties.

Even though LC tandem diode-array, UV absorbance, or fluorescence detection may be a cost-effective technology, these techniques have been progressively replaced by mass spectrometric detection in the determination of pharmaceuticals in environmental matrices, as it provides high selectivity, specificity, and sensitivity [9,71,73].

MS methods applied to environmental matrices comprise diverse technologies, such as single-quadrupole MS [21,25,33], tandem MS (MS/MS), triple-quadrupole MS/MS [52], and time-of-flight MS [22,23,74].

LC–MS has been successful to analyze ibuprofen metabolites [25], and propranolol [37] in fish plasma and macrolide antibiotics in fish muscle [33]. However, LC–MS/MS is preferred to LC–MS for the measurement of pharmaceuticals in biota samples, as the fragmentation ions allow to increase the specificity of the analysis. Consequently, most of the analytic methods developed for the analysis of pharmaceuticals in biota are based on LC–MS/MS [24,26,39,45,50,54,55,58,59,75–77].

The most commonly used ionization method for LC interfaces is atmospheric pressure ionization (API), which includes electrospray ionization (ESI) and atmospheric pressure chemical ionization (APCI). ESI appears to be the most employed mode of ionization in pharmaceutical determination, since it is particularly suitable for both polar and nonpolar analytes and for

thermolabile substances. However, it is known to be more susceptible to signal suppression than APCI [71]. Drastic matrix effects in the use of ESI were observed in a study by Schlüsener and Bester [78], whereas they were less pronounced with APCI. Unfortunately, only a few pharmaceutical compounds can be efficiently ionized by APCI, and most of them have to be analyzed by ESI.

4 MATRIX EFFECTS

A much known downside of LC–MS and LC–MS/MS is that coextracted matrix components tend to interfere with API interfaces. The matrix effects could be caused by coeluted matrix components that have common ions with target analytes, as a competition can occur between matrix coeluents and analyte ions for gas-phase emission during the ionization in samples with high protein and lipid content. The matrix effects result in suppression or enhancement of the signal of the target analyte during the ionization process, which can acutely compromise the accuracy of quantitative data and affect the LOD in real samples (see Figure 2). Even when working with a specific kind of matrix, that is, fish muscle, matrix effects can be highly variable and difficult to predict. Matrix effects are particularly acute at low analyte concentration, which is a common situation for pharmaceuticals in biota [39,79]. Chu and Metcalfe [52] reported signal suppression between 19% and 39% when analyzing paroxetine, fluoxetine, and norfluoxetine in whole fish, whereas Dussault et al. [39] reported effects ranging from 14% signal suppression to 25% signal enhancement in the analysis of selected pharmaceuticals in

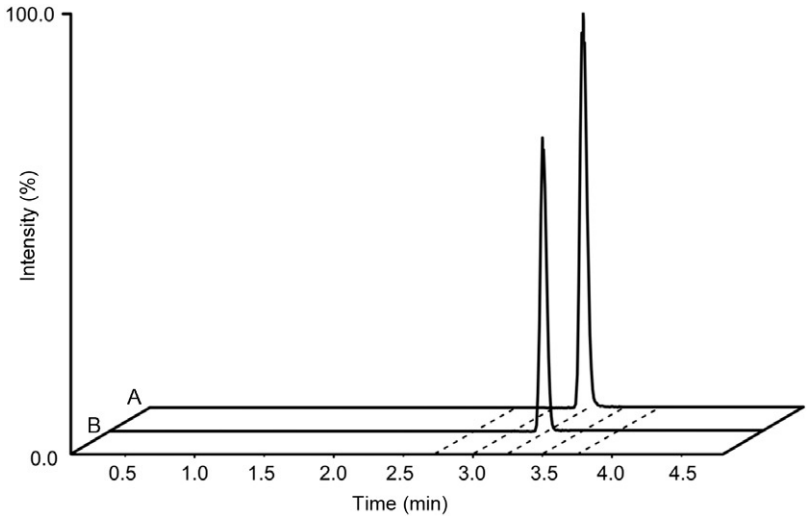

FIGURE 2 Suppression of signal for carbamazepine spiked into (a) pure solvent and (b) fish liver and analyzed by LC–ESI-MS/MS.

invertebrates. Another study reported 83% of signal suppression and 19% of signal enhancement for some compounds in fish liver [59].

Different approaches have been used to correct these variations on ionization efficiency. Some authors have obtained good results when quantifying with external sample calibration (spiked standards in pure solvent), probably because the analysis was reduced to a small number of compounds and the extraction methods applied were very selective [21,44,48]. Standard addition is considered the most effective approach for compensating matrix effects, although it can be difficult and time-consuming. A standard addition method was used to evaluate the influence of matrix effects on the analysis of psychiatric drugs (and their corresponding labeled compounds) in fish tissues by Chu and Metcalfe [52]. Since results showed that the internal standards were subjected to the same recoveries and matrix effects as the target compounds, the reduced response of the analyte was compensated for by the internal standard response.

In fact, the most popular approach consists in the addition of internal standards (isotopically labeled compounds), which can correct variations during instrumental detection [15,46,52,55,58]. Ideally, there should be one internal standard for each analyte that elutes from the chromatographic column at the same time as the native compounds, as there is a gradual decrease in the matrix effect with increasing retention time [80]. However, this option is not always possible, as they are quite expensive and not available in all cases [37,53]. Furthermore, when the labeled compounds behave in a different way in its interaction with the matrix than the target analytes [81], the use of this approach would result in quantification errors [39].

For this reason, other corrective measures are becoming increasingly used, such as matrix-matched calibration [23,24,33,40,54,82]. This also has some drawbacks, as uncontaminated matrix for the preparation of a matrix-matched calibration is usually difficult to obtain and the generation of a calibration curve for each sample is unfeasible when analyzing a large number of samples [83]. An alternative strategy has been applied, namely, internal sample calibration [84,85], where the quantification uses a calibration curve prepared with spiked sample extracts and internal standard addition. This method has shown to correct the matrix effects for all compounds targeted in a method, even though their internal standard does not correct completely the matrix effects or it is not available [59].

5 ENVIRONMENTAL OCCURRENCE

As a result of the continuous input of pharmaceuticals in the environment, aquatic organisms inhabiting receiving waters have also shown the capacity to bioconcentrate amounts of these compounds in their tissues, despite their relatively low concentrations in water and their physicochemical properties [52,59,76,86]. It is generally accepted that substances with octanol–water partition coefficient (log K_{OW}) values higher than or equal to 3 have the potential to bioaccumulate in biological tissues, which is not the case for

many pharmaceuticals, which are in general quite polar compounds [87]. However, when considering bioaccumulation of these compounds in aquatic organisms, one must take other factors into consideration, such as the different rates of metabolism of xenobiotic compounds in various organisms, the accumulation behavior of the metabolites, and the uptake and depuration kinetics [88]. However, unlike other trace organic pollutants, such as polychlorinated biphenyls, which can easily reach concentrations in the microgram per gram range in aquatic organisms of polluted sites [89], pharmaceuticals can be found at levels not higher than nanogram per gram range.

Fish biological traits might make them potentially more susceptible to pharmaceutical bioaccumulation [10], and most of the studies about pharmaceutical accumulation in biota have been focused on them. Bioaccumulation of drug residues in aquatic invertebrates has been less frequently investigated, and only a few studies have reported their presence in shrimps and mussels [35,50]. Nonetheless, organisms such as invertebrates and algae are intrinsically involved in the natural flow of energy and nutrients in aquatic systems. They also are able to integrate swift environmental variations, which validates their position as indicator species.

A summary of the studies about accumulation of pharmaceuticals in wild biota is presented in Table 2. Presence of pharmaceuticals in wild fish tissues was first reported by Brooks et al. [51], who found concentrations of 30 ng/g of some psychiatric drugs such as fluoxetine, sertraline, and metabolites in brain tissue of fish from aquatic environments heavily impacted by wastewater effluents in Texas, United States. Subsequently, this study was extended to a longer list of pharmaceuticals [54], and it detected the accumulation of diphenhydramine, diltiazem, and carbamazepine in fish collected from the same stream. Also in Canada, two studies revealed the presence of psychiatric drugs and the synthetic hormone 17α-ethinylestradiol at concentrations between 1 and 2 ng/g (wet weight) in wild fish [52,93].

In Europe, research about the presence of pharmaceuticals in biota has been mostly limited to fish or seafood intended for human consumption and regulation demands related to antibiotic compounds. Levels reported for antibiotics in fish tissues reached in some cases up to 100 ng/g [30,33,82,94]. In the last couple of years, four studies in different European countries confirmed that wild fish exposed to low quantities of pharmaceuticals (i.e., psychiatric drugs, antihypertensives, and analgesics) in river water accumulated these compounds. Pharmaceuticals were detected at a concentration up to 18 ng/g in whole fish and fish liver and muscle [15,59,76]. In particular, in the study performed by Brodin et al., the concentration of the psychiatric drug oxazepam found in fish muscle was more than six times higher than in water [15]. Brozinski et al. on the other hand, who studied the presence of anti-inflammatories diclofenac, ibuprofen, and naproxen in fish bile, detected a maximum concentration of 150 ng/mL [55].

TABLE 2 Occurrence and Concentration of Pharmaceutical Compounds in Aquatic Biota

Therapeutic Class	Compounds	Matrix	Concentration (ng/g)[a]	Location	References
Analgesics/anti-inflammatories	Diclofenac	Fish homogenate	4.1–8.8[b]	Spain	[59]
	Diclofenac, naproxen, ibuprofen	Fish bile	nd[c]–148[d]	Finland	[55]
Antibiotic	Erythromycin A	Fish muscle	nd–87	Spain	[33]
	Sulfonamides, tetracyclines, penicillin, amphenicols	Fish muscle	<MQL	Spain	[82]
	Florfenicol	Fish muscle	0.6–3.4	Spain	[40]
	Tetracyclines	Fish muscle	2.1–152.2	Spain	[90]
	Quinolones, sulfonamides, macrolides	Mollusk homogenate	nd–1575	China	[91]
Antiplatelet agent	Clopidogrel	Fish homogenate	<MQL	Spain	[59]
Antihistamine	Diphenhydramine	Fish muscle	0.66–1.32	United States	[54]
		Fish muscle	0.14–0.31	Utah, United States	[58]
		Fish muscle	0.04–0.07	Germany	[76]
		Fish liver	<MDL–8.6	Utah, United States	[58]
β-blockers	Carazolol, propranolol, sotalol	Fish homogenate	<MQL–4.2[b]	Spain	[59]
	Atenolol, metoprolol, propranolol	Fish muscle	0.11–0.27	United States	[54]
Calcium channel blocker	Diltiazem	Fish liver	<MDL–0.86	Utah, United States	[58]

Hormones	17α-Ethinylestradiol	Fish homogenate	1.4–2.0	Canada	[38]
		Mussel homogenate	3–38[b]	Italy	[42]
	17β-Estradiol	Fish muscle	0.81–1.6	Spain	[40]
	Estrone	Fish muscle	0.52–1.3	Spain	[40]
Lipid regulator	Gemfibrozil	Fish liver	11–34	Utah, United States	[58]
Psychiatric drugs	Paroxetine, fluoxetine, norfluoxetine	Fish homogenate	nd[c]–1.1	Canada	[52]
	Venlafaxine, citalopram, sertraline, *N*-desmethylvenlafaxine, desmethylsertraline	Fish homogenate	nd–7	Ontario, Canada	[48]
	Citalopram, venlafaxine	Fish homogenate	0.6–0.8[b]	Spain	[59]
	Carbamazepine, fluoxetine, norfluoxetine, sertraline	Fish muscle	0.83–5.14	United States	[54]
	Desmethylsertraline	Fish muscle	1.65–3.28	Germany	[76]
	Fluoxetine, sertraline, norfluoxetine, and desmethylsertraline	Fish muscle	0.1–1.07	United States	[51]
	Carbamazepine, paroxetine, norfluoxetine, fluoxetine, desmethylsertraline, sertraline, diazepam	Fish muscle	nd–12	Utah, United States	[58]
	Oxazepam	Fish muscle	0.39–13	Sweden	[15]
	Carbamazepine	Fish liver	0.77 ± 0.15	Texas, United States	[46]
		Fish liver	17.9[b]	Spain	[59]
		Fish plasma	693.0 ± 228.6[d]	Texas, United States	[46]
		Fish muscle	1.03 ± 0.51	Texas, United States	[46]

Continued

TABLE 2 Occurrence and Concentration of Pharmaceutical Compounds in Aquatic Biota—Cont'd

Therapeutic Class	Compounds	Matrix	Concentration (ng/g)	Location	References
	Diazepam	Fish liver	23–110	California, United States	[92]
	Fluoxetine, sertraline, norfluoxetine, and desmethylsertraline	Fish liver	0.8–12	United States	[51]
		Fish brain	1.58–15.6	United States	[51]
	Carbamazepine, paroxetine, norfluoxetine, fluoxetine, desmethylsertraline, sertraline, diazepam	Fish liver	<MDL—600	Utah, United States	[58]
	Fluoxetine, norfluoxetine, sertraline, norsertraline, paroxetine, citalopram, fluvoxamine, duloxetine, venlafaxine, bupropion	Fish brain	nd–6.1	United States	[49]
	Fluoxetine	Mollusk homogenate	nd–79.1	United States	[50]
To treat asthma	Salbutamol	Fish homogenate	2.6[b]	Spain	[59]

[a]*Results expressed in wet weight, unless otherwise indicated.*
[b]*Results expressed in dry weight.*
[c]*nd, nondetected.*
[d]*ng/mL for bile and plasma analysis.*

A consideration when analyzing biological samples is that not all tissues retain all the compounds at the same level, and a differential accumulation might imply marked effects due to long-term exposure to pharmaceuticals. As an example, various studies on the uptake of selected pharmaceuticals by fish after controlled exposure showed that the concentration of antidepressants and the analgesic diclofenac exhibited a 25-fold increase in tissues such as liver and brain versus muscle [21,95]. It is noteworthy that many of the studies have focused on muscle tissue, whereas the prevalence and concentration range of some psychiatric drugs and metabolites is much higher in fish liver and brain tissues according to various studies [51,58,92].

This might suggest that bioaccumulation studies should consider the most probable target organ for each family of pharmaceuticals, probably according to their predetermined mode of action in animals and humans. In this scenario, higher bioconcentration factors could be related to their therapeutic mode of action, that is, a psychiatric drug like carbamazepine should be expected to be present at higher levels in the brain. However, in a comparative study performed by Garcia et al., carbamazepine was found at the same concentration range in multiple tissues [46].

Another issue to consider in further work is the relevance of metabolites and transformation products when studying bioaccumulation of pharmaceuticals as they could have equal or higher bioaccumulation capability. Only some published studies have included metabolites as target analytes [48,49,51,52,58,59,76,77], and in some cases, the metabolite was six times more concentrated than the parent compound [58], while in others, only the metabolite was detected [76].

6 CONCLUSIONS

Analytic methodology for the determination of pharmaceutical compounds in complex matrices such as aquatic organisms has advanced greatly over the past few years, particularly the development of highly sensitive analytic tools and instrumentation in combination with selective extraction and purification. However, further advances are required to address analytic challenges, such purification of highly fatty extracts and the consequences associated, that is, matrix effects. Understanding why a compound accumulates in a nontarget organism is poorly developed, so more information on uptake by organisms, absorption, metabolism, and elimination of specific pharmaceuticals is necessary. The study of not only the parent drug but also their metabolites and transformation products is becoming highly relevant and the exposure studies to mixtures of compounds, which emphasizes the need for the corresponding analytic method development to cover this area. A thorough understanding of the presence of pharmaceutical residues on aquatic life on a broad scale is thus necessary to support efforts characterizing ecological and human health risks of pharmaceuticals in the environment.

ACKNOWLEDGMENTS

This work has been cofinanced by Spanish Ministry of Economy and Competitiveness through the project SCARCE (Consolider-Ingenio 2010 CSD2009-00065), the EU Project ECSafeSEAFOOD [FP7-KBBE 311820], and by the European Union through the European Regional Development Fund (FEDER). Prof. Barceló acknowledges King Saud University for his visiting professorship. This work was partly supported by the Generalitat de Catalunya (Consolidated Research Group: Water and Soil Quality Unit 2009-SGR-965).

REFERENCES

[1] B. Halling-Sørensen, S. Nors Nielsen, P.F. Lanzky, F. Ingerslev, H.C. Holten Lützhøft, S.E. Jørgensen, Chemosphere 36 (1998) 357.
[2] D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, H.T. Buxton, Environ. Sci. Technol. 36 (2002) 1202.
[3] D.J. Lapworth, N. Baran, M.E. Stuart, R.S. Ward, Environ. Pollut. 163 (2012) 287.
[4] M. Gros, M. Petrovic, D. Barceló, Talanta 70 (2006) 678.
[5] C. Daughton, Lancet 360 (2002) 1035.
[6] W.W. Buchberger, J. Chromatogr. A 1218 (2011) 603.
[7] A. Pal, K.Y.-H. Gin, A.Y.-C. Lin, M. Reinhard, Sci. Total. Environ. 408 (2010) 6062.
[8] C. Daughton, in: M. Petrovic, D. Barceló (Eds.), Analysis, Fate and Removal of Pharmaceuticals in the Water Cycle, Elsevier, Oxford, 2007, p. 1.
[9] S.D. Richardson, T.A. Ternes, Anal. Chem. 83 (2011) 4614.
[10] J. Corcoran, M.J. Winter, C.R. Tyler, Crit. Rev. Toxicol. 40 (2010) 287.
[11] C. Daughton, T. Ternes, Environ. Heal. Perspect. 107 (Suppl. 6) (1999) 907–938.
[12] J.P. Sumpter, Toxicol. Lett. 82–83 (1995) 737.
[13] S. Jobling, R. williams, A. Johnson, A. Taylor, M. Gross-Sorokin, M. Nolan, C. Tyler, Environ. Heal. Perspect. 114 (Suppl. 1) (2006) 32.
[14] J.L. Oaks, M. Gilbert, M.Z. Virani, R.T. Watson, C.U. Meteyer, B.A. Rideout, H.L. Shivaprasad, S. Ahmed, M.J. Iqbal Chaudhry, M. Arshad, S. Mahmood, A. Ali, A. Ahmed Khan, Nature 427 (2004) 630.
[15] T. Brodin, J. Fick, M. Jonsson, J. Klaminder, Science 339 (2013) 814.
[16] D.M. Pavlović, S. Babić, A.J.M. Horvat, M. Kaštelan-Macan, Trends Anal. Chem. 26 (2007) 1062.
[17] H. Runnqvist, S.A. Bak, M. Hansen, B. Styrishave, B. Halling-Sørensen, E. Björklund, J. Chromatogr. A 1217 (2010) 2447.
[18] M.D. Marazuela, S. Bogialli, Anal. Chim. Acta 645 (2009) 5.
[19] B. Kinsella, J. O'Mahony, E. Malone, M. Moloney, H. Cantwell, A. Furey, M. Danaher, J. Chromatogr. A 1216 (2009) 7977.
[20] M. Moreno-Bondi, M. Marazuela, S. Herranz, E. Rodriguez, Anal. Bioanal. Chem. 395 (2009) 921.
[21] J. Schwaiger, H. Ferling, U. Mallow, H. Wintermayr, R.D. Negele, Aquat. Toxicol. 68 (2004) 141.
[22] A.C. Mehinto, E.M. Hill, C.R. Tyler, Environ. Sci. Technol. 44 (2010) 2176.
[23] J.-M. Kallio, M. Lahti, A. Oikari, L. Kronberg, Environ. Sci. Technol. 44 (2010) 7213.
[24] M. Lahti, J.-M. Brozinski, A. Jylha, L. Kronberg, A. Oikari, Environ. Toxicol. Chem. 30 (2011) 1403.

[25] G.C. Nallani, P.M. Paulos, L.A. Constantine, B.J. Venables, D.B. Huggett, Chemosphere 84 (2011) 1371.
[26] V. Contardo-Jara, C. Lorenz, S. Pflugmacher, G. Nützmann, W. Kloas, C. Wiegand, Aquat. Toxicol. 105 (2011) 428.
[27] R. Cueva-Mestanza, M.E. Torres-Padrón, Z. Sosa-Ferrera, J.J. Santana-Rodríguez, Biomed. Chromatogr. 22 (2008) 1115.
[28] A. Kaufmann, P. Butcher, K. Maden, Anal. Chim. Acta 711 (2012) 46.
[29] R. Fernandez-Torres, M.A.B. Lopez, M.O. Consentino, M.C. Mochon, M.R. Payan, J. Pharm. Biomed. Anal. 54 (2011) 1146.
[30] A. Kirbiš, J. Marinšek, V.C. Flajs, Biomed. Chromatogr. 19 (2005) 259.
[31] A.C. Huet, C. Charlier, G. Singh, S.B. Godefroy, J. Leivo, M. Vehniäinen, M.W.F. Nielen, S. Weigel, P. Delahaut, Anal. Chim. Acta 623 (2008) 195.
[32] R. Delepee, H. Pouliquen, H. Le Bris, Sci. Total. Environ. 322 (2004) 243.
[33] H. Berrada, F. Borrull, G. Font, R.M. Marcè, J. Chromatogr. A 1208 (2008) 83.
[34] D. Faroongsarng, A. Chandumpai, S. Chiayvareesajja, Y. Theapparat, Aquaculture 269 (2007) 89.
[35] U. Schröder, A. Machetzki, Eur. Food Res. Technol. 225 (2007) 627.
[36] M. Meredith-Williams, L.J. Carter, R. Fussell, D. Raffaelli, R. Ashauer, A.B.A. Boxall, Environ. Pollut. 165 (2012) 250.
[37] S.F. Owen, D.B. Huggett, T.H. Hutchinson, M.J. Hetheridge, L.B. Kinter, J.F. Ericson, J.P. Sumpter, Aquat. Toxicol. 93 (2009) 217.
[38] A.M. Al-Ansari, A. Saleem, L.E. Kimpe, V.L. Trudeau, J.M. Blais, J. Chromatogr. B 879 (2011) 3649.
[39] E.B. Dussault, V.K. Balakrishnan, K.R. Solomon, P.K. Sibley, Can. J. Chem. 87 (2009) 662.
[40] A. Azzouz, B. Souhail, E. Ballesteros, Talanta 84 (2011) 820.
[41] J.H. Writer, J.N. Ryan, L.B. Barber, Environ. Sci. Technol. 45 (2011) 7275.
[42] G. Pojana, A. Gomiero, N. Jonkers, A. Marcomini, Environ. Int. 33 (2007) 929.
[43] C. Mimeault, A.J. Woodhouse, X.S. Miao, C.D. Metcalfe, T.W. Moon, V.L. Trudeau, Aquat. Toxicol. 73 (2005) 44.
[44] J.W. Kwon, K.L. Armbrust, D. Vidal-Dorsch, S.M. Bay, J. AOAC Int. 92 (2009) 359.
[45] A. Lajeunesse, G. Vernouillet, P. Eullaffroy, C. Gagnon, P. Juneau, S. Sauve, J. Environ. Monit. 11 (2009) 723.
[46] S.N. Garcia, M. Foster, L.A. Constantine, D.B. Huggett, Ecotoxicol. Environ. Saf. 84 (2012) 207.
[47] B. Subedi, M.A. Mottaleb, C.K. Chambliss, S. Usenko, J. Chromatogr. A 1218 (2011) 6278.
[48] C.D. Metcalfe, S. Chu, C. Judt, H. Li, K.D. Oakes, M.R. Servos, D.M. Andrews, Environ. Toxicol. Chem. 29 (2010) 79.
[49] M.M. Schultz, E.T. Furlong, D.W. Kolpin, S.L. Werner, H.L. Schoenfuss, L.B. Barber, V.S. Blazer, D.O. Norris, A.M. Vajda, Environ. Sci. Technol. 44 (2010) 1918.
[50] R.B. Bringolf, R.M. Heltsley, T.J. Newton, C.B. Eads, S.J. Fraley, D. Shea, W.G. Cope, Environ. Toxicol. Chem. 29 (2010) 1311.
[51] B.W. Brooks, C.K. Chambliss, J.K. Stanley, A. Ramirez, K.E. Banks, R.D. Johnson, R.J. Lewis, Environ. Toxicol. Chem. 24 (2005) 464.
[52] S. Chu, C.D. Metcalfe, J. Chromatogr. A 1163 (2007) 112.
[53] M. Wang, B. Guo, Z. Huang, J. Duan, Z. Chen, B. Chen, S. Yao, J. Chromatogr. A 1217 (2010) 2821.
[54] A.J. Ramirez, M.A. Mottaleb, B.W. Brooks, C.K. Chambliss, Anal. Chem. 79 (2007) 3155.

[55] J.-M. Brozinski, M. Lahti, A. Meierjohann, A. Oikari, L. Kronberg, Environ. Sci. Technol. 47 (2013) 342.
[56] O.P. Togunde, K.D. Oakes, M.R. Servos, J. Pawliszyn, Environ. Sci. Technol. 46 (2012) 5302.
[57] O.P. Togunde, K.D. Oakes, M.R. Servos, J. Pawliszyn, J. Chromatogr. A 1261 (2012) 99.
[58] B. Du, P. Perez-Hurtado, B.W. Brooks, C.K. Chambliss, J. Chromatogr. A 1253 (2012) 177.
[59] B. Huerta, A. Jakimska, M. Gros, S. Rodríguez-Mozaz, D. Barcelò, J. Chromatogr. A 1288 (2013) 63.
[60] M. Gros, S. Rodríguez-Mozaz, D. Barceló, J. Chromatogr. A 1248 (2012) 104.
[61] J. Radjenovic, M. Petrovic, D. Barceló, Trends Anal. Chem. 26 (2007) 1132.
[62] C. Vannini, G. Domingo, M. Marsoni, F. De Mattia, M. Labra, S. Castiglioni, M. Bracale, Aquat. Toxicol. 101 (2011) 459.
[63] S. Wang, K.D. Oakes, L.M. Bragg, J. Pawliszyn, G. Dixon, M.R. Servos, Chemosphere 85 (2011) 1472.
[64] S.N. Zhou, K.D. Oakes, M.R. Servos, J. Pawliszyn, Environ. Sci. Technol. 42 (2008) 6073.
[65] V. Matamoros, D. Calderón-Preciado, C. Domínguez, J.M. Bayona, Anal. Chim. Acta 722 (2012) 8.
[66] R. Carabias-Martinez, E. Rodriguez-Gonzalo, P. Revilla-Ruiz, J. Hernandez-Mendez, J. Chromatogr. A 1089 (2005) 1.
[67] N. Pastor-Navarro, Á. Maquieira, R. Puchades, Anal. Bioanal. Chem. 395 (2009) 907.
[68] L. Kantiani, M. Farré, D. Barceló, Trends Anal. Chem. 28 (2009) 729.
[69] C. Cháfer-Pericás, Á. Maquieira, R. Puchades, J. Miralles, A. Moreno, Anal. Bioanal. Chem. 396 (2010) 911.
[70] A.-C. Huet, P. Delahaut, T. Fodey, S.A. Haughey, C. Elliott, S. Weigel, Trends Anal. Chem. 29 (2010) 1281.
[71] M.S. Díaz-Cruz, D. Barceló, in: M. Petrovic, D. Barceló (Eds.), Analysis, Fate and Removal of Pharmaceuticals in the Water Cycle, Elsevier, Oxford, 2007.
[72] M. Rambla-Alegre, J. Peris-Vicente, J. Esteve-Romero, S. Carda-Broch, Food Chem. 123 (2010) 1294.
[73] K. Kümmerer, J. Environ. Manage. 90 (2009) 2354.
[74] R.J.B. Peters, Y.J.C. Bolck, P. Rutgers, A.A.M. Stolker, M.W.F. Nielen, J. Chromatogr. A 1216 (2009) 8206.
[75] G. Vernouillet, P. Eullaffroy, A. Lajeunesse, C. Blaise, F. Gagné, P. Juneau, Chemosphere 80 (2010) 1062.
[76] B. Subedi, B.W. Du, C.K. Chambliss, J. Koschorreck, H. Rudel, M. Quack, B.W. Brooks, S. Usenko, Environ. Sci. Technol. 46 (2012) 9047.
[77] J. Jeon, D. Kurth, J. Hollender, Chem. Res. Toxicol. 26 (2013) 313.
[78] M.P. Schlüsener, K. Bester, Rapid Commun. Mass Spectrom. 19 (2005) 3269.
[79] M.D. Celiz, J. Tso, D.S. Aga, Environ. Toxicol. Chem. 28 (2009) 2473.
[80] X.S. Miao, C.D. Metcalfe, in: M. Petrovic, D. Barceló (Eds.), Analysis, Fate and Removal of Pharmaceuticals in the Water Cycle, Elsevier, Oxford, 2007.
[81] V.K. Balakrishnan, K.A. Terry, J. Toito, J. Chromatogr. A 1131 (2006) 1.
[82] R. Fernandez-Torres, M.A. Bello Lopez, M.O. Consentino, M.C. Mochón, Anal. Lett. 44 (2011) 2357.
[83] C. Hao, X. Zhao, P. Yang, Trends Anal. Chem. 26 (2007) 569.
[84] R.K. Boyd, Rapid Commun. Mass Spectrom. 7 (1993) 257.
[85] M. Stüber, T. Reemtsma, Anal. Bioanal. Chem. 378 (2004) 910.

[86] A.J. Ramirez, R.A. Brain, S. Usenko, M.A. Mottaleb, J.G. O'Donnell, L.L. Stahl, J.B. Wathen, B.D. Snyder, J.L. Pitt, P. Perez-Hurtado, L.L. Dobbins, B.W. Brooks, C.K. Chambliss, Environ. Toxicol. Chem. 28 (2009) 2587.
[87] C. Franke, G. Studinger, G. Berger, S. Böhling, U. Bruckmann, D. Cohors-Fresenborg, U. Jöhncke, Chemosphere 29 (1994) 1501.
[88] R. van der Oost, J. Beyer, N.P.E. Vermeulen, Environ. Toxicol. Pharmacol. 13 (2003) 57.
[89] J.C. Colombo, N. Cappelletti, M.C. Migoya, E. Speranza, Chemosphere 69 (2007) 1253.
[90] C. Chafer-Pericas, Ã.n. Maquieira, R. Puchades, J. Miralles, A. Moreno, N. Pastor-Navarro, F. Espinós, Anal. Chim. Acta 662 (2011) 177.
[91] W. Li, Y. Shi, L. Gao, J. Liu, Y. Cai, Environ. Pollut. 162 (2012) 56.
[92] K.A. Maruya, D.E. Vidal-Dorsch, S.M. Bay, J.W. Kwon, K. Xia, K.L. Armbrust, Environ. Toxicol. Chem. 31 (2012) 2683.
[93] A.M. Al-Ansari, A. Saleem, L.E. Kimpe, J.P. Sherry, M.E. McMaster, V.L. Trudeau, J.M. Blais, Environ. Pollut. 158 (2010) 2566.
[94] C. Cháfer-Pericás, Á. Maquieira, R. Puchades, B. Company, J. Miralles, A. Moreno, Aquac. Res. 41 (2010) e217.
[95] A. Lajeunesse, C. Gagnon, F. Gagne, S. Louis, P. Cejka, S. Sauve, Chemosphere 83 (2011) 564.

Chapter 7

Application of Bioassays/ Biosensors for the Analysis of Pharmaceuticals in Environmental Samples

Enrique Valera[*,†], Ruth Babington[*,†], Marta Broto[*,†], Salvador Petanas[*,†], Roger Galve[*,†] and Maria-Pilar Marco[*,†]

[*]*Nb4D–Nanobiotechnology for Diagnostics, IQAC-CSIC, Barcelona, Spain*

[†]*Centro de Investigación Biomédica en Red en Bioingeniería Biomateriales y Nanomedicina (CIBER-BBN), Zaragoza, Spain*

Chapter Outline

1 INTRODUCTION

The worldwide pharmaceutical market has been growing for decades and the environmental impact of pharmaceutically active compounds has increased in parallel. Pharmaceutical drugs or medicines are diverse groups of chemical substances used in humans, animals, and plants for the medical diagnosis, cure, treatment, and prevention of different diseases. They can be classified by their chemical properties, the mode of administration, the biological system affected, or their therapeutic effects. An elaborate and widely used classification system, made by the World Health Organization (WHO), is the Anatomical Therapeutic Chemical Classification System (ATC system) [1]. The purpose of this classification is to serve as a tool for drug utilization research in order to improve quality of drug use. Some of the main

Comprehensive Analytical Chemistry, Vol. 62. http://dx.doi.org/10.1016/B978-0-444-62657-8.00007-0

pharmaceutical families included in this classification are antibiotics, hormones, analgesics, antipyretics, antiseptics, cytostatics, and β-blockers.

Global pharmaceutical sales were around $880 billion in 2011, thanks to robust growth in emerging markets, especially China. In Europe, the European Federation of Pharmaceutical Industries and Association estimated in 2011 a production of around €200,000 million in drugs [2]. Obviously, the presence of these pharmaceuticals has become an important parameter of the impact of human activity in the environment. Since the 1990s, water contamination by pharmaceuticals has been an environmental issue of concern. Most pharmaceuticals are deposited in the environment through human and animal consumption and excretion and are often filtered ineffectively by wastewater treatment plants (WWTPs), which are not designed to manage them [3]. In 2009, an investigative report by the Associated Press concluded that US manufacturers had legally released around 270 million pounds of drugs into the environment and estimated that 250 million pounds of pharmaceuticals and contaminated packaging was discarded by hospitals and long-term care facilities.

The US Environmental Protection Agency (EPA) [4] started calling the pharmaceuticals and personal care products (PPCPs) only a few years ago. The PPCPs refers, in general, to any product used by individuals for personal health or cosmetic reasons or used by agribusiness to enhance growth or health of livestock. PPCPs comprise a diverse collection of thousands of chemical substances, including therapeutic drugs, veterinary drugs, fragrances, and cosmetics. Human and veterinary PPCPs can be released into the environment unaltered, metabolized to new hazardous compounds, or excreted as glucuronide or sulfate conjugates that can be easily hydrolyzed to obtain the active parent compounds. While the full effects of most PPCPs on the environment are not understood, there is concern about the potential they have for harm when they act unpredictably by synergisms with other chemicals from the environment or when concentrated in the food chain. Additionally, some PPCPs are active at very low concentrations and are often released continuously in large or widespread quantities (i.e., steroids) [5]. Because of the high solubility of most PPCPs, aquatic organisms are especially vulnerable to their effects. In 2012, Brausch et al. [6] published a review summarizing the toxicity of a large amount of pharmaceutical families and their effects in the aquatic organisms. They conclude that, although there is a large amount of information and studies involving aquatic life, no "intelligent" well-designed aquatic toxicology studies that consider comparative pharmacokinetics and pharmacodynamics (mechanisms-of-action, MOA) have been performed. Concerning this idea, Ankley et al. [7] proposed adverse outcome pathways (AOP) as a conceptual framework to support ecological risk assessments of contaminants. The goal of the AOP is to create a stepwise linkage between molecular initiating events and the resulting adverse outcomes that occur in the organism and population levels. In

addition, certain PPCPs can be deposited in sediments because of their lipophilic characteristics. These substances take a long time or cannot be degraded biologically, and finally can make their way up the food chain and bioaccumulate in the organisms, or produce resistant bacteria in the environment (i.e., antibiotics) [8].

In light of this emerging problem, through early warnings by the scientific community, the authorities and governmental bodies have established several regulations. Concerning the European Commission [9], it has been proposed a new Environment Action Programme for the EU entitled "Living well, within the limits of our planet" that will guide environment policy up to 2020. The proposal aims to enhance Europe's ecological resilience and transform the EU into an inclusive and sustainable green economy. The fundamentals of this proposal are based on a set of directives around the use of pharmaceuticals and their release into the environment. Thus, as an example, Directive 2008/98/EC sets the basic concepts and definitions related to waste management, and Directive 2010/75/EU aims to control and prevent the industrial emissions. In 2006, the European Community defined the regulation (EC/1907/2006) about chemicals and their safe use. It deals with the Registration, Evaluation, Authorisation and Restriction of Chemical substances (REACH) [9]. The aim of REACH is to improve the protection of human health and the environment through the better and earlier identification of the intrinsic properties of chemical substances. At the same time, REACH aims to enhance innovation and competitiveness of the chemical industries. A clear example of what REACH proposed is the list of environmentally classified pharmaceuticals made in 2012 by the Stockholm County Council [10]. This list shows the environmental risks of pharmaceuticals based on the ratio between predicted environmental concentrations and the highest concentrations of the substances that does not have a harmful effect on the environment in terms of persistence, bioaccumulation, and toxicity (PBT). Each of these characteristics is assigned a numerical value. Finally, the total of these values constitutes the PBT index for a specific substance, and it is possible to create an informative sheet for each one.

Because of the activity of these pharmaceuticals and their impact on the environment and the public health, it is mandatory to provide highly sensitive and robust analytical methodologies to control them and their active metabolites, at trace levels. In the food safety field, Council Directive 96/23/EC and 2377/90/EC, Decision 97/747/EC, and Commission Regulation 37/2010 establish the groups of substances to be monitored, the maximum residue limits (MRLs) permitted, and the requirements of the analytical methods that should be used by the veterinary and public health control laboratories to detect residues.

This chapter is focused on the application of bioassays, biochemical assays, and biosensors for the analysis of pharmaceuticals in the environment, in addition to the usual chromatographic methodologies coupled to mass

spectrometry detectors. These types of analytical methodologies can offer important advantages as screening methods due to their simplicity and high-throughput capabilities. The content of this chapter is an update of a previous manuscript published in 2007 [11]. To not reproduce literally the first version, primarily based on general descriptions of the main bioanalytical methodologies, we decided to focus this update on the bioanalytical identification of pharmaceuticals in the environment from 2007 until today, mentioning briefly the techniques used and describing those which have been developed more recently (i.e., microarray technology). Basically, most of the literature found is focused on the main pharmaceutical families that, owing to their actual use and activity, may have a more strong negative environmental impact. Thus, some of the actors will be antibiotics, hormones, analgesics, antipyretics, cytostatic agents, or psychiatric drugs. For some of these substances, there are bioanalytical tools available that have never been applied in the environmental analytical field. Nevertheless, often these bioreagents have been applied to complex biological matrices; therefore, the application to environmental water samples can be considered straightforward. Figure 1 shows the most common pharmaceuticals found in the environment [12].

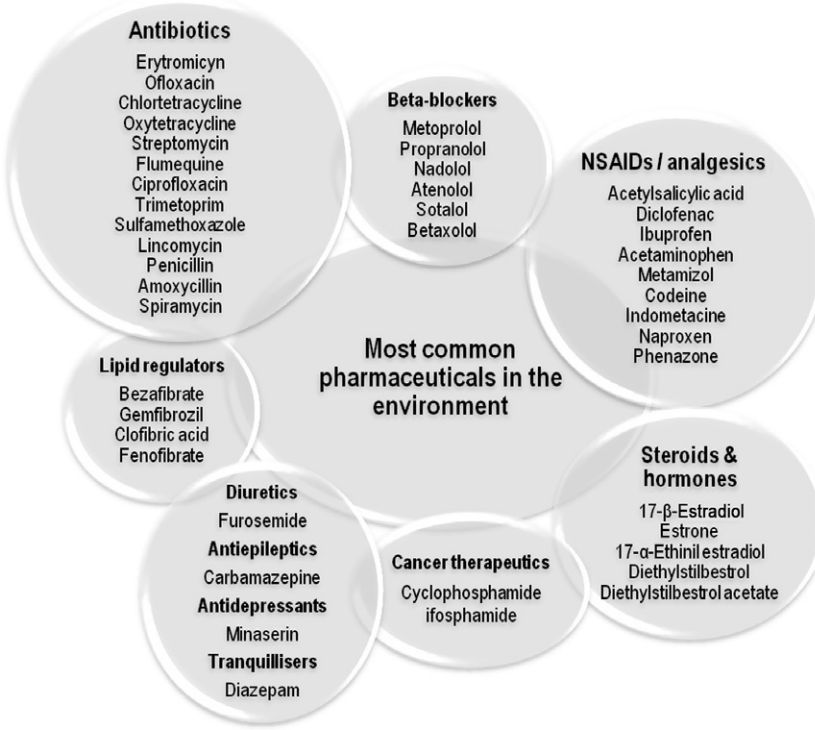

FIGURE 1 Most common pharmaceuticals found in the environment in 2007 [12].

Antibiotics are chemical substances that are able to suppress or kill the growth of microorganisms. There are nine main families including penicillins, cephalosporins, fluoroquinolones, tetracyclines, sulfonamides, amphenicols, aminoglycosides, macrolides, and glycopeptides. Antibiotics are used extensively in human and veterinary medicine, as well as aquaculture, for the purpose of preventing (prophylaxis) or treating microbial infections [13]. This overuse shows a clear correlation between antibiotic consumption and the emergence of resistance strains, being one of the major public health problems [14]. Consumption of these medicines is measured through the defined daily dose (DDD) unit, as recommended by the WHO Collaborating Center for Drug Statistics, and it shows the assumed average maintenance dose per day for a medicine used for its main indication in adults. The use of antibiotics is very difficult to calculate because it varies across European countries. Nevertheless, in 2010, it was possible to calculate an estimation of 20 DDDs per 1000 people per day of the total consumption of antibiotics [15,16]. Clearly, more than 10,000 tonnes of antibiotics are consumed in Europe each year, and 30–60% passes through animals and humans completely unchanged.

Steroid hormones are a group of biologically active compounds controlling human body functions related to the endocrine system and the immune system. Steroids are synthesized from cholesterol and have in common a cyclopentanoperhydrophenanthrene ring. Natural steroids are secreted by the adrenal cortex, testis, ovaries, and placenta in humans and animals and include progestogens, corticoids, androgens, and estrogens [17]. The widespread occurrence of steroid hormones and their metabolites in the natural water resources as well as drinking water is gaining as a growing concern [18]. As a result of the continuous growth of the population and of livestock farming, the level of endogenous hormones excreted into the environment has gradually increased, particularly due to the overuse of synthetic oral contraceptives (SOCs) [19]. As an example, the estimated yearly usage in the United Kingdom of SOCs is about 1700 kg/year, much greater than other estrogens and androgens (about 700 kg/year) [20]. The fraudulent use of hormones in animals to enhance growth and as reproductive aids caused the EU to ban the employment of these substances in food-producing animals (Directive 2003/74/EC). Other international organizations such as US Food and Drug Administration (FDA) [21] and Food and Agriculture Organization (FAO/WHO) [22] have also regulated the misuse of these drugs.

Nonsteroidal anti-inflammatory drugs (NSAIDs) are medicines with analgesic, antipyretic, and anti-inflammatory effects, and one of the reasons for their popularity is that, unlike opioids, they do not produce sedation, respiratory depression, or addiction. NSAIDs have long been used in human medicine and have become accepted as relatively safe. Some of the most prominent members of this group of drugs are aspirin, ibuprofen, diclofenac, and naproxen, all of which are available over the counter (OTC) without

prescription in most countries. Paracetamol has also antipyretic and analgesic properties, but has no anti-inflammatory properties and is therefore not classified as an NSAID. The worldwide consumption of these medicines is very high. Only for aspirin, over 80 billion tablets are taken each year around the world. It has been estimated that more than 30 billion doses of NSAIDs are consumed every year in the United States alone [23]. The use of NSAIDs, and in particular chronic use, increases with age, with an estimated 10–40% of people aged over 65 years using NSAIDs daily [24]. Owing to their hydrophilicity and stability, NSAIDs tend to remain in the aqueous phase and are not totally eliminated by sewage treatment plants (STPs) or WWTPs. As a consequence, these drugs and their metabolites are frequently detected in surface waters [25,26].

Approximately 3000 substances are used as pharmaceutical ingredients. Added to the aforementioned, we can also find antidiabetics, antihypertensives, antidepressants, or cytotoxic drugs, but only a few of them have been included in environmental studies [27]. Liquid chromatography coupled with mass spectrometry (LC–MS) has become the most powerful analytical tool for screening and identification of drugs in environmental samples. However, adequate sample preparation is a key prerequisite aspect of successful quantitative and qualitative analysis. A current trend in pharmaceutical analysis is the reduction of the analysis time and the increase in sample throughput without sacrificing the separation selectivity. In this sense, bioassays and biosensors are techniques that can provide complementary analytical solutions to the chromatographic ones. As mentioned previously, throughout this chapter, we will present some of these techniques currently used for the determination of the pharmaceuticals in the environment. Previously, we should comment that often, some literature reports apply the terms biosensor, biochemical assay, and bioassay indistinctly. We think it is important to correctly define these techniques in order to use a criterion to identify each one.

A *bioassay* is a tool for the determination of a biological activity or the quantification of a target analyte based on this activity, using as a recognition element a bacteria, cell, or tissue. This recognition event is mainly determined by physical or indirect measurement methods. In the food industry, the majority of antibiotic residues are determined through bioassays (i.e., microbial tests for quality control). By *biochemical assay*, we understand an assay where the biorecognition element is a biomolecule such as an enzyme, an oligonucleotide, a protein, or an antibody. Several types of biochemical assays have been described for the detection of small organic molecules (antibiotics, hormones, etc.). A *biosensor* is a self-contained integrated device, consisting of a biological recognition element in direct contact with a transduction element, which converts the biological recognition event into a useable output signal. Biosensors can be classified according to either the method of signal transduction or the biorecognition principle.

2 BIOASSAYS

A bioassay is defined as "the determination of the relative strength of a substance (such as a drug) by comparing its effect on a test organism or an isolated organ preparation with that of a standard preparation." There are a wide variety of bioassays available for the detection of pharmaceuticals in the environment [28]. These depend on the type of observed effect, for example, inhibition of growth, and the biorecognition element (whole cells, tissues, etc.). Bioassays are widely used in drug production when they are used among the standard battery of tests for the evaluation of toxicity of such drugs. For environmental monitoring, bioassays have been used to assess the toxicity of different chemical substances on environmental living organisms (see Figure 2).

One of the most important bioassays developed within the last century has been the Ames test or the *Salmonella* mutagenicity test. Developed by Bruce Ames in 1973 [29] and recently discussed by Mortelmans and Zeiger [30], the Ames *Salmonella* microsome mutagenicity assay is a short-term bacterial reverse mutation assay designed to detect chemical substances that can produce genetic damage leading to gene mutations. The test uses several histidine-dependent Salmonella strains that each carry different mutations in various genes in the histidine operon. When the *Salmonella* tester strains are grown on minimal agar plates containing a trace of histidine, only those

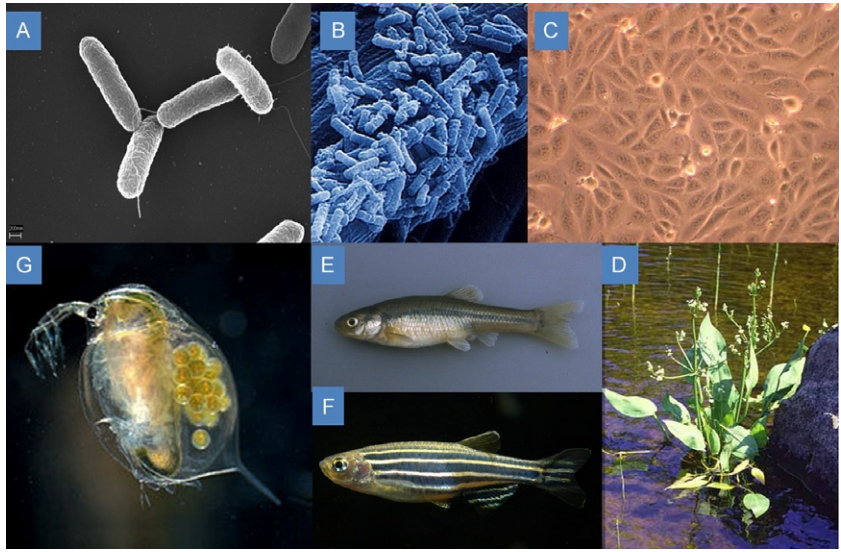

FIGURE 2 Variety of species used in bioassays. (A) *Salmonella typhimurium*; (B) *Bacillus stearothermophilus*; (C) Madin–Darby canine kidney MDCK cells; (D) *Alisma plantago-aquatica*; (E) fathead minnow *Pimephales promelas*; (F) zebra fish *Danio rerio*; (G) *Daphnia magna*.

bacteria that revert to histidine independence (his^+) are able to form colonies. Spontaneously induced revertant colonies occur but when a mutagen is added to the plate, the number of colonies per plate is greatly increased, often in a dose-related manner. While this is a standard test for drug production, its use for environmental monitoring is limited. It has been used for the detection of mutagens/carcinogens in the workplace [31,32]. The US EPA [33] and the Organisation for Economic Co-operation and Development (OECD) [34] both accept the Ames assay as a standard test for mutagenicity and have published standard protocols.

Microbial bioassays can be used for environmental monitoring by observing transformations, growth or mortality, respiration inhibition, and luminescence [35]. The most commonly used assays are the microbial inhibition tests used for the detection of antibiotics. A variety of commercial microbial inhibition tests are available. Strains of bacteria are grown on agar plates. The addition of a sample containing an antibiotic will inhibit the growth. Certain strains have been produced, which are specific to one antibiotic. The incorporation of a color indicator (i.e., bromocresol purple) within the medium allows for easier determination. During the incubation, the growing test culture reduces the specific indicator or modifies the pH of the medium, leading to a color change. A summary of commercially available microbial inhibition tests for testing in milk or meat tissue can be seen in Table 1. These microbial inhibition tests have not been applied to environmental monitoring but rather milk. Microbial tests are cheap, are easily performed on a large scale, and do not require specialized equipment or harmful solvents. The major disadvantages are the possibility of false positives or negatives because of the matrix. The use of confirmatory techniques is always necessary.

Other test organisms applied in bioassays include plants, invertebrates, and fish as well as cell or tissue cultures. Table 2 gives an outline of the types of organisms and tests that have been performed for the evaluation of pharmaceutical contamination. Tests based on the growth response of plants are sensitive but require a long time for growth to occur, for example, 4–6 days for length measurement of root and shoot of plants, 14–30 days for fresh or dry weight measurement, and 21 days for germination of spores [36]. The *Tradescantia micronucleus* (Trad-MCN) bioassay is used for testing environmental mutagenesis. It was first developed as a test system for the gaseous mutagen 1,2-dibromoethane [37]. Klumpp et al. applied this test to the monitoring of urban atmospheres and the test showed an elevated genotoxic potential mainly at sites exposed to severe car traffic emissions [38]. Using the aquatic flowering plants *Ceratophyllum oryzetorum*, *Ranunculus trichophyllus*, and *Alisma plantago-aquatica*, it was able to develop a bioassay for simazine detection with high sensitivities [36].

The invertebrate-based bioassays are standardized tests for the evaluation of the effects of drug exposure on the environment. The two main freshwater toxicity tests with invertebrates that are routinely used are the 21-day *Daphnia*

TABLE 1 Commercially Available Microbial Inhibition Tests

Test	Analyte	Bacterial Strain	Indicator	Time Analysis	LOD ($\mu g\ kg^{-1}$)	Supplier	Reference
BRT MRL test	Penicillins Cephalosporins Macrolides Tetracyclines Sulfonamides Aminoglycosides Amphenicols	*B. stearothermophilus*	Brilliant black	2 h	2–10 4–100 25–200 100–250 100 100–500 2500	AiM (Munchen, Germany)	[55]
Charm cowside	Penicillin Amoxicillin Ampicillin Cloxacillin Ceftiofur Oxytetracycline Sulfamethazine Sulfadimethoxine Gentamicin Tylosin Pirlimycin	*B. stearothermophilus*	Bromocresol purple	2 h	3–4 6 5 30–50 50–100 200–300 100–200 50 300–400 75–100 100–200	Charm Sciences Inc. (MA, United States)	[56,57]
Delvotest SP-NT	Penicillins Sulfonamides Macrolides Aminoglycosides Trimethoprim Dapsone	*B. stearothermophilus*	Bromocresol purple	3 h	1–25 25–250 30–400 50–100 50 0.5	DSM (Delft, the Netherlands)	[55,56,58]

Continued

TABLE 1 Commercially Available Microbial Inhibition Tests—Cont'd

Test	Analyte	Bacterial Strain	Indicator	Time Analysis	LOD ($\mu g\ kg^{-1}$)	Supplier	Reference
Eclipse farm	Penicillins Sulfonamides Tetracyclines Aminoglycosides Cephalosporins	*B. stearothermophilus*	Bromocresol purple	2 h	5–40 100 150 200 8–75	Zeu-Inmunotec (Zaragoza, Spain)	[55]
EuroClone Kalidos TB	Penicillins Aminoglycosides Macrolides Tetracyclines Amphenicols Sulfonamides	*B. stearothermophilus*	Bromocresol purple	3 h	2–30 50–400 200 100 2500 25	EuroClone Spa (Milan, Italy)	[59]
Valio T 101 test	Penicillins Aminoglycosides Macrolides Tetracyclines Quinolones Sulfonamides Novobiocin Chloramphenicol Trimethoprim Dapsone	*S. thermophilus* T101 strain	pH	4 h	2–150 300–1000 30–150 200 1000 200–500 1000 500 2000 5000	Valio Ltd. (Valio, Finland)	[56]

TABLE 2 Bioassays for the Detection of Pharmaceuticals in the Environment Samples Classified According to Biorecognition Element

Organism	Species	Analyte	Sensitivity	Matrix	Time	Observations	Reference
Plant	*Tradescantia hirsutiflora* *Tradescantia subacaulis*	Chlorite Chlorate	0.8 mg L^{-1} 0.4 mg L^{-1}	Water	48 h	Mean frequency of micronuclei in early tetrads of *Tradescantia* inflorescences exposed to solutions for 24 h followed by 24 h recovery	[60]
	Vicia faba	Benzalkonium Dimethyldioctadecylammonium	10 mg L^{-1} 1 mg L^{-1}	Water	7 days	Significant mutagenic effects	[61]
Invertebrates	*Daphnia magna*	Trimethoprim 4-Hydroxyandrostenedione	8.21 mg L^{-1} 4.26 mg L^{-1}	Water	21 days 48 h	Rate of reproduction Acute toxicity testing (growth of daphnids) and chronic toxicity testing (reproduction). Results from acute toxicity tests	[40]
		Ibuprofen	51.4 mg L^{-1}	Water	48 h	Acute toxicity tests	[42]

Continued

TABLE 2 Bioassays for the Detection of Pharmaceuticals in the Environment Samples Classified According to Biorecognition Element—Cont'd

Organism	Species	Analyte	Sensitivity	Matrix	Time	Observations	Reference
Algae	*Pseudokirchneriella subcapitata* *Lemna minor*	Trimethoprim	83.8 mg L^{-1} 27.4 mg L^{-1}	Water		Algal-growth inhibition test Growth inhibition test	[40]
Fish	*Poecilia reticulata*	Trimethoprim	92.6 mg L^{-1}	Water	14 days	Behavior, swimming activity—total traveled distance in 2 min	[40]
	Dreissena polymorpha	Norfluoxetine	0.3 μg L^{-1}	Water	4 h	Effect on spawning	[62]
	Mytilopsis leucophaeata	Fluoxetine	30.9 ng L^{-1}	Water	4 h	Effect on spawning	[62]
	Oreochromis niloticus	Ibuprofen	300 ng L^{-1}	Water	48 h	Acute toxicity tests	[48]
Mollusks	*Sphaerium striatinum*	Norfluoxetine	2.95 μg L^{-1}	Water	4 h	Parturition in clams	[62]
Cell lines	PLHC-1 *Poeciliopsis lucida* hepatoma cell	Doxorubicin Diclofenac Atorvastatin Diazepam Fluoxetine Tamoxifen	1.4 μg L^{-1} 67.6 μg L^{-1} 46 μg L^{-1} 103 μg L^{-1} 6.34 μg L^{-1} 7.43 μg L^{-1}	Water	2–3 days	MTT uptake test (cell viability)	[63]

		Doxorubicin Diclofenac Atorvastatin Diazepam Fluoxetine Tamoxifen	1.18 μg L^{-1} 74.8 μg L^{-1} 43.6 μg L^{-1} 125.3 μg L^{-1} 7.48 μg L^{-1} 7.2 μg L^{-1}	Water	2–3 days	Neutral red uptake test	[63]
	RTG-2, rainbow trout gonadal cell line	Doxorubicin Diclofenac Atorvastatin Diazepam Fluoxetine Tamoxifen	2.55 μg L^{-1} 495 μg L^{-1} 169 μg L^{-1} 172 μg L^{-1} 3.3 μg L^{-1} 7.1 μg L^{-1}	Water	2–3 days	MTT uptake test (cell viability)	[63]
	MELN, MDA-kb2, HG5LN-GR, HG5LN-MR, HELN-PR B	Estrogens Androgens	Different sensitivities (ng L^{-1})	Sewage, sediments			[64,65]
	Transfected reporter cell lines	Estrogens Androgens Xenobiotics	Different sensitivities (ng L^{-1})	River water			[66]
	Human cell-derived CALUX reporter gene	Estrogens, androgens, progesterone, glucocorticoids	Different sensitivities (ng L^{-1})	Wastewater, surface water, sediments			[67,68]
Yeast		Estrogens	1 ng EEQ L^{-1}	Water		REA (RIKILT yeast estrogen bioassay)	[69]
		Estrogens	ng L^{-1}	River water, wastewater, sluge		YES (yeast estrogen screen) YAS (yeast androgen screen)	[70–75]

and the 7-day *Ceriodaphnia* survival and reproduction tests. *Daphnia magna* is used in aquatic toxicology because of its easy culturing, its high sensitivity to toxins, and its clonal method of reproduction. Two types of bioassays are commonly performed with it: 48-h acute tests using neonates that are <24 h old and 21-day chronic life-cycle tests that are run from birth. In the former, the toxicological effect is death; in the latter, it is the inhibition of normal reproduction [39]. It has been employed in toxicity studies of antibiotics [40], steroids [41], analgesics [42], and many other pollutants [43]. These tests are practical but require skilled personnel for culturing and maintaining the organisms. The results are based on visual or microscopic examination and therefore are not suitable alone.

Fish bioassays are very important for environmental monitoring as they are a reflection of the true state of the environment. Fish have distinct physiological and behavioral responses to low levels of pollutants and are therefore good indicators of water quality. Tests are based on larval growth and survival where newly hatched fish of the population after 96 h is measured. Other fish assays measure ATP (adenosine triphosphate) as a biochemical indicator of energy stress in white muscle tissue [44–46]. Commonly used species include zebra fish (*Danio rerio*), atlantic salmon (*Salmo salar*), fathead minnow (*Pimephales promelas*), or rainbow trout (*Oncorhynchus mykiss*). These tests have been performed to determine the toxicity of antibiotics [47], analgesics [48], and psychoactive drugs [49]. In the last few years, the feminization of fish has been observed and many fish bioassays have been performed to assess the role of human pharmaceutical contamination on this phenomenon [50,51]. Lately, the emphasis has been on reducing and replacing acute fish assays with *in vitro* assays using cultured fish cell lines. Cell lines include RTG-2 fibroblasts from rainbow trout, BF-2 fibroblasts from the bluegill sunfish fry, BB fibroblasts from brown bullhead catfish, or FHM epithelioid cells from fathead minnow [35]. For example, the cytotoxicity and genotoxicity of 11 organic fractions from STPs were evaluated using the RTG-2 rainbow trout permanent cell line. An automated *in vitro* micronucleus assay developed for the cell line was used to test the genotoxicity, whereas neutral red uptake, kenacid blue protein assay, and ATP content were used to evaluate cytotoxicity [46].

In vitro assays are not exclusive to fish species. The OECD has published protocols regarding several *in vitro* toxicity assays. The fluorescein leakage (FL) assay is an *in vitro* test that can be used to classify chemicals as ocular corrosives and severe irritants. In the FL test, toxic effects after a short exposure time to test substances are measured by an increase in the permeability of sodium fluorescein through the epithelial monolayer of Madin–Darby canine kidney (MDCK) cells cultured on permeable inserts. The amount of FL is proportional to the chemical-induced damage to the tight junctions, desmosomal junctions, and cell membranes and can be used to estimate the ocular toxicity of a test substance [52]. The MTT assay developed by Mosmann [53] in 1983 determines cell viability. Viability is measured by the enzymatic conversion

of the vital dye MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide into a blue formazan salt that is quantitatively measured after extraction from tissues. Therefore, cytotoxic agents will inhibit this process. *In vitro* assays are numerous and there will be ever-increasingly specific tests being developed depending on cell lines, endpoints, etc. The use of *in vitro* tests for environmental monitoring is still in its infancy. These tests are not capable of successfully detecting one agent from another and are therefore only suitable for evaluating the quality of the environment as a whole. However, for ethical reasons, these tests are more viable than the whole organism tests.

The use of bioassays for chemical monitoring is becoming increasingly doubtful as reflected by the recent decision of the European Commission to consider one such bioassay, the mouse bioassay as having "shortcomings and not considered an appropriate tool for control purposes because of the high variability in results, the insufficient detection capability and the limited specificity" [54]. These disadvantages are not exclusive to the mouse bioassay. As already mentioned, the bioassays are often not specific for an individual chemical substance. The effect observed cannot be conclusively attributed to one chemical substance without further confirmation methods. What bioassays do afford is the ability to observe whether the environment as a whole is observing toxic or adverse effects by substances present within it.

3 BIOCHEMICAL ASSAYS

The main purpose of this manuscript is to update the book chapter written in 2007. In the first edition, we thoroughly delved into the description of the different bioanalytical techniques and its application in the determination of pharmaceuticals in samples, almost all of them, from food safety control (milk, tissue, honey, etc.). When we wrote the first version of this chapter, we realized that almost no biochemical tests applied to the analysis of environmental samples were virtually published, only samples from animal origin. In these last few years, the scientific community and international organizations are increasingly more aware on environmental monitoring, and that is why more and more articles related to the detection of drugs in the environment are being published.

As mentioned earlier, in a biochemical assay, the biorecognition element has been isolated. The biorecognition element consists in a biomolecule such as an enzyme, a nuclear or membrane receptor, or an antibody that recognizes selectively the analyte of interest. The mode of action of each molecule depends on different mechanisms. In the case of enzymes, the mechanism involves the catalytic transformation of the pollutants. Regarding the nuclear receptors, their affinity versus particular endogenous and exogenous substances is exploited. For instance, the affinity of the estrogen receptor (ER) for estrogenic compounds such as estradiol, estrone, and ethinyl estradiol has been used to develop

a variety of methods. One of the most important biorecognition elements are antibodies. Because of the broad variety of specificities that can be achieved, several immunochemical assays have been developed for a great variety of substances. However, the use of these biochemical assays for the detection of pharmaceuticals in the environment has not been frequently reported. The ideal assay should be specific, sensitive, easy to perform, reliable and reproducible, inexpensive, rapid and suitable for automation, able to make a high-throughput screening [76], and with the possibility to quantify multiple analytes in a single assay (multiplexed capabilities) [77,78].

Basically, we can differentiate the biochemical assays depending on their biorecognition element (receptors and antibodies). Many biochemical processes, essential for the functioning and survival of cells (and the organism), are regulated by hormones, neurotransmitters, cytokines, and other "messenger" molecules. This regulation proceeds by interaction of these naturally occurring molecules with receptors that are either embedded in the cell membrane or present in the cytoplasm or in the nucleus of the cell. Receptor-screening methodologies can be based on either the determination of a functional response (i.e., cell proliferation), the production of second messengers (i.e., Ca^{2+}), or the interaction of a ligand with its receptor. Regarding detection methods, receptor assay formats usually require labeling of either the ligand or the receptor. Some of the most common technologies are based on either colorimetric (ELRA, enzyme-linked receptor assay), fluorescence (FRET, fluorescence resonance energy transfer), or chemo/bioluminescence detection systems. Very few examples are described in the literature for environmental detection applications. In 2009, Kase et al. developed an ELRA assay using the human ER-alpha for the detection of 17β-estradiol in sediments with very low sensitivities [79]. In this case, the authors used a secondary anti-ER antibody biotin-labeled for the signal detection.

Alternatively, immunochemical techniques are based on the affinity of the antibody against an antigen. The complex formed has a high-affinity constant that can reach values of around $10^{-10}\,M^{-1}$. This interaction is specific between the antigen and the corresponding antibody. The immunochemical techniques use this characteristic as a powerful tool for the detection of pollutants at low concentrations. Several immunochemical techniques have been developed for the determination of small molecules. The reader can be addressed to several reviews to find more information on immunochemical technologies for residue analysis [11,80–82]. Immunoassays (IAs) are the most frequently used methodologies for the detection of pollutants such as pesticides and other industrial residues at trace levels. They have been applied to the analysis of environmental samples (wastewaters, river waters, sediments, and other kinds of matrices) and complex biological matrices (urine, serum, and saliva). In IAs for small organic molecules such as pharmaceuticals, the reaction antigen–antibody (Ag–Ab) is quantified under competitive

conditions. Therefore, most of these techniques rely on the use of labels that are responsible for the signal generated. In the beginning, the label was always a radioisotope (RIA, radioimmunoassay). However, the use of RIAs was hazardous and there quickly appeared safer strategies such as fluorescent labels (FIA, fluoroimmunoassay) and enzyme labels (EIA, enzyme IA). The EIA offers the possibility to increase detectability by amplifying the signal produced by a substrate. Enzymes commonly used are horseradish peroxidase (HRP), alkaline phosphatase, and glucose oxidase.

The biochemical assays can work under homogeneous or heterogeneous conditions. Enzyme-linked immunosorbent assays (ELISAs) are the most well-known and frequently used heterogeneous formats, where one of the immunoreagents is immobilized onto a solid support. Table 3 summarizes the most recent biochemical assays (since 2007) applied for the detection of pharmaceuticals in the environment. As can be seen, many of them are ELISAs or similar, such as FIA or CLEIA (chemiluminescent enzyme immunoassay). With the dramatic progress in material science, nanotechnology, and bioconjugation techniques, a great diversity of nanomaterials with desirable superior properties have been designed, synthesized, and tailored to facilitate high-performance detections for advanced IAs. Recently, great attention has been focused on the amplification of detectable signals using nanoparticle (NP)-based probes. One major merit of using NPs is that one can control and tailor their properties in a very predictable manner to meet the needs of the specific application. For example, NPs can provide unique chemical and physical properties enabling new advanced functionality such as good biocompatibility, high surface-to-volume ratio, and unique optical properties [83,84]. NPs are usually employed as affinity supports for the immobilization of biomolecules or for the labeling of biomolecules for the amplification of a detectable signal. The processes used to generate, manipulate, and deploy NPs can provide exciting new possibilities for advanced development of new analytical tools and instrumentation for bioanalytical and bionanotechnological applications. Examples of biochemical assays using NPs are fluorescent quantum dot-based IAs [85]; colloidal nanomaterial-based IAs (optical-based agglutination IAs) [86]; or the use of gold [87], colored latex, or carbon NPs [88] as labeling materials (i.e., lateral flow assays [89]). But, for the moment, none of these technologies have been applied yet for the detection of pharmaceuticals in the environment. Nevertheless, a couple of NP-based IAs have been used in wastewater sample detection. The use of magnetic particles has been described to get better kinetics for the detection of sulfamethazine (SMZ) in wastewater samples [90]. In this case, the antibodies are coated on a magnetic particle and a direct competitive IA is carried out. In another example, the secondary antibody can be coated on a magnetic particle surface and used to capture the complex antigen–antibody; this strategy has been applied in a CLEIA for the detection of 17β-estradiol in water [91].

TABLE 3 Biochemical Assays for the Detection of Pharmaceuticals in the Environment Samples

Assay Type	Analyte	Sensitivity	Matrix	Miscellaneous	Reference
ELISA	Monensin	LOD 1.5 μg L^{-1}	Water, soil, manure	Kit commercially available (MaxSignal®)	[97]
ELISA	Norfloxacin	2.2 μg L^{-1}	Water	Indirect competitive assay with pAb	[98]
ELISA	Ofloxacin	1.2 μg L^{-1}	Water, manure, sludge	pAb	[99]
TRFIA	Sulfamethazine Sulfamethoxazole Sulfadiazine	LOD 9.8 ng L^{-1} LOD 6.1 ng L^{-1} LOD 5.4 ng L^{-1}	Water	mAb	[100]
MP-ELISA	Sulfamethazine	<0.03 μg L^{-1}	Waste	Ab conjugated to magnetic particles	[90]
ELISA	Sulfamethoxazole	0.255 μg L^{-1}	Water	Kit commercially available (Abraxis)	[101]
ELISA	Sulfamethoxazole	0.75 μg L^{-1}	Waste	Indirect competitive assay with pAb	[102]
ELISA	Sulfamethoxazole Sulfamethazine	0.25 μg L^{-1} 0.88 μg L^{-1}	Water	Kit commercially available (Abraxis)	[90]
ELISA	Fluoroquinolones	2.5 μg L^{-1}	Water	Indirect competitive assay	[103]
ELISA	Indomethacin	12 μg L^{-1}	Water	Indirect competitive assay with pAb	[104]
ELISA	Carbamazepine	LOQ 0.03 μg L^{-1}	Water, ground, surface	mAb	[105]
ELISA	Carbamazepine	LOD 0.024 μg L^{-1}	Water	Direct competitive assay with mAb	[106]
ELISA	Cotinine	2.5 μg L^{-1}	Water	Direct competitive assay	[107]

ELISA	Levonorgestrel	0.9 μg L^{-1}	Water	Indirect competitive assay with pAb	[108]
ELISA	Levonorgestrel	3.3 μg L^{-1}	Water	Indirect competitive assay with pAb	[109]
LFIA	Progesterone	0.6 μg L^{-1}	Water	mAb	[110]
ELISA	Estradiol	21 ng L^{-1}	Water		[111]
ELISA	Estradiol	2.5 ng L^{-1}	Water	Kit commercially available (Abraxis)	[112]
ELISA	17-Estradiol	243 μg L^{-1}	Water	Indirect competitive assay with pAb	[113]
MP-CLEIA	17β-Estradiol	LOD 2 ng L^{-1}	Water	Indirect competitive chemiluminescent assay with pAb conjugated particles	[91]
FIA	17β-Estradiol	5.4 ng L^{-1}	Water	Indirect competitive assay with pAb	[114]
ELISA	17-Estradiol	18 μg L^{-1}	Water	Indirect competitive assay with pAb	[113]
ELISA	Estrone 17β-Estradiol Estriol	0.1–3 μg L^{-1}	Water	Kit commercially available (Biosense Laboratories AS)	[115]
RIA	Estrone Estradiol Estriol		Water		[116]
ELISA	Estrone 17β-Estradiol Ethinyl estradiol		Wastewater	Comparison with YES assays	[75]
IA	17β-Estradiol	0.32 μg L^{-1}	Water		[117]
ELISA	17β-Estradiol 17α-Ethinyl estradiol	0.5 ng L^{-1}	Water	Indirect competitive microarray assay	[94]
ELRA	17β-Estradiol	0.05 μg L^{-1}	Sediments	Competitive assay using a labeled anti-ER Ab	[79]

Different strategies for rapid and on-site assays are being developed to deal with the growing concerns related to chemical contamination. Research on microarrays as multianalyte biosystems has generated increased interest in the last decade. The main feature of the microarray technology is the ability to simultaneously detect multiple analytes in one sample by an affinity-binding event at a surface interface. Thus, microarray-based analytical systems are attractive alternatives to the classic immunochemical strategies due to their high throughputs, high density, high sensitivity, enhanced reproducibility, low sample consumption, reduced analytical time, and ease of automation. Using microprinting, microspotting, or microstructuring, each probe molecule is patterned on a chosen support to form a highly ordered matrix. The target analytes from samples can be recognized and identified either semiquantitatively or quantitatively. For recognition of target molecules on microarrays, antibody molecules are most commonly used, providing high specificities and sensitivities. A great variety of target analytes capable of interacting selectively with a biomolecular receptor have been adapted to microarrays [92,93]. For example, microarrays have been reported in a DNA-/dye-based competitive IA for the detection of several pollutants including 17β-estradiol in water samples [94]. Basically, the antigen is coated to the microarray surface, and after the immunologic detection, the signal comes from a secondary antibody labeled with a fluorescent DNA probe. This

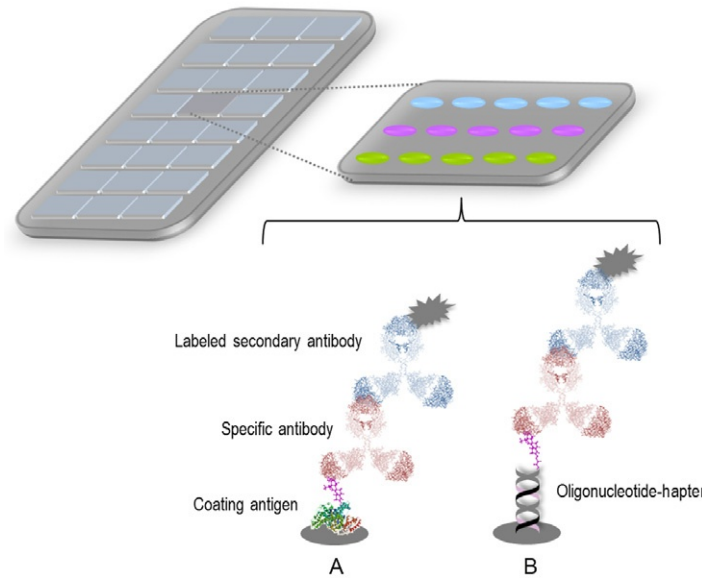

FIGURE 3 Scheme of a couple of microarrays described in the literature. (A) Protein functionalized competitive immunoassay. (B) DNA functionalized competitive immunoassay.

strategy allows multiplexing analysis by site-encoded coating antigens added to the use of different DNA probes. A very similar strategy (see Figure 3) has been used for the detection of androgenic steroids. In that case, the strategy consisted in the direct immobilization of DNA, where one of the oligonucleotide chains is linked to the hapten [95]. It is noteworthy that Rivas et al. published in 2008 an antibody microarray for the environmental monitoring of hundreds of biomarkers simultaneously [96]. Although it is not applied for pharmaceuticals, once the platform has been developed, it is straightforward to add new specific antibodies. This chapter shows clearly the true potential of the technique.

4 BIOSENSORS

This section aims to illustrate with recent examples the development of new biosensor devices for the detection of pharmaceuticals (e.g., antibiotics, hormones, analgesics, and anti-inflammatories), especially in environmental samples.

As was commented earlier, this manuscript is an update of the book chapter written in 2007 by authors from the group. Regarding the biosensor section, in the previous edition, the different transduction techniques and biorecognition principles were explained, and to each technique/principle, examples of biosensors were commented. However, although several new biosensor techniques were exposed, not many of them were applied to the detection of pharmaceuticals in environmental samples. From 2007 to nowadays, the scientific community has increased the number of contributions related to the detection of drugs in the environment. Thus, in this update, the more recent publications related to the analysis of the different types of pharmaceuticals in environmental samples are presented.

As is well known, biosensors are analytical devices consisting of a specific biological element and a transducer. The aim of the specific biological element is to recognize a specific analyte. Thus, the biological element is responsible for the selectivity of the biosensor. On the other hand, the aim of the transducer is to convert the biorecognition process into a measurable signal (Figure 4).

The specific biological element may be an enzyme, antibody, antigen, living cells, tissues, etc. The use of enzymes and antibodies is very popular. Tissue and microbial cells are more complicated to use because they must be kept alive. In the same way, a broad variety of transducers exist, such as Au electrode, interdigitated electrodes, carbon paste electrode, screen-printed electrode, graphite–epoxy composite, piezoelectric crystal, surface plasmon resonance (SPR), fiber-optic, FRET, and bioluminescence resonance energy transfer. These transducers are capable of converting the changes in the biomolecule into different measurable signals such as, electric current, electric potential, conductance, impedance, intensity and phase of

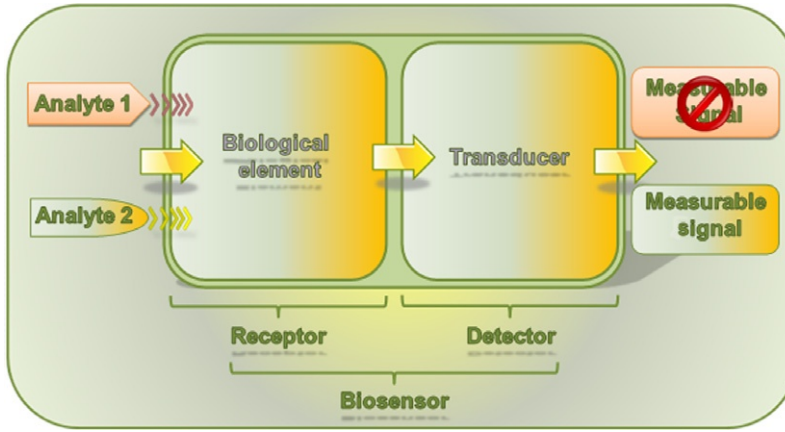

FIGURE 4 Schematic view of the biosensor operation. In the figure, only analyte 2 is recognized by the biological element. As consequence, the transducer converts the biorecognition process into measurable signal.

electromagnetic radiations, absorption, fluorescence, and chemiluminescence. A biosensor classification depending on their biological recognition element or the signal transduction principle used can be seen in Table 4.

In this section, we describe novel nanotechnological and biotechnological approaches. The physical principles and nanotechnological approaches behind the examples should be considered universal and generally applicable to the analysis of other chemical or biological hazards as soon as a bioreceptor is available to detect these substances specifically.

Currently, there are few papers related to the development of biosensors for the analysis of pharmaceuticals applied to environmental samples. However, in the last few years, several biosensors for the analysis of pharmaceuticals in other matrices such as foodstuff have been reported. Hence, in this chapter, the review of biosensors for the analysis of pharmaceuticals applied to environmental samples is complemented with some new biosensor techniques applied to pharmaceuticals detection, especially devoted to the multiplexation, but in other complex matrices.

According to the transduction mechanism, most common classes of biosensors for environmental studies include electrochemical and optical biosensors. For the recognition element, the most common classes include those based on the use of enzymes, whole cells, and immunosensors (see Table 5).

4.1 Hormones

Regarding the analysis of pharmaceuticals applied to environmental samples, using biosensors, researcher interest has mostly been focused on the hormones. Thus, one hormone of great interest in the literature is 17β-estradiol.

TABLE 4 Two Biosensor Classifications According Biological Element and Signal Transduction

Biological recognition element		
Single molecule or molecular complexes	Enzymes	Oxidases, esterases, etc. (i.e., glucose biosensor)
	Antibodies	Monoclonal/polyclonal antibodies (immunosensors) Based on specific Ab–antigen interactions
	Nucleic acids	Genosensors
Cell-based biosensors	Whole cells	Cells are sensitive to environment and can respond to all kinds of stimulants (microbial sensors)
	Tissues	Tissues contains abundance of enzymes
	Organelles	Lysosomes, chloroplasts, and mitochondria
Signal transduction		
Electrochemical	Amperometric	Detection of electroactive species (e.g., electroactive labels) present in biological test samples
	Conductometric	Measure of changes in conductance
	Impedimetric	Based on the change in impedance produced close to transducer surface
	Potentiometric	Determination of the potential difference between an indicator and a reference electrode
Optical	Absorbance	Based on changes in absorbance on an indicator compound
	Evanescent wave	Evanescent wave biosensors use waveguides where the propagation through the waveguide changes due the absorption of molecules to the waveguide surface (e.g., dual polarization interferometry)
	Fluorescence	Based on changes in fluorescence on an indicator compound
	Surface plasmon resonance (SPR)	Electron waves (surface plasmons) on the gold surface are highly dependent on the surface of the gold, then the binding of a target analyte to a receptor on the gold surface is detectable
Thermal	Measure of the absorption or evolution of heat of biological reactions (e.g., enzyme thermistor)	

Continued

TABLE 4 Two Biosensor Classifications According Biological Element and Signal Transduction—Cont'd

Resonant	Measure of the change in the resonance frequency. This change is produced by the change in the refractive index and/or thickness of a resonant waveguide grating due to the association rate between the analyte and its receptor
Ion-sensitive FETs (ISFETs)	The ISFET devices are fabricated using microelectronic technology compatible with CMOS processes (e.g., H^+, K^+, Ca^{2+}, Cl^{2-})
Piezoelectric	Measure of changes in the resonance frequency produced by the binding of a target analyte to a receptor (e.g., surface acoustic waves—SAW)

Estradiol is a sex hormone that is present in females but also in males. The serum levels of estradiol in males (14–55 ng L^{-1}) are comparable to those of postmenopausal women (<35 ng L^{-1}). Estradiol not only has a critical impact on reproductive and sexual functioning but also affects other organs, including the bones.

In 2008, Habauzit et al. [118] demonstrated the direct detection of 17β-estradiol at concentrations above 1.4 μg L^{-1}. The presence of estradiol was monitored by SPR. The ligand-activated ER dimer was detected by its interaction with a specific DNA consensus sequence estrogen response element. The concentration and the nature of the estrogenic compounds modified the SPR signal and were characteristic of the ligand-dependent homodimerization of ER. Although the running buffer for all experiments was 50 mM Tris–HCl, 150 mM NaCl, 10 mM $MgCl_2$, and 0.05% Tween 20 at pH 7.5, the authors said that the results show that SPR-based technology used can be successfully applied to the quantification of estrogenic compounds in water. In 2009, Liu et al. [119] also reported a biosensor for the detection of the hormone 17β-estradiol, but in this case, the transduction was electrochemical. The developed immunosensor features a gold NP|protein G-(LC-SPDP)[1] scaffold, to which a monoclonal antiestradiol capture antibody was immobilized to facilitate a competitive IA between sample 17β-estradiol and a HRP-labeled 17β-estradiol conjugate. Amperometric detection was applied to monitor the reduction current of benzoquinone produced from a catalytic reaction of HRP. Thus, the authors reached a LOD of 3.5 ng L^{-1}. More recently, in 2012, again Liu et al. [120] detected estradiol using an electrochemical immunosensor, but in this case, the calibration of the immunosensor was performed in wastewater samples spiked with 17β-estradiol. In this approach, a competitive IA was conducted between the estradiol–bovine

TABLE 5 Biosensors for the Detection of Pharmaceuticals in the Environment Samples

Pharmaceutical Family	Analyte	Biosensor	Sensitivity	Matrix	Miscellaneous	Reference
Antibiotics	Sulfamethazine	Dipstick	20–5000 μg L^{-1}	River water	Polyclonal antibody	[131]
	Sulfathiazole	Fluorescence	0.11 μg L^{-1} 0.85 μg L^{-1}	Bottled, source, and tap water Honey	Polyclonal antibody	[130]
	Sulfapyridine	Amperometric	0.11 μg L^{-1}	Honey	Polyclonal antibody	[132]
	Enrofloxacin	Colorimetric	100 μg L^{-1}	PBS	Enzyme	[133]
	Enrofloxacin Sulfapyridine Chloramphenicol	SPR	0.34 μg L^{-1} 0.43 μg L^{-1} 0.22 μg L^{-1}	Milk	Polyclonal antibody	[134]
	Enrofloxacin	SPR	0.07 μg L^{-1}	PBS	Polyclonal antibody	[135]
	Tiamulin	Optical SPR	10.8 μg L^{-1} 2.4 μg L^{-1}	Grass Groundwater		[137]
Steroids	17β-Estradiol	SPR	1.4 μg L^{-1}	Buffer	DNA	[118]
		Amperometric	3.5 ng L^{-1}	PBS, serum	Monoclonal antibody	[119]
		Impedimetric	1 ng L^{-1}	River water	Bilayer lipid membrane (s-BLM) modified with Au nanoparticles	[121]
		Electrical conductivity	50–200 ng L^{-1}	Synthetic stream water	Sulfur-oxidizing bacteria	[122]
		Amperometric	12 ng L^{-1}	Wastewater	Monoclonal antibody	[120]
		Fluorescence	0.6 μg L^{-1}	Wastewater	DNA aptamer	[123]

Continued

TABLE 5 Biosensors for the Detection of Pharmaceuticals in the Environment Samples—Cont'd

Pharmaceutical Family	Analyte	Biosensor	Sensitivity	Matrix	Miscellaneous	Reference
	Ethinyl estradiol	Amperometric	0.09 ng L^{-1}	River water	Polyclonal antibody	[124]
	Estriol	SPR	14 ng L^{-1}	Liquid media	Polyclonal antibody	[127]
	Estradiol Ethinyl estradiol Estriol	Fluorescence	0.139 nM 0.191 nM 0.066 nM	River water	ERα-LBD	[128]
	17β-Estradiol Testosterone	Bioluminescent	10 nM 0.1 μM	PBS	Whole cell	[129]
Analgesics Anti-inflammatory	Paracetamol	Amperometric	1 μM	River water	FeTPyPz catalyst	[138]
		Voltammetric	2.5 nM	Serum, urine	Reduction of N-acetyl-p-benzoquinoneimine	[139]
		Voltammetric	0.21 μM	Neutral buffer	Nafion-/TiO_2–graphene-modified GCE	[140]
	Morphine	Voltammetric	0.02 μM	Human urine, injection solutions	ILs (n-hexyl-3-methylimidazolium hexafluorophosphate)	[141]
	Codeine Morphine	Voltammetric	0.041 μg L^{-1} 0.043 μg L^{-1}	Pharmaceutical formulations	DNA	[142]
	5-Aminosalicylic acid	Amperometric	20–600 μM	PBS	Enzyme	[143]
	Diclofenac	Voltammetric	0.04 μM	Buffer, serum, seawater	MWCNTs/$Cu(OH)_2$ nanoparticles/IL-GCE	[145]

serum albumin (BSA) conjugate and the free estradiol for the limited binding sites of estradiol antibody. Square wave voltammetry (SWV) was employed to monitor the electrochemical reduction current of ferrocenemethanol and the SWV current decreased with the increase of estradiol–BSA conjugate concentration at the immunosensor surface. Hence, a detection limit of 12 ng L^{-1} was quantified in wastewaters. In 2010, Xia et al. [121] also developed an electrochemical biosensor for the detection of the natural estrogen 17β-estradiol but in this case applied to river water samples. The authors developed a nanostructure electrochemical biosensor to directly detect and screen estrogenic substances based on ER binding without the use of radio or enzyme-labeled compounds. The biosensor was fabricated by immobilization of ERs in supported bilayer lipid membrane modified with Au NPs, and the detection limit for 17β-estradiol was 1 ng L^{-1}. In addition, estrogenic activity of river water samples determined by this biosensor was in good agreement with that determined by MCF-7 cell proliferation assay. Also in 2010, Van Ginkel et al. [122] reported a novel toxicity detection methodology based on sulfur-oxidizing bacteria (SOB) for the detection of endocrine disrupting compounds in water. In this work, the authors demonstrated this system in compounds such as bisphenol A, nonylphenol, diethylstilbestrol, tributyltin, and estradiol. The SOB biosensor was able to detect these chemicals in the 50–200 ppb range. Besides the biological elements commented before, aptamers have also been applied for the analysis of 17β-estradiol. In 2012, Yildirim et al. [123] developed a reusable evanescent wave aptamer-based optical biosensor for the detection of this hormone in wastewater samples. In this system, β-estradiol 6-(O-carboxymethyl)oxime-BSA was covalently immobilized onto the optical-fiber sensor surface. Then, the samples and the fluorescence-labeled DNA aptamer were premixed, and an indirect competitive assay was performed. The LOD was determined as 0.6 μg L^{-1}. Another hormone of great interest due to its high utilization as part of oral contraceptive is the ethinyl estradiol (EE2). Martínez et al. [124] developed an electrochemical immunosensor based on competitive direct immunoassay between the EE2 present in the river water sample and the EE2-HRP conjugated for the immobilized anti-EE2 polyclonal antibody. The HRP, in the presence of hydrogen peroxide (H_2O_2), catalyzes the oxidation of catechol (Q) whose back electrochemical reduction was detected on gold electrode at 0.0 V. By means of this technique, the detection limit was calculated in 0.09 ng L^{-1}. Estriol (E3), which is only produced in significant amounts during pregnancy, is one of the three main estrogens produced by the human body. Although E3 has demonstrated to reduce the symptomatology of multiple sclerosis [125], it has also been found to be associated with breast cancer [126]. In 2009, Jiang et al. [127] developed a SPR immunosensor for the quantitative evaluation of low levels of an estriol metabolite of estriol (estriol-16-glucuronide, E3-16G) in liquid media. E3-16G was conjugated to ovalbumin (OVA) through an oligoethylene glycol (OEG) linker to form

protein conjugates (E3-16G-OEG-OVA). Then, the bioconjugate was immobilized on a carboxymethyl dextran-coated sensor chip via amine coupling to develop inhibition immunoassays, reaching a limit of detection of 76 ng L^{-1}, using a rabbit antisheep primary antibody as a binding agent. However, this LOD was further improved by using synthesized gold colloids (15 nm) as high mass labels conjugated to the primary antibody. Thus, the LOD reached was 14 ng L^{-1}. In 2009, Le Blanc et al. [128] reported an analytical tool for quantification of estrogenic compounds in river water based on fluorescence-labeled ER-α. The system was based on an advanced labeling procedure for ER-α-ligand-binding domain (ERα-LBD), where the produced protein material was shown to have high affinities towards natural estrogens as well as xenoestrogens, similar to nonlabeled ERα. Using this approach, the authors detected estradiol, EE2, and estrone and reached LODs of 0.139, 0.191, and 0.066 nM, respectively. The system was applied to the analyses of EE2 in spiked river water samples. Although the paper published in 2011 by Roda et al. [129] has not been demonstrated in environmental samples, their contribution is noticeable due to the development of a cell-based biosensor for the simultaneous detection of 17β-estradiol and testosterone. Consequently, a portable biosensing device relying on lensless contact imaging was developed. The device comprises a disposable cartridge containing immobilized bioluminescent (BL) whole-cell biosensors coupled with a CCD detector via a fiber-optic-based taper. For the simultaneous detection, two cell populations, a green-emitting androgen-responsive strain and a red-emitting estrogen-responsive strain, were combined in the same well and dose–response curves for testosterone and 17β-estradiol were obtained. In the case of the testosterone, the EC_{50} was 1×10^{-7} M, while the EC_{50} for the 17β-estradiol was 1×10^{-8} M.

4.2 Antibiotics

Besides hormones, other pharmaceutical compounds of great interest in environmental analysis are the antibiotics.

Sulfonamides antibiotics are a kind of pharmaceutical that are widely used. These antibiotics are employed in the treatment and prevention of bacterial infection in veterinary and human medicine [130]. In 2007, Kandimalla et al. [131] reported a dipstick immunoassay for the detection of SMZ in water, milk, and pig manure. In this approach, the dipstick assay was optimized in terms of the immunoreagent concentration, blocking agents, and incubation times in order to develop intense dot blots on a nitrocellulose membrane for the visual detection test for SMZ. In the case of water, spiked river water was used as a sample (0, 20, 50, 100, 1000, and 5000 μg L^{-1}). In 2010, Jornet et al. [130] developed two optical immunosensors for the selective detection of sulfathiazole (STZ). One of them is based on an immunocomplex capture format, and the other makes use of the HH immunoanalysis mode. In both cases, the signal—fluorescence peak area was related to the analyte concentration. Using the first strategy, the LOD reached was

0.11 μg L^{-1} (total assay time of 18 min). With the second configuration, the LOD was 0.85 μg L^{-1}, but the time of each whole assay was reduced to only 2 min. The authors applied both systems to the analysis of commercial bottled water, source water, and tap water, as native and spiked with STZ at levels from 0 to 50 μg L^{-1}. A new approach for the detection of sulfonamide antibiotic residues was recently reported by Valera et al. [132]. In this work, the detection of sulfapyridine (SPY) was reached using an electrochemical immunosensor based on electrochemical nanoprobes prepared by labeling the specific antibodies with CdS NPs. The system was applied to honey samples reaching a LOD of 0.11 μg/kg. The authors also commented that the use of different electrochemical nanoprobes opens the possibility to obtaining multiplexed electrochemical immunosensors.

Fluoroquinolones are a subset of the family of synthetic broad-spectrum antibacterial drugs, quinolones. Fluoroquinolones are broad-spectrum antibiotics that play an important role in the treatment of serious bacterial infections, in human as well as veterinary medicine. In 2009, Kim et al. [133] developed an immuno-strip biosensor system to detect enrofloxacin (ERFX) residues. The biosensor was based on the combination of immuno-chromatography assay and ELISA techniques. The LOD obtained was 100 ppb in PBS buffer. In 2010, Fernández et al. [134] demonstrated portable multichannel SPR immunosensor for on-site analysis of antibiotics. Although the system was applied to milk samples, this work is noticeable due to the simultaneous detection of enrofloxacin, SPY, and chloramphenicol (CAP) reported. The chips were covalently biofunctionalized with haptenized proteins by means of a previously formed mixed self-assembled monolayer (m-SAM) prepared using two types of mercapto alkyl reagents containing polyethylene glycol units. The samples or standards were mixed with specific polyclonal antibodies and injected into the sensor device. The LODs reached were 0.34, 0.43, and 0.22 μg L^{-1} for ERFX, SPY, and CAP, respectively. More recently, in 2012, Fernández et al. [135] reported a nanogold probe enhanced SPR immunosensor for improved detection of antibiotic residues. By this enhancement, the LOD of ERFX was improved to 0.07 μg L^{-1}, reducing at the same time the amount of primary antibody used.

Another approach is the use of molecularly imprinted polymer (MIPs), not for the detection, but for the removal of pharmaceutical. As an example, in 2013, Tan et al. [136] used molecularly imprinted polymer nanoparticles (nanoMCN@MIPs) for the selective removal of fluoroquinolones in spiked seawater. The nanoMCN@MIPs were prepared by covalent grafting of ofloxacin-imprinted polymer onto the surface of mesoporous carbon nanoparticles (MCNs). The adsorption capacity of the NPs for ofloxacin was 40.98 mg g^{-1}. Other antibiotic drug that is used in veterinary medicine is the tiamulin (TIA). In 2007, Wilson et al. [137] presented the development and validation of a screening method, based on a SPR biosensor, for the TIA determination in grass and groundwater, reaching LODs of 10.8 and 2.4 μg L^{-1}, respectively.

4.3 Analgesics and Anti-Inflammatory Compounds

As it is well known, an analgesic is any member of the diverse group of drugs used to relieve pain and to achieve analgesia, paracetamol (acetaminophen) and the NSAIDs being the most common. On the other hand, anti-inflammatory refers to the property of a substance or treatment that reduces inflammation. In 2010, Oliveira et al. [138] developed a biosensor for the determination of paracetamol using a biomimetic sensor coupled to a flow injection analysis FIA system, based on a modified glassy carbon electrode surface with a Nafion® membrane doped with iron tetrapyridinoporphyrazine (FeTPyPz). Thus, the LOD reached was 1×10^{-6} M. The presented system was applied to the analysis of river water enriched with paracetamol. In 2011, electrochemical sensors for the paracetamol were also reported. Ozcan and Sahin [139] published a system based on the reduction of N-acetyl-p-benzoquinoneimine formed on the electrochemically treated pencil graphite electrode (PGE). The LOD obtained was 2.5 nM in buffer. The developed system was also applied to the detection of paracetamol in human blood serum and urine samples. On the other hand, Fan et al. [140] demonstrated the electrochemical behavior of paracetamol at the Nafion/TiO_2–GR composite film-modified glassy carbon electrode. The LOD reached was 2.1×10^{-7} M in neutral buffer. In 2012, Ensafi et al. [141] demonstrated the voltammetric detection of morphine using a N-hexyl-3-methylimidazolium hexafluorophosphate/multiwall carbon nanotubes paste electrode as a biosensor. The LOD reached was 0.02 μM and the sensor was applied for the determination of morphine in biological and pharmaceutical samples such as human urine and injection solution. Again Ensafi et al. [142] reported in 2013 the simultaneous determination of codeine and morphine. In this work, a DNA-based biosensor was constructed through layer-by-layer technique. Thus, MWCNTs–PDDA was immobilized on the surface of electrochemically pretreated PGE to increase the electron transfer characteristics of the electrode surface. Finally, the dsDNA polyions were immobilized at the surface of MWCNTs–PDDA/PGE. The detection limits were 0.041 and 0.043 μg L^{-1} for codeine and morphine, respectively. The biosensor was applied to validate its capability for the analysis of codeine and morphine in blood serum, urine samples, and pharmaceutical formulations. In 2009, Akkaya et al. [143] reported a catalase–peroxidase-based biosensor for the determination of 5-aminosalicylic acid (5-ASA, mesalazine), which is an anti-inflammatory drug used to treat inflammation of the digestive tract (Crohn's disease). This compound is an aspirin derivative, which is a very effective form of treatment of inflammatory bowel disease. While the linear concentration range was 20–600 μM, the biosensor was applied to the determination of 5-ASA level in Salofalk (medicine 500 mg tablet) but by dissolving the tablet first in phosphate buffer (pH 6.5, 50 mM). Diclofenac is one of the most frequently applied NSAID, and due to its extensive use as an analgesic and anti-rheumatic, diclofenac residues can nowadays be regularly detected in surface

waters throughout the world [144]. In 2012, an electrochemical sensor for the determination of diclofenac was reported by Arvand et al. [145]. This sensor consists of $Cu(OH)_2$ NPs, hydrophobic ionic liquid 1-ethyl-3-methylimidazolium fhexafluorophosphate ($EMIMPF_6$), and multiwalled carbon nanotubes for glassy carbon electrode modification, and the LOD obtained was 0.04 μM. This system was applied to blood serum and seawater using diclofenac sodium—25 tablets and ampoules.

ACKNOWLEDGMENTS

This work has been supported by the European Community (EC, VII. Food, Agriculture and Fisheries, and Biotechnology, KBBE2007-211326) and by the Ministry of Science and Innovation (Contract number CTQ2011-29163-C03-01). Nanobiotechnology for Diagnostics group (Nb4D) is a consolidated *Grup de Recerca de la Generalitat de Catalunya* and has support from the Departaments d'Universitats, Recerca i Societat de la Informació from the Generalitat de Catalunya (expedient 2009 SGR 1343). CIBER-BBN is an initiative funded by the VI National R&D&I Plan 2008–2011, Iniciativa Ingenio 2010, Consolider Program, and CIBER Actions and financed by the Instituto de Salud Carlos III with assistance from the European Regional Development Fund.

REFERENCES

[1] World Health Organization (WHO), The Anatomical Therapeutic Chemical Classification System (ATC), (2003), www.who.int/classifications/atcddd/en/.
[2] European Federation of Pharmaceutical Industries and Associations (FPIA). www.efpia.eu/.
[3] D.S. Aga, Fate of Pharmaceuticals in the Environment and in Water Treatment Systems, CRC Press, Boca Raton, FL, 2008.
[4] US Environmental Protection Agency (EPA). www.epa.gov/.
[5] C.P. Silva, M. Otero, V. Esteves, Environ. Pollut. 165 (2012) 38.
[6] J.M. Brausch, K.A. Connors, B.W. Brooks, G.M. Rand, Human pharmaceuticals in the aquatic environment: a review of recent toxicological studies and considerations for toxicity testing. Rev. Environ. Contam. Toxicol. 218 (2012) 1–99. Ed. D.M. Whitacre. Springer US.
[7] G.T. Ankley, R.S. Bennett, R.J. Erickson, D.J. Hoff, M.W. Horning, R.D. Johnson, D.R. Mount, J.W. Nichols, C.L. Russom, P.K. Schmieder, J.A. Serrano, J.E. Tietge, D.L. Villeneuve, Environ. Toxicol. Chem. 29 (2010) 730.
[8] A. Lupo, S. Coyne, T.U. Berendonk, Front. Microbiol. 3 (2012) 1.
[9] European Commission (EC). http://ec.europa.eu/environment/chemicals/reach/pdf/2007_02_reach_in_brief.pdf.
[10] Janusinfo. Stockholm County Council. http://www.janusinfo.se/Beslutsstod/Miljo-och-lakemedel/About-the-environment-and-pharmaceuticals/Environmentially-classified-pharmaceuticals/.
[11] J.-P. Salvador, J. Adrian, R. Galve, D.G. Pinacho, M. Kreuzer, F. Sánchez-Baeza, M.-P. Marco, in: D. Barceló, M. Petrovic (Eds.), Analysis, Fate and Removal of Pharmaceuticals in the Water Cycle, Elsevier, Amsterdam, The Netherlands, 2007, p. 279.
[12] A. Nikolaou, S. Meric, D. Fatta, Anal. Bioanal. Chem. 387 (2007) 1225.

[13] K. Kümmerer, Chemosphere 75 (2009) 417.
[14] K. Kümmerer, Chemosphere 75 (2009) 435.
[15] OECD, Unmet Health Care Needs, Health at a Glance: Europe 2012, OECD Publishing, Paris, 2012, http://dx.doi.org/10.1787/9789264183896-39-en.
[16] ECDC, Eurosurveillance 18 (2013), www.ecdc.europa.eu.
[17] G.G. Ying, R.S. Kookana, Y.J. Ru, Environ. Int. 28 (2002) 545.
[18] T. Deksissa, UCOWR Conference Paper 17, 2008.
[19] Z.-H. Liu, J.A. Ogejo, A. Pruden, K.F. Knowlton, Sci. Total Environ. 409 (2011) 5149.
[20] T.J. Runnalls, L. Margiotta-Casaluci, S. Kugathas, J.P. Sumpter, Hum. Ecol. Risk Assess. 16 (2010) 1318.
[21] FDA. http://www.fda.gov/AnimalVeterinary/default.htm.
[22] FAO. http://www.fao.org/ag/portal/index_en/en/.
[23] IMS Institute for Healthcare Informatics, The Use of Medicines in the United States: Review of 2010. www.theimsinstitute.org, 2011.
[24] P.G. Conaghan, Rheumatol. Int. 32 (2012) 1491.
[25] A. Gentili, Anal. Bioanal. Chem. 387 (2007) 1185.
[26] M. Farré, M. Petrovic, D. Barceló, Anal. Bioanal. Chem. 387 (2007) 1203.
[27] B. Huerta, S. Rodríguez-Mozaz, D. Barceló, Anal. Bioanal. Chem. 404 (2012) 261.
[28] R.E. Connon, J. Geist, I. Werner, Sensors 12 (2012) 12741.
[29] B.N. Ames, F.D. Lee, W.E. Durston, Proc. Natl. Acad. Sci. U. S. A. 70 (1973) 782.
[30] K. Mortelmans, E. Zeiger, Mutat. Res., Fundam. Mol. Mech. Mutagen. 455 (2000) 29.
[31] M.A. Brewster, Ann. Clin. Lab. Sci. 18 (1988) 306.
[32] J.M. Daisey, F. Mukai, Am. Ind. Hyg. Assoc. J. 40 (1979) 823.
[33] Environmental Protection Agency (EPA), Microbial Bioassay for Toxic and Hazardous Materials (Ames Test for Mutagenicity), EPA-330/9-80-002, (1980).
[34] OECD, Test No. 471: Bacterial Reverse Mutation Test, OECD Publishing, Paris, 1997.
[35] I.E. Tothill, A.P.F. Turner, Trends Anal. Chem. 15 (1996) 178.
[36] Z.Q. Ou, T.H. Sun, H.R. Zhang, Pestic. Sci. 42 (1994) 173.
[37] T.-H. Ma, A.H. Sparrow, L.A. Schairer Anne, F. Nauman, Mut. Res./Genet. Toxicol. 58 (1978) 251.
[38] A. Klumpp, W. Ansel, G. Klumpp, V. Calatayud, J.P. Garrec, S. He, J. Peñuelas, A. Ribas, H. Ro-Poulsen, S. Rasmussen, M.J. Sanz, P. Vergne, Environ. Pollut. 139 (2006) 515.
[39] D. Baird, I. Barber, M. Bradley, P. Calow, A.V.M. Soares, Hydrobiologia 188–189 (1989) 403.
[40] M. De Liguoro, V. Di Leva, M. Dalla Bona, R. Merlanti, G. Caporale, G. Radaelli, Ecotoxicol. Environ. Saf. 71 (2012) 757.
[41] I.R. Barbosa, A.J.A. Nogueira, A. Soares, Ecotoxicol. Environ. Saf. 71 (2008) 757.
[42] S. Han, K. Choi, J. Kim, K. Ji, S. Kim, B. Ahn, J. Yun, J.S. Khim, X.W. Zhang, J.P. Giesy, Aquat. Toxicol. 98 (2010) 256.
[43] Y. Ikenaka, M. Sakamoto, T. Nagata, H. Takahashi, Y. Miyabara, T. Hanazato, M. Ishizuka, T. Isobe, J.-W. Kim, K.-H. Chang, J. Toxicol. Sci. 38 (2013) 131.
[44] P. Couture, C. Blaise, D. Cluis, C. Bastien, Water Air Soil Pollut. 47 (1989) 87.
[45] D.W. Hohreiter, R.E. Reinert, P.B. Bush, Arch. Environ. Contam. Toxicol. 21 (1991) 325.
[46] M.T. Llorente, J.M. Parra, S. Sanchez-Fortun, A. Castano, Water Res. 46 (2012) 6351.
[47] S.P. Carraschi, N.S. Shiogiri, F.P. Venturini, C. de Cruz, A.C.F. Girio, J.G.M. Neto, Boletim Inst. Pesca 37 (2011) 115.
[48] M. Ragugnetti, M.L. Adams, A.T.B. Guimaraes, G. Sponchiado, E.C. de Vasconcelos, C. M.R. de Oliveira, Water Air Soil Pollut. 218 (2011) 361.

[49] H. Richendrfer, S.D. Pelkowski, R.M. Colwill, R. Creton, Behav. Brain Res. 228 (2012) 99.
[50] M.R. Perez, J.I. Fernandino, P. Carriquiriborde, G.M. Somoza, Environ. Toxicol. Chem. 31 (2012) 941.
[51] T. Iguchi, Y. Katsu, H. Urushitani, A. Lange, C.R. Tyler, J. Mar. Sci. Technol. – Taiwan 15 (2007) 29.
[52] OECD, Test No. 460: Fluorescein Leakage Test Method for Identifying Ocular Corrosives and Severe Irritants, OECD Publishing, 2012.
[53] T. Mosmann, J. Immunol. Methods 65 (1983) 55.
[54] European Commission (EC), Commission Regulation (EU) No. 15/2011 Amending Regulation (EC) No 2074/2055 as Regards Recognised Testing Methods for Detecting Marine Biotoxins in live Bivalve Molluscs, (2011).
[55] D. Sierra, A. Contreras, A. Sánchez, C. Luengo, J.C. Corrales, C.T. Morales, C. de la Fe, I. Guirao, C. Gonzalo, J. Dairy Sci. 92 (2009) 4200.
[56] S.M. Andrew, A. Frobish, M.J. Paape, L.J. Maturin, J. Dairy Sci. 80 (1997) 3050.
[57] A. Carlsson, L. Björck, G. Johnsson, Int. Dairy J. 2 (1992) 109.
[58] S.L. Stead, H. Ashwin, S.F. Richmond, M. Sharman, P.C. Langeveld, J.P. Barendse, J. Stark, B.J. Keely, Int. Dairy J. 18 (2008) 3.
[59] Euroclone. www.euroclone.net.
[60] D. Feretti, I. Zerbini, E. Ceretti, M. Villarini, C. Zani, M. Moretti, C. Fatigoni, G. Orizio, F. Donato, S. Monarca, Water Res. 42 (2008) 4075.
[61] F. Ferk, M. Misík, C. Hoelzl, M. Uhl, M. Fuerhacker, B. Grillitsch, W. Parzefall, A. Nersesyan, K. Micieta, T. Grummt, V. Ehrlich, S. Knasmüller, Mutagenesis 22 (2007) 363.
[62] P.P. Fong, N. Molnar, Bull. Environ. Contam. Toxicol. 81 (2008) 535.
[63] D. Caminada, C. Escher, K. Fent, Aquat. Toxicol. 79 (2006) 114.
[64] V. Bellet, G. Hernandez-Raquet, S. Dagnino, L. Seree, P. Pardon, C. Bancon-Montiny, H. Fenet, N. Creusot, S. Aït-Aïsa, V. Cavailles, H. Budzinski, J.-P. Antignac, P. Balaguer, Water Res. 46 (2012) 1912.
[65] S. Kinani, S. Bouchonnet, N. Creusot, S. Bourcier, P. Balaguer, J.M. Porcher, S. Aït-Aïssa, Environ. Pollut. 158 (2010) 74.
[66] W. Mnif, I. Zidi, A.I.H. Hassine, E. Gomez, A. Bartegi, B. Roig, P. Balaguer, Soil Sediment Contam. 21 (2012) 815.
[67] S.C. Van der Linden, M.B. Heringa, H.Y. Man, E. Sonneveld, L.M. Puijker, A. Brouwer, B. Van der Burg, Environ. Sci. Technol. 42 (2008) 5814.
[68] C.J. Houtman, P.E.G. Leonards, W. Kapitejin, J.F. Bakker, A. Brouwer, M.H. Lamoree, J. Legler, H.J.C. Klamer, Sci. Total Environ. 386 (2007) 134.
[69] M.T. Nguyen, R. van der Oost, T.F.H. Bovee, Toxicol. In Vitro 25 (2011) 2003.
[70] X.J. Tang, N. Ullah, C.N. Yu, Z.H. Qin, R.L. Huang, X.L. Zhu, C.F. Shen, Y.X. Chen, Bull. Environ. Contam. Toxicol. 89 (2012) 816.
[71] S. Schmitt, G. Reifferscheid, E. Claus, M. Schlusener, S. Buchinger, Environ. Sci. Pollut. Res. 19 (2012) 3350.
[72] Y.X. Fang, G.G. Ying, J.L. Zhao, F. Chen, S. Liu, L.J. Zhang, B. Yang, Environ. Toxicol. Chem. 31 (2012) 1273.
[73] J. Citulski, K. Farahbakhsh, Chemosphere 87 (2012) 498.
[74] M. Bistan, M. Podgorelec, R.M. Logar, T. Tisler, Food Technol. Biotechnol. 50 (2012) 427.
[75] M. Allinson, F. Shiraishi, S.A. Salzman, G. Allinson, Arch. Environ. Contam. Toxicol. 58 (2010) 576.
[76] R. Macarrón, R.P. Hertzberg, Mol. Biotechnol. 47 (2011) 270.
[77] H. Jiang, M.T. Fan, Anal. Lett. 45 (2012) 1347.

[78] C.A. Marquette, B.P. Corgier, L.J. Blum, Bioanalysis 4 (2012) 927.
[79] R. Kase, P.D. Hansen, B. Fischer, W. Manz, P. Heininger, G. Reifferscheid, Environ. Sci. Pollut. Res. 16 (2009) 54.
[80] L.A.A. de Jong, D.R.A. Uges, J.P. Franke, R. Bischoff, J. Chromatogr. B 829 (2005) 1.
[81] M.-C. Estévez, H. Font, M. Nichkova, J.-P. Salvador, B. Varela, F. Sánchez-Baeza, M.-P. Marco, in: D. Barceló (Ed.), Emerging Organic Pollutants in Waste Waters and Sludge, Springer-Verlag, Berlin Heidelberg, 2005, p. 181.
[82] M.-C. Estévez, H. Font, M. Nichkova, J.-P. Salvador, B. Varela, F. Sánchez-Baeza, M.-P. Marco, in: D. Barceló (Ed.), Emerging Organic Pollutants in Waster Waters and Sludge, Springer-Verlag, Berlin Heidelberg, 2005, p. 119.
[83] D. Tang, Y. Cui, G. Chen, Analyst 138 (2013) 981.
[84] W.H. Hu, C.M. Li, Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol. 3 (2011) 119.
[85] F.A. Esteve-Turrillas, A. Abad-Fuentes, Biosens. Bioelectron. 41 (2013) 12.
[86] B. Teste, S. Descroix, Nanomedicine 7 (2012) 917.
[87] L. Yu, A. Andriola, Talanta 82 (2010) 869.
[88] G.A. Posthuma-Trumpie, J.H. Wichers, M. Koets, L.B.J.M. Berendsen, A. van Amerongen, Anal. Bioanal. Chem. 402 (2012) 593.
[89] G.A. Posthuma-Trumpie, J. Korf, A. van Amerongen, Anal. Bioanal. Chem. 393 (2009) 569.
[90] W.L. Shelver, N.W. Shappell, M. Franek, F.R. Rubio, J. Agric. Food Chem. 56 (2008) 6609.
[91] T.B. Xin, X. Wang, H. Jin, S.X. Liang, J.M. Lin, Z.J. Li, Appl. Biochem. Biotechnol. 158 (2009) 582.
[92] M. Seidel, R. Niessner, Anal. Bioanal. Chem. 391 (2008) 1521.
[93] Z. Zhang, P. Li, X. Hu, Q. Zhang, X. Ding, W. Zhan, Sensors 12 (2012) 9234.
[94] Z.Y. Fan, Y.S. Keum, Q.X. Li, W.L. Shelver, L.H. Guo, J. Environ. Monit. 14 (2012) 1345.
[95] N. Tort, J.P. Salvador, M.P. Marco, R. Eritja, M. Poch, E. Martínez, J. Samitier, Trends Anal. Chem. 28 (2009) 718.
[96] L.A. Rivas, M. Garcia-Villadangos, M. Moreno-Paz, P. Cruz-Gil, J. Gomez-Eivira, V. Parro, Anal. Chem. 80 (2008) 7970.
[97] H. Dolliver, K. Kumar, S. Gupta, A. Singh, J. Environ. Qual. 37 (2008) 1220.
[98] J.L. Cui, K. Zhang, Q.X. Huang, Y.Y. Yu, X.Z. Peng, Anal. Chim. Acta 688 (2011) 84.
[99] X.L. Hou, K.J. Guo, J. Bao, Y.N. Wu, H.R. Li, J. Food Agric. Environ. 9 (2011) 779.
[100] Z. Zhang, J.F. Liu, B. Shao, G.B. Jiang, Environ. Sci. Technol. 44 (2010) 1030.
[101] I. Cernoch, M. Franek, I. Diblikova, K. Hilscherova, T. Randak, T. Ocelka, L. Blaha, J. Environ. Monit. 14 (2012) 250.
[102] N. Pastor-Navarro, E.M. Brun, E. Gallego-Iglesias, A. Maquieira, R. Puchades, J. Environ. Monit. 11 (2009) 1094.
[103] J.Q. Jiang, H.T. Zhang, Z.L. Wang, in: X. Zhou (Ed.), 2011 International Conference on Energy and Environmental Science-ICEES 2011, Elsevier Science BV, Amsterdam, 2011, p. 2756.
[104] N. Skalka, A. Krol, H. Schlesinger, M. Altstein, Anal. Bioanal. Chem. 400 (2011) 3491.
[105] V. Calisto, A. Bahlmann, R.J. Schneider, V.I. Esteves, Chemosphere 84 (2011) 1708.
[106] A. Bahlmann, M.G. Weller, U. Panne, R.J. Schneider, Anal. Bioanal. Chem. 395 (2009) 1809.
[107] S. Nicolardi, S. Herrera, M.J.M. Bueno, A.R. Fernandez-Alba, Anal. Methods 4 (2012) 3364.
[108] C. Pu, Y.F. Wu, H. Yang, A.P. Deng, Anal. Chim. Acta 628 (2008) 73.
[109] M. Shalev, M. Bardugo, A. Nudelman, A. Krol, H. Schlesinger, A. Bronshtein, M. Altstein, Anal. Chim. Acta 665 (2010) 176.
[110] G.A. Posthuma-Trumpie, J. Korf, A. van Amerongen, Anal. Bioanal. Chem. 392 (2008) 1215.
[111] S.H. Wang, H.S. Zhuang, L.Y. Du, S.L. Lin, C.T. Wang, Anal. Lett. 40 (2007) 887.
[112] M. Farre, M. Kuster, R. Brix, F. Rubio, M.J.L. de Alda, D. Barcelo, J. Chromatogr. A 1160 (2007) 166.

[113] E. Caron, C. Sheedy, A. Farenhorst, J. Environ. Sci. Health, Part B 45 (2010) 145.
[114] M.M. Sun, L.Y. Du, S.Q. Gao, Y.H. Bao, S.H. Wang, Steroids 75 (2010) 400.
[115] L. Vigano, E. Benfenati, A. van Cauwenberge, J.K. Eidem, C. Erratico, A. Goksoyr, W. Kloas, S. Maggioni, A. Mandich, R. Urbatzka, Chemosphere 73 (2008) 1078.
[116] S. Surujlal-Naicker, F. Bux, J. Environ. Sci. Health A: Toxic/Hazard. Subst. Environ. Eng. 48 (2013) 37.
[117] C. Yang, B. Hu, A.D. Wheatley, Quantification of Steroid Oestrogens in Wastewater Treatment by EMSA Kits with an Improved Sample Pretreatment Protocol, Shaanxi Science and Technology Publishing House, Xi An, 2008.
[118] D. Habauzit, J. Armengaud, B. Roig, J. Chopineau, Anal. Bioanal. Chem. 390 (2008) 873.
[119] X. Liu, D.K.Y. Wong, Talanta 77 (2009) 1437.
[120] X. Liu, X. Wang, J. Zhang, H. Feng, X. Liu, D.K.Y. Wong, Biosens. Bioelectron. 35 (2012) 56.
[121] W. Xia, Y. Li, Y. Wan, T. Chen, J. Wei, Y. Lin, S. Xu, Biosens. Bioelectron. 25 (2010).
[122] S.W. Van Ginkel, S.H.A. Hassan, S.E. Oh, Chemosphere 81 (2010) 294.
[123] N. Yildirim, F. Long, C. Gao, M. He, H.C. Shi, A.Z. Gu, Environ. Sci. Technol. 46 (2012) 3288.
[124] N.A. Martínez, R.J. Schneider, G.A. Messina, J. Raba, Biosens. Bioelectron. 25 (2010) 1376.
[125] N.L. Sicotte, S.M. Liva, R. Klutch, P. Pfieffer, S. Bouvier, S. Odesa, T.C.J. Wu, R.R. Voskuhl, Ann. Neurol. 52 (2002) 421.
[126] P.E. Lonning, P. Skulstad, J. Steroid Biochem. 33 (1989) 565.
[127] X. Jiang, M. Waterland, L. Blackwell, Y.F. Wu, K.P. Jayasundera, A. Partridge, Steroids 74 (2009) 819.
[128] A.F. Le Blanc, C. Albrecht, T. Bonn, P. Fechner, G. Proll, F. Proll, M. Carlquist, G. Gauglitz, Anal. Bioanal. Chem. 395 (2009) 1769.
[129] A. Roda, L. Cevenini, E. Michelini, B.R. Branchini, Biosens. Bioelectron. 26 (2011) 3647.
[130] D. Jornet, M.A. González-Martínez, R. Puchades, A. Maquieira, Talanta 81 (2010).
[131] V.B. Kandimalla, N. Kandimalla, K. Hruska, M. Freanek, Vet. Med. 52 (2007) 445.
[132] E. Valera, A. Muriano, I. Pividori, F. Sánchez-Baeza, M.-P. Marco, Biosens. Bioelectron. 43 (2013).
[133] Y.K. Kim, H. Kim, J. Ind. Eng. Chem. 15 (2009) 229.
[134] F. Fernández, K. Hegnerová, M. Piliarik, F. Sánchez-Baeza, J. Homola, M.-P. Marco, Biosens. Bioelectron. 26 (2010) 1231.
[135] F. Fernández, F. Sánchez-Baeza, M.-P. Marco, Biosens. Bioelectron. 34 (2012) 151.
[136] F. Tan, D. Sun, J. Gao, Q. Zhao, X. Wang, F. Teng, X. Quan, J. Chen, J. Hazard. Mater. 244–245 (2013) 750.
[137] S. Wilson, T.L. Fodey, I. Traynor, C.T. Elliott, Food Agric. Immunol. 18 (2007) 213.
[138] M.C.Q. Oliveira, M.R.V. Lanza, A.A. Tanaka, M.D.P.T. Sotomayor, Analyt. Methods 2 (2010) 507.
[139] A. Ozcan, Y. Sahin, Anal. Chim. Acta 685 (2011) 9.
[140] Y. Fan, J.H. Liu, H.T. Lu, Q. Zhang, Colloids Surf. B Biointerfaces 85 (2011) 289.
[141] A.A. Ensafi, M. Izadi, B. Rezaei, H. Karimi-Maleh, J. Mol. Liq. 174 (2012) 42.
[142] A.A. Ensafi, E. Heydari-Bafrooei, B. Rezaei, Biosens. Bioelectron. 41 (2013) 627.
[143] A. Akkaya, C. Altug, N.K. Pazarlioglu, E. Dinckaya, Electroanalysis 21 (2009) 1805.
[144] B. Hoeger, B. Köllner, D.R. Dietrich, B. Hitzfeld, Aquat. Toxicol. 75 (2005).
[145] M. Arvand, T.M. Gholizadeh, M.A. Zanjanchi, Mater. Sci. Eng. C: Mater. Biol. Appl. 32 (2012) 1682.

Chapter 8

Removal of Pharmaceuticals by Conventional Wastewater Treatment Plants

Paola Verlicchi[*,†], Elena Zambello[*,†] and Mustafa Al Aukidy[*,†]
[*]*Department of Engineering, University of Ferrara, Ferrara, Italy*
[†]*Terra & AcquaTech Technopole of the University of Ferrara, Ferrara, Italy*

Chapter Outline

Comprehensive Analytical Chemistry, Vol. 62. http://dx.doi.org/10.1016/B978-0-444-62657-8.00008-2

1 INTRODUCTION

More than 15,000 prescription pharmaceutical compounds (PhCs) and over-the-counter (OTC) drugs are registered and approved for use today, corresponding to about 1300 active ingredients [1]. Attention is currently paid to the "origin" of PhCs, as set in the regulations issued by the US FDA [2] and the European Community Directive 2004/27/EC [3], which contains a community code relating to medicinal products for human use, and Regulation 726/2004 [4], which lays down community procedures for the authorization and supervision of medicinal products for human and veterinary use, and an environmental assessment of each *new* compound is mandatory before its launch onto the market and use. Additionally, in June 2007, the European Community Regulation Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) [5] came into force, with the aim of safeguarding human health and the environment through better and earlier identification of the intrinsic properties of chemical substances. As a result, information about the composition of administered PhCs is readily available. Nevertheless, data on their consumption in terms of annual quantity administered in a specific area (country, region, etc.) or for particular users (households, hospitals, healthcare structures, etc.) are much more difficult to obtain, in particular for OTC drugs. Furthermore, consumption patterns vary between study areas (local, regional, and countrywide), making prediction of PhC consumption extremely difficult. Consumption patterns may vary due to the local economic situation, national and local healthcare system organization, and drug prescription guidelines and behavior (recommended average dose and treatment duration), as well as geographic prevalence of certain diseases at particular times. A rough estimate of the global consumption of human PhCs showed that about 100,000 tons of PhCs is used each year, which corresponds to a worldwide average consumption of 15 g/(year *per capita*) [6]. Although more detailed analyses of PhC consumption of specific therapeutic classes by area and by country are available in terms of sales [7], these data do not aid evaluation of the mass flow of PhCs consumed in a specific area over a specific period of time.

Although many investigations have pointed out the environmental risks correlated to the occurrence of pharmaceuticals in aquatic environments (surface and groundwaters) [8] and that the main source is due to wastewater treatment plant (WWTP) discharges [9], up to now, legal limits regarding PhCs have not thus far been set, and no technical guidelines or suggestions as to most suitable treatments for reducing their concentrations in final effluent are yet available [10]. However, recent studies evidenced that hospital effluents can be considered hot-spot sources and the search for appropriate management and treatment of this kind of effluent is an extremely pressing issue [11–13].

Conventional activated sludge (CAS) processes have been employed extensively in WWTPs all over the world, predominantly because they produce a secondary effluent that complies with global and national quality standards for discharge into surface water bodies, and they entail reasonable construction, operating, and maintenance costs. WWTPs were built and upgraded with the principal aim of removing easily or moderately biodegradable carbon, nitrogen, and phosphorus compounds and microbiological organisms, which regularly arrive at the treatment plant in concentrations of the order of mg/L and at least 10^6 MPN/100 mL, respectively. In raw domestic wastewaters, PhCs generally range considerably from 10^{-3} to 10^{-6} mg/L [14], and their chemical and physical properties, namely, solubility, volatility, adsorbability, absorbability, biodegradability, polarity, and stability, also vary greatly [15,16], with obvious repercussions on their behavior during the treatments and consequently their removal efficiencies [17].

Among the many factors governing the complex interactions in wastewaters and treatment systems, trace lipophilic pollutants are likely to be associated with colloids, due to their organic coating [18], on which some PhCs can sorb. In addition, positively charged molecules can become associated to these colloids by means of low-strength van der Waals bonds.

To get an overview of the current situation, a literature search was performed, and the findings are reported in the graph of Figure 1. They were obtained by searching Scopus with the following variables: document type, all; data range, 1997–2012 (included); subject areas, all; and search for, "pharmaceutical activated sludge" or "drug municipal wastewater treatment" or "pharmaceutical sewage." It is quite evident that in the last 6 years, the number of studies dealing with occurrence of PhCs in wastewater and removal by CAS systems has greatly increased. To refine the search, these studies were screened for the terms: "pharmaceutical mass load," "environmental risk assessment," and "pharmaceutical prediction concentration" (Figure 2) and for "activated sludge modeling pharmaceutical compound," "pharmaceutical concentration secondary sludge," and "removal mechanism pharmaceuticals activated sludge" (Figure 3).

These graphs show that in recent years, the main focus of such studies has been environmental risk assessment (636 items), followed by pharmaceutical mass loads (168 items), activated sludge modeling pharmaceutical compound (144), pharmaceutical prediction concentration (143), removal mechanisms

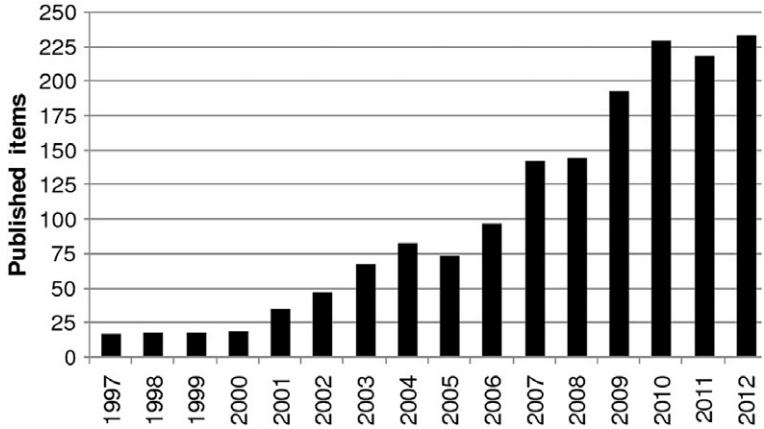

FIGURE 1 Scopus search for relevant publications, reported by year.

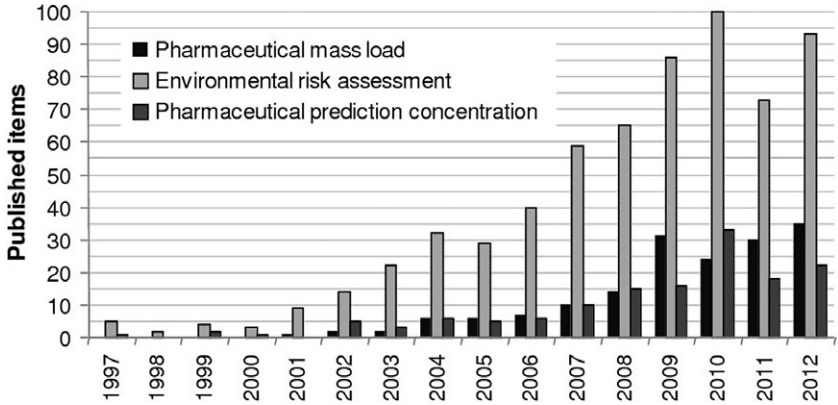

FIGURE 2 Refined search within the results for Pharmaceutical mass load, Environmental risk assessment and Pharmaceutical prediction concentration.

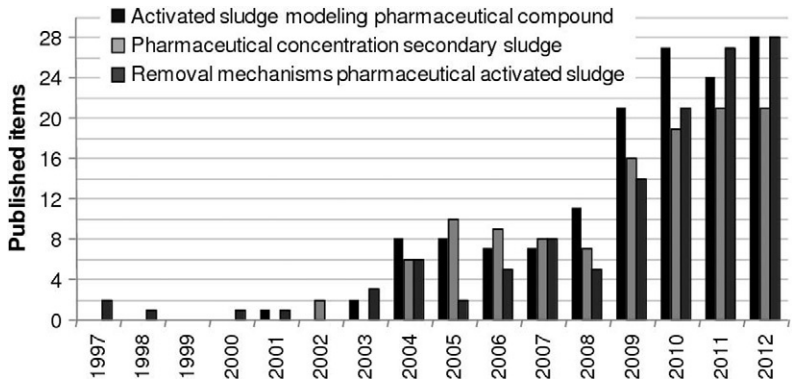

FIGURE 3 Refined search within the results for the three terms reported in the legend.

(124), and, in last place, pharmaceutical concentrations in secondary sludge (119). The following sections will present and discuss the major findings on these topics for selected pharmaceuticals belonging to a wide spectrum of therapeutic classes.

2 CHAPTER FRAMEWORK

The first part of the chapter presents a brief description of the CAS process, focusing on the most common treatment trains for both wastewater and sludge (Section 3). The historical development of the activated sludge process is then discussed, in order to identify the most common reactor configurations, which will then be considered as the chapter progresses. The selection criteria for compounds to include in this study are outlined in Section 4, which also reports the list of selected PhCs grouped according to their therapeutic class.

The occurrence of the selected PhCs in domestic raw influent and CAS effluent is reported in Section 5, while their occurrence in the primary, excess, and treated sludge is detailed in Section 6. Aqueous and overall pharmaceutical removal efficiencies are discussed in Section 7, as well as their percentage partitions (where data available) among effluent, sludge, and removed fraction during secondary biological treatment. How PhC removal efficiencies can be affected by the main chemical and physical properties of selected compounds and operational parameters within the biological reactors is discussed, respectively, in Sections 8 and 9.

The average mass load rankings, based on the collected data pertaining to the secondary effluent and the corresponding average flow rate, are reported and discussed in Section 10. Section 11 outlines an environmental risk assessment of secondary effluent as well as treated sludge and in particular reports results in terms of risk quotient both for the two kinds of CAS outlets. The PhCs are then ranked according to their presence in secondary effluent and sludge, highlighting those with the highest risk and enabling identification of the most critical compounds in terms of load and environmental risk. The aim is to contribute to the debate by raising issues to consider further to reducing the impact of PhCs in secondary effluent and treated sludge, which are generally directly discharged into surface water bodies or applied to the land, respectively. Some indications about the available tools for modeling the behavior of PhCs in CAS are also reported (Section 12).

The chapter concludes with a focus on a special kind of wastewater that contains a great amount of PhCs: the effluent from pharmaceutical manufacturing facilities. The observed concentration ranges of such micropollutants, the treatments commonly adopted for this kind of effluent (mainly CAS), and the lack of specific regulations for the discharge of these contaminants are discussed (Section 13).

All reported concentration data are measured rather than predicted, but it is important to note that they (PhC occurrence in water and sludge, removal

efficiency, and mass load) were reported in a host of previous investigations carried out in different countries and at different times. Hence, the findings are unavoidably affected by uncertainty. For instance, measured PhC concentrations will depend on protocols used for sampling, preparation, conservation, and chemical analysis. Furthermore, removal efficiency is strictly correlated to measured influent and effluent concentrations, while mass load will depend on assumed (average) flow rate and (average) concentration, and the risk quotients are calculated using assumed measured concentrations and predicted no-effect concentrations, and so on [19–22]. Hence, for in-depth analysis of the reported data, the specific cited studies should be consulted. Nevertheless, the data reported and analyzed in this study should provide a snapshot of the current state of affairs and provide a springboard for further debate on this crucial issue.

3 CONVENTIONAL WASTEWATER TREATMENTS

Domestic (also known as urban) wastewaters are generally subjected to a treatment sequence including preliminary treatments (screening, grit removal, and oil and grease removal), a primary gravity settling (sometimes this step is absent), secondary biological treatment (by activated sludge, fixed-film reactors, lagoon systems, and/or sedimentation), and finally tertiary steps, sometimes including advanced treatments (chemical coagulation, flocculation, sedimentation, activated carbon filtration, disinfection, and chemical oxidation). Figure 4 reports the sequences generally adopted for raw wastewater and the resulting sludge.

For the secondary step, activated sludge treatment is that most extensively employed all over the world for processing both urban wastewaters from small and large communities and industrial effluents. This type of treatment was developed by two English researchers, Ardern and Lockett, in 1914, and since then, it has been implemented on a global scale. Activated sludge treatment consists mainly of flocculating microorganisms held in suspension and contact with wastewater in a mixed aerated tank. The so-called CAS system consists of a biological reactor (where activated sludge may develop and

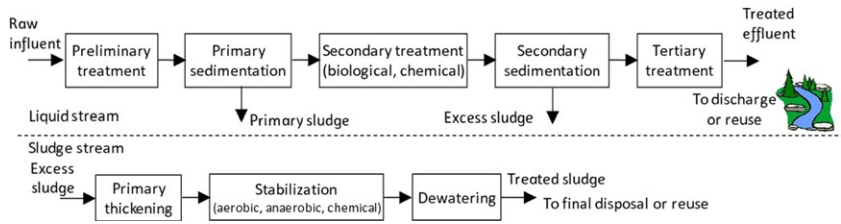

FIGURE 4 Common treatment sequences adopted for domestic effluent and sludge produced during their treatment.

grow) followed by a secondary clarifier: The simplest diagram of this process is that shown in Figure 5, and subsequent configurations developed over the years are shown in Figure 6.

The biological reactor may consist of one (Figures 5 and 6A) or more compartments (Figure 6B–F). Multiple compartments provide different operational conditions, namely, aerobic, anoxic, and anaerobic, and enable C, N, and P removal. Adsorption, absorption, flocculation, oxidation–reduction reactions, and sedimentation are the main physical and biochemical processes occurring within the activated sludge process. Biochemical reactions (anabolic, catabolic, and cometabolic reactions) take place within the biological reactor and bring about the degradation of the organic compounds in the influent wastewater. The reactions are performed by the microorganisms suspended in the liquid, namely, bacteria, protozoa, rotifers, and fungi, which together form the *biomass* (see image on the left in Figure 5), which develops and grows as these reactions take place. Organic compounds subject to biodegradation include not only lipids, proteins, and carbohydrates, which occur at the order of mg/L, but also micropollutants (i.e., pharmaceuticals and personal care products), occurring at concentrations of ng/L or μg/L.

After enough time for the appropriate biochemical reactions, the mixed liquor is transferred to a settling tank (secondary clarifier) to allow gravity separation of the suspended solids (in form of floc particles) from the treated effluent. Some of the settled solids are returned to the biological reactor (return activated sludge) in order to maintain the desired biomass concentration inside (about 3–4 g/L). The remainder is considered waste (the so-called excess sludge) and is subjected to thickening, by removing a portion of the liquid fraction in order to increase its solid content. Through the processes of stabilization, dewatering, drying, and combustion, both the water and organic fractions are considerably reduced, and the processed solids (treated or digested sludge) are suitable for reuse or disposal.

Over the years, different configurations of the activated sludge process were developed to promote nitrification, denitrification, and phosphorus removal. More recent evolutions in CAS include membrane bioreactors (MBRs, Figure 6E) and moving bed biological reactors (MBBRs, Figure 6F). MBRs were developed with the primary aim not only to improve effluent quality but also to upgrade existing WWTPs by replacing the previous secondary settler with a membrane compartment able to better separate the solid from the liquid phase. They generally operate at higher biomass concentrations and higher sludge ages with respect to CAS. MMBRs were designed to enhance biological processes by promoting the growth of both suspended and attached (on the surface of carriers present in the biological reactor) biomass, thereby increasing the biomass concentration in the aeration tank. One of the main advantages of the two new configurations is that they are able to treat a higher pollutant load in the "original" reactor volume [23]. Although these two treatments are becoming more diffuse, CAS is still by far the most common in operation (and most studied).

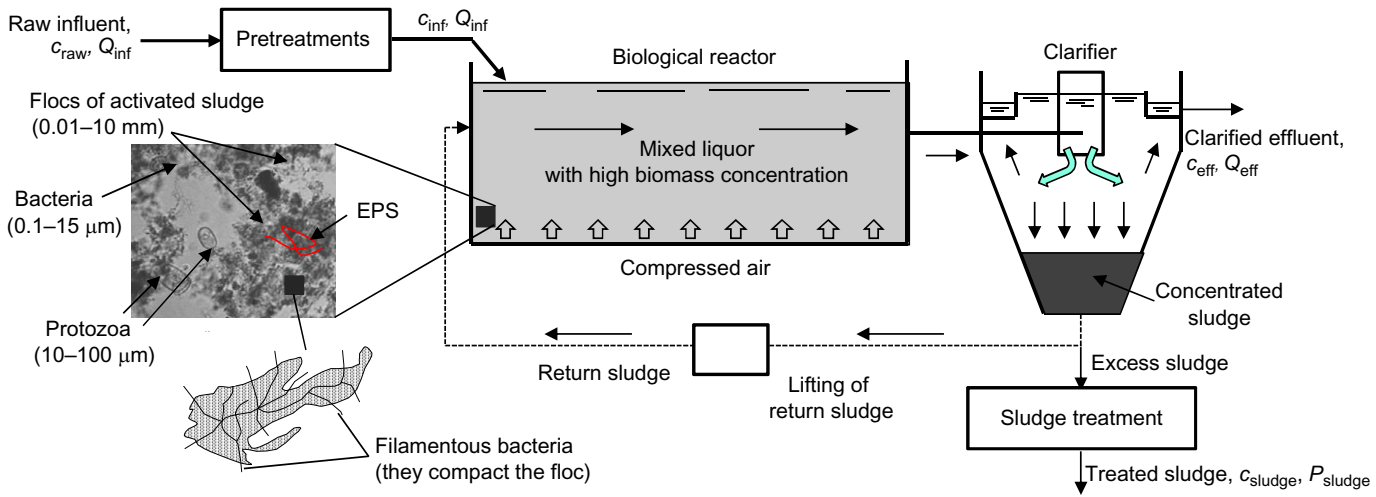

FIGURE 5 Simplified layout of the activated sludge process. On the left, an image obtained by optical microscopy of activated sludge in the presence of protozoa; bottom left, an image and a schematic of a sludge floc containing filamentous bacteria, which make it more robust. EPS, extracellular polymeric substance that acts as a bond between flocs.

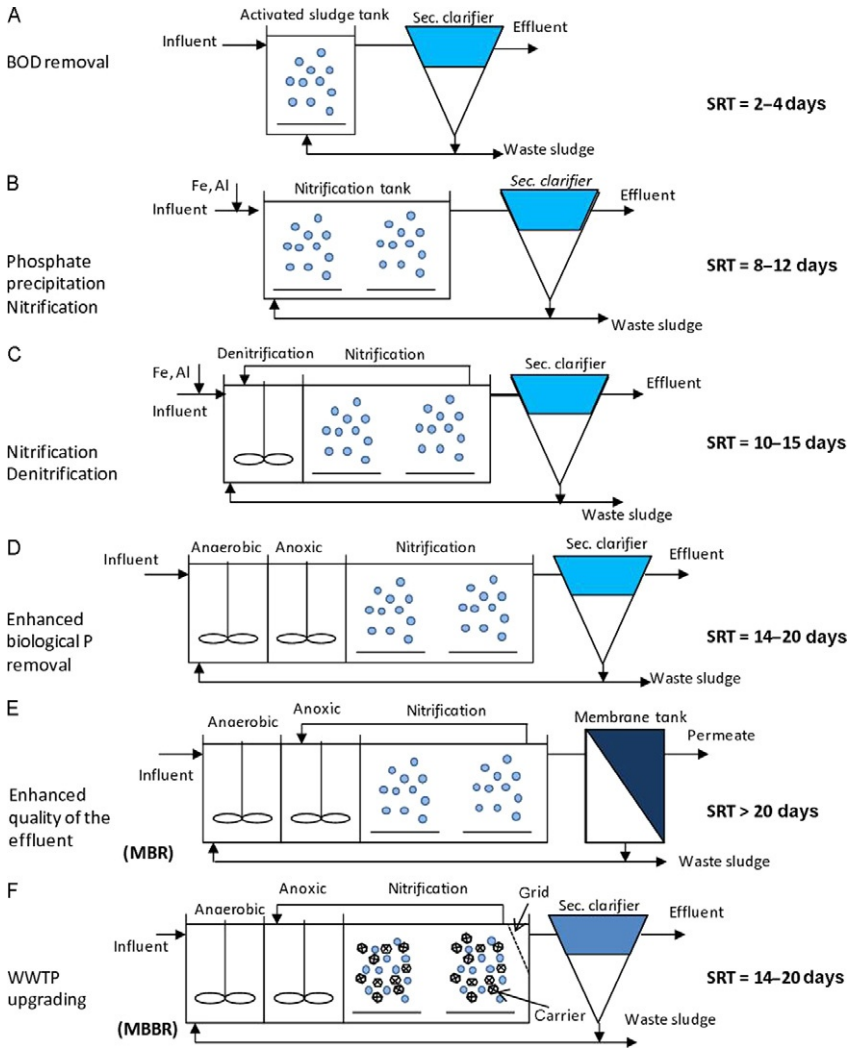

FIGURE 6 Historical development of the activated sludge process through the six schematics: A for BOD removal, B for phosphate precipitation and nitrification, C for nitrification and denitrification, D for enhanced biological P removal, E includes membrane bioreactor, F includes a moving bed bioreactor. The last two schematics (MBR and MBBR) are designed for enhancing the quality of the final effluent and upgrading the existing CAS while maintaining or reducing the existing footprint.

4 PHARMACEUTICAL COMPOUNDS INCLUDED IN THE STUDY

PhCs include a wide spectrum of highly active substances designed to interact with receptors in humans and animals. They are generally grouped into therapeutic classes according to their physiological activity. However, it is worth

noting that these compounds, even if they belong to the same therapeutic class, may have very different chemical structures and chemical–physical properties, resulting in very different behaviors during wastewater treatment.

To narrow the field somewhat, a group of PhCs was selected according to the following criteria: high consumption, widespread occurrence in urban wastewater and treated effluent all over the world, as documented by the recent studies (see in particular [13,14,24–28]), and available analytical methods. By these means, 74 PhCs were selected, spanning the following 15 therapeutic classes: analgesics and anti-inflammatories (A), antibiotics (B), antidiabetics (C), antihypertensives (D), beta-blockers (E), diuretics (F), lipid regulators (G), psychiatric drugs (H), receptor antagonists (I), hormones (J), beta-agonists (K), antineoplastics (L), topical products (M), antiseptics (N), and contrast agents (O). Among these compounds, data pertaining to 64 in water and 54 in sludge were considered, as shown in Table 1.

5 OCCURRENCE IN THE INFLUENT AND IN THE EFFLUENT

Figure 7 shows the occurrence of the selected PhCs, grouped according to their therapeutic class, reported for raw municipal WWTP influent (on the left) and CAS effluent (on the right). These graphs are plotted from data collated in the review by Verlicchi et al. [14] of 244 full-scale CAS systems of different nominal capacities operating in various global locations. The bars of the graph show the variability range observed for each PhC and the corresponding average values measured in the raw influent and secondary effluent. As discussed in [14], measured concentrations generally refer to 24 h composite, flow-proportional, or time-proportional water samples. As reported and discussed in [20–22], the sampling mode may greatly influence the reliability of experimental data.

Referring to the influent, six compounds had an average concentration >10 μg/L, 21 PhCs were detected in the range 1–10 μg/L, and the remaining 37 had a mean concentration below 1 μg/L. The highest average values were found for the analgesics/anti-inflammatories acetaminophen, ibuprofen, and tramadol (all about 30 μg/L), followed by the psychiatric drugs diazepam and gabapentin (on average, respectively, 21 and 13 μg/L) and then the analgesic salicylic acid (17 μg/L). The antibiotics cefalexin, ciprofloxacin, clarithromycin, erythromycin, and sulfapyridine were, on average, detected at concentrations higher than 1 μg/L. The widest variability ranges were observed for the analgesic/anti-inflammatory, antibiotic, and lipid regulator classes. As discussed in [14], and elsewhere, influent concentrations may vary over the course of the day [29], the week [30], and the year [27], depending on many factors, including differences in the nature and consumption patterns of the PhCs in question, as well as CAS influent flow rate.

In general, CAS effluent contains smaller average concentrations than its influent, but they are, nonetheless, far from negligible. Indeed, for

TABLE 1 Selected Compounds Included in This Study

Therapeutic Class	Compounds	Water	Sludge
Analgesics/anti-inflammatories (A)	Acetaminophen, *acetylsalicylic acid*, codeine, diclofenac, *fenoprofen*, ibuprofen, *indomethacin*, ketoprofen, mefenamic acid, naproxen, *phenazone*, *propyphenazone*, salicylic acid, *tramadol*	14	8
Antibiotics (B)	Azithromycin, *cefalexin*, *chloramphenicol*, chlortetracycline, ciprofloxacin, clarithromycin, doxycycline, enrofloxacin, erythromycin, fleroxacin, gatifloxacin, lomefloxacin, *metronidazole*, minocycline, moxifloxacin, norfloxacin, ofloxacin, oxytetracycline, roxithromycin, sarafloxacin, sparfloxacin, *sulfachloropyridazine*, *sulfadimethoxine*, sulfamethoxazole, sulfanilamide, *sulfapyridine*, *sulfasalazine*, *sulfathiazole*, tetracycline, trimethoprim	20	22
Antidiabetics (C)	Glibenclamide, metformin	1	2
Antihypertensives (D)	Diltiazem, hydrochlorothiazide	2	2
Beta-blockers (E)	Atenolol, *bisoprolol*, *celiprolol*, *metoprolol*, propranolol, sotalol	6	3
Diuretics (F)	Furosemide	1	1
Lipid regulators (G)	Bezafibrate, clofibric acid, *fenofibric acid*, gemfibrozil, *pravastatin*	5	3
Psychiatric drugs (H)	Carbamazepine, diazepam, fluoxetine, *gabapentin*, paroxetine	4	4
Receptor antagonists (I)	Cimetidine, famotidine, loratadine, ranitidine	2	4
Hormones (J)	Estradiol E2, estriol E3, estrone E1, ethinyl estradiol EE2	4	4
Beta-agonists (K)	*Salbutamol*	1	0
Antineoplastics (L)	*Ifosfamide*	1	0
Topical products (M)	*Crotamiton*	1	0
Antiseptics (N)	Triclosan	1	1
Contrast agents (O)	Iopromide	1	0

Compounds whose data are only available for water phase are in italics, and compounds whose data are only available in sludge phase are underlined.

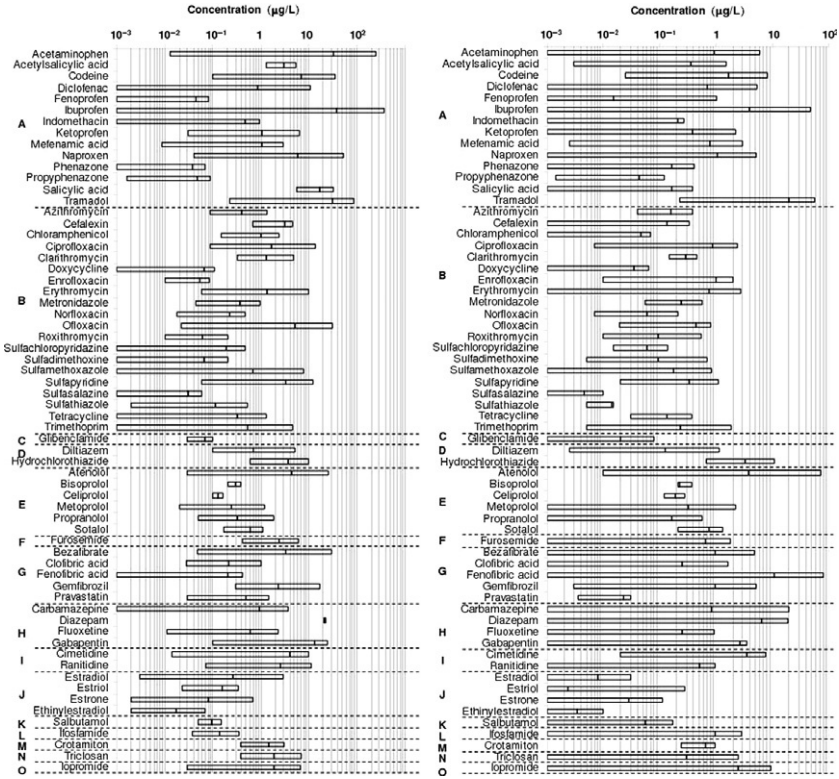

FIGURE 7 Occurrence of the 64 selected PhCs from 15 therapeutic classes in the influent (left side) and effluent (right side) of a conventional activated sludge system.

2 compounds, the mean concentrations were still >10 µg/L (tramadol and fenofibric acid); for 9, they were between 1 and 10 µg/L; and only for the remaining 63 substances were detected effluent levels below 1 µg/L. The highest average values were found for tramadol (20 µg/L as reported by Kasprzyk-Hordern et al. [31] and Wick et al. [32]), fenofibric acid (10 µg/L), diazepam (6.5 µg/L), ibuprofen (3.90 µg/L), atenolol (3.74 µg/L), and cimetidine (3.47 µg/L). Differences in the values observed in the CAS effluent are due not only to different influent concentrations values and the characteristics of the compounds but also to the design and operational characteristics of the WWTP, as will be discussed later.

6 OCCURRENCE IN SEWAGE SLUDGE

Investigations on the occurrence of selected PhCs in sewage sludges from different stages of their treatment have been carried out less often than wastewater investigations. As a result, data pertain to a smaller number of compounds

and a limited number of full-scale treatment plants. The analysis reported here includes 54 common PhCs that were investigated in the major studies on the issue, among them [25,27,28,33–37]. Collected data refer to (generally grab) samples of primary (diverting from the primary clarifier), excess (secondary), and treated (thickened) sludges. The main results, in terms of concentration variability and means of the selected PhCs (grouped according to their therapeutic class), are reported in Figure 8. The number in brackets after the name in the *X*-axis corresponds to the logarithm of solid–liquid distribution coefficient of the compound $\text{Log}\, K_d$ (with K_d in L/kg_{ss}). As discussed in Section 8.6, in an initial analysis, the affinity of a compound for the solid phase is expressed by K_d, which is experimentally determined as the ratio between the concentration of compound sorbed to solid and the concentration of compound in the liquid phase at equilibrium. For most PhCs, removal by sorption is negligible in comparison with the total mass balance, as evidenced by the relatively low K_d values ($K_d < 500\ \text{L/kg}_{ss}$), corresponding to $\text{Log}\, K_d < 2.6$ [38].

In general, data on the presence of PhCs in sludges are few and far between. Antibiotics have been the most analyzed and found to be the most abundant. Other classes investigated in sludges are analgesics and anti-inflammatories, hormones, lipid regulators, psychiatric drugs, and receptor antagonists. An interesting study recently published by Martin et al. [27] details the evolution of the concentration levels of 16 common PhCs (analgesics and anti-inflammatories, antibiotics, lipid regulators, psychiatric drugs, hormones, and beta-blockers) in the sludge treatment sequence over the course of the year. These authors found that the time of year may influence the concentration of PhCs in sludge, mainly due to different seasonal consumption (as for ibuprofen and salicylic acid or some antibiotics) and, to a lesser extent, the changes in degradation rates at the elevated temperatures during the summer season. This was found to apply to PhCs such as carbamazepine and ethinyl estradiol, whose consumption is not influenced by the season. Gao et al. [54] found similar results regarding the concentrations of three types of antibiotics: fluoroquinolones, sulfonamides, and macrolides, whose concentrations were slightly higher in winter than in spring and autumn, due to both a greater consumption and a decline in water use in winter. They concluded that the antibiotics in raw sewage are more prone to transfer from the aqueous to the solid phase in winter, causing an increase in the amount of antibiotics in the sludge.

Martin et al. [27] found that the concentrations of most of the selected compounds increased between primary and secondary sludges, with the exception of diclofenac, ibuprofen, and salicylic acid. They ascribed this behavior to the different physical–chemical properties of the investigated compounds (namely, chemical structures, pK_a, and K_{ow} values) and the different chemical compositions of primary and secondary sludges, which resulted in different absorption/adsorption patterns. The highest concentration of PhCs

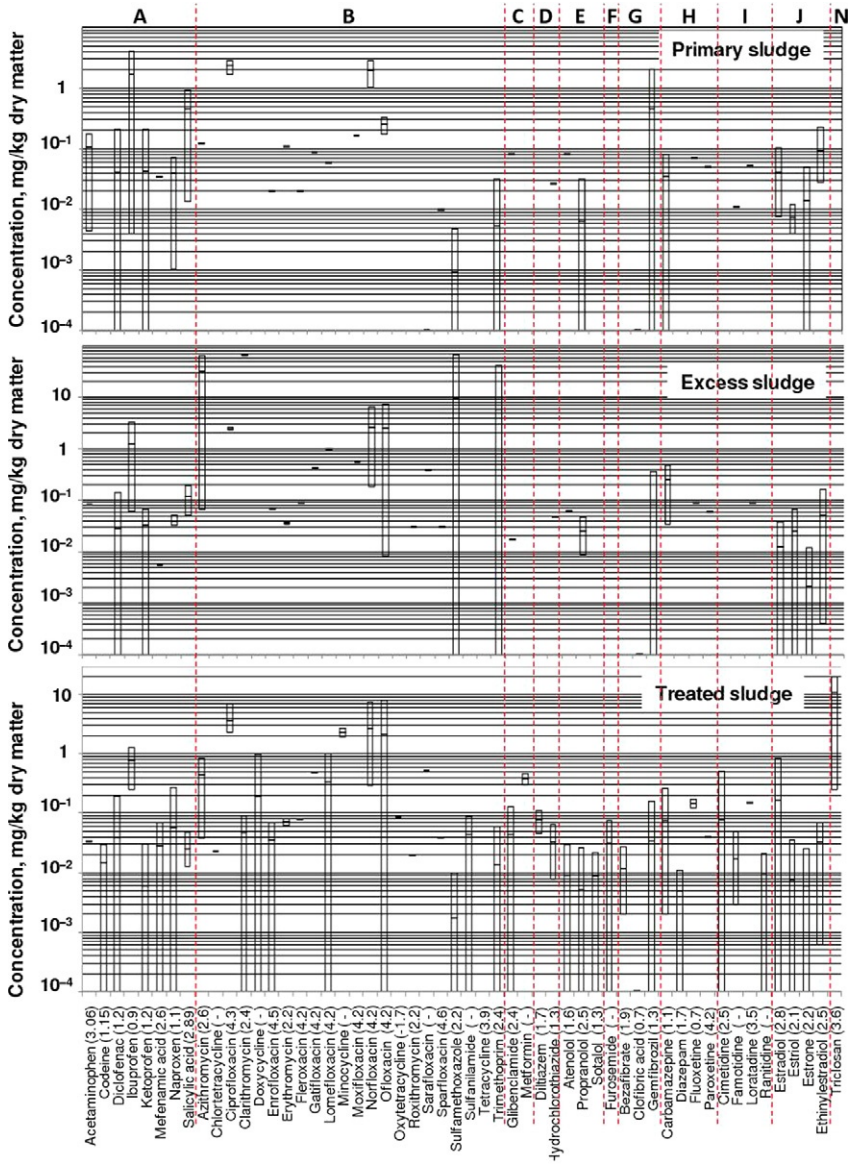

FIGURE 8 Occurrence of selected compounds in primary (top), excess (middle), and treated sludge (bottom). Number in brackets after the name of the compound corresponds to Log K_d value reported in the literature (K_d is in L/kg$_{ss}$). K_d data from [39–41] and sludge concentration data from [25,27,28,36,37,42–53].

found in secondary sludge could be explained by the hydrolysis of conjugates or by the higher organic matter content of secondary sludge, which is mainly composed of biomass, considering that the retention of PhCs occurs mainly in the organic fraction of sewage sludge [42,55]. The higher concentration of diclofenac, ibuprofen, and salicylic acid found in primary sludge could be due to a retention mechanism based on electrostatic interactions [56]. Despite their hydrophilic potential (negative Log K_{ow}), the fluoroquinolones ciprofloxacin and norfloxacin have a high tendency for sorption due to their zwitterionic character ($pK_{a,COOH} = 5.9–6.4$ and $pK_{a,NH2} = 7.7–10.2$) [46].

Martin et al. [27] also noted that the concentrations of most of the investigated PhCs (ibuprofen, naproxen, ketoprofen, salicylic acid, sulfamethoxazole, carbamazepine, propranolol, ethinyl estradiol, and estriol) decrease in an anaerobically treated sludge, contrasting with data reported by Radjenović et al. [26], who detected an increase in ibuprofen, diclofenac, gemfibrozil, loratadine, and glibenclamide. In any case, biodegradation of pharmaceutically active compounds is influenced by desorption of pharmaceuticals from the sludge matrix and microbial activity, and the final outcome will depend on the balance between these two processes in each particular case [52]. An increase in the concentrations of compounds such as ibuprofen, diclofenac, gemfibrozil, loratadine, and glibenclamide could be explained by lower biodegradation potential of the sludge. Triclosan is present at high concentrations in digested sludge; it has a Log K_{ow} of 4.8 and a pK_a of 7.9 and under wastewater conditions (pH about 7) can be considered a hydrophobic compound prone to sorption onto sludge. Gao et al. [57] found that tetracyclines manifest strong sorption to sludge via complexation with metals associated with the sludge and cation-exchange reactions. Their sorption removal is affected by the temperature, pH, and Ca^{2+} and Mg^{2+} concentrations of the sludge, as well as its organic matter content.

As for the psychiatric drugs, paroxetine and fluoxetine were the antidepressants most retained on sludge (they have a high sorption potential as shown by their Log $K_d > 4$), whereas carbamazepine showed a wide variability, but in general, its partition to solids remained quite low.

7 PhC REMOVAL BY CONVENTIONAL WWTPs

Over the last decade, most studies have dedicated more attention to the liquid than the solid phase, assessing its impact on the environment following discharge of the effluent from the treatment plant. For this reason, authors have predominantly evaluated the efficiency of selected PhC removal from the *liquid phase*, considering the raw influent and the treated liquid effluent, but not the sludge produced during either primary or secondary treatment. This removal efficiency can therefore legitimately be termed

the "apparent removal" or "aqueous phase removal," to distinguish it from the overall removal efficiency, which also takes into account the sludge phase.

According to many authors [12,58–63], preliminary treatments and primary settling are generally fairly inefficient at removing PhCs (almost always <10%) from wastewaters. Removal depends mostly on sorption potential to suspended solids deposited during primary sedimentation. In some cases, compounds may even be released during this process, presumably due to the simultaneous presence of deconjugable substances, that is, human metabolites, of these compounds in the raw influent [45,64].

A relatively high removal efficiency has been found for norfloxacin, reported at 28% [46] and even as high as 40% [63]. This latter study also reported high efficiency of removal of tetracycline, 40%, and oxytetracycline, 35%. As regards tetracycline, this has been tentatively ascribed to a strong tendency of the compound to form complexes with iron (III) ions, which may enhance removal by coagulation and flocculation during sedimentation [65].

Leung et al. [63] found that mechanical coarse screening (>6 mm) combined with a very short hydraulic retention time (HRT) (<0.5 h) should not be expected to remove micropollutants. Chemically enhanced sedimentation moderately increased the removal of norfloxacin (47%) and tetracycline (41%) alone.

No significant reduction was found for ibuprofen, ketoprofen naproxen, mefenamic acid, or gemfibrozil [64,66]. This can be correlated to their acidic structures (negative charge of the molecule at pH 7), accompanied by a very low solid–liquid partition coefficient K_d, which results in their presence mainly in the aqueous phase. For the hormone estrone, a higher concentration was observed at the end of primary sedimentation with respect to the influent [64], very likely due to the oxidation of the estradiol present, which would explain the high negative removal efficiencies seen for estrone and the positive reduction of estradiol.

Whatever the configuration of the biological reactor, the main removal mechanisms invariably include biological degradation, adsorption, absorption, flocculation, and sedimentation. Chemical transformations may also occur within the biological reactor and generally consist of deconjugation of certain micropollutants, which is conversion back to their original compounds, but this is not a particularly influential occurrence [67].

The different mechanisms that occur within the biological reactor may be favored by different operational conditions (namely, redox, pH, temperature, sludge retention time (SRT), and HRT) and different reactor configurations (plug-flow or complete-mix reactors, single-tank reactors, or reactors in series with alternate anoxic–oxic–anaerobic compartments), as discussed in Section 9.

CAS processes are not able to efficiently remove all the different kinds of PhCs [68] for various reasons. In particular, PhCs are designed to be

biologically stable, and their sorption tendency depends on the types and properties of both the suspended solids (sludge) and the PhC molecule, not to mention the conditions inside the bioreactor, mainly pH, redox potential, and temperature.

As preliminary and primary treatments are fairly inefficient at removing PhCs, raw influent can be considered to possess the same pollutant load as the influent to the biological tank (hence, $c_{raw} = c_{inf}$ in Figure 5). Moreover, Q_{inf} can be assumed as equal to Q_{eff}. As a consequence, removal from the liquid phase $\eta_{aqueous}$ can be evaluated by applying Equation (1):

$$\eta_{aqueous} = \frac{Q_{inf}\,c_{inf} - Q_{eff}\,c_{eff}}{Q_{inf}\,c_{inf}} \times 100 = \frac{c_{inf} - c_{eff}}{c_{inf}} \times 100 \quad (1)$$

where Q is the average influent (subscript inf) or effluent (subscript eff) flow rate expressed in terms of L/d and c is the average concentration in the influent (subscript inf) or in the effluent (subscript eff), μg/L, as shown in Figure 5.

A limited number of investigations have thus far considered the WWTP as a whole: a black box with only *one* inlet (influent water) and *two* outlets (namely, effluent water and treated sludge). Accordingly, the overall removal efficiency can be evaluated by means of Equation (2):

$$\eta_{overall} = \frac{Q_{inf}\,c_{inf} - \left(Q_{eff}\,c_{eff} + P_{sludge}\,c_{sludge}\right)}{Q_{inf}\,c_{inf}} \times 100 \quad (2)$$

where P_{sludge} is the sludge production rate (tons/d) and c_{sludge} is the concentration of PhC in the treated sludge (ng/g dry matter).

The difference between overall and aqueous removal is the fraction that is sorbed to sludge matter; as a consequence, $\eta_{aqueous}$ is expected to be higher than $\eta_{overall}$.

Figure 9 shows the variability ranges and the mean value of the removal efficiencies $\eta_{aqueous}$ for the 64 selected PhCs (listed in Table 1) based on data presented in the review by Verlicchi et al. [14]. The graph only reports PhC removal and does not show any release that may occur. An in-depth analysis of this is reported in the cited review, whereas in this chapter, only a few cases will be discussed.

Out of the 64 compounds, data are not available for four PhCs: the antibiotic sulfasalazine, the beta-blockers bisoprolol and celiprolol, and the antineoplastic ifosfamide. The best average removal efficiencies (>75%) were found for 15 PhCs, with the highest values (>95%) for salicylic acid, estriol, and chloramphenicol. Twenty-three compounds showed good removal, in the range 50–75%, whereas for 17 compounds, the removal was modest (25–50%) and quite low for the remaining compounds, as in the case of metoprolol, fenofibric acid, tramadol, carbamazepine, and diazepam.

As mentioned earlier, the extent to which a compound can be removed in a CAS system depends on many factors: the chemical and physical properties of

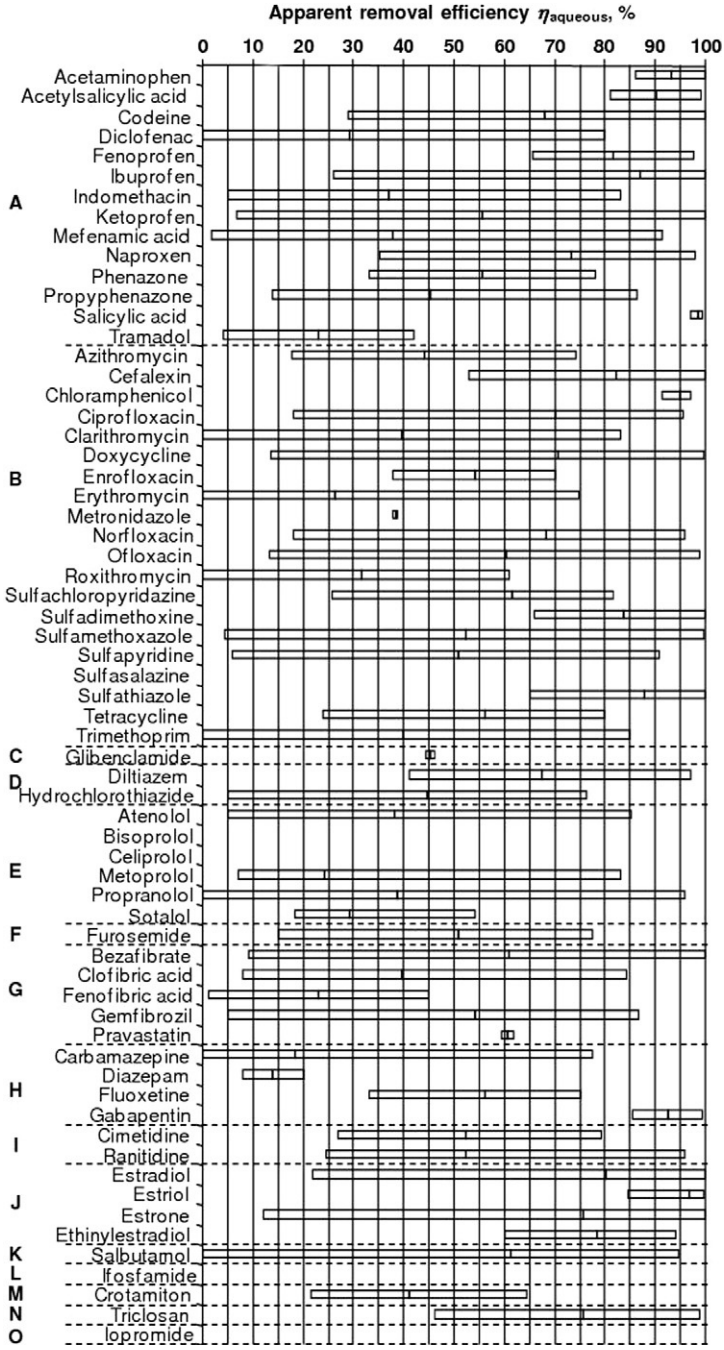

FIGURE 9 Average removal efficiencies from the liquid phase for the selected compounds.

the compound, wastewater composition, operational conditions, and reactor configurations. Hence, high variations in reported removal were observed for most compounds (e.g., diclofenac, ketoprofen, clarithromycin, atenolol, propranolol, and salbutamol, as shown in Figure 9), and no clear and definitive conclusions can be drawn on their removal, and even less can be stated about the fate of a particular therapeutic group.

Among the influential operating parameters (HRT, SRT, T, and redox and recirculation ratio), SRT seems to be the most critical for activated sludge design, as it affects the treatment process performance, aeration tank volume, sludge production, and oxygen requirements. It has been proven that longer SRT improves the removal of most of the PhCs during sewage treatments [35,69]. Indeed, WWTPs with high SRTs allow the enrichment of slowly growing bacteria and consequently the establishment of a more diverse biocoenosis with broader physiological capabilities (e.g., nitrification or the capacity for certain pathways) than WWTPs with low SRTs [70]. All of these parameters will be taken into consideration in the following discussion of the behavior of specific compounds under particular conditions.

Acetaminophen, ibuprofen, acetylsalicylic acid, salicylic acid, estrone, estriol, and estradiol were efficiently removed by CAS systems. Biodegradation of both acetaminophen [71] and ibuprofen [72] is known to be rapid. Diclofenac, on the other hand, was one of the selected PhCs that showed a modest removal efficiency (<29%). This may be due to the combination of degradation in wastewater and the liberation of additional diclofenac molecules by deconjugation of glucuronidated or sulfated diclofenac and/or its desorption from particles [61]. According to Cirja et al. [73], compounds with chlorine groups within the molecule may more readily persist during biological treatment. This could explain the poor average removal efficiencies reported for diclofenac and clofibric acid (on average <40%).

For fluoroquinolones (namely, norfloxacin, ciprofloxacin, enrofloxacin, and ofloxacin), adsorption is a potentially major elimination process. Although these compounds are very hydrophilic and zwitterionic [53], their higher concentrations in sludge (Figure 8) and their percentage partition onto sludge (Table 2) support this conclusion.

As regards sulfamethoxazole, [35] observed that in some cases, a release occurred due to the presence of metabolites in the influent that can subsequently be transformed into their parent compounds during biological processes.

Macrolides, namely, erythromycin, clarithromycin, and roxithromycin, were removed to a lesser extent in CAS systems. One possible reason is that sometimes, particles larger than 0.45 μm are not included in the analysis. This may lead to an underestimation of the concentrations of these compounds in the influent [54]. Gobel et al. [35] also proposed a gradual release of the macrolides from fecal particles during biological treatment as an explanation for the possible negative removal efficiencies sometimes observed. According to [53], the conjugated metabolites in raw influent samples can

TABLE 2 Fractions of Selected PhCs Removed via Sorption to Sludge and Discharge with Secondary Effluent During Biological Treatment, with Respect to the Influent Mass Load

Class	Compound	SRT (Days)	% Biodegraded	% Sorbed	% in Effluent	References
	Acetaminophen	–	>99	<0.01	<0.2	[57]
Analgesics and anti-inflammatories A	Diclofenac	4–60	5–45	<5	55–95	[33]
		6	25	<5	70–75	[25]
		16	10	5	85	[25]
		<20	5	0	95	[34]
		>50	10–30	0	70–90	[34]
	Ibuprofen	4–60	90–100	<5	0–10	[33]
		2	<5	<5	95–100	[72]
		<20	35–40	0	60–65	[34]
		>50	95	0	5	[34]
		>20	96	0	4	[75]
	Indomethacin	6	27	0	73	[25]
		16	40	<5	58–60	
	Ketoprofen	6	70	0	30	[25]
		16	<95		5–10	
	Mefenamic acid	6	65	7	28	[25]
		16	55–58	<30	<20	

	Naproxen	10–30	55–85	<5	15–45	[33]
		6	77	0	23	[25]
		16	95–98	0	<5	[25]
		<20	5	0	95	[34]
		>50	85–90	0	10–15	[34]
		>20	91	0	9	[75]
Antibiotics B	Azithromycin	10–30	<40	<10	60–90	[35]
	Chloramphenicol	6	0	0	100	[25]
	Chlortetracycline	–	100			[57]
	Ciprofloxacin	10–12	<10	70–80	≤30	[46]
		20	<10	77	<4	[37]
	Clarithromycin	<20	<10	<5	75–90	[35]
		>50	90	<5	10	[35]
		<20	<10	≤10	>90	[35]
		6	0	18	82	[25]
		16	0	<45	55–60	[25]
	Doxycycline	–	47	3	50	[57]
	Enrofloxacin	20–25	19	65	17	[36]
	Erythromycin	<20	20	0	80	[34]
		>20	93	0	7	[75]

Continued

TABLE 2 Fractions of Selected PhCs Removed via Sorption to Sludge and Discharge with Secondary Effluent During Biological Treatment, with Respect to the Influent Mass Load—Cont'd

Class	Compound	SRT (Days)	% Biodegraded	% Sorbed	% in Effluent	References
	Lomefloxacin	20–25		60	40	[36]
	Metronidazole	6			100	[25]
		16	15–18		82–85	
	Norfloxacin	10–12	<10	80–90	≤20	[46]
		20	<10	72	<4	[37]
	Ofloxacin	20–25		60	40	[36]
	Oxytetracycline	–	37	2.2	61	[57]
	Roxithromycin	4–30	<60	<5	>35	[35]
		<20	18	2	80	[34]
		>20	93	0	7	[75]
	Sulfamethoxazole	–	>89	<0.1	11	[57]
		4–12	50–90	<5	10–50	[35]
		<20	20	0	80	[34]
	Sulfapyridine	10–30	≤70	<10	≥30	[35]
	Tetracycline	–	93	7.1		[57]

	Trimethoprim	<50	~90	≤5	~10	[35]
		<20	<10	≤5	>90	[35]
		6	40	<5	<60	[25]
		16	38–40	5–10	50–55	[25]
		<20	18	0	72	[34]
		>20	78	0	22	[75]
Antidiabetics C	Glibenclamide	6		<10	90–95	[25]
		16		60	40	[25]
		15	73	7	20	[25]
Antihypertensives E	Hydrochlorothiazide	6		100		[25]
		16		100		
Beta-blockers G	Atenolol	6	<70	<5	<35	[25]
	Metoprolol	6	~35	0	~65	[25]
		16	0	0	100	
	Sotalol	6	10	<5	<90	[25]
		16	<50	<5	50	
Diuretics H	Furosemide	6	35–40	<5	60–65	[25]
		16	75–80	2–5	20	
Lipid regulators I	Bezafibrate	6	12	2	86	[25]
		16	<80	<5	20–25	[25]
		2	45–50	<5	50	[72]

Continued

TABLE 2 Fractions of Selected PhCs Removed via Sorption to Sludge and Discharge with Secondary Effluent During Biological Treatment, with Respect to the Influent Mass Load—Cont'd

Class	Compound	SRT (Days)	% Biodegraded	% Sorbed	% in Effluent	References
	Gemfibrozil	6	0	3	97	[25]
		16	90	<5	5–10	[25]
	Pravastatin	6	45	0	55	[25]
		16	62	2	<40	
Psychiatric drugs J	Carbamazepine	–	−41	0.6	141	[57]
		4–60	<40	<5	>60	[76]
		6	22	3	75	[25]
		16	0	5	95	[25]
	Diazepam	6	0	42	58	[25]
		16		65	35	
	Fluoxetine	<20	80	0	20	[34]
		>50	90	0	10	[34]
		>20	78	2	20	[75]
Receptor antagonists K	Cimetidine	6	42	4	54	[25]
		16	60	5–8	32–35	

	Famotidine	6	<10	10	85	[25]
		16	80	20	0	
	Ranitidine	6	<20	<5	80	[25]
		16	75	<5	20–25	
Hormones L	Estradiol, E2	10–30	85–99	<5	<15	[76]
		5–15	93	0	7	[77]
	Estrone, E1	10–30	35–97	≤5	5–60	[76]
		5–15	95	0	5	[77]
	Ethinyl estradiol, EE2	10–30	45–95	≤5	5–50	[76]
		<20	25	5	70	[34]
		>50	80–90	0	10–20	[34]
		5–15	25	63	12	[77]
	Estriol, E3	5–15	100			[77]
Beta-agonists M	Salbutamol	6	<60	<5	<45	[25]
		16	40–42	2	55–60	
Contrast agents Q	Iopromide	10–30	20–95	<5	5–80	[76]

be deconjugated during the treatment. They also propose that analyte behavior, such as adsorption to particles, may be altered by changing physical–chemical parameters during the treatment process, thus influencing the removal efficiency.

Modest to good removal efficiencies were found for the lipid regulators, which, however, displayed quite wide variability ranges, in particular for bezafibrate, gemfibrozil, and clofibric acid. Modestly average removals were observed for the beta-blockers, in particular for metoprolol (<20%). It is possible that microbial clearance of conjugates could be responsible for an underestimation of its removal efficiency, as this is well known to influence the balance in WWTPs [42].

Carbamazepine is quite a stable compound and may even be considered an anthropogenic marker [74]. Due to its hydrophilic nature, it is removed from wastewater by sorption onto sludge. It has quite often been detected at a higher concentration in the CAS effluent. This may be due to conversion of carbamazepine glucuronides and other conjugated metabolites to the parent compounds by enzymatic processes in the CAS [68].

It is important to observe that the term *removal* in CAS quite often implies conversion of the original PhC (parent compound) to other different compounds (metabolites) rather than complete mineralization (*elimination*). Moreover, it is important to note that low removal efficiencies could also be due to the fact that contaminants are present at *very low* concentrations in the influent, and unavoidable instrumental errors may affect their "observed" removal values [14,72]. At the other extreme, high removal efficiencies, >99%, which corresponds to a reduction of the influent concentration of two orders of magnitude, may nevertheless not be enough to consistently reduce the PhC concentrations to a low level of risk to aquatic life. For instance, if ibuprofen presents an influent concentration of 350 μg/L, even if 99% is removed, its final concentration would still amount to 3.5 μg/L, that is, a considerable mass load when discharged by the WWTP, as discussed in the succeeding text.

7.1 Solid–Liquid Partition and Pharmaceutical "Loss" Through Biodegradation

As reported in the preceding text, sludge tends to concentrate poorly degradable micropollutants. These are quite often hydrophobic substances with a high sorption potential. High aqueous removal efficiencies for some PhCs would seem to indicate very efficient removal during the treatments. However, only a certain fraction of the total mass is really lost (degraded); for some compounds, a considerable portion of the influent mass load could accumulate onto the sludge. Thus, determining the mass balance at a particular WWTP requires evaluation of the percentage mass loads of the selected PhCs discharged with the effluent, sorbed onto to sludge, and removed during

biological treatment, with respect to the influent mass load. Table 2 reports the corresponding fractions reported by different investigations that performed both liquid- (raw influent and CAS effluent) and solid-phase (sludge) analyses. Where available, the SRT of the investigated plant is reported. This parameter seems to be one of the factors that can influence the behavior of micropollutants in biological reactors, as will be discussed later (Section 9).

7.2 Considerations About Biological Degradation and Sorption Removal Mechanisms

Biodegradation of PhCs may occur through (i) metabolic reactions in which the pollutant is used as a source of primary carbon or nutrients for microorganism growth (anabolic reactions) and/or as an energy source (catabolic reactions) or (ii) cometabolic reactions in which the pollutants are transformed by the action of extracellular polymeric enzymes (called EPS in Figure 5) produced by the cells, but without any benefit for the microorganisms. It is less probable that the biological compartment contains specific microorganisms able to metabolize micropollutants exclusively. For instance, Forrez et al. [78] found that the enzyme ammonium monooxygenase, which is involved in the nitrification processes, was responsible for the degradation of the hormone ethinyl estradiol. In any case, CAS systems operating at high SRTs could promote a higher and more specific enzymatic activity through increased cell lysis [67]. The enzymatic mechanism responsible for the degradation of certain PhCs may be not activated as long as there are more readily degradable carbon or nutrient sources available, as may be the case in conventional municipal WWTPs. In this context, Drillia et al. [79] found that the antibiotic sulfamethoxazole can serve as a source of both carbon and nitrogen for enriched consortia but is only biodegraded whenever there is a depletion of carbon and nitrogen or both in the medium. In the presence of acetate and ammonium nitrogen, however, the antibiotic was not degraded and remained unaltered. For this reason, sulfamethoxazole is expected to be detected in many municipal WWTP effluents, only in extended aeration systems will a depletion of carbon and nitrogen source occur, making sulfamethoxazole degradation more likely.

Few studies have investigated the long-term effects of PhCs on the performance of biological reactors, namely, removal of COD, nitrogen and phosphorus compounds, and bacteria. Schmidt et al. [80] investigated the influence of a mixture of ciprofloxacin, gentamicin, sulfamethoxazole, trimethoprim, and vancomycin, up to a final concentration up to 30–40 mg/L, on the removal of COD, ammonia, and bacteria by activated sludge processes in lab-scale WWTPs. These concentrations are unlikely to be found in urban and hospital wastewater [12], but they may be a feature of pharmaceutical industry wastewaters, as will be discussed in Section 13. Schmidt and colleagues observed that at 30 mg/L of the total antibiotic concentration, the nitrification ended at nitrite, while no nitrification at all occurred at 40 mg/L antibiotic

concentration. They also determined that the nitrifiers were more sensitive to antibiotics than heterotrophic bacteria. COD removal in antibiotic-stressed lab plants was not influenced by $\leq$20 mg/L antibiotics, and antibiotics were not found to negatively affect the total viable count of bacteria. Furthermore, removal of antibiotics varied during the observation period, and these fluctuations were not strictly influenced by the total antibiotic concentrations.

Gao et al. [54] investigated the potential effect of fluoroquinolones on microorganisms in CAS and concluded that these compounds are unlikely to have adverse effects as their concentrations did not generally exceed the threshold of 8 μg/L at which genotoxic effects may occur. Discussion of the behavior of some other common PhCs is reported in Section 8.5.

Sorption mechanisms are quite difficult to assess and to predict [81]. As discussed in Section 8.6, these will depend not only on the sorbate in question but also on the sorbent, that is, the composition of the solid phase, in particular its organic carbon fraction (f_{oc}) and cation-exchange capacity (CEC) [82]. Indeed, compounds may absorb into/adsorb onto bacterial lipid structures and the fat fraction of sewage sludge through hydrophobic interactions (this is the case of aliphatic and aromatic groups); may adsorb onto polysaccharide structures, which often feature a negative charge, on the outside of bacterial cells through electrostatic interactions (this is the case of amino groups); and/or can bind chemically to bacterial proteins and nucleic acids. The partitioning between the aqueous and the solid phase is described by the solid–water distribution coefficient K_d, that is, the ratio of the equilibrium concentration of the chemical on the solids to the corresponding equilibrium concentration in the aqueous fraction, as discussed in Section 8.6, which analyzes different case studies and specific PhCs.

8 PROPERTIES PREDICTING REMOVAL IN CAS

As mentioned previously, the behavior of a PhC in conventional WWTPs will depend upon many factors, including the chemical and physical properties of the compound and the configuration and operational conditions of the biological reactor and the settling tank. The properties of a particular compound will influence whether it will remain in the aqueous phase (like many acidic, neutral, and basic compounds), degrade (such as ibuprofen and acetaminophen), or interact with solid particles (such as certain antibiotics, which have a higher potential for adsorption onto sewage sludges). In this context, the chemical structure, volatility, acidity, lipophilicity, biodegradability, and sorption potential of PhCs are the main properties investigated up to now by different research teams and are therefore those that are reported in the succeeding text, with particular focus on their significance, values, and reliability as predictors, based on knowledge about their behavior in a CAS. The popular *rules of thumb* defining threshold values of each of these properties are also reported, alongside the limitations plaguing their application.

8.1 Chemical Structure

Poor removal efficiencies in CAS systems have been documented for compounds with complex molecular structures, like those featuring aromatic rings (as in naproxen and ketoprofen), and for small PhC molecules containing halogens groups (like clofibric acid and diclofenac) [83]. Very small differences in chemical structure can result in very different behavior in the CAS. Take, for example, the hormones estradiol and ethinyl estradiol. Although they have basically the same chemical structure, the latter features an ethinyl group, which results in a great difference in biodegradability. Indeed, microorganisms in biological reactors are able to degrade estradiol quite easily, while ethinyl estradiol is more persistent.

8.2 Volatility

Volatility is the tendency of a compound to volatilize—that is, to evaporate from the liquid phase into the gaseous phase. This property is strictly correlated to the Henry coefficient H of a compound, defined as the ratio between the concentration of this compound in solution and its concentration in the gas above the solution, at the equilibrium. In fact, Ternes and Joss [58] found that a significant amount of compound will be stripped in a bioreactor with fine bubble aeration if $H > 10^{-3}$. However, most PhCs are characterized by H values $<10^{-5}$ (often $<10^{-10}$), since they are designed to take effect in an aqueous environment (for instance blood) and are therefore rather hydrophilic. As a consequence, the amount of PhCs stripped in the aeration tank of a CAS system is very low (Table 3).

8.3 Acidity

Acidity indicates whether or not a specific ionic interaction is relevant for the sorption potential of a given PhC. It is measured through the dissociation constant pK_a of the compound. pK_a can be used to determine the fraction of the dissolved chemical that exists in a neutral, nonionized state at the system pH. Since pK_a is the negative logarithm of K_a ($pK_a = -\log K_a = pH - \log([A^-]/[AH])$), it follows that the *lower* the value of pK_a, the *stronger* the acid and that a difference in the unit in pK_a on a log scale reflects a tenfold

TABLE 3 Henry Coefficient: Rule of Thumb

Parameter	Conditions	Rule of Thumb	References
H	$<10^{-3}$	Low volatility	[58]
H	$>10^{-3}$	High volatility	[58]

difference in acid strength. On the other side, the *higher* the value of pK_a, the *stronger* the base. Common acidic drugs are ketoprofen (pK_a = 3.88) and acetaminophen (pK_a = 9.5), which are mainly present in anionic form at pH 7, and common basic drugs are diazepam (pK_a = 3.3) and nadolol (pK_a = 9.76), which are mainly present in their cationic form. Verlicchi et al. [14] provide values of pK_a for most common PhCs.

The complex molecule of a PhC often contains heteroatoms and multifunctional groups and can be polar and ionizable. These properties are arguably closely linked to and influenced by the pH of the mixture. Moreover, many compounds have more than one ionizable functional group (for instance, ciprofloxacin; see Figure 10), which will generate several equilibrium constants that have to be considered separately. The degree of ionization is correlated to the pH of the solution containing the compound, and as ionized and nonionized species typically behave differently, this is a crucial factor. For instance, an ionized molecule will generally be more water-soluble and less likely to partition to lipid-like substances than its nonionized form.

Naturally, the potential of a molecule to participate in the environmental ion-exchange processes ubiquitous in soil and sludge systems will also be affected by whether the charge is positive or negative [84]. At the pH of wastewater, compounds tend to be classified as either nonionized (neutral) or ionized (basic or acidic). Acidic compounds may carry a negative charge, while basic compounds may carry a positive charge. As reported in detail in the supplementary data of the review by Verlicchi et al. [14], at pH 7, some of the selected PhCs may have a positive charge overall, some may have a negative charge, and some will be neutral.

Table 4 reports the rules of thumb usually adopted for pK_a.

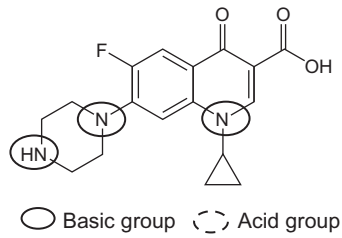

FIGURE 10 Chemical structure of ciprofloxacin containing both acid and basic.

TABLE 4 pK_a: Rule of Thumb

Parameter	Conditions	Rule of Thumb
pK_a	2–12	Low acidity
pK_a	<2	High acidity

8.4 Hydrophobicity/Lipophilicity

Hydrophobicity is the physical property of a compound that allows it to be repelled by a mass of water. Different coefficients have been used to evaluate the tendency of a substance to stay in the aqueous phase, and the most common parameters are the octanol–water *partition* coefficient (K_{ow}) and the octanol–water *distribution* coefficient (D_{ow}). In the past, K_{ow} was generally used for evaluating and predicting PhC behavior in the aquatic compartment by considering high K_{ow} values as characteristics of hydrophobic substances, poor water solubility, and in some cases a high potential to sorb on organic material of sludge ([85], as reported in Table 5).

Nonetheless, PhCs are complex multifunctional organic compounds, which, in some cases, are ionized in the aquatic environment. Thus, one PhC may generate nonionized species, which will predominate in partition into octanol from water, and ionized species, which will generally remain in the aqueous compartment. Hence, the pH at which measurements are made for evaluating K_{ow} is a crucial parameter, prompting Cunningham [84] to recently state that K_{ow} does not properly describe environmental partitioning or dynamic interactions in the environment of polar and ionizable compounds such as PhCs. He suggested that for these compounds, the coefficient D_{ow} is more suitable, as it is pK_a-dependent at environmental pH. D_{ow} is defined by Equation (3) and, according to Schwarzenbach et al. [86], evaluated through Equation (4) for acidic compounds and Equation (5) for basic ones:

$$D_{ow} \equiv \frac{\text{concentration in } n-\text{octanol}}{\text{concentration in water}} \tag{3}$$

$$\text{Log}D_{ow} = \text{Log}K_{ow} + \text{Log}\frac{1}{1+10^{\text{pH}-\text{p}K_a}} \text{ (acidic compound)} \tag{4}$$

$$\text{Log}D_{ow} = \text{Log}K_{ow} + \text{Log}\frac{1}{1+10^{\text{p}K_a-\text{pH}}} \text{ (basic compound)} \tag{5}$$

where $\text{Log}D_{ow} = \log_{10}D_{ow}$.

TABLE 5 Lipophilicity: Rule of Thumb

Parameter	Conditions	Rule of Thumb	References
Log K_{ow}	<2.5	Low sorption	[85]
Log K_{ow}	>4	High sorption	[85]
Log D_{ow}	<1	Low sorption	[84]
Log D_{ow}	>3	High sorption	[84]

In the case of neutral moieties, the two previous correlations result in Equation (6):

$$\mathrm{Log}D_{\mathrm{ow}} = \mathrm{Log}K_{\mathrm{ow}} \tag{6}$$

According to [87], since most water treatments are conducted at a pH between 7 and 8, and as D_{ow} simultaneously embodies the concepts of hydrophobicity and ionogenicity, D_{ow} at pH 7–8 is an appropriate physicochemical parameter for understanding and regulating water treatment of PhCs. Table 5 reports the rule of thumb when using lipophilicity to predict PhC behavior in aquatic compartments.

However, the parameter Log D_{ow} assumes that any charged species is completely water-soluble and that only the neutral fraction of an acidic or basic trace organic contaminant can partition to the solid phase. In fact, charged species can participate in interactions that are not necessarily electrostatic; hence, sorption of those analytes carrying a charge is likely to be a function of both the electrostatic properties of sorbent and sorbate [34] and the van der Waals interactions between them.

8.5 Biodegradability

The biodegradability of a compound is measured using the experimentally determined kinetic constant k_{biol} [88]. The constant k_{biol} is influenced by many factors: the biochemical versatility of the sludge (correlated to SRT), the bioavailability and chemical structure of the substance to degrade (i.e., the potential of microorganisms to interact with them, which is correlated to its concentration in the aqueous phase, generally very low), the availability of a cosubstrate, and the fraction of inert matter contained in the sludge (influenced by influent composition and sludge age) [58]. The degradation rate may also be influenced by temperature, biological reactor configuration, and sludge floc dimension and characteristics. Values may vary in a wide range, for instance, 0.002 L/(g_{ss} d) for roxithromycin and 350 L/(g_{ss} d) for estradiol [39].

Temperature can be accounted for by the known model based on Arrhenius equation in Equation (7):

$$k_{\mathrm{biol},T} = k_{\mathrm{biol},T_0} \mathrm{e}^{\theta(T-T_0)} \tag{7}$$

where $k_{\mathrm{biol},T}$ is the constant (L/g_{ss} d) at the desired temperature T (°C), k_{biol,T_0} is the constant at the reference temperature T_0 (°C), and θ is the temperature coefficient (0.03–0.09).

Biomass is usually approximated by the amount of total or volatile suspended solids (respectively, TSS and VSS), which can easily be determined by routine measurements. However, a major drawback of utilizing TSS is that only a fraction of them can be considered as viable biomass, while an inert

fraction is also present [89]. Although this has been successfully overcome, for instance, for COD and ammonia transformation, by classifying activated sludge bacteria into heterotrophic and autotrophic fractions, the issue of identifying bacteria responsible for PhC degradation still remains to be addressed [90].

The sludge characteristics that may influence the values of k_{biol} are as follows:

- Floc size: The CAS floc has a smaller dimension than that found in MBRs. Çiçek et al. [91] found that the average diameter of particles in the MBR was about 3.5 μm, with 97% of the particles being smaller than 10 μm. Most of the surface area was made up of particles in the size range of 3 to 5 μm in diameter. In a CAS system, only 88% of the particles were smaller than 10 μm, and a large number of particles ranging from 20 to 120 μm were detected. In this case, the main contribution to the total surface area was provided by particles in the size range of 80–120 μm. Their analysis showed that the CAS sludge contains large size flocs, while the MBR sludge is primarily composed of single bacteria and small flocs. Ternes and Joss [58] found that diffusion limits transformation of the compound, which occurs only in the outer floc layers, not contributing to the biological activity. As a result, for many PhCs, the k_{biol} in a CAS is smaller than the corresponding k_{biol} determined for an MBR [58,88].
- Diversity of the activity of the biomass due to differences in either the microbial population or the enzyme activity expressed (i.e., sludge age, as reported by Clara et al. [70]).
- The fraction of active biomass within the total suspended solids [88].

Furthermore, a complex structure and the presence of toxic groups in the compound will make breaking down the molecule more difficult [67]. Table 6 reports the rule of thumb for evaluating biodegradability of a PhC.

TABLE 6 Biodegradability: Rule of Thumb

Parameter	Conditions	Rule of Thumb [88]
k_{biol}	<0.01 L/g_{ss} d	No removal by biodegradation (<20% for strongly sorbing compounds with K_d > 1 L/g_{ss}, due to transfer to sludge)
k_{biol}	0.1–10 L/g_{ss} d	Partial removal (20–90%)
k_{biol}	>10 L/g_{ss} d	Removal >90%. Degradation strongly depends on reactor configuration

To give a few examples, high values of k_{biol} have been found for ibuprofen (9–35 l/g_{ss} d), paracetamol (58–80 L/g_{ss} d), estradiol (350 L/g_{ss} d), and estrone (600 L/g_{ss} d), while very low k_{biol} levels have been reported for the recalcitrant carbamazepine (0.08 L/g_{ss} d), iopamidol (<0.36 L/g_{ss} d), and tetracycline (0.44 L/g_{ss} d). Values of k_{biol} for many common PhCs are listed in the review by Pomiès et al. [39], along with the corresponding references.

8.6 Sorption Potential

Sorption of an organic contaminant mainly occurs by *absorption*, which involves hydrophobic interactions between the aliphatic and aromatic groups of a compound with the lipophilic cell membrane of the microorganisms and the fat fractions of the sludge, and by *adsorption*, where positively charged groups on the PhC (e.g., amino groups) electrostatically interact with the negatively charged surfaces of the microorganisms. These positively charged groups can also bind chemically to bacterial proteins and nucleic acids. As a result, sorption depends on the characteristics not only of the compound (presence of amino groups, COOH groups, etc., in the molecule) but also of the sludge, namely, the organic compound fraction (f_{oc}), cation-exchange capacity (CEC), suspended solid size, and SRT.

While primary sludge contains few microorganisms and a large fat fraction, microorganisms make up the greatest portion of suspended solids in the secondary sludge. Interestingly, Hyland et al. [82] found that f_{oc} appears to be fairly similar in different activated sludge solids (43–47%, on average 44%), appearing relatively unaffected by the location and operational conditions of the treatment plants investigated. Likewise, the CEC of the sludge solids is consistent across sludges (CEC = 54–75 meq/100 g). These authors also confirmed that SRT has no significant impact on the sorption potential of a compound. Instead, sorption potential is often correlated to the solid–water distribution coefficient K_d (=X/S), which describes the ratio between the concentration sorbed onto sludge and the dissolved concentration S at equilibrium. The pertinent rule of thumb for predicting PhC behavior is reported in Table 7.

TABLE 7 Sorption Potential: Rule of Thumb

Parameter	Conditions	Rule of Thumb	References
K_d	>500 L/kg	High sorption	[58]
Log K_d	>2.67		
K_d	<500 L/kg	Low sorption	[58]
Log K_d	<2.67		

The coefficient K_d of various PhCs has been experimentally evaluated for different primary, activated, and digested sludges, as well as for soils and sediments [28,32,52,55,92]. Among these, activated sludges have been investigated the most, and a recent review by Pomiès et al. [39] reports K_d data for a great number of compounds. Some authors found that for some compounds, K_d values are greater in secondary sludge than in primary [46,58], for example, ciprofloxacin, whose K_d was found to be equal to 2000 L/kg$_{ss}$ in primary sludge and 2×10^4 L/kg$_{ss}$ in activated sludge. Despite being an extremely polar compound, it sorbs readily onto the suspended solids in the sewage sludge [46]. At a neutral pH, this sorption is likely to rely mainly on electrostatic interactions between the positively charged amino group (Figure 10) and the negatively charged surfaces of the microorganisms. As microorganisms in the secondary sludge make up the greatest proportion of the suspended solids, a relatively high sorption constant of $K_d \approx 20$ L/g of suspended solids and a relatively high sorbed fraction were observed. In contrast, primary sludge contains few microorganisms and has a large fat fraction, so the K_d of ciprofloxacin in the primary sludge is only ≈ 2 L/gSS. This means that $\sim$20% of the ciprofloxacin is sorbed onto the primary sludge, whereas more than double this load partitions onto the secondary sludge [93].

When employing literature values for K_d, great care must be taken to choose the right ones. This is because in evaluating K_d, some studies have used PhC concentration in the range µg/l to mg/L [32,55], which are higher orders of magnitude than those usually observed in raw municipal wastewaters for many compounds. Moreover, as reported by Stevens-Garmon et al. [81], some studies have relied on single-point calculation rather than sorption isotherms, which may not be suitable at other PhC concentration ranges.

Hyland et al. [82] suggest that for hydrophobic, nonionized compounds, partition to organic matter in activated sludges can be estimated using K_d derived from K_{ow} values. The assumption is that the chemical will partition solely into the organic fraction of the solid. However, in general, the sorption of polar compounds and/or compounds with charged functional groups may be governed by a combination of different mechanisms, including electrostatic interactions, van der Waals forces, cation exchange, cation bridging, surface complexation, and hydrogen bonding [84]. The extent of sorption does not correlate with their hydrophobicity (hence K_{ow}) as can be seen for neutral compounds. This implies that some electrostatic interactions or others may be driving the specific sorption of these species, but no conclusions can yet be drawn as to the specific nature of these mechanisms and how they may differ between analytes.

Specific sorption coefficients generally decrease with increasing temperature, and the measured effect of temperature on sorption isotherms is ascribable to a combination of the temperature dependence of both sorption coefficient and solubility [94]. K_d may also be influenced by pH [95]. For instance, many psychiatric drugs (fluoxetine and carbamazepine) present basic properties with

their amine moieties (pK_a around 9). Having a higher pH value close to 8 would result in a higher ratio of undissociated, and hence more hydrophobic, molecules in the sludge and consequently higher K_d values.

As reported in Table 2, norfloxacin is mainly removed by sorption onto sludge. It has a high sorption potential (Log $K_d \sim 4$) and a high hydrophobic potential (Log $D_{ow} = 1$–3), and, being a positively charged compound, it partly sorbs to solid sludge surfaces by electrostatic interactions. This behavior can be explained by the fact that microorganisms have a negatively charged surface acting as a cation exchanger, meaning a stronger association will occur between this surface and a positively charged species than with a neutral one [86]. That being said, atenolol ($K_d \sim 30$ L/kg$_{ss}$, Log $K_d \sim 1.4$), another positively charged molecule at pH 7, was observed to possess a noticeably lower potential to sorb onto sludge solids. However, the compound is less hydrophobic than norfloxacin (Log $D_{ow} = -2.14$), suggesting that hydrophobic sorption interactions are still important for positively charged compounds [81].

The neutral hormones ethinyl estradiol, estradiol, and estrone have high Log K_d (2.6–3.2) and high Log K_{ow} (3.7–4.3), but they are not removed by sorption, as they have very high k_{biol} (ethinyl estradiol $\sim$10 L/(g$_{ss}$ d)) and one order of magnitude higher the other two hormones. The negatively charged compounds atorvastatin and gemfibrozil have Log K_d values in the range 1.5–1.7 and 2–2.3 and Log D_{ow} values of 1.9 and 2.8, respectively. Other negatively charged substances, namely, ibuprofen, diclofenac, naproxen, sulfamethoxazole, and enalapril, have very low Log K_d (<1.4) and Log $D_{ow} < 1.7$. For neutral and negatively charged compounds, increasing Log D_{ow} is indicative of increasing sorption potential. For nonionic compounds, sorption is assumed to be governed by partitioning to the organic phase in the activated sludge [81].

9 OPERATIONAL FACTORS AFFECTING PhC REMOVAL

There are a number of operational factors likely to influence the biological removal of PhCs in CAS. These include carbon load, HRT, solid retention time (SRT), food–microorganism ratio (F/M), mixed liquor-suspended solids (MLSS), pH, temperature, redox potential, and reactor configuration. The following section discusses these factors through interesting case studies found in the literature:

9.1 Initial Organic Carbon Concentration and Applied Organic Load

Urase and Kikuta [55] found higher degradation rates of selected PhCs (hormones, analgesics, lipid regulators, and psychiatric drugs) with lower initial organic carbon concentrations. Their investigations, carried out in batch

experiments in lab reactors fed with synthetic wastewaters, showed that microorganisms in the activated sludge degrade the target compounds more rapidly in the absence of easily biodegradable substances such as glucose and peptones. The lower Total Organic carbon operational condition was found to be preferable for the removal of target substances in the batch experiment, as under these conditions, microorganisms are forced to utilize micropollutants as sources of C and N.

Gabet Giraud et al. [62] found that in low-loaded activated sludge with an applied F/M ratio below 0.1 kg BOD_5 (kg MMLVSS d)$^{-1}$, higher removal was achieved for the ten selected beta-blockers and the investigated estrogens (estrone, estradiol, estriol, and ethinyl estradiol) than in medium-loaded activated sludge processes (0.5 kg BOD_5 (kg MMLVSS d)$^{-1}$).

9.2 Hydraulic Retention Time

This parameter determines the mean residence time of soluble compounds within the biological compartment. In this time, PhCs may biodegrade to a greater or lesser extent, depending on their biological degradation kinetics. Based on literature data pertaining to PhC removal collected in their database, Miège et al. [96] revealed that higher PhC removal occurs at higher HRT. Unsurprisingly, therefore, Yang et al. [97] found that the contact time required for activated sludge to degrade sulfamethoxazole and sulfadimethoxine is longer than the HRT of 4–6 h usually provided by CAS processes in urban WWTPs.

Gros et al. [98] and Garcia-Galan et al. [99] found that those compounds with a half-life, $t_{1/2}$, less than WWTP HRT generally exhibited high removal efficiencies, concluding that $t_{1/2}$ can give us an idea of the time the compounds need to remain in the biological reactor to ensure their efficient removal. In particular, they found that three different situations applied: (a) for compounds with high removal efficiency and high degradation rate (low $t_{1/2}$), like ibuprofen, naproxen, salicylic acid, acetaminophen, and enalapril, and (b) for compounds with poor or no elimination and low degradation (high $t_{1/2}$), like carbamazepine, clofibric acid, and diclofenac, HRT does not influence compound removal; (c) for compounds with medium removal and moderate degradation rate (including famotidine, ranitidine, and pravastatin), HRT seems to play a role, as their removal efficiencies were higher at increased HRT. Gros et al. [98] concluded that substances that are biodegradable (high k_{biol} or low $t_{1/2}$) and have low Log K_d (low sludge–water distribution coefficient, corresponding to low tendency to adsorb on sewage sludge) are more influenced by HRT, while compounds with high Log K_d and low k_{biol} are more influenced by SRT. However, there are other PhCs like ibuprofen with high k_{biol} and low Log K_d that are efficiently removed, irrespective of HRT and SRT.

Based on experimental findings on Canadian WWTPs (SRT from 2 to 10 days), Metcalfe et al. [100] proposed the following correlation for naproxen and ibuprofen, between HRT and percentage PhC removal η:

$$\eta = 1.735e^{0.886HRT} \quad (8)$$

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

9.3 Sludge Retention Time

Many authors (among them [101]) have found that a long SRT promotes the adaptation of different kinds of microorganisms, as well as the presence of slower growing species that could have a greater capacity for removing xenobiotics while simultaneously greatly improving suspended solid separation. This is the case for ibuprofen and diclofenac, as reported by Suárez et al. [69], who found removal only after the growth of specific bacteria.

For compounds with a significant sorption potential, such as estrogens and sulfamethoxazole, SRT is known to exert a significant effect only on the degree of their transformation [69,70], while no clear correlation was found between SRT and the removal of beta-blockers, carbamazepine, ciprofloxacin, ofloxacin, and norfloxacin [102].

For lipophilic substances, in general, the retention time inside the reactor may be more strongly influenced by the SRT rather than the HRT of the plant, which could explain how compounds with relatively slow kinetics can be biologically transformed during secondary treatment steps operating at high SRTs. Varying SRT in a secondary biological treatment system may influence the biological activity of the activated sludge, as well as potentially affecting the nature of the organic matter [82]. SRT may potentially be indicative of the degree of oxidation of the organic matter present, or it might influence the composition and activity of the biomass or even of the active fraction of the biomass [88].

A minimum SRT of 10–15 days has been suggested as necessary to ensure the development of a diverse biocoenosis, comprising nitrification, denitrification, and phosphorus removal [70].

An increase in SRT may also cause differences in sludge characteristics and performance. Indeed, Massé et al. [103] observed a deterioration of sludge settleability and CAS effluent quality in the presence of filamentous bacteria and therefore an increase in protein and polysaccharide release.

Clara et al. [70] found that if a specific substance is degraded in an SRT-dependent fashion, a critical value for the sludge age can be determined. In WWTPs operating SRTs below this critical value, effluent concentrations in the range of influent concentrations or a distribution according to the adsorption equilibrium must be expected, whereas degradation will occur in WWTPs operating at SRTs higher than the critical value. Generally speaking, high removal efficiencies and low effluent concentrations are achieved at SRTs higher than 10 days at an environmental temperature of 10 °C. This

corresponds to the requirements for WWTPs situated in sensitive areas, according to the urban wastewater directive of the European Community 91/271/EEC [104] in moderate climatic zones.

9.4 Sludge Characteristics (Floc Size, Biomass Concentration, and Acclimation)

Few studies have thus been carried out on this issue. Nonetheless, microscopic analysis carried out by Çiçek et al. [91] showed that with respect to the sludge of a MBR, CAS sludge is composed of larger flocs, fewer free-swimming bacteria, greater amounts of filamentous organisms inside the flocs (see Figure 5, left bottom), and higher concentrations of nematodes and crawling or free-swimming ciliates. Biomass in CAS has a lower viable fraction than in the MBR. Moreover, metabolic activity and specific enzymatic activity tests showed that overall activity is lower in the CAS than in the MBR sludge. The CAS contains fewer enzymes in the soluble phase than found in the MBR, and CAS cultures are capable of degrading a narrower spectrum of carbon substrates than MBR cultures.

Microbial communities evolve according to the prevailing environmental conditions and therefore largely depend on the composition of the incoming wastewater, including its organic loading rate. Kraigher et al. [105] showed that a significant structural shift in the bacterial community caused by permanent PhC presence occurred only at a concentrations >50 μg/L, which are unlikely to occur in municipal WWTPs receiving urban effluents. However, interesting considerations are raised by the long-term study conducted by Suárez et al. [69] on a CAS pilot plant fed by a synthetic mixture containing selected PhCs. They revealed that the removal efficiency observed for naproxen was directly proportional to the concentration of the mixed liquor volatile suspended solids (MLVSS) in the bioreactor. The removal efficiency increased from 27% to 99% during the first 300 d of investigation, when the VSS increased from 1 to 4 g/L, and remained stable during the following 300 days. This initial enhancement could be attributable to a possible acclimation of bacteria to this compound. Similarly, in an aerobic pilot reactor, diclofenac removal increased from 0 to 25% during the first 170 days, which coincides with the death and wash of heterotrophic bacteria and the development of strictly nitrifying biomass. Removal of ibuprofen in an anoxic reactor increased gradually with time from below 16% (up to day 200) to 75% (on day 340) [67]. These examples confirm that the type of bacteria flourishing in biological systems can influence the behavior of micropollutants to a very significant extent.

According to Ternes et al. [93], existing microorganisms could acclimate to the presence of PhCs by broadening their enzyme spectrum in response to the lower sludge loading with bulk organics when working at higher SRT. Suarez et al. [69] confirmed that biological transformation of PhCs follows pseudo-first-order kinetics, the transformation rate being directly

proportional to the soluble substance concentration, as well as to the sludge concentration, although the effect of the latter will only be significant for compounds with moderate biological degradation constants. Hence, an increase in SRT will cause an increase in the relative amount of inert mass in the activated sludge [88]. Majewsky et al. [90] found that active heterotrophic bacteria, known to govern COD removal, could be considered a determining factor for biological PhC removal.

9.5 Internal Recirculation Ratio

Suarez et al. [69] found that the effect of an increase in the internal recirculation ratio from 3 to 4 (from the aerobic to the anoxic compartment of the pilot reactor) was relevant for substances with moderate biological degradation constants, such as the psychiatric drug citalopram (0.41 L/g_{ss} d), whose removal efficiency increased from 25% to 50%. A slighter improvement (about 10%) was found in the removal efficiency of compounds with higher k_{biol}, including ibuprofen ($k_{biol} = 3.7$ L/g d), naproxen ($k_{biol} = 3.3$ L/g_{ss} d), and fluoxetine ($k_{biol} = 1.6$ L/g d). Nonetheless, these three compounds were already transformed to a high extent (70–80%) at a recirculation ratio equal to 3.

9.6 Temperature

The effect of temperature on the efficiency of PhC removal has been investigated by many authors. Among them, Vieno et al. [102] reported that at low winter temperatures, nitrification did not occur in the investigated activated sludge plants in Finland and far lower removal efficiencies were observed for analgesics (naproxen, ibuprofen, ketoprofen, and diclofenac) and lipid regulators (bezafibrate). Likewise, Vader et al. [106] found that removal of ethinyl estradiol in activated sludges ceased when the sludge lost its nitrification capacity due to falling temperatures. Suarez et al. [69] concluded that the influence of temperature is inversely proportional to the biological degradation rate constants of PhCs. As a consequence, temperature is a significant factor for substances with moderate to low k_{biol} that undergo transformation through mechanisms involving microbial activity.

9.7 pH Value

pH may influence the removal of micropollutants from wastewater by influencing both the physiology of microorganisms (optimal pH for microbial enzyme activities) and the solubility of the micropollutants present in wastewater. Depending on their pK_a values, PhCs can exist in various protonation states as a consequence of pH variation in the aquatic compartments. At pH 6–7, tetracyclines are neutral molecules, and for them, adsorption becomes the most incisive removal mechanism. Moreover, Horsing et al. [95] found that pH can be an important factor for the partition coefficient K_d.

9.8 Redox Potential

Suarez et al. [69] found that anoxic conditions (corresponding to a redox potential range from about −50 mV to about +50 mV) favor the removal of fluoxetine, trimethoprim, and erythromycin, while aerobic conditions (corresponding to a redox potential greater than 50 mV) are better for the removal of naproxen, ibuprofen, hormones, citalopram, sulfamethoxazole, and roxithromycin. Their investigations confirmed that operating at different redox conditions could result in an increased microbial diversity and a broader enzyme spectrum inside the biological reactor.

9.9 Reactor Configuration

Joss et al. [88] found that where sorption levels are high ($K_d > 100$ L/kg$_{ss}$), the impact of dividing the reactor volume into cascades becomes less significant (i.e., in the removal of the plug flow, the configuration becomes increasingly similar to a single completely mixed tank, even for compounds with high degradation constant k_{biol}). This is due to the fact that with increasing K_d, the soluble concentration is increasingly controlled by sorption/desorption, while the influent load has limited impact.

Clara et al. [70] and McAdam et al. [107] found that high removal efficiencies and low effluent concentrations of ibuprofen and bezafibrate are achieved at the design criteria for nitrogen removal. Relatively high removal efficiencies for estrogens may be observed in the absence of nitrogen removal, implying that effective biodegradation can proceed in heterotrophically dominated microbial consortia. Vieno et al. [108] found that atenolol and sotalol were slightly more efficiently eliminated in the WWTPs where nitrogen removal was greater than 60%, compared with those that removed less than 30% nitrogen. Similarly, Lajeunesse et al. [94] found that biological nutrient reactors, including anoxic–oxic–anaerobic tanks operating at different redox conditions, and microbial environments may contribute to the decomposition of more persistent compounds such as the antidepressants carbamazepine and fluoxetine.

That being the case, it is still not entirely clear how the type of technology affects micropollutant removal, as in many cases, discussion is based on data referring to activated sludge reactors, which differ in their configurations, operational conditions, and concentration of the influent wastewater. Nonetheless, Behera et al. [66] found that carbamazepine, metoprolol, and triclosan were more efficiently removed in a modified CAS called Daewoo nutrient removal (DNR) treatment, consisting of a sludge denitrification tank and anaerobic, anoxic, and aerobic zones, which help in the simultaneous removal of nitrogen and phosphorus. The same authors found improved removal efficiencies for clofibric acid, gemfibrozil, atenolol, estriol, and estradiol in WWTPs adopting a Symbio treatment, wherein both aerobic and anoxic conditions coexist in a single stage, within a single tank. They ascribed the

increase in the removal of those PhCs with the development of a dual zone within the sludge floc, brought about by a controlled air supply to the aeration tank maintaining dissolved oxygen at the desired low level. In this scenario, the outer region of the floc has access to the dissolved oxygen and promotes nitrification, while the inner part is oxygen-depleted and maintained under anoxic (denitrifying) condition, resulting in simultaneous nitrification and denitrification in a single tank.

Suarez et al. [34] divided PhCs into three groups according to their potential to be removed in a biological reactor. In this system, ibuprofen, fluoxetine, and natural estrogens were classed as highly biodegradable compounds under aerobic and anoxic conditions; diclofenac, naproxen, ethinyl estradiol, roxithromycin, and erythromycin as highly biodegradable compounds under aerobic conditions but persistent in anoxic conditions; and finally sulfamethoxazole, trimethoprim, carbamazepine, and diazepam as resistant to biological transformation.

10 MASS LOAD DISCHARGED BY CAS SYSTEMS

Up to now, attention has been paid to the behavior of PhCs during their passage through a CAS system and how chemical and physical properties as well as operational and design conditions influence the removal of selected compounds in order to improve it. The amount of compounds not degraded during the treatment still remains in the treated effluent or in the sludge. An attempt to quantify the mass load for selected PhCs discharged by means of municipal CAS effluent has been made in order to define the most critical compounds, according to the amount discharged into the environment.

Mass loads L_i were evaluated for selected PhCs i on the basis of the data (PhC mass load and average flow rate and PhC concentrations in many WWTPs) collected in the review by Verlicchi et al. [14]. These data are reported in the graph in Figure 11 in terms of variability range and average value. L_i was evaluated via Equation (9), using the effluent concentration $c_{i,j,h}$ (h = min, max, and average observed value) from the WWTP j, the average treated flow rate Q_j, and the population served by the WWTP j. Each mass load is expressed in mg/1000 inhabitants/day:

$$L_{i,j,h} = \frac{c_{i,j,h} Q_j}{\text{served population}} \times 1000 \tag{9}$$

The graph in Figure 11 reports, in descending order, the range of variability of mass loads L_i.

As discussed in [14], these findings may be affected by different sources of uncertainty, as pointed out in [20], and for this reason, they have to be considered with caution. That being said, the highest average mass loads (>200 mg/1000 inh/d) were found for the antihypertensive hydrochlorothiazide (368 mg/1000 inh/day), the psychiatric drug carbamazepine (364 mg/

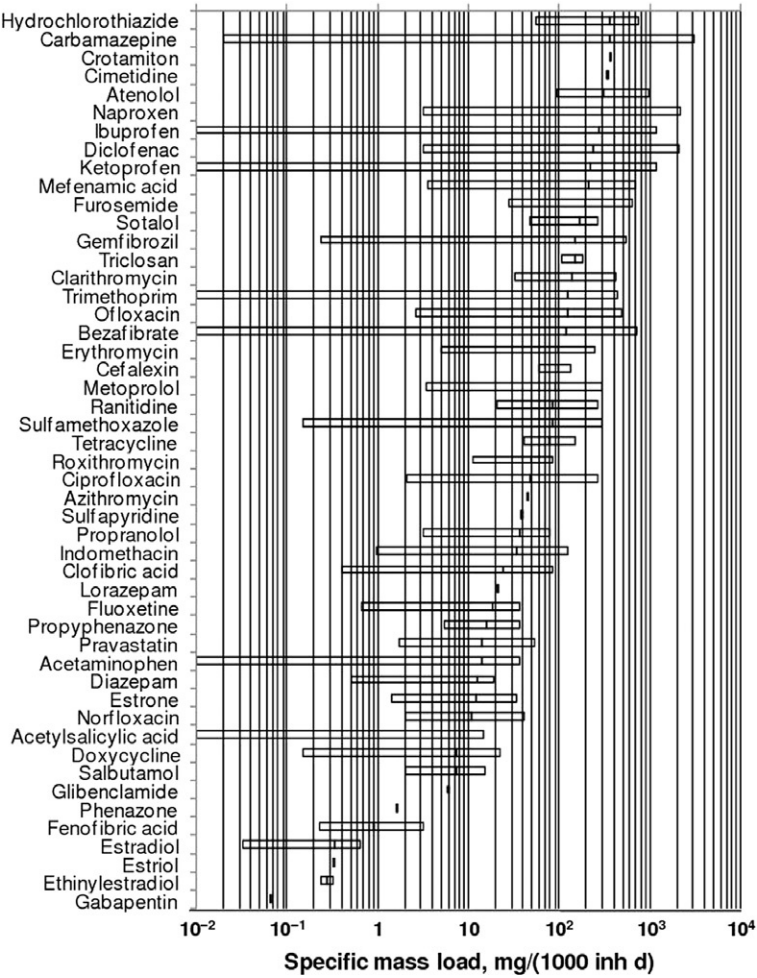

FIGURE 11 Mass load in CAS effluent discharged into the environment mg/(1000 inh d).

1000 inh/day), the receptor antagonist cimetidine (332 mg/1000 inh/day), and the beta-blocker atenolol (316 mg/1000 inh/day), followed by the analgesics/anti-inflammatories: naproxen (295), ibuprofen (273), diclofenac (241), ketoprofen (217), and mefenamic acid (211). The antibiotics clarithromycin (140), trimethoprim (124), ofloxacin (123), and erythromycin (100) exhibited lower average daily mass loads.

It was not possible to correlate the mass load to the sludge production due to lack of data for each WWTP. However, this is a pressing issue as an increase of sewage sludge production has taken place in Europe in recent years. The amount of sludge generated in European countries in 2006 was

estimated to be more than 8 million tons, of which 50% was land-applied. Estimates of sewage sludge annual production are of 11.6 million for 2012 (42% land-applied) and more than 13 million for 2020 (44% land-applied) [109]. Although land disposal is regulated by European directives and national laws, none of these regulations take into account the problem of PhCs, which can be transferred to soil after land application of biosolids. This gives them the potential to enter surface water, leach groundwater, or accumulate in vegetation or other living microorganisms. For this reasons, further research is necessary to complete the mass balance and to identify the most urgent mitigation measures required to reduce the impact of this widespread practice on the environment.

11 ENVIRONMENTAL RISK OF RESIDUAL PhCs IN TREATED EFFLUENT AND SLUDGE

11.1 Environmental Risk Assessment for Water and Sludge

Ecotoxicological risk assessment was performed for PhCs in secondary effluents and treated sludge by means of risk quotient RQ, which is evaluated by means of Equation (10):

$$\mathrm{RQ} = \frac{\mathrm{MEC}_i}{\mathrm{PNEC}_i} \quad i = 1\,(\mathrm{water}),\ 2(\mathrm{digested\,sludge}) \tag{10}$$

where MEC_i is the measured environmental concentration of the PhC in the secondary effluent ($i = 1$) or digested sludge ($i = 2$) and PNEC_i is the corresponding predicted no-effect concentration in water ($i = 1$) or sludge ($i = 2$). In [110,111], $\mathrm{PNEC}_{\mathrm{water}}$ values were estimated from the lowest acute or chronic toxicity data reported in literature from toxicological studies using bacteria, algae, or fish species as target organisms and applying an assessment factor of 1000, which takes into account interspecies variations in sensitivity, intraspecies variability, and laboratory data to field impact extrapolation, as already discussed and reported in [14]. A different approach was adopted for estimating $\mathrm{PNEC}_{\mathrm{sludge}}$. As to date, little toxicological data regarding PhCs in terrestrial organisms have been reported in the literature, and $\mathrm{PNEC}_{\mathrm{sludge}}$ values were estimated from $\mathrm{PNEC}_{\mathrm{water}}$ values by applying the equilibrium partition approach, as suggested by the European Commission [110] and according to [28,112] as follows:

$$\mathrm{PNEC}_{\mathrm{sludge}} = \mathrm{PNEC}_{\mathrm{water}} \times K_{\mathrm{d}} \times 1000 \tag{11}$$

where K_{d}, the solid–water partition coefficient referred to the sludge, is in $\mathrm{L/kg_{ss}}$ and $\mathrm{PNEC}_{\mathrm{sludge}}$ in μg/L.

Common criteria for interpreting RQ values in risk assessment studies establish different risk levels: low risk ($\mathrm{RQ} \leq 0.1$), medium risk ($0.1 < \mathrm{RQ} < 1$), and high risk ($\mathrm{RQ} \geq 1$) [113].

RQ in treated effluent—Figure 12 reports the RQ ranges and the corresponding average values of some of the selected compounds found in the secondary effluent, taken from graph on the right in Figure 7. PhCs are listed in descending order of risk on the Y-axis, alongside their $PNEC_{water}$ (μg/L). PNEC values used for secondary effluent are those used in [14,40,41].

As shown in Figure 12, out of the 49 selected PhCs, average effluent concentration data yield high environmental risk figure for 12 compounds (from erythromycin to azithromycin), while a moderate risk is posed by 14 substances (from acetaminophen to metronidazole) and a low risk by the remaining 23 compounds.

The most critical compounds are antibiotics (6 pose a high risk and 2 a moderate one), psychiatric drugs (fluoxetine and diazepam present a high

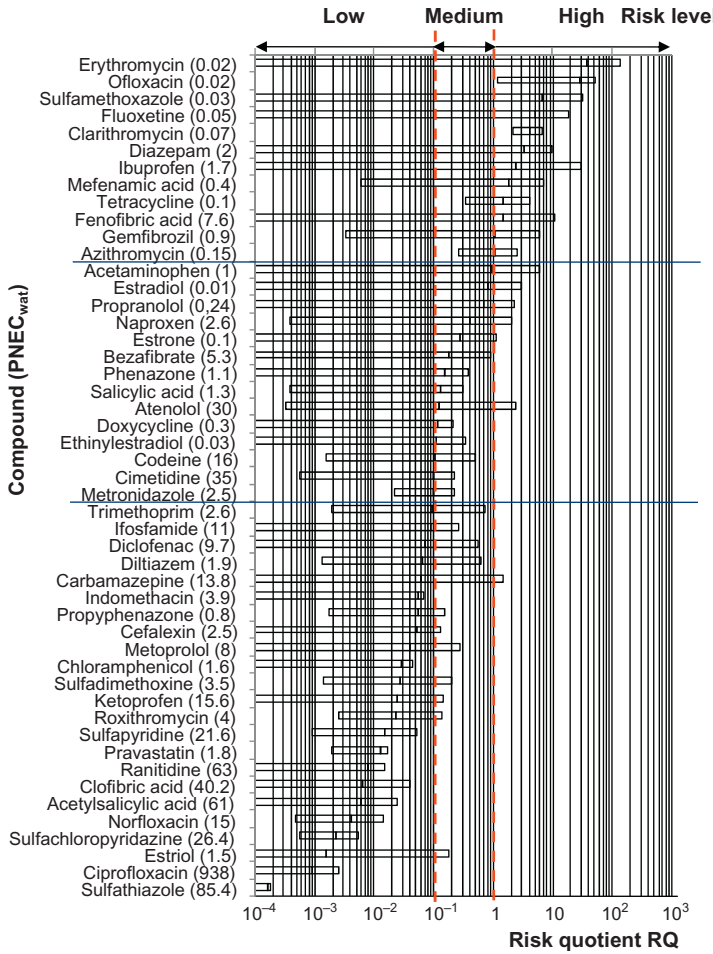

FIGURE 12 Risk quotient of selected PhCs, in descending order of risk, in secondary effluent.

risk), analgesics and anti-inflammatories (for two compounds, RQ > 1, and for 5 compounds, RQ is between 0.1 and 1), and the lipid regulators gemfibrozil and fenofibric acid.

Once the effluent is discharged into the surface water body, dilution occurs, and its extent will depend on the receiving body flow rate. This will result in some decrease in the concentration of the pharmaceutical compounds. If a dilution factor equal to 100 can be assumed, the risk quotient in surface water for all the compounds decreases by two orders of magnitude. According to data reported in Figure 12, only two compounds (erythromycin and ofloxacin) still have RQ > 0.1 (medium risk), on the basis of the average PhC concentration, the remaining compounds having an RQ < 0.1. However, if the environmental risk assessment is based, more prudently, on the maximum PhC concentration measured, the risk level is still high for erythromycin and medium for ofloxacin, sulfamethoxazole, fluoxetine, diazepam, ibuprofen, and fenofibric acid.

The dilution effect is vital for mitigating the adverse effects posed by the presence of micropollutants in receiving water bodies. In this context, Al Aukidy et al. [24] show the importance of the hydrodynamic characteristics of the receiving water body (mainly flow rate) and the risk related to effluent dominant rivers for which the dilution effect is quite modest (about 1 or less), resulting therefore in an equally modest mitigation of the risk. In any case, it is important to remember, as remarked by Martín et al. [28], that even if acute toxic effects in the aquatic environment may seem unlikely, chronic environmental exposure to toxic chemicals may still harm aquatic species with a long life cycle.

RQ in treated sludge—Figure 13 reports the RQ ranges for treated sludge, based on the concentration data reported in Figure 8 and available $PNEC_{water}$ data. K_d values are those reported in brackets, after the name of each substance, on the *X*-axis in Figure 8. The resulting PNEC values for the sludge are those in brackets, after the name of each compound in the Y-axis of Figure 13. The compounds responsible for the highest environmental risks in digested sludges (based on average concentrations detected in digested sludge sample) are the six antibiotics, oxytetracycline, erythromycin, azithromycin, ofloxacin, tetracycline, and clarithromycin; the two analgesics/anti-inflammatories ibuprofen and naproxen; the two hormones estradiol and ethinyl estradiol; the lipid regulator gemfibrozil; and the psychiatric drug fluoxetine.

The risk posed by the presence of PhCs in digested sludge applied to land can be evaluated according to European Commission Technical Guidance on Risk Assessment EUR 20418 EN/2 [110] as the ratio between their predicted environmental concentration in soil (PEC_{soil}) and the corresponding $PNEC_{soil}$. This document recommends evaluating PEC_{soil} 1 year after one sludge-dose application by means of Equation (12):

$$PEC_{soil} = \frac{c_{sludge} \times APP_{sludge}}{DEPTH_{soil} \times RHO_{soil}} \tag{12}$$

where c_{sludge} is the measured concentration in digested sludge (μg/kg dry matter), APP_{sludge} is the application rate of dry sludge onto soil (0.5 kg/m^2 for

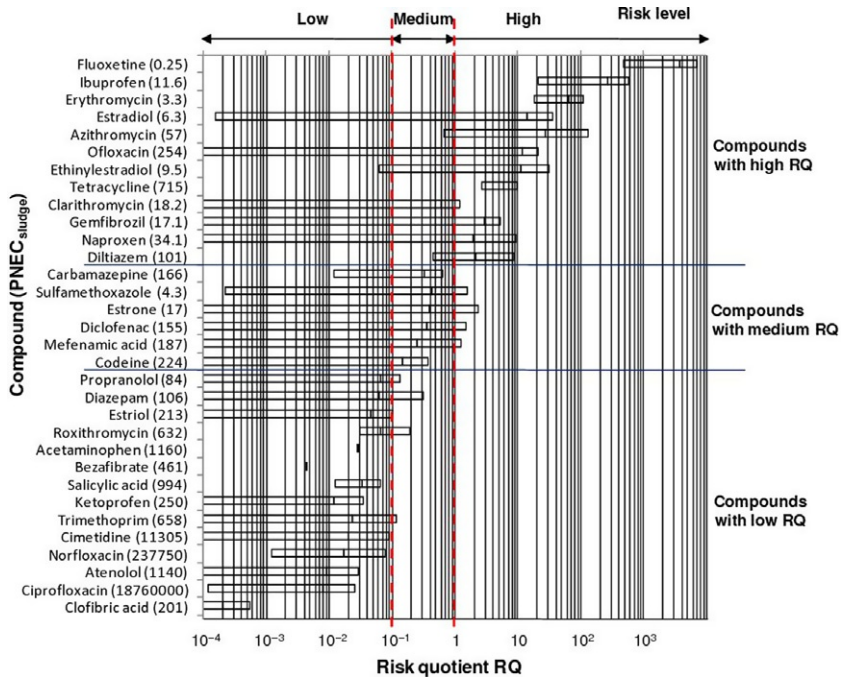

FIGURE 13 Risk quotient of selected PhCs, in descending order of risk, in digested sludge.

agricultural soils), $DEPTH_{soil}$ is the mixing depth (0.20 m for agricultural soils), and RHO_{soil} is the bulk density of wet soil (1700 kg/m^3 for agricultural soils).

$PNEC_{soil}$ is evaluated by means of an equation formally similar to Equation (11), using K_d values for soil.

Very few values for soil are available in the literature, and as remarked in Section 8.6, a considerable difference has been found between K_d in sludges and soils in some cases, as reported by Martín et al. [28]. In that study, they found a drastic decrease of RQ values after sludge application onto soil. The only toxic effect expected is the one caused by estradiol, since its RQ has been calculated as 2.7. This means that an ecotoxic risk is still present to terrestrial ecosystem in spite of the significant decrease in the concentration of estradiol from digested to amended digested sludge.

Additionally, Yang et al. [97] found that sorption onto sludge of sulfonamide antibiotics like sulfamethoxazole and sulfadimidine is reversible. This implies that they can be released from the sludge upon their release into the natural environment, highlighting the fact that these compounds pose a potential risk for the environment if there are no suitable processes to eliminate them from the sludge.

Recent studies investigated the occurrence and distribution of PhCs in soil irrigated with reclaimed water [114] and soil that received biosolids from urban sewage treatment plants [115]. They confirmed that conventional WWTPs, currently adopted all over the world, are not efficient enough to remove these micropollutants from wastewaters and sludge, and as a result, they found their way into the environment. Once in the environment, pharmaceutically active compounds can produce subtle effects on aquatic and terrestrial organisms, especially on the former since they are exposed to long-term continuous influx of WW effluents as remarked in [14] and biosolids as pointed out in [27].

The most critical compounds—The current study highlights the fact that the most critical PhCs, namely, those posing a high risk to the environment, will depend on the matrix investigated: secondary effluent or treated sludge. If we compare these groups of compounds with those with the highest mass load discharged into the environment reported in Figure 11, we find that the two groups do not overlap, as shown in Figure 14. In fact, this graph shows the RQ of the selected compounds in both sludge and water (the two series of histograms previously shown in Figures 11 and 12) together with their corresponding mass load (the black line, data from Figure 13). Compounds are reported from the highest to the lowest mass load.

Using these criteria, the most critical compounds are found to be ibuprofen (high RQ_{water}, high RQ_{sludge}, and high load); fluoxetine, ofloxacin, erythromycin, tetracycline, and azithromycin (high RQ_{water} and high RQ_{sludge}); and gemfibrozil, estradiol, and ethinyl estradiol (high RQ_{water} and medium RQ_{sludge}).

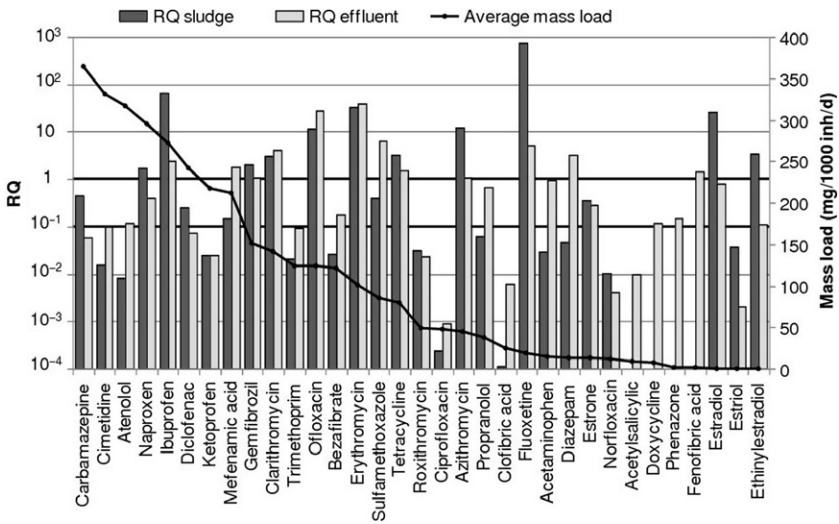

FIGURE 14 Comparison of average-specific mass load discharged by CAS effluent and RQs for secondary effluent and sludge.

11.2 Risk of Antibiotic-Resistant Bacteria and Genes

In addition to the environmental risk based on PNEC values for effluents as well as for sludge, there is another source of risk linked to the occurrence of the class of antibiotics both in the effluent and sludges: the development and release of antibiotic-resistant bacteria (ARB) and genes (ARG). ARG and ARB have been found to be several orders of magnitude higher in raw WWTP influents than in treated effluents, but, due to their high bacterial content, digested sludges also represent a significant environmental contamination route [116]. It has been reported in the literature that the percentage antibiotic resistance in a treated wastewater effluent was generally higher than the percentages in the river water, but these were observed to increase downstream of a WWTP [117]. WWTPs play a vital role in the elimination or spread of ARB and ARG, as the treatment systems and their operational conditions are likely to influence their fate. While it is likely that treated effluents with trace amount of ARGs and ARB from the treatment plants discharged into rivers or streams can add to the contamination of the environment, comparison of release loads of ARGs and ARB, Munir et al. [116] showed that land application of biosolids from WWTPs seems to be the main source of entry of ARGs and ARB into the natural environment. Further research is necessary to determine how best to reduce the spread of such bacteria.

12 MODELING

Various attempts have been made to create and propose a model able to simulate the fate and behavior of selected pharmaceuticals in a CAS in support of their design process. In this context, Plósz et al. [118] recently suggest using mechanistic models, that is, ASM-X, in regional risk assessment. Pomiés et al. [39] reviewed 18 different literature models describing micropollutant removal in CAS and remarked that an explanation for the validity of proposed models is often lacking, and for this reason, future developments are necessary to improve modeling of micropollutant removal in WWTP. Indeed, in their current form, they are not ready to be used in process design.

13 CAS: TREATMENT OF PHARMACEUTICAL INDUSTRY WASTEWATERS

Wastewaters generated by pharmaceutical manufacturers contain a variety of organic and inorganic constituents including spent solvents, catalysts, additives, reagents, and small amounts of intermediates, by-products, raw materials, and active pharmaceutical ingredients, which makes them particularly difficult to treat [119]. In addition, concentrations of COD, BOD, SS, and nitrates are generally very high, of the order of tens–hundreds mg/L. The ratio BOD/COD is about 0.45–0.60, and pH may vary in the range 5–8.

For instance, pharmaceutical wastewater investigated by Sreekanth et al. [119] contained as follows: 8500–9000 mg/L total dissolved solids; 2800–3000 mg/L TSS; 13,000–15,000 mg/L COD; 7000–7500 mg/L BOD; 600–750 volatile fatty acids; 2500–3000 mg/L alkalis, such as $CaCO_3$; 200–250 mg/L chlorides; 120–170 mg/L nitrates; 300–450 mg/L sulfates; and 100–120 mg/L phosphates, and the pH of the bulk drug in pharmaceutical wastewater was 7.0–7.5. In this effluent, the target PhC was carbamazepine, which was detected at levels of 10–15 mg/L. In some areas, PhC concentration may be even higher: Sirtori et al. [120] reported a concentration of 45 mg/L of nalidixic acid (a fluoroquinolone-type antibiotic was found) in an industrial effluent, and Chelliapan et al. [121] found tylosin concentrations of up to 20–200 mg/L in pharmaceutical effluent they investigated. Indeed, it is estimated that approximately half of the pharmaceutical wastewaters produced worldwide are discharged without specific treatment [122]. When treated, they are generally subjected to physicochemical processes [123] and then to aerobic biological steps [124].

The operational parameters most influential in the removal of pollutants from pharmaceutical effluent are HRT, temperature, pH, dissolved oxygen, organic load, microbial community, presence of toxic and persistent compounds, and batch operation of pharmaceutical production facilities [124]. Hence, activated sludge processes for the pharmaceutical industry effluent are generally designed with long HRT [125], operational temperature not greater than 30 °C (between 30 and 60 °C, the number of bacterial species decline with temperature, and activated sludge process fail at temperatures above 60 °C [126]). In fact, cooling of pharmaceutical effluent may even be necessary. Suman Raj and Anjaneyulu [124] found that pharmaceutical wastewater can be biologically treated using mixed consortia by integrating chemical coagulation as a pretreatment. They found that a chemical coagulation with lime followed by aerobic oxidation with activated sludge increased the biodegradability through reduction in sulfate concentration (down to 44–48%). They also found that the best results in the biological step were achieved at a mixed liquor concentration of about 4000 mg/L, confirming earlier results by Suman Raj et al. [127].

Unfortunately, the impact of high concentrations of PhCs in activated sludges, as seen in pharmaceutical wastewaters, has not been yet investigated, and the worry is that their concentrations may inhibit biological processes. In any case, biological treatments are not able to complete removal of PhCs and other pollutants, and so complementary treatments should be used in conjunction with the traditional methods. These additional treatments include membrane filtration, reverse osmosis, and activated carbon. In this context, Larsson et al. [128] monitored the effluent of a WWTP situated in Patancheru, near Hyderabad, in India. This plant receives about 1500 m^3/d of wastewaters, mainly from 90 bulk drug facilities ($BOD_5 = 1300$ mg/L; COD = 6000 mg/L; SS = 500 mg/L ; and dissolved solids = 9000 mg/L), and the treatment sequence consists of an equalization tank (HRT = 2 days), a chemically assisted SS

removal tank, a biological reactor (HRT = 4 days) in which 20% of domestic wastewaters are added to improve the removal efficiency, and a secondary clarifier. Excess sludge is subjected to centrifugation. The final effluent (BOD = 270 mg/L; COD = 1300 mg/L; SS = 300 mg/L; and dissolved solids = 5000 mg/L) is discharged into surface water bodies, and the treated sludge is disposed of in landfill. An investigation on the occurrence of some PhCs in the final effluent of this plant showed the following concentration ranges: 28–31 mg/L for ciprofloxacin, 0.8–0.95 mg/L for metoprolol, 0.7–0.9 for enrofloxacin, 0.39–0.42 for norfloxacin, 0.15–0.30 for enoxacin, 0.15–0.16 for ofloxacin, and 0.09–0.16 for ranitidine.

Deegan et al. [129] review many common treatments (traditional as well as advanced) and conclude that the problem of pharmaceuticals in wastewater cannot be solved merely by adopting end-of-pipe treatments, but source measures such as replacement of critical chemicals and reduction in raw material consumption also need to be adopted.

14 CONCLUSIONS

Most of the municipal WWTPs consist of preliminary, primary, and secondary treatments, mainly activated sludge systems with the final effluent being discharged into a surface water body and often indirectly reused for irrigation purposes or recreational activities and the treated sludge often land-applied. Many PhCs are usually present in raw influent at concentrations in the range 10^{-3}–10^{2} μg/L and even more, and common WWTPs are not able to efficiently remove all of them from liquid effluent as well as sludge. Observed removal efficiencies vary in a wide range for the different compounds, as well as for the same substance, due to the different chemical and physical characteristics of PhCs and to operational conditions.

This study highlights the fact that the occurrence of some PhCs in the secondary effluent discharged into surface water bodies may pose a medium–high (acute) risk to aquatic life. Furthermore, many other compounds, even if their environmental risk was found to be low, are discharged at high daily mass loads, which could contribute to negative effects on aquatic organisms in the long term due to chronic and mixture toxicity. For these reasons, it would be more prudent to begin monitoring the most frequently and most persistent administered PhCs, as well as those with the highest environmental risk, namely, antibiotics (including erythromycin, ofloxacin, sulfamethoxazole, clarithromycin, amoxicillin, tetracycline, and azithromycin), psychiatric drugs (like fluoxetine, diazepam, and carbamazepine), analgesics/anti-inflammatories (ibuprofen, mefenamic acid, naproxen, diclofenac, and ketoprofen), and lipid regulators (fenofibric acid, fenofibrate, and gemfibrozil). Unfortunately, up to now, PhCs are not included among those compounds to be monitored, notwithstanding their occurrence has been documented since more than 20 years in many European countries. For this reason, further

researches are necessary (i) to analyze the occurrence of scarcely investigated PhCs in the influent and outlets of municipal WWTPs, (ii) to evaluate the environmental impact of mixtures of different PhCs, (iii) to evaluate the best end-of-pipe measures for the existing WWTPs to guarantee better removal of the most persistent compounds, and (iv) to suggest source control options to reduce the quantity and variety of PhCs in the water cycle.

REFERENCES

[1] FDA, Approved Drug Products with Therapeutic Equivalence Evaluations, 2012. Available at the web site http://www.fda.gov/downloads/Drugs/InformationOnDrugs/ucm086233.pdf (last access on 15 February 2013).
[2] FDA, Guidance for Industry: Environmental Assessment of Human Drug and Biologics Applications, 1998. http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/ucm070561.pdf (last access on 15 February 2013).
[3] Directive 2004/27/EC of the European Parliament and of the Council of 31 March 2004 amending Directive 2001/83/EC on the Community code Relating to Medicinal Products for Human Use.
[4] Regulation (EC) n. 726/2004 of the European Parliament and of the Council of 31 March 2004 Laying Down Community Procedures for the Authorisation and Supervision of Medicinal Products for Human and Veterinary Use and Establishing a European Medicines Agency.
[5] EC, 2006. Regulation of the European Parliament and of the Council of 18 December 2006, (1907/2006), Concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), Establishing a European Chemicals Agency, Amending Directive 1999/45/EC and Repealing Council Regulation (EEC) No 793/93 and Commission Regulation (EC) No 1488/94 as well as Council Directive 76/769/EEC and Commission Directives 91/155/EEC, 93/67/EEC, 93/105/EC and 2000/21/EC.
[6] K. Kummerer, Pharmaceutical in the Environment: Sources, Fate Effects, and Risks, Springer, Berlin, 2004.
[7] WHO, 2004. The World Medicines Situation, Report WHO/EDM/PAR/2004.5, available at the web site http://apps.who.int/medicinedocs/en/d/Js6160e/6.html#Js6160e.6 (last access on 15 February 2013).
[8] J. Fick, H. Söderström, R.H. Lindberg, C. Phan, M. Tysklind, D.G.J. Larsson, Environ. Toxicol. Chem. 28 (2009) 2522–2527.
[9] C.G. Daughton, I.S. Ruhoy, Environ. Toxicol. Chem. 28 (2009) 2459–2521.
[10] P. Verlicchi, A. Galletti, M. Petrovic, D. Barcelo, in: D. Barcelo (Ed.), Emerging Organic Contaminants and Human Health, Springer-Verlag, Berlin, Heidelberg, 2012, pp. 139–172.
[11] P. Verlicchi, A. Galletti, L. Masotti, Water Sci. Technol. 61 (2010) 2507–2519.
[12] P. Verlicchi, A. Galletti, M. Petrovic, D. Barceló, J. Hydrol. 389 (2010) 416–428.
[13] P. Verlicchi, M. Al Aukidy, A. Galletti, M. Petrovic, D. Barceló, Sci. Total Environ. 430 (2012) 109–118.
[14] P. Verlicchi, M. Al Aukidy, E. Zambello, Sci. Total Environ. 429 (2012) 123–155.
[15] N. Le Minh, S.J. Khan, J.E. Drewes, R.M. Stuetz, Water Res. 44 (2010) 4295–4323.
[16] A. Ziylan, N.H. Ince, J. Hazard. Mater. 187 (2011) 24–37.
[17] D. Cairns, Essentials of Pharmaceutical Chemistry, Pharmaceutical Press, London, 2012.

[18] W. Stumm, J.J. Morgan, Aquatic Chemistry – Chemical Equilibria and Rates in Natural Waters, third ed., Wiley-Interscience, New York, 1996.
[19] M. Liebig, J. Moltmann, T. Knacker, Environ. Sci. Pollut. Res. 13 (2006) 110–119.
[20] C. Ort, W. Gujer, Water Sci. Technol. 54 (2006) 169–176.
[21] C. Ort, M.G. Lawrence, J. Reungoat, J.F. Mueller, Environ. Sci. Technol. 44 (2010) 6289–6296.
[22] C. Ort, M.G. Lawrence, J. Rieckermann, A. Joss, Environ. Sci. Technol. 44 (2010) 6024–6035.
[23] Metcalf & Eddy, Wastewater Engineering Treatment Disposal Reuse, McGraw Hill International, Singapore, 2003.
[24] M. Al Aukidy, P. Verlicchi, A. Jelic, M. Petrovic, D. Barcelò, Sci. Total Environ. 438 (2012) 15–25.
[25] A. Jelic, M. Gros, A. Ginebreda, R. Cespedes-Sánchez, F. Ventura, M. Petrovic, D. Barceló, Water Res. 45 (2011) 1165–1176.
[26] J. Radjenović, M. Petrovic, D. Barceló, Water Res. 43 (2009) 831–841.
[27] J. Martín, M.D. Camacho-Muñoz, J.L. Santos, I. Aparicio, E. Alonso, J. Environ. Manage. 102 (2012) 18–25.
[28] J. Martín, M.D. Camacho-Munoz, J.L. Santos, I. Aparicio, E. Alonso, J. Hazard. Mater. 239–240 (2012) 40–47.
[29] B.G. Plósz, H. Leknes, H. Liltved, K.V. Thomas, Sci. Total Environ. 408 (2010) 1915–1924.
[30] R. Salgado, R. Marques, J.P. Noronha, J.T. Mexia, G. Carvalho, A. Oehmen, M.A.M. Reis, Environ. Pollut. 159 (2011) 2359–2367.
[31] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Water Res. 43 (2009) 363–380.
[32] A. Wick, G. Fink, A. Joss, H. Siegrist, T.A. Ternes, Water Res. 43 (2009) 1060–1074.
[33] A. Joss, E. Keller, A. Alder, A. Göbel, C. Mcardell, T. Ternes, H. Siegrist, Water Res. 39 (2005) 3139–3152.
[34] S. Suárez, J.M. Lema, F. Omil, Water Res. 44 (2010) 3214–3224.
[35] A. Göbel, C.S. Mcardell, A. Joss, H. Siegrist, W. Giger, Sci. Total Environ. 372 (2007) 361–371.
[36] A. Jia, Y. Wan, Y. Xiao, J. Hu, Water Res. 46 (2012) 387–394.
[37] R.H. Lindberg, U. Olofsson, P. Rendahl, M.I. Johansson, M. Tysklind, B.A.V. Andersson, Environ. Sci. Technol. 40 (2006) 1042–1048.
[38] T.A. Ternes, N. Herrmann, M. Bonerz, T. Knacker, H. Siegrist, A. Joss, Water Res. 38 (2004) 4075–7084.
[39] M. Pomiès, J. Choubert, C. Wisniewski, M. Coquery, Sci. Total Environ. 443 (2013) 733–748.
[40] T.A. Roepke, M.J. Snyder, G.N. Chen, Aquat. Toxicol. 71 (2005) 155–173.
[41] T.H. Hutchinson, N.A. Pounds, M. Hampel, T.D. Williams, Sci. Total Environ. 233 (1999) 167–179.
[42] H. Andersen, H. Siegrist, B. Halling-Sørensen, T.A. Ternes, Environ. Sci. Technol. 37 (2003) 4021–4026.
[43] O. Braga, G.A. Smythe, A.I. Schäfer, A.J. Feitz, Environ. Sci. Technol. 39 (2005) 3351–3358.
[44] M. Esperanza, M.T. Suidan, R. Marfil-Vega, C. Gonzalez, G.A. Sorial, P. McCauley, R. Brenner, Chemosphere 66 (2007) 1535–1544.
[45] A. Göbel, A. Thomsen, C.S. McArdell, A. Joss, W. Giger, Environ. Sci. Technol. 39 (2005) 3981–3989.

[46] E. Golet, I. Xifra, H. Siegrist, A. Alder, W. Giger, Environ. Sci. Technol. 37 (2003) 3243–3249.
[47] S.J. Khan, J.E. Ongerth, Water Sci. Technol. 46 (2002) 105–113.
[48] R.H. Lindberg, P. Wennberg, M.I. Johansson, M. Tysklind, B.A.V. Andersson, Environ. Sci. Technol. 39 (2005) 3421–3429.
[49] S. Martin Ruel, M. Esperanza, J.M. Choubert, I. Valor, H. Budzinski, M. Coquery, Water Sci. Technol. 62 (2010) 2970–2978.
[50] K. McClellan, R.U. Halden, Water Res. 44 (2010) 658–668.
[51] X. Miao, J. Yang, C.D. Metcalfe, Environ. Sci. Technol. 39 (2005) 7469–7475.
[52] J. Radjenović, A. Jelić, M. Petrović, D. Barceló, Anal. Bioanal. Chem. 393 (2009) 1685–1695.
[53] W. Xu, G. Zhang, X. Li, S. Zou, P. Li, Z. Hu, J. Li, Water Res. 41 (2007) 4526–4534.
[54] L. Gao, Y. Shi, W. Li, H. Niu, J. Liu, Y. Cai, Chemosphere 86 (2012) 665–671.
[55] T. Urase, T. Kikuta, Water Res. 39 (2005) 1289–1300.
[56] M.S. Diaz-Cruz, M.J. López De Alda, D. Barcelo, Trends Anal. Chem. 22 (2003) 340–351.
[57] P. Gao, Y. Ding, H. Li, I. XagorarakiI, Chemosphere 88 (2012) 17–24.
[58] T.A. Ternes, A. Joss, Human Pharmaceuticals, Hormones and Fragrances, the Challenge of Micropollutants in Urban Water Management, IWA Publishing, London, 2006.
[59] M. Yasojima, N. Nakada, K. Komori, Y. Suzuki, H. Tanaka, Water Sci. Technol. 53 (2006) 227–233.
[60] A.J. Watkinson, E.J. Murby, S.D. Costanzo, Water Res. 41 (2007) 4164–4176.
[61] S. Zorita, L. Martensson, L. Mathiasson, Sci. Total Environ. 407 (2009) 2760–2770.
[62] V. Gabet-Giraud, C. Miège, J.M. Choubert, S.M. Ruel, M. Coquery, Sci. Total Environ. 408 (2010) 4257–4269.
[63] H.W. Leung, T.B. Minh, M.B. Murphy, J.C.W. Lam, M.K. So, M. Martin, P.K.S. Lam, B.J. Richardson, Environ. Int. 42 (2012) 1–9.
[64] M. Carballa, F. Omil, J.M. Lema, M. Liompart, C. Garcia-Jares, I. Rodriguez, M. Gómez, T. Ternes, Water Res. 38 (2004) 2918–2926.
[65] C. Gu, K.G. Karthikeyan, Environ. Sci. Technol. 39 (2005) 2660–2667.
[66] S.K. Behera, H.W. Kim, J.E. Oh, H.S. Park, Sci. Total Environ. 409 (2011) 4351–4360.
[67] F. Omil, S. Suarez, M. Carballa, R. Reif, J.M. Lema, in: D. Fatta-Kassinos (Ed.), Xenobiotics in the Urban Water Cycle: Mass, Flows, Environmental Process, Mitigation and Treatment Strategies, Environmental pollution, Vol. 16, Springer Science and Business Media B.V., Dordrecht, London, New York, 2010, pp. 283–305.
[68] S.C. Monteiro, A.B.A. Boxall, Environ. Contam. Toxicol. 202 (2010) 53–154.
[69] S. Suárez, R. Reif, J.M. Lema, F. Omil, Chemosphere 89 (2012) 164–171.
[70] M. Clara, N. Kreuzinger, B. Strenn, O. Gans, H. Kroiss, Water Res. 39 (2005) 97–106.
[71] M.J. Gómez, M.J. Martínez Bueno, S. Lacorte, A.R. Fernández-Alba, A. Agüera, Chemosphere 66 (2007) 993–1002.
[72] M. Clara, B. Strenn, O. Gans, E. Martinez, N. Kreuzinger, H. Kroiss, Water Res. 39 (2005) 4797–4807.
[73] M. Cirja, P. Ivashechkin, A. Schaeffer, P.F.X. Corvini, Rev. Environ. Sci. Biotechnol. 7 (2008) 61–78.
[74] M. Clara, B. Strenn, M. Ausserleitner, N. Kreuzinger, Water Sci. Technol. 50 (2004) 29–36.
[75] E. Fernandez-Fontaina, F. Omil, J.M. Lema, M. Carballa, Water Res. 46 (2012) 5434–5444.

[76] A. Joss, H. Andersen, T. Ternes, P.R. Richle, H. Siegrist, Environ. Sci. Technol. 38 (2004) 3047–3055.
[77] Y. Zhou, J. Zha, Z. Wang, Environ. Monit. Assess. 184 (2012) 6799–6813.
[78] I. Forrez, M. Carballa, N. Boon, W. Verstraete, J. Chem. Technol. Biotechnol. 84 (2008) 119–125.
[79] P. Drillia, S.N. Dokianakis, M.S. Fountoulakis, M. Kornaros, K. Stamatelatou, G. Lyberatos, J. Hazard. Mater. 122 (2005) 259–265.
[80] S. Schmidt, J. Winter, C. Gallert, Arch. Environ. Contam. Toxicol. 63 (2012) 354–364.
[81] J. Stevens-Garmon, J.E. Drewes, S.J. Khan, J.A. McDonald, E.R.V. Dickenson, Water Res. 45 (2011) 3417–3426.
[82] K.C. Hyland, E.R.V. Dickenson, J.E. Drewes, C.P. Higgins, Water Res. 46 (2012) 1958–1968.
[83] K. Kimura, H. Hara, Y. Watanabe, Desalination 178 (2005) 135–140.
[84] V.L. Cunningham, in: K. Kummerer (Ed.), Pharmaceutical in the Environment – Sources, Fate, Effects and Risks, third ed., Springer, Berlin, Heidelberg, 2008, pp. 23–34.
[85] H.R. Rogers, Sci. Total Environ. 185 (1–3) (1996) 3–26.
[86] R.P. Schwarzenbach, P.M. Gschwend, D.M. Imboden, Environmental Organic Chemistry, Wiley-Interscience, Hoboken, 2003.
[87] M.J.M. Wells, Environ. Chem. 3 (2006) 439–449.
[88] A. Joss, S. Zabczynski, A. Göbel, B. Hoffmann, D. Löffler, C.S. McArdell, T.A. Ternes, A. Thomsen, H. Siegrist, Water Res. 40 (2006) 1686–1696.
[89] G.L. Cronje, A.O. Beeharry, M.C. Wentzel, G.A. Ekama, Water Res. 36 (2002) 439–444.
[90] M. Majewsky, T. Gallé, V. Yargeau, K. Fischer, Bioresour. Technol. 102 (2011) 7415–7421.
[91] N. Çiçek, J.P. Franco, M.T. Suidan, V. Urbain, J. Manem, Water Environ. Res. 71 (1999) 64–70.
[92] M. Carballa, G. Fink, F. Omil, J.M. Lema, T. Ternes, Water Res. 42 (2008) 287–295.
[93] T.A. Ternes, A. Joss, H. Siegrist, Environ. Sci. Technol. 38 (2004) 393A–398A.
[94] A. Lajeunesse, S.A. Smyth, K. Barclay, S. Sauvé, C. Gagnon, Water Res. 46 (2012) 5600–5612.
[95] M. Hörsing, A. Ledin, R. Grabic, J. Fick, M. Tysklind, J.L. Jansen, H.R. Andersen, Water Res. 45 (2011) 4470–4482.
[96] C. Miège, J.M. Choubert, L. Ribeiro, M. Eusèbe, M. Coquery, Environ. Pollut. 157 (2009) 1721–1726.
[97] S. Yang, C. Lin, A. Yu-Chen Lin, P. Andy Hong, Water Res. 45 (2011) 3389–3397.
[98] M. Gros, M. Petrović, A. Ginebreda, D. Barceló, Environ. Int. 36 (2010) 15–26.
[99] M.J. García-Galán, M.S. Díaz-Cruz, D. Barceló, Environ. Int. 37 (2011) 462–473.
[100] C.D. Metcalfe, B.G. Koenig, D.T. Bennie, M. Servos, T.A. Ternes, R. Hirsch, Environ. Toxicol. Chem. 22 (2003) 2872–2880.
[101] S. Weiss, T. Reemtsma, Water Res. 42 (2008) 3837–3847.
[102] N.M. Vieno, T. Tuhkanen, L. Kronberg, Environ. Sci. Technol. 39 (2005) 8220–8226.
[103] A. Massé, M. Spérandio, C. Cabassud, Water Res. 40 (2006) 2405–2415.
[104] Council Directive 1991/271/EEC of 21 May 1991 Concerning Urban Waste-Water Treatment.
[105] B. Kraigher, T. Kosjek, E. Heath, B. Kompare, I. Mandic-Mulec, Water Res. 42 (2008) 4578–4588.
[106] J.S. Vader, C.G. van Ginkel, F.M.G.M. Sperling, J. de Jong, W. de Boer, J.S. de Graaf, M. van der Most, P.G.W. Stokman, Chemosphere 41 (2000) 1239–1243.

[107] E.J. McAdam, J.P. Bagnall, Y.K.K. Koh, T.Y. Chiu, S. Pollard, M.D. Scrimshaw, J.N. Lester, E. Cartmell, Chemosphere 81 (2010) 1–6.
[108] N. Vieno, T. Tuhkane, L. Kronberg, Water Res. 41 (2007) 1001–1012.
[109] Milieu Ltd, WRc, RPA, Environmental, Economic and Social Impacts of the Use of Sewage Sludge on Land, Final Report for the European Commission, DG Environment Under Study Contract DG ENV.G.4/ETU/2008/0076r.
[110] EC, 2003. Technical Guidance Document on Risk Assessment, Part II. EUR 20418 EN/2. European Commission, Joint Research Centre.
[111] European Agency for the Evaluation of Medical Products (EMEA). Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use, The European Agency for the Evaluation of Medicinal Products: Committee for Medicinal Products for Human Use. EMEA/CHMP/SWP/4447/00, 2006.
[112] M.M. González, J. Martín, J.L. Santos, I. Aparicio, E. Alonso, Sci. Total Environ. 408 (2010) 563–570.
[113] M.D. Hernando, M. Mezcua, A.R. Fernández-Alba, D. Barceló, Talanta 69 (2006) 334–342.
[114] T.A. Ternes, M. Bonerz, N. Herrmann, B. Teiser, H.R. Andersen, Chemosphere 66 (2007) 894–904.
[115] G.J.H.P. Gielen, M.R. van den Heuvel, P.W. Clinton, L.G. Greenfield, Chemosphere 74 (2009) 537–542.
[116] M. Munir, K. Wong, I. Xagoraraki, Water Res. 45 (2011) 681–693.
[117] T. Iwane, T. Urase, K. Yamamoto, Water Sci. Technol. 43 (2001) 91–99.
[118] B.G. Plósz, K.H. Langford, K.V. Thomas, Biotechnol. Bioeng. 109 (2012) 2752–2769.
[119] D. Sreekanth, D. Sivaramakrishna, V. Himabindu, Y. Anjaneyulu, Bioresour. Technol. 100 (2009) 2534–2539.
[120] C. Sirtori, A. Zapata, I. Oller, W. Gernjak, A. Agüera, S. Malato, Water Res. 43 (2009) 661–668.
[121] S. Chelliapan, T. Wilby, P.J. Sallis, Water Res. 40 (2006) 507–516.
[122] O.V. Enick, M.M. Moore, Environ. Impact Assess. 27 (2007) 707–729.
[123] N. Kulik, M. Trapido, A. Goi, Y. Veressinina, R. Munter, Chemosphere 70 (2008) 1525–1531.
[124] D.S. Suman Raj, Y. Anjaneyulu, Process Biochem. 40 (2005) 165–175.
[125] N. Oz, O. Ince, B. Ince, J. Environ. Sci. Health A 39 (2004) 2029–2042.
[126] T.M. LaPara, C.H. Nakatsu, L.M. Pantea, J.E. Alleman, Water Res. 35 (2001) 4417–4425.
[127] D.S. Suman Raj, N.S. Chary, V.H. Bindu, M.R.P. Reddy, Y. Anjaneyulu, Int. J. Environ. Stud. 61 (2004) 99–111.
[128] D.G.J. Larsson, C. de Pedro, N. Paxeus, J. Hazard. Mater. 148 (2007) 751–755.
[129] A.M. Deegan, B. Shaik, K. Nolan, K. Urell, M. Oelgemöller, J. Tobin, A. Morrissey, Int. J. Environ. Sci. Technol. 8 (2011) 649–666.

Chapter 9

Removal of Pharmaceuticals by Membrane Bioreactor (MBR) Technology

Ruben Reif, Francisco Omil and Juan M. Lema
Department of Chemical Engineering, School of Engineering, University of Santiago de Compostela, Santiago de Compostela, Spain

Chapter Outline

1 INNOVATIVE TECHNOLOGIES FOR EMERGING ISSUES

Combating water scarcity is undoubtedly a global priority. Different factors such as increasing population, climate change, more intensive agricultural practices, and urbanization constitute a challenge that will require a transformation of the water industry based on the combination of innovative technologies and new management approaches, with the aim to supply, protect, and reuse water in agricultural, industrial, and urban contexts. Twenty years ago, technologies based on membrane separation for wastewater treatment were first commercialized for special applications like the treatment of high-strength wastewater such as landfill leachate or industrial effluents. The most common membrane processes for wastewater treatment use pressure

Comprehensive Analytical Chemistry, Vol. 62. http://dx.doi.org/10.1016/B978-0-444-62657-8.00009-4

as the driving force, acting as selective permeable barriers, which permit the passage of water and can reject a wide range of particulate and dissolved compounds present in the wastewater [1]. Membrane design usually consists of polymeric materials with pores or molecular channels incorporated on its structure, being its molecular weight cutoff (MWCO) the main characteristic. In this sense, Schäfer et al. [2] distinguished two main categories: porous (ultrafiltration (UF) and microfiltration (MF)) and dense membranes (reverse osmosis (RO)), being nanofiltration (NF) modules between porous and dense. This classification strongly influences the type of application for each module, being MF/UF modules often employed in combination with biological treatment processes, the so-called membrane bioreactor (MBR) and NF/RO for effluent polishing, although RO membranes are most commonly used in drinking water purification from seawater due to its extremely high selectivity and ability to separate ions.

Focusing on the use of porous membranes, the first systems developed were based on cross-flow units placed outside the activated sludge tank and equipped with high-flow circulation pumps. Energy requirements were substantially high, so they were considered uneconomical for municipal wastewater applications. A first example of an early pilot project, which assessed the performance of membranes coupled with biological processes, was described in Knoblock et al. [3]. This work shows the development of design information for a system treating wastewater from two General Motors facilities. These types of studies provided a solid basis for the design of full-scale demonstration systems for the treatment of complex wastewater, characterized by a high variability in its composition. A recent review by Mutamin et al. [4] shows the knowledge available on the use of MBRs to treat high-strength industrial wastewater, confirming that, after more than 20 years of research, this technology has been extremely successful for industrial applications. Further research showed that the high operational cost, mainly attributed to energy consumption, eventually became the main constraint for the widespread implementation of membrane solutions, since their process specificities directly impact the energy demand. More specifically, aeration constitutes the main limiting factor, since it still accounts for $\sim$80% of the total energy demand. Aiming at overcoming this limitation, the more recent developments of a new generation of low-pressure/submerged filtration systems boosted the implementation of MBR technologies. This new operational strategy showed lower costs and consequently, applications to municipal wastewater treatment gained relevance. The operation of those immersed systems consists of the positioning of the membrane units in the activated sludge tank, requiring a lower transmembrane pressure. Air blowers, which have high energy consumption, could be simultaneously used for biological sludge aeration and membrane module scouring, to avoid fouling or pore clogging. Therefore, such systems are less costly to install and operate, making the technology more viable for the treatment of both municipal and industrial wastes.

In spite of this improvement, direct comparisons based on real operation still show lower costs for conventional treatments. For example, Fenu et al. [5] calculated an overall energy consumption of 0.64 kW h/m^3 of permeate, necessary for the operation of a full-scale MBR, with this demand being substantially higher than the estimated energy cost for processes based on conventional activated sludge (CAS) systems (0.3 kW h/m^3 of effluent). Considering the practitioner's point of view, Kraemer et al. [6] showed the main advantages and disadvantages of the MBR technology. Indeed, higher operational costs were mentioned as a main drawback, although other factors were highlighted, such as the lack of equipment standardization, their poor capacity facing flow peaks, and the greater mechanical performance, which make the exploration of new or expanded systems difficult. Therefore, ongoing research is still focused on improving systems to reduce energy consumption, with the aim to promote MBRs as definitive cost-effective answers to a growing range of treatment requirements.

In spite of the aforementioned drawbacks, MBRs have been gradually implemented in the market, and nowadays, they cannot be considered just as a promising wastewater treatment alternative, thus representing a mature technology. The review of Santos and Judd [7] analyzed the status of membrane products for MBRs with specific reference to municipal wastewater treatment, showing how the MBR market doubled in the 5 years between 2000 and 2005 to reach $217 million, being expected to increase its value from $296 million in 2008 to $488 million in 2013. In the survey carried out by Huisjes et al. [8], it was reported that by the end of the year 2008, about 800 MBR plants with an installed capacity greater than 20 m^3 d^{-1} (industrial applications) and 100 m^3 d^{-1} (municipal applications) were commissioned in Europe, of which 566 were built up for industrial applications and 229 for municipal applications. In the same study, Spain and Italy were pointed out as the most dynamic countries, since together doubled the parks of MBR units installed from 2005 to 2008. Indeed, this commercial success can be explained by MBR numerous advantages such as their small footprint (expanding an MBR-based treatment plant only requires the addition of new modules to existing basins, instead of installing another large clarifier), high-quality effluent (meeting very strict discharge limits particularly in terms of suspended solid and pathogen elimination), and high level of automation, being their capital costs comparable to conventional technologies when both are designed to achieve similar effluent quality [6]. It is also important to mention their low space requirements, due to the avoidance of the use of secondary settlers and, therefore, bulking issues. Thus, membrane technology is considered a useful technology for upgrading obsolete facilities.

In parallel with the gradual implementation of MBRs in the wastewater market, during the last decade, several studies have reported the worldwide occurrence of pharmaceutically active compounds (PhACs) in different

environmental compartments (surface waters, groundwaters, soils, sediments, etc.). This emerging environmental issue has been widely discussed on a scientific level, and it is evidently perceived in a comparable way in different countries. Within the context of the European Water Framework Directive (Directive 2000/60/EC), which has the aim of achieving a good status of all water bodies in Europe for 2015, current legislation is drifting toward the inclusion of new pollutants in the list of priority substances. More concretely, the inclusion of three pharmaceuticals of concern (diclofenac, estradiol, and ethinyl estradiol) in the list might imply a paradigm shift in the European wastewater management due to the substantial changes that many facilities should undertake in order to comply with the new regulations. For example, in Germany, the first full-scale applications of suitable technologies for trace pollutant removal are already being used or are under construction [9] since conventional water treatment processes were designed to remove organic matter and nutrients in some cases, but they cannot fully and systematically remove PhACs to a high extent, mainly due to their poor biodegradability. In this context, it is obvious that some of the aforementioned advantages of the MBR technology, particularly those related to effluent quality, might contribute to mitigate the continuous release of pharmaceuticals into the aquatic environment. Consequently, MBRs were soon targeted by researchers within the wastewater treatment field since it was relevant to assess the influence of some specific features in order to determine the potential of MBRs for an enhanced elimination of recalcitrant compounds:

- MBRs allow an accurate control of the sludge retention time (SRT). Previous works in this line point out that this parameter exerts a significant influence in the adaptation of the microorganisms to a continuous input of PhACs [10,11]. Longer SRTs would allow the growth of slowly growing bacteria, subsequently leading to the formation of a broader ecology of microorganisms with a wider spectrum of physiological and adaptation characteristics.
- MBRs are normally operated using a high suspended biomass concentration, which allows a more intense biological treatment within a reduced space. MBR biomass shows different physical properties compared with CAS, such as higher specific surface area and smaller particle size. Since biological sludge also acts as a sorbent for some pharmaceuticals, depending on their physicochemical properties (pKa and hydrophobicity), an enhanced sorption potential might be expected.
- Although expensive, posttreatment processes have achieved excellent results eliminating pharmaceuticals from sewage. Increased efficiency might be expected treating MBR permeate with technologies such as NF, ozonation, or filtration through activated carbon columns due to its significantly lower number of interfering substances (organic matter, colloids, suspended solids, etc.). In fact, MBRs can rightly be called the most important pretreatment solution before further advanced treatment [9].

2 MBRs FOR THE ELIMINATION OF PHARMACEUTICALS: 10 YEARS OF RESEARCH

Considering the aspects indicated, the availability of scientific literature on this topic has been growing during the last years. This section synthesizes the research on MBRs applied to remove pharmaceuticals from wastewater. In particular, we analyze past and current research in the field, providing a critical review of results attained, operational strategies adopted by researchers, and MBR configurations employed. Those are crucial aspects in considering how reliable and representative data are regarding the potential improved effectiveness of MBRs compared with conventional approaches.

An extensive survey carried out by Santos et al. [12] analyzes different topics that constitute the core of the research into MBRs. Briefly, their research survey was conducted using a web-based search engine, using five different primary research terms combined with another six secondary terms. Publications concerning membrane fouling were the most prominent of all those analyzed, but published studies of micropollutants were the ones growing faster, this obviously being driven by MBR current market size, growth projections, and the obvious impact of future regulations. A similar surveying approach was conducted by Hughes et al. [13], who carried out a global-scale analysis identifying all studies that had detected pharmaceuticals in either STP effluent or receiving waters across 41 countries. Their wide search criteria, also based on a review via a search engine for scientific literature, yielded more than 18,000 results, and consequently, the study was further constrained only to common journals, using in the end 236 papers. Obviously, the topic addressed in this chapter represents only a small picture within the vast number of scientific literature available dealing with the environmental issue of pharmaceuticals in the water cycle. Nevertheless, the use of a similar approach has allowed us to identify the most considered aspects regarding the use of MBRs for pharmaceuticals elimination as well as current trends and knowledge gaps. The web of knowledge search engine (http://apps.webofknowledge.com) was used for this survey, considering the topics "MBR," "membrane bioreactor," "pharmaceuticals," and PPCPs (Pharmaceutical and Personal Care Products), since pharmaceuticals are quite often grouped within this category. No restrictions based on time span were considered for this query, and we only included scientific papers published in journals belonging to the Science Citation Index, dismissing technical reports, short communications, or contributions to conferences. In total, 115 research papers dealing with aspects related to the topic were found and classified for this review. The first papers were published in 2003, and since then, their number has been growing exponentially. Obviously, the majority of them deal with the effectiveness of MBRs at removing different pharmaceutical compounds. According to the different research lines found on this topic, we

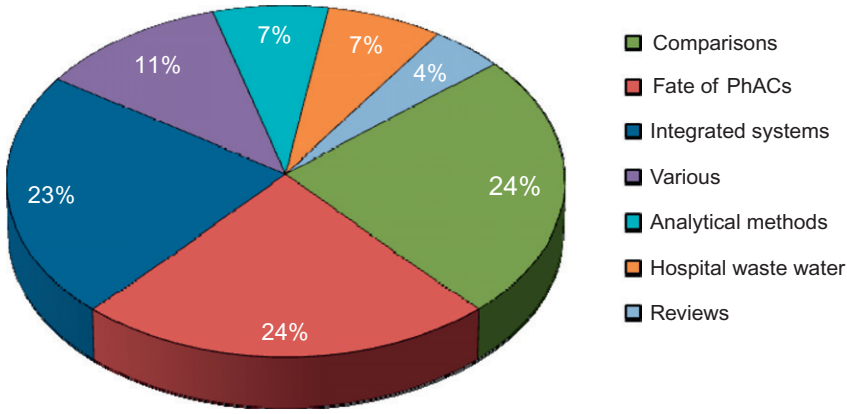

FIGURE 1 Main topics addressed in the research available on pharmaceuticals removal by MBRs.

have grouped them into different categories. Figure 1 shows the importance of each category according to the number of papers available online.

The most numerous studies were those that establish direct comparisons of the performance of MBRs with other technologies in terms of PhAC removal and those assessing the fate of different pharmaceuticals in MBRs, under the influence of different operational parameters. It is particularly interesting to highlight the growing use of integrated systems combining MBRs with other approaches (26 papers found), this category being the one that has gained more relevance in the last 3 years. For this type of works, it is important to clarify that the terminology employed in the literature is confusing and the terms "hybrid" or "integrated" are randomly used, very often mixed with "posttreatment." In the wastewater treatment field, it can be considered that a bioreactor is based on a hybrid configuration when a combination of two or more processes is taking place simultaneously within the same treatment unit, enhancing the overall quality of treatment thanks to synergistic effects. Often, this definition includes the involvement of two different types of biomass (suspended and fixed) within the same process.

Actually, MBR process can be considered as hybrid itself, since it combines within the same unit a biological treatment with a filtration step. In this case, it is obvious that the combination of both processes could provide a more advantageous treatment. On the contrary, two consecutive processes placed in a treatment train, for example, MBR followed by a polishing step using ozonation, should not be considered as a typical hybrid process. Therefore, we have grouped both types of approaches under the single term "integrated," which we consider more appropriate, although the majority of papers grouped within this category consisted of a further posttreatment of the MBR permeate. A comparatively lower number of papers classified as

"various" carried out different approaches, such as the elucidation of biodegradation kinetics, role of specific strains of bacteria (nitrifiers in most cases), studies about sorption and distribution of PhACs in wastewater and sewage sludge, or the effect of different PhACs on the behavior of microbial communities. Some studies were mainly focused on the development and validation of analytical methods for measuring different PhACs in wastewater, permeate, and sludge, which, in some cases, provided specific insights on the performance of the MBR used. A similar number of papers studied the overall performance of MBRs treating hospital wastewater, which is also an interesting application of the MBR process due to its complexity. For example, Beier et al. [14] found that 34% of antibiotics found in municipal wastewaters were originated from a hospital. Five reviews were found, and given the relative novelty of the topic, they were mostly focused on the comparison of data available for several technologies that provided information on the relevance of the main removal mechanisms influencing the elimination of PhACs.

In spite of the number of papers published, a general consensus regarding the reasons and the extent to which MBRs can improve the elimination of pharmaceuticals compared with conventional systems still has not been reached. As it will be shown in the following section, comparison between different studies is difficult due to the substantial differences in terms of operational parameters and size (lab, pilot, or full-scale), which add more uncertainty to the vast list of issues that researchers face trying to get reliable and consistent data (different sampling strategies, analytical methods, lack of reproducibility of results, etc.). A clear example of these challenges can be found in the calculation methodologies described in Carballa et al. [15] to perform mass balances of pharmaceuticals and personal care products in sewage treatment plants. This work showed how the method used for mass balance calculations (the use of measured data or solid-water distribution coefficients to calculate concentrations in sludge) could significantly affect the conclusions concerning the efficiency of a wastewater treatment process.

3 EFFICIENCY OF MBRs TO REMOVE PHARMACEUTICALS FROM WASTEWATER

During biological treatment, a vast number of factors could affect the process performance for removal of pharmaceutical compounds. Although their influence has been widely studied throughout literature, most of studies were focused on conventional systems. Nevertheless, valuable information can be extracted from such studies for a better understanding of PhAC elimination in MBRs. The review of Suarez et al. [16] showed that four main removal mechanisms govern the elimination of PPCPs during conventional treatment: volatilization, sorption to solids, biodegradation, and chemical transformation. Their individual contribution to elimination efficiencies is strongly determined by the physicochemical properties of each specific PhAC. Given the

distinctive features of the MBR technology, the assessment of biodegradation and sorption is particularly interesting to elucidate how the use of membranes might enhance removal efficiencies.

Biodegradation of PhACs can in principle be driven either by metabolism, when microbial growth is achieved using the micropollutant as a source of primary carbon or nutrients, or by cometabolism, which implies that transformation is carried out by the action of extracellular enzymes produced by the cells, not leading to cellular growth or energy production [1]. The relevance of this second pathway might be greater than expected due to higher availability of other pollutants in sewage at much higher concentrations, which are more likely to act as primary substrates. How biodegradation is achieved might be independent of the technology employed, but in the case of MBRs, subtle differences might be expected. For example, Jones et al. [17] reported that systems operating at high SRT, which is a common characteristic of most MBRs, could favor a higher and less specific enzymatic activity due to the increased cell lysis.

Sorption takes place by two very different mechanisms: Absorption, which is strongly dependent on PhAC lipophilicity, is driven by their interactions with the lipophilic cell membrane of the microorganisms and with the lipid fractions of the sludge. On the contrary, adsorption proceeds by the electrostatic interactions of positively charged groups of PhACs with the negatively charged surfaces of microorganism, and thus, it is related to the tendency of a substance to be ionized in aqueous phase. Since smaller floc sizes and surface area have been reported for MBR biomass [18], a slightly different behavior might be expected in terms of sorption potential. The most common approach to determine the fraction of PhACs sorbed onto solids is the use of solid-water distribution coefficients (K_d, in L kg^{-1}), whereas biodegradability is estimated through pseudo first-order degradation kinetics (K_{biol}) as shown in Joss et al. [11]. Apparently, PhACs with high values of both parameters will be successfully eliminated during the biological treatment, whereas those compounds presenting low values will not be removed nor biotransformed at a significant extent. In both situations, the influence of operating parameters of the plant will be rather limited [16]. Therefore, intermediate situations with one high value, either K_d or K_{biol}, are of interest for MBRs, due to the aforementioned capacity to operate at extended SRT (a feature typically associated with high sludge concentrations), independently of the hydraulic retention time (HRT) applied. Unfortunately, the availability of K_d and K_{biol} data specifically measured for MBRs is extremely scarce. Table 1 classifies PhACs into four elimination ranges using information gathered from a selection of 16 research papers focused on MBR technology, also showing K_{biol} and K_d data. Since there are potentially hundreds of pharmaceutical compounds present in the aquatic environment, for the purposes of this chapter, we constrained the selection of substances of interest to 12 representative PhACs from five therapeutic classes. The selection was based on the following

TABLE 1 Efficiency of PhAC Removal in MBRs According to the Reviewed Papers

					Elimination Range (%)				
Therapeutic Group	**PhACs**	**Acronym**	**K_{biol}**	**K_d**	***0–20***	***20–50***	***50–80***	***80–100***	**References**
Antibiotics	Erythromycin	ERY	0.31	10.2	0	0	1	2	[20–22]
	Roxithromycin	RXT	0.51	21.8	0	0	4	1	[20,22–24]
	Sulfamethoxazole	SMX	0.3	8.6	0	0	5	1	[20,22–26]
	Trimethoprim	TMP	0.05	25.4	1	2	0	3	[20–24,26]
Antidepressant	Fluoxetine	FLX	1.98	355	1	0	0	1	[20,26]
Antiepileptic	Carbamazepine	CBZ	0.00	<2.7	6	2	1	0	[20–23,25–28]
Anti-inflammatories	Diclofenac	DCF	<0.10	78.5	5	4	0	0	[20,22–24,26,28–31]
	Ibuprofen	IBP	38.07	112	0	0	0	8	[20,22,23,26,28,29,31,32]
	Naproxen	NPX	4.23	35.5	0	0	4	4	[20,22–24,26,29,31,32]
Hormones	Estradiol	E2	800	*250–630*	0	1	0	3	[21,26,27,33]
	Ethinyl estradiol	EE2	8	*316–630*	0	1	0	5	[21,23,27,33–35]
Tranquilizer	Diazepam	DZP	0	32.4	2	1	0	0	[20,22,26]

K_{biol} (L (g VSS d)$^{-1}$)and K_d (L kg^{-1}). Data in italics belong to CAS systems.
Data were obtained from [11,16,19] and removal data were from references shown on the table.

criteria: to consider a wide range of substances found at measurable levels in STP effluents, with high prescription rates and belonging to different therapeutic groups. Simultaneously, it was preferred to work with substances comprising different physicochemical properties and therefore behavior/fate throughout sewage treatment processes and with an availability of reliable analytical methods to detect them in complex matrices such as wastewater.

Although the fate and behavior of PhACs during MBR treatment is the main aspect addressed in the reviewed papers, some of them also provide information on the assessment of operating conditions (pH, temperature, MLSS concentration, HRT, and SRT), elucidation of removal mechanisms, and other relevant findings, as shown in Table 2.

From Table 1, it can be seen that IBP was the PhAC most efficiently transformed in MBRs closely followed by NPX, in good agreement with their reported K_{biol} and K_d values. E2, EE2, and ERY were also easily removed PhACs (although the availability of information was slightly limited for E2 and ERY), showing a consistent trend among different studies. It is interesting to highlight that, in spite of similar removal efficiencies, their behavior is substantially different. According to both constants, E2 and EE2 removal is mainly driven by sorption, whereas ERY is biologically transformed. Therefore, it is expected that operation parameters might influence differently the extent of their elimination. Data available for CBZ are fairly consistent and well correlated with K_{biol} and K_d and show the opposite fate, with very poor eliminations reported. DZP and DCF eliminations are similarly low. The availability of data was again limited for DZP, although the range of eliminations reported is again in good agreement with kinetic and sorption data. In the case of DCF, the extent of its removal ranges from 0 to 50%. This high variability can be attributed to its moderate sorption behavior and low biodegradability, which might enhance or reduce its removal depending on MBR operating conditions. TMP also shows the same variability, which also confirms the importance of varying operational aspects on its removal. RXT and SMX show low to moderate K_{biol} and K_d. Accordingly, most of the reviewed papers placed its removal in the 50–80% range. Data available for FLX were scarce (two papers) and contradictory (lowest and highest range of removal reported). The fate and behavior of this compound should be considered for future studies in MBRs. However, according to K_{biol} (moderate) and K_d (high), its elimination should be placed in the upper range.

From this assessment, it can be stated that the fate of recalcitrant or easily transformed pharmaceuticals in MBRs has been well elucidated, and further research efforts on this topic should shift toward other aspects. In the case of easily removed PhACs, the fate of their generated degradates during the treatment should be assessed as well. For recalcitrant PhACs, the exploration of new approaches based on integrated configurations is indeed the key to find feasible mitigation options. However, the optimization of operating parameters and the elucidation of other aspects that might help to understand

TABLE 2 Characteristics and Operating Conditions Applied in the MBRs Assessed for PhAC Elimination

Configuration	Topics Covered	Scale	Feeding	HRT (h)	SRT (days)	Redox	VSS ($g\ L^{-1}$)	References
Two MBRs hollow-fiber submerged MF membranes	Fate study relating removal with the chemical structure	Pilot	Real	9	–	Aerobic	10	Kimura et al. [20]
Submerged plate module (MF)	Identification of microbial metabolites	Lab	Real	8.8–10	37	Aerobic	20–30	Quintana et al. [21]
Four flat-sheet submerged modules (MF)	Fate and behavior of two differently radiolabeled forms of ethinyl estradiol	Lab	Synthetic	15	25	Aerobic	8	Cirja et al. [33]
Hollow-fiber submerged UF module	Assessment of membrane module performance	Pilot	Synthetic	12	44–72	Aerobic	8	Reif et al. [34]
Submerged hollow-fiber (MF)	Influence of adaptation, pH, and HRT	Lab	Synthetic	1–8	Extended	Aerobic	2.3–4.6	Bo et al. [27]
Submerged	Decentralized wastewater treatment using a single-house MBR	Full	Real	3.4/6.3	150/100	An–Anox–Aerob	3.8/6.2	Abegglen et al. [29]
Three submerged plate membranes made of chlorinated polyethylene	Degradation of ethinyl estradiol using a nitrifier enrichment culture	Lab	Synthetic	0.6–96	Extended	Aerobic	0.1–0.7	De Gusseme et al. [23]

Continued

TABLE 2 Characteristics and Operating Conditions Applied in the MBRs Assessed for PhAC Elimination—Cont'd

Configuration	Topics Covered	Scale	Feeding	HRT (h)	SRT (days)	Redox	VSS ($g\ L^{-1}$)	References
Submerged hollow-fiber PVDF membranes (UF)	Use of a full-scale multiredox system. Adsorption/ biodegradation kinetics	Full	Real	12	20	An–Anox–Aerob	11.5	Xue et al. [30]
Submerged hollow-fiber (UF)	Relevance of adsorption and biodegradation mechanisms	Pilot	Real	9	50	Aerobic	5–6.3	Dialynas et al. [24]
Six flat-sheet submerged modules (MF)	Use of isotopically labeled diclofenac and metabolites	Lab	Synthetic	8	28	Aerobic	10	Bouju et al. [31]
Three submerged polysulfone membranes (UF)	Fate and distribution of estrogens between the solid and liquid phases	Lab	Synthetic	7–12	35–95	Aerobic	5–8	Estrada-Arriaga et al. [35]
Two hollow-fiber submerged modules (UF)	Enantiospecific fate of ibuprofen, ketoprofen and naproxen	Lab	Synthetic	24	70	Aerobic	8.6–10	Hashim et al. [22]

Submerged hollow-fiber (MF)	Study of CBZ degradation in anoxic conditions	Lab	Synthetic	24	Extended	Anox/ Aerob	10.5	Hai et al. [28]
Hollow-fiber modules (UF)	Efficiency of two MBRs operated at different SRTs	Pilot	Real	9–13	15 and 30	–	12	Schroeder et al. [25]
Submerged hollow-fiber (UF)	Decentralized MBR to characterize the removal of 48 trace organics	Full	Real	24	10–15	Anox/ Aerob	7.5–8.5	Trinh et al. [32]
Submerged hollow-fiber (UF)	Estimation of K_{biol}, K_d and liquid–solid partition coefficients for 10 PhACs in an SBR and an MBR	Pilot	Real	24	125	Aerobic	4.3	Fernandez-Fontaina et al. [19]

how MBRs can help to attenuate the release of PhACs in the aquatic environment should still be assessed for those compounds of moderate biodegradability and/or sorption potential.

Table 2 provides relevant information from the selected studies regarding different parameters applied, type of MBR used, and main topics covered. It can be observed that many papers studied the performance MBRs operated at lab scale and using synthetic feeding. Although the information that can be obtained from those experiments is indeed valuable to provide a better understanding of some specific characteristics of this technology, they do not necessarily reflect the real situation at full scale. Considering that nowadays it is easier to find full-scale facilities implementing MBR technology, further research should fill this gap. A careful revision of Table 2 illustrates one of the main drawbacks that researchers face trying to find conclusive information: a considerably high uncertainty, since available data are subjected to the influence of a large set of variables (scale factor, applied conditions, experimental designs, configurations, sewage characteristics, sampling strategies, analytical uncertainty, etc.). Accordingly, this leads to a high variability on the removal data found for specific PhACs.

3.1 Relevance of Operational Parameters and Other Factors

3.1.1 Hydraulic Retention Time

HRT indicates the mean residence time of the wastewater within a biological reactor, thus determining the contact time between the pollutant and the microorganisms. The HRT usually applied for conventional processes ranges from 5 to 24 h. According to Table 2, MBRs usually apply a similar range, and theoretically, conclusions from studies testing different HRTs should not vary when compared to those obtained from conventional systems. Nevertheless, the relevance of this parameter on the elimination of pharmaceuticals is not completely elucidated yet, although it is suspected that a minimum HRT is needed to accomplish the complete removal of a specific pollutant. This minimum value might vary depending on the biodegradability of each pollutant and other operating conditions, which also influence the reaction kinetics (e.g., temperature). For example, Bo et al. [27] showed low or no influence of different HRTs (1 day, 3 days, and 8 h) tested in an MBR for removal of ibuprofen, carbamazepine, and diclofenac, whereas Tauxe-Wuersch et al. [26] determined the influence of HRT on the removal of acidic drugs in full-scale conventional plants, showing a different behavior of ibuprofen, with efficiencies varying from 0% to 79% depending on the HRT. Apparently, a correlation was obtained indicating that an increased HRT resulted in higher ibuprofen degradation. Abegglen et al. [29] indicated that this parameter might influence the efficiencies to a certain extent in MBRs, but only for compounds of moderate biodegradability with the premise of operating at long SRT.

3.1.2 Sludge Retention Time

SRT determines the mean residence time that bacteria remain inside a biological reactor and greatly affects the development of microbial diversity. Different studies have shown that the SRT of biological reactors may influence the removal efficiency of degradable pharmaceuticals such as ibuprofen, naproxen, or ethinyl estradiol [10,36]. In general, a critical value of 10 days has been observed to exert a positive effect on their removal, which is in good correlation with the minimum SRT of 10–15 days proposed as necessary to ensure the development of a diverse biocenosis able to achieve nitrification, denitrification, and phosphorus removal. The development of an enriched nitrifier population, typically associated with longer SRTs, can enhance the elimination of some specific PhACs. For example, De Geusseme et al. [23] found high elimination of ethinyl estradiol in an MBR using a nitrifier enrichment culture. Moreover, a linear relationship between specific micropollutant biodegradation rate and the nitrification rate was found in an enriched nitrifying bioreactor [19]. Often, MBRs are operated with extended values of SRT, which implies no sludge withdrawal from the bioreactor. Lesjean et al. [37] observed a substantial higher elimination of PhACs operating at SRT = 26 days than at 8 days. Apparently, once the growth of bacteria involved in the treatment process is ensured, SRTs longer than 20 days might not further enhance micropollutant removal [10]. Again, the literature shows some contradictions, since other studies have shown that SRTs longer than 2 months can improve the removal efficiencies for compounds such as mefenamic acid, indomethacin, and diclofenac [38]. Therefore, it is not easy to extract further conclusions comparing different works due to the aforementioned variability of conditions (from 10 days to extended). Since SRT has been pointed out as the most influential parameter on PhAC removal and is easy to modify in an MBR, its influence will be explained in detail in a subsequent section of this chapter, showing the operation of a parallel-operated MBR–CAS system, under strictly similar conditions.

3.1.3 Redox Conditions

Table 2 shows that most of the MBR studies were carried out in aerobic conditions, which are supposed to be adequate to maximize pharmaceuticals removal. However, specific compounds might be better removed by incorporating varying redox conditions such as anoxic or anaerobic stages within the same process, as shown in Joss et al. [39]. A study carried out in lab-scale CAS by Suarez et al. [40] showed that fluoxetine and estradiol were transformed to a large extent (>65%) under anoxic conditions, whereas carbamazepine, diazepam, sulfamethoxazole, and trimethoprim were not biodegraded. The number of studies testing anoxic/anaerobic conditions in MBRs is particularly scarce, although it is possible to find some examples. In Abargues et al. [41], the elimination of hormones and nonionic surfactants

was tested in an anaerobic MBR and compared versus an aerobic conventional plant and an MBR. The three systems were similarly effective in removing hormones, and the main differences were found for surfactants. In this case, anaerobic conditions proved to be less favorable for surfactant degradation. Hai et al. [28] found that near-anoxic conditions (dissolved oxygen of about 0.5 mg L^{-1}) were a favorable operating regime for removal of carbamazepine by MBR treatment, which is in contradiction with the aforementioned data from Suarez et al. [40]. Considering this, it is obvious that the influence of different redox conditions has not been sufficiently studied in MBRs and should deserve further attention.

3.1.4 Biomass Characteristics

The MBR biological sludge characteristics experience changes during the operation due to factors such as the complete retention of solids inside the bioreactor, extended SRT operation, or the effect of the membrane filtration process [42]. Early studies on MBR biomass properties were carried out to extend the understanding of membrane-fouling mechanisms, considered a significant drawback for MBR implementation. For example, Massé et al. [18] found different structural conformations of biomass in MBRs, which influence its settling properties. Other differences were found for properties such as the specific cake resistance, floc size, viscosity, hydrophobicity, and surface charge [43–45].

As an example, Figure 2 shows the morphology of MBR and CAS sludge using a scanning electron microscope. The sludge structure observed consisted of compact and well-defined macroflocs, but it illustrates important differences between both morphologies. Focusing on the influence of these aspects on PhACs, Kimura et al. [46] found larger specific sorption capacities for diclofenac during batch experiments with MBR sludge. It was hypothesized that MBR sludge also had a larger specific surface area. However, in Cirja et al. [47], it is mentioned that some enzymatic activities increase

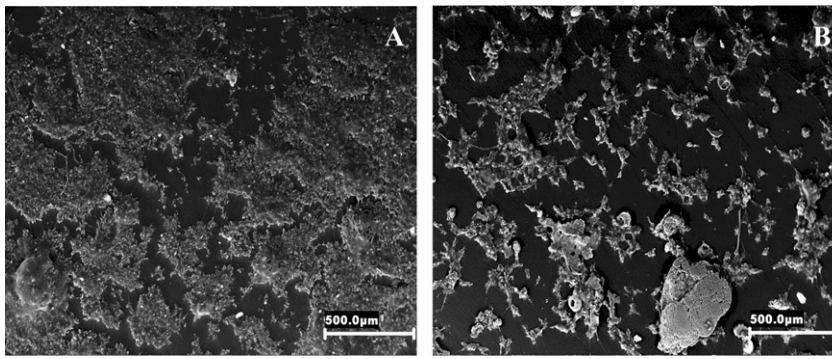

FIGURE 2 SEM scans obtained with biomass from (A) MBR and (B) CAS.

proportionally to the higher specific surface area of the floc structure. According to this, the smaller particle sizes usually found in MBR sludge might favor reactive processes as well. However, all these explanations are highly speculative, and there is little conclusive research to support a link between sludge surface area and sorption potential. To date, only a few works have estimated sorption coefficients for both CAS and MBR systems. Radjenovic et al. [38] compared the sorption of various pharmaceuticals using sludge from two MBRs (operated at extended SRT) and one CAS. Apparently, PhACs tended to sorb less onto the aged MBR sludge compared with primary and CAS sludge, and it was pointed out that such results were likely due to a higher biodegradation potential in MBR biomass rather than to a diminished sorption potential. However, most of the studied PhACs in that work had low tendency to be associated with the particulate phase based on their estimated distribution coefficients (K_d). As a consequence, sorption was found to be a minor removal pathway. Yi et al. [48] determined K_d values of 0.33–0.57 L g^{-1}, equal to or larger than those of a CAS (0.25–0.33 L g^{-1}) for ethinyl estradiol. In this case, a clear correlation between biomass characteristics and sorption potential was found. Interestingly, the modification of the SRT was not considered an effective strategy to modify the particle size. Li et al. [49] carried out experiments with MBR and CAS lab-scale bioreactors fed with synthetic feeding spiked with ethinyl estradiol to investigate its removal, mineralization, and bioincorporation. Similar parameters were simultaneously applied in both systems (HRT of 12 h and SRT of 20 days). The K_d of ethinyl estradiol determined for an MBR sludge was 0.64 L g^{-1}, which was higher than the value of 0.52 L g^{-1} found in the CAS. Although a different sorption potential was observed, it was only relevant at EE2 concentrations >50 μg L^{-1}. It appears that further research in more realistic conditions is still required to understand how MBRs might enhance the removal of PhACs undergoing a sorption mechanism.

3.1.5 Membrane Filtration Step: Role of pH and Natural Organic Matter

Only few studies were focused on the influence of the membrane filtration step on PhAC removal (Table 2). Often, researchers point out that the rejection mechanism due to size exclusion is not expected. This hypothesis is based on the pore size of the UF or MF membranes (ranging between 50 and 10,000 nm for MF and 1 and 100 nm for UF), substantially larger than the average pharmaceuticals MWCO. For example, Yoon et al. [50] mentioned that pollutants of molecular weight lower than 400 g mol^{-1} cannot be retained even by the lowest MWCO membranes. In Table 2, we observe that both types of membranes (MF and UF) are indistinctly used for this type of research studies. However, the trend for wastewater treatment applications is to focus onto UF modules, due to a key advantage: they are able to remove

bacteria and most viruses, providing the treatment with an additional disinfection step. Since the hypothesis based on molecular sizes is commonly accepted, few studies have compared levels of pharmaceuticals present on the mixed liquor compared with levels found in permeate, discarding other feasible interactions that might exert influence on the removal of pharmaceuticals. For example, in Semião et al. [1], it is highlighted that membrane adsorption might be relevant to compounds such as hormones, although sorption capacity of the membrane might be easily exhausted once adsorption sites saturate. Indeed, this situation might occur easily during the sewage treatment process, due to the large number of compounds present in the mixed liquor (extracellular polymeric substances, proteins, colloids, etc.). It is also mentioned that the development of a fouling layer onto the membrane surface might alter its MWCO, making this layer able to provide partial rejection of macromolecular organic carbon to which some pharmaceuticals are adsorbed to. Other factors such as pH [51] and the presence of natural organic matter [52] might also exert a high influence on observed retentions. More specifically, pH can promote or decrease sorption through the formation of H-bonds, whereas natural organic matter can acts as a competitor decreasing available sorption sites. Bouju et al. [31] provide a deeper insight on this matter thanks to the use of isotopically labeled compounds. This novel methodology can help to identify PhACs sorbed onto the membrane surface and/or sludge. In the aforementioned paper, the fate of diclofenac and its most relevant human metabolite, 4′-hydroxydiclofenac, was assessed in an MBR. Spiking with a single pulse of ^{14}C-radiolabeled diclofenac, they could demonstrate that the presence of this compound onto the membrane surface was negligible. However, diclofenac is not characterized by a high sorption potential. In this sense, a wider number of PhACs, particularly those with more hydrophobic characteristics (e.g., azithromycin, with a $K_{ow} = 4$), should be assessed in further works. Of course, the use of other types of membranes (NF or RO) has provided quite better results in terms of pharmaceuticals rejection and water quality in general, but their use is usually restricted to polishing applications or drinking water production.

3.2 Innovative Hybrid Configurations Using Activated Carbon

From the analysis of the available data, it is obvious that MBRs cannot provide a complete elimination of the load of pharmaceuticals present in wastewater. As a consequence, new studies appeared during the last few years attempting to overcome this limitation with other approaches, many of them based on a further posttreatment of the MBR permeate with integrated systems (Figure 1). Since the use of such alternatives (ozonation, advanced oxidation processes, and the use of NF and RO membranes) has shown great effectiveness improving the removal efficiency of different PhACs, they will be particularly addressed in Chapters 10 and 11 of this book.

Therefore, the emphasis of this section will be put on systems integrating MBR treatment and sorption onto activated carbon, which have shown promising results.

The use of activated carbon for removing specific pharmaceuticals has been widely studied, showing its usefulness to mitigate their release. Baumgarten et al. [53] studied the elimination of antibiotics from MBR permeate dosing different amounts of powdered activated carbon (PAC), showing an increased elimination with a parallel increase of PAC dosage. Nguyen et al. [54] used an integrated system consisting of a lab-scale MBR treating synthetic sewage followed by a column filled with granular activated carbon (GAC), where the MBR permeate was pumped in an upflow mode. The GAC posttreatment led to a substantial increase in the removal of carbamazepine and diclofenac among other compounds, in spite of their moderate hydrophobicity. Mechanisms highlighted to explain the high removal achieved were ion exchange, surface complexation, and hydrogen bonding. In parallel, new research studies are starting to show the advantage of seeding the mixed liquor with adsorbents, in a similar manner to the use of charcoal amendments for sediment and soil bioremediation, and it has been demonstrated that direct PAC addition into the MBR mixed liquor can also lead to increased retention of pharmaceuticals. In this sense, the MBRs are particularly useful since the sorbent can be successfully separated from the treated permeate thanks to the filtration step. A first approach of this strategy was described by Guo et al. [55], although in this case the use of this type of amendments was studied in relation to membrane-fouling mitigation, since the activated carbon might have additional benefits for the membrane performance and integrity, facilitating the operation with a sustainable transmembrane pressure. Li et al. [56] found improved removal of sulfamethoxazole and carbamazepine by a PAC-amended MBR system. The removal of these compounds was dependent on their hydrophobicity and loading as well as the PAC dosage, achieving maximum removal efficiencies for sulfamethoxazole and carbamazepine of 82% and 92%, respectively. However, to maintain such eliminations, the application of a high PAC dosage (1 g L^{-1}) was imperative to sustain the high micropollutant loading, which suggests a quick depletion of available sorption sites due to the high pharmaceutical concentration in the synthetic sewage (750 μg L^{-1}). A similar PAC dose was applied by Serrano et al. [57] in a sequential MBR treating synthetic sewage spiked with nine PhACs. After a single addition of PAC directly into the aeration tank, the more recalcitrant PPCPs carbamazepine, diazepam, diclofenac, and trimethoprim reached removal efficiencies in the range of 93–99%. A very recent study [58] compares the performance of both approaches (GAC posttreatment vs. PAC addition). Both strategies were successful for complementing MBR treatment to obtain high overall elimination of biologically resistant PhACs, although PAC addition was more efficient since it showed improved efficiency in terms of activated carbon consumption. Therefore, the next steps

should involve the optimization of the PAC dose considering the use of real wastewater and a more complete assessment of the effects of PAC addition on membrane module performance.

3.3 Comparison with Conventional Processes

3.3.1 Compilation of Removal Efficiencies from the Literature

Figure 1 shows that the number of papers devoted to a direct comparison between CAS and MBR systems in terms of PhAC removal is prominent compared with other type of approaches. The main conclusion tends to be common in most of them: MBRs show improved performance eliminating PhACs. This can be easily confirmed by analyzing review papers, which handled a large quantity of data. For example, in Omil et al. [1], it is mentioned that reported eliminations in comparison studies tend to be higher for MBRs (>25%), although this increase might be attributed mainly to the optimum conditions set in those systems, more specifically the SRT. The recent review of Verlicchi et al. [59] presented data pertaining to 244 CAS systems and 20 pilot-scale MBRs. Although this vast compilation confirmed that there is a high variability range, the observed trend also confirms that MBRs guarantee higher removal efficiencies for some PhACs, apart from a better permeate quality. Similar conclusions are found in the review of Sipma et al. [60], which used a similar approach to compare data from both technologies and concluded that MBRs seem to be superior for most pharmaceuticals of moderate biodegradability, but not for those that are well degradable or resistant to biological treatment. However, it is obvious that the data available need more precise and critical assessment. In this sense, the high variability of the removal efficiencies observed for many PhACs constitutes a major drawback in understanding how MBRs outperform conventional systems. It is also difficult to gather reliable conclusions when data reviewed do not belong to research specifically carried out to compare both technologies. Although the premise of analyzing a large set of data from MBRs and CAS using statistical tools might be valid, the number of papers dealing with MBRs is comparatively low, and there are even less papers devoted to carrying out direct MBR–CAS comparisons. Therefore, this section analyzes removal data gathered from a more limited number of research papers (16), which carried out a direct comparison between simultaneously operated bioreactors. The average removal efficiencies from those studies are summarized in Figure 3, which complements Tables 3 and 4, where more detailed information is provided. The availability of data for FLX and DZP was fairly limited, which explain their low variability shown in the figure. In fact, the few data available for DZP in other types of studies reveal that it is a recalcitrant compound, although in Martin Ruel et al. [68], an efficiency of 80% was achieved. In this comparison, it can be clearly observed that with no exception, MBR

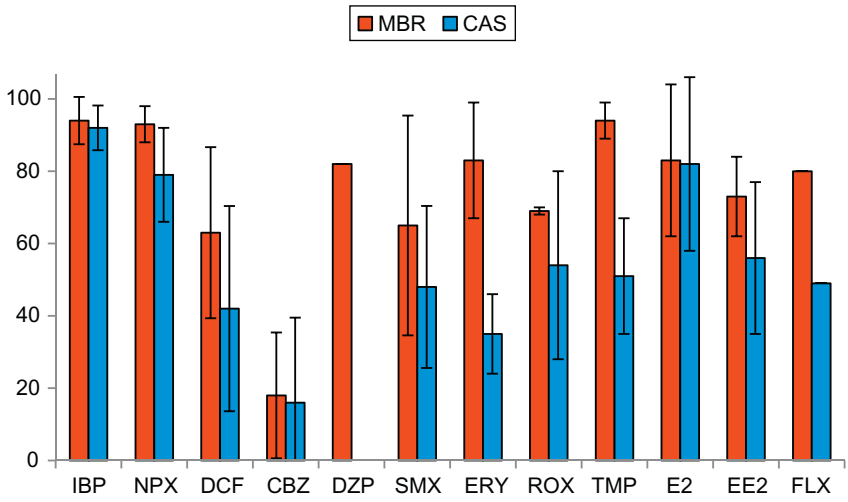

FIGURE 3 Average CAS and MBR removal efficiencies estimated from 16 selected publications.

performance is slightly or substantially better than that of CAS. Analyzing the different conclusions found in the assessed papers, the high SRT used in MBRs is pointed out as the main reason explaining the observed differences. However, other feasible explanations are frequently mentioned throughout the literature, most of them related to sludge characteristics.

As shown in Tables 3 and 4, in most cases, the works consisted in a simultaneous operation of two reactors using the same feeding. Although this approach is indeed correct, we consider that a more adequate comparison in terms of overall performance should be carried out operating both systems at their maximum capacity, which might not be true in many tested CAS systems. Moreover, many studies were carried out comparing pilot- or lab-scale MBRs with full-scale sewage treatment works already in operation. Those full-scale facilities are not easily controllable for developing accurate sampling strategies and long-term experiments, and their operation is not fully devoted to the purposes of this type of research. These points should be considered for further experimentation, not only aiming at identifying more unequivocally the potential strengths of the MBR technology those associated with the high SRT or MLSS concentrations achieved but also showing how the appropriate operation of CAS systems might enhance the elimination of many PhACs. In Tables 3 and 4, it can be observed that only three papers assessed systems operated in similar conditions. In order to gain a deeper knowledge on this topic, the direct operation of parallel systems under strictly similar operating conditions trying to attain the maximum capacity of each is strongly advised.

TABLE 3 Comparative Performance of MBR–CAS Systems for PhAC Removal (Eliminations Observed)

	Removal (%)				Removal (%)				Removal (%)		
PhAC	*MBR*	*CAS*	References	PhAC	*MBR*	*CAS*	References	PhAC	*MBR*	*CAS*	References
ROX	0/34/73	0/44/41	[10]	CBZ	12/44/0	14/0/0	[10]	IBP	98/99/97	100/100/99	[10]
	68	80	[61]		13	7	[62]		99	97	[62]
SMX	61	65	[10]		0	0	[63]		95/98	98	[46]
	60	56	[63]		3	10	[64]		100	82	[63]
	75	0–66	[65]		0	0	[66]		83/98	50/70/90	[67]
	88	52	[68]		51/32	67	[69]		84/82	88	[69]
	70	52	[61]	DCF	0/51/33	53/63/47	[10]	NPX	96	64	[46]
	100	–	[69]		58	24	[62]		99	85	[63]
	52/55	46	[70]		51/82	42	[46]		57/83/69	5/38/69	[67]
TMP	97	29	[64]		87	50	[63]		97/95/99	97	[69]
	95	0–49	[65]		8	0	[71]	E2	>41	>41	[65]
	99	45	[61]		78	8	[64]		98	98	[72]
	53–98	0–77	[66]		58	9	[68]		88	92	[69]
EE2	>92	>92	[65]		0/58/77	0/87/71	[67]		99	99	[73]
	52–76	30–68	[49]		61	37	[66]	ERY	67	24	[63]
	67	42	[72]		88/76/91	92	[69]		61	71	[61]
	80/83	49	[69]	FLX	80	50	[68]	DZP	82	0	[68]

TABLE 4 Comparative Performance of MBR–CAS Systems for PhAC Removal (References Used and Additional Comments)

References	Parallel Operation	Comments
Clara et al. [10]	No	SRT was pointed out as the main influencing parameter
Bernhard et al. [62]	No	Improved adaptation rates resulting from SRT above 14 days
Kimura et al. [46]	No	Two MBRs operated at different SRTs
		Larger adsorption capacity of MBR sludge for DCF
Radjenovic et al. [63]	No	Greater fluctuations observed in CAS
		CAS removal more sensitive to changes in operating conditions
De Wever et al. [71]	Yes	High SRT achieved also in CAS (>100 days)
		Reduced lag phases and stronger memory effect for the MBR
Celiz et al. [64]	No	Development of an analytical method
Le-Minh et al. [65]	No	Full-scale conventional system with MBR added as sidestream
Martin Ruel et al. [68]	No	Wide study comparing six CAS, one MBR and six tertiary treatment technologies
		MBR showed increased efficiency (average 20% for 22 compounds)
Reif et al. [67]	Yes	Smaller particle size was found in the MBR
		MBR showed better performance than CAS at low SRT (6 days)
Sahar et al. [61]	No	Comparison of CAS+UF (full-scale) and a pilot-scale MBR
		The incorporation of UF after CAS improved the antibiotics removal
		Biofilm formed on membrane might explain the enhanced removal
Sui et al. [66]	No	Study of seasonal variations in full-scale facilities
		MBR was less susceptible to ambient temperatures and operational perturbations

Continued

TABLE 4 Comparative Performance of MBR–CAS Systems for PhAC Removal (References Used and Additional Comments)—Cont'd

References	Parallel Operation	Comments
Yi et al. [49]	Yes	Enhanced EE2 removal in the MBR at high concentrations (300–500 μg L^{-1})
		Similar removal when the influent EE2 concentration was 24.5 μg L^{-1}
Zhou et al. [72]	No	Lab-scale MBR compared with a sequencing batch reactor (SBR)
		Critical SRT of 10 days (minimum) for an efficient EDC removal
Camacho-Muñoz et al. [69]	No	Comparison of different MBR configurations/ modules with CAS
		Unusually high removal of DCF and even for CBZ after RO treatment
		Low differences between the three systems
García Galan et al. [70]	No	Two pilot-scale MBRs with different submerged modules compared with a CAS
		Low amount of sulfonamide antibiotics (<3%) on digested sludge
Lopez-Fernandez et al. [73]	Yes	SRT >10 days is enough for efficient E2 removal in both MBR and CAS systems

3.3.2 *Demonstration of a Case Study: Parallel Operation of CAS and MBR Systems*

In this section, the performance of two parallel-operated systems, a pilot-scale MBR and a lab-scale activated sludge unit, was compared in terms of PhAC removal (Figure 4). This study was intended to truly simulate the operation of both technologies in the conditions applied in full-scale facilities, strictly monitoring their main operational parameters (sludge concentration, HRT, SRT, pH, and temperature) in order to ensure that they were maintained at similar values in both bioreactors. Feeding consisted of municipal wastewater spiked with PhACs in concentrations within their environmental range (1–10 μg L^{-1}). The impact of a substantial SRT decrease was assessed, and the particle size of the biomass present in both systems was also monitored. The setup for the development of this study was located at the premises of

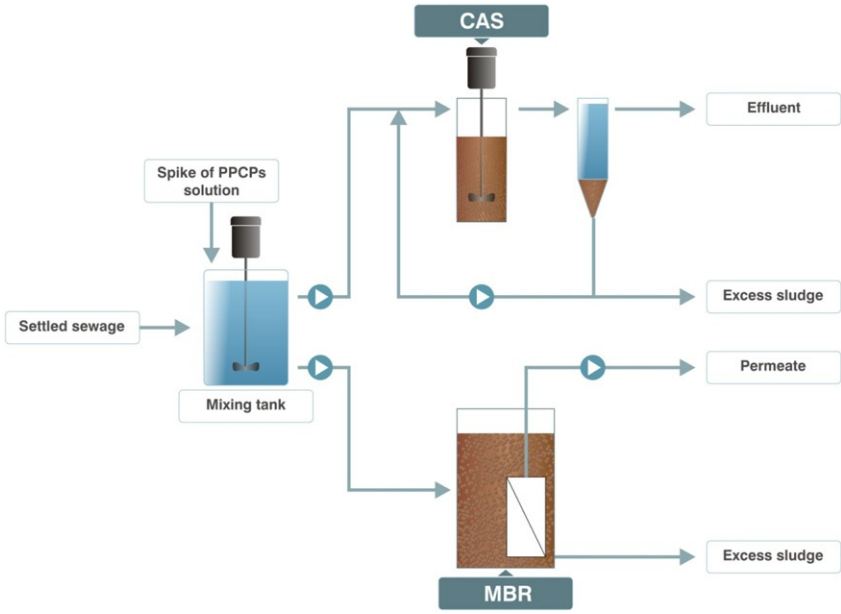

FIGURE 4 Flow diagram of the setup to compare the performance of CAS and MBRs for PhAC removal.

a municipal WWTP. It consisted of a primary settling step followed by a mixing tank where PPCPs were continuously spiked. The biomass was completely adapted to a continuous input of PhACs, since the MBR was previously operated and fed with the same spiked sewage during an extended period (>1 year) and was used as inoculum to start up the parallel CAS bioreactor. An additional PhAC, the antidepressant citalopram (CTL), was also considered in this study. Initially, SRT was set at a long value, above 20 days, high enough to guarantee a successful nitrification in both systems. After 5 months of operation, sludge was steadily removed from both systems in a daily basis, until SRT <8 days (low) was achieved. Figure 5 shows the comparison of removal data under these conditions. No strong differences were found between both systems for any of the studied PhACs during the operation at high SRT. Interestingly, slightly higher removals were observed in the CAS, especially for DCF, for which eliminations were 20% in the MBR versus 45% in the CAS, SMX (42% vs. 66%), and TMP (65% vs. 82%). After decreasing the SRT, the removal efficiency of many substances was severely reduced, more intensely in the case of the CAS. The elimination of IBP and E2 was always higher than 85% in both systems and the variation of SRT did not affect its removal from sewage to any significant extent. The biodegradability of NPX is moderate and consequently its removal can be particularly affected by operating conditions and factors such as microorganism's

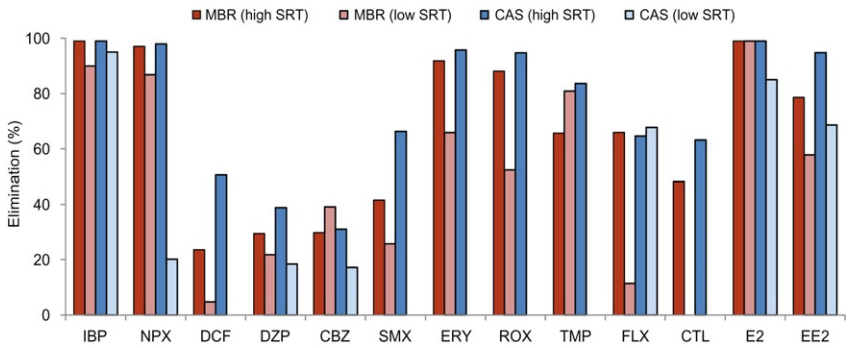

FIGURE 5 PhAC elimination in a CAS and an MBR system at high and low SRTs.

adaptation [1]. In this study, its removal slightly decreased in the MBR after changing the SRT, whereas in the CAS, the reduction was much more significant (−78%). In the case of DCF, its recalcitrant characteristics have been well documented in the literature [10,62,74], although some works have also reported high removals during conventional treatment [63,75]. In fact, data previously presented on Figure 3 show an average efficiency of 60% in MBRs. Nevertheless, at low SRT, its elimination decreased to 20% in the MBR and completely stopped in the CAS. The fate of DZP was fairly similar in both systems. Its efficiency was the lowest among the PhACs considered in this study and only slightly decreased at low SRT. This might be expected for recalcitrant compounds, since this type of behavior entails that the biological performance of the system will exert neither positive nor negative impact on its removal. Figure 3 shows that CBZ is similarly persistent. In our experiments, its removal was similarly poor, although experiencing a minor increase in the MBR at low SRT (+9%), whereas the CAS showed a reduction of −14%. Antibiotics (SMX, ERY, ROX, and TMP) elimination ranged from moderate to high, in good agreement with the reviewed literature. At low SRT, eliminations abruptly stopped in the CAS and decreased moderately (−20% to −30%) in the MBR, with the exception of TMP, whose removal slightly increased in the MBR, in a similar manner to CBZ. Previous research linked the presence of nitrifying bacteria with the removal of TMP [76,77], being this information fairly consistent with the results from the CAS, but not from the MBR. This finding is interesting, since it has been already mentioned that MBRs can be less susceptible to operational perturbations [66], which can explain the trend followed by most of the considered PhACs. The hormone EE2 showed a moderate impact after reducing the SRT (−21% and −26% for the MBR and CAS, respectively), with slightly improved efficiencies in the CAS. In a similar manner to TMP, nitrification during an aerobic process appears to be positive for EE2 removal, although observed efficiencies did not experience a dramatic decrease. Estrogens have also shown a moderate

tendency to partition onto the sludge. As estimated by Suarez et al. [16], 33–64% of these compounds are sorbed onto sludge in a CAS process, and this additional removal might attenuate the lower biodegradation due to decreased SRT. Antidepressants FLX and CTL were the only substances whose elimination was strongly affected at low SRT also in the MBR. In the CAS, the elimination of FLX remained almost unchanged at low SRT and completely stopped in the case of CTL. To our knowledge, there are few studies regarding the behavior of both compounds during conventional or modern sewage treatment, although there is increasing evidence regarding FLX tendency to partition onto sludge [78]. Fernandez-Fontaina et al. [19] also found moderate K_{biol} values for this compound, which might explain the influence of SRT on its elimination, although it is unclear why the CAS removal was unaffected. Since the behavior of some PhACs (more specifically CBZ, DCF, IBP, and NPX) during MBR and CAS treatment has been widely studied, further research in this topic should consider other pharmaceuticals of concern, such as the aforementioned antidepressants.

Considering the possible influence of sorption on the removal of specific compounds, it was also interesting to corroborate that biomass properties were modified during the operation of both bioreactors. Therefore, the particle size distribution was determined and compared along the operational period (Figure 6). This was particularly interesting in this study, since normally MBRs are inoculated with CAS biomass and, afterward, some of its characteristics evolve during operation. In this work, the opposite strategy was followed (CAS inoculated with MBR biomass). The first measurements of the particle size were performed after the starting up of the CAS, when biomass properties were similar to the ones of the MBR sludge. Median values were 34.8 and 47.1 μm for the MBR and CAS, respectively. Interestingly, the CAS median particle size increased with operation time, until typical value for conventional [18] was achieved. In this sense, after more than 6 months of operation, median values of CAS biomass particle size almost doubled those measured in the MBR (74.2 and 134.2 μm, respectively). According to Massè et al. [18], the decreased floc size may also be associated with a more compact floc structure,

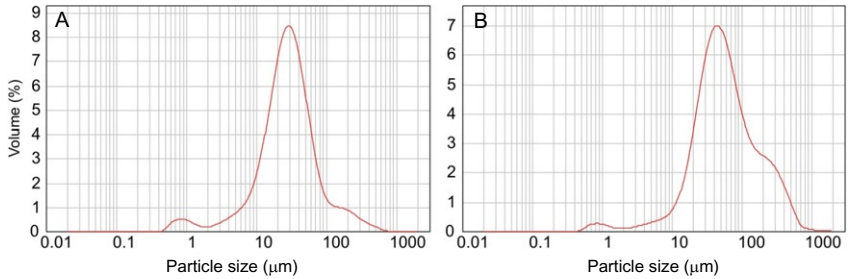

FIGURE 6 Particle size distribution for (A) MBR and (B) CAS biomass after 6 months of operation.

due to fact that small particles (dispersed bacteria and small colonies) have a higher density than the large flocs, with more bridging between biopolymers. Wisniewski et al. [79] found that the tangential flow along the membrane is a relevant factor that contributes to increase the shear stress, inducing changes in the settleability of the sludge. Since the operational parameters were similar in the studied bioreactors, these characteristics might explain at a certain extent the different performance in terms of PhAC elimination, although further research is essential to provide conclusive information.

4 CONCLUSIONS

Concerning PhAC removal, the main characteristics of the operation of MBRs are their ability to operate with higher biomass concentrations, longer SRTs, and the generation of a final permeate with very low concentration of solids. Operation at long SRT may favor a higher and less specific enzymatic activity due to the increased cell lysis and the development of a broader biocenosis, leading to an improved adaptation and less susceptibility to operational perturbations. However, the extent to which these factors might enhance PhAC removal is still unclear.

Taking into account the different behavior of a selected group of pharmaceuticals (expressed by their K_d and K_{biol} values), the following statements can be expected:

- Compounds with high K_{biol} and K_d values, such as ibuprofen, achieve a high degree of elimination, independently of operating conditions or the technology used.
- Compounds with intermediate K_{biol} and K_d values, such as ethinyl estradiol, are moderately transformed during biological treatment, being the removal efficiency positively particularly affected by higher SRT.
- Compounds with low K_{biol} and K_d values, such as carbamazepine, are not removed and not biotransformed regardless of operational conditions. However, the use of integrated MBR processes with activated carbon has resulted in their high removal.

According to the available knowledge, the benefits of the use of MBRs to eliminate PhACs are not pronounced enough to serve as a sole argument for upgrading conventional wastewater treatment facilities with membrane technology, and CAS systems correctly operated with nitrogen removal might be able to remove these micropollutants at a similar degree. However, the degree of quality achieved in permeate is outstanding in terms of solid and pathogen removal, as well as for a further posttreatment in order to obtain a final effluent suitable for discharge in sensitive receiving waters or for reuse purposes. Moreover, very promising results are currently being obtained with hybrid processes that combine sorption onto activated carbon within a single MBR unit, achieving also the removal of recalcitrant compounds by adsorption (and perhaps by a further degradation). In this sense, future

research should be focused on understanding how MBRs can help to attenuate the release of PhACs of moderate biodegradability and/or sorption potential in the aquatic environment. For recalcitrant PhACs, the exploration of new approaches based on integrated configurations might be the key to find feasible mitigation options. Additionally, the lack of studies carried out in more realistic conditions (including the use of full-scale facilities) for the optimization of operating parameters and the elucidation of other influencing aspects, particularly those related with MBR biomass properties, have been also identified as relevant knowledge gaps.

Considering these aspects, the conclusions of this chapter should not constitute an obstacle for the widespread of the MBR technology. On the contrary, this chapter has been intended to show the future trends in MBR research and identify knowledge gaps that should be filled in order to optimize their ability to remove not only PhACs but also a wider range of micropollutants.

ACKNOWLEDGMENTS

This research was supported by the Spanish Ministry of Economy and Competitiveness through NOVEDAR_Consolider (CSD2007-00055) and INNOTRAZA (CTQ2010-20240) projects. The authors belong to the Galician Competitive Research Group GRC2010/37.

REFERENCES

[1] D. Fatta-Kassinos, K. Bester, K. Kümmerer (Eds.), Xenobiotics in the Urban Water Cycle, Springer, Dordrecht, 2010.
[2] A.I. Schäfer, A.G. Fane, T.D. Waite (Eds.), Nanofiltration: Principles and Applications, Elsevier, Oxford, 2005.
[3] M.D. Knoblock, P.M. Sutton, P.N. Mishra, K. Gupta, A. Janson, Water Environ. Res. 66 (2) (1994) 133–139.
[4] N.S.A. Mutamim, Z.Z. Noor, M.A.A. Hassan, G. Olsson, Desalination 305 (2012) 1–11.
[5] A. Fenu, J. Roels, T. Wambecq, K. De Gussem, C. Thoeye, G. De Gueldre, B. Van De Steene, Desalination 262 (2010) 121–128.
[6] J.T. Kraemer, A.L. Menniti, Z.K. Erdal, T.A. Constantine, B.R. Johnson, G.T. Daigger, G.V. Crawford, Bioresour. Technol. 122 (2012) 2–10.
[7] A. Santos, S. Judd, Sep. Sci. Technol. 45 (2010) 850–857.
[8] E. Huisjes, K. Colombel, B. Lesjean, MBR-Network Workshop "Salient Outcomes of the European R&D Projects on MBR Technology", Book of Proceedings: 81–82, 2009.
[9] S. Koester, S. Beier, F.F. Zhao, Q. Sui, G. Yu, J. Pinnekamp, Water Sci. Technol. 66 (5) (2012) 942–952.
[10] M. Clara, B. Strenn, O. Gans, E. Martinez, N. Kreuzinger, H. Kroiss, Water Res. 39 (19) (2005) 4797–4807.
[11] A. Joss, S. Zabczynski, A. Göbel, B. Hoffmann, D. Löffler, C.S. McArdell, T.A. Ternes, A. Thomsen, H. Siegrist, Water Res. 40 (8) (2006) 1686–1696.
[12] A. Santos, W. Ma, S.J. Judd, Desalination 273 (1) (2011) 148–154.
[13] S.R. Hughes, P. Kay, L.E. Brown, Environ. Sci. Technol. 47 (2) (2013) 661–677.
[14] S. Beier, C. Cramer, S. Köster, C. Mauer, L. Palmowski, H.F. Schröder, J. Pinnekamp, Water Sci. Technol. 65 (9) (2011) 1648–1653.
[15] M. Carballa, F. Omil, J.M. Lema, Environ. Sci. Technol. 41 (3) (2007) 884–890.

[16] S. Suarez, M. Carballa, F. Omil, J.M. Lema, Rev. Environ. Sci. Biotechnol. 7 (2008) 125–138.
[17] O.A.H. Jones, N. Voulvoulis, J.N. Lester, Environ. Pollut. 145 (2007) 738–744.
[18] A. Massé, M. Spérandio, C. Cabassud, Water Res. 40 (2006) 2405–2415.
[19] E. Fernandez-Fontaina, I. Pinho, M. Carballa, F. Omil, J.M. Lema, Biodegradation 24 (2013) 165–177.
[20] K. Kimura, H. Hara, Y. Watanabe, Desalination 178 (1–3) (2005) 135–140.
[21] J.B. Quintana, S. Weiss, T. Reemtsma, Water Res. 39 (2005) 2654–2664.
[22] N.H. Hashim, L.D. Nghiem, R.M. Stuetz, S.J. Khan, Water Res. 45 (18) (2011) 6249–6258.
[23] B. De Gusseme, B. Pycke, T. Hennebel, A. Marcoen, S.E. Vlaeminck, H. Noppe, N. Boon, W. Verstraete, Water Res. 43 (2009) 2493–2503.
[24] E. Dialynas, E. Diamadopoulos, J. Chem. Technol. Biotechnol. 87 (2012) 232–237.
[25] H.F. Schröder, J.L. Tambosi, R.F. Sena, R.F. Moreira, H.J. José, J. Pinnekamp, Water Sci. Technol. 65 (5) (2012) 833–839.
[26] A. Tauxe-Wuersch, L.F. de Alencastro, D. Grandjean, J. Tarradellas, Water Res. 39 (9) (2005) 1761–1772.
[27] L. Bo, T. Urase, X. Wang, Front. Environ. Sci. Eng. China 3 (2) (2009) 236–240.
[28] F.I. Hai, X. Li, W. Price, L.D. Nghiem, Bioresour. Technol. 102 (22) (2011) 10386–10390.
[29] C. Abegglen, A. Joss, C.S. McArdell, G. Fink, M.P. Schlüsener, T.A. Ternes, H. Siegrist, Water Res. 43 (2009) 2036–2046.
[30] W. Xue, C. Wu, K. Xiao, X. Huang, H. Zhou, H. Tsuno, H. Tanaka, Water Res. 44 (2010) 5999–6010.
[31] H. Bouju, G. Hommes, T. Wintgens, F. Malpei, P.F.X. Corvin, Water Sci. Technol. 63 (12) (2011) 2878–2885.
[32] T. Trinh, B. van den Akker, R.M. Stuetz, H.M. Coleman, P. Le-Clech, S.J. Khan, Water Sci. Technol. 66 (9) (2012) 1856–1863.
[33] M. Cirja, S. Zuehlke, P. Ivashechkin, J. Hollender, A. Schäffer, P.F. Corvini, Water Res. 41 (19) (2007) 4403–4412.
[34] R. Reif, S. Suarez, F. Omil, J.M. Lema, Desalination 221 (2008) 511–517.
[35] E.B. Estrada-Arriaga, P. Mijaylova, Water Sci. Technol. 64 (11) (2011) 2158–2168.
[36] A. Joss, E. Keller, A.C. Alder, A. Göbel, C.S. McArdell, T. Ternes, H. Siegrist, Water Res. 3 (2005) 3139–3152.
[37] B. Lesjean, R. Gnirss, H. Buisson, S. Keller, A. Tazi-Pain, F. Luck, Water Sci. Technol. 52 (10–11) (2005) 453–460.
[38] J. Radjenovic, M. Petrovic, D. Barcelo, Water Res. 43 (2009) 831–841.
[39] A. Joss, H. Andersen, T. Ternes, P.R. Richle, H. Siegrist, Environ. Sci. Technol. 38 (11) (2004) 3047–3055.
[40] S. Suarez, J.M. Lema, F. Omil, Water Res. 44 (10) (2010) 3214–3224.
[41] M.R. Abargues, A. Robles, A. Bouzas, A. Seco, Water Sci. Technol. 65 (12) (2012) 2242–2250.
[42] S.J. Judd (Ed.), The MBR Book. Principles and Applications of Membrane Bioreactors in Water and Wastewater Treatment, Elsevier, Oxford, 2006.
[43] I.S. Chang, S.N. Kim, Process Biochem. 40 (2005) 1307–1314.
[44] H.P. Chu, X.Y. Li, Biotechnol. Bioeng. 90 (2005) 323–331.
[45] A. Pollice, C. Giordano, G. Laera, D. Saturno, G. Mininni, Water Res. 41 (2007) 1832–1840.
[46] K. Kimura, K.H. Hara, Y. Watanabe, Environ. Sci. Technol. 41 (2007) 3708–3714.
[47] M. Cirja, P. Ivashechkin, A. Schäffer, P.F.X. Corvini, Rev. Environ. Sci. Biotechnol. 7 (2008) 61–78.
[48] T. Yi, W.F. Harper Jr., Water Res. 41 (7) (2007) 1543–1553.
[49] T. Yi, S. Mackintosh, D.S. Aga, W.F. Harper Jr., Water Res. 45 (3) (2011) 1369–1377.

[50] Y. Yoon, P. Westerhoff, S.A. Snyder, E.C. Wert, J. Membr. Sci. 270 (2006) 88–100.
[51] S. Lyko, T. Wintgens, T. Melin, Desalination 178 (2005) 95–105.
[52] A.I. Schäfer, L.D. Nghiem, N. Oschmann, J. Membr. Sci. 283 (2006) 233–243.
[53] S. Baumgarten, H.F. Schröder, C. Charwath, M. Lange, S. Beier, J. Pinnekamp, Water Sci. Technol. 56 (5) (2007) 1–8.
[54] L.N. Nguyen, F.I. Hai, J. Kang, W.E. Price, L.D. Nghiem, Bioresour. Technol. 113 (2012) 169–173.
[55] W. Guo, S. Vigneswaran, H.H. Ngo, W. Xing, P. Goteti, Bioresour. Technol. 99 (2008) 1012–1017.
[56] X. Li, F.I. Hai, L.D. Nghiem, Bioresour. Technol. 102 (2011) 5319–5324.
[57] D. Serrano, S. Suárez, J.M. Lema, F. Omil, Water Res. 45 (2011) 5323–5333.
[58] L.N. Nguyen, F.I. Hai, J. Kang, L.D. Nghiem, W.E. Price, W. Gu, H.H. Ngo, K. Tung, Bioresour. Technol. 130 (2013) 412–417.
[59] P. Verlicchi, M. Al Aukidy, E. Zambello, Sci. Total Environ. 429 (2012) 123–155.
[60] J. Sipma, B. Osuna, N. Collado, H. Monclús, G. Ferrero, J. Comas, I. Rodriguez-Roda, Desalination 250 (2) (2010) 653–659.
[61] E. Sahar, R. Messalem, H. Cikurel, A. Aharoni, A. Brenner, M. Godehardt, M. Jekel, M. Ernst, Water Res. 45 (16) (2011) 4827–4836.
[62] M. Bernhard, J. Müller, T.P. Knepper, Water Res. 40 (2006) 3419–3428.
[63] J. Radjenovic, M. Petrovic, D. Barceló, Anal. Bioanal. Chem. 387 (2007) 1365–1377.
[64] M.D. Celiz, S. Pérez, D. Barceló, D.S. Aga, J. Chromatogr. Sci. 47 (1) (2009) 19–25.
[65] N. Le-Minh, H.M. Coleman, S.J. Khan, Y. van Luer, T.T.T. Trang, G. Watkins, R.M. Stuetz, Water Sci. Technol. 61 (5) (2010) 1081–1088.
[66] Q. Sui, J. Huang, S. Deng, W. Chen, G. Yu, Environ. Sci. Technol. 45 (8) (2011) 3341–3348.
[67] R. Reif, A. Besancon, K. Le Corre, B. Jefferson, J.M. Lema, F. Omil, Water Sci. Technol. 63 (10) (2011) 2411–2417.
[68] S. Martin Ruel, J.M. Choubert, M. Esperanza, C. Miège, P. Navalón Madrigal, H. Budzinski, K. le Ménach, V. Lazarova, M. Coquery, Water Sci. Technol. 63 (11) (2011) 2486–2497.
[69] D. Camacho-Muñoz, J. Martín, J.L. Santos, E. Alonso, I. Aparicio, T. De la Torre, C. Rodriguez, J.J. Malfeito, J. Environ. Monit. 14 (5) (2012) 1428–1436.
[70] M.J. García Galán, M.S. Díaz-Cruz, D. Barceló, Anal. Bioanal. Chem. 404 (5) (2012) 1505–1515.
[71] H. De Wever, S. Weiss, T. Reemtsma, J. Vereecken, J. Müller, T. Knepper, O. Rördend, S. Gonzalez, D. Barceló, M.D. Hernando, Water Res. 41 (2007) 935–945.
[72] Y. Zhou, X. Huang, H. Zhou, J. Chen, W. Xue, Water Sci. Technol. 64 (10) (2011) 2096–2102.
[73] R. López-Fernández, F.V.F. Tavares, M. Gómez, R. Irusta, P. Le-Clech, Desal. Water Treat. 51 (10–12) (2013) 2336–2342.
[74] J.T. Yu, E.J. Bouwer, M. Coelhan, Agric. Water Manag. 86 (1–2) (2006) 72–80.
[75] M. Stumpf, T.A. Ternes, R.D. Wilken, S.V. Rodrigues, W. Baumann, Sci. Total Environ. 225 (1–2) (1999) 135–141.
[76] A.L. Batt, S. Kim, D.S. Aga, Environ. Sci. Technol. 40 (23) (2006) 7367–7373.
[77] A. Göbel, C.S. McArdell, A. Joss, H. Siegrist, W. Giger, Sci. Total Environ. 372 (2–3) (2007) 361–371.
[78] D.J. Johnson, H. Sanderson, R.A. Brain, C.J. Wilson, J. Ketut, T. Bestari, K.R. Solomon, Exposure assessment and microcosm fate of selected selective serotonin reuptake inhibitors, Regul. Toxicol. Pharmacol. 42 (3) (2005) 313–323.
[79] C. Wisniewski, A. Grasmick, Colloids Surf. A Physicochem. Eng. Asp. 138 (2–3) (1998) 403–411.

Chapter 10

Removal of Pharmaceuticals by Ultrafiltration (UF), Nanofiltration (NF), and Reverse Osmosis (RO)

Davor Dolar and Krešimir Košutić
Department of Physical Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Zagreb, Croatia

Chapter Outline

1 INTRODUCTION TO THE MEMBRANE PRESSURE PROCESSES

The membrane processes are characterized by the fact that the feed is divided into two streams, that is, into the retentate and the permeate, where both streams may be of interest. However, in practice of water treatment, permeate is considered as the main product. The heart of any membrane process is a

Comprehensive Analytical Chemistry, Vol. 62. http://dx.doi.org/10.1016/B978-0-444-62657-8.00010-0

membrane that has the ability to transport one component from the feed more readily than the other, due to differences in physical and/or chemical properties between the membrane and the permeating components [1].

In pressure-driven membrane processes, feed water is forced through a membrane by pressure exerted on the feed membrane side. Depending of the value of the applied pressure, we distinguish microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), and reverse osmosis (RO). As we go from MF through UF to NF/RO, the size (or molecular weight (MW)) of the separated solutes diminishes, and consequently, the membrane pore size is smaller. This implies that the membrane resistance to mass transfer increases, and hence, the applied pressure (driving force) has to be increased (from 1 bar for UF up to 60–80 bar for RO) to obtain the same flux of the same order of magnitude [1]. Figure 1 represents cross-sectional illustration of RO/NF thin-film composite (TFC) membranes.

The pore sizes of UF membranes range from 0.05 μm to 2 nm and are typically used to retain macromolecules and colloids from a solution, the lower limit being solutes with MW of a few thousand Daltons (Da). UF is used over a wide field of application involving situations where high molecular components have to be separated from low molecular components (pharmaceutical, food and dairy industry, etc.) [1,2].

NF (pore size range ~0.5–2 nm) and RO (~0.2–1 nm) are used when low-molecular-weight solutes such as inorganic salts and small organic molecules

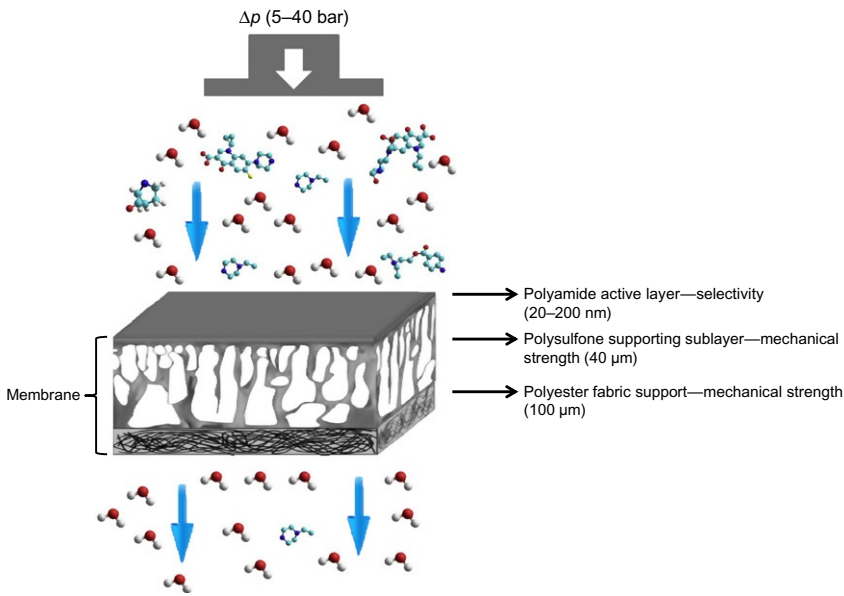

FIGURE 1 Cross-sectional illustration of RO/NF thin-film composite membranes.

have to be separated from a solvent. RO is primarily used for desalination of sea and brackish water in order to obtain drinking water including purification of the water and production of ultrapure water, while NF was initially used for water softening and recently in wastewater treatment and in the removal of different class of organic micropollutants, etc. [1,3,4].

2 REMOVAL OF PHARMACEUTICALS BY UF

UF is rarely used in the removal of pharmaceuticals research as a single step due to the fact that the molecular weight cutoff (MWCO) of UF membranes (10–100 kDa) is at least one order of magnitude above the MW of most micropollutants (<1 kDa) [5] but is very often applied in hybrid systems (UF/RO treatments [6–9], conventional activated sludge system (CAS)/UF [9,10], coagulation–UF [11,12], etc.). Nevertheless, some papers about the removal of pharmaceuticals with UF membranes were found.

Jermann et al. [5] investigated influence of natural organic matter (NOM) on the removal of estradiol and ibuprofen with polyethersulfone (PES, Biomax) and regenerated cellulose (RC) UF membranes. Firstly, the retentions, without NOM, was relatively low (8%) for estradiol and insignificant for ibuprofen with hydrophilic RC membrane, while retention of both micropollutants was significantly greater with hydrophobic (Biomax) membrane (for ibuprofen and estradiol, 25% and 80%, respectively).

Acero et al. [13] investigated the removal of 11 emerging contaminants (acetaminophen, metoprolol, caffeine, antipyrine, sulfamethoxazole, flumequine, ketorolac, atrazine, isoproturon, 2-hydroxybiphenyl, and diclofenac) dissolved in ultrapure water and in municipal secondary effluent by UF (GK, PT, and PW membranes by GE Osmonics, United States). Retention coefficients in ultrapure water were up to 50%, except for hydroxybiphenyl (>84%). As expected, retention coefficients in municipal secondary effluent were higher than those obtained with ultrapure water, due to adsorption of hydrophobic compounds on the NOM of the secondary effluent or by the formation of the cake layer.

UF was also used for the removal of 52 compounds (endocrine-disrupting compounds (EDCs)/pharmaceuticals and personal care products (PPCPs)) from one model water and three natural rivers in paper published by Yoon et al. [14]. They used GM (Desal–Osmonics, United States) UF membrane at dead-end stirred-cell filtration system. The used UF membrane had retention less than 40%, except a few compounds (triclosan 87%, oxybenzone 77%, and progesterone 56%).

In their next paper, Yoon et al. [15] used same conditions for the removal of 27 EDC/PPCPs, different in properties (solute size/structure/polarity/hydrophobicity), with GM UF (MWCO 8000 ± 1000 Da) membrane. Retention of investigated membranes were <30%, except a few compounds (triclosan

85%, oxybenzone 70%, estrone 45%, progesterone 55%, and erythromycin 60%) having log $K_{O/W}$ (octanol–water partitioning coefficient) higher than 3.

Heo et al. [16] investigated the removal of bisphenol A (BPA) and 17β-estradiol (E2) in single-walled carbon nanotubes–ultrafiltration (SWNTs–UF) membrane systems. All of the membranes showed significant retention of E2 (>80%) and BPA (>40%), while a significant decrease in retention (30–70%) was observed for BPA and E2 in the presence of NOM only (no SWNTs).

The removal of hormones (estradiol, estrone, progesterone, and testosterone) was investigated by Neale et al. [17]. The used RC UF membranes (MWCO ranged from 1 to 100 kDa) showed removal up to 28% with increasing removal with decreasing membrane MWCO.

Sui et al. [18] used UF (ZeeWeed 1000 membrane, Zenon GE) for the removal of 13 pharmaceuticals and 2 consumers from secondary effluent (wastewater treatment plant (WWTP) of Beijing, China). The elimination by UF was low (<50%) for all the investigated compounds. Due to MWCO much higher than 1000 Da, UF membranes showed poor retention of the compounds that had MW <400 Da.

Real et al. [19] used PT UF membrane with MWCO of 5000 Da, as a pretreatment to chemical oxidation stages (O_3 and Cl_2), in different water systems (groundwater, surface water, and secondary effluent from municipal WWTP), for the elimination of five pharmaceuticals (amoxicillin, hydrochlorothiazide, metoprolol, naproxen, and phenacetin). Rejections were between 4.1% and 35.1%, whereas the lowest was for amoxicillin, and highest for metoprolol, both in surface water.

Ionic UF containing two Norit membrane cassettes was used for the removal of a broad range of representative EDCs and PPCPs from secondary effluent from a municipal WWTP. The results presented by Snyder et al. [20] showed that the vast majority of compounds were not rejected by used membranes. The authors did not explain why some compounds were significantly removed, but they showed that steroids were well removed probably due to their relatively lower water solubility.

2.1 Removal Mechanisms for UF Membranes

As stated before, MWCO of UF membranes is much higher than MW of most pharmaceuticals; therefore, size exclusion as a retention mechanism cannot be consider as a main removal mechanism [5,13,16,17]. Therefore, removal might be due to the adsorption onto the membrane [5,13–18,21,22] while retention increases with increasing log $K_{O/W}$, due to a greater affinity of the membrane [15,16]. Jermann et al. [5] stated that although adsorption onto some UF membranes can lead to their retention in the initial filtration period, it cannot be considered as a long-term removal mechanism. Yoon et al. [14]

confirmed this statement and claimed that once steady-state operation is achieved, size exclusion can be dominant for EDC/PPCPs retention by UF.

3 REMOVAL OF PHARMACEUTICALS BY NF AND RO

3.1 Removal from Ultrapure and Model Waters

Acero et al. [13] investigated the removal of 11 emerging contaminants from ultrapure water with HL and DK poly(piperazine-amide) NF membranes and cellulose acetate CK membrane. The removal of most compounds was above 70%, except for acetaminophen between 11% and 34%.

Dolar et al. [23] investigated the application of RO (LFC-1 and XLE) and NF (NF90, NF270, NF, and HL) membranes for the removal of veterinary antibiotics (sulfamethoxazole, trimethoprim, ciprofloxacin, dexamethasone, and febantel) and their mixture from Milli-Q water. This work achieved a high level of retention (>95%) of all selected antibiotics with the RO and tight NF90 membranes. Other NF membranes showed lower rejection (15–100%) of the individual compounds depending on MW.

The removal of 22 EDCs and pharmaceutically active compounds (PhACs) from Milli-Q water with RO (X20), tight NF (TS80), and loose NF (NF270) membranes was investigated by Comerton et al. [24]. Results showed that the RO membrane provided high (>91%) rejection of all the investigated compounds. Conversely, the loose NF membrane generally offers poor and variable (1–69%) rejection. Finally, the tight NF membrane showed rejections between 0 and 95% and for most compounds was higher than for loose NF membrane.

The removal of estrone, estradiol, and salicin was treated with NF membranes by Braeken and Van der Bruggen [25]. Estradiol retentions of, respectively, 75% (UTC-20) and 85% (NF270), and estrone retention of 83% (UTC-20) and 65% (NF270) were obtained. These retentions were lower than expected based on the MWCO and size exclusion mechanism and also lower than the retention of salicin (>90%), with comparable MW but more hydrophilic.

Koyuncu et al. [26] obtained retention of several hormones (estradiol, estrone and testosterone amounted 64%, 80% and 62%, respectively) and antibiotics (sulfamethoxazole, sulfathiazole, tetracycline, and oxytetracycline amounted 60%, 88%, 100%, and 100%, respectively) from deionized (DI) water. Results showed increase in removal efficiencies with MW increase and were higher than 95% after MW of 300 Da, due to membranes' MWCO of 200–300 Da. Changes in the solution chemistry, organic matter, and salinity increased rejections of hormones, while for sulfonamides varied greatly.

The removal of cyclophosphamide (CP) by NF (Desal 5 DK) and RO (YMAKSP3001) membranes from ultrapure water was investigated by Wang

et al. [27]. In this study, RO membrane provided excellent rejection (>90%), while with NF membrane rejection was poor, that is, in the range of 20–40%.

Nanofiltration NF200 and NF90 membranes were used by Yangali-Quintanilla et al. [28] for the removal of organic contaminants. The rejection of neutral compounds by NF200 was low to moderate (22–42%) when equivalent width was smaller (<0.6 nm) than other equivalent widths of compounds with comparable or larger lengths. NF90 membrane showed greater rejection (>71%) of neutral compounds.

3.2 Removal from Various Water Matrices

Dolar et al. [29] explored the removal of five veterinary pharmaceuticals from different water matrices using NF (NF90, NF270 NF, and HL) and RO (LFC-1 and XLE) membranes (presented in Figure 2). Milli-Q, model and tap water, and real pharmaceutical wastewater were used as water matrices. Rejections in Milli-Q water with RO and tight NF90 membranes were >97%, confirming size exclusion as a main rejection mechanism, while for NF270, NF, and HL membrane were in the range from 15% to >99.9%, with an impact of two other mechanism (charge exclusion and physicochemical interactions). In general, the rejection of investigated compounds was higher in model and tap water than in Milli-Q water, but the water flux was lower, probably due to ion adsorption inside the membrane pores.

Yoon et al. [15] presented average retentions of 27 compounds by NF membrane ~30–90%. The retention varied depending on source water, model, and three surface waters. General conclusion was increase of the retention with increase log $K_{O/W}$ value, indicating that retention for hydrophobic membranes is influenced by hydrophobic interaction (adsorption).

Comerton et al. [24] investigated the removal of 22 EDCs and PhAC from raw and filtered (5 μm) Lake Ontario water and membrane bioreactor effluent and compared this to the removal from Milli-Q water with X20, TS80, and NF270 membranes as RO, tight, and loose NF membranes, respectively. Rejections with RO, tight, and loose NF membranes were >82%, 46–100%, and 0–93%, respectively, and were higher than from the Milli-Q water, indicating that water matrix may influence rejection. Also, membrane fouling and compound interactions with the water matrix resulted on increase of rejections, while the presence of divalent cations, calcium in particular, caused decrease in the rejection from natural waters.

Wang et al. [27] investigated the removal of CP from membrane bioreactor (MBR) effluent and also influence of water matrix on rejection. The rejection of CP with RO and NF membranes was >90% and around 60%, respectively, and was much higher than in ultrapure water. The authors concluded that membrane fouling and compound interactions with the water matrix likely contributed to the higher rejection.

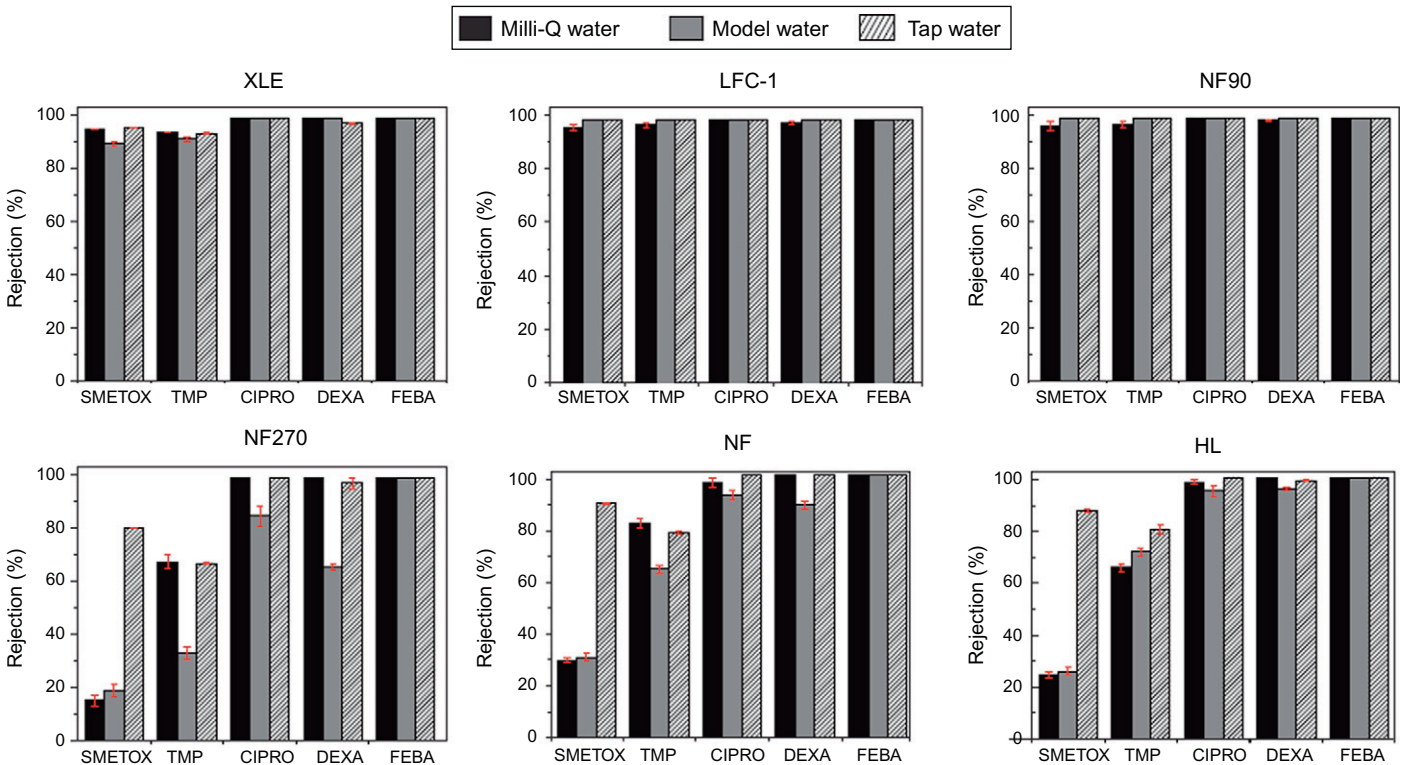

FIGURE 2 The rejection of veterinary pharmaceuticals dissolved individually in different water matrices (Milli-Q, model, and tap water).

Fourteen pharmaceuticals, 6 hormones, 2 antibiotics, 3 PPCPs, and 1 flame retardant were monitored, while 17 were found in WWTP effluents in South Korea by Kim et al. [30]. RO (RE4040-FL) and NF (NE4040-90-RF) membranes were used for the removal of detected compounds in effluent. These membranes showed high removal rates (>95%) for all detectable analytes.

The removal of 12 pharmaceuticals during NF and RO applied in a full-scale drinking water treatment plant (DWTP) using ground water was investigated by Radjenović et al. [31]. Both NF (NF90) and RO (BW30LE) showed high rejection percentages (>85%) for almost all investigated pharmaceuticals. Deteriorations in retention on used membrane were observed for acetaminophen (44.8–73%), gemfibrozil (50–70%), and mefenamic acid (30–50%). In the case of acetaminophen, retention was lower probably due to its small molecular size (i.e., MW < MWCO), while for gemfibrozil and mefenamic acid, authors found no plausible explanation.

Nanofiltration (NF270, DK, and DL) and low-pressure RO (CG) membranes were used for the rejection of four polar trace organic substances (terbutaline, atrazine, clofibric acid, and metamitron) in DI and surface water [32]. Clofibric acid showed the highest rejection (>90%) in DI and surface (river) water for all investigated membranes, while the lowest was for metamitron, ranging between 5% and 60%. In the case of clofibric acid, highest rejection was probably due to electrostatic repulsion between the negatively charged membrane surface and the negatively charged dissociated organic acid molecule. In addition, steric and electrostatic effects were found to be the most important factors influencing retention of the organic substances. Atrazine and metamitron were rejected at an equal amount in both water matrices, whereas terbutaline was significantly less rejected in DI water in case of the DL and CG membranes. Attractive forces between the positively charged molecule and the negatively charged membrane surface may lead to a higher passage of terbutaline in DI water, whereas those interactions could be shielded by negatively charged water constituents (NOM, anions) during filtration of surface water.

The removal of target contaminants from saline groundwater feed with RO pilot system was investigated by Snyder et al. [20]. Only one or two compounds were detected in the saline groundwater; therefore, they were spiked. Feed concentrations were in the range of 118–458 ng L^{-1}, and used RO membranes well-rejected target analytes, that is, concentrations in final permeate, were <25 ng L^{-1} for all compounds, except for caffeine and pentoxifylline 52 and 45 ng L^{-1}, respectively.

Röhricht et al. [33] used relatively low pressure (0.7 bar) for the removal of carbamazepine, diclofenac, and naproxen from municipal WWTP effluent with submerged NF flat sheet module. Low pressure was used because at such low-pressure membrane does not retain salts to a great extent, and they noticed that this is advantageous in wastewater treatment because no salt concentrate is produced. At 0.7 bar, the removal of carbamazepine and diclofenac

was around 12% and 65%, respectively, while for naproxen was hard to define due to low influent concentration.

The study by Sanches et al. [34] showed the efficiency of NF (Desal 5-DK) in laboratory scale to remove different pesticides and hormones from surface water and groundwater. Used compounds were spiked, and prior NF treatment natural water matrices were ultrafiltrated. High rejections (67.4–99.9%) were obtained often independently of the water composition, except pentachlorophenol (57.5–83.5%). The lower rejection for pentachlorophenol could be explained by its increased solubility at the waters' pH.

3.3 Removal Mechanism for NF and RO Membranes

Molecular weight, molecular size (length and width), acid disassociation constant (pK_a), hydrophobicity/hydrophilicity ($\log K_{O/W}$), and diffusion coefficient (D_p) were indentified to primarily affect solute rejection [35].

Solute can be rejected on NF and RO membranes by one or combination of three basic mechanisms: size exclusion (sieving, steric effect), charge exclusion (electrical, Donnan), and physicochemical interactions between solute, solvent, and membrane. The rejection of uncharged trace organics by NF and RO membranes is considered to be predominantly influenced by steric hindrance (size exclusion), while the rejection of polar trace organics is mainly governed by electrostatic interactions with charged membranes [35,36].

In most cases stated in Section 3.1, size exclusion, that is, steric hindrance, was the main rejection mechanism, due to low MWCO of RO (100 Da) and NF (100–300 Da) membranes and larger compounds with MW higher than 200 Da [9,13,23,24,27,28,34]; hence membrane pore size is larger relative to compound MW, that is, size [24]. When indicating rejection and MWCO, it has to be very careful, because, as stated by Comerton et al. [37], the standard measurement for MWCO has limitation for predicting the rejection of compounds that have MW close to the membranes' MWCO value. Consequently, Comerton et al. [24] found that rejection from Milli-Q water was most influenced by compounds volume when compared to the other size parameters, that is, width and length, and it is necessary to investigate the removal of EDC/PhAC from natural waters, which will provide an accurate estimation of how a membrane will perform at full scale [37].

Verliefde et al. [38] showed high removal efficiencies for all pharmaceuticals (positive, negative, and neutral) with both TS80 and HL membranes. The removal of the pharmaceuticals was partly determined by size exclusion, but the charge of the solute also played important role. For neutral solute, rejection was governed by size exclusion, while higher rejection for negatively charged solute was explained by charge repulsion, and lower rejection for positively charged solute by charge interactions, more precisely electrostatic attraction [38–40]. In details, for negatively charged solutes, charge repulsion exists between the solutes and the negatively charged membrane

surface. For neutral solutes, no charge interactions with the membrane surface exist, but for positively charged solutes, the charges promote charge attraction. The authors explained this with an increased concentration of positively charged solutes at the membrane surface compared to the bulk solution (solutes are attracted toward the oppositely charged membrane), which results in lower observed rejection values. In the case of negatively charged solutes, the opposite holds the charge repulsion results in a lower concentration of negatively charged solutes at the membrane surface and thus a higher rejection. This concept was called "charge concentration polarization." The same work [38] obtained rejection decrease with increasing solute hydrophobicity for neutral and positively charged pharmaceuticals. For negatively charged compounds, relationship could not be concluded. This was due to hydrophobic interactions and adsorption of solutes on the membrane surface because negatively charged solutes cannot approach the membrane surface due to charge repulsion, whereas neutral and positively charged compounds can approach membrane surface and adsorb onto polymer matrix [38,39].

Sahar et al. [9] stated that relatively high polarities of sulfonamides and trimethoprim may even increase the removal rate to high levels >93%, probably due to electrostatic repulsion mechanism. Also, Acero et al. [13] and Yangali-Quintanilla et al. [28] showed that electrostatic interactions had contribution to size exclusion of ionic/negative compounds due to the effect of electrostatic interactions between the negative charge of the membrane surface and the negative charge of the ionic species. Therefore, rejections of ionic compounds were higher than neutral.

Dolar et al. [23] investigated the rejection of febantel and sulfamethoxazole in their mixture and the mixture of five veterinary antibiotics compared to single antibiotic. For RO membranes, rejections showed negligible increase, while for loose NF membranes, the rejection of febantel and sulfamethoxazole increased for 15–70%. Therefore, results presented in this work showed a higher level of rejection than that of the single solute. This proved the synergistic effect, that is, physicochemical interactions.

Braeken and Van der Bruggen [25] concluded that both molecular size and hydrophobicity influenced the retention of a dissolved organic compound. It can be expected that retention of organic compounds is governed by several mechanisms and not only one. Along with the aforementioned mechanisms, adsorption is also important for the removal of organic compounds [9,13,24,27,34]. Sahar et al. [9] found that some hydrophobic interactions between macrolides and the membrane surface may also occur and contribute to the high total removal rate. Acero et al. [13] showed that 2-hydroxybiphenyl was efficiently adsorbed on the membrane. Comerton et al. [22] investigated adsorption of 22 EDCs and PhACs by UF, NF, and RO membranes. Adsorption was strongly correlated with log $K_{O/W}$ of compounds and membrane pure water permeability and moderately correlated with compound water solubility. It was highest for UF membrane followed by the NF and RO membranes,

because membranes with larger pores allow compound to access the membrane's internal adsorption sites, whereas access to these internal sites may be limited with tighter membranes. Therefore, the more porous, in their case of UF membrane, may allow more compound adsorption within its structure in addition to its surface, when compared to RO membrane. Furthermore, an increase in pore size results in an increase in compound adsorption. In the case of gemfibrozil and carbamazepine in particular, adsorption was lower than expected based on their log $K_{O/W}$, as a result of charge repulsion caused by deprotonation, due a higher water pH than compound pK_a value. In their next work, Comerton et al. [24] observed differences in the adsorption of several organic compounds (including alachlor, estriol, 17β-estradiol, 17α-ethinyl estradiol, and estrone) in different water matrices and found that adsorption was generally higher in a surface water matrix, comparatively to Milli-Q water. Wang et al. [27] analyzed CP concentration in the first 100 mL and the second 100 mL of permeate and found that the concentration was always lower in the first 100 mL than in the second 100 mL. This behavior was attributed to weak adsorption of CP onto membrane, in spite of hydrophilic character of the compounds. In addition, rejection was higher for more hydrophobic compounds due to higher adsorption [24].

Size exclusion and hydrophobic interactions were found to highly influence the rejections obtained by Sanches et al. [34]. The overall NF efficiency to remove the selected compounds was not found to be considerably affected by the preadsorption of the compounds on the membrane under static and dynamic conditions, except for 17α-ethinyl estradiol, estrone, and estriol in surface water. Adsorption effects and size exclusion are therefore expected to govern the rejection of investigated compounds, since the selected hormones present similar and high molecular weights. Adsorption interactions took place since these are hydrophobic compounds (high log $K_{O/W}$), and the structure of the hormones comprises hydroxyl and carbonyl groups that may form hydrogen bonding between the oxygen atoms of the molecule and the membrane polymer. In general, the adsorbed mass increased with the time needed to achieve equilibrium, and the time needed to achieve equilibrium increased with the solubility of the target compounds. As expected, the most hydrophobic compounds (with higher log $K_{O/W}$ values and lower solubility) showed higher affinity to the membrane and achieved the equilibrium concentrations sooner. As a general trend, the mass adsorbed decreased with the decrease of log $K_{O/W}$, for all the target compounds except pentachlorophenol and atrazine. Generally, the time needed to achieve equilibrium, as well as the level of adsorption of the compounds on the membrane, was higher in the surface water matrix than in ground water. The fact that the adsorption equilibrium took longer in the surface water may be related to the competition between the selected compounds and the NOM present in this matrix for the membrane adsorption sites or due to possible interactions between the NOM and the target analytes.

Uncharged molecules of glibenclamide ($\log K_{O/W} = 4.79$, $pK_a = 6.3$), used by Radjenovic et al. [31], could potentially adsorb onto the membrane surface and inside the pores. Therefore, molecules accumulated on the membrane surface due to size exclusion could eventually diffuse through the membrane polymer matrix toward the permeate side. For example, experiments with membrane cells showed that pharmaceuticals can adsorb and subsequently diffuse through the NF/RO membrane polymer [41–43]. Therefore, adsorption and diffusion may be a possible explanation for slightly lower rejections of glibenclamide in NF and RO treatments ($R \sim 85\%$), compared to rejections of other uncharged pharmaceuticals (carbamazepine, acetaminophen, hydrochlorothiazide, and propyphenazone) with MW larger than the MWCO of the membranes.

It can be expected theoretically that the more the compound adsorbs on the membrane, the easier it will dissolve into the membrane and thus be transported to the permeate side. Higher $\log K_{O/W}$ value (i.e., a higher hydrophobicity) should thus lead to a higher transport of solute and a lower rejection [36,38]. Hence, Dolar et al. [41] confirmed that adsorption has to be taken into account together with size exclusion and charge attraction or repulsion when considering the removal of pharmaceuticals with RO and NF membranes. They used four compounds with relatively weak hydrophobicities ($1 < \log K_{O/W} < 3$). For hydrocortisone and dexamethasone ($\log K_{O/W} < 2$), a decrease in feed concentrations was observed and was associated with the irreversible adsorption on NF270 and CPA3 membranes. Additional indicators of adsorption were decrease in flux and permeate concentrations and therefore an increase in rejection. For procaine and lidocaine (smaller and slightly hydrophobic pharmaceuticals, i.e., $\log K_{O/W} > 2$), feed concentration increased probably due to instantaneous adsorption to the membrane polymer matrix and then diffusion through investigated RO and NF membranes. This confirms that for compounds with higher hydrophobicity, initial adsorption will be high, causing a high initial rejection, which eventually will drop to an equilibrium concentration when breakthrough was observed [36].

4 INFLUENCES ON PHARMACEUTICALS REJECTION BY NF AND RO

As systematized by Bellona et al. [35], the rejection of solute on RO and NF membranes will be affected by solute and membrane properties, feed water composition, and operating conditions.

4.1 Operating Conditions

The influence of transmembrane pressure (TMP), as a driving force in pressure-driven processes, was investigated by Acero et al. [13], Zazouli et al. [44], and Wang et al. [27]. Work by Acero et al. [13] showed that

rejection increased slightly with increase of the TMP, with the explanation that increase of TMP increases the permeate flux, being this permeate more diluted. In next work [44], the authors used two NF (SR2 and SR3) membranes for the removal of tetracycline. Results showed that increasing TMP increased the rejection of tetracycline with SR2 membrane, while for SR3 membrane, rejection showed a plateau value in the range of 95–98%. That suggests that the separation mechanism of both membranes is different. For membrane SR2, the separation is not mainly determined by size exclusion mechanism, while in the case of SR3 membrane size exclusion, mechanism can be more obviously observed. Also Wang et al. [27] obtained no obvious difference in the rejection of CP for NF and RO membranes when TMP was changed.

The increase of temperature led to decrease of rejection [13,45]. According to Acero et al. [13], the increase of temperature provided a slight decrease in the rejection of emerging contaminant probably due to decrease in the water viscosity, which increases the permeation flux through the membrane and decreases the rejection. On the other hand, Fujioka et al. [45] obtained that an increase in the feed temperature (from 20 to 30 °C) led to a significant decrease in the rejection of all *N*-nitrosamines, and the impact was more pronounced for the small molecular weight *N*-nitrosamines. For example, an increase in the feed temperature in the range from 20 to 30 °C caused a significant drop in the rejection of *N*-nitrosodimethylamine (NDMA), *N*-nitrosomethylethylamine (NMEA), and *N*-nitrosopyrrolidine (NPYR) from 49% to 24%, 81% to 62%, and 90% to 74%, respectively.

Acero et al. [13] also investigated the influence of turbulence on the rejection of emerging contaminants. Rejection increased slightly with tangential velocity because turbulence increases with increase of velocity due to removing part of the accumulated solutes in the cake layer by hydrodynamic forces, leading to a lower concentration of compounds on the membrane surface and, thus, higher retention.

4.2 Feed Water Composition

Solution pH, ionic strength, hardness, and the presence of organic matter belong to feed water composition and may affect the solute rejection.

Influence of solution pH was shown by several papers [13,39,44–46]. Acero et al. [13] obtained higher rejection at pH 9 than at pH 3, 5, and 7 in both ultrapure water and WWTP effluent. This positive effect was more pronounced for the negatively charged compounds at pH 9 (sulfamethoxazole, flumequine, ketorolac, and diclofenac) because of electrostatic repulsion at high pH.

TriSep TS90 membrane was used for the removal of pharmaceuticals under various pH by Verliefde et al. [39]. In general, the rejection of all acids in Milli-Q water at pH 8 was above 93% due to electrostatic repulsion

between negatively charged membrane and the completely dissociated organic acids. The rejection at the same pH also increases with increasing MW. Conversely, at pH 5, dissociation is incomplete (except for malonic acid), and the rejection of all organic acids was lower than at pH 8.

Zazouli et al. [44] used acetaminophen for investigating influence of solution pH on rejection. As the pH increased, solute rejection increased with the explanation that the membrane charge would be more negative due to deprotonation of carboxylic acid group under alkaline conditions. At the same time (when moving to higher pH), the phenolic group of acetaminophen would be deprotonated, leading to more negatively charged character of the solute. Under these conditions, the electrostatic repulsions between acetaminophen and membrane will be larger, leading to larger rejection.

For two smallest compounds (NDMA and NMEA), Fujioka et al. [45] showed the same behavior, that is, rejection decreased with the decreasing pH probably due to changes in the membrane pore size. For other *N*-nitrosamines with MW larger than that of NMEA, 88 g mol^{-1} was expected to be negligible.

Last paper by Ahmad et al. [46] examined the retention of atrazine and dimethoate with NF membranes (NF90, NF200, NF270, and DK) under pH 4, 7, and 9. Other parameters, operating pressure, feed pesticide, and stirring rate, were constant. It was found that the rejection for these two compounds by NF200, NF270, and DK increased as the pH was increased, while the rejection for NF90 was almost constant regardless of pH. For example, the rejection of dimethoate increased from 20% to 45% and from 40% to 65% for NF270 and NF200 membranes, respectively. The authors deduced that the trend of atrazine and dimethoate rejection obtained for NF200, NF270, and DK in this experiment was due to the changes of the membrane structures caused by the solution's pH.

Results by Wang et al. [27] showed that presence of salt (NaCl), that is, changes in ionic strength, did not affect CP retention by NF membrane probable because CP mainly existed in neutral form therefore salting-out and electrostatic repulsive interactions had a negligible effect on uncharged CP.

The influence of ionic strength, adjusting with NaCl (10 and 20 mM) on cephalexin rejection, was investigated by Zazouli et al. [44]. For the first NF membrane SR2, increasing the ionic strength of the feed solution decreased the rejection of cephalexin. By contrast, for membrane SR3, the rejection increased as the ionic strength was increased. For SR2 membrane, both the charge of the solute and the membrane are reduced due to double layer compression, leading to a decrease in electrostatic repulsion between the cephalexin and active layer of the membrane, while for SR3 membrane, this explanation is not good. The authors stated that the drug separation mechanism is different for both membranes, that is, much stronger influence of Donnan exclusion on rejection has been evoked for SR2 membrane than for SR3 membrane. Fujioka et al. [45] changed ionic strength from 26 to

260 mM and showed that it affected the rejection of NDMA (for TFC-HR decreased from 52% to 34%), while for other organic compounds, rejection decrease was small (e.g., from 90% to 83% for NPYR). Explanation was that increase in ionic strength can increase the membrane pore size (or porosity) and reduce the size of neutral solutes [47,48].

Wang et al. [27] obtained no changes in the rejection of CP when feed concentration was changed. The same behavior was observed by Fujioka et al. [45] when investigating the removal of *N*-nitrosamines (trace organic chemicals formed during chlorination in drinking water and indirect potable water reuse) with one NF (NF90) and two RO (TFC-HR and SWC5) membranes.

GE NF/RO membranes (DL, CK, AK and CG) were used for the rejection of steroid hormone from treated sewage effluent by Jin et al. [49]. Also, effect of effluent organic matter (EfOM) on rejection was investigated. Firstly, the rejection of estrone from electrolyte background solution (1 mM $NaHCO_3$ and 8 mM NaCl, pH 7) showed that rejection was initially higher than 90% and decreased dramatically and then stabilized at later filtration stage. The excellent removal performances at the initial filtration stage were attributed to estrones adsorption capabilities and steric hindrance. However, the adsorption effect can only contribute to the short-term removal of estrone. As the feed solution is continuously filtered through the membrane, more and more available sites on the membrane are occupied by adsorbed estrone. When the partition of estrone between feed solution and membrane reaches equilibrium, there is no further net adsorption effect taking place, and thus, the contribution from adsorption would be negligible. Under this condition, size exclusion would become the overriding removal mechanism at the later filtration stage. Therefore, all experiments were conducted for 24 h. The removal of estrone was higher (6.5–32.5%) in MF-treated secondary effluent than in electrolyte background solution. First reason for increase rejection was in flux decline, that is, the authors suggest that the membranes were fouled by EfOM, and the second reason was that estrone may bind to some fractions of EfOM in bulk solution and retained together by the membranes. Experiments with EfOM showed that hydrophobic acid made a crucial contribution to rejection, that is, "enhancement effect," hydrophobic base could also improve rejection, while hydrophobic neutral and hydrophilic acid with low aromaticity had little effects.

Comerton et al. [37] investigated the impact of NOM and cations on the rejection of five EDCs and PhACs (acetaminophen, carbamazepine, estrone, gemfibrozil, and oxybenzone) by NF (TS80) membrane. They used various water matrices (MBR effluent, Lake Ontario water, and laboratory prepared waters modeled to represent the characteristics of the Lake Ontario water). First of all, rejection of mentioned compounds in Milli-Q water ranged from $28.9 \pm 2.5\%$ for acetaminophen to $95.2 \pm 3.0\%$ for gemfibrozil. Final conclusions of NOM and cations influence on rejection were as follows: (1) the

presence of Suwannee River NOM spiked into laboratory-grade water resulted in an increase in compound rejection; (2) rejection was higher from the Lake Ontario water and MBR effluent when compared to Milli-Q water; (3) the presence of cations alone did not have a significant impact on compound rejection, with the exception of gemfibrozil (the most polar compound); and (4) the presence of cations results in a rejection decrease in the association of EDCs and PhACs with NOM.

The influence of feed water composition (presence of surfactant sodium dodecyl sulfate (SDS), NOM, and cellulose) to the removal of estrone with loose NF TFC-SR2 membrane was also investigated by Schäfer et al. [50]. Results showed that in the presence of cellulose, retention of estrone increased due to estrone–cellulose partitioning. In the case of SDS and NOM, retention reduced at low and neutral pH, while no significant effect was visible at alkaline pH when solute–solute interaction were minimal.

4.3 Influence of Membrane Fouling

Membrane fouling is inevitable in membrane filtration during long-term operation [51]. Fouling can be divided into biofouling (microbial), organic (accumulation of NOM on the membrane surface), colloidal, or particulate fouling (accumulation of small colloidal particles in the feed water on the membrane surface), and scaling (inorganic deposition on the membrane surface when the solubility product of sparingly soluble salts is exceeded). According to Vincent Vela et al. [52] and Hermia model, there are four main types of membrane blocking: complete blocking, intermediate blocking, standard blocking, and cake formation. Complete blocking occurs when the size of foulants is similar to the membrane pore size, which results in reducing the number of open pores without particles depositing on the membrane surface in the first place. Intermediate blocking is somewhat similar to complete blocking, that is, a single particle can precipitate on other particles to form multilayers, and it can directly block some membrane surfaces, resulting in an increase in cake thickness. Standard blocking is similar to adsorption, by which the particles approaching the membrane are adsorbed and deposited on the internal pore wall, thereby reducing the pore volume. In cake formation, foulants deposit on the particles that already block the pores and result in cake formation.

Recently, many reports have indicated that fouling, that is, deposition of fouling layer on the membrane surface, may change membrane surface properties, that is, contact angle [40,53–56], zeta potential [40,54,57,58], and surface morphology [40,57], which could affect the rejection mechanisms of the NF/RO membranes [44,58,59].

Chang et al. [53] showed that fouling (with humic acid and humic acid together with Ca^{2+}-ions) of NF270 and NTR7450 membranes changed hydrophobicity of these membranes. More precisely, NF270 membrane became

more hydrophobic, while on the other hand, NTR7450 became more hydrophilic. Also Bellona et al. [54] indicated that the NF90 membrane became more hydrophilic and more negatively charged, whereas the NF270 became more hydrophobic and less negatively charged. Plakas et al. [55] suggested that the fouling on membrane hydrophobicity was not the same for three investigated (NF270, NF90, and XLE) membranes. Membrane separation of humic substances with the denser NF90 and XLE membranes resulted in small changes of the membrane surface contact angle. However, the NF270 membrane became less hydrophilic due to fouling. Experiments with humic substances–calcium complexes showed significant increase in hydrophobicity of the NF270 membrane but altered slightly the hydrophobic character of the XLE and NF90 membranes.

Comerton et al. [57] investigated changes in zeta potential of the NF270 membrane depending on feed water, that is, various water matrices. Experiment with Milli-Q water did not show changes in zeta potential and was -87 mV for both virgin membrane and after filtration with Milli-Q water. Experiments with other prepared waters suppressed the membrane's negative charge. After filtration, zeta potentials were -67, -70, -67, -62, and -49 mV for Milli-Q with cations, Milli-Q with NOM, Milli-Q with cations and NOM, Lake Ontario water, and MBR effluent, respectively. In the same paper, the authors measured surface roughness and showed changes after filtration. Root mean square (RMS) and mean (R_a) roughness for virgin NF270 membrane were RMS $= 6.8 \pm 1.6$ nm and $R_a = 5.3 \pm 1.3$ nm, while filtration with Milli-Q water resulted in the smoothest layer (RMS $= 44.3 \pm 12.9$ nm and $R_a = 32.2 \pm 11.8$ nm) compared to other feed waters. In addition, the MBR effluent and Milli-Q with cations and NOM resulted in a rough foulant layer and amounted RMS $= 142.1 \pm 29.7$ nm, $R_a = 105.5 \pm 25.8$ nm and RMS $= 140.4 \pm 9.0$ nm, $R_a = 105.8 \pm 3.9$ nm, respectively. It is very well known and shown in previous sections that retention of trace organics by NF membranes can be governed by steric hindrance together with electrostatic and hydrophobic interactions [60]. However, Nghiem and Hawkes [60] showed that steric hindrance (or size exclusion) appears to be the most prevalent mechanism, controlling not only trace organic retention but also the membrane fouling retention.

As a result, the solute–membrane interactions that determine organic micropollutant rejection will also be affected, and thus, the rejection of the organic micropollutants will change. It is clear that NF membrane fouling may change the surface properties and therefore may affect the rejection mechanisms, that is, size exclusion, electrostatic exclusion, and adsorption. Although many studies found that pharmaceuticals may be removed by NF membranes, the effect of fouling on the performance of the process must be considered [40,44,57–59]. For example, Comerton et al. [57] showed changes in MWCO. Results showed that NOM caused a statistically significant reduction in effective MWCO, whereas neither the influence of cations nor the

interaction of NOM and cations was significant. In addition, the MWCO of the NF270 membrane was reduced from 385 ± 13 Da to 222 ± 46 Da and 348 ± 28 Da following filtration with the MBR effluent and Lake Ontario water, respectively.

Investigations in the last few years have reported that membrane fouling can both increase and decrease solute rejection, depending on the solute, membrane, and foulant.

Both tertiary treated effluent and several model fouling solutions (containing sodium alginate, bovine serum albumin, humic acid, or colloidal silica) were used for investigation of membrane fouling [56]. In this chapter, changes in the rejection of *N*-nitrosamines were studied. Fujioka et al. [56] showed that in general the rejection of *N*-nitrosamines increased when membranes were fouled with tertiary effluent, while fouling with model foulants had noticeably less effect on rejection. The highest increase was for compounds with small MW, in particular NDMA, where rejection increased from 34% to 73% by the ESPA2 membrane. ESPAB membrane, that is, membrane with the lowest permeability, showed smallest impact, and rejection of *N*-nitrosamines was over 82% regardless of membrane fouling.

In a work by Yangali-Quintanilla et al. [58], rejections of nine pharmaceuticals and five endocrine disruptors with clean and fouled NF (NF90 and NF200) membranes were compared. Membranes were fouled with sodium alginate. For clean membranes, rejection varied from 35% to 75% and 62% to 96% for NF200 and NF90 membranes, respectively. Fouling of NF200 membrane decreased the rejection of hydrophilic neutral, as well as hydrophilic and hydrophobic ionic compounds, due to restricted back diffusion to the bulk solution and subsequent transport across the membrane. The rejection of hydrophobic neutral compounds with the same fouled membrane increased (5–38%) due to the incipient interaction of the solutes with the membranes and increased interaction with the alginate fouling cake layer, thus resulting in less partitioning and diffusion across the membrane. On the other hand, the rejection of hydrophobic compounds by NF90 membrane was not changed, while for hydrophilic neutral compounds increased by 7–30% due to the domination of an enhanced sieving effect.

In addition, Chang et al. [53] obtained changes in pharmaceuticals rejection with fouled membranes. For small and neutral-charged target compound, acetaminophen, the presence of humic acid and calcium ions increased rejection due to an extra hindrance layer provided by the foulant. Conversely, the rejection of larger compounds (sulfamethoxazole and triclosan) decreased with membrane fouling because concentration polarization was enhanced by presence of foulants (in this case humic acid and calcium ions). The same conclusion, that is, that the rejection of larger compounds decreased and of the partially rejected compounds increased by activated sludge fouled membrane, was found by Agenson and Urase [61]. They stated that the adsorption and diffusion across the fouled membrane played a prominent role in lowering

rejection for larger compounds, while the narrower pores of the fouled membranes resulted in the more dominant size exclusion.

The rejection of the nonionic organic contaminants investigated by the NF90 membrane was greater than 80% and was relatively unaffected by organic fouling. Furthermore, Bellona et al. [54] provided variable rejections of the same contaminants with the NF270 membrane and had markedly lower rejection for acetaminophen, bisphenol A, and phenacetin after membrane fouling with effluent organic matter.

Nghiem et al. [59] showed significant enhancement in the rejection of triclosan, compound with very high hydrophobicity ($\log K_{O/W} = 5.17$), when membranes (NF270, NF90, and BW30) were prefouled with bovine serum albumin, alginate, and humic acid, while no discernible variation in rejection was observed when the membranes were prefouled with hydrophilic silica colloids compared to the clean membranes.

Nghiem and Hawkes [62] investigated the role of membrane pore size on the rejection of PhACs with fouled NF (NF270, NF90, and TFC-SR2) membranes. Membranes were fouled with a foulant cocktail containing model organic foulant in a background electrolyte solution. NF NF90 membrane had the smallest pore sizes, while TFC-SR2 had the largest. Fouling was more pronounced for the membranes with larger pore size, that is, for TFC-SR2 (1.28 nm) and NF270 (0.84 nm), compared to the membrane with smaller pore size NF90 (0.68 nm). For NF90 membrane, there were no changes in rejection except for very small decrease of sulfamethoxazole and carbamazepine at pH 6 in amount of 5% and 1%, respectively. The highest changes were for TFC-SR2 for all compounds and both pHs (6 and 8) prevailed by pore restriction, except for ibuprofen at pH 8 no changes was observed. The smallest increase (19%) was for carbamazepine, and highest for sulfamethoxazole (37%), both at pH 6. For NF270 membrane, changes in rejection were variable, that is, positive and negative. In the case of sulfamethoxazole, rejection increased for 16% and 3% at pH 6 and 8, respectively, while for ibuprofen and carbamazepine decreased for 3–7%. The authors found that the influence of membrane fouling on the retention of PhACs was largely dependent upon membrane pore size. They assumed that this was governed by modification of the membrane charge surface, pore restriction, and cake-enhanced concentration polarization.

As mentioned before, fouling of RO/NF membranes is unavoidable in full-scale plant; therefore, extensive feed water pretreatment is normally used to remove foulant material in order to prevent fouling of membranes. Hence, Verliefde et al. [40] investigated the influence of feed water pretreatment on membrane fouling and the effect on the rejection of organic micropollutant. In their work, untreated surface water was compared with surface water pretreated with an anionic fluidized ion exchange (FIX) and surface water pretreated with UF. Ion exchange resin was used to remove negatively charged NOM components, and in the second case, UF was used to remove

colloidal particles. Consequently, fouling of used NF (TriSep TS80 TSF and Desal HL) membranes was different. The fouling layer on the used membranes, caused by the filtration of untreated surface water, was a combination of both colloids and NOM, while the treatment of anionic ion exchange resin effluent resulted in the deposition of a mainly colloidal fouling layer, with a rough morphology. Treatment of UF effluent resulted in the deposition of a smooth fouling layer, containing mainly NOM. Second part of their study was the influence of different membrane fouling on the rejection of positive, neutral, and negative pharmaceuticals. Both clean membranes showed relatively high rejection ($\geq$70%) and was slightly higher for TS80 due to larger pore size of Desal HL membrane reflected by larger MWCO. Results with fouled membranes showed that rejection decreased significantly for positively charged pharmaceuticals. Rejection values decreased up to 43% with the HL membrane, fouled with ion exchange effluent (compared to the clean Desal HL membrane). Conversely, rejections of almost all negatively charged pharmaceuticals increased on all fouled membranes. For neutral pharmaceuticals, rejections stayed approximately equal to the rejection values on the clean membranes. Also, the authors compared the influence of the different types of fouling and concluded that the largest difference compared to the clean membrane was for membranes fouled with FIX effluent and was caused by a combination of cake-enhanced concentration polarization and electrostatic (charge) effect. For other two water types, changes were smaller and were caused by a combination of steric and electrostatic effect.

Huang et al. [63] investigated influence of different pretreatment (UF, magnetic ion exchange (MIEX)–UF, and MIEX–coagulation–UF) on the removal of 16 EDC and PPCPs, because NOM fouling affects the adsorption and diffusion of organic substances through RO membrane and thus their rejection. Final results showed that RO was effective in removing organic microconstituents when MW was higher than MWCO and that used pretreatments were not effective for EDC and PPCPs removal.

Influence of biofouling on 23 pharmaceuticals (neutral, positively, and negatively charged) rejection in NF membrane filtration was investigated by Botton et al. [64]. They used Desal HL 2521 TFC module. As expected, biofilm slightly changed membrane surface, that is, surface charge became more negative, while hydrophobicity became higher. Biofilm layer was negatively charged, and the presence of this layer induced accumulation of positively charged pharmaceuticals within the biomass layer, which probably also hindered back diffusion. Hence, the rejection efficiency of positively charged solutes decreased (up to 17% absolute decrease in rejection), but did not have impact on the rejection of neutral and negatively charged pharmaceuticals. Probably, combination of different phenomena caused rejection decrease of positively charged solutes. In the case of biofouling, concentration polarization was probably enhanced by the attractive forces occurring between negatively charged biomass and positively charged pharmaceuticals.

Real wastewaters usually contain a large amount of organic and inorganic matter [65–67], resulting, as mentioned before, in formatting of organic and colloidal fouling, biofouling, and inorganic scales on membranes. Therefore, it is necessary to regularly clean membranes. In full-scale NF plant, cleaning of the fouled elements is recommended by membrane manufacturers when normalized pressure drop increase (PDi) reaches 10–15% over the entire installation [68] or if permeability has reached ~10% of the original value [69]. Membrane flux, usually expressed as normalized flux, can show if membranes are fouled. Many studies [56,61,70–72] reported flux and normalized flux decrease due to membrane fouling. Hence, some papers investigated influence of membrane cleaning on the removal of pharmaceuticals.

Firstly, Simon et al. [69] presented changes of the NF270 membrane surface after various cleaning. Exposure to acidic and SDS cleaning, negative charge slightly decreased, while both caustic and acidic cleaning resulted in increased hydrophobicity. In addition, permeability increased after caustic cleaning, while acidic cleaning had opposite effect [69,73]. Caustic cleaning led to a significant decrease (around 35%) in carbamazepine rejection because at pH 11.5 and 12 enhanced interactions among the ionizable functional groups of the membrane polymeric matrix resulted in an increase in membrane pore size. Consequently, lower rejection of neutral carbamazepine was observed. At acidic cleaning, opposite effect happened. Chemical cleaning did not affect the rejection of sulfamethoxazole (pH 8–10), but below pH 8 considerable effect of caustic cleaning on sulfamethoxazole rejection was observed. Both acidic and SDS cleaning resulted in a small increase in the rejection of carbamazepine with no effect on sulfamethoxazole rejection. Nanofiltration NF270 membrane was also used by Simon et al. [73] for investigating changes in the rejection of nine trace organic contaminants after chemical cleaning with MC11 and PC-98 cleaning reagents. Results showed dramatic decrease in the retention of all organics, which was correlated with the dramatic increase in permeability. More precisely, the impact of chemical cleaning on the retention of neutral/hydrophobic contaminants was more severe than for negatively charged compounds. The authors hypothesized that changes in membrane hydrophobicity had impact on adsorption–desorption behavior of the trace organic contaminants.

Commercially available RO and NF membranes in most cases are polyamide TFC membranes. They contain three separate layers. First is nonwoven polyester inner web on which a polysulfone (PS) layer is casted. The last layer is an ultrathin polyamide layer. Membranes can be in contact to chlorine (typically in the form of hypochlorite solution) or monochloramine because these chemicals are used to suppress biological growth in the feed water. However, according to manufacturers, free chlorine tolerance is $<0.1\ mg\ L^{-1}$. Therefore, Simon et al. [74] investigated effect of membrane degradation on the rejection of PhACs. They soaked one RO (BW30) and three NF (TFC-SR2, NF90, and NF270) membranes to sodium hypochlorite solutions. One of the

conclusions was that the effect of membrane degradation on PhAC rejection was strongly membrane dependent. The RO, BW30 and tight NF90 membranes were much more resistant to hypochlorite solution than the TFC-SR2 and NF270 membrane, which have larger pore size. The rejection of all three compounds (sulfamethoxazole, carbamazepine, and ibuprofen) by the BW30 was unchanged. In contrast, chlorine exposure to TFC-SR2 and NF270 membranes resulted in rejection decrease of PhACs, while small increase was observed when a more diluted hypochlorite solution was used. Also, Urase and Sato [75] used loose and tight NF membranes to study the effect of deterioration of NF membranes on retention of pharmaceuticals due to exposure to chlorine. Retention of chloride is mainly affected by the electric repulsion affect; therefore, the authors stated that the exposure of membrane to chlorine spoiled to a certain extent the electric exclusion characteristics of the membrane. The retention of the pharmaceuticals (8 acidic and 2 neutral) by the virgin membrane was very high (>98%). In the case of loose NF membrane, the retention of pharmaceuticals was more sensitive to the chlorine than salt retention. In general, after degradation of the membrane, the retention of pharmaceuticals decreased especially in the lower pH range, though the retention of acidic pharmaceuticals in the neutral pH was maintained above 99.55%.

5 HYBRID SYSTEMS WITH UF, NF, AND RO

Sahar et al. [9] used CAS–UF/RO and MBR/RO system for the removal of various organic micropollutants treating raw sewage of the Tel-Aviv WWTP. Macrolides (hydrophobic compounds, log $K_{O/W} \sim 3$) were efficiently removed by CAS–UF treatment (72–93%), while sulfonamides (hydrophilic compounds, log $K_{O/W} \sim 1$) have been removed during CAS–UF for 60–74%. Contribution of UF for the removal of clarithromycin, erythromycin, roxithromycin, trimethoprim, and sulfamethoxazole was 80%, 64%, 55%, >99.9%, and 55%, respectively. Despite significant molecular differences between the selected micropollutants, high removal rates were achieved after the RO stage (>99% for macrolides, pharmaceuticals, cholesterol, and BPA, 95% for diclofenac, and >93% removal of sulfonamides).

Various hybrid systems (UF/RO, MBR/RO, MF/RO, MF/RO/UV, and MF/RO/RO) were used by Snyder et al. [20] for the removal of EDCs and PPCPs. Microfiltration and UF were not effective in removing these target compounds. In all systems where RO membranes were used, concentrations of almost all compounds were below method reporting limits (1.0 ng L^{-1}). In the MF/RO/UV full-scale experiments, some compounds (oxybenzone, DEET, galaxolide, and TCEP) had concentrations up to 11 ng L^{-1} and were additionally decreased up to 65% with UV.

Dolar et al. [76] used MBR–RO pilot plant (Figure 3) for the removal of twenty multiple-class pharmaceuticals found in municipal wastewater of a

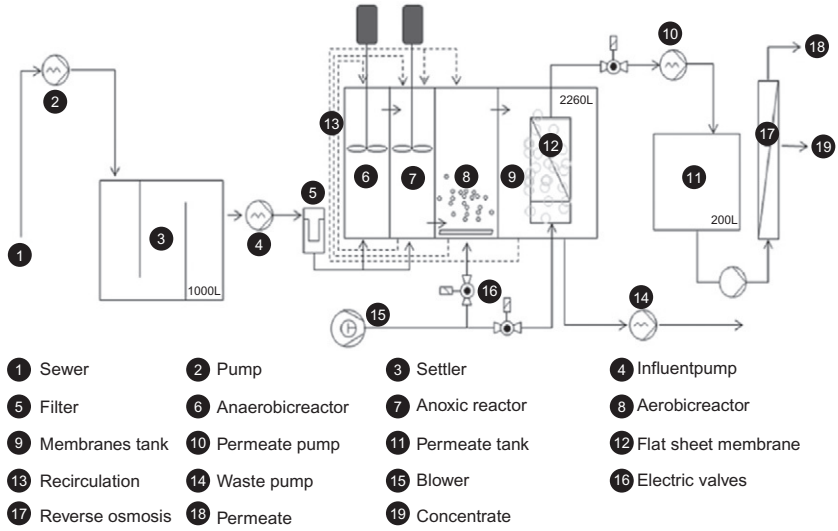

FIGURE 3 Schematic representation of the MBR–RO pilot plant.

costal WWTP (Castell-Platja d'Aro, Spain). This combination treatment showed high overall removal of all the investigated compounds, above 99%. RO (TR70-4021-HF) membrane complemented MBR treatment very well, since the majority of compounds studied in the influent were completely removed, or concentrations were below limit of quantification after RO membrane. Contribution of RO membrane was between 50% and 100%, depending on compound. One of the possible removal mechanisms was steric hindrance but with influence of electrostatic attraction or repulsion forces, due to the negative charge of the membrane and charge of some compounds. Alturki et al. [77] combined MBR with NF or RO membranes for the removal of 40 trace organic contaminants. Rejection with MBR varied quite significantly (from 0% to 100%), but more precisely, it was effective (>50%) for hydrophobic ($\log D \geq 3$) and biodegradable compounds. For example, the removal of nonylphenol, triclocarban, and triclosan was around 85%, 83%, and 65%, respectively. Additional RO step resulted in more than 95% removal or removal to below analytical detection limit.

The laboratory-scale MBR coupled with NF (NE40, NE70, and NE90) membranes was tested to demonstrate the performance of treating 11 pharmaceuticals and PPCPs in municipal wastewater by Chon et al. [78]. Removal varied between 15% and 100%, and the lowest was for the membrane NE40 with highest MWCO (1000 Da), and the highest for NE90 membrane with lowest MWCO (210 Da). Removal for acetaminophen was lowest for all investigated membranes in amount of 15%, 17%, and 30% for NE40, NE70, and NE90 membrane, respectively. For NE40 membrane, the highest removal

was for glimepiride (50%), and complete removal of diclofenac, ibuprofen, and naproxen with NE90 membrane. The negatively charged PPCPs were more effectively removed by the negatively charged NF membranes compared with nonionic or positively charged PPCPs. This is due to electrostatic repulsion between the negatively charged PPCPs and the negatively charged membrane surface.

Effluent from the conventional WWTP in Brisbane, Australia, was treated with MF/RO in order to investigate the removal of 28 human and veterinary antibiotics by Watkinson et al. [79]. Overall removal of antibiotics was 92%, where MF stage removed approximately 43% of total antibiotics from liquid phase, and RO membrane reduced concentration approximately 94%. Only eight antibiotics were present in the RO permeate, with nalidixic acid, the most prominent (0.045 $\mu g\ L^{-1}$), followed by enrofloxacin, roxithromycin, norfloxacin, oleandomycin, trimethoprim, tylosin, and lincomycin in concentration below 0.01 $\mu g\ L^{-1}$.

REFERENCES

[1] M. Mulder, Basic Principles of Membrane Technology, Kluwer Academic Publisher, Dordrecht, Netherlands, 1996.
[2] L.J. Zeman, A.L. Zydney, Microfiltration and Ultrafiltration: Principles and Applications, Marcel Dekker, New York, USA, 1996.
[3] A.I. Schäfer, A.G. Fane, T.D. Waite, Nanofiltration: Principles and Application, Elsevier Advanced Technology, Oxford, UK, 2005.
[4] Z. Amjad, Reverse Osmosis: Membrane Technology, Water Chemistry, and Industrial Application, Van Nostrand Reinhold, New York, USA, 1993.
[5] D. Jermann, W. Pronk, M. Boller, A.I. Schäfer, J. Membr. Sci. 329 (2009) 75–84.
[6] M.R. Boleda, M.T. Galceran, F. Ventura, Environ. Pollut. 159 (2011) 1584–1591.
[7] V. Bonnélye, L. Guey, J. Del Castillo, Desalination 222 (2008) 59–65.
[8] G.K. Pearce, Desalination 222 (2008) 66–73.
[9] E. Sahar, I. David, Y. Gelman, H. Chikurel, A. Aharoni, R. Messalem, A. Brenner, Desalination 273 (2011) 142–147.
[10] E. Sahar, R. Messalem, H. Cikurel, A. Aharoni, A. Brenner, M. Godehardt, M. Jekel, M. Ernst, Water Res. 45 (2011) 4827–4836.
[11] R. Bergamasco, L.C. Konradt-Moraes, M.F. Vieira, M.R. Fagundes-Klen, A.M.S. Vieira, Chem. Eng. J. 166 (2011) 483–489.
[12] K. Konieczny, D. Sąkol, J. Płonka, M. Rajca, M. Bodzek, Desalination 240 (2009) 151–159.
[13] J.L. Acero, F.J. Benitez, F. Teva, A.I. Leal, Chem. Eng. J. 163 (2010) 264–272.
[14] Y. Yoon, P. Westerhoff, S.A. Snyder, E.C. Wert, J. Membr. Sci. 270 (2006) 88–100.
[15] Y. Yoon, P. Westerhoff, S.A. Snyder, E.C. Wert, J. Yoon, Desalination 202 (2007) 16–23.
[16] J. Heo, J.R.V. Flora, N. Her, Y.-G. Park, J. Cho, A. Son, Y. Yoon, Sep. Purif. Technol. 90 (2012) 39–52.
[17] P.A. Neale, A.I. Schäfer, Sep. Purif. Technol. 90 (2012) 31–38.
[18] Q. Sui, J. Huang, S. Deng, G. Yu, Q. Fan, Water Res. 44 (2010) 417–426.
[19] F.J. Real, F.J. Benitez, J.L. Acero, G. Roldan, J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng. 47 (2012) 522–533.

[20] S.A. Snyder, S. Adham, A.M. Redding, F.S. Cannon, J. DeCarolis, J. Oppenheimer, E.C. Wert, Y. Yoon, Desalination 202 (2007) 156–181.
[21] J.L. Acero, F. Javier Benitez, F.J. Real, F. Teva, Chem. Eng. J. 210 (2012) 1–8.
[22] A.M. Comerton, R.C. Andrews, D.M. Bagley, P. Yang, J. Membr. Sci. 303 (2007) 267–277.
[23] D. Dolar, A. Vuković, D. Ašperger, K. Košutić, Water Sci. Technol. 65 (2012) 317–323.
[24] A.M. Comerton, R.C. Andrews, D.M. Bagley, C. Hao, J. Membr. Sci. 313 (2008) 323–335.
[25] L. Braeken, B. Van der Bruggen, Desalination 240 (2009) 127–131.
[26] I. Koyuncu, O.A. Arikan, M.R. Wiesner, C. Rice, J. Membr. Sci. 309 (2008) 94–101.
[27] L. Wang, C. Albasi, V. Faucet-Marquis, A. Pfohl-Leszkowicz, C. Dorandeu, B. Marion, C. Causserand, Water Res. 43 (2009) 4115–4122.
[28] V. Yangali-Quintanilla, S.K. Maeng, T. Fujioka, M. Kennedy, G. Amy, J. Membr. Sci. 362 (2010) 334–345.
[29] D. Dolar, A. Vuković, D. Ašperger, K. Košutić, J. Environ. Sci. 23 (2011) 1299–1307.
[30] S.D. Kim, J. Cho, I.S. Kim, B.J. Vanderford, S.A. Snyder, Water Res. 41 (2007) 1013–1021.
[31] J. Radjenović, M. Petrović, F. Ventura, D. Barceló, Water Res. 42 (2008) 3601–3610.
[32] A.M. Klüpfel, F.H. Frimmel, Desalination 250 (2010) 1005–1007.
[33] M. Röhricht, J. Krisam, U. Weise, U.R. Kraus, R.-A. Düring, Desalination 250 (2010) 1025–1026.
[34] S. Sanches, A. Penetra, A. Rodrigues, E. Ferreira, V.V. Cardoso, M.J. Benoliel, M.T. Barreto Crespo, V.J. Pereira, J.G. Crespo, Sep. Purif. Technol. 94 (2012) 44–53.
[35] C. Bellona, J.E. Drewes, P. Xu, G. Amy, Water Res. 38 (2004) 2795–2809.
[36] A. Verliefde, E. Cornelissen, G. Amy, B. Van der Bruggen, H. van Dijk, Environ. Pollut. 146 (2007) 281–289.
[37] A.M. Comerton, R.C. Andrews, D.M. Bagley, Water Res. 43 (2009) 613–622.
[38] A.R.D. Verliefde, S.G.J. Heijman, E.R. Cornelissen, G. Amy, B. Van der Bruggen, J.C. van Dijk, Water Res. 41 (2007) 3227–3240.
[39] A.R.D. Verliefde, E.R. Cornelissen, S.G.J. Heijman, J.Q.J.C. Verberk, G.L. Amy, B. Van der Bruggen, J.C. van Dijk, J. Membr. Sci. 322 (2008) 52–66.
[40] A.R.D. Verliefde, E.R. Cornelissen, S.G.J. Heijman, I. Petrinic, T. Luxbacher, G.L. Amy, B. Van der Bruggen, J.C. van Dijk, J. Membr. Sci. 330 (2009) 90–103.
[41] D. Dolar, K. Košutić, D. Ašperger, Water Air Soil Pollut. 224 (2013) 1377.
[42] H.Y. Ng, M. Elimelech, J. Membr. Sci. 244 (2004) 215–226.
[43] L.D. Nghiem, A.I. Schäfer, M. Elimelech, Environ. Sci. Technol. 38 (2004) 1888–1896.
[44] M.A. Zazouli, H. Susanto, S. Nasseri, M. Ulbricht, Water Res. 43 (2009) 3270–3280.
[45] T. Fujioka, L.D. Nghiem, S.J. Khan, J.A. McDonald, Y. Poussade, J.E. Drewes, J. Membr. Sci. 409–410 (2012) 66–74.
[46] A.L. Ahmad, L.S. Tan, S.R. Abd. Shukor, J. Hazard. Mater. 154 (2008) 633–638.
[47] A. Escoda, P. Fievet, S. Lakard, A. Szymczyk, S. Déon, J. Membr. Sci. 347 (2010) 174–182.
[48] J. Luo, Y. Wan, J. Membr. Sci. 372 (2011) 145–153.
[49] X. Jin, J. Hu, S.L. Ong, Water Res. 44 (2010) 638–648.
[50] A.I. Schäfer, L.D. Nghiem, A. Meier, P.A. Neale, Sep. Purif. Technol. 73 (2010) 179–187.
[51] B. Van der Bruggen, M. Mänttäri, M. Nyström, Sep. Purif. Technol. 63 (2008) 251–263.
[52] M.C. Vincent Vela, S. Álvarez Blanco, J. Lora García, E. Bergantiños Rodríguez, Chem. Eng. J. 149 (2009) 232–241.
[53] E.E. Chang, Y.-C. Chang, C.-H. Liang, C.-P. Huang, P.-C. Chiang, J. Hazard. Mater. 221–222 (2012) 19–27.
[54] C. Bellona, M. Marts, J.E. Drewes, Sep. Purif. Technol. 74 (2010) 44–54.
[55] K.V. Plakas, A.J. Karabelas, Sep. Purif. Technol. 80 (2011) 246–261.

[56] T. Fujioka, S.J. Khan, J.A. McDonald, R.K. Henderson, Y. Poussade, J.E. Drewes, L.D. Nghiem, J. Membr. Sci. 427 (2013) 311–319.
[57] A.M. Comerton, R.C. Andrews, D.M. Bagley, J. Membr. Sci. 327 (2009) 155–163.
[58] V. Yangali-Quintanilla, A. Sadmani, M. McConville, M. Kennedy, G. Amy, Water Res. 43 (2009) 2349–2362.
[59] L.D. Nghiem, P.J. Coleman, Sep. Purif. Technol. 62 (2008) 709–716.
[60] L.D. Nghiem, S. Hawkes, Desalination 236 (2009) 273–281.
[61] K.O. Agenson, T. Urase, Sep. Purif. Technol. 55 (2007) 147–156.
[62] L.D. Nghiem, S. Hawkes, Sep. Purif. Technol. 57 (2007) 176–184.
[63] H. Huang, H. Cho, K. Schwab, J.G. Jacangelo, Desalination 281 (2011) 446–454.
[64] S. Botton, A.R.D. Verliefde, N.T. Quach, E.R. Cornelissen, Water Res. 46 (2012) 5848–5860.
[65] N.S.A. Mutamim, Z.Z. Noor, M.A.A. Hassan, G. Olsson, Desalination 305 (2012) 1–11.
[66] E. Garcia-Castello, A. Cassano, A. Criscuoli, C. Conidi, E. Drioli, Water Res. 44 (2010) 3883–3892.
[67] J.A. Perdigón-Melón, J.B. Carbajo, A.L. Petre, R. Rosal, E. García-Calvo, J. Hazard. Mater. 181 (2010) 127–132.
[68] J.S. Vrouwenvelder, J.A.M. van Paassen, J.C. Kruithof, M.C.M. van Loosdrecht, J. Membr. Sci. 338 (2009) 92–99.
[69] A. Simon, W.E. Price, L.D. Nghiem, Sep. Purif. Technol. 88 (2012) 208–215.
[70] A. Widjaya, T. Hoang, G.W. Stevens, S.E. Kentish, Sep. Purif. Technol. 89 (2012) 270–281.
[71] K.L. Tu, A.R. Chivas, L.D. Nghiem, Desalination 279 (2011) 269–277.
[72] Y.-N. Wang, C.Y. Tang, J. Membr. Sci. 376 (2011) 275–282.
[73] A. Simon, W.E. Price, L.D. Nghiem, Procedia Eng. 44 (2012) 464–467.
[74] A. Simon, L.D. Nghiem, P. Le-Clech, S.J. Khan, J.E. Drewes, J. Membr. Sci. 340 (2009) 16–25.
[75] T. Urase, K. Sato, Desalination 202 (2007) 385–391.
[76] D. Dolar, M. Gros, S. Rodriguez-Mozaz, J. Moreno, J. Comas, I. Rodriguez-Roda, D. Barceló, J. Hazard. Mater. 239–240 (2012) 64–69.
[77] A.A. Alturki, N. Tadkaew, J.A. McDonald, S.J. Khan, W.E. Price, L.D. Nghiem, J. Membr. Sci. 365 (2010) 206–215.
[78] K. Chon, H. KyongShon, J. Cho, Bioresour. Technol. 122 (2012) 181–188.
[79] A.J. Watkinson, E.J. Murby, S.D. Costanzo, Water Res. 41 (2007) 4164–4176.

Chapter 11

Removal of Pharmaceuticals from Environmentally Relevant Matrices by Advanced Oxidation Processes (AOPs)

Irene Michael, Zacharias Frontistis and Despo Fatta-Kassinos
NIREAS, International Water Research Centre, University of Cyprus, Nicosia, Cyprus

Chapter Outline

1 INTRODUCTION

Occurrence, impact, and removal of pharmaceuticals from waters, which are considered as contaminants of emerging concern [1], have been the target of many studies in the last decades [2,3]. The particularity of these compounds lies in the fact that conventional biological treatment is not able to completely remove them. Thus, pharmaceuticals may end up in the environment, where they can cause adverse effects due to their inherent biological potency toward organisms [1]. Advanced oxidation processes (AOPs) have shown great potential in the treatment of pharmaceuticals, either in high or in low concentrations, and have found various applications in the wastewater treatment field [4,5]. AOPs have been studied over the past 30 years and the scientific literature surrounding their development and application is quite extensive.

In general, the AOP systems generate *in situ* $HO^{\bullet}$ in very mild experimental conditions. Second to fluorine ($E^0 = 3.03$ V), the hydroxyl radical is the

Comprehensive Analytical Chemistry, Vol. 62. http://dx.doi.org/10.1016/B978-0-444-62657-8.00011-2

strongest known oxidant with a potential of 2.80 V. Rate constants (k_{HO}, $r = k_{HO}$ [HO•] C) for most reactions involving HO• in aqueous solutions are usually on the order of 10^6–10^9 M^{-1} s^{-1} [6]. The versatility of the AOPs is enhanced by the fact there are different ways of producing HO•, facilitating compliance with the specific treatment requirements. Table 1 lists those AOPs that have been developed so far, and while the list is not of course exhaustive, it does highlight the variety of the main processes developed, which have applications in water and wastewater treatment. The most common AOPs that have been widely used and evaluated in the water/wastewater remediation field are photolysis under ultraviolet (UV) or solar irradiation; combinations of hydrogen peroxide (H_2O_2), ozone (O_3), and UV irradiation; homogeneous photocatalysis with Fenton reagent; and heterogeneous photocatalysis with semiconductor materials (e.g., TiO_2). In addition, process integration is conceptually advantageous in wastewater treatment since it can eliminate the disadvantages associated with each individual process and provide treatment efficiencies that are greater than the sum of efficiencies that could be achieved by the individual processes applied alone. Special emphasis is given on the research combining AOPs (as a pretreatment or posttreatment stage) and biological systems for the decontamination of wastewater [23]. Even though photo-driven AOPs for wastewater treatment have been proven to be highly efficient, their operation is currently quite expensive. As a means of reducing treatment cost, scientific interest has focused on photocatalytic processes driven by solar irradiation since the latter is a renewable energy source.

In this chapter, an overview of the various photochemical and non-photochemical AOPs with respect to their efficiency in removing pharmaceuticals from various water matrices is given, together with recent relevant literature. Limitations, advantages, and drawbacks are pointed out for each process. Given that the subject is very extensive, the purpose of this chapter is not to provide a complete literature review on this topic, but rather to give a critical evaluation on key parameters associated with the efficiency of each process regarding the removal of pharmaceuticals. Finally, relevant knowledge gaps are discussed while future challenges are also highlighted.

2 ASSESSMENT OF AOPs PERFORMANCE FOR PHARMACEUTICAL REMOVAL

Several papers have been published discussing the capability of AOPs for removing pharmaceuticals in various water matrices and illustrating examples of successful bench- and pilot-scale studies. This is reflected in the increasing number of scientific journal articles published in recent years (more than 5500 articles, Scopus). AOPs are divided into photochemical and non-photochemical processes. In this chapter, technologies included in both groups are reviewed regarding their efficiency for removing pharmaceuticals in a comprehensive way.

TABLE 1 AOPs Used for Water and Wastewater Treatment

AOPs	Key Reactions	Fundamentals
UV	$R—R + h\nu \rightarrow R—R^* \rightarrow 2R^\bullet$ $R—R^* + O_2 \rightarrow R—R^{\bullet+} + O_2^{\bullet-}$ $^3DOM^* + {}^3O_2 \rightarrow DOM + {}^1O_2$	• Direct irradiation leads to the promotion of a molecule from the fundamental state to an excited singlet state. The formed radicals initiate chain reactions; for example, the carbon-centered radicals ($R^\bullet$) react with dissolved oxygen leading to peroxyl ($RO_2^\bullet$) and oxy ($RO^\bullet$) radicals • Photolysis (indirect or sensitized) may be favored in the presence of naturally occurring substances in the system (e.g., dissolved organic matter that can act as photosensitizers generating strong reactive agents, e.g., singlet oxygen ($^1O_2$) and hydroxyl radicals ($HO^\bullet$)) • Disadvantages: UV irradiation with lamps is expensive
UV/H_2O_2	$H_2O_2 + h\nu \rightarrow HO^\bullet + HO^\bullet$ $HO^\bullet + H_2O_2 \rightarrow HO_2^\bullet + H_2O$ $HO_2^\bullet + H_2O_2 \rightarrow HO^\bullet + H_2O + O_2$	• Hydroxyl radicals are formed through the photolytic cleavage of H_2O_2 • High concentration of H_2O_2 scavenges the radicals, making the process less effective • Disadvantages: low radical formation through low molar extinction coefficient of H_2O_2 (18.7 mol cm^{-1} at 254 nm)
O_3	$O_3 + R \rightarrow R_{ox}$ $2O_3 + 2H_2O \rightarrow 2HO^\bullet + O_2 + 2HO_2^\bullet$	• In the absence of light, ozone can react directly with an organic substrate (R) through a slow and selective reaction or through a fast and non-selective radical reaction that produces hydroxyl radicals • Disadvantages: low solubility of O_3 in water, O_3 is selective, formation of by-products (bromates), elevated costs
H_2O_2/O_3	$O_3 + H_2O_2 \rightarrow HO^\bullet + O_2 + 2HO_2^\bullet$	• H_2O_2 initiates O_3 decomposition by electron transfer • Disadvantages: additional cost of H_2O_2 in comparison to O_3 alone
UV/O_3	$O_3 + h\nu + H_2O \rightarrow H_2O_2 + O_2$ $O_3 + h\nu \rightarrow O_2 + O\ (^1D)$ $O\ (^1D) + H_2O \rightarrow 2HO^\bullet$	• The generated hydrogen peroxide is photolyzed (see UV/H_2O_2 process), generating hydroxyl radicals, and also reacts with the excess of ozone • If $\lambda < 300$ nm, photolysis of O_3 takes place, generating additional hydroxyl radicals and other oxidants, with a subsequent increase in the efficiency • Disadvantages: high operating costs

Continued

TABLE 1 AOPs Used for Water and Wastewater Treatment—Cont'd

AOPs	Key Reactions	Fundamentals
UV/H_2O_2/O_3	$O_3 + H_2O_2 + h\nu \rightarrow O_2 + HO^{\bullet} + HO_2^{\bullet}$	• The addition of light to the H_2O_2/O_3 process produces a net increase in the efficiency through the additional generation of hydroxyl radicals • Disadvantages: elevated costs
UV/TiO_2	$TiO_2 + h\nu \rightarrow TiO_2\ (e_{CB}^- + h_{VB}^+)$ $HO^- + h_{VB}^+ \rightarrow HO^{\bullet}$ $O_2 + e_{CB}^- \rightarrow O_2^{\bullet -}$	• When a particle of semiconductor is excited by light energy higher than that of the band gap, electron–hole pairs are formed • The valence holes (h_{VB}^+) are strong oxidants and are able to oxidize various contaminants, as well as water, resulting in the formation of hydroxyl radicals, while the conduction band electrons (e_{CB}^-) are good reductants, reducing the dissolved oxygen to $O_2^{\bullet -}$ • Disadvantages: low quantum yield, need for catalyst removal and regeneration
Fenton	$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + HO^{\bullet}$	• The Fenton process (or dark Fenton) involves the use of H_2O_2 and a catalyst, usually iron (in the form of ferrous or ferric ions) in acidic medium • Fe^{2+} oxidation leads to the formation of hydroxyl radicals • Disadvantages: low pH (2.8–3.0) and iron removal are required
Photo-Fenton	$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^- + HO^{\bullet}$ $Fe^{3+} + H_2O \rightarrow Fe^{2+} + H^+ + HO^{\bullet}$	• The photo-Fenton process involves irradiation with sunlight or from an artificial light source. In the presence of light, the process can be more efficient, by photoreducing the Fe^{3+} to Fe^{2+} and the generation of additional hydroxyl radicals • Disadvantages: low pH (2.8–3.0) and iron removal are required. Additional cost for the UV irradiation • Solar Fenton has gained increasing attention due to its prospect of operating under solar irradiation, hence lowering the operation cost considerably

Electro-Fenton	$Fe^{3+} + e^- \rightarrow Fe^{2+}$ $O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$	• There are two main types of Fenton process involving the use of electrochemically produced reagents • In cathodic process, iron is added as a Fe^{2+} (or Fe^{3+}) salt. The source of H_2O_2 may be either via direct H_2O_2 addition or produced by reduction of oxygen at the cathode • In anodic Fenton process, the source of the iron is a sacrificial iron anode • Disadvantages: elevated costs, requirement for high iron concentration (g L^{-1})
Sonolysis	$H_2O \rightarrow H^{\bullet} + HO^{\bullet}$	• The sonochemical degradation in aqueous phase involves several reaction pathways and zones such as pyrolysis inside the bubble and/or at the bubble–liquid interface and hydroxyl radical-mediated reactions at the bubble–liquid interface and/or in the liquid bulk • Pyrolytic reactions inside or near the bubble and solution radical chemistry are the two major pathways of sonochemical degradation • Disadvantages: high operational cost
Wet air oxidation	Substrate + O_2 → degradation products	• WAO is defined as the oxidation of substances in an aqueous solution by means of oxygen or air at elevated temperatures and pressures (T = 100–372 °C; P = 20–200 bar) • Disadvantages: high operational cost

References: [4,7–22].

Tables 2 and 3 provide an overview of the recent work undertaken in this field describing the most frequently detected pharmaceuticals in the aquatic environment that have been treated so far by AOPs along with comprehensive information related to the treatment method, the aqueous matrix, and the main findings. Among these compounds, diclofenac, amoxicillin, clofibric acid, acetaminophen, ibuprofen, carbamazepine, sulfamethoxazole, and fluoxetine (all belonging to different therapeutic pharmaceuticals classes) are the most widely examined pharmaceuticals since they have become ubiquitous in surface waters and wastewater [7,135]. It is notable that several publications have been devoted to the treatment of pharmaceuticals by AOPs in various aqueous matrices (e.g., pure water, wastewater effluents, surface water, seawater, and water with inorganic ions) with the main focus, however, on ultrapure water. In addition, although the environmental concentrations of pharmaceuticals are in the ng–μg L^{-1} range, the degradation of pharmaceuticals at higher concentration level (mg L^{-1}) was examined in most studies to allow the accurate determination of residual substrate concentrations with the analytical techniques employed.

According to Tables 2 and 3, AOPs were found to be effective treatment processes for removing the selected pharmaceutical contaminants. However, this was not necessarily found to be accompanied by total mineralization. The determination of the total or dissolved organic carbon (TOC or DOC) removal during the application of the advanced treatment is generally used to assess the degree of mineralization in the treated samples. In most studies, mineralization was found to be low compared to the degradation/removal of a specific pharmaceutical, a fact that clearly implies that a considerable organic load remains attributed to the presence of persistent oxidation products. If a substance is not completely eliminated, a number of transformation products can eventually reach the environment with the potential of adversely affecting aquatic and terrestrial organisms rendering, thus toxicity measurements as an indispensable task [136]. However, ecotoxicity assessment of the treated samples by AOPs is beyond the scope of this chapter.

Among the various AOPs, homogenous and heterogeneous photocatalyses have been extensively used with success for the oxidation of many classes of pharmaceuticals due to their high efficiency to generate hydroxyl radicals during the decomposition of H_2O_2 by Fe^{2+} in acidic medium and the activation of a semiconductor by light irradiation, respectively. Other processes that have been used include photolysis under UV or solar irradiation and combinations of hydrogen peroxide (H_2O_2), ozone (O_3), and UV irradiation. Ultrasound irradiation (or sonolysis), electrolysis, and wet air oxidation are relatively new processes in water and wastewater treatment and, therefore, have unsurprisingly received less attention than other AOPs. This is also reflected by the small number of publications concerning the treatment of pharmaceutical compounds. Moreover, as indicated in Tables 2 and 3, AOPs have been studied mainly at a bench scale but many of the processes are being developed and tested at a pilot scale during the last 5 years.

TABLE 2 Examples Taken from the Recent Literature on the Photochemical Advanced Oxidation Treatment (Under Solar or Artificial Irradiation) of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices

Photochemical Advanced Oxidation Technologies						
Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
1. Photolysis						
Carbamazepine, ibuprofen, ketoprofen, 17α-ethinylestradiol	10–40 mg L^{-1}	Distilled water River water Seawater	Bench	Direct natural sunlight, simulated sunlight (507.5 W m^{-2}, $\lambda = 300$–80 nm)	Ketoprofen was rapidly transformed via direct photolysis in all the water matrices under both direct ($t_{1/2} = 2.4$ min) and simulated ($t_{1/2} = 0.54$ min) sunlight. Under simulated radiation, ibuprofen and 17α-ethinylestradiol were photodegraded at moderate rate ($t_{1/2} = 1$–5 h). Carbamazepine had the lowest photodegradation rate ($t_{1/2} = 8$–39 h). Their elimination was strongly dependent on the DOC concentration present in water matrix	[24]
Ciprofloxacin	100 μg L^{-1}	Deionized water	Bench	Medium-pressure mercury lamp (150 W)	The elimination of ciprofloxacin (∼2 min) is very rapid and depends on pH. The fastest degradation ($t_{1/2} = 0.15$ h) was found at pH 7, which is very close to the isoelectric point of ciprofloxacin	[25]

Continued

TABLE 2 Examples Taken from the Recent Literature on the Photochemical Advanced Oxidation Treatment (Under Solar or Artificial Irradiation) of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices—Cont'd

Photochemical Advanced Oxidation Technologies						
Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
Diclofenac	45.5 mg L^{-1}	Demineralized water Reconstructed standard freshwater	Bench	Natural sunlight	Diclofenac undergoes spontaneous phototransformation under solar illumination in both water matrices (100% removal, ~80 min). However, important differences were observed between experiments with respect to pH evolution. Photolysis in demineralized water showed a drop in pH due to chloride release that gave rise to low TOC and diclofenac removal	[26]
Difloxacin, sarafloxacin	10 μg mL^{-1}	Deionized water	Bench	Simulated sunlight (xenon lamp, 500 W m^{-2}, $\lambda = 290–800$ nm)	Both drugs degraded completely (100% removal, ~4 h)	[27]
Fenofibric acid	na	Distilled water	Bench	Low-pressure mercury-vapor lamp (15 W, $\lambda = 254$ nm), $[H_2O_2] =$ 50 mg L^{-1}	The degradation of fenofibric acid was 100% during UV and UV/H_2O_2 processes with UV doses below 1 J cm^{-3}	[28]

Ketoprofen	0.1 mM	Acetonitrile–water (1:1)	Bench	UVA lamp (6 W, $\lambda_{max} = 254$ nm)	Ketoprofen was rapidly decomposed (60 min)	[29]
Oxytetracycline, doxycycline, ciprofloxacin	5 μM	Buffered ultrapure water Surface water Drinking water Wastewater	Bench	Low-pressure Hg vapor lamp (11 W), $[H_2O_2] =$ 0–0.35 mM	The efficiency of UV and UV/H_2O_2 process was affected by water composition. For all of the three selected antibiotics, the fastest degradation was observed in drinking water and the slowest degradation occurred in wastewater. For all compounds, the rate constants increased linearly with the applied H_2O_2	[30]]
Paracetamol	1.5×10^{-5} mol dm^{-3}	Deionized water	Bench	Low-pressure monochromatic lamp (254 nm), $[H_2O_2] =$ 1.5×10^{-5} mol dm^{-3}	UV irradiation resulted into a moderate substrate removal (20%). On the other hand, the addition of H_2O_2 allowed a complete abatement (100%)	[7]
Propranolol	50 mg L^{-1}	Demineralized water	Bench/ pilot	Xenon short-arc lamp (1000 W)	Propranolol removal after 240 min was 77% and 71% for the pilot- and the bench-scale setup, respectively. However, mineralization accomplished resulted to be negligible (7% and 2%)	[3]

Continued

TABLE 2 Examples Taken from the Recent Literature on the Photochemical Advanced Oxidation Treatment (Under Solar or Artificial Irradiation) of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices—Cont'd

Photochemical Advanced Oxidation Technologies

Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
Propranolol, atenolol, metoprolol	3×10^{-3}–10 mg L^{-1}	Deionized water	Bench	Xenon arc lamp (1 kW, $\lambda = 290$–800 nm)	The measured half-lives of propranolol, atenolol, and metoprolol were approximately 16, 350, and 630 h, respectively. The half-lives were related to daylight surface conditions by comparing the light intensity of the lamp and the sun at different latitudes and seasons	[31]
Propranolol, metronidazole	100 mg L^{-1}	Demineralized water	Bench	UV-254 germicidal lamp (UVC) UV-365 black-light lamp (UVA)	After 8 h of irradiation, direct UVA photolysis promoted insignificant pharmaceutical removal (propranolol UVA, 0%, and metronidazole UVA, 22%). Under UVC radiation, substrate removal was increased (near 50%)	[32]
Sulfamethoxazole, trimethoprim	1 μM	Wastewater	Bench	Solar simulator with a UV-Suprax optical filter (765 W m^{-2})	Photolysis could be apportioned into direct photolysis (48% for sulfamethoxazole and 18% for trimethoprim) reaction with hydroxyl radicals (36% and 62%, respectively) and reaction with triplet excited effluent organic matter (16% and 20%, respectively)	[33]

Sulfamethoxazole, sulfamethazine, sulfadiazine, trimethoprim, diclofenac	4 μM	Demineralized water Lake water Wastewater	Bench/ pilot	Low-pressure UV lamps, $[H_2O_2] = 10$ mg L^{-1}	The removal efficiency increases with the order: wastewater < lake water < demineralized water. For sulfonamides, pH-related differences in transformation rates were mainly due to differences in the photolysis rate between the neutral and anionic species. For trimethoprim, the reaction rate between the substrate and HO• was pH-dependent and the protonated form reacted more readily than the neutral form. For the UV + H_2O_2 process, the required UV dose to achieve >90% was <860 mJ cm^{-2} (sulfamethoxazole), <330 mJ cm^{-2} (diclofenac), and >900 mJ cm^{-2} (sulfamethazine, sulfadiazine, trimethoprim)	[34]
Trimethoprim	20 mg L^{-1}	Demineralized water Simulated seawater	Pilot	Simulated sunlight (250 W m^{-2})	Direct photolysis yielded a similar, slow trimethoprim (TMP) complete degradation rate in both water matrices (demineralized water in 1100 min, simulated seawater in 1400 min)	[35]

Continued

TABLE 2 Examples Taken from the Recent Literature on the Photochemical Advanced Oxidation Treatment (Under Solar or Artificial Irradiation) of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices—Cont'd

Photochemical Advanced Oxidation Technologies

Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
Norfloxacin, doxycycline, mefenamic acid	10^{-4} M	Demineralized water	Bench	Low-pressure mercury-vapor lamp using UVC radiation (150 W, $\lambda = 254$ nm) in the presence or absence of hydrogen peroxide or sodium monopersulfate	After 2 h of treatment, approximately 50% of norfloxacin and mefenamic acid were removed, while roughly 20% of doxycycline was eliminated. Inorganic peroxides considerably enhanced the contaminant conversion (100%), although no appreciable mineralization could be obtained	[36]
Acetaminophen, atenolol, carbamazepine, ibuprofen, ifenprodil, indomethacin, propranolol, mefenamic acid	100 mg L^{-1}	Demineralized water	Bench	Direct natural sunlight	Propranolol, indomethacin, and ifenprodil were easily photodegraded ($t_{1/2} < 24$ h), whereas the other five pharmaceuticals were stable against sunlight	[37]
Difloxacin, sarafloxacin	10 μg mL^{-1}	Demineralized water River water	Bench	Suntest CPS + photoreactor equipped with xenon lamp (500 W m^{-2}, $\lambda = 290$–800 nm)	The degradation rate in pure water dropped sharply for sarafloxacin ($t_{1/2} = 0.84$ h) in comparison to difloxacin ($t_{1/2} = 2.62$ h).The degradation	[38]

					rate was rapid in river water for both sarafloxacin ($t_{1/2}=0.34$ h) and difloxacin ($t_{1/2}=0.49$ h). The difference in the degradation rate was predicted to be from the influence of river water pH (6.3) after addition of substrate and also from the dissolved organic matters and inorganic matters that could have possibly aided the dissipation process	
Ofloxacin	20 mg L^{-1}	Demineralized water	Bench	Medium-pressure mercury-vapor lamp (150 W)	Ofloxacin was not present after 32 min of irradiation and this was accompanied with a 9% DOC removal. After 64 min of treatment, 15% of DOC was removed	[39]
Clofibric acid, diclofenac, fenoprofen, isopropylantipyrine, ketoprofen, phenytoin, triclosan	100 μg L^{-1}	Demineralized water	Bench	Low-pressure UV mercury lamp (10 W, $\lambda=254$ nm), $[H_2O_2]=$ 0–1.47 mM	Clofibric acid, diclofenac, fenoprofen, isopropylantipyrine, ketoprofen, phenytoin and triclosan were removed very efficiently (>96%) by ultraviolet photolysis alone. Hydrogen peroxide addition to ultraviolet photolysis was not worthy for majority of the tested compounds as their removal did not increase significantly	[40]

Continued

TABLE 2 Examples Taken from the Recent Literature on the Photochemical Advanced Oxidation Treatment (Under Solar or Artificial Irradiation) of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices—Cont'd

Photochemical Advanced Oxidation Technologies						
Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
2. Homogeneous photocatalysis (photo-Fenton)						
Bezafibrate, gemfibrozil, metformin, carbamazepine, gabapentin, diclofenac, ibuprofen, ketoprofen, mefenamic acid, naproxen, paracetamol, primidone, atenolol, metoprolol, sotalol, azithromycin, ciprofloxacin, clarithromycin, metronidazole, norfloxacin, ofloxacin, sulfamethoxazole, iopamidol trimethoprim	25–1737 ng L^{-1}	Wastewater	Bench/ pilot	Low-pressure mercury lamp ($\lambda = 254$ nm), solar silumator (550 W m^{-2}), $[Fe^{2+}] = 5$ mg L^{-1}, $[H_2O_2] = 10$–50 mg L^{-1}	Photo-Fenton employing sunlight simulator reached low removals. Meanwhile, the removal improved noticeably when photo-Fenton was developed using UVA light. Global percentages of pharmaceutical removal achieved were 100%, after 90 min of treatment ($[Fe^{2+}] = 5$ mg L^{-1}, $[H_2O_2] = 50$ mg L^{-1})	[41]
Amoxicillin, paracetamol	0.1 mM	Distilled water Wastewater	Bench/ pilot	Black-light (15 W, $\lambda = 365$ nm) and natural solar irradiation, $[H_2O_2] = 2.0$ mM, [ferrioxalate or $Fe(NO_3)_3$] = 0.20 mM, pH 2.5	The degradation of amoxicillin was not influenced by the source of the irradiation. Under black-light irradiation, 90% and 89% of amoxicillin oxidation were obtained after 1 min of irradiation in distilled water and wastewater,	[42]

					respectively, while under solar irradiation, 96% and 85% were reached after the same time. The use of solar irradiation favored the degradation of paracetamol, achieving complete degradation in a shorter time than that obtained with black-light irradiation. The photodegradation of paracetamol was influenced by the iron source (higher degradation in the presence of potassium ferrioxalate (FeO_x) in comparison to $Fe(NO_3)_3$)	
Amoxicillin	50 mg L^{-1}	Distilled water	Bench	Solar simulator with xenon arc lamp (1100 W), $[FeSO_4 \cdot 7H_2O] =$ 0.05 mM, $[FeO_x] =$ 0.05 mM, $[H_2O_2] =$ 120 mg L^{-1}	Total oxidation of amoxicillin in the presence of FeO_x was obtained after 5 min, while 15 min was necessary using $FeSO_4$	[43]
Amoxicillin	30 mg L^{-1}	Distilled water Surface water	Bench	Black-light irradiation (13 W m^{-2}, $\lambda = 365$ nm), $[Fe^{2+}] =$ 0.0179–0.0895 mM, $[H_2O_2] =$ 1–10 mM	In all cases, complete amoxicillin degradation occurred within 5 min and this was accompanied by lower mineralization rates	[44]

Continued

TABLE 2 Examples Taken from the Recent Literature on the Photochemical Advanced Oxidation Treatment (Under Solar or Artificial Irradiation) of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices—Cont'd

Photochemical Advanced Oxidation Technologies						
Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
Acetaminophen, atenolol	10 mg L^{-1}	Distilled water Synthetic wastewater	Pilot	Direct natural sunlight, $[Fe^{2+}] = 5$ mg L^{-1}, $[H_2O_2] = 10$ mg L^{-1}	Total disappearance of the parent compounds and discreet mineralization were attained in all experiments (acetaminophen, 12 min in distilled water and 21.8 min in wastewater; atenolol, 3.8 min in distilled water and 30 min in wastewater)	[45]
Penicillin	na	Wastewater	Bench	UV light ($\lambda = 253.7$ nm), $[H_2O_2] = 20$ mM, $[Fe^{2+}] = 1$ mM; $[Fe^{3+}] =$ 1 mM	After 60 min of treatment, the COD removal during photo-Fenton and photo-Fenton-like was 56% and 66%, respectively, while the respective TOC removal was 51% and 42%	[46]
Penicillin	na	Pharmaceutical wastewater	Bench	Microwave power = 100–500 W, radiation time = 2–10 min, pH 1–11, $[H_2O_2] =$ 3200–19,000 mg L^{-1}, $[Fe_2(SO_4)_3] =$ 2000–8000 mg L^{-1}	Under the optimum conditions (microwave power = 300 W; radiation time = 6 min; pH 4.42; $[H_2O_2] = 1300$ mg L^{-1}; $[Fe_2(SO_4)_3] = 4900$ mg L^{-1}), penicillin degradation was 55.1% that was accompanied with 57.5% and >40% of COD and TOC removal, respectively	[47]

Acetaminophen, antipyrine, carbamazepine, diclofenac, flumequine, hydroxybiphenyl, ibuprofen, ketorolac, ofloxacin, progesterone, sulfamethoxazole	100 μg L^{-1}	Synthetic water Wastewater	Pilot	Direct natural sunlight, $[Fe^{2+}] = 5$ mg L^{-1}, $[H_2O_2] = 50$ mg L^{-1}, $t_{30W} = 102$ min	The drugs can be successfully degraded to negligible concentrations without adjusting the pH. The degradation was found to depend on the presence of CO_3^{2-} and HCO_3^- ($HO^\bullet$ scavengers) and on the type of water matrix	[48]
Ofloxacin	10 mg L^{-1}	Wastewater	Bench	Solar simulator (1 kW xenon lamp), $[Fe^{2+}] =$ 1–5 mg L^{-1}, $[H_2O_2] =$ 1.357–8.142 mmol L^{-1}	The complete degradation of the examined substrate and DOC reduction (50%) were achieved in 30 min of the photocatalytic treatment ($[Fe^{2+}] = 5$ mg L^{-1}, $[H_2O_2] = 2.714$ mmol L^{-1})	[49]
Ofloxacin, trimethoprim	100 μg L^{-1}	Wastewater	Pilot	Direct natural sunlight, $[Fe^{2+}] = 5$ mg L^{-1}, $[H_2O_2] = 75$ mg L^{-1}	The complete degradation of the drugs was achieved at $t_{30WT,n} = 38.7$ min (ofloxacin) and $t_{30WT,n} = 20.1$ min (trimethoprim)	[50]
Sulfamethazine	50 mg L^{-1}	Deionized water	Bench	Sunlight lamp (300 W), $[Fe^{2+}] = 40$ mg L^{-1}, $[H_2O_2] = 600$ mg L^{-1}	Sulfamethazine was completely removed in less than 2 min of treatment	[51]
Sulfamethoxazole	200 mg L^{-1}	Distilled water	Bench	Three black-light blue lamps (8 W each), $[Fe^{2+}] = 10$ mg L^{-1}, $[H_2O_2] = 300$ mg L^{-1}	The complete antibiotic removal was achieved for a H_2O_2 dose over 300 mg L^{-1}	[52]

Continued

TABLE 2 Examples Taken from the Recent Literature on the Photochemical Advanced Oxidation Treatment (Under Solar or Artificial Irradiation) of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices—Cont'd

Photochemical Advanced Oxidation Technologies

Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
Trimethoprim	10 mg L^{-1}	Distilled water Simulated water Simulated effluent Pretreated wastewater	Pilot	Direct natural sunlight, $[Fe^{2+}] = 2$ mg L^{-1}, $[H_2O_2] = 2.5$ mg L^{-1}	The extent of mineralization decreases in the order: distilled water > simulated water > simulated effluent > pretreated wastewater	[53]
Tetracycline	24 mg L^{-1}	Deionized water Surface water	Bench	Black-light (15 W) and solar irradiation, $[H_2O_2] = 1$–10 mM, [Ferrioxalate or $Fe(NO_3)_3$] = 0.20 mM, pH 2.5	The photo-Fenton process under black or solar irradiation is very efficient for the degradation of tetracycline in pure water, achieving total degradation after approximately 1 min irradiation. Under black-light irradiation, higher efficiency is obtained using iron nitrate than when ferrioxalate is used. When tetracycline was dissolved in surface water, similar results were obtained indicating no significant interference of this matrix on the degradation process	[18]

Acetaminophen, atenolol, diclofenac, iopromide sulfamethoxazole, naproxen, fluoxetine	1 μg L^{-1}	Wastewater	Bench	Low-intensity interior lighting, $[Fe^{2+}]$ = 20 mg L^{-1}, $[H_2O_2]/[Fe^{2+}]$ = 0.5–3.0	All tested drugs, except iopromide, were completely removed by Fenton treatment carried out using a 2.5 $[H_2O_2]/[Fe^{2+}]$ molar ratio	[54]
Diclofenac	50 mg L^{-1}	Distilled water	Pilot	Direct natural sunlight, $[Fe^{2+}]$ = 0.05 mM, $[H_2O_2]$ = 20 mM	A rapid and complete oxidation of diclofenac after 60 min and total mineralization after 100 min of treatment were achieved	[55]
4-Methylaminoantipyrine[a]	0.56 mg L^{-1}	Demineralized water	Pilot	Direct natural sunlight, $[Fe^{2+}]$ = 2 mg L^{-1}, $[H_2O_2]$ = 200–500 mg L^{-1}	Complete disappearance of the drug and 10% of TOC removal were observed during the dark Fenton reaction within 15 min. Once solar radiation started to enter the reactor, TOC decreased rapidly to reach the final TOC of 2.5 mg L^{-1} after 120 min	[56]
Ibuprofen	0.87 mM	Distilled water	Bench	Xenon lamp (1 kW, λ = 290–400 nm), $[Fe^{2+}]$ = 0.15–1.2 mM, $[H_2O_2]$ = 0.04–0.32 mM	The degradation of ibuprofen was direct proportional to the amount of hydrogen peroxide used between 80% and 100% for 0.04and 0.32 mM of H_2O_2, respectively, in the presence of 1.2 mM of Fe^{2+}. In regard to the mineralization, photo-Fenton reached 40% of TOC removal	[57]

Continued

TABLE 2 Examples Taken from the Recent Literature on the Photochemical Advanced Oxidation Treatment (Under Solar or Artificial Irradiation) of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices—Cont'd

Photochemical Advanced Oxidation Technologies

Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
Melatonin	20 mg L^{-1}	Distilled water	Bench	8 W low-pressure mercury lamp, $[Fe^{2+}]$ = 0.05–0.2 mM, $[H_2O_2]$ = 5–15 mM	Melatonin was degraded completely in 60 min under the optimum experimental conditions ($[Fe^{2+}]$ = 0.1 mM, $[H_2O_2]$ = 10 mM)	[58]
Nalidixic acid	na	Demineralized water Simulated industrial effluent Saline water	Pilot	Direct natural sunlight, $[Fe^{2+}]$ = 2 or 20 mg L^{-1}, $[H_2O_2]$ = 200–400 mg L^{-1}	Nalidixic acid was completely eliminated in all water matrices. The water composition altered the mineralization rate, which was slower the more complex the matrix is ($DOC_{removal}$ = 86% in demineralized water, $DOC_{removal}$ = 73% in saline water, $DOC_{removal}$ = 20% in simulated industrial effluent)	[59]
Clarithromycin, roxithromycin	1.34 μM	River water	Bench	Medium-pressure mercury lamp, $[Fe^{2+}]$ = 37.7 μM	The findings suggest that photodegradation with Fe^{3+} involves the Fe^{3+}–substrate complexes and not hydroxyl radicals photogenerated by Fe^{3+} (clarithromycin, $t_{1/2}$ = 1.25 h; roxithromycin, $t_{1/2}$ = 1.63 h)	[60]

Flumequine, nalidixic acid	20 mg L^{-1}	Distilled water	Pilot	Direct natural sunlight, $[Fe^{2+}] = 2$ mg L^{-1}, $[H_2O_2] =$ 150–350 mg L^{-1}	Photo-Fenton degradation of both substances was very quick (flumequine, 18 min, and nalidixic acid, 11 min), and the same mineralization level (76–77%) was reached in both cases	[61]
Acetaminophen, antipyrine, diclofenac, progesterone, sulfamethoxazole, triclosan	100 μg L^{-1}	Distilled water Standard fresh water Standard fresh water without $NaHCO_3$	Pilot	Direct natural sunlight, $[Fe^{2+}] = 5$–55 mg L^{-1}, $[H_2O_2] = 50$ mg L^{-1}	The degradation of all compounds in distilled water was achieved within 20 min illumination time. Acetaminophen, sulfamethoxazole, and triclosan were completely degraded in fresh water while antipyrine and progesterone were still present after 270 min. All the compounds were degraded in fresh water without $NaHCO_3$ after 55 min	[62]
3. Heterogeneous photocatalysis (TiO_2)						
Amoxicillin, cloxacillin	138 mg L^{-1}	Wastewater	Bench	UV lamp (6 W, $\lambda = 365$ nm), $[TiO_2] =$ 0–1000 mg L^{-1}, $[H_2O_2] =$ 50–350 mg L^{-1}	Under the optimum conditions ($[TiO_2] = 1000$ mg L^{-1}, $[H_2O_2] =$ 250 mg L^{-1}, pH 5), complete degradation of both substrates was achieved in 30 min	[63]
Amoxicillin, diclofenac, carbamazepine	2.5–10 mg L^{-1}	Wastewater	Bench	Black-light fluorescent lamp (125 W, 300–420 nm), $[TiO_2] =$ 0.2–0.8 g L^{-1}	All the drugs were completely removed within 120 min of treatment ($[TiO_2] = 0.8$ g L^{-1})	[64]

Continued

TABLE 2 Examples Taken from the Recent Literature on the Photochemical Advanced Oxidation Treatment (Under Solar or Artificial Irradiation) of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices—Cont'd

Photochemical Advanced Oxidation Technologies

Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
Sulfamethoxazole, sulfamethizole, sulfathiazole, sulfisoxazole	100 μM	Demineralized water	Bench	Xenon arc lamp system (450 W), $[TiO_2] = 0.1\ g\ L^{-1}$	Results demonstrate that UVA–TiO_2 photocatalysis can be a very effective approach for degrading sulfonamides, particularly in natural waters exhibiting either alkaline pH or low concentrations of NOM or both conditions (sulfamethoxazole, sulfathiazole, and sulfisoxazole, removal >95% in 60 min, and sulfamethizole removal, >80% in 60 min)	[65]
Sulfamethoxazole	$10\ mg\ L^{-1}$	Wastewater	Bench	9 W UVA lamp (Radium Ralutec, 9 W/78, 350–400 nm), $[TiO_2] = 500\ mg\ L^{-1}$	Sulfamethoxazole and TOC removal decreased with decreasing catalyst loading and dissolved oxygen concentration and increasing substrate concentration and solution pH. Within ~20 min and at pH 4.8 < pH < 5.6, a complete removal of the substrate was observed while higher treatment time (60 min) was needed for >99% removal at pH 7.5 < pH < 8.2	[66]

Acetaminophen	100 μM	Bidistilled water	Bench	Metal-halide lamp (250 W, $\lambda > 365$ nm), $[TiO_2] = 1.0$ g L^{-1}	After 100 min irradiation, about 95% of the substrate was decomposed. The effect of adsorption at three different pH values has also been analyzed and it has been conducted that pH 3.5, at which acetaminophen was readily adsorbed also degraded at a faster rate	[67]
Acetaminophen	4.0 mM	Demineralized water	Bench	Black-light blue UVA lamp ($\lambda = 365$ nm), UVC (15 W, $\lambda = 254$ nm), $[TiO_2] = 0.4$ g L^{-1}	A much faster degradation and effective mineralization of acetaminophen took place under UVC irradiation in 300 min (>99% UVC, ~40% UVA). Experimental results showed that the rate constants decrease with an increase in the initial concentration of paracetamol but increase with increase in light intensity and additional oxygen	[68]
Trimethoprim	20 mg L^{-1}	Deminelized water Simulated seawater	Pilot	Direct natural sunlight, $[TiO_2] = 200$ mg L^{-1}	During TiO_2 photocatalysis, trimethoprim was completely eliminated in both water matrices (demineralized water, 29 min, and simulated seawater, ~50 min); however, the mineralization rate was appreciably reduced in seawater, which can be explained by the presence of inorganic species acting as hydroxyl radical scavengers	[35]

Continued

TABLE 2 Examples Taken from the Recent Literature on the Photochemical Advanced Oxidation Treatment (Under Solar or Artificial Irradiation) of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices—Cont'd

Photochemical Advanced Oxidation Technologies

Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
Trimethoprim, sulfamethoxazole	100 mg L^{-1}	Demineralized water	Bench	$[TiO_2] = 0.1–2.0$ g L^{-1}	Sulfamethoxazole was removed by 92% ($[TiO_2] = 2.0$ g L^{-1}, 6 h) whereas trimethoprim was completely eliminated ($[TiO_2] > 0.2$ g L^{-1}, 6 h)	[69]
Flumequine, nalidixic acid	20 mg L^{-1}	Distilled water	Pilot	Direct natural sunlight, $[TiO_2] = 200$ mg L^{-1}	Degradation efficiency by heterogeneous photocatalysis was similar for both compounds, which were completely degraded after 25 min of illumination	[61]
Ciprofloxacin	100 μM	Demineralized water	Bench	Xenon arc lamp system (450 W, (Vis: $\lambda > 400$ nm, UVA: $\lambda > 324$ nm), $[TiO_2] = 0.5$ g L^{-1}	The experiments conducted in deionized water yielded greater deactivation energy efficiency for UVA–TiO_2 photocatalysis relative to Vis–TiO_2	[70]
Carbamazepine, clofibric acid, iomeprol, iopromide	1.0–5.4 mg L^{-1}	Demineralized water	Bench	Xe short-arc lamp (1000 W), $[TiO_2] = 0.1–1000$ g L^{-1}, TiO_2 (Aeroxide P25 and Hombikat UV100)	Kinetic studies showed that P25 had a better photocatalytic activity for clofibric acid and carbamazepine than Hombikat UV100. For photocatalytic degradation of iomeprol, Hombikat UV100 was more suitable than P25 due to its higher adsorption capacity	[71]

Propranolol	50 mg L^{-1}	Demineralized water	Bench/ pilot	Xe short-arc lamp (1000 W), direct natural sunlight, [TiO_2] = 0.1–0.4 g L^{-1}	Propranolol degradation percentages achieved after 240 min were 81% at the pilot- and 94% at bench-scale setup. Meanwhile, mineralization reached was 30% and 41% in pilot plant and laboratory device, respectively	[3]
Atenolol, metoprolol, propranolol	100 μM	Demineralized water	Bench	High-pressure mercury lamp (125 W, λ_{max} = 365 nm), [TiO_2] = 1.0 g L^{-1}	The results showed that propranolol degraded much more efficiently than atenolol and metoprolol. The half-lives of three β-blockers are 18.9, 19.9, and 7.8 min for atenolol, metoprolol, and propranolol, respectively	[72]
Ofloxacin, atenolol	10 mg L^{-1}	Demineralized water Groundwater Wastewater	Bench	9 W UVA lamp (λ = 350–400 nm), [TiO_2] = 250 mg L^{-1}, [H_2O_2] = 0.07 mmol L^{-1}	Ofloxacin (∼85% removal, 30 min) is generally more susceptible to photocatalytic degradation than atenolol (∼60% removal, 30 min). When H_2O_2 added to the photocatalytic system, 79% and 60% of DOC removals were achieved for ofloxacin and atenolol, respectively. The effect of solution pH was substrate-specific while the extent of mineralization decreases in the order demineralized water > groundwater > wastewater	[73]]

Continued

TABLE 2 Examples Taken from the Recent Literature on the Photochemical Advanced Oxidation Treatment (Under Solar or Artificial Irradiation) of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices—Cont'd

Photochemical Advanced Oxidation Technologies

Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
Ofloxacin	10 mg L^{-1}	Wastewater	Bench	Solar simulator (1 kW xenon lamp), $[TiO_2]$ = 0.25–4.0 g L^{-1}, $[H_2O_2]$ = 1.357–8.142 mmol L^{-1}, pH 2–10	Under the optimum experimental conditions ($[TiO_2]$ = 3 g L^{-1}, 120 min), 60% of ofloxacin removal was observed while the addition of H_2O_2 ($[H_2O_2]$ = 5.428 mmol L^{-1}) enhanced the substrate degradation (67%). The degradation of ofloxacin depends strongly on the pH of the solution and is substantially reinforced at acidic conditions, while hindered at alkaline conditions	[49]
Carbamazepine, ibuprofen	10 mg L^{-1}	Demineralized water Wastewater	Bench	9 W UVA lamp (λ = 350–400 nm, photon flux = 3.37×10^{-6} einstein s^{-1}), $[TiO_2]$ = 50–3000 mg L^{-1}, $[H_2O_2]$ = 0.07–1.4 mmol L^{-1}, pH 3–10	The removal of carbamazepine in pure water was 74% (120 min, $[TiO_2]$ = 100 mg L^{-1}), while ibuprofen was degraded by 65% (120 min, $[TiO_2]$ = 500 mg L^{-1}). Process performance was lower when drugs were spiked in wastewater. DOC removal was enhanced (56–58%) using 1.4 mM of H_2O_2. The degradation decreased in either acidic or alkaline conditions compared to experiments at ambient pH	[74]

Oxolinic acid	20 mg L^{-1}	Demineralized water	Bench	Black-light lamp (14 W m^{-2}, λ_{max} = 365 nm), [TiO_2] = 0.2–1.5 g L^{-1}	The substrate was eliminated within 30 min and [TiO_2] = 1.0 g L^{-1}. At the same conditions, 53% of both initial DOC and COD remain in the solution	[75]
Erythromycin	10 mg L^{-1}	Demineralized water	Bench	9 W UVA lamp (λ = 350–400 nm, photon flux = 4.69 × 10^{-6} einstein s^{-1}), [TiO_2] = 100–750 mg L^{-1}	Erythromycin was completely removed in 120 min and [TiO_2] = 500 mg L^{-1}. Mineralization was favored at solution's natural pH of 5, while near neutral conditions (pH 7) impeded degradation. The degradation expectedly increased with decreasing concentration (2.5–30 mg L^{-1})	[76]
Estrone, 17β-estradiol	0.1–1.0 μg L^{-1}	Demineralized water	Bench	Reactor 1 (150 W, λ = 253 nm), reactor 2 (λ = 238–579 nm), [TiO_2] = 1.0 g L^{-1}	In reactor 1 (150 W), 97% of compounds were degraded within 4 h of irradiation. In reactor 2 (15 W), 98% of both compounds disappeared within 1 h	[77]
Diclofenac, naproxen, and ibuprofen	200 mg L^{-1}	Demineralized water	Bench	Xe-OP lamp (1 kW, photon flux = 6.9 meinstein s^{-1} (290–400 nm), [TiO_2] = 0.1–1.0 g L^{-1}	The results showed that the optimum amount of TiO_2 to achieve maximum degradation (98%) of ibuprofen was 1.0 g L^{-1} whereas the maximum degradation for diclofenac and naproxen was observed at a TiO_2 loading of 0.1 g L^{-1}	[78]

Continued

TABLE 2 Examples Taken from the Recent Literature on the Photochemical Advanced Oxidation Treatment (Under Solar or Artificial Irradiation) of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices—Cont'd

Photochemical Advanced Oxidation Technologies						
Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
4-Methylaminoantipyrine[a]	0.56 mg L^{-1}	Demineralized water	Pilot	Direct natural sunlight, $[TiO_2] = 200$ mg L^{-1}	The substrate decreased rapidly from 95% to 30%, during the first 20 min of treatment, and completely disappeared after 60 min	[56]
Norfloxacin	na	Demineralized water	Bench	12 Low-pressure mercury lamps ($\lambda = 420$ nm), $[TiO_2] =$ 2.0 g L^{-1}	The influences of catalyst dosage, initial compound concentration, and solution pH levels on the decay performance and reaction kinetics were investigated. The optimum dosage of catalyst was found to be 2.0 g L^{-1}, in which norfloxacin was completely degraded in 20 min	[79]
Levofloxacin	20 mg L^{-1}	Water after reverse osmosis	Bench	Hg–Ar UVC lamp (254 nm), $[TiO_2] =$ 0.05–0.5 g L^{-1}	At 120 min of irradiation and $[TiO_2] = 0.2$ g L^{-1}, 97% of levofloxacin was removed and it was no longer detected at 180 min of irradiation	[80]

Tetracycline	40 mg L^{-1}	Demineralized water	Bench	Three light sources: 1: 125 W, UV ($\lambda > 254$ nm) 2: 6 W × 20 W lamps ($\lambda = 300$–400 nm) 3: 160 W black light ($\lambda = 365$ nm), $[TiO_2] = 0.5$ g L^{-1}	Close to 50% of its initial concentration was eliminated after 10, 20, and 120 min when the irradiation source used was a UV lamp, a solarium device, and a UVA lamp, respectively. Significant mineralization was also obtained when the UV lamp and solarium were used	[81]
Fluoxetine, paroxetine, diclofenac, clotrimazole, azithromycin, lorazepam, propranolol, furosemide, hydrochlorothiazide, carbamazepine, bisoprolol, fenofibrate, ofloxacin, losartan, ketoprofen, norfloxacin, carvedilol, fluconazole, ciprofloxacin, gemfibrozil, alprazolam, terbinafine	12 ng L^{-1} to 24 µg L^{-1}	Wastewater	Pilot	Direct natural sunlight, $[TiO_2] = 200$ mg L^{-1}	All the pharmaceutical compounds were completely removed except ciprofloxacin (35%), ketoprofen (61%), and bisoprolol (77%) with a total accumulated UV energy of approximately 32 kJ L^{-1}	[82]

na, not available; DOC, dissolved organic carbon; TOC, total organic carbon; NOM, natural organic matter. TiO_2 refers to Aeroxide® TiO_2 P25 (anatase–rutile 75:25, particle size 21 nm and 50 m^2 g^{-1} BET area).

[a] *Dipyrone is readily hydrolyzed to 4-methylaminoantipyrine after dissolving in water for a few minutes.*

TABLE 3 Examples Taken from the Recent Literature on the Non-photochemical Advanced Oxidation Treatment of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices

Non-photochemical Advanced Oxidation Technologies						
Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
1. Ozonation						
Diclofenac, sulfamethoxazole, caffeine	10 mg L^{-1}	Wastewater	Bench	Inlet ozone concentration = 10 mg L^{-1}, gas flow rate = 30 L h^{-1}, UV-A lamp 700 W, [TiO_2] = 1.5 g L^{-1}	The pharmaceuticals studied were rapidly removed by ozone processes within 5–10 min for diclofenac and sulfamethoxazole and 10–15 min for caffeine. The fastest process was photocatalytic ozonation. O_3, O_3/TiO_2, and O_3/UVA systems led to between 20% and 40% mineralization in 2 h	[83]
Acetaminophen, metoprolol, caffeine, antipyrine, sulfamethoxazole, ketorolac, atrazine, hydroxybiphenyl, diclofenac, flumequine	10 mg L^{-1}	Distilled water Wastewater	Bench	Inlet ozone concentration = 10–40 mg L^{-1}	Ozone dosage exerted a positive effect on TOC and COD removal. This statement is not applicable to individual contaminants. An optimum ozone concentration can be found with no further improvement of the rate of depletion of the organics as ozone inlet concentration is increased	[84]

Tetracycline	1.13–2.08 mM	Distilled water	Bench	Inlet ozone concentration = 0.53–2.08 mmol L^{-1}	The direct ozonation of tetracycline was the dominant process. A 35% COD removal obtained after 90 min ozonation, indicating that tetracycline could not be mineralized completely by ozone	[85]
Triclocarban	100 mg L^{-1}	Distilled water	Bench	Inlet ozone gas concentration = 10–60 mg L^{-1}, gas flow rate = 0.4 L min^{-1}	The degradation rate increased by about 16 times at pH 7 as compared to that at pH 2. The results showed that the oxidation rates of tetracycline with ozone increased significantly by increasing pH, temperature, and ozone gas concentration	[86]
Iopamidol diatrizoate, iopromide, iomeprol, 17β-estradiol , 17α-ethinylestradiol, ibuprofen, bezafibrate, naproxen gemfibrozil, clofibric acid, indomethacin, sulfadiazine, sulfathiazole sulfapyridine sulfamethoxazole, roxithromycin, clarithromycin	0.5–5.0 μg L^{-1}	Wastewater	Pilot	Ozone dose = 0–5 mg L^{-1}, gas flow rate = 200 L h^{-1}	Macrolide and sulfonamide antibiotics, estrogens, and the acidic pharmaceuticals diclofenac, naproxen, and indomethacin were oxidized by more than 90–99% for O_3 doses 2 mg L^{-1}. In all effluents, X-ray contrast media and a few acidic pharmaceuticals were only partly oxidized, but no significant differences were observed among the three effluents	[87]

Continued

TABLE 3 Examples Taken from the Recent Literature on the Non-photochemical Advanced Oxidation Treatment of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices—Cont'd

Non-photochemical Advanced Oxidation Technologies

Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
Sulfamethoxazole	0.1–60 mg L^{-1}	Distilled water Wastewater	Batch	Ozone production = 3.3 g h^{-1}	The complete antibiotic removal was achieved for an ozone dose over 15 mg L^{-1}. Wastewater matrix had no significant effect on the amount of ozone required to remove sulfamethoxazole	[88]
Carbamazepine	2–20 mg L^{-1}	Distilled water	Batch	Ozone concentration = 0.7–2.5 mg L^{-1}, gas flow = 4.5–44.8 L h^{-1}	Complete carbamazepine (6 mg L^{-1}) reduction in less than 5 min was observed. Optimum combination of the three studied variables 55 L h^{-1}, 0.4 mg L^{-1} and 18 mg L^{-1} for ozone flow, ozone concentration and carbamazepine concentration, respectively	[89]
Oxytetracycline	100 mg L^{-1}	Distilled water	Batch	Inlet ozone concentration = 11 mg L^{-1}, gas flow rate = 20 L h^{-1}	Oxytetracycline was degraded completely in 20 min. At pH 3, the decomposition of oxytetracycline was slower than under neutral and basic conditions	[90]

Ampicillin	200 mg L^{-1}	Distilled water	Batch	Ozone dose = 10 mg L^{-1} min^{-1}, gas flow rate = 0.5 L min^{-1}	COD removal was about 58%, 68%, and 74% for pH 5, 7.2, and 9, respectively. No significant differences in the depletion of TOC at the given pH levels. Only 35–42% of TOC was mineralized after 90 min of ozonation (250–280 mg L^{-1} of consumed ozone dose)	[91]
Sulfamethoxazole	200 mg L^{-1}	Distilled water	Bench	Ozone production = 2.04 g h^{-1}	Ozone dosage of 0.4 g L^{-1} (15 min of reaction) was enough to achieve almost complete sulfamethoxazole abatement (up to 98.6%). At the end of the ozonation time (60 min), only 18% of TOC was removed	[92]
17α-Ethinyl estradiol	100–200 μg L^{-1}	Synthetic wastewater	Bench	Ozone stock solution = 2–10 mg L^{-1}	17α-Ethinyl estradiol was shown to be effectively degraded by ozonation in the conditions of low pH (6), NOM (10 mg L^{-1}), carbonate (50 mg L^{-1}), but high suspended solids (20 mg L^{-1}) and initial ozone concentration (9 mg L^{-1})	[93]
Ranitidine	10–52 mg L^{-1}	Distilled water	Bench	Inlet ozone concentration = 5–35 mg L^{-1}, gas flow rate = 40 L h^{-1}	10 mg L^{-1} of inlet ozone (gas flow rate 40 L h^{-1}) was sufficient to completely eliminate approximately 33 mg L^{-1} of ranitidine in 10 min. Only alkaline conditions (pH 11) were capable of increasing TOC conversion up to values close to 70%	[94]

Continued

TABLE 3 Examples Taken from the Recent Literature on the Non-photochemical Advanced Oxidation Treatment of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices—Cont'd

Non-photochemical Advanced Oxidation Technologies

Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
17α-Estradiol, 17β-estradiol, 17α-dihydroequilin, 17α-ethinyl estradiol, estriol, estrone, equilin	100 μg L^{-1}	Distilled water Tap water	Bench	Ozone weight percent = 3–13 wt%	The reaction was very fast for all the studied steroid hormones with a half-life ($t_{1/2}$) of approximately 30 s in the deionized water solution. The successful removal (>99%) of all steroids in mixture in 1 min required higher concentrations of ozone (5 mg L^{-1})	[95]
Iomeprol	1 μg L^{-1} to 10 mg L^{-1}	Distilled water, wastewater	Bench	Ozone dose = 3 mg L^{-1}	70% degradation for 1 μg L^{-1} iopromide and 3 mg L^{-1} ozone dose in 10 min. No significant mineralization of iomeprol can be achieved at pH 7 and 9, whereas at pH 12 approximately 40% of the initial iomeprol concentration was mineralized after 20 min of contact with an ozone dose at 3 mg L^{-1}	[96]
Ciprofloxacin	0.1–10 mg L^{-1}	Wastewater	Bench	0–5 mg L^{-1} ozone solution	Ciprofloxacin was degraded readily and removed almost completely when the ozone concentration was increased to 2.0 mg L^{-1} (or within 4 min of treatment)	[97]

Carbamazepine, fluoxetine, diclofenac, inuprofen, naproxen, gemfibrozil, atorvastatin	na	Drinking water	Pilot	Ozone dose = 2–2.3 mg L^{-1}, $[H_2O_2]$ = 0.2 mg L^{-1}	Almost complete degradation for inuprofen, naproxen, gemfibrozil, diclofenac, atorvastatin, and carbamazepine; 40% and 60% removal for ibuprofen and fluoxetine, respectively	[98]
Paracetamol	157–1000 mg L^{-1}	Distilled water	Batch	Ozone production = 1.0 g O_3 h^{-1}	Complete degradation of 156 mg L^{-1} paracetamol in less than 6 min. Ozonation yielded a slow and progressive decontamination up to reach 39% of TOC removal at 4 h. For the O_3/UVA system, pollutants were more rapidly degraded and TOC was finally reduced by 96%	[99]
Tylosin, sulfamethoxazole, *N*(4)-acetyl-sulfamethoxazole, trimethoprim, ciprofloxacin, enrofloxacin, penicillin, cephalexin, tetracycline, amikacin	1 μM	Wastewater	Batch	Ozone dose = 0–5 mg L^{-1}	Ozone dose equal to 3 mg L^{-1} resulted in complete degradation of all pharmaceuticals. Most substrates reacted predominantly with ozone than with hydroxyl radicals	[100]
Bezafibrate	0.2–0.5 mM	Distilled water	Batch	Inlet ozone concentration = 1 mM, gas flow rate = 0.38 L min^{-1}	Complete abatement of 0.5 mM bezafibrate was achieved, after 10 min of treatment (ozone dose = 0.73 mM). TOC removal was <40% in 120 min	[101]
Acebutolol, atenolol, metoprolol, sotalol, carbamazepine,	na	Surface river water	Pilot	Ozone dose = 1–1.3 mg L^{-1},	The applied ozone doses (1–1.3 mg L^{-1}) were sufficiently high to result in an elimination of	[102]

Continued

TABLE 3 Examples Taken from the Recent Literature on the Non-photochemical Advanced Oxidation Treatment of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices—Cont'd

Non-photochemical Advanced Oxidation Technologies

Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
bezafibrate, diclofenac, ibuprofen, ketoprofen, naproxen, ciprofloxacin, norfloxacin, ofloxacin				gas flow rate = 0.1 $m^3\ h^{-1}$	most of the studied compounds. Ciprofloxacin was the most recalcitrant compound to ozonation as its concentration was reduced by an average of 16% only. Ibuprofen, naproxen, bezafibrate, and sotalol were occasionally detected in the ozonated water	
2. Ultrasonic						
Carbamazepine	42 μM	Distilled water Tap water	Bench	40 kHz, 240 W	Complete destruction of carbamazepine at pH 3 after less than 30 min of reaction. Almost 90% carbamazepine disappearance was reached at pH 5 after 1 h of reaction with 100 μL H_2O_2 additives and 200 mg Fe^{2+} load. Higher carbamazepine degradation rate for double distilled water (82%), a less pronounced rate with deionized water (60%) and lower rate for tap water (24%)	[103]

Diclofenac	30–420 μM	Deionized water	Bench	20, 577, 861, 1145 kHz, 0.20 W mL^{-1}	Complete elimination of diclofenac with 22% and 9% reduction in COD and TOC after 60 min. The reactions were most rapid at non-buffered pH 3.0, the catalyst (zero valent iron nanoparticles) was more effective at high frequency irradiation, and the rate of degradation was negligible at pH 10	[104]
	15–130 μM	Demineralized water Reconstructed standard freshwater	Bench	577, 861, and 1145 kHz, 108 W	Mineralization after 90 min sonication of diclofenac in the presence of 8.9 mM zero valent iron, 0.01 mM divalent iron and 0.001 mM iron superoxide nanoparticles were 22%, 43%, and 30%. The reaction was not affected by pH within the acidic–neutral range, but decelerated at the alkali level. 861 kHz was the optimum frequency for the degradation of diclofenac	[105]
Carbamazepine	1–50 μg L^{-1}	Distilled water	Bench	Hydrodynamic-acoustic cavitation, 24 kHz, 200 W	Carbamazepine was transformed by pseudo first-order kinetics to an extent of >96% within 15 min (27% by hydrodynamic cavitation, 33% by acoustic cavitation). A synergistic effect of 63% based on the sum of the single methods was calculated. With increasing temperature, the conversion of carbamazepine increased up to 90% at 25 °C. Further increase led to a slight decrease of carbamazepine conversion	[106]

Continued

TABLE 3 Examples Taken from the Recent Literature on the Non-photochemical Advanced Oxidation Treatment of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices—Cont'd

Non-photochemical Advanced Oxidation Technologies

Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
Ciprofloxacin	15 mg L^{-1}	Deionized water	Bench	520 kHz, 92 W L^{-1}	Degradation at pH 3 was almost four times faster than at pH 7. The degradation constant at pH 10 was also significantly higher than at pH 7. BOD/COD ratio increases from 0.06 to 0.60, 0.17 and 0.18 after 120 min of treatment at pH 3, 7, and 10, respectively. The antibiotic activity against *Escherichia coli* (G^-) and *Bacillus coagulans* (G^+) of the treated solutions also reduced after sonolysis	[107]
Levofloxacin	20–80 mg L^{-1}	Distilled water	Bench	20 kHz, 200 W	The decomposition rate of levofloxacin enhanced about seven times by the addition of 0.02 mL of CCl_4, where the decomposition of levofloxacin in a 50 mL solution was finished within 35 min ultrasound irradiation. The BOD/COD ratio increases from 0 to 0.41 after 35 min of treatment	[108]

Ciprofloxacin	0.15–50 mg L^{-1}	Distilled water	Bench	544, 801, and 1081 kHz, 200 W	544 kHz, was the most favorable frequency for ciprofloxacin degradation in comparison with 801 and 1081 kHz. The degradation constant increased significantly with increasing temperature from 0.0055 min^{-1} at 15 °C to 0.0105 min^{-1} at 45 °C	[109]
Levodopa, paracetamol	25–150 mg L^{-1}	Distilled water	Bench	574, 860, and 1134 kHz, 99–281 W	95% and 91% degradation after 4 h of ultrasonic irradiation for levodopa and paracetamol, respectively. The best results were obtained with 574 kHz frequency. Addition of H_2O_2 had a positive effect on degradation rate, but the optimum concentration of hydrogen peroxide was found to depend on the pollutant	[110]
Ibuprofen	2–21 mg L^{-1}	Distilled water	Bench	300 kHz, 20–80 W	98% degradation of ibuprofen was achieved in 30 min. The conversion of ibuprofen follows the order: pH 3 > pH 5 > pH 11. Under air and oxygen, the degradation rate of ibuprofen was higher than when argon was used. The initial BOD/COD ratio increased from 0 to 0.36 after 120 min of treatment	[78]

Continued

TABLE 3 Examples Taken from the Recent Literature on the Non-photochemical Advanced Oxidation Treatment of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices—Cont'd

Non-photochemical Advanced Oxidation Technologies						
Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
17α-Ethinyl estradiol	25–110 μg L^{-1}	Wastewater	Bench	80 kHz, 150 W	Degradation in the range 25–110 μg L^{-1} follows first-order kinetics. The reaction rate increased linearly with applied power and decreased exponentially with temperature. The addition of H_2O_2 (8.6 and 86 mg L^{-1}), Fe^{2+} (2.5–25 mg L^{-1}) or TiO_2 (50–2000 mg L^{-1}) had no or, in some cases, adverse effect on kinetics. Continuous sparging of air or oxygen had a little effect on the kinetics relative to air-equilibrated conditions, while helium had a marginally positive effect. The intrinsic matrix of the wastewater appeared to promote degradation	[111]
Pharmaceutical wastewater	10.330 mg L^{-1}	Industrial wastewater	Bench	30 kHz	Sonolysis yielded a 41% COD reduction after 60 min. A maximum COD reduction (83%) was observed with the addition of CCl_4 (100 mg L^{-1}) and activated carbon (2 g L^{-1}). The COD reduction was strongly influenced by the initial pH and a better reduction was observed at pH 6. The sonochemical degradation of pharmaceutical wastewater was found to follow the Langmuir–Hinshelwood kinetics	[112]

Ofloxacin	5–20 mg L^{-1}	Distilled water	Bench	20 kHz, 130–640 W L^{-1}	Final conversion increases with increasing power density but remains unchanged at higher densities. Increasing initial substrate concentration results in reduced conversion. The degradation was found to be enhanced under an argon atmosphere	[113]
Diclofenac	2.5–80 mg L^{-1}	Distilled water	Bench	20 kHz, 25–100 W L^{-1}	Diclofenac conversion was enhanced at increased applied power densities and liquid bulk temperatures, acidic conditions and in the presence of dissolved air or oxygen. The reaction rate increased with increasing diclofenac concentration in the range 2.5–5 mg L^{-1} but it remained constant in the range 40–80 mg L^{-1}. H_2O_2 production rates in pure water were higher than those in diclofenac solutions. Toxicity to *D. magna* increased during the early stages of the reaction and then decreased progressively upon degradation of reaction by-products. Complete toxicity elimination was not achieved	[114]

Continued

TABLE 3 Examples Taken from the Recent Literature on the Non-photochemical Advanced Oxidation Treatment of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices—Cont'd

Non-photochemical Advanced Oxidation Technologies						
Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
Triclosan	5 μg L^{-1}	Deionized water Seawater Wastewater	Bench	80 kHz, 135 W	Triclosan degradation followed pseudo first-order kinetics with the rate constant being (min^{-1}): 0.2284 for seawater >0.1051 for 3.5% NaCl in deionized water >0.0597 for centrifuged urban runoff ~0.0523 for untreated urban runoff >0.0272 for deionized water >0.0063 for wastewater influent. Degradation was not accompanied by the formation of toxic metabolites	[115]
17α-Ethinyl estradiol	10 μg L^{-1}	Distilled water	Bench	20 kHz, 640 W L^{-1}	The degradation of estrogens followed a pseudo first-order rate kinetics, and the order of degradation was 17α dihydroequilin > equilin > 17α-ethinyl estradiol > 17α-estradiol > 17β-estradiol > estrone > estriol. The presence of salinity (0.17 M) enhanced the estrogen degradation except for the equilin compounds. At alkalinity concentration of 10 mM, no adverse effect on the degradation was observed but significant inhibitory effects at high alkalinity concentration of 120 mM were observed	[116]

3. (Catalytic) Wet air oxidation						
Paracetamol	1000 mg L^{-1}	Distilled water	Bench	Temperature = 150 °C, pressure = 20 bar, activated carbon	Paracetamol and COD were reduced by 98% and 62% in 2 h, respectively. T = 150 °C, C_0 = 1000 mg L^{-1}, 1 g L^{-1} activated carbon and P_{Oxygen} = 3.2 bar	[117]
Enrofloxacin	0.2 mM	Distilled water	Bench	Temperature = 150 °C, pressure = 0.5 MPa	99.5% degradation, 37% COD removal, and 51% TOC conversion obtained when 100 mol% $FeCl_3$ and 25 mol% $NaNO_2$ at 150 °C under 0.5 MPa oxygen pressure after 120 min. The BOD/COD increased from 0.01 to 0.12 after 120 min of reaction time. The inhibition of bioluminescence of the marine bacteria *V. fischeri* decreased from 43% to 12%	[118]
Fosfomycin pharmaceutical wastewater	60,000–80,000 mg L^{-1}	pharmaceutical wastewater	Bench	Temperature = 125–250 °C, pressure = 1–6 MPa	When the temperature increased from 125 to 200 °C, the COD removal increased from 10% to 58%. At the temperatures of 200 and 225 °C, the COD removal results were comparable. When the temperature increased from 225 to 250 °C, the COD removal efficiency was promoted from 58% to 80%. COD removal increased from 57% to 67% with the increase of oxygen partial pressure from 1.0 to 4.0 MPa after 180 min. As the	[119]

Continued

TABLE 3 Examples Taken from the Recent Literature on the Non-photochemical Advanced Oxidation Treatment of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices—Cont'd

Non-photochemical Advanced Oxidation Technologies						
Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
					initial pH value increased from 7.0 to 11.2, the COD removal was significantly enhanced from 44% to 57% in 180 min	
Amoxicillin, naproxen, phenacetin	100 mg L^{-1}	Distilled water Reservoir water groundwater wastewater	Bench	Temperature = 120–140 °C, pressure = 20–40 bar, Pt/activated carbon catalyst, synthesized Pt/carbon nanotubes catalyst	For a synthesized catalyst dosage of 0.025 g Pt/carbon nanotubes, oxygen pressure of 20 bar, and temperature 140 °C, final removals of naproxen, amoxicillin, and phenacetin after 30 min of reaction were 96.8%, 98.3%, and 24%, respectively. Varying the stirring speed from 500 to 1000 rpm was found to have no effect on the initial reaction rate	[120]
4. Electrochemical oxidation						
Ketoprofen	5 μM	Distilled water 0.1 M Na_2SO_4	Bench	Si–BDD electrode, 11.25 cm^2, 4.4–13.3 mA cm^{-2}	The influence of pH on mineralization was very marginal. DOC was completely removed in 8 h (13.3 mA cm^{-2})	[121]

Chlortetracycline	10 mg L^{-1}	Distilled water 100 mg L^{-1} NaCl, 100 mg L^{-1} Na_2SO_4	Bench	Ti/IrO_2 and Ti/PbO_2, 65 cm^2, 2 A	Current intensity and treatment time were the most influent parameters on the electrochemical oxidation of chlortetracycline. Chlortetracycline was almost completed removed in 49 min of treatment and 2 A at Ti/PbO_2 electrode	[122]
Iohexol	3.65 mM	Distilled water 0.05 M $NaClO_4$	Bench	BDD—DiaCell PS, 33–66 mA cm^{-2}	COD removal >90% in 210 min (66 mA cm^{-2}). The amount of transformation products was lower when working at the limiting current of iohexol oxidation	[123]
Diclofenac	175 mg L^{-1}	Distilled water 0.05 M Na_2SO_4/ 0.05 M KH_2PO_4 + 0.05 M Na_2SO_4 + NaOH (pH 6.5)	Bench	Pt and BDD, 3 cm^2, 50–450 mA	TOC was only reduced by 46% after 6 h of treatment with Pt electrode at neutral solution. BDD allowed almost overall mineralization of the drug solution (>97% TOC removal) at the same time (300 mA, pH 6.5)	[124]
Progesterone	0.1–100 mg L^{-1}	Distilled water 0.035 mol L^{-1} Na_2SO_4 or NaCl	Bench	p-Si–BDD, 78 cm^2, 15–100 mA cm^{-2}	100% removal of 10 mg L^{-1} progesterone at 120 min (15 mA cm^{-2}). Kinetic constant decreases with the initial concentration of progesterone. Increase in current density led to less efficient processes, indicating mass transfer control of the process rate	[125]

Continued

TABLE 3 Examples Taken from the Recent Literature on the Non-photochemical Advanced Oxidation Treatment of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices—Cont'd

Non-photochemical Advanced Oxidation Technologies						
Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
Sulfamethoxazole	0.16–1.0 mM	Distilled water 0.025 M Na_2SO_4	Bench	BDD, two compartments, 13–107 mA cm^{-2}	For the electrolysis of 1.0 mM sulfamethoxazole at 10 mA current after 210 min, the loss of TOC was 32% when the loss of sulfamethoxazole was 73%. Electrolyses at a constant current of 10 mA but different starting concentrations gave mixed zero- and first-order behavior	[126]
Trimethoprim	1.72×10^{-4} M	Distilled water 0.1 M Na_2SO_4	Bench	Si–BDD electrode, 11.25 cm^2, 4.4–13.3 mA cm^{-2}	Under the optimal conditions (i.e., pH 3, solution flow rate = 1.25 cm^3 min^{-1}, current density = 207 mA cm^{-2} and supporting electrolyte = 0.493 mol L^{-1}). Trimethoprim was completely removed, while COD and TOC removals were 20.1% and 50.9%, respectively	[127]
Paracetamol	78–948 mg L^{-1}	Distilled water 0.05 M Na_2SO_4	Bench	Si–BDD 3 cm^2, 100–450 mA	Almost complete mineralization (98%) of 157 mg L^{-1} paracetamol after 4 h of treatment at 450 mA and 35 °C. The TOC removal was found to be pH-independent	[128]

Ibuprofen	0.05–0.2 mM	Distilled water 0.05 M Na_2SO_4 or NaCl	Bench	Pt, BDD 25 cm^2, 50–500 mA	Complete destruction of 0.2 mM ibuprofen at 500 mA in about 90 and 180 min for BDD and Pt, respectively. Ibuprofen removal increased when NaCl was used instead of Na_2SO_4 due to the electrogeneration of active chlorine. Almost complete mineralization (>96%) of ibuprofen was obtained with BDD in 480 min of electrolysis	[129]
Atenolol, metoprolol, propranolol	0.15–0.3 mM	Distilled water 0.05 M Na_2SO_4	Bench	Pt, BDD 4.5, 25 cm^2, 30–300 mA	BDD anodic oxidation led to almost complete mineralization (25 mg L^{-1} TOC) in 300 min at 300 mA and pH 3. With Pt/carbon felt cell electro-Fenton process led to complete degradation of 0.15 mM atenolol in <15 min at 60 mA and 0.2 mM Fe^{2+} at pH 3. Electro-Fenton reactivity was found to increase in the order: atenolol < metoprolol < propranolol	[130]
Atenolol	2.25 μM	Distilled water 0.1 M Na_2SO_4	Bench	Pt, BDD 11.25 cm^2, 4.4–13.3 mA cm^{-2}	Maximum removal of TOC was achieved using BDD in the presence of Na_2SO_4. About 96 and 84.6% of TOC removals were achieved using Na_2SO as electrolyte with BDD and Pt anodes, respectively	[131]

Continued

TABLE 3 Examples Taken from the Recent Literature on the Non-photochemical Advanced Oxidation Treatment of the Most Commonly Detected Pharmaceuticals in Various Aqueous Matrices—Cont'd

Non-photochemical Advanced Oxidation Technologies

Pharmaceutical	*Initial Concentration*	*Water Matrix*	*Scale*	*Treatment Process Information*	*Main Findings*	*References*
Acetylsalicylic acid	100–900 mg L^{-1}	Distilled water 0.05–0.5 M Na_2SO_4	Bench	PbO_2, 10–90 mA cm^{-2}	The removal of acetylsalicylic acid and COD attained 93% and 65%, respectively, under the initial concentration of 100 mg L^{-1} after 150 min (current density 50 mA cm^{-2}). The removal rate of different hydrogen peroxide concentration was 68%, 75%, 86%, 89%, 94%, and 92% with 0 mg/L H_2O_2, 100 mg L^{-1} H_2O_2, 200 mg L^{-1} H_2O_2, 500 mg $L^{-1}H_2O_2$, 1000 mg $L^{-1}H_2O_2$, and 1200 mg L^{-1} H_2O_2, respectively (C_0 = 500 mg L^{-1}, current density 50 mA cm^{-2})	[132]

Salicylic acid	164 mg L^{-1}	Distilled water 0.05 M Na_2SO_4	Bench	Pt, BDD 3 cm^2, 33–150 mA cm^{-2}. Graphite or O_2-diffusion cathode	14% and 81% TOC removal in 180 min with 150 mA cm^{-2} for Pt and BDD electrode, respectively. The oxidation power of EAOPs for a given anode increases in the order: anodic oxidation < anodic oxidation—H_2O_2 < electro-Fenton < photoelectro-Fenton < solar photoelectro-Fenton	[133]
17α-Ethinyl estradiol	100–800 μg L^{-1}	Wastewater 0.1 M NaCl	Bench	BDD, 19 cm^2, 0.9–2.6 mA cm^{-2}	Conversion increases with increasing current density. Complete removal can be achieved within 5–7 min at 2.6–2.1 mA cm^{-2}. 85% COD removal occurred after 30 min implying high levels of mineralization	[134]

na, not available; TOC, total organic carbon; BOD, biochemical oxygen demand; COD, chemical oxygen demand; NOM, natural organic matter; BDD, boron-doped diamond.

2.1 Photochemical Advanced Oxidation Technologies

2.1.1 Photolysis

Irradiation with either artificial light source (usually performed with low- or medium-pressure mercury-vapor lamps) or natural sunlight is a potential means to limit the release of pharmaceuticals via wastewater effluents into the aquatic environment. Laboratory studies using sunlight and lamps with varying characteristics have shown that several pharmaceuticals are sensitive to photodegradation in different water matrices [24,137]. Photolysis can be evolved through a direct or indirect mechanism. While direct photolysis of chemical species is caused by direct absorption of solar light (which leads to the promotion of a molecule from the fundamental state to an excited singlet state), the indirect photolysis occurs via light absorption by photosensitizers such as dissolved organic matter (DOM). During the indirect mechanism, strong reactive agents, for example, singlet oxygen ($^1O_2$), hydroxyl radicals ($HO^\bullet$), or alkyl peroxyl radicals ($^\bullet OOR$), are generated *in situ* that can significantly enhance the oxidation in the chemical system [8,137].

The degradation of a compound under irradiation conditions is affected by the UV energy absorption and the quantum yield of the specific compound [4]. UV energy absorption is expressed as molar extinction coefficient, which is a measure of how strongly a chemical species absorbs light at a given wavelength that can be used for its degradation [138,139]. In addition, the inorganic and organic contents present in the water matrix, UV type and dose, and contact time are considered as important factors governing the removal efficiency of pharmaceuticals during photolysis. High concentrations of DOC and other inorganic substances (i.e., carbonates/bicarbonates and chlorides) can render mineralization of pharmaceuticals quite inefficient [35]. UVC irradiation, which is widely used for disinfection purposes, has been shown to be more efficient in degrading pharmaceuticals compared to UVA [32]. Regarding the UV dose, limited information is provided in the scientific literature, while in some cases, the heterogeneity on the data does not allow comparison among the various studies conducted. Typical UV doses applied for the efficient removal of pharmaceuticals range between 1 and 10 J cm^{-3}, whereas the treatment time is strongly dependent on the water–matrix composition.

Photolysis is not efficient in treating pharmaceuticals in matrices containing high amounts of solids in suspension, because the quantum efficiency decreases through loss of light, dispersion, and/or by competitive light absorption. It is worthwhile to point out that under the aforementioned conditions, elimination of pharmaceuticals can be achieved at longer times of treatment; nonetheless, this is accompanied by an insignificant DOC removal. In addition, some pharmaceutical compounds (i.e., hydrophobic compounds) may also evade photochemical degradation through sorption to suspended particles. In general, photolytic treatment appears to be efficient when it is applied

to waters with low organic concentrations (e.g., surface and drinking waters) [30,34,140]. Generally, direct photolysis has proved to be less effective in degrading pharmaceuticals in wastewater effluents compared to other AOPs (photo-Fenton and TiO_2 photocatalysis) [9,49].

The efficiency of the photolytic process can be significantly enhanced when UV irradiation is combined with H_2O_2. The oxidizing power of H_2O_2 can be sensibly improved by $HO^{\bullet}$ generation through cleavage of the O—O union with photons of adequate energy (higher than 213 kJ mol^{-1}, the energy bond, which corresponds to wavelengths lower than 280 nm). It is important, however, that a low concentration of the oxidant is used during this application in order to reduce the treatment cost. During the application of UV/H_2O_2, the degradation rate depends on the oxidant concentration, increasing to an optimum value, beyond which an inhibitory effect takes place. At high $HO^{\bullet}$ concentration, competitive reactions occur because these radicals are prone to recombination, regenerating H_2O_2 [141].

2.1.2 Homogeneous Photocatalysis

In the recent years, photo-Fenton process has gained increasing attention due to its environmentally friendly application and the prospect of operating under solar irradiation, hence lowering the operation cost considerably [9,57]. It remains one of the most applied AOPs for its ability to degrade high loading of organic compounds including pharmaceuticals in water matrices of increased complexity [142,143]. A vast number of literature [142,144–146] have provided a comprehensive review of the basic understanding and clarity of the principles underlying the photo-Fenton reaction. However, the mechanisms and the key intermediates in the Fenton chemistry are still under intense and controversial discussion.

The capacity of the photo-Fenton system to degrade a great variety of pharmaceuticals in water matrices is affected by several operating parameters, such as hydrogen peroxide and iron concentrations, iron type (ferrous or ferric iron), pH, light intensity, temperature, and solution salinity. The hydrogen peroxide use with respect to the theoretically needed stoichiometric amount of the oxidant is highly dependent on the substrate concentration [147]. With increasing H_2O_2 concentration, the reaction rates increase due to the additionally produced $HO^{\bullet}$. This increase of the reaction rate continues up to a level that corresponds to the optimum peroxide concentration. On the other hand, the use of excessive oxidant concentration leads to an adverse effect on the substrate degradation. Additionally, the increase of iron concentration causes an increase in the reaction rate. It has been demonstrated that low iron concentrations (i.e., <20 mg L^{-1}) are high enough to degrade several pharmaceuticals dissolved in water or wastewater [148]. In this respect, the final separation of soluble iron species from the treated water in order to comply with the regulatory limits for effluent discharge is not necessary. Optimization of the catalyst and oxidant

ratio renders the process suitable to treat complex water matrices or effluents from pharmaceutical manufacturing. According to the available literature, the use of ferrous or ferric ions as the catalyst source in Fenton reactions is not critical, as results are comparable for both in terms of degradation of specific pharmaceuticals and mineralization. Ferrous ions are slightly more active than ferric, which can be attributed to the Fe^{2+}/Fe^{3+} redox cycle inducing fast formation of reactive radicals [149]. However, some studies have demonstrated that the iron source can influence considerably the degradation of different pharmaceuticals [42,43].

The temperature significantly increases the activity of the photo-Fenton system and has a beneficial effect on the degradation rate [150]. In the photo-Fenton reaction, the formation of the highly photoactive iron complexes is highly dependent on the water pH. It is well known that the pH 2.8 is the frequent optimum pH for photo-Fenton process since iron precipitates at higher pH [151] that constitutes the main disadvantage of the process. Such a low optimum pH 2.8 is not cost-effective for operation as it requires high chemical cost for pH rectification [152]. A new approach aimed at performing photo-Fenton treatment at neutral pH has been proposed by De la Cruz et al. [41], Klamerth et al. [48], and Moncayo-Lasso et al. [153]. The efficiency of the modified photo-Fenton system is based on the reaction of DOM present in wastewaters with Fe^{2+} leading to the formation of soluble iron complexes. These complexes have the advantage of being soluble in the wastewater and so preventing the Fe^{3+} precipitation at neutral pH conditions. Another remarkable point is that they have typically higher molar absorption coefficients in the near-UV and visible regions than the aquo-Fe^{3+} complexes do [145]. However, pharmaceutical degradation and mineralization during photo-Fenton tend to be slower at neutral pH than at the optimum pH value.

The occurrence of inorganic anions (i.e., Cl^- and SO_4^{2-}) in the water matrix influences the degradation of pharmaceuticals during the photo-Fenton treatment since they compete with organic contaminants for hydroxyl radical reactions; however, the process efficiency is still sufficient even if salt concentrations are high [143]. De Laat et al. [154] presented a rather comprehensive review of the additional reactions in the Fenton system in the presence of significant amounts of chlorides and sulfates. In another study by Klamerth et al. [62], the negative effect of carbonate species (CO_3^{2-} and HCO_3^-) on the degradation efficiency of various pharmaceuticals was confirmed.

The pilot-scale application of the photo-Fenton through the technology of concentrating parabolic collectors has been increasingly used for the treatment of pharmaceuticals in various environmental matrices. In this respect, natural solar light can be exploited that dramatically lowers the costs of the process and, thus, provides a major step toward industrial application. The results obtained from the pilot-scale applications are quite satisfactory regarding the pharmaceutical removal; however, in most of the studies, the authors failed to include economic data [23].

2.1.3 Heterogeneous Photocatalysis (TiO_2)

In a heterogeneous photocatalytic system, photo-induced molecular transformations or reactions take place at the surface of a catalyst, normally a wide-band semiconductor [155]. Photocatalytic reactions on semiconductor powders have attracted much attention because of their applicability to the treatment of a variety of pharmaceuticals and utilization of solar energy [9]. Among the various semiconductors that have been so far tested, titanium dioxide (TiO_2) has generally been demonstrated to be the most active. To date, the most widely applied photocatalyst in the research of water treatment is the Aeroxide® TiO_2 P25 due to specific features that possess related to its structure and photocatalytic activity.

Given the results of Table 2, it is obvious the efficiency of a heterogeneous photocatalytic system depends on a number of factors that govern the kinetics of photocatalysis such as the catalyst concentration and solution pH, the addition of oxidant, and initial substrate concentration. The degradation rate is generally found to increase with catalyst concentration toward a limiting value at high TiO_2 concentration. This limit depends on the reactor geometry and operating conditions. The total active surface area increases by increasing catalyst dosage up to a level that corresponds to the optimum of light absorption [156]. Furthermore, at high concentrations of the catalyst, agglomeration (particle–particle interactions) can also take place resulting to the loss of surface area available for light absorption [157].

The pH of the water matrix to which pharmaceuticals are spiked in significantly affects the efficiency of the process, since it dictates the charge of the catalyst particles and consequently the adsorption of the substrates onto the catalyst surface. Of course, the effect of pH on the process efficiency strongly depends on the chemical structure of the specific pharmaceutical compound and its ionization constants. In many cases, the effect of the pH on the photocatalytic degradation of pharmaceuticals cannot be explained in terms of the ionization state of the catalyst and the substrate alone, and this may be due to the relative contribution of various complex reactions [49,66,75,76]. Heterogeneous photocatalytic reactions usually obey to Langmuir–Hinshelwood kinetic model, which is reduced to pseudo first- or zero-order kinetics depending on the operating conditions. Generally, the degradation rate constant is found to increase by decreasing the substrate concentration.

One practical problem in using TiO_2 as a photocatalyst is the electron–hole recombination that, in the absence of proper electron acceptors, is extremely efficient and thus represents a major energy-wasting step, limiting the achievement of a high quantum yield. The use of inorganic powerful oxidizing species such as H_2O_2 has been demonstrated to enhance the photodegradation rate of a variety of organic compounds using TiO_2 [9,74]. The oxidant accepts a photogenerated electron from the conduction band and thus promotes the charge separation and the production of $HO^{\bullet}$.

From an engineering point of view, the use of slurry TiO_2 system requires an additional process step to be entailed for postseparation of the catalyst. This separation process is crucial to avoid the loss of catalyst particles and introduction of the new pollutant of contamination of TiO_2 in the treated water [152]. The catalyst recovery can be achieved through membrane filtration [158]. Nevertheless, several important operating issues with slurry TiO_2 still remain even with a membrane integration process. These include the types of membrane, pore size, regeneration or backwashing, and fouling [152]. A new approach that has been developed with the aim to avoid the catalyst separation step has focused on an immobilized titanium dioxide deposit film. The main problems with regard to this process relate to the efficiency due to the limited mass transfer and/or technical effort leading to high operational cost.

2.2 Non-photochemical Advanced Oxidation Technologies

2.2.1 Sonochemistry

Ultrasound with frequencies in the range of 20–1000 kHz generates cavitation phenomena comprising the creation, expansion, and collapse of bubbles in extremely small intervals of time, a process that releases large quantities of energy over a tiny "hot-spot" location. In general, sonochemical degradation in aqueous phase involves several reaction pathways and zones such as pyrolysis inside the bubble and/or at the bubble–liquid interface and hydroxyl radical-driven reactions at the bubble–liquid interface and/or in the liquid bulk [159].

According to the results provided in Table 3, the sonochemical process depends on a number of parameters such as the power density, ultrasound frequency, pH, water matrix, temperature, addition of catalyst or promoters, and finally the initial concentration and the physicochemical properties of the pharmaceutical under investigation.

In general, most researchers agree that the reaction is not affected by pH within the acidic–neutral range but is strongly decelerated under alkaline conditions. At pH values greater than the pharmaceutical pK_a value, the more hydrophilic ionic state prevails and reactions are likely to occur in the liquid bulk, where there is a lower concentration of hydroxyl radicals considering the fact that only a small fraction of the latter can reach the liquid bulk [114]. Moreover, under alkaline conditions, the rate of hydrogen peroxide production is greater compared to that in acidic conditions. Consequently, the degradation rate decreases at alkaline pH due to the fact that a higher number of hydroxyl radicals recombine to hydrogen peroxide and do not interact with the pharmaceuticals' substrate [78].

Sonochemical degradation of various pharmaceuticals was found to obey a pseudo first-order reaction kinetics with kinetic constant values decreasing

when increasing the initial substrate concentration [105,110]. In addition, the use of inorganic promoters such as H_2O_2 has been demonstrated to enhance the sonodegradation rate of pharmaceuticals, as more radicals are generated until an optimum oxidant dose beyond excess peroxide can act as a scavenger, which can limit the system effectiveness [103,113].

An interesting observation is that when the ionic strength increases, a beneficial effect on degradation is observed. The enhancement in the degradation rates can be attributed to the salting out effect, where the solute is expected to migrate from the liquid bulk inside or near the cavitation bubble where degradation via pyrolysis and/or hydroxyl radical-induced reactions is likely to occur [115,116]. On the other hand, the presence of bicarbonates, a well-known hydroxyl radical scavenger, leads to conflicting results depending on their concentration [111,116].

2.2.2 Electrochemical Oxidation

The electrochemical degradation of micropollutants is a relatively new technology for the treatment of wastewater effluents. The main advantage of this technology is attributed to the fact that no chemical reagents are used throughout the process. In fact, only electrical energy is consumed for the decomposition of organic pollutants on high oxidation power anodes. An ideal anode for this type of treatment is the boron-doped diamond (BDD) electrode that is characterized by a high reactivity toward organic oxidation and efficient use of electrical energy [160]. Two mechanisms are considered responsible for organic matter electrochemical degradation, namely, (a) direct anodic oxidation where the pollutants are adsorbed on the anode surface and destroyed by the anodic electron transfer reaction and (b) indirect oxidation in the liquid bulk that is mediated by the oxidants that are formed electrochemically; such oxidants include chlorine, hypochlorite, hydroxyl radicals, ozone, and hydrogen peroxide [5,161].

According to Table 3, it is obvious that the efficiency of electrochemical oxidation depends on a number of parameters that govern the electrochemical process such as the anode material and surface, the current density, the concentration and nature of the electrolyte and solution pH, and finally the initial concentration and the physicochemical properties of a specific substrate.

The degradation rate is generally found to increase with the current density towards a limiting value. This can be related to the consequent generation of more hydroxyl radicals on the electrode surface. However, after a limiting value, the degradation rate is not increased proportionally as the current increases, indicating the progressive enhancement at high current of the parasitic reactions, mainly oxygen evolution [129]. The electrochemical oxidation is strongly pH-dependent. Even though there are many scientific reports on the influence of pH, the results are controversial due to the different organic structures and electrode materials that have been examined. In addition, the redox potential for most of the organic compounds is affected by the solution

pH. Usually, the oxidation potential in acidic medium is higher than that in alkaline medium. Therefore, the electrochemical degradation of most pollutants is influenced by the alteration of the initial pH value [162].

Skoumal et al. [99] have reported on the oxidation power of different anodes. In summary, high oxidation power anode such as BDD was found to demonstrate a greater ability to produce active radicals (i.e., loosely adsorbed BDD (OH)) compared to a low oxidation power anode such as Pt, IrO_2, or SnO_2 [121,124]. The occurrence of inorganic anions (i.e., Cl^- and SO_4^{2-}) and organic matter in the water matrix influences the degradation of pharmaceuticals during electrolysis. In general, there is a competition for the electrogenerated hydroxyl radicals and other reactive oxygen species between micropollutants and effluent organic matter, and this behavior is more pronounced during the early stages. Moreover and after a critical charge, the increased production of active chlorine due to the electrolysis of chlorides intrinsically present in the effluent is expected to enhance pharmaceutical conversion [134].

2.2.3 Ozonation

Ozone is industrially applied for wastewater and drinking water treatment either alone or in combination with hydrogen peroxide. Ozone is a strong oxidant that either decomposes in water to form hydroxyl radicals thus inducing the so-called indirect oxidation or attacks selectively certain functional groups of organic molecules through an electrophilic mechanism (ozonolysis) [5,92]. As shown in Table 3, the efficiency of the process depends on various parameters such as ozone dose, ozone flow rate, treatment time, pH, addition of hydrogen peroxide, water matrix, and the type and the concentration of pharmaceuticals.

By increasing the ozone dose, a much faster degradation of pharmaceuticals is achieved in both water and wastewater matrices. However, it is important to note that ozone dose as low as $5\ mg\ L^{-1}$ (typical value used for disinfection) can lead to very rapid and complete removal of a large number of pharmaceuticals [87,100,102].

The effect of pH is quite important during ozonation. The decomposition of ozone is reduced to acidic pH, which leads to increased efficiency in the process in case that the main pathway is ozonolysis, while if the substances do not react with molecular ozone and the main path is the reaction with hydroxyl radicals (e.g., the X-ray contrast), increasing the pH to 8–9 leads to increased efficiency [86,90].

In general, ozonation follows second-order kinetics (first-order with respect to both ozone, hydroxyl radicals, and substrate); however, often a pseudo steady-state condition for ozone and hydroxyl radicals can be assumed. Therefore, the process in most studies appears to follow pseudo first-order kinetics where the kinetic constant is reduced when pharmaceuticals are in high concentration.

The simultaneous addition of hydrogen peroxide (the process O_3/H_2O_2 is known as peroxone) also leads to an increase in process efficiency due to

the production of additional hydroxyl radicals to an optimal concentration of peroxide after which the hydrogen peroxide acts as a scavenger for hydroxyl radicals [98].

The effect of the aqueous matrix was found to be negligible for pharmaceuticals that react directly with ozone while it becomes more essential where the dominant path is the oxidation with hydroxyl radicals and the pharmaceutical substrate must compete with the remaining organic matter from the matrix and the inorganic ions for the nonselective radicals [83,88].

In recent years, many researchers have used various catalysts (mainly MnO_x/Al_2O_3) to enhance the production of oxidative species and thus the efficiency of the process [163]. Although the results in many cases are satisfactory, there are several technical issues (such as stability/reuse of the catalyst) to be solved in order to use the catalytic ozonation in large scale.

In general, ozonation seems to be a promising technology for the removal of pharmaceuticals. Nevertheless, the possibility of producing undesirable bromate ions in the case where the aqueous matrix contains bromide ions should not be omitted.

2.2.4 (Catalytic) Wet Air Oxidation

There are very few articles dealing with the degradation of pharmaceuticals by (catalytic) wet air oxidation. This is not surprising since wet air oxidation is a process requiring extreme conditions and thus increased costs compared to the mild conditions under which other AOPs are performed (i.e., photocatalysis). From this perspective, treatment of micropollutants by wet air oxidation is not an economically viable option as it would result in excessive specific energy consumption (i.e., energy per unit mass of pollutant destroyed) [164]. Nevertheless, there are some applications for the treatment of hospital/pharmaceutical wastewaters where the main aim is to increase the biodegradability of wastewater prior to conventional biological treatment [119,165,166].

In general, the efficiency of the process depends on the pressure, temperature, initial substrate concentration, and the physicochemical characteristics of the pharmaceutical under investigation. In many cases, when temperature increases, the efficiency of the process with regard to substrate degradation increases [120]. However, this increase continues up to a level that corresponds to the optimum value [119]. In addition, the use of catalysts (mainly activated carbon) can improve significantly the efficacy of the process that simultaneously can be used for the regeneration of the catalyst [117].

3 CONCLUSIONS

The large number of studies reviewed herein is indicative of the extensive and intense research that has been carried out in the field of AOPs for the removal of pharmaceuticals in various environmentally relevant matrices. Among the

various AOPs, homogenous and heterogeneous photocatalysis, ozonation, and photolysis under UV irradiation have been extensively used with success for the oxidation of many classes of pharmaceuticals. Different operational parameters were studied to select the optimum conditions for each process. The process efficiency mainly depends on the water matrix composition, reagent doses, pH, and the pharmaceutical molecular structure and its concentration. Nevertheless, it must be stated that total mineralization seldom is attained during the application of AOPs indicating the formation of persistent oxidation products that may exhibit toxic effects. Hence, toxicological tests to control the formation of these products along the process pathway are mandatory.

More pilot plant- and field-scale studies are required to demonstrate the removal efficiencies of AOPs that can be achieved under different water quality conditions and operational parameters and the limitations associated with their implementation. A balance between efficiency and cost is what should be mostly pursued. One important aspect usually not dealt with by the studies relates to the estimation of the cost of the processes (capital and operational). It is often stated that AOPs are expensive processes compared to the biological ones and this is one of their major drawbacks. However, this comparison does not do justice to the processes as these are able to remove recalcitrant compounds in a higher degree than the biological treatments. Therefore, great attention should be directed to the water industry and policymakers to reconsider the growing problem of the deterioration of water quality with respect to the presence of pharmaceutical compounds, the removal of which is difficult to achieve without the application of advanced technologies. Finally, a unified costing approach would enable a potential comparison of the various technologies for specific water quality requirements.

ACKNOWLEDGMENTS

This work was cofunded by the European Regional Development Fund and the Republic of Cyprus through the Research Promotion Foundation (NIREAS Strategic Infrastructure Project ΝΕΑΥΠΟΔΟΜΗ/ΣΤΡΑΤΗ/0308/09).

REFERENCES

[1] D. Fatta-Kassinos, S. Meric, A. Nikolaou, Anal. Bioanal. Chem. 399 (2011) 251–275.
[2] A. Nikolaou, S. Meric, D. Fatta, Anal. Bioanal. Chem. 387 (2007) 1225–1234.
[3] N. De la Cruz, R. Dantas, J. Giménez, S. Esplugas, Appl. Catal. B Environ. 130 (2013) 249–256.
[4] O. Legrini, E. Oliveros, A. Braun, Chem. Rev. 93 (1993) 671–698.
[5] M. Klavarioti, D. Mantzavinos, D. Kassinos, Environ. Int. 35 (2009) 402–417.
[6] R. Andreozzi, V. Caprio, A. Insola, R. Marotta, Catal. Today 53 (1999) 51–59.
[7] R. Andreozzi, M. Raffaele, P. Nicklas, Chemosphere 50 (2003) 1319–1330.
[8] M.I. Litter, Introduction to photochemical advanced oxidation processes for water treatment, Environmental Chemistry, vol. 2, Part M, 2005, pp. 325–366.

[9] S. Malato, P. Fernández-Ibáñez, M. Maldonado, J. Blanco, W. Gernjak, Catal. Today 147 (2009) 1–59.
[10] J. Herrmann, C. Guillard, P. Pichat, Catal. Today 17 (1993) 7–20.
[11] R. Goslich, R. Dillert, D. Bahnemann, Water Sci. Technol. 35 (1997) 137–148.
[12] I. Arslan, I.A. Balcioğlu, Dyes Pigments 43 (1999) 95–108.
[13] P.L. Huston, J.J. Pignatello, Water Res. 33 (1999) 1238–1246.
[14] D. Mantzavinos, M. Sahibzada, A.G. Livingston, I.S. Metcalfe, K. Hellgardt, Catal. Today 53 (1999) 93–106.
[15] I.A. Balcıoglu, M. Otker, Chemosphere 50 (2003) 85–95.
[16] S. Sakthivel, H. Kisch, Angew. Chem. Int. Ed. 42 (2003) 4908–4911.
[17] S. Parsons (Ed.), Advanced Oxidation Processes for Water and Wastewater Treatment, IWA Publishing, London, 2004.
[18] I.R. Bautitz, R.F.P. Nogueira, J. Photochem. Photobiol. A Chem. 187 (2007) 33–39.
[19] S. Esplugas, D.M. Bila, L.G. Krause, M. Dezotti, J. Hazard. Mater. 149 (2007) 631–642.
[20] V. Naddeo, S. Meriç, D. Kassinos, V. Belgiorno, M. Guida, Water Res. 43 (2009) 4019–4027.
[21] K. Ikehata, N.J. Naghashkar, M.G. El-Din, Ozone Sci. Eng. 28 (2006) 353–414.
[22] N. Le-Minh, S. Khan, J. Drewes, R. Stuetz, Water Res. 44 (2010) 4295–4323.
[23] I. Oller, S. Malato, J. Sánchez-Pérez, Sci. Total Environ. 409 (2011) 4141–4166.
[24] V. Matamoros, A. Duhec, J. Albaigés, J.M. Bayona, Water Air Soil Pollut. 196 (2009) 161–168.
[25] T.G. Vasconcelos, D.M. Henriques, A. König, A.F. Martins, K. Kümmerer, Chemosphere 76 (2009) 487–493.
[26] A. Agüera, L. Perez Estrada, I. Ferrer, E. Thurman, S. Malato, A. Fernández-Alba, J. Mass Spectrom. 40 (2005) 908–915.
[27] S. Kusari, D. Prabhakaran, M. Lamshöft, M. Spiteller, Environ. Pollut. 157 (2009) 2722–2730.
[28] J. Santiago, A. Agüera, M. del Mar Gómez-Ramos, A.R. Fernández Alba, E. García-CalvoR, R. Rosal, J. Hazard. Mater. 194 (2011) 30–41.
[29] A. Nakajima, M. Tahara, Y. Yoshimura, H. Nakazawa, J. Photochem. Photobiol. A Chem. 174 (2005) 89–97.
[30] F. Yuan, C. Hu, X. Hu, D. Wei, Y. Chen, J. Qu, J. Hazard. Mater. 185 (2011) 1256–1263.
[31] Q. Liu, H.E. Williams, Environ. Sci. Technol. 41 (2007) 803–810.
[32] R.F. Dantas, O. Rossiter, A.K.R. Teixeira, A.S. Simões, V.L. da Silva, Chem. Eng. J. 158 (2010) 143–147.
[33] C.C. Ryan, D.T. Tan, W.A. Arnold, Water Res. 45 (2011) 1280–1286.
[34] C. Baeza, D.R. Knappe, Water Res. 45 (2011) 4531–4543.
[35] C. Sirtori, A. Agüera, W. Gernjak, S. Malato, Water Res. 44 (2010) 2735–2744.
[36] J. Rivas, O. Gimeno, T. Borralho, M. Carbajo, J. Hazard. Mater. 174 (2010) 393–397.
[37] H. Yamamoto, Y. Nakamura, S. Moriguchi, Y. Nakamura, Y. Honda, I. Tamura, Y. Hirata, A. Hayashi, J. Sekizawa, Water Res. 43 (2009) 351–362.
[38] D. Prabhakaran, P. Sukul, M. Lamshöft, M.A. Maheswari, S. Zühlke, M. Spiteller, Chemosphere 77 (2009) 739–746.
[39] M. Vasquez, M. Garcia-Käufer, E. Hapeshi, J. Menz, K. Kostarelos, D. Fatta-Kassinos, K. Kümmerer, Sci. Total Environ. 450–451 (2013) 356–365.
[40] R. Giri, H. Ozaki, Y. Takayanagi, S. Taniguchi, R. Takanami, Int. J. Environ. Sci. Technol. 8 (2011) 19–30.

[41] N. De la Cruz, J. Giménez, S. Esplugas, D. Grandjean, L. de Alencastro, C. Pulgarin, Water Res. 46 (2012) 1947–1957.
[42] A.G. Trovó, S.A.S. Melo, R.F.P. Nogueira, J. Photochem. Photobiol. A Chem. 198 (2008) 215–220.
[43] A.G. Trovó, R.F. Pupo Nogueira, A. Agüera, A.R. Fernandez-Alba, S. Malato, Water Res. 45 (2011) 1394–1402.
[44] C. Mavronikola, M. Demetriou, E. Hapeshi, D. Partassides, C. Michael, D. Mantzavinos, D. Kassinos, J. Chem. Technol. Biotechnol. 84 (2009) 1211–1217.
[45] J. Radjenović, C. Sirtori, M. Petrović, D. Barceló, S. Malato, Appl. Catal. B Environ. 89 (2009) 255–264.
[46] I. Arslan-Alaton, S. Dogruel, J. Hazard. Mater. 112 (2004) 105–113.
[47] Y. Yang, P. Wang, S. Shi, Y. Liu, J. Hazard. Mater. 168 (2009) 238–245.
[48] N. Klamerth, L. Rizzo, S. Malato, M.I. Maldonado, A. Agüera, A. Fernández-Alba, Water Res. 44 (2010) 545–554.
[49] I. Michael, E. Hapeshi, C. Michael, D. Fatta-Kassinos, Water Res. 44 (2010) 5450–5462.
[50] I. Michael, E. Hapeshi, C. Michael, A. Varela, S. Kyriakou, C. Manaia, D. Fatta-Kassinos, Water Res. 46 (2012) 5621–5634.
[51] M. Pérez-Moya, M. Graells, G. Castells, J. Amigó, E. Ortega, G. Buhigas, L.M. Pérez, H.D. Mansilla, Water Res. 44 (2010) 2533–2540.
[52] O. Gonzalez, C. Sans, S. Esplugas, J. Hazard. Mater. 146 (2007) 459–464.
[53] I. Michael, E. Hapeshi, V. Osorio, S. Perez, M. Petrovic, A. Zapata, S. Malato, D. Barceló, D. Fatta-Kassinos, Sci. Total Environ. 430 (2012) 167–173.
[54] W. Li, V. Nanaboina, Q. Zhou, G.V. Korshin, Water Res. 46 (2012) 403–412.
[55] L.A. Pérez-Estrada, S. Malato, W. Gernjak, A. Agüera, E.M. Thurman, I. Ferrer, A. R. Fernández-Alba, Environ. Sci. Technol. 39 (2005) 8300–8306.
[56] L.A. Pérez-Estrada, S. Malato, A. Agüera, A.R. Fernández-Alba, Catal. Today 129 (2007) 207–214.
[57] F. Méndez-Arriaga, S. Esplugas, J. Giménez, Water Res. 44 (2010) 589–595.
[58] X. Xu, X. Li, X. Li, H. Li, Sep. Purif. Technol. 68 (2009) 261–266.
[59] C. Sirtori, A. Zapata, W. Gernjak, S. Malato, A. Lopez, A. Agüera, Water Res. 45 (2011) 1736–1744.
[60] D. Vione, J. Feitosa-Felizzola, C. Minero, S. Chiron, Water Res. 43 (2009) 1959–1967.
[61] C. Sirtori, A. Zapata, S. Malato, W. Gernjak, A.R. Fernández-Alba, A. Agüera, Photochem. Photobiol. Sci. 8 (2009) 644–651.
[62] N. Klamerth, N. Miranda, S. Malato, A. Agüera, A. Fernández-Alba, M. Maldonado, J. Coronado, Catal. Today 144 (2009) 124–130.
[63] E.S. Elmolla, M. Chaudhuri, Desalination 272 (2011) 218–224.
[64] L. Rizzo, S. Meric, M. Guida, D. Kassinos, V. Belgiorno, Water Res. 43 (2009) 4070.
[65] L. Hu, P.M. Flanders, P.L. Miller, T.J. Strathmann, Water Res. 41 (2007) 2612–2626.
[66] N.P. Xekoukoulotakis, C. Drosou, C. Brebou, E. Chatzisymeon, E. Hapeshi, D. Fatta-Kassinos, D. Mantzavinos, Catal. Today 161 (2011) 163–168.
[67] X. Zhang, F. Wu, X. Wu, P. Chen, N. Deng, J. Hazard. Mater. 157 (2008) 300–307.
[68] L. Yang, L.E. Yu, M.B. Ray, Water Res. 42 (2008) 3480–3488.
[69] M. Abellán, J. Giménez, S. Esplugas, Catal. Today 144 (2009) 131–136.
[70] T. Paul, M.C. Dodd, T.J. Strathmann, Water Res. 44 (2010) 3121–3132.
[71] T.E. Doll, F.H. Frimmel, Water Res. 38 (2004) 955–964.
[72] H. Yang, G. Li, T. An, Y. Gao, J. Fu, Catal. Today 153 (2010) 200–207.

[73] E. Hapeshi, A. Achilleos, M. Vasquez, C. Michael, N. Xekoukoulotakis, D. Mantzavinos, D. Kassinos, Water Res. 44 (2010) 1737–1746.
[74] A. Achilleos, E. Hapeshi, N. Xekoukoulotakis, D. Mantzavinos, D. Fatta-Kassinos, Sep. Sci. Technol. 45 (2010) 1564–1570.
[75] A.L. Giraldo, G.A. Peñuela, R.A. Torres-Palma, N.J. Pino, R.A. Palominos, H.D. Mansilla, Water Res. 44 (2010) 5158–5167.
[76] N.P. Xekoukoulotakis, N. Xinidis, M. Chroni, D. Mantzavinos, D. Venieri, E. Hapeshi, D. Fatta-Kassinos, Catal. Today 151 (2010) 29–33.
[77] Y. Zhang, J.L. Zhou, B. Ning, Water Res. 41 (2007) 19–26.
[78] F. Méndez-Arriaga, S. Esplugas, J. Giménez, Water Res. 42 (2008) 585–594.
[79] M. Chen, W. Chu, J. Hazard. Mater. 219–220 (2012) 183–189.
[80] D. Nasuhoglu, A. Rodayan, D. Berk, V. Yargeau, Chem. Eng. J. 189–190 (2012) 41–48.
[81] C. Reyes, J. Fernández, J. Freer, M. Mondaca, C. Zaror, S. Malato, H. Mansilla, J. Photochem. Photobiol. A Chem. 184 (2006) 141–146.
[82] M. Sousa, C. Gonçalves, V.J. Vilar, R.A. Boaventura, M. Alpendurada, Chem. Eng. J. 198–199 (2012) 301–309.
[83] F.J. Beltrán, A. Aguinaco, J.F. García-Araya, Ozone Sci. Eng. 34 (2012) 3–15.
[84] J. Rivas, Á. Encinas, F. Beltrán, N. Graham, J. Environ. Sci. Health A 46 (2011) 944–951.
[85] Y. Wang, H. Zhang, J. Zhang, C. Lu, Q. Huang, J. Wu, F. Liu, J. Hazard. Mater. 192 (2011) 35–43.
[86] C. Tizaoui, N. Grima, N. Hilal, Chem. Eng. Process. 50 (2011) 637–643.
[87] M.M. Huber, A. GÖbel, A. Joss, N. Hermann, D. LÖffler, C.S. McArdell, A. Ried, H. Siegrist, T.A. Ternes, U. von Gunten, Environ. Sci. Technol. 39 (2005) 4290–4299.
[88] A. Rodayan, R. Roy, V. Yargeau, J. Hazard. Mater. 177 (2010) 237–243.
[89] P. Palo, J.R. Domínguez, J. Sánchez-Martín, Water, Air, Soil Pollut. 223 (2012) 5999–6007.
[90] K. Li, A. Yediler, M. Yang, S. Schulte-Hostede, M.H. Wong, Chemosphere 72 (2008) 473–478.
[91] Y.J. Jung, W.G. Kim, Y. Yoon, T. Hwang, J. Kang, Ozone Sci. Eng. 34 (2012) 156–162.
[92] R.F. Dantas, S. Contreras, C. Sans, S. Esplugas, J. Hazard. Mater. 150 (2008) 790–794.
[93] Z. Zhang, H. Zhu, X. Wen, X. Si, J. Environ. Sci. 24 (2012) 228–233.
[94] J. Rivas, O. Gimeno, A. Encinas, F. Beltrán, Chemosphere 76 (2009) 651–656.
[95] E.V. Rokhina, N.S. Vattikonda, C. Johnson, R.P. Suri, Chemosphere 92 (2012) 1064–1066.
[96] W. Seitz, J. Jiang, W. Schulz, W.H. Weber, D. Maier, M. Maier, Chemosphere 70 (2008) 1238–1246.
[97] C. Liu, V. Nanaboina, G.V. Korshin, W. Jiang, Water Res. 46 (2012) 5235–5246.
[98] M.O. Uslu, M.F. Rahman, S.Y. Jasim, E.K. Yanful, N. Biswas, Water Air Soil Pollut. 223 (2012) 3173–3180.
[99] M. Skoumal, P. Cabot, F. Centellas, C. Arias, R.M. Rodríguez, J.A. Garrido, E. Brillas, Appl. Catal. B Environ. 66 (2006) 228–240.
[100] M.C. Dodd, M. Buffle, U. Von Gunten, Environ. Sci. Technol. 40 (2006) 1969–1977.
[101] R.F. Dantas, M. Canterino, R. Marotta, C. Sans, S. Esplugas, R. Andreozzi, Water Res. 41 (2007) 2525–2532.
[102] N.M. Vieno, H. Härkki, T. Tuhkanen, L. Kronberg, Environ. Sci. Technol. 41 (2007) 5077–5084.
[103] A. Ghauch, H. Baydoun, P. Dermesropian, Chem. Eng. J. 172 (2011) 18–27.
[104] A. Ziylan, Y. Koltypin, A. Gedanken, N.H. Ince, Ultrason. Sonochem. 20 (2012) 580–586.

[105] G.T. Güyer, N.H. Ince, Ultrason. Sonochem. 18 (2011) 114–119.
[106] P. Braeutigam, M. Franke, R.J. Schneider, A. Lehmann, A. Stolle, B. Ondruschka, Water Res. 46 (2012) 2469–2477.
[107] E. De Bel, J. Dewulf, B.D. Witte, H. Van Langenhove, C. Janssen, Chemosphere 77 (2009) 291–295.
[108] W. Guo, Y. Shi, H. Wang, H. Yang, G. Zhang, Ultrason. Sonochem. 17 (2010) 680–684.
[109] E. De Bel, C. Janssen, S. De Smet, H. Van Langenhove, J. Dewulf, Ultrason. Sonochem. 18 (2011) 184–189.
[110] Q. Isariebel, J. Carine, J. Ulises-Javier, W. Anne-Marie, D. Henri, Ultrason. Sonochem. 16 (2009) 610–616.
[111] Z. Frontistis, D. Mantzavinos, Ultrason. Sonochem. 19 (2012) 77–84.
[112] R. Sivakumar, K. Muthukumar, CLEAN – Soil, Air, Water 39 (2011) 136–141.
[113] E. Hapeshi, A. Achilleos, A. Papaioannou, L. Valanidou, N. Xekoukoulotakis, D. Mantzavinos, D. Fatta-Kassinos, Water Sci. Technol. 61 (2010) 3141.
[114] V. Naddeo, V. Belgiorno, D. Kassinos, D. Mantzavinos, S. Meric, Ultrason. Sonochem. 17 (2010) 179–185.
[115] L. Sanchez-Prado, R. Barro, C. Garcia-Jares, M. Llompart, M. Lores, C. Petrakis, N. Kalogerakis, D. Mantzavinos, E. Psillakis, Ultrason. Sonochem. 15 (2008) 689–694.
[116] R.P. Suri, T.S. Singh, S. Abburi, Environ. Sci. Technol. 44 (2010) 1373–1379.
[117] I. Quesada-Peñate, C. Julcour-Lebigue, U. Jáuregui-Haza, A. Wilhelm, H. Delmas, J. Hazard. Mater. 221–222 (2012) 131–138.
[118] Y. Li, F. Zhang, X. Liang, A. Yediler, Chemosphere 90 (2013) 284–291.
[119] G. Qiu, Y. Song, P. Zeng, S. Xiao, L. Duan, Chemosphere 84 (2011) 241–246.
[120] F.J. Benitez, J. García, J.L. Acero, F.J. Real, G. Roldan, Process. Saf. Environ. Prot. 89 (2011) 334–341.
[121] M. Murugananthan, S. Latha, G. Bhaskar Raju, S. Yoshihara, J. Hazard. Mater. 180 (2010) 753–758.
[122] F. Zaviska, P. Drogui, J. Biais, G. Mercier, S. De La Rochebrochard d'Auzay, J. Environ. Eng. 139 (2012) 810–821.
[123] G. Tissot, A. Anglada, P. Dimitriou-Christidis, L. Rossi, J.S. Arey, C. Comninellis, Electrochem. Commun. 23 (2012) 48–51.
[124] E. Brillas, S. Garcia-Segura, M. Skoumal, C. Arias, Chemosphere 79 (2010) 605–612.
[125] M.J. Martín de Vidales, C. Sáez, P. Cañizares, M.A. Rodrigo, J. Chem. Technol. Biotechnol. 87 (2012) 1173–1178.
[126] S. Li, D. Bejan, M. McDowell, N.J. Bunce, J. Appl. Electrochem. 38 (2008) 151–159.
[127] T. González, J.R. Domínguez, P. Palo, J. Sánchez-Martín, E.M. Cuerda-Correa, Desalination 280 (2011) 197–202.
[128] E. Brillas, I. Sirés, C. Arias, P.L. Cabot, F. Centellas, R.M. Rodríguez, J.A. Garrido, Chemosphere 58 (2005) 399–406.
[129] S.L. Ambuludi, M. Panizza, N. Oturan, A. Özcan, M.A. Oturan, Environ. Sci. Pollut. Res. 20 (2012) 2381–2389.
[130] I. Sirés, N. Oturan, M.A. Oturan, Water Res. 44 (2010) 3109–3120.
[131] M. Murugananthan, S. Latha, G. Bhaskar Raju, S. Yoshihara, Sep. Purif. Technol. 79 (2011) 56–62.
[132] Q. Dai, Y. Xia, L. Jiang, W. Li, J. Wang, J. Chen, Int. J. Electrochem. Sci. 7 (2012) 12895–12906.
[133] E. Guinea, C. Arias, P.L. Cabot, J.A. Garrido, R.M. Rodríguez, F. Centellas, E. Brillas, Water Res. 42 (2008) 499–511.

[134] Z. Frontistis, C. Brebou, D. Venieri, D. Mantzavinos, A. Katsaounis, J. Chem. Technol. Biotechnol. 86 (2011) 1233–1236.
[135] M. Petrovic, M. Gros, D. Barcelo, J. Chromatogr. A 1124 (2006) 68.
[136] L. Rizzo, Water Res. 45 (2011) 4311–4340.
[137] D. Fatta-Kassinos, M. Vasquez, K. Kümmerer, Chemosphere 85 (2011) 693–709.
[138] I. Kim, N. Yamashita, H. Tanaka, J. Hazard. Mater. 166 (2009) 1134–1140.
[139] I. Michael, L. Rizzo, C. McArdell, C. Manaia, C. Merlin, T. Schwartz, C. Dagot, D. Fatta-Kassinos, Water Res. 43 (2012) 957–995.
[140] V. Homem, L. Santos, J. Environ. Manag. 92 (2011) 2304–2347.
[141] T.M. El-Morsi, M.M. Emara, H.M. Abd El Bary, M.H. Hassan, A.S. Abd-El-Aziz, K.J. Friesen, Chemosphere 47 (2002) 343–348.
[142] E. Neyens, J. Baeyens, J. Hazard. Mater. 98 (2003) 33–50.
[143] J. Bacardit, J. Stötzner, E. Chamarro, S. Esplugas, Ind. Eng. Chem. Res. 46 (2007) 7615–7619.
[144] F. Buda, B. Ensing, M. Gribnau, E.J. Baerends, Chem. Eur. J. 7 (2001) 2775–2783.
[145] J.J. Pignatello, E. Oliveros, A. MacKay, Crit. Rev. Environ. Sci. Technol. 36 (2006) 1–84.
[146] P.R. Gogate, A.B. Pandit, Adv. Environ. Res. 8 (2004) 501–551.
[147] I. Oller, S. Malato, J. Sánchez-Pérez, W. Gernjak, M. Maldonado, L. Pérez-Estrada, C. Pulgarín, Catal. Today 122 (2007) 150–159.
[148] J. Farias, E.D. Albizzati, O.M. Alfano, Catal. Today 144 (2009) 117–123.
[149] A. Zapata, T. Velegraki, J. Sánchez-Pérez, D. Mantzavinos, M. Maldonado, S. Malato, Appl. Catal. B Environ. 88 (2009) 448–454.
[150] W. Gernjak, M. Fuerhacker, P. Fernández-Ibañez, J. Blanco, S. Malato, Appl. Catal. B Environ. 64 (2006) 121–130.
[151] J.J. Pignatello, Environ. Sci. Technol. 26 (1992) 944–951.
[152] M.N. Chong, B. Jin, C.W. Chow, C. Saint, Water Res. 44 (2010) 2997–3027.
[153] A. Moncayo-Lasso, C. Pulgarin, N. Benítez, Water Res. 42 (2008) 4125–4132.
[154] J. De Laat, G. Truong Le, B. Legube, Chemosphere 55 (2004) 715–723.
[155] A.L. Linsebigler, G. Lu, J.T. Yates Jr., Chem. Rev. 95 (1995) 735–758.
[156] N. Daneshvar, D. Salari, A. Khataee, J. Photochem. Photobiol. A Chem. 162 (2004) 317–322.
[157] S. Sakthivel, B. Neppolian, M. Shankar, B. Arabindoo, M. Palanichamy, V. Murugesan, Sol. Energy Mater. Sol. Cells 77 (2003) 65–82.
[158] Y. Zhao, J. Zhong, H. Li, N. Xu, J. Shi, J. Membr. Sci. 208 (2002) 331–341.
[159] Y.G. Adewuyi, Ind. Eng. Chem. Res. 40 (2001) 4681–4715.
[160] C. Comninellis, A. Kapalka, S. Malato, S.A. Parsons, I. Poulios, D. Mantzavinos, J. Chem. Technol. Biotechnol. 83 (2008) 769–776.
[161] M. Panizza, G. Cerisola, Chem. Rev. 109 (2009) 6541–6569.
[162] M. Wu, G. Zhao, M. Li, L. Liu, D. Li, J. Hazard. Mater. 163 (2009) 26–31.
[163] R. Rosal, M.S. Gonzalo, A. Rodriguez, E. Garcia-Calvo, J. Hazard. Mater. 183 (2010) 271–278.
[164] J. Levec, A. Pintar, Catal. Today 124 (2007) 172–184.
[165] J. Melero, F. Martínez, J. Botas, R. Molina, M. Pariente, Water Res. 43 (2009) 4010–4018.
[166] G. Wang, D. Wang, X. Xu, L. Liu, F. Yang, J. Hazard. Mater. 217–218 (2012) 366–373.

Chapter 12

Removal of Pharmaceutical Compounds from Wastewater and Surface Water by Natural Treatments

Víctor Matamoros and Josep M. Bayona
Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

Chapter Outline

1 INTRODUCTION

The presence of pharmaceutical compounds in the aquatic environment is ubiquitous due to their incomplete removal in wastewater treatment plants (WWTPs). Consequently, they enter into the water cycle with unknown consequences to wildlife and humans [1,2]. Natural water treatments constitute an attractive technology to mitigate the contamination of pharmaceutical and other active compounds at the same time that they are able to proportionate effluent with better biological quality. Natural treatments are eco-engineered filter systems, which simulate the ability of the natural ecosystems to attenuate the occurrence of pollutants from water. They can be used in the purification of wastewater, agricultural runoff, storm water, and industrial effluents. In most of the cases, these systems are supported by the action of

Comprehensive Analytical Chemistry, Vol. 62. http://dx.doi.org/10.1016/B978-0-444-62657-8.00012-4

plants, as in constructed wetlands (CWs) and buffer strips, whereas in other systems like ponds, plants are less frequent [3]. Natural water treatments rely on the capacity of physical, chemical, and biological processes for removing pollutants. Among them, photochemical oxidation, sorption, and biological degradation are the most predominant [4,5], but other processes such as hydrolysis, sedimentation, plant uptake, phytovolatilization, contaminant accumulation, and metabolic transformation can also take place [6]. Photodegradation is related to the sunlight exposure and is able to remove pharmaceutical compounds by direct or indirect photolysis [7]. Biodegradation is the most extended removal process, which is carried out by bacteria or exudates from other organisms (including plant roots and fungi). Sorption can be either adsorption to the particles or matrix or absorption (e.g., plant uptake). Accumulation and metabolic transformation of pharmaceutical compounds in plant tissues have already been observed for carbamazepine [8], whereas hydrolysis has only been reported for some antibiotics such as tetracyclines [9].

This chapter reviews the effectiveness of natural treatment technologies such as CWs, ponds, buffer strips, and restored wetlands for removing pharmaceutical compounds from wastewater and surface waters impacted by WWTP effluents.

2 CONSTRUCTED WETLANDS

CWs are land-based wastewater treatment systems that consist of shallow ponds, beds, or trenches that contain floating or emergent rooted wetland vegetation [10]. Polluted water is treated by percolation induced by gravity through a vegetated bed, which is insulated by a geomembrane protected with a geotextile or compacted clay to avoid groundwater pollution. CWs are effective in treating polluted waters arising from a wide range of domestic, industrial, and agricultural operations. Such ecotechnology enables the water to be reused in a cost-effective way while at the same time creating small areas of wetland wildlife habitat [11]. CWs can be classified according to the various parameters, but the two most important criteria are the water flow regime (surface and subsurface) and the type of macrophytic growth [12]. Afterward, CWs are classified as either surface flow (SF) systems or subsurface flow (SSF) systems. Figure 1 shows the different CW configurations that will be reviewed in this section in accordance with their capacity for removing pharmaceutical compounds.

2.1 Subsurface Flow Constructed Wetlands

Subsurface flow constructed wetlands (SSFCWs) constitute an alternative, cost-effective technology for the treatment of urban wastewater that has attracted increasing interest over the last decades in the context of small communities with <2000 population equivalent [13]. In this regard, it is possible to distinguish between the influence of different design parameters such as wastewater flow direction, the effect of hydraulic residence time (HRT), batch

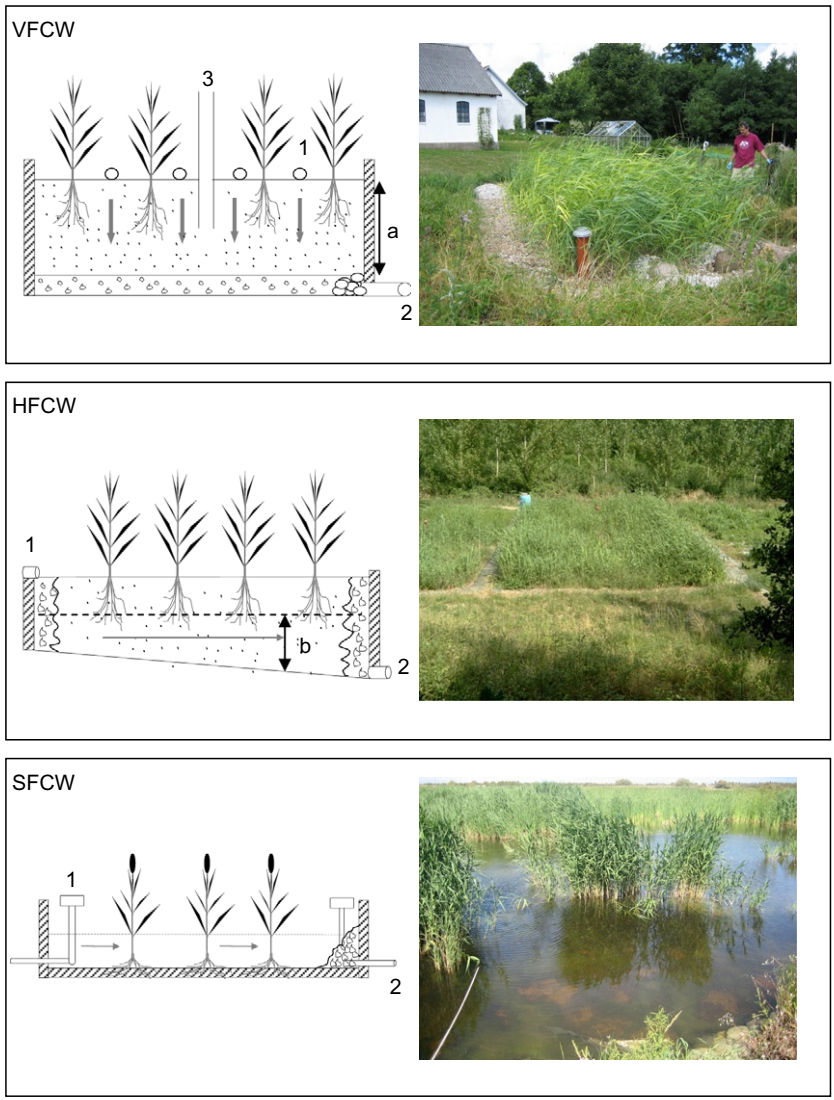

FIGURE 1 Longitudinal section of three most used CW configurations. VFCW picture was taken in the eastern part of Jutland (Denmark). HFCW picture was taken in Les Franqueses del Valles (Spain). SFCW picture was taken in Empuriabrava WWTP facilities (Spain). Arrows indicate the water flow direction. 1, Influent; 2, effluent; and 3, aeration pipe. (a) Sand layer depth and (b) water depth.

versus continuous feeding, clogging, the effect of primary treatment, water depth or sand layer depth, the presence of plants, the presence of a supporting matrix, hybrid systems, and seasonality or temperature on the removal efficiency of pharmaceutical compounds.

2.1.1 Wastewater Flow Direction

SSFCWs are subdivided with vertical flow (VF) and horizontal flow (HF), depending on the direction that the wastewater flows through the wetland bed (Figure 1). HFCWs are continuously fed, and the wastewater flows slowly due to the gravity through a vegetated, gravel bed. Conversely, VFCWs are intermittently fed from the wetland surface, combining the feeding with a resting period. In this regard, the HFCW works in saturated water conditions, and the removal of organic matter is mostly by anaerobic pathways, whereas the VFCW system works in unsaturated water conditions and the aerobic environment prevails [5]. Nevertheless, aerobic microenvironments in the rhizosphere exist even in saturated systems such as the HFCW, and therefore, aerobic biodegradation pathways cannot be ruled out. Table 1 shows that the VFCWs are able to remove biodegradable pharmaceutical compounds (e.g., ibuprofen and naproxen) more efficiently than HFCWs, which is in accordance with laboratory-scale studies carried out by Zwiener and Frimmel [14] and Conkle et al. [15] who found that aerobic pathways are, in general, more effective at removing pharmaceutical compounds than anaerobic pathways. Furthermore, some authors [16,17] have used the enantiomeric factor (EF) of chiral pharmaceutical compounds (R and S) to differentiate between the aerobic and anaerobic conditions of VFCWs and HFCWs, respectively. Thus, naproxen biodegradation has been reported as enantiomer-specific in both CWs, but ibuprofen biodegradation was only reported as enantiomer-specific in VFCWs.

2.1.2 The Effect of HRT/HLR

There is a concern about the feasibility of wetlands as a cost-effective method because wetlands typically require a low hydraulic loading rate (HLR) and a long HRT to achieve efficient pollutant removal. This means wetland treatment method may need a large land area [18]. Different studies published by Matamoros et al. [5,19] proved that the removal of pharmaceutical compounds in CWs follows the same general trend. Figure 2 shows the mass loading rate against the mass removal rate of some analgesic drugs in a VFCW. Whereas ibuprofen was not affected by HLR, diclofenac removal increases with the decrease of the HLR. This effect has been attributed to the first-order reaction rate of biological processes involved in the degradation of these compounds. Ranieri et al. [20] found that paracetamol removal in a pilot plant HFCW varied from 52% at HLR of 240 mm d^{-1} to 87% at HLR of 120 mm d^{-1} and 99% at HLR of 30 mm d^{-1}. Similarly, Zhang et al. [21] found that naproxen and ibuprofen were removed more efficiently at HRT of 4 days (80–91%) as opposed to at HRT of 2 days (71–83%).

TABLE 1 Removal Efficiency of Pharmaceutical Compounds in the CWs Reported in the Chapter

Type	Configuration	Influent Water Quality	HRT (Days)	Removal Efficiency (%)	References
HFCW	0.27 m water depth	Primary-treated wastewater	4.8	Caffeine (99), ibuprofen (71), naproxen (85), diclofenac (15), ketoprofen (38), carbamazepine (16), clofibric acid (nr)	[27,31]
	0.5 m water depth	Primary-treated wastewater	6.5	Caffeine (90), ibuprofen (34), naproxen (24), diclofenac (6), ketoprofen (nr), carbamazepine (26), clofibric acid (nr)	
	Five systems designed for 80–280 population equivalents	Primary-treated wastewater	–	Caffeine (97), ibuprofen (65), naproxen (45), diclofenac (21)	[43]
	Microcosm, 0.5 m water depth	Synthetic wastewater	8	Ibuprofen (51%), carbamazepine (5%), clofibric acid (nr)	[85]
	Microcosm, 0.3 m gravel depth	Synthetic wastewater	4	Ibuprofen (80), naproxen (91), diclofenac (55), carbamazepine (27)	[21]
	2HFCW + HFCW	Primary-treated wastewater	3.5	Ibuprofen (98–99), naproxen (99), diclofenac (97–98)	[86]
	Microcosm; surface area, 1 m^2; water depth, 0.4 m	Primary-treated wastewater	2.2	Caffeine (60–95), ibuprofen (35–70), naproxen (50–80), diclofenac (30–50), ketoprofen (10–60), carbamazepine (20–40)	[87]
	Microcosm, LECA	Primary-treated wastewater	4	Atenolol (93–95)	[88]
VFCW	Microcosm, 7.5 cm filter layer depth	Secondary-treated wastewater	0.1	Estrone (68), 17β-estradiol (84), 17α-ethynylestradiol (75)	[33]
	Four systems designed for 2–4 population equivalents	Decentralized domestic wastewater	–	Caffeine (99), ibuprofen (89), naproxen (92)	[43]
	Pilot plant, 1 m sand layer depth	Primary-treated wastewater	0.4	Caffeine (99), ibuprofen (96), naproxen (92), diclofenac (96), ketoprofen (99), carbamazepine (26)	[19]

Continued

TABLE 1 Removal Efficiency of Pharmaceutical Compounds in the CWs Reported in the Chapter—Cont'd

Type	Configuration	Influent Water Quality	HRT (Days)	Removal Efficiency (%)	References
SFCW	Surface area: 2 ha	Secondary-treated wastewater	30	Ibuprofen (96a/95b), naproxen (92a/52b), diclofenac (96a/73b), ketoprofen (99a/97b), carbamazepine (30a/47b), flunixin (nr[a]/30b)	[52]
	(28 ha/2.7 ha/24 ha/2 ha)	Secondary-treated wastewater	7/2/6/8	Atenolol (27/53/53/53), bisoprolol (26/22/29/36), citalopram (45/84/63/97), codeine (29/75/74/47), diclofenac (31/24/36/30), diltiazem (30/74/68/88), dipyridamole (80/100/94/98), eprosartan (75/39/48/24), ibuprofen (38/80/88/5), irbesartan (58/8/3/27), ketoprofen (56/3/32/19), memantine (5/29/5/32), naproxen (34/46/75/50), telmisartan (73/51/4/87), trimetoprim (25/51/69/86), venlafaxine (40/17/18/65)	[55]
	Pilot plant, sandy soil:0.6 m and surface water:0.15 m	Artificial agricultural wastewater	2.2	Monensin (32), salinomycin (34), narasin (36)	[56]
	0.36 ha	Secondary-treated wastewater	0.3	Sulfamethoxazole (30–50), atenolol (95–100), dilantin (10–40), carbamazepine (nr-60), diazepam (nr), diclofenac (40–80), naproxen (75–80), triclosan (78–100),	[89]
	Herbaceous marsh containing plants	Secondary-treated wastewater	1	Cotinine (nr), caffeine (nr), carbamazepine (105), atenolol (6), nadolol (23), metoprolol (8), sotalol (52), sulfapyridine (24), sulfamethoxazole (1), acetaminophen (nr), naproxen (1), ibuprofen (nr), gemfibrozil (31)	[62]

a, warm season; b, cold season; nr, no removal.

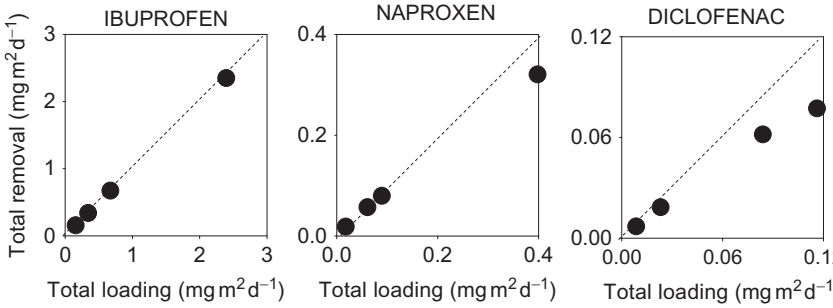

FIGURE 2 Pharmaceutical average mass removal rates in a VFCW against their mass loading rates. From left to right, the circles represent a HLR of 13, 30, 70, and 160 mm day^{-1}, respectively. Discontinuous line represents 100% removal.

2.1.3 Batch Versus Continuous Feeding

Previous studies have hypothesized that drain and fill cycles in batch mode operation can introduce air into the pore spaces of the soil matrix and thereby enhance microbial oxidation of organic matter [22,23]. Zhang et al. [24] observed that the values of the area-based decay rate were constant for the batch-fed wetlands (drain and fill) and the removal of eight pharmaceutical compounds (i.e., carbamazepine, naproxen, diclofenac, ibuprofen, caffeine, salicylic acid, ketoprofen, and clofibric acid) was higher than for the continuous-fed system (with the exception of naproxen). This observation indicates that the batch-feeding regime for most pharmaceutical compounds does attain its highest removal efficiency faster than continuous-fed systems. In a similar study, Ávila et al. [25] observed that the removal efficiencies were always greater for the batch feeding than for the continuous feeding, and in this respect, statistically significant differences were found for ibuprofen and diclofenac. As an example, ibuprofen, whose major removal mechanism has been reported to be biodegradation under aerobic conditions, showed a higher removal in the batch line (85%) than in the control continuous lines (63%). The same authors demonstrated that the mode of operation in batch feeding resulted in a prevailing higher redox status, when compared to functioning under saturated conditions, which in turn significantly enhanced the elimination of pharmaceutical compounds.

2.1.4 Clogging

System clogging is attributed to biological, physical, and chemical treatment processes that occur in CWs, which can result in the gradual obstruction of the porous medium. Clogging may result in hydraulic malfunction and/or reduced treatment performance of the CWs [26]. SSFCW clogging leads to a decrease of the removal efficiency of pharmaceutical compounds, among other undesirable effects. Matamoros and Bayona [27] reported that

biodegradable compounds such as ibuprofen and naproxen were highly affected by clogging and suggested that the reduction of oxygen transfer due to the clogging may be the explanation.

2.1.5 Effect of Primary Treatment

Most of the CWs include a primary pretreatment step, which consists of a sedimentation or septic tank. During recent years, a lot of research has been undertaken to improve these pretreatments in order to reduce the demonstrated drawbacks that clogging has on SSFCW performance. This is the case for upflow anaerobic sludge blanket (UASB) and hydrolytic upflow sludge blanket (HUSB) reactors. These reactors work under anaerobic conditions that are reported to increase the removal efficiency of recalcitrant organic compounds [28]. Hijosa-Valsero et al. [29] studied the effect of this pretreatment on the removal of pharmaceutical compounds and found that there were no differences in comparison to conventional sedimentation tanks. Ávila et al. [25] found that the sedimentation tank offered slightly better removal values throughout the experimental period than the HUSB. Reyes-Contreas et al. [30] found that UASB was able to remove recalcitrant pharmaceutical compounds such as carbamazepine and ketoprofen at 10 and 50%, respectively. Overall, it is difficult to conclude that these pretreatments may favor the removal of pharmaceutical compounds. This is because all studies done up to now have been focused on polar pharmaceutical compounds, which have a low interaction with suspended solids. Therefore, it is plausible that hydrophobic pharmaceutical compounds, with high interaction with the suspended solids, may be eliminated by wastewater pretreatments, most of which have the ability to remove these suspended solids.

2.1.6 Water Depth/Sand Filter Depth

Water depth and sand filter depth are two relevant design parameters in HFCWs and VFCWs, respectively. Matamoros et al. [27,31] compared the removal efficiency of pharmaceutical compounds in HFCWs with 0.5 m water depth with those observed in 0.27 m. The results indicated that the shallow system, with a less negative redox potential, was more efficient than the deep one at removing biodegradable pharmaceutical compounds such as ibuprofen and naproxen. García et al. [32] demonstrated that the predominant biochemical reactions responsible for the degradation of organic matter in those systems were caused by different metabolic pathways. Whereas methanogenesis and sulfate reduction were predominant in deep HFCWs, denitrification predominated in the shallower one. Song et al. [33] evaluated the effectiveness of VFCWs at the polishing step in conventional wastewater treatment and studied the removals of estrogens at different sand layer depths (i.e., 7.5, 30, and 60 cm filter layer depth). The highest removal efficiencies were achieved in the shallowest wetland (i.e., $68\% \pm 28\%$, $84\% \pm 15\%$, and

75% ± 18% for estrone, 17β-estradiol and 17α-ethynylestradiol, respectively). The highest efficiency achieved in the extremely shallow wetland was not only a result of the presumable enhancement of aerobic conditions but also likely to be due to the high root density increasing the surface area available for sorption processes and the enhancing effects of root exudates on estrogen removal. In summary, water and sand depth play a relevant role in the removal of pharmaceutical compounds as well as organic matter, with shallow systems being the most suitable to achieve an effluent with a high chemical water quality.

2.1.7 Plant Effect

The presence of plants in CWs is generally considered beneficial as they can take up and assimilate nutrients, stabilize the bed surface, act as an anchoring surface for biofilm, release exudates that can aid the biodegradation processes, pump and release oxygen to the bottom of the systems, provide good conditions for physical filtration, and insulate against low temperatures [34–36]. Nowadays, there are different studies in SSFCWs that indicate that the vegetation plays a relevant role in the removal of pharmaceutical compounds [5,16]. Ranieri et al. [20] observed that acetaminophen removal efficiencies in an unplanted HFCW were on average 12% lower than those in planted beds with *Phragmites* sp. and *Typha* sp. Zhang et al. [21] observed that ibuprofen and naproxen removal efficiencies were on average 20% and 40% higher in planted HFCWs, respectively. However, no differences between planted and unplanted beds were observed for carbamazepine and diclofenac due to their recalcitrance to the biodegradation. Hijosa-Valsero et al. [16] suggested that the ability of a plant to enhance pollutant removal depends not only on the typical species' characteristics but also on many other factors like the microbial communities related to them, wastewater nature, and climate conditions. Same authors observed that the presence of *Typha* sp. and *Phragmites* sp. contributed to the removal of naproxen, ibuprofen, diclofenac, carbamazepine, and caffeine, whereas Matamoros et al. [19] demonstrated that the presence of *Phragmites* sp. increases the mass loading capacity of the system due to the ability of plants to reduce clogging effects. Dordio et al. [8] observed in a laboratory-scale study that *Typha* sp., present in most of the SSFCWs, has the capacity to actively participate in the removal of carbamazepine. Zhang et al. [37–39] determined that noncharged compounds such as caffeine are easily incorporated by aquatic plants (*Scirpus validus*), whereas negatively charged compounds such as diclofenac are not. This can be explained due to the electrical repulsion between the negative charge of anions and the negative charge of the biomembrane [17]. Furthermore, taking into account the experimental studies in neutral compounds, we may speculate that neutral pharmaceuticals compounds with a $\log K_{ow}$ between 1 and 3 may be uptaken by plants [22]. In conclusion, the direct

plant uptake of the pharmaceuticals and the enhancement of biodegradation processes are described as the most important elimination processes due to the presence of plants.

2.1.8 *Effect of the Supporting Matrix*

Coarse granite gravel or sand media are the regular support matrix for CWs, but in recent years, the use of alternative sorbents has increased. Dordio et al. [40–42] carried out different laboratory studies in which they found that the use of light expanded clay aggregates (LECA) or biosorbents such as cork can improve the removal efficiency of some pharmaceutical compounds in CWs. Figure 3 shows the different removal capacity of granulated cork sorbent for eliminating pharmaceutical compounds from ultrapure water and wastewater. Although this material was shown to be unaffected by water composition, it has a higher capacity than LECA for the sorption of pharmaceutical compounds and is capable of extensively removing ibuprofen and carbamazepine (2 out of 3 compounds studied). Nevertheless, the sorbent capacity could be limited by the saturation of all the sorbent active sites by biofilm development. Preliminary studies existing in the use of LECA for wastewater treatment gave similar removal efficiencies of pharmaceutical compounds to biofilters filled with regular media [43], so further field studies are needed for further insight.

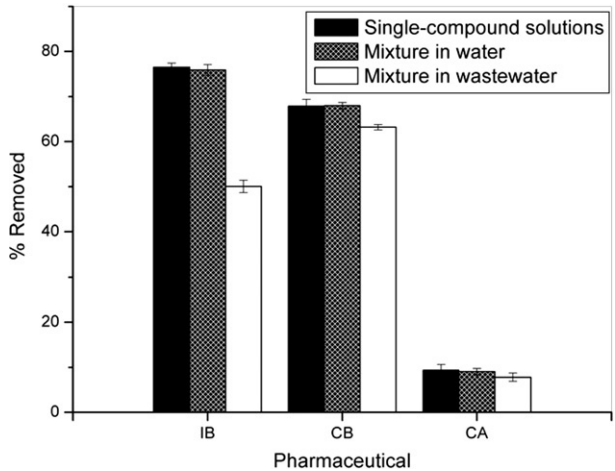

FIGURE 3 Comparison of removal efficiencies of clofibric acid (CA), ibuprofen (IB), and carbamazepine (CB) by granulated cork (after 144 h of contact time) for initial concentrations of 35 mg L^{-1} of each compound in single-compound aqueous solutions, pharmaceutical-mixture aqueous solutions, and wastewater spiked with the pharmaceutical mixture. *Reprinted from Dordio et al. [40].*

2.1.9 Hybrid Systems

Due to the difficulty in removing contaminants from wastewaters in a single-stage system, hybrid systems, which consist of various types of CWs staged in series, have been introduced to exploit the different degradation pathways between systems [44]. For example, the use of a VFCW located as a first step would be able to nitrify the ammonia species, whereas a HFCW afterward is able to denitrify the previously produced nitrates. Consequently, the combination of different redox potentials in CWs can be a suitable solution for removing compounds with certain recalcitrance. For example, Masi et al. [45] reported a removal efficiency of estrogens up to 90% in a CW composed by a first-stage HFCW and a second-stage VFCW.

2.1.10 Seasonality and Temperature Dependence

Temperature and sunlight radiation intensity changes throughout the year in places with mid and high latitudes are relevant factors to take into consideration in those regions with such seasonality. Different authors have found that biodegradable pharmaceutical compounds such as naproxen and ibuprofen are more likely to be affected by temperature changes, which cause changes to the biodegradation rates. For example, Dordio et al. [41] studied winter (12 °C) and summer (26 °C) removal efficiencies of ibuprofen, carbamazepine, and clofibric acid in planted microcosm CWs and observed better efficiencies in summer (96% for ibuprofen, 97% for carbamazepine, and 75% for clofibric acid) than in winter (82% for ibuprofen, 88% for carbamazepine, and 48% for clofibric acid). However, Hijosa-Valsero et al. [46] found in a pilot study, involving different HFCW configurations, that high temperatures had a significant positive effect on the degradation of caffeine, naproxen, and salicylic acid, but no statistical differences were observed for the degradation of ibuprofen, carbamazepine, ketoprofen, and diclofenac. Zhang et al. [21] suggested that the high temperatures in the tropics increased plant productivity and biodegradation kinetics and decreased the time necessary for biodegradation. Additionally, tropical conditions (e.g., warmth, plant activity, and sunlight) can enhance the removal of pharmaceuticals, as microorganisms living in the CWs usually reach their optimal activity at warm temperatures (15–25 °C) [47].

Overall, it seems that VFCWs, which present aerobic removal pathways, are the most suitable CW technology for removing pharmaceuticals from urban wastewaters. Nevertheless, the limited number of pharmaceuticals assessed until now prevents further generalization of this statement.

2.2 Surface Flow Constructed Wetlands

Surface flow constructed wetlands (SFCWs) consist of basins or channels, of soil, or another suitable medium that supports the rooted vegetation and water

at a relatively shallow depth flowing through the unit over compacted clay to prevent groundwater pollution [12] (Figure 1). Usually, unplanted deeper zones are combined with planted shallow zones, which prevent typical flow shortcuts occurring in stabilization or facultative ponds promoted by the predominant winds. According to the type of macrophytes growing in the wetland, SFCW systems can be further classified into systems of free-floating, submerged, and emerging macrophytes [48,49].

As discussed in the former section about SSFCWs, the presence of rooted plants in the SFCWs increases their capacity to remove biodegradable and hydrophobic organic pollutants [50,51]. Besides biodegradation, sorption, and phytoremediation, photodegradation plays a relevant role in SFCWs due to the direct exposure of the water to sunlight. In this regard, Matamoros et al. [52,53] found that emerging macrophyte SFCWs (one with a HRT of 8 and the other with a HRT of 30 days) were able to remove up to 90% photodegradable compounds such as diclofenac or ketoprofen (Table 1). Moreover, temperature and sunlight irradiation changes in SFCWs were more relevant for photolabile compounds (e.g., naproxen, diclofenac, and ketoprofen) than biodegradable compounds (e.g., ibuprofen and naproxen) (Figure 4B). They also highlighted that the seasonal development of aquatic plants, algae and *Lemna* sp., in the SFCW system (mainly in summer and spring) may also have a high impact on the removal efficiency of pharmaceutical compounds. This agrees with other research reporting that the presence of *Lemna* sp. and a developed rhizosphere from Typha and Phragmites plants enhances biodegradation processes and the uptake of these contaminants [8,54]. Breitholtz et al. [55] investigated the removal efficiency of 92 pharmaceuticals in four SFCWs located in Sweden, with a HRT of between 2 and 8 days, and observed that the average estimated removal efficiencies ranged from 42% to 52%. The effects observed in the ecotoxicity tests with the macroalga (EC50s in the range of 8–46%) and the crustacean (LOECs in the range of 11–90%) could not be assigned to either pharmaceutical residues or metals, but generally showed that these treatment facilities release water with a relatively low toxic potential comparable to water that has been treated with advanced tertiary treatments. The soil employed to grow the emerging macrophytes in SFCWs is another interesting design parameter that should be taken into consideration, since it is proven that the removal of pharmaceutical compounds depends on its composition. Hussain et al. [56] found better removal efficiencies of ionophoretic antibiotics in SFCWs filled with sandy soil (32–36%) than in those filled with sandy clay loam soil (27–30%). This was explained by water being able to infiltrate the sandy soil more, providing greater solute-to-substrate interaction and oxidation conditions. The same authors found a significant correlation of antibiotic removal with soil temperature and oxidation–reduction potential indicating that microbial degradation was most likely involved in the attenuation of these compounds in SFCWs.

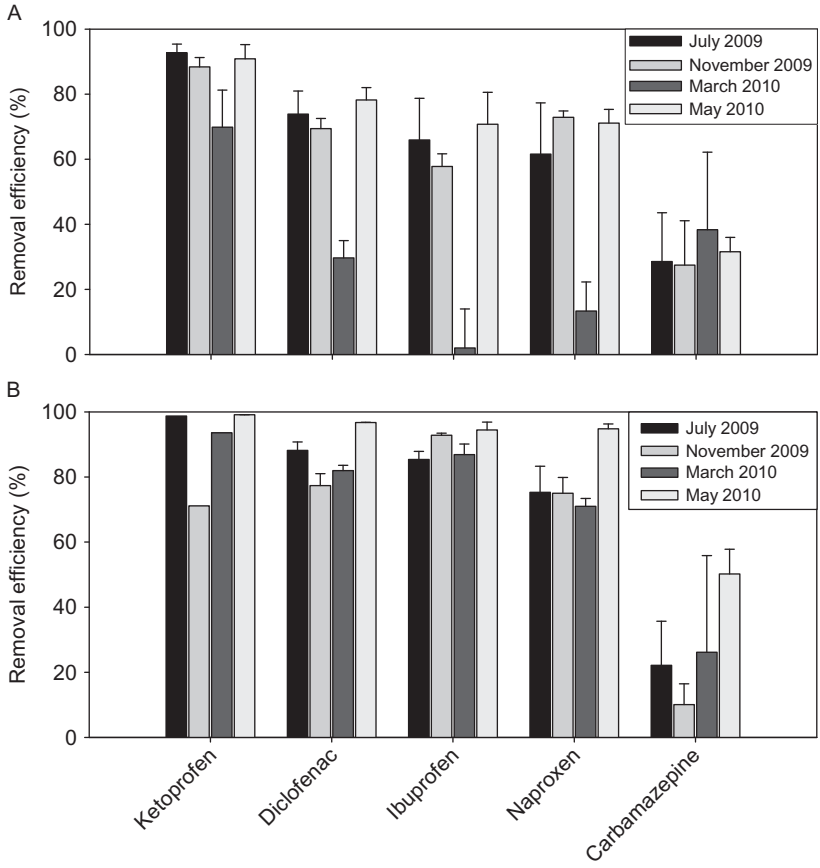

FIGURE 4 Seasonal removal efficiency of pharmaceutical compounds in a polishing pond (A) and SFCW (B) from Empuriabrava WWTP with a total surface area of 7 ha and a HRT of 4 and 9 days, respectively (Spain).

Finally, Xian et al. [57] studied the capacity of free-floating plants in CWs for removing antibiotics from swine wastewater and found that their presence was able to enhance the removal of sulfamethoxazole at higher rates than in unplanted control reactors (Figure 5). Further studies are needed to establish the effectiveness of this technology in removing other pharmaceutical compounds.

3 PONDS

3.1 Waste Stabilization Ponds

Waste stabilization ponds are large, shallow or deep basins in which raw sewage is treated entirely by natural processes involving both algae and

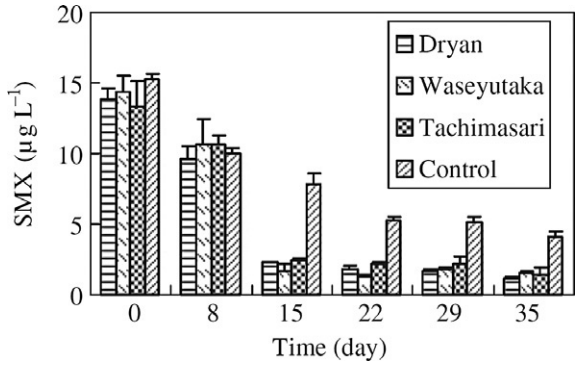

FIGURE 5 Levels of sulfamethoxazole (SMX) in swine wastewater during 35-day growth of macrophytes in floating bed system. *Reprinted from Q. Xian, L. Hu, H. Chen, Z. Chang, H. Zou, J. Environ. Manage., Copyright (2012), with permission from Elsevier.*

bacteria, which comprise a single or several series of anaerobic, facultative, or maturation ponds [58,59]. The main removal processes occurring in these systems are the biodegradation, photodegradation, and sorption of the settled organic matter.

Hijosa-Valsero et al. [16] reported that the capacity of *anaerobic and facultative pond* systems for removing pharmaceutical compounds in a decentralized WWTP located in the northwest Spain achieved removal efficiencies ranging from 70% to 80%. The anaerobic pond accounted for more than 80% of the total removal, which was explained by the high algal proliferation. Camacho-Muñoz et al. [60] studied two conventional lagoon systems and observed that most of the pharmaceutical compounds were slightly better removed by conventional activated sludge or oxidation ditch WWTPs in which higher water aeration takes place. Nevertheless, the slight difference between mean removal rates achieved in conventional treatments (64%) and mean removal rates in low-cost treatments (55%) was not considered sufficient to distinguish between both kinds of technologies. Li et al. [61] tested the use of a series of aerated lagoons for treating wastewater and found that, except for carbamazepine, the removal efficiencies of other detected pharmaceutical compounds (i.e., naproxen, ibuprofen, gemfibrozil, sulfamethoxazole, and diphenhydramine) by the lagoon system were relatively high in warm season, with the overall removal efficiencies ranging from 88% to 100%. Furthermore, they concluded that elimination mainly occurred in the first lagoon, at an efficiency of 70–100%. A similar study carried out by Conkle et al. [62] showed that three aerated lagoons in series were capable of removing up to 90% of pharmaceutical compounds, except carbamazepine, nadolol, sotalol, sulfapyridine, and gemfibrozil (see Table 2). Looking at these results, it appears that aerated lagoons are a suitable alternative for removing several pharmaceuticals occurring in urban wastewaters.

TABLE 2 Removal Efficiency of Pharmaceutical Compounds in the Ponds, Hybrid Systems, and Restored Wetlands Reported in the Chapter

Type	Configuration	Influent Water Quality	HRT (Days)	Removal Efficiency (%)	References
Ponds	Three aerated lagoons in series	Raw wastewater	27	Cotinine (99), caffeine (99), carbamazepine (−53), atenolol (99), nadolol (77), metoprolol (92), sotalol (30), sulfapyridine (76), sulfamethoxazole (91), acetaminophen (100), naproxen (99), ibuprofen (99), gemfibrozil (64)	[62]
	Two aerated lagoons in series	Raw wastewater	–	Caffeine (99a/99b), carbamazepine (−350a/−120b), naproxen (99a/98b), ibuprofen (99a/81b), gemfibrozil (62a/−82b), sulfamethoxazole (99a/81b), diphenhydramine (98a/68b)	[61]
	Lagoon	Primary-treated wastewater	–	Caffeine (77), ibuprofen (96), naproxen (99), carbamazepine (31), propranolol (nr), estriol (29%), gemfibrozil (46)	[60]
	Four polishing ponds in series (pilot plant)	Secondary-treated wastewater	4	Caffeine (49a/19b), ibuprofen (86a/58b), naproxen (72a/35b), diclofenac (93a/78b), ketoprofen (99a/96b), carbamazepine (5a/15b)	[53]
Combined hybrid systems	Two polishing ponds in series + SFCW	Secondary-treated wastewater	15	Caffeine (67), ibuprofen (92), naproxen (88), diclofenac (93), ketoprofen (98), carbamazepine (43)	[16]
	UASB + SFCW + HFCW	Raw wastewater	2.3	Caffeine (65a/99b), ibuprofen (50a/25b), naproxen (65a/45b), ketoprofen (65a/30b), carbamazepine (25a/10b)	[30]
	HFCW + VFCW	Raw wastewater	3	Estrogens >90%	[45]
Natural and restored wetlands	The Prado wetland (130 ha)	Four WWTP effluents	2–4	Ibuprofen (47), gemfibrozil (58)	[80]
	Restored wetland (100 ha)	WWTP effluents	7	Caffeine (4), ibuprofen (16), naproxen (7), diclofenac (6), carbamazepine (5), furosemide (53)	[79]

a, warm season; b, cold season; nr, no removal.

Polishing ponds are ponds that are located after a secondary treatment system, which can be either a maturation pond or an activated sludge WWTP. As has been mentioned earlier, their main characteristic in comparison with CWs is their higher water depth and the lower presence of plants in them (Figure 6). García et al. [9] carried out different laboratory-scale studies for assessing the capacity of algae and aquatic plants present in polishing ponds for removing sulfonamides and tetracyclines. They observed that tetracycline was mainly removed by photodegradation, oxytetracycline was removed by biodegradation or hydrolysis, and sulfonamides were mainly eliminated by biodegradation or indirect photodegradation. They concluded that the presence of aquatic vegetation such as *Spirogyra* sp. and *Zannichellia palustris* had no significant influence on the removal of sulfonamides and tetracyclines. Nevertheless, other laboratory studies pointed out that antibiotics such as tetracycline and oxytetracycline can be oxidized by root exudates produced by the aquatic plant roots such as *Myriophyllum aquaticum* and *Pistia stratiotes* [63]. This last finding was later confirmed by the decrease in the antibiotic removal rates upon addition of the antioxidant ascorbic acid, suggesting that reactive oxygen species are involved in the antibiotic modification process [64]. Therefore, although the presence of aquatic plants is normally beneficial, it depends on the plant species. Matamoros and Salvadó [53] found that the presence of *Lemna* sp. covering a polishing pond in summer may reduce the removal efficiency of pharmaceutical compounds (Figure 4A). This difference resulted in light being blocked out resulting in decreased photodegradation in the polishing pond. Furthermore, algae growth ceased during spring due to the lack of photosynthesis, and the resulting degradation of the algae consumed all the available oxygen from the polishing pond, causing sulfate reduction and a decrease in the elimination of aerobic

FIGURE 6 Picture showing a typical polishing pond located in the northeast Spain. *Lemna* sp. proliferation can be observed.

biodegradable compounds such as ibuprofen. Bearing this in mind, the adequate control and election of the vegetation present in polishing ponds seems to be a key design factor to achieve good pharmaceutical removal efficiencies.

3.1.1 Biological Filtration Systems Based on Macroinvertebrates

During recent years, the concept of improving the ecological and chemical water quality gained importance. Bearing this in mind, a new strategy, which has been named "waterharmonica," has recently been proposed by Claassen [65], building on the idea formulated by Mitsch [66] by which ecology and engineering are integrated. This technology is based on the presence of Daphnia and its capacity for removing particles [67]. Matamoros et al. [68] showed that biological filtration based on macroinvertebrates (e.g., Chydoridae, Daphnia, and Ostracoda) and algae is a reliable technology for removing some pharmaceutical compounds from treated wastewater. Figure 7 compares the removal efficiency of pharmaceutical compounds at different mass loading rates in summer and winter. The authors found that there is a high

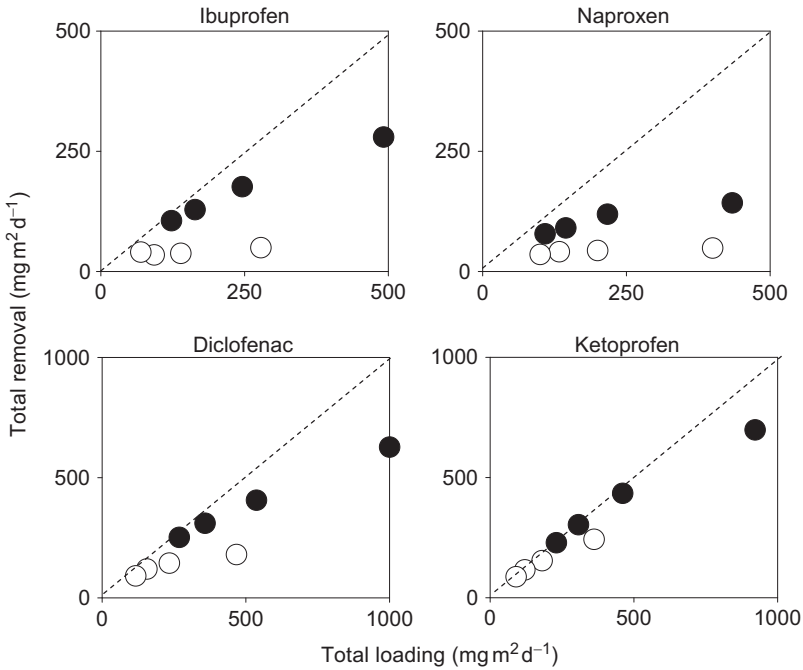

FIGURE 7 Average removal rate of pharmaceuticals in a biological filtration systems based on macroinvertebrates in summer and winter against their mass loading rates. From right to left, the circles represent a HRT of 1, 2, 3, or 4 days, respectively. Dotted lines represent 100% removal. *Reprinted from Matamoros et al. [68], Copyright (2012), with permission from Elsevier.*

dependence on seasonality and mass loading rate for compounds that were removed predominantly by photodegradation and biodegradation such as ibuprofen, naproxen, diclofenac, and ketoprofen.

3.2 High-Rate Algal Ponds

High-rate algal ponds (HRAPs) were described for the first time by Osward et al. [69] as shallow, open raceway ponds that have been used for treating municipal, industrial, or agricultural wastewater with the aim of producing algal biofuel [70]. Due to the rising prices of fuels in the recent decades, the suitability of using HRAP has been open for discussion, and today, it seems to be a promising technology, which may simultaneously meet the requirements of cleaning wastewater and producing energy. Even so, little attention has been paid to the capacity of this technology in removing pharmaceutical compounds. The first results demonstrate that the shallow HRAP design is advantageous to support the photodegradation of antibiotics such as tetracyclines during wastewater biological treatment [71], but as yet, there is no information regarding the relevance of microalgae present in the HRAPs and its effect on the removal of pharmaceutical compounds.

4 BUFFER STRIPS

Buffer strips along watercourses are primarily designed to protect surface water quality from agricultural runoff, but they also have a role in promoting biodiversity and landscape integration. A buffer strip normally implies a strip of vegetation that acts as a filter for sediment and its attached nutrients and pollutants. In this way, it improves or maintains the quality of water further downslope [72]. Lin et al. [73] recently evaluated the suitability of buffer strips for removing herbicides and veterinary antibiotics such as sulfamethazine, tylosin, and enrofloxacin. Figure 8 illustrates the effectiveness of vegetative buffer strips at reducing sulfamethazine transport. With 4–8 m of vegetated buffer strip, sulfamethazine loads in surface runoff were reduced by more than 70%, but the effectiveness between plant species was not significantly different. On the other hand, unvegetated control beds removed only 20–40%. Hence, a good solution for attenuating pharmaceutical discharge into the river waters may be the inclusion of buffer strips, which would simultaneously facilitate the landscape integration of the WWTP facility.

5 NATURAL AND RESTORED WETLANDS

The intensification of agriculture, with high fertilizer and pesticide usage rates, has increased the discharge of nutrients and agrochemicals into the

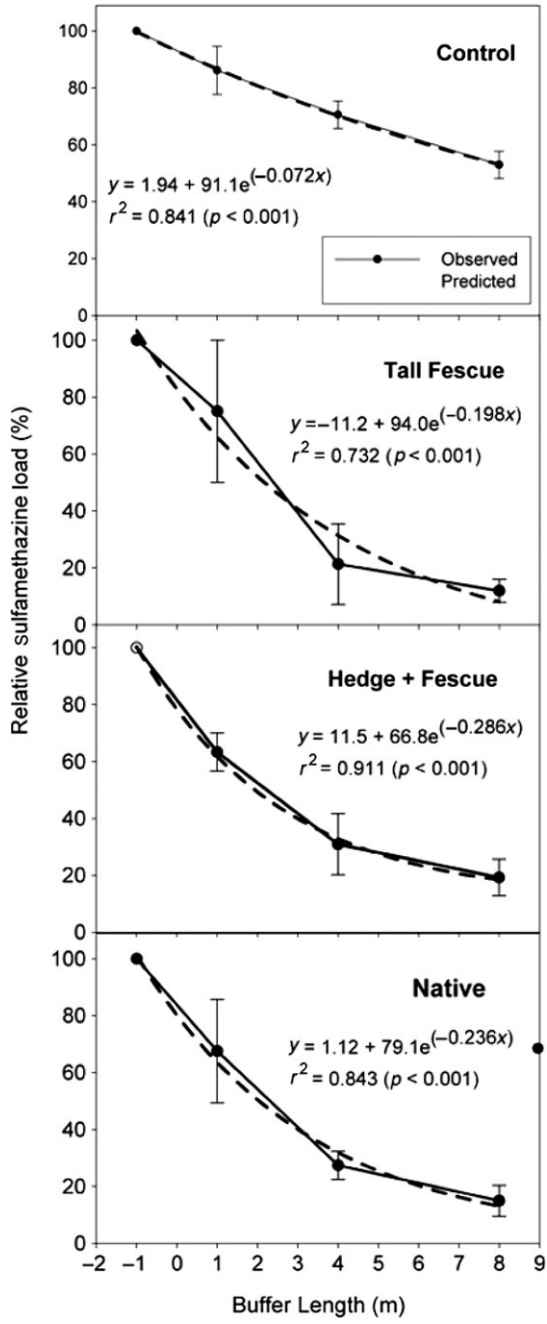

FIGURE 8 Relationship between relative dissolved sulfamethazine load reduction (y) and buffer length (x). Error bars represent 95% confidence interval. y-Intercept, load reduction at the interface between source and buffer; x-intercept, buffer length required to completely remove the antibiotic. *Reprinted from Lin et al. [73].*

aquatic ecosystems. However, in some countries, the discharge of nutrients from point sources such as WWTPs and industry still contributes significantly to riverine pollution loading [74]. Enhanced levels of nutrients in aquatic ecosystems have led to increased primary production, and the consequences derived from this eutrophication are algal blooms, increased water turbidity, oxygen depletion, and fish deaths [75]. In order to reduce sediments, nutrients, and pollutants entering streams, lakes, groundwater, and coastal waters, several countries such as Sweden, Denmark and the United States have created or restored wetlands with the aim to reinstall ecosystem services that were lost after wetlands were drained and converted into agricultural land [66,76,77]. Restored and natural wetlands are capable of improving the chemical and ecological status of the river waters before their discharge to the seas or indirect reuse. For example, Figure 9 shows the restored wetland created at the Aarhus River upstream of the Brabrand Lake (Denmark). HRTs in the restored wetland ranged between 3 and 20 days over the year (average 7 days) with an average water depth of 0.5 m. Hoffmann et al. [78] observed that these systems were capable to remove total nitrogen at a rate of 195 kg ha^{-1} $year^{-1}$. Conversely, Matamoros et al. [79] found that the attenuation efficiency of pharmaceutical compounds was compound-dependent and ranged from 4% for caffeine to 53% for furosemide. Nevertheless, it is worth mentioning that since the sampling campaigns of this study were undertaken during the

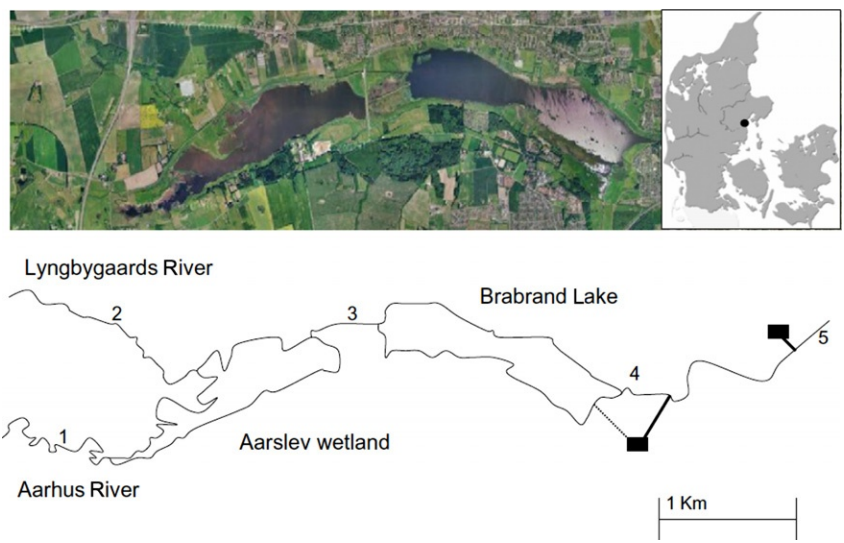

FIGURE 9 Map of Denmark showing a restored wetland (1. Aarhus River, 2. Lyngbygaards River, 3. Restored wetland outlet, 4. Brabrand outlet, and 5. Aarhus channel). Black boxes represent the WWTPs (from Google Earth). *Reprinted from Matamoros et al. [79], Copyright (2012), with permission from Elsevier.*

cold season, greater attenuations are expected during the warm season in which the high temperature and sunlight radiation increase the biodegradation and photodegradation rates, respectively. In this sense, Gros et al. [80] sampled a wetland system of 200 ha highly impacted by WWTP effluents from April to December and found significant removal efficiencies during wetland treatment for ibuprofen (47%) and gemfibrozil (58%) but noticed that the wetland treatment is difficult to assess due to the low concentrations in the affluent.

6 FUTURE TRENDS

The competitiveness of natural treatments with existing technologies, such as activated sludge wastewater treatment, has always been compromised by the land space they require. However, they still possess two main advantages: their low energy requirements and landscape integration. Brix [81] compared the energy requirement of biological treatments and conventional systems and found, for example, that CWs require $<0.1\ \text{kWh m}^{-3}$, whereas extended aeration sewage requires $2.39\ \text{kWh m}^{-3}$ and sequencing batch reactors require $1.13\ \text{kWh m}^{-3}$. CWs perform favorably in comparison to other treatment technologies according to their sustainability when using life-cycle assessment tools [82]. Furthermore, the combination of different natural treatments, which has already been proven to be efficient for removing nutrients, can favor pharmaceutical removal. Matamoros and Salvadó [53] demonstrated that the combined use of polishing ponds with SFCWs aids pharmaceutical attenuation, with mass removal of microcontaminants of up to 80% on average.

In addition to the treatment technologies that have been described in this chapter, there are other natural treatments, which may be a good alternative for removing pharmaceuticals, but for which, no information or study has been carried out until now. An example is the use of Willow Systems. The removal, uptake, and accumulation of phenol in willow trees (*Salix viminalis*) have already been determined in hydroponic cultures at a phytoremediation percentage of 90% [83], showing that Willow Systems are a suitable option for removing these compounds from industrial waste. Bearing this in mind, this would be a good technology for removing pharmaceutical compounds based on phenol groups such as paracetamol. Another example of a natural treatment is the use of floating mat wetlands, which employ floating matrices for plant support and are able to achieve removal rates of phosphorous and nitrogen up to 40% and 98%, respectively [84].

7 CONCLUDING REMARKS

This chapter shows that natural treatment systems (i.e., ponds, buffer strips, and constructed, natural, or restored wetlands) are an interesting option to

consider for removing pharmaceutical residues from a variety of waters, including domestic and industrial wastewaters as well as continental surface waters. Photodegradation, biodegradation, and sorption have been elucidated as the most plausible mechanisms involved in the elimination of pharmaceutical compounds from natural systems. Their removal is increased by increasing the HRT or by decreasing the HLR of the natural treatment system. The presence of vegetation in general favors the elimination of pharmaceutical compounds due to direct plant uptake or the indirectly increasing biodegradation processes. Temperature and sunlight radiation are relevant factors for removing pharmaceutical compounds in natural treatment technologies due to the high dependence of biodegradation and photodegradation, respectively. It has been reported that VFCWs are more efficient at removing pharmaceutical compounds than HFCWs due to the more aerobic conditions in them. Furthermore, it has been demonstrated that greater removal rates of pharmaceutical compounds are achieved in SSFCWs with lower water and sand layer depths. Unfortunately, clogging manifests a negative factor on the performance of SSFCWs at removing pharmaceutical compounds, requiring the use of a primary treatment to minimize this effect. Finally, the use of combined treatment technologies for removing pharmaceuticals appears to be a good strategy to increase the overall removal rate due to the combination of removal mechanisms. In conclusion, although the use of natural treatment technologies is a suitable alternative solution for removing pharmaceutical compounds from water, the land limitation is still a major issue that this technology needs to solve. Nevertheless, the landscape integration and low energy consumption are big advantages for decision makers to take into consideration, and therefore, it is clear that these systems can be competitive with other market technologies for many specific applications.

ACKNOWLEDGMENTS

The financial support of the Spanish Ministry of Economy and Innovation through project CTM2012-33547 is gratefully acknowledged. Dr. V. M. would like to acknowledge a JAE-Doc contract from the CSIC and the European Social Fund.

ABBREVIATIONS

CW	constructed wetland
HF	horizontal flow
HLR	hydraulic loading rate
HRAP	high-rate algal ponds
HRT	hydraulic residence time
HUSB	hydrolytic upflow sludge blanket
LECA	light expanded clay aggregates

SF	surface flow
SSF	subsurface flow
UASB	upflow anaerobic sludge blanket
VF	vertical flow
WWTPs	wastewater treatment plants

REFERENCES

[1] M. Cleuvers, Toxicol. Lett. 142 (2003) 185–194.
[2] K. Kümmerer, Annu. Rev. Environ. Resour. 35 (2010) 57–75.
[3] J. Persson, N.L.G. Somes, T.H.F. Wong, Water Sci. Technol. 40 (1999) 291–300.
[4] J.R. White, M.A. Belmont, C.D. Metcalfe, Sci. World J. 6 (2006) 1731–1736.
[5] V. Matamoros, J.M. Bayona, in: Handbook of Environmental Chemistry, Water Pollution, vol. 5, Springer Verlag, Berlin, 2008, pp. 199–217.
[6] G.l. Imfeld, M. Braeckevelt, P. Kuschk, H.H. Richnow, Chemosphere 74 (2009) 349–362.
[7] A.L. Boreen, W.A. Arnold, K. McNeill, Aquat. Sci. 65 (2003) 320–341.
[8] A.V. Dordio, M. Belo, D. Martins Teixeira, A.J. Palace Carvalho, C.M.B. Dias, Y. Picó, A.P. Pinto, Bioresour. Technol. 102 (2011) 7827–7834.
[9] A. Garcia-Rodríguez, V. Matamoros, C. Fontàs, V. Salvadó, Chemosphere 90 (2013) 2297–2302.
[10] S. Cole, Environ. Sci. Technol. 32 (1998) 218A–223A.
[11] P.F. Randerson, Environ. Biotechnol. 2 (2006) 78–79.
[12] J. Vymazal, L. Kröpfelova, Wastewater Treatment in Constructed Wetlands with Horizontal Sub-Surface Flow, Environmental Pollution, Springer, Netherlands, 2008.
[13] J. Puigagut, J. Villaseñor, J.J. Salas, E. Bécares, J. García, Ecol. Eng. 30 (2007) 312–319.
[14] C. Zwiener, F.H. Frimmel, Sci. Total Environ. 309 (2003) 201–211.
[15] J.L. Conkle, J. Gan, M.A. Anderson, J. Soils Sediments 12 (2012) 1164–1173.
[16] M. Hijosa-Valsero, V. Matamoros, J. Martín-Villacorta, E. Bécares, J.M. Bayona, Water Res. 44 (2010) 1429–1439.
[17] V. Matamoros, M. Hijosa, J.M. Bayona, Chemosphere 75 (2009) 200–205.
[18] M.S. Fountoulakis, S. Terzakis, A. Chatzinotas, H. Brix, N. Kalogerakis, T. Manios, Sci. Total Environ. 407 (2009) 2996–3003.
[19] V. Matamoros, C. Arias, H. Brix, J.M. Bayona, Environ. Sci. Technol. 41 (2007) 8171–8177.
[20] E. Ranieri, P. Verlicchi, T.M. Young, J. Hydrol. 404 (2011) 130–135.
[21] D.Q. Zhang, S.K. Tan, R.M. Gersberg, S. Sadreddini, J. Zhu, N.A. Tuan, Ecol. Eng. 37 (2011) 460–464.
[22] H. Brix, H. Schierup, in: B.C. Findlater, P.F. Cooper (Eds.), Constructed Wetlands in Water Pollution Control, Pergamon Press, Oxford, 1990, pp. 53–66.
[23] P.S. Burgoon, K.R. Reddy, T.A. DeBusk, Water Environ. Res. 67 (1995) 855–862.
[24] D.Q. Zhang, R.M. Gersberg, J. Zhu, T. Hua, K.B.S.N. Jinadasa, S.K. Tan, Environ. Pollut. 167 (2012) 124–131.
[25] C. Ávila, C. Reyes, J.M. Bayona, J. García, Water Res. 47 (2013) 315–325.
[26] J. Nivala, P. Knowles, G. Dotro, J. García, S. Wallace, Water Res. 46 (2012) 1625–1640.
[27] V. Matamoros, J.M. Bayona, Environ. Sci. Technol. 40 (2006) 5811–5816.
[28] B. Schink, Environmental Biotechnology, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, 2005229–257.

[29] M. Hijosa-Valsero, V. Matamoros, A. Pedescoll, J. Martín-Villacorta, E. Bécares, J. García, J.M. Bayona, Int. J. Environ. Anal. Chem. 91 (2011) 632–653.
[30] C. Reyes-Contreras, V. Matamoros, I. Ruiz, M. Soto, J.M. Bayona, Chemosphere 84 (2011) 1200–1207.
[31] V. Matamoros, J. García, J.M. Bayona, Environ. Sci. Technol. 39 (2005) 5449–5454.
[32] J. García, P. Aguirre, J. Barragán, R. Mujeriego, V. Matamoros, J.M. Bayona, Ecol. Eng. 25 (2005) 405–418.
[33] H.-L. Song, K. Nakano, T. Taniguchi, M. Nomura, O. Nishimura, Bioresour. Technol. 100 (2009) 2945–2951.
[34] C.C. Tanner, Water Sci. Technol. 44 (2001) 9–17.
[35] J. Kyambadde, F. Kansiime, L. Gumaelius, G. Dalhammar, Water Res. 38 (2004) 475–485.
[36] H. Brix, Water Sci. Technol. 35 (1997) 11–17.
[37] D.Q. Zhang, T. Hua, R.M. Gersberg, J. Zhu, W.J. Ng, S.K. Tan, Ecol. Eng. 49 (2012) 59–64.
[38] D.Q. Zhang, T. Hua, R.M. Gersberg, J. Zhu, W.J. Ng, S.K. Tan, Chemosphere 91 (2013) 14–21.
[39] D.Q. Zhang, T. Hua, R.M. Gersberg, J. Zhu, W.J. Ng, S.K. Tan, Chemosphere 90 (2013) 1568–1572.
[40] A.V. Dordio, P. Gonçalves, D. Texeira, A.J. Candeias, J.E. Castanheiro, A.P. Pinto, A.J.P. Carvalho, Int. J. Environ. Anal. Chem. 91 (2011) 615–631.
[41] A. Dordio, A.J.P. Carvalho, D.M. Teixeira, C.B. Dias, A.P. Pinto, Bioresour. Technol. 101 (2010) 886–892.
[42] A.V. Dordio, A.J. Estevao Candeias, A.P. Pinto, C. Teixeira da Costa, A.J. Palace Carvalho, Ecol. Eng. 35 (2009) 290–302.
[43] V. Matamoros, C. Arias, H. Brix, J.M. Bayona, Water Res. 43 (2009) 55–62.
[44] J. Vymazal, Ecol. Eng. 25 (2005) 478–490.
[45] F. Masi, G. Conte, L. Lepri, T. Martellini, M. Del Bubba, 9th IWA International Conference on Wetland Systems for Water Pollution Control, IWA, Avignon, France, 2004, pp. 461–468.
[46] M. Hijosa-Valsero, V. Matamoros, R. Sidrach-Cardona, J. Martín-Villacorta, E. Bécares, J.M. Bayona, Water Res. 44 (2010) 3669–3678.
[47] M. Truu, J. Juhanson, J. Truu, Sci. Total Environ. 407 (2009) 3958–3971.
[48] A. Ghermandi, D. Bixio, C. Thoeye, Sci. Total Environ. 380 (2007) 247–258.
[49] H. Brix, in: G.A. Moshiri (Ed.), Constructed Wetlands for Water Quality Improvement, Lewis Publishers, Boca Raton, 1993, pp. 9–22.
[50] M. Winkler, J.R. Lawrence, T.R. Neu, Water Res. 35 (2001) 3197–3205.
[51] K. Lin, J. Gan, Chemosphere 83 (2011) 240–246.
[52] V. Matamoros, J. García, J.M. Bayona, Water Res. 42 (2008) 653–660.
[53] V. Matamoros, V. Salvadó, Chemosphere 86 (2012) 111–117.
[54] D. Reinhold, S. Vishwanathan, J.J. Park, D. Oh, F. Michael Saunders, Chemosphere 80 (2010) 687–692.
[55] M. Breitholtz, M. Näslund, D. Sträe, H. Borg, R. Grabic, J. Fick, Ecotoxicol. Environ. Saf. 78 (2012) 63–71.
[56] S.A. Hussain, S.O. Prasher, R.M. Patel, Ecol. Eng. 41 (2012) 13–21.
[57] Q. Xian, L. Hu, H. Chen, Z. Chang, H. Zou, J. Environ. Manage. 91 (2010) 2657–2661.
[58] S. Kayombo, T.S.A. Mbwette, J.H.Y. Katima, S.E. Jorgensen, Water Res. 37 (2003) 2937–2943.
[59] D. Mara, in: D. Mara, N. Horan (Eds.), The Handbook of Water and Wastewater Microbiology, Academic Press, London, 2003.

[60] D. Camacho-Muñoz, J. Martín, J.L. Santos, I. Aparicio, E. Alonso, Water Air Soil Pollut. 223 (2012) 2611–2621.
[61] X. Li, W. Zheng, W.R. Kelly, Sci. Total Environ. 445–446 (2013) 22–28.
[62] J.L. Conkle, J.R. White, C.D. Metcalfe, Chemosphere 73 (2008) 1741–1748.
[63] N.P. Gujarathi, B.J. Haney, J.C. Linden, Int. J. Phytoremediation 7 (2005) 99–112.
[64] N.P. Gujarathi, B.J. Haney, H.J. Park, S.R. Wickramasinghe, J.C. Linden, Biotechnol. Prog. 21 (2005) 775–780.
[65] T.H.L. Claassen, Ecol. Eng. 30 (2007) 176–186.
[66] W.J. Mitsch, Ecol. Eng. 10 (1998) 119–130.
[67] C. Pau, T. Serra, J. Colomer, X. Casamitjana, L. Sala, R. Kampf, Water Res. 47 (2013) 181–186.
[68] V. Matamoros, L. Sala, V. Salvadó, Bioresour. Technol. 104 (2012) 243–249.
[69] W.J. Oswald, C.G. Golueke, W.U. Wayne, Advances in Applied Microbiology, Academic Press, New York, 1960223–262.
[70] J.B.K. Park, R.J. Craggs, A.N. Shilton, Bioresour. Technol. 102 (2011) 35–42.
[71] I. de Godos, R. Múñoz, B. Guieysse, J. Hazard. Mater. 229–230 (2012) 446–449.
[72] R. Barling, I. Moore, Environ. Manage. 18 (1994) 543–558.
[73] C. Lin, R. Lerch, K. Goyne, H. Garrett, J. Environ. Qual. 40 (2011) 791–799.
[74] B. Kronvang, J.P. Jensen, C.C. Hoffmann, P. Boers, in: R.F. Follett, J.L. Hatfield (Eds.), Nitrogen in the Environment: Sources, Problems and Management, Elsevier Science, Amsterdam, 2001, pp. 183–206.
[75] J. Kalff, Limnology: Inland Water Ecosystems, Prentice Hall, New Jersey, 2002, p. 592.
[76] C.C. Hoffmann, A. Baattrup-Pedersen, Ecol. Eng. 30 (2007) 157–166.
[77] G. Thiere, S. Milenkovski, P.E. Lindgren, G. Sahlén, O. Berglund, S.E.B. Weisner, Biol. Conserv. 142 (2009) 964–973.
[78] C. Hoffmann, B. Kronvang, J. Audet, Hydrobiologia 674 (2011) 5–24.
[79] V. Matamoros, C.A. Arias, L.X. Nguyen, V. Salvadó, H. Brix, Chemosphere 88 (2012) 1083–1089.
[80] B. Gross, J. Montgomery-Brown, A. Naumann, M. Reinhard, Environ. Toxicol. Chem. 23 (2004) 2074–2083.
[81] H. Brix, Water Sci. Technol. 40 (1999) 45–50.
[82] A. Dixon, M. Simon, T. Burkitt, Ecol. Eng. 20 (2003) 297–308.
[83] A.S. Ucisik, S. Trapp, Environ. Toxicol. Chem. 25 (2006) 2455–2460.
[84] R.H. Kadlec, S. Wallace, Treatment Wetlands, CRC Press, Boca Raton, 2009, p. 1016.
[85] V. Matamoros, A. Caselles-Osorio, J. García, J.M. Bayona, Sci. Total Environ. 394 (2008) 171–176.
[86] C. Ávila, A. Pedescoll, V. Matamoros, J.M. Bayona, J. García, Chemosphere 81 (2010) 1137–1142.
[87] C. Reyes-Contreras, M. Hijosa-Valsero, R. Sidrach-Cardona, J.M. Bayona, E. Bécares, Chemosphere 88 (2012) 161–167.
[88] A. Dordio, J. Pinto, C.B. Dias, A.P. Pinto, A.J.P. Carvalho, D.M. Teixeira, Int. J. Environ. Anal. Chem. 89 (2009) 835–848.
[89] N. Park, B.J. Vanderford, S.A. Snyder, S. Sarp, S.D. Kim, J. Cho, Ecol. Eng. 35 (2009) 418–423.

Chapter 13

Removal of Pharmaceuticals by Bank Filtration and Artificial Recharge and Recovery

Sung Kyu Maeng*, Carlos N.A. Salinas Rodriguez* and Saroj K. Sharma†

**Department of Civil and Environmental Engineering, Sejong University, Seoul, Republic of Korea*

†UNESCO-IHE Institute for Water Education, Delft, The Netherlands

Chapter Outline

Comprehensive Analytical Chemistry, Vol. 62. http://dx.doi.org/10.1016/B978-0-444-62657-8.00013-6

1 INTRODUCTION

1.1 Bank Filtration and Artificial Recharge and Recovery as Water Treatment Systems

Managed aquifer recharge (MAR) systems like bank filtration (BF) and artificial recharge and recovery (ARR) have been successfully employed in many countries to augment groundwater and increase quantity of water available as well as to improve water quality. These systems are robust, reliable, and capable of removing multiple contaminants. Depending upon the quality of the water source used for recharge (river or lake water, stormwater, and wastewater effluent) and local hydrogeological conditions, MAR systems can serve at least as a pretreatment or sometimes even as a total treatment system [1,2].

BF is a traditional, efficient, and well-accepted method of surface water treatment in many countries in Europe including Slovak Republic, Germany, the Netherlands, Hungary, and France [3–5]. BF systems have also been supplying drinking water to several communities in the United States for nearly half a century [6]. In Asia, BF systems have recently been implemented in South Korea (Changwon city 80,000 m^3/day and Kimhae city 180,000 m^3/day). BF can occur with water extracted indirectly, either from a river BF or from a lake BF (LBF) according to what is available in the specific site location, by drawing it through the subsurface prior to use. Extraction is accomplished by an infiltration gallery or line of wells (horizontal, vertical, or sloped) located at a short to intermediate distance from the bank of a river or lake. During extraction, the groundwater discharge into the river decreases and the groundwater table near the waterline may decrease below the river water level. Consequently, surface water enters the aquifer and flows to the gallery or wells. BF systems are generally not operated to provide storage of surface water underground; however, they provide water quality improvements during subsurface transport.

ARR is an engineered system designed for intentional treatment, storage, and withdrawal of water in aquifers; storage depends on infiltration versus pumping rates. ARR methods are employed when the local geologic conditions and/or water quality in the river/lake is not suitable for induced infiltration (BF) or when different source waters are available (e.g., stormwater). ARR can be employed without pretreatment of the source or the source water can be treated to the required level (e.g., filtration) prior to recharge [2]. ARR is also practiced to control seawater intrusion into coastal aquifers, control land subsidence caused by declining groundwater levels, maintain base flow in some streams, and raise water levels to reduce the cost of groundwater pumping. ARR can be accomplished by (1) surface spreading, (2) infiltration through the unsaturated zone (vadose zone injection wells), or (3) direct injection into groundwater. ARR systems are also further categorized as (1) aquifer storage and recovery (ASR) that involves injection of water into a well for storage and recovery from the same well and (2) aquifer storage transfer

and recovery (ASTR) that involves injection of water into a well for storage and recovery from a different well, generally to provide additional water treatment. ASTR differs from ASR in that it is more applicable to confined aquifers where infiltration is not feasible.

1.2 Advantages and Limitations of BF and ARR Systems

The following are the main advantages and limitations of employing BF and ARR systems for water treatment [2,7].

1.2.1 Advantages

1. BF and ARR are multiple contaminant removal systems that improve water quality by removing suspended solids, microorganisms, heavy metals, nitrogen, and organic micropollutants.
2. BF and ARR are sustainable natural treatment processes that avoid or reduce the use of chemicals and produce biologically stable water.
3. BF and ARR dampen concentration peaks and temperature fluctuations.
4. BF and ARR replace or support other treatment processes by providing a robust barrier for multiple contaminants and reduce the overall cost of water treatment.

1.2.2 Limitations

1. The performance of BF and ARR systems is site-specific and their feasibility depends on the local hydrogeological conditions.
2. Aquifer materials may leach under certain reducing conditions (e.g., reductive dissolution when the redox shifts from oxic to anoxic), leading to increased concentrations of iron and manganese in extracted water.
3. There can be clogging of the river/lake or infiltration basins due to accumulation of suspended matter, if the systems are not properly designed.
4. BF and ARR may be only a limited barrier for certain contaminants such as persistent organic micropollutants. Furthermore, some contaminants may not be removed by these systems if the conditions are not favorable.

2 FACTORS INFLUENCING THE BEHAVIOR OF PHARMACEUTICALS IN BANK FILTRATION AND ARTIFICIAL RECHARGE AND RECOVERY

When the rivers and lakes are relatively clean and unpolluted, BF and ARR could be the only method of water treatment, and water extracted from these systems is directly supplied to the consumers with no or minimal posttreatment (mainly disinfection) [5,8,9]. Due to the escalating use of organic chemicals and increasing impact of wastewater treatment plant and industrial effluents worldwide, the concentration of organic micropollutants, namely, pharmaceutically

active compounds (pharmaceuticals), endocrine-disrupting compounds (EDCs), and other persistent organic compounds including pesticides, is increasing in water sources worldwide [10,11]. Under these circumstances, some additional treatment steps after the MAR systems may be required to reduce the health risks to the consumers and to ensure that the treated water meets the local water quality guidelines and standards. In this context, proper understanding of the factors affecting the removal of these micropollutants during soil passage is essential in order to improve the application of these natural treatment systems and to optimize the subsequent posttreatment systems.

BF and ARR reduce the concentrations of organic micropollutants in water including pesticides, pharmaceutical and personal care products, EDCs, and pharmaceuticals to some extent [12–14]. The fate of pharmaceuticals in BF and ARR system depends on several factors including (i) redox conditions, (ii) travel time and travel distance, (iii) background bulk organic matter, (iv) other source water characteristics, (v) type of the pharmaceuticals present and their concentrations, (vi) presence of other competing micropollutants, and (vii) pretreatment applied (specifically in the case of an ARR system) [15,16]. Some of these factors are elaborated further in the following texts.

2.1 Redox Conditions

Redox conditions in the aquifer significantly influence behavior of pharmaceuticals during BF and ARR and are considered to be the master variable in its attenuation [17]. Some micropollutants are more degradable under anoxic conditions than under aerobic conditions and vice versa. The redox conditions along the flow path are determined by the type and quantity of available degradable organic matter and the electron acceptors. Mainly, the oxygen concentration in the source water and the concentration of oxygen-consuming substances (biodegradable organic matter) present in the water influence the redox conditions. The removal of oxygen during percolation might be favorable to anoxic conditions in the saturated zone. Furthermore, mixing of the recharge water into the aquifer may create conditions that are not in thermodynamic equilibrium, consequently driving several redox reactions in the aquifer.

During BF, oxygen in the water depletes with the travel distance and ultimately may lead to iron and manganese reduction [18]. In ARR systems, the oxygen concentrations in the aquifer can be increased by aeration and removal of oxygen-consuming contaminants during pretreatment (before recharge); therefore, aerobic conditions could be more dominant. Furthermore, existence of both aerobic and anoxic conditions in different zones may also favor the removal of specific micropollutants as the removal of trace organics is highly dependent on the redox conditions and concentration of bulk organic matter present [12,13]. In the case of ARR, intermittent application of the recharge water (wet/dry cycle) also helps to influence the redox conditions as oxygen can pass deeper in the soil layer during the dry period.

2.2 Travel Time and Travel Distance

The travel time of water in the aquifer (before abstraction), which is a function of travel distance, significantly affects the removal of pharmaceuticals during BF and ARR as many of the removal mechanisms (biodegradation, adsorption, retardation, and dispersion) are time-dependent [19]. Travel time depends on the distance of the abstraction well from the recharge source, type of the wells used (horizontal or vertical), spacing between the wells, number of wells, and the pumping rate (production capacity). In general, longer travel time increases the removal of contaminants during soil passage [2,9,16]. Furthermore, depending upon the travel time, the water will pass through different redox zones (oxic and anoxic) and several regions with different mineralogical characteristics facilitating adsorption, chemical precipitation, and ion-exchange reactions, which may ultimately influence the removal of pharmaceuticals.

2.3 Background Bulk Organic Matter

Bulk organic matter present in the source water serves as a primary substrate/carbon source for the biodegradation of pharmaceuticals during soil passage, especially if oligotrophic conditions are present [14,20]. Microbial activity is one of the dominant sources for mineralization, or the complete conversion of organic compounds into inorganic products, in waters and soils [21]. Furthermore, if the majority of the bulk organic matter in the source water is only absorbable and not biodegradable (humic substances), there can be competition for adsorption sites between bulk organic matter and adsorbable and slowly biodegradable pharmaceuticals. Another factor to be considered for the fate of pharmaceuticals during their soil passage is their potential for mineralization.

2.4 Type of the Pharmaceutical Present

Removal of pharmaceuticals during soil passage is highly influenced by its characteristics, namely, molecular size, polarity and surface charge, concentration, and solubility in water (hydrophobicity/hydrophilicity). Depending on their log K_{ow} values (octanol–water partition coefficient, an index of compound hydrophobicity), removal rates for different pharmaceuticals could range from low to very high. log K_{ow} reflects both hydrophobicity affecting adsorption and compound complexity affecting biodegradation. Two main characteristics are common to persistent compounds after soil passage; they are hydrophilic (polar) and they have structural features that prevent enzymatic attack and render them resistant to biodegradation [10,22]. Compounds such as primidone, carbamazepine, and clofibric acid have been reported to be partly recalcitrant during underground passage. Polar organic molecules, such as complexing agents, pesticides, industrial products like aromatic sulfonates, pharmaceutical compounds, and personal care products, are of recent concern [23].

2.5 Presence of Other Competing Organic Micropollutants

Biodegradation, adsorption, and dilution are the main mechanisms of removal of pharmaceuticals during soil passage. If there is high concentration of other inorganic contaminants or organic micropollutants present in the water, there is likely competition for carbon source and oxygen (for biodegradation) and adsorption sites. Relative competition depends on the size, concentration, solubility, and polarity of the competing molecules.

2.6 Pretreatment Applied

In case of ARR, some pretreatment (coagulation, sedimentation, and filtration) may be applied before the recharge in order to increase infiltration rate and to decrease clogging of the aquifer [24]. Some of the pharmaceuticals may be partially removed during this pretreatment. Furthermore, in some cases, ozonation or advanced oxidation may be applied as pretreatment, which will considerably improve the removal of pharmaceuticals present in the source water during soil passage [25–27]. A pretreatment system for ARR is elaborated further in Section 4.

3 ATTENUATION OF PHARMACEUTICALS DURING BANK FILTRATION AND ARTIFICIAL RECHARGE AND RECOVERY

As previously mentioned in Section 2, behavior of pharmaceuticals in MAR relies upon their physicochemical properties or specific geographic characteristics of BF and ARR sites, which ultimately means that pharmaceuticals are affected in a different manner according to the type MAR systems (BF or ARR) applied; therefore, it is necessary to understand which and how the parameters governing the attenuation of pharmaceuticals in MAR systems are. Table 1 shows the comparison of both systems and the behavior of most important parameters that influence the removal of pharmaceuticals. Among MAR systems, BF has been more practiced and studied than ARR. A summary of BF an ARR sites reported in following Sections 3.1 and 3.2 is presented in Table 2.

3.1 Bank Filtration

Most of the previous studies on the fate of pharmaceuticals during BF are referred to research conducted in Lake Tegel or Lake Wannsee in Germany, with study period of 2.5 years [9,12,31–33,38]. There are also recent studies conducted in United States at BF sites along different rivers that enrich the literature available about this topic [29]. Table 3 summarizes the fate and occurrence of pharmaceuticals at different BF sites that were included with their respective removal efficiencies grouped into four categories (low (<25%),

TABLE 1 Comparison Between Bank Filtration and Artificial Recharge and Recovery

Parameters	Artificial Recharge and Recovery	Bank Filtration
Travel time	Short (>50 days)	Longer (>3 months)
Selective intake and pretreatment	Yes	No
Redox conditions	Mainly oxic zone	Short oxic zone followed by a more reduced zone (anoxic conditions)
Temperature change (seasonality)	High	Low

Source: Modified from Maeng et al. [19]; Grunheid et al. [9].

moderately low (26–50%), relatively high (51–79%), and high (>80%)). Eight major groups of pharmaceutical compounds (e.g., antibiotics, nonsteroidal anti-inflammatory drugs and analgesics, anticonvulsants, antidepressants, beta-blockers, lipid regulators, X-ray contrast media, and steroid hormones) were analyzed with respect to their removal efficiencies after BF is applied. For better understanding, only production wells were included in the analysis, and more detailed information (monitoring wells) can be found in cited references.

Heberer et al. [31] investigated 19 targeted antibiotics at a LBF site located in Berlin, which is well characterized and instrumented in terms of production wells and transects of monitoring wells where travel distances, travel times, and redox conditions are well defined. They detected 7 out of 19 target antibiotics in Lake Wannsee water used for BF: sulfamethoxazole, acetyl sulfamethoxazole, anhydroerythromycin, clarithromycin, roxithromycin, trimethoprim, and clindamycin. However, all antibiotics were completely attenuated after less than 2–4 months of travel times except for sulfamethoxazole. In other German site, Lake Tegel in Berlin, Grünheid et al. [9] compared bulk organic matter and organic micropollutant removal between BF and ARR sites. The BF site exhibited oxic conditions followed by prolonged anoxic conditions.

Schmidt et al. [37] also investigated some antibiotics, named sulfamethoxazole, clarithromycin, trimethoprim, and clindamycin at four BF sites located along the river Rhine (Rhine A and Rhine B), Elbe, and Ruhr. Similarly to other researchers, they also found that removal efficiency for selected pharmaceuticals was higher than 70%, except for sulfamethoxazole that exhibited relatively low removal (0–25%) at Rhine A and Rhine B sites, which can be a result of the oxic conditions present. Different values were estimated at Ruhr BF site that

TABLE 2 Summary of Bank Filtration and Artificial Recharge and Recovery Sites

Site (Location)	Type	Source	Aquifer Thickness (m)	Travel Distance (m)	Travel Time (d)	Study Period	References
Platte River (United States)	BF	River	<40			NR	[28]
South Platte River (United States)	BF	River	10.7			~1 year	[29]
Cedar River (United States)	BF	River	25–30			~1 year	[29]
Ohio River (United States)	BF	River	33			~1 year	[29]
Lake Wannsee (Germany)	BF	Lake	NR		<30	31 months	[30]
Lake Wannsee (Germany)	BF	Lake	NR		60–120	32 months	[31–33]
Lake Tegel (Germany)	BF	Lake	<40	90	135		[9,34–36]
Lake Wannsee (Germany)	BF	Lake	<40	33	45		[34]
Rhine A (Germany)	BF	River	12–15	160	7–20	26 months	[37]
Rhine B (Germany)	BF	River	10–12	70	12–60		[37]
Elbe (Germany)	BF	River	40–55	270	45–300		[37]
Ruhr (Germany)	BF	River	5	125	5–15		[37]
Lake Tegel (Germany)	ARR	Lake	<40	50	50		[9,12,38,39]

BF, bank filtration; NR, not reported; ARR, artificial recharge and recovery.
Source: Modified from Maeng et al. [16].

TABLE 3 Removal Efficiencies of Pharmaceuticals During Bank Filtration

Therapeutic Use	Compound	Removal Efficiencies *[▼]Low (<25%), [◆]Moderately Low (26–50%), [◇]Relatively High (51–79%), [△]High (>80%)*	References
Antibiotics	Sulfamethoxazole	▼: Rhine A (20d,160m)[a], Rhine B (60d,70m); ◇: Elbe (300d,270m); △: Lake Wannsee (45d,33m), Lake Tegel (135d,90m), Ruhr (15d, 125m)	[9,32,34,37]
	Acetyl sulfamethoxazole	△: Lake Wannsee (120d,75m)	[31]
	Clarithromycin	◇: Rhine A (20d,160m), Elbe (300d,270m), Ruhr (15d, 125m); △: Lake Wannsee (120d,75m)	[31,37]
	Roxithromycin	▼: Ruhr (15d, 125m); ◇: Ruhr (15d, 125m); △: Lake Wannsee (120d,75m)	[31,37]
	Trimethoprim	△: Lake Wannsee (120d,75m), Rhine A (20d,160m), Elbe (300d,270m), Ruhr (15d,125m)	[31,37]
	Clindamycin	◇: Rhine B (60d,70m), Elbe (300d,270m), Ruhr (15d,125m); △: Lake Wannsee (120d,75m), Rhine A (20d,160m)	[31,37]
Nonsteroidal anti-inflammatory drugs (NSAIDs) and analgesics	Diclofenac	◇: Lake Wannsee (120d,75m), Lake Tegel (135d,90m); △: Rhine A (20d,160m), Rhine B (60d,70m), Elbe (300d,270m), Ruhr (15d,125m)	[37,40]
	Ibuprofen	△: Rhine A (20d,160m), Elbe (300d,270m), Ruhr (15d,125m)	[37]
	Indomethacin	◇: Rhine A (20d,160m), Elbe (300d,270m); △: Lake Wannsee (120d,75m), Lake Wannsee (45d,33m)	[34,37,40]
	Naproxen	◇: Elbe (300d,270m), Ruhr (15d,125m), △: Rhine A (20d,160m)	[37]
	Pentoxifylline	△: Rhine A (20d,160m), Elbe (300d,270m)	[37]

Continued

TABLE 3 Removal Efficiencies of Pharmaceuticals During Bank Filtration—Cont'd

Therapeutic Use	Compound	Removal Efficiencies *[▼]Low (<25%), [◆]Moderately Low (26–50%), [◇]Relatively High (51–79%), [△]High (>80%)*	References
Anticonvulsants	Carbamazepine	▼: Rhine A (20d,160m), Rhine B (60d,70m); ◇: Elbe (300d,270m); △: Ruhr (15d,125m)	[37]
	Primidone	◆: Lake Wannsee (120d,75m)	[40]
Beta-blockers	Atenolol	△: Rhine A (20d,160m), Elbe (300d,270m), Ruhr (15d,125m)	[37]
	Metoprolol	△: Rhine A (20d,160m), Rhine B (60d,70m), Elbe (300d,270m), Ruhr (15d,125m)	[37]
	Bisoprolol	◇: Rhine A (20d,160m), Ruhr (15d,125m)	[37]
Lipid regulators	Bezafibrate	◇: Lake Tegel (135d,90m)[a]; △: Rhine A (20d,160m), Rhine B (60d,70m), Elbe (300d,270m), Ruhr (15d,125m), Lake Wannsee (45d,33m)	[34,37,40]
	Fenofibric acid	◇: Rhine A (20d,160m), Rhine B (60d,70m)	[37]
	Clofibric acid	▼: Lake Wannsee (120d,75m), Lake Tegel (135d,90m); △: Ruhr (15d,125m)	[37,40]
X-ray contrast media	AOI	◇: Lake Tegel (135d,90m)	[9]
	Iopromide	△: Lake Tegel (135d,90m), Rhine A (20d,160m), Rhine B (60d,70m), Elbe (300d,270m), Ruhr (15d,125m)	[9,37]
	Iopamidol	▼: Rhine A (20d,160m); ◆: Rhine B (60d,70m); ◇: Elbe (300d,270m); △: Ruhr (15d,125m)	[37]
	Iomeprol	△: Rhine A (20d,160m), Rhine B (60d,70m), Elbe (300d,270m), Ruhr (15d,125m)	[37]
	Iohexol	△: Rhine A (20d,160m), Rhine B (60d,70m), Elbe (300d,270m), Ruhr (15d,125m)	[37]

[a]*Site name (travel time, travel distance).*

has relatively short travel times compared to Rhine A and Rhine B but mainly exhibited anaerobic conditions. Specific behavior of each compound has to be analyzed, especially with respect to presence of anoxic/anaerobic conditions, as suggested by Grünheid et al. [9]. The removal of antibiotics was gradually increased as travel time and travel distance increased.

Nonsteroidal anti-inflammatory drug and analgesics, NSAIDs, also known as pain killers, are commonly used for symptoms of arthritis, bursitis, gout, swelling, stiffness, and joint pain around the world [41]. Large amounts of NSAIDs are sold by prescription or nonprescription worldwide (i.e., over-the-counter drugs) [10]. High concentrations of NSAIDs have been detected in aquatic environments and wastewater due to their high consumption in human medical care and, to some degree, because of their persistent characteristics [42]. Removal efficiencies of NSAIDs are reported in values greater than 50% during soil passage, and a possible explanation is the occurrence of biodegradation or biotransformation (biotic) and sorption (abiotic). Studies on phenazone (redox-sensitive compounds), an NSAID-type compound, have shown that its removal is more likely to happen under oxic conditions [12]. Massmann et al. [12] suggest that biodegradation by aerobic bacteria was the main removal mechanism of phenazone-type pharmaceuticals during soil passage. Monitoring of pH is also important to have better understanding of removal of many of NSAIDs because many of them remain as ionic species in the aquatic environment.

Anticonvulsant pharmaceuticals are the most persistent type during BF. Carbamazepine is the anticonvulsant most frequently detected in the environment [12,33,37,39,43–45]; and it has shown a persistent behavior in the aquatic environment, with low removal (<10%) due to its poor biodegradability [46–49]. A number of laboratory and field studies on BF have also revealed low removals of primidone and dilantin during infiltration [39,40]. Drewes et al. [43] found that there was no change in carbamazepine and primidone concentrations during soil aquifer treatment for estimated travel times of up to 6 years. Based on the performance of selected anticonvulsants in these studies, neither BF nor ARR is effective in the removal of anticonvulsants.

In a study conducted by Schmidt et al. [37], they suggest removal of beta-blockers is due to sorption and/or biodegradation during soil passage. The removal efficiency for beta-blockers (atenolol, metoprolol, bisoprolol, and sotalol) in BF sites located along the rivers Rhine (at two different locations), Elbe, and Ruhr was higher than 70%.

Metabolites of lipid regulators (e.g., clofibric acid and fenofibric acid) are important to monitor during soil passage because they are derived from "prodrugs," administered in an inactive form. A prodrug undergoes metabolic conversion of the parent compound to an active metabolite, which cures the symptoms, not the parent compound, which is an inactive form (e.g., clofibrate and fenofibrate). Like the NSAIDs, many lipid regulators remain as ionic species in the aquatic environment [50]. Clofibric acid, fenofibric acid, and

salicylic acid are common metabolites originating from clofibrate, fenofibrate, and aspirin, respectively. Clofibric acid is one of the most common metabolites studied for MAR systems, and it is often detected in the aquatic environment, wetlands, and wastewater treatment plants. In Berlin, at the Lake Tegel BF site, concentration of clofibric acid was found to have an increment with time [40], which is explained by the high consumption of the fibrate-based lipid regulators during the 1990s. Although the use of fibrate-based lipid regulators has been significantly reduced over the late 1990s and early 2000s, the presence of clofibric acid still is detected in deeper layers of the aquifer [40].

X-ray contrast agents, measured as adsorbable organic iodine (AOI), have been reported by Grünheid et al. [9] at sites located in Lake Tegel. Along the pathway flow for BF site, a gradual change in redox conditions was detected, from oxic to prolonged anoxic conditions. AOI removal efficiency was 60%. Grünheid et al. [9] found that AOI removal efficiencies and oxidation–reduction potential were inversely correlated and dehalogenation of AOI was enhanced under anoxic conditions (i.e., redox-sensitive compound). As a result of long-term monitoring of AOI, seasonal effect was detected in AOI concentrations expressed as variations of dilution in discharges [51]. In a different study, Schittko et al. [52] obtained similar results at the same BF site, with removal value of AOI at 63%. Four individual iodinated X-ray contrast agents (iopromide, iopamidol, iomeprol, and iohexol) were measured at different BF sites located in Germany, and those compounds have been found to be easily removable, with values higher than 80% [9,37,51,52].

3.2 Artificial Recharge and Recovery

ARR sites are less frequently reported; also, the compounds usually reported are lesser than in the case of BF sites. A summary of the most important studies conducted at ARR sites is shown in Table 4.

With respect to antibiotics, the removal of sulfamethoxazole at the ARR site in Lake Wannsee was lower than that at BF site (50% and 75%, respectively), but the difference in residence times should be taken into account as factor for control of redox conditions [31]. The ARR site showed a shorter residence time with oxic conditions.

With respect to beta-blockers, five compounds of this type (atenolol, sotalol, celiprolol, propranolol, and metoprolol) were found to be below the limit of quantification (0.025 μg/L) after ARR using agricultural fields in Braunschweig, Germany, with water coming from secondary effluent [46].

In the case of NSAIDs, Massmann et al. [12] found the removal of phenazone at an ARR site varied by season and was relatively high during winter compared to summer. This was due to oxic conditions that mainly occurred at the ARR site during winter when temperatures were low. It is equally important to monitor redox conditions during BF and ARR as it plays a key role for pharmaceutical removal.

TABLE 4 Removal Efficiencies of Pharmaceuticals During Artificial Recharge and Recovery

Therapeutic Use	Compound	Removal Efficiencies *[▼]Low (<25%), [◆] Moderately Low (26–50%), [◇]Relatively High (51–79%), [△]High (>80%)*	References
Antibiotics	Sulfamethoxazole	◇: Lake Tegel (50d,50m)[a]	[9]
Nonsteroidal anti-inflammatory drugs (NSAIDs) and analgesics	Diclofenac	△: Lake Tegel (50d,90m)	[39]
	Indomethacin	△: Lake Tegel (50d,90m)	[39]
Anticonvulsants	Primidone	◆: Lake Tegel (50d,90m)	[39]
Lipid regulators	Bezafibrate	△: Lake Tegel (50d,90m)	[39]
	Clofibric acid	◇: Lake Tegel (50d,90m)	[39]
X-ray contrast media	AOI	◆: Lake Tegel (50d,50m)	[9]
	Iopromide	△: Lake Tegel (50d,50m)	[9]
Steroid hormones	Estrone (E1)	△: Lake Tegel (50d,90m)	[38]

[a]*Site name (travel time and travel distance).*

In the ARR site located at Lake Tegel, authors have found that the removal of clofibric acid was greater than 70% [37,39,40]. Moreover, bezafibrate was found to be significantly removed during subsurface infiltration [10]. Thus, the removal performance of lipid regulators in ARR systems varied from site to site, but most of lipid regulators from MAR systems reviewed in this study were removed greater than 50%.

As mentioned in the previous section, Grünheid et al. [9] conducted field studies at BF and ARR sites (Lake Tegel, Berlin, Germany) to investigate the fate of X-ray contrast agents. At the ARR site, oxic conditions prevailed differently to the case of BF. AOI removal efficiency at ARR site was 30% (which is the half of efficiency found at BF site), because AOI compounds are sensitive to redox conditions and the presence of anoxic conditions are favorable to their removal.

Steroid hormones such as synthetic estrogens (e.g., 17α-ethinylestradiol (EE2)) and natural estrogens (e.g., estrone (E1) and 17β-estradiol (E2)) are of special concern because of potential adverse effects on human health and aquatic life at very low concentration (ng/L) [53,54]. A number of laboratory-scale and field studies on the fate of estrogens during soil passage have been carried out [28,38,55,56]. A field study carried out by Zuehlke et al. [38]

showed that E2 and EE2 were not detected in the surface water from Berlin (LOQ, 0.2 ng/L) and E1 was removed greater than 80% at a monitoring well located close to the lake shore. This study demonstrated that a significant removal of E1 was possible during soil passage even within a short distance. Estrogen compounds are generally hydrophobic compounds that are typically neutral; thus, adsorption is likely to be the main removal mechanism. Mansell et al. [56] found that steroids were removed below the detection limit by a combination of adsorption and biodegradation; thus, the removal of estrogen compounds not only is dependent on adsorption but also is affected by biodegradation.

4 HYBRIDIZATION OF BANK FILTRATION AND ARTIFICIAL RECHARGE AND RECOVERY IN MULTIBARRIER TREATMENT

The occurrence of organic micropollutants, such as pharmaceuticals, in water resources including wastewater effluent is a major constraint to either indirect potable reuse or direct potable reuse purposes. Many individual advanced water treatment systems have been studied to enhance the removal of organic micropollutants, and there is a great interest in a multibarrier approach by providing synergies in which two or more systems can function as a hybrid system. Each treatment system in a hybrid system is based on different removal mechanisms (e.g., granular activated carbon (GAC), sorption; membranes, size exclusion and electrostatic interactions; and ozone/advanced oxidation process (AOP), oxidation). In particular, the hybrids of BF or ARR with advanced treatments such as AOP/ozone, nanofiltration (NF), or GAC gains much of interest since ARR and BF are more sustainable treatment. BF and ARR can be as a pretreatment process to NF (BF/ARR-NF), GAC (BF/ARR-GAC), AOP (BF/ARR-AOP), ozone (BF/ARR-ozone), and UV (BF/ARR-UV). BF and ARR reduce target compounds and bulk organic matter that reduces the performance of membranes, GAC, ozone, and AOP processes. There are a number of plants operating ARR or BF as a pretreatment to advanced treatment systems. ARR can be also used as a posttreatment to oxidation treatment systems (AOP-ARR and ozone-ARR). The problem associated with AOP or ozone is that metabolites are produced during oxidation; therefore, biodegradation during soil passage is a great option to reduce the metabolites.

5 RESEARCH NEEDS

Several researches and field studies have shown that properly designed and operated BF and ARR have high potential for removal of pharmaceuticals. Further research are needed to facilitate and optimize the removal of some selected pharmaceuticals which are partially removed or not removed during soil passage. Some of the key research needs for further development of these natural treatment systems are listed below:

- Investigate the fate of metabolites produced by oxidation processes (e.g., AOP (UV/H_2O_2) or ozone as pretreatment to ARR systems) during ARR (posttreatment to oxidation treatment systems).
- Investigate transformation products of pharmaceuticals during BF and ARR.
- Investigate the attenuation of pharmaceuticals under copiotrophic conditions or oligotrophic conditions during soil passage by providing more insight on biodegradation of pharmaceutical via BF or ARR.
- Evaluate the ecotoxicity of organic micropollutants during BF and ARR using different species (e.g., bacteria, algae, daphnia, and fish).
- Determine the effect of interactions between pharmaceuticals on their removal during BF and ARR.
- Investigate hybridization of BF and ARR by combining with advanced water treatment processes (UV/H_2O_2, ozone, GAC, membranes, etc.).

6 CONCLUSIONS

BF and ARR offer wide possibilities for their implementation in different treatment schemes, especially for removal of pharmaceuticals for indirect potable reuse, with a sustainable and natural approach. The removal of such compounds is governed by different factors, but usually higher removal efficiencies are reached if values of travel time and travel distance are favorable, and both oxic and anoxic conditions are present in the system. Finally, the versatility of BF and ARR has to be taken for possible hybridization with other advanced water treatments, either as pretreatment or as posttreatment process, for enhancing the overall performance of treatment schemes with respect to the attenuation of organic micropollutants such as pharmaceuticals.

REFERENCES

[1] K. Schütz, PhD University of Basel, 2008.
[2] S.K. Sharma, G. Amy, in: James K. Edzwald (Ed.), Water Quality and Treatment: Handbook of Community Water Supply, American Water Works Association, McGraw Hill, 2010.
[3] T. Grischek, D. Schoenheinz, E. Worch, K. Hiscock, in: P. Dillon (Ed.), Management of Aquifer Recharge for Sustainability, A.A. Balkema, Lisse, The Netherlands, 2002, pp. 485–488.
[4] J. Schubert, J. Hydrol. 266 (2002) 145–161.
[5] P. Hiemstra, R. Kolpa, J.M.J.M. van Eekhout, T.A.L. van Kessel, E.D. Adamse, J.A.M. van Paassen, J. Water Supply Res. Technol. 52 (2003) 37–47.
[6] C. Ray, Clean Technol. Environ. Policy 10 (2008) 223–225.
[7] C. Ray, T. Grischek, J. Schubert, J. Wang, T. Speth, J. Am. Water Works Assoc. 94 (2002) 12.
[8] J. Schubert, in: C. Ray, G. Melin, R. Linsky (Eds.), Riverbank Filtration, Springer, The Netherlands, 2003, pp. 267–277.

[9] S. Grünheid, G. Amy, M. Jekel, Water Res. 39 (2005) 3219–3228.
[10] T. Heberer, Toxicol. Lett. 131 (2002) 5–17.
[11] R.P. Schwarzenbach, B.I. Escher, K. Fenner, T.B. Hofstetter, C.A. Johnson, U. von Gunten, B. Wehrli, Science 313 (2006) 1072–1077.
[12] G. Massmann, J. Greskowiak, U. Dünnbier, S. Zuehlke, A. Knappe, A. Pekdeger, J. Hydrol. 328 (2006) 141–156.
[13] P.J. Stuyfzand, W. Segers, N. van Rooijen, in: 6th International Symposium on Managed Aquifer Recharge, ISMAR6, Phoenix, Arizona, USA, 2007, pp. 231–245.
[14] S.K. Maeng, S.K. Sharma, C.D. Abel, A. Magic-Knezev, G.L. Amy, Water Res. 45 (2011) 4722–4736.
[15] S.K. Maeng, S.K. Sharma, G. Amy, in: C. Ray, M. Shamrukh (Eds.), Riverbank Filtration for Water Security in Desert Countries, Springer, Dordrecht, 2011.
[16] S.K. Maeng, S.K. Sharma, K. Lekkerkerker-Teunissen, G.L. Amy, Water Res. 45 (2011) 3015–3033.
[17] G. Massmann, A. Nogeitzig, T. Taute, A. Pekdeger, Environ. Geol. 54 (2008) 53–65.
[18] S.K. Maeng, S.K. Sharma, A. Magic-Knezev, G. Amy, Water Sci. Technol. 57 (2008) 1999–2007.
[19] S.K. Maeng, E. Ameda, S.K. Sharma, G. Grutzmacher, G.L. Amy, Water Res. 44 (2010) 4003–4014.
[20] S.K. Maeng, S.K. Sharma, C.D. Abel, A. Magic-Knezev, K.G. Song, G.L. Amy, J. Contam. Hydrol. 140–141 (2012) 139–149.
[21] M. Alexander, Science 211 (1981) 132–138.
[22] National Research Council of the National Academies, Prospects for Managed Underground Storage of Recoverable Water, The National Academies Press, Washington, DC, 2008.
[23] C.K. Schmidt, F.T. Lange, H.-J. Brauch, W. Kühn, Experiences with Riverbank Filtration and Infiltration in Germany. http://www.tzw.de/pdf/bankfiltration.pdf, 2008.
[24] M.W.M. Tielemans, in: IWA Regional Conference on Groundwater Management in the Danube River Basin and Other Large River Basins, Belgrade, Serbia, 2007.
[25] K.-D. Balke, Y. Zhu, J. Zhejiang Univ. Sci. B 9 (2008) 221–226.
[26] K. Lekkerkerker, J. Scheideler, S.K. Maeng, J. Ried, J.C. Verberk, A.H. Knol, G. Amy, J.C. van Dijk, Water Sci. Technol. 9 (2009) 643–651.
[27] U. Hübner, U. Miehe, M. Jekel, Water Res. 46 (2012) 6059–6068.
[28] T. Heberer, I. Verstraeten, M.T. Meyer, A. Mechlinski, R. Kirsten, J. Contemp. Water Resour. Res. Educ. 120 (2011) 14.
[29] C. Hoppe-Jones, G. Oldham, J.E. Drewes, Water Res. 44 (2010) 4643–4659.
[30] G. Massmann, U. Dünnbier, T. Heberer, T. Taute, Chemosphere 71 (2008) 1476–1485.
[31] T. Heberer, G. Massmann, B. Fanck, T. Taute, U. Dünnbier, Chemosphere 73 (2008) 451–460.
[32] S. Grünheid, M. Jekel, in: 5th International Symposium on Managed Aquifer Recharge, Berlin, 2005.
[33] A. Mechlinski, T. Heberer, in: 5th International Symposium on Management of Aquifer Recharge, Berlin, 2005.
[34] A. Pekdeger, Hydrogeological–Hydrogeochemical Processes During Bank Filtration and Groundwater Recharge Using a Multi-tracer Approach, NASRI Project, Berlin, 2006.
[35] T. Scheytt, P. Mersmann, M. Leidig, A. Pekdeger, T. Heberer, Ground Water 42 (2004) 767–773.
[36] I. Verstraeten, T. Heberer, T. Scheytt, in: C. Ray, G. Melin, R. Linsky (Eds.), Riverbank Filtration, Springer, The Netherlands, 2003.

[37] C.K. Schmidt, F.T. Lange, H.-J. Brauch, Water Sci. Technol. 7 (2007) 1–7.
[38] S. Zuehlke, U. Duennbier, T. Heberer, B. Fritz, Ground Water Monit. Remediat. 24 (2004) 78–85.
[39] T. Heberer, M. Adam, Environ. Chem. 1 (2004) 22–25.
[40] T. Heberer, A. Mechlinski, B. Fanck, A. Knappe, G. Massmann, A. Pekdeger, B. Fritz, Ground Water Monit. R. 24 (2004) 70–77.
[41] C. Metcalfe, X.-S. Miao, W. Hua, R. Letcher, M. Servos, Pharmaceuticals in the Canadian Environment, Springer, Berlin, 2004.
[42] J.L. Zhou, Z.L. Zhang, E. Banks, D. Grover, J.Q. Jiang, J. Hazard. Mater. 166 (2009) 655–661.
[43] J.E. Drewes, T. Heberer, R. Kirsten, Water Sci. Technol. 46 (2002) 73–80.
[44] T. Heberer, K. Reddersen, A. Mechlinski, Water Sci. Technol. 46 (2002) 81–88.
[45] G.E. Cordy, N.L. Duran, H. Bouwer, R.C. Rice, E.T. Furlong, S.D. Zaugg, M.T. Meyer, L.B. Barber, D.W. Kolpin, Ground Water Monit. R. 24 (2004) 58–69.
[46] T.A. Ternes, Water Res. 32 (1998) 3245–3260.
[47] K. Stamatelatou, C. Frouda, M.S. Fountoulakis, P. Drillia, M. Kornaros, G. Lyberatos, Water Sci. Technol. 3 (2003) 131–137.
[48] Y. Zhang, S.-U. Geißen, C. Gal, Chemosphere 73 (2008) 1151–1161.
[49] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Water Res. 43 (2009) 363–380.
[50] S. Mompelat, B. Le Bot, O. Thomas, Environ. Int. 35 (2009) 803–814.
[51] M. Jekel, S. Grünheid, Water Sci. Technol. 5 (2005) 9.
[52] S. Schittko, A. Putschew, M. Jekel, Water Sci. Technol. 50 (2004) 261–268.
[53] C.E. Purdom, P.A. Hardiman, V.V.J. Bye, N.C. Eno, C.R. Tyler, J.P. Sumpter, Chem. Ecol. 8 (1994) 275–285.
[54] C.R. Tyler, C. Spary, R. Gibson, E.M. Santos, J. Shears, E.M. Hill, Environ. Sci. Technol. 39 (2005) 2599–2607.
[55] J. Mansell, J.E. Drewes, Ground Water Monit. R. 24 (2004) 94–101.
[56] J. Mansell, J.E. Drewes, T. Rauch, Water Sci. Technol. 50 (2004) 229–237.

Chapter 14

Fate and Occurrence of Pharmaceuticals in the Aquatic Environment (Surface Water and Sediment)

Guang-Guo Ying, Jian-Liang Zhao, Li-Jun Zhou and Shan Liu
State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, China

Chapter Outline

1 INTRODUCTION

Pharmaceuticals are used in treatment and prevention of diseases in humans and animals. After use, these pharmaceuticals are released into the receiving environments via wastewater treatment plants (WWTPs) or via direct disposal (Figure 1). The first detected pharmaceutical is clofibric acid at the concentration of 0.8–2 μg/L in treated wastewater from the United States [1]. Since then, especially in the last decade, many monitoring studies showed

Comprehensive Analytical Chemistry, Vol. 62. http://dx.doi.org/10.1016/B978-0-444-62657-8.00014-8

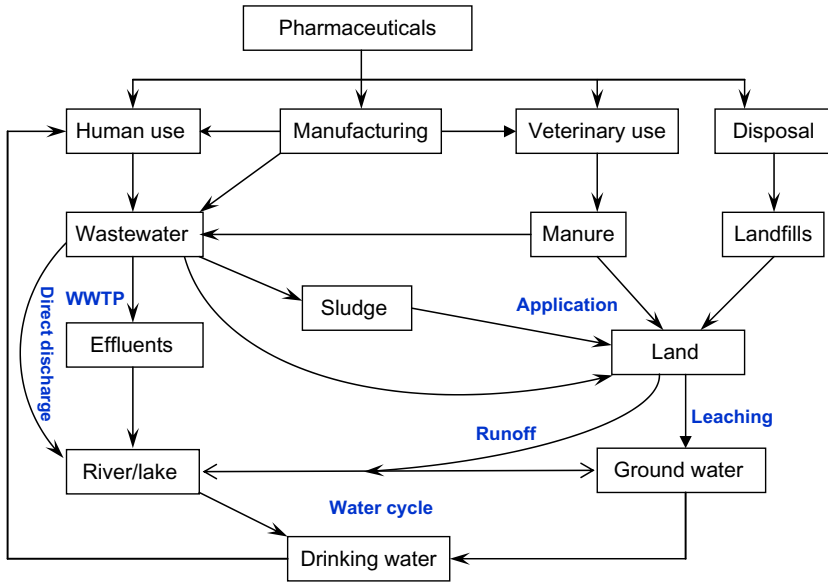

FIGURE 1 Pathway of pharmaceuticals in the environment.

widespread presence of various pharmaceutical residues in sewage treatment plant effluents and surface water and sediments in some countries. The pharmaceuticals in the aquatic environment could pose potential risks to organisms since they are designed to be bioactive with a certain kind of mode of action. For example, feminization of male fish caused by synthetic estrogenic steroid 17α-ethinylestradiol (EE2) [2] and increased bacterial resistance to antibiotics due to sewage discharge have been reported [3,4]. Because of these reported field observations, it has become an increasing concern for the general public and scientific community. Hence, there is a need to understand the fate and occurrence of pharmaceuticals in the aquatic environment in order to properly assess the risks posed by these pharmaceutical residues. This chapter will compile and critically review the current knowledge of environmental occurrence and fate of pharmaceuticals in the aquatic environment and identify major gaps in current knowledge and future research needs. Here, we mainly focus on the pharmaceuticals of environmental importance, which include antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs), lipid regulators, psychoactive drugs, and steroid hormones.

1.1 Antibiotics

Antibiotics refer to synthetic and semisynthetic products that are used to kill or inhibit bacteria, fungi, or viruses. Antibiotics can be grouped by their chemical structure into different classes such as sulfonamides, tetracyclines, quinolones, macrolides, and β-lactams (Table 1). Antibiotics are used

TABLE 1 The Physicochemical Properties of Antibiotics Commonly Used for Humans and Animals

Class	Compound	CAS No.	MW	Formula	Solubility (in water, mg/L)	Log K_{ow} [a]	pK_a [b]	Solid–Water Distribution		
								Matrices	K_d *(L/kg)*	K_{oc} *(L/kg)*
Sulfonamides	Sulfacetamide	144-80-9	214.2	$C_8H_{10}N_2O_3S$	12,500 [c]	−0.96 [c]	5.4 [5]			
	Sulfachlorpyridazine	80-32-0	284.7	$C_{10}H_9ClN_4O_2S$			1.87, 5.45 [6]	Clay loamy, sandy loam	0.9–1.8 [18]	
	Sulfadiazine	68-35-9	250.3	$C_{10}H_{10}N_4O_2S$	77 [18]	−0.09 [18]	1.6 6.4 [18]	Whole soil, clay, sand fraction	1.4–2.8 [18]	37–125
	Sulfadoxine	2447-57-6	310.3	$C_{12}H_{14}N_4O_4S$	2700 [c]	0.7 [c]	3.15 6.16 [7]			
	Sulfadimethoxine	122-11-2	310.3	$C_{12}H_{14}N_4O_4S$	343 [c]	1.63 [c] 1.4 [8]	6.3 [5] 2.13, 6.08 [6]	Whole soil, clay, sand fraction	2.3–4.6 [18]	89–144
	Sulfaguanidine	57-67-0	214.2	$C_7H_{10}N_4O_2S$	2200 [c]	−1.22 [c]	11.3 [5]			
	Sulfamethazine	57-68-1	278.3	$C_{12}H_{14}N_4O_2S$	1500 [18]	0.80 [18]	2.07 7.49 [6] 2.65 [5]	Sand, loamy sand, sandy loam	0.6–3.2 [18]	82–208
	Sulfamethoxazole	723-46-6	253.3	$C_{10}H_{11}N_3O_3S$	610 [c]	0.89 [c]	1.85 5.6 [c] 5.9 [5]			
	Sulfameter	651-06-9	280.3	$C_{11}H_{12}N_4O_3S$	730 [c]	0.41 [c]	1.48 6.49 [7]			
	Sulfamonomethoxine	1220-83-3	280.3	$C_{11}H_{12}N_4O_3S$	4030 [c]	0.7 [c]				

Continued

TABLE 1 The Physicochemical Properties of Antibiotics Commonly Used for Humans and Animals—Cont'd

Class	Compound	CAS No.	MW	Formula	Solubility (in water, mg/L)	Log K_{ow}	pK_a	Solid–Water Distribution		
								Matrices	K_d *(L/kg)*	K_{oc} *(L/kg)*
	Sulfanilamide	63-74-1	172.2	$C_6H_8N_2O_2S$	7500 [c]	−0.62 [c]	10.58 [c]	Slit loam	1.7 [9]	104
	Sulfapyridine	144-83-2	249.3	$C_{11}H_{11}N_3O_2S$	270 [18]	0.35 [18]	2.58 8.43 [18] 8.4 [5]	Silty loam	1.6–7.4 [18]	101–308
								Whole soil, clay, sand fraction	3.1–3.5 [18]	80–218
	Sulfaquinoxaline	59-40-5	300.4	$C_{14}H_{12}N_4O_2S$	7.5 [c]	1.68 [c]				
	Sulfisoxazole	127-69-5	267.3	$C_{11}H_{13}N_3O_3S$	300 [c]	1.01 [c]		Clay loam	1.5 [9]	48
	Sulfathiazole	72-14-0	255.3	$C_9H_9N_3O_2S_2$	373 [c]	0.05 [c]	2.0 [5] 2.01, 7.11 [6]	Loamy sand	4.9 [10]	200
Diaminopyrimidines	Ormetoprim	6981-18-6	274.3	$C_{14}H_{18}N_4O_2$	1540 [c]	1.23 [c]				
	Trimethoprim	738-70-5	290.3	$C_{14}H_{18}N_4O_3$	400 [c]	0.91 [c]	3.23 6.76 [c]	Sewage sludge	76 [9]	205
Tetracyclines	Chlortetracycline	57-62-5	478	$C_{22}H_{23}ClN_2O_8$	630 [c]	−0.62 [c]	3.33 7.55 9.33 [6]	Clay loam, sandy loam	1280–2386 [18]	
	Doxycycline	564-25-0	444.4	$C_{22}H_{24}N_2O_8$	630 [c]	−0.02 [c]	3.02 7.97 9.15 [6]			
	Methacycline	914-00-1	442.4	$C_{22}H_{22}N_2O_8$	7550 [c]	−1.37 [c]	4.05 6.87 9.59 [6]			
	Oxytetracycline	79-57-2	460.4	$C_{22}H_{24}N_2O_8$	1000 [10]	−1.22 [10]	3.22 7.46 8.94 [6]	Loamy sand, sand	417–1026 [18]	42,506–9337

	Tetracycline	60-54-8	444.4	$C_{22}H_{24}N_2O_8$	1700 [10]	−1.19 [10]	3.32 7.78 9.58 [6]	Clay loam, sandy loam	1147–2370[f]	
								Aldrich humic acid	1430–2060 [10]	
Fluoroquinolones	Ciprofloxacin	85721-33-1	331.3	$C_{17}H_{18}FN_3O_3$	30,000 [10]	0.4 [10]	3.01 6.14 8.70 10.58 [6]	Loamy sand	427[18]	61,000
								Sewage sludge	417[18]	1127
	Danofloxacin	112398-08-0	357.4	$C_{19}H_{20}FN_3O_3$		1.85 [18]	2.73 9.13 [18]	Humic acid from a soil	630 [10]	
	Difloxacin	98106-17-3	399.4	$C_{21}H_{19}F_2N_3O_3$	1330 [c]	0.89 [c]				
	Enrofloxacin	93106-60-6	359.4	$C_{19}H_{22}FN_3O_3$	130,000 [c]	1.1 [10]	3.85 6.19 7.59 9.86 [6]	Clay, loam, loamy sand	260–5612 [18]	16,510–99,980
								Humic acid from a soil	110 [10]	
	Fleroxacin	79660-72-3	369.34	$C_{17}H_{18}F_3N_3O_3$	7320 [c]	0.24 [c]				
	Lomefloxacin	98079-51-7	351.3	$C_{17}H_{19}F_2N_3O_3$	27,200 [c]	−0.3 [c]				
	Marbofloxacin	115550-35-1	362.4	$C_{17}H_{19}FN_4O_4$						
	Norfloxacin	70458-96-7	319.3	$C_{16}H_{18}FN_3O_3$	17,800 [c]	−1.03 [c]	3.11 6.10 8.6 10.56 [6]			

Continued

TABLE 1 The Physicochemical Properties of Antibiotics Commonly Used for Humans and Animals—Cont'd

Class	Compound	CAS No.	MW	Formula	Solubility (in water, mg/L)	Log K_{ow}	pK_a	Solid–Water Distribution		
								Matrices	K_d *(L/kg)*	K_{oc} *(L/kg)*
	Ofloxacin	82419-36-1	361.3	$C_{18}H_{20}FN_3O_4$	2830 [c]	0.36 [9]	5.97 8.28 [11]	Humic acid from a soil	100 [10]	
								Loamy sand	309 [9]	44,140
	Pefloxacin	70458-92-3	333.4	$C_{17}H_{20}FN_3O_3$	1140 [c]	0.27 [c]				
	Sarafloxacin	98105-99-8	385.4	$C_{20}H_{17}F_2N_3O_3$	100 [10]	1.07 [c]	6.0 8.6 [10]	Aldrich humic acid	18,700–52,700 [10]	55,000–155,000
	Carbadox	6804-7-5	262.2	$C_{11}H_{10}N_4O_4$	15,000 [c]	−1.37 [c]				
Macrolides	Clarithromycin	81103-11-9	748.0	$C_{38}H_{69}NO_{13}$	0.342 [c]	3.16 [c]	8.99 [c]			
	Erythromycin	114-07-8	733.9	$C_{37}H_{67}NO_{13}$	2000 [12]	3.06 [c]	8.9 [6]			
	Erythromycin-H_2O	23893-13-2	715.9	$C_{37}H_{65}NO_{12}$						
	Leucomycin	1392-21-8	771	$C_{39}H_{65}NO_{14}$						
	Oleandomycin	3922-90-5	687.9	$C_{35}H_{61}NO_{12}$	15.5 [c]	1.69 [c]	3.31 7.50 [6] 8.84 [c]			
	Roxithromycin	80214-83-1	837.0	$C_{41}H_{76}N_2O_{15}$		2.75 [c]	9.17 [6]			
	Tylosin	1401-69-0	916.1	$C_{46}H_{77}NO_{17}$	5 [c]	1.63 [c]	7.73 [c]	Loamy sand, sand	8.3–128	553–7990

Ionophores	Salinomycin	53003-10-4	751	$C_{42}H_{70}O_{11}$	17-905 [13]	8.53 [c] 5.15 [13]	4.5,6.4 [13]	
	Monensin	17090-79-8	670.9	$C_{36}H_{62}O_{11}$	63 [14] 5–63 [13]	2.75 [14] 2.8–4.1 [13]	6.65 [14] 6.7 [13]	
	Narasin	55134-13-9	765.0	$C_{43}H_{72}O_{11}$	102–681 [13]	4.9–6.2 [13]	7.9 [13]	
Aminocoumarins	Novobiocin	303-81-1	612.6	$C_{31}H_{36}N_2O_{11}$[15]		2.45 [c]	4.3 [c]	
Polypeptides	Bacitracin	1405-87-4	1422.7	$C_{66}H_{103}N_{17}O_{16}S$				
Lincosamides	Lincomycin	154-21-2	406.5	$C_{18}H_{34}N_2O_6S$	927 [c]	0.56 [c]		
Chloramphenicol derivatives	Florfenicol	73231-34-2	358.2	$C_{12}H_{14}Cl_2FNO_4S$		−0.04 [15]	–	
	Chloramphenicol	154-75-2	323.1	$C_{11}H_{12}Cl_2FN_2O_5$	2500 [c]	1.14 [c]		Fresh water– sediment 0.4 [10]
β-Lactams	Ceftiofur	80370-57-6	523.6	$C_{19}H_{17}N_5O_7S_3$				
	Cloxacillin	61-72-3	435.9	$C_{19}H_{18}ClN_3O_5S$		2.48[c]	2.78[c]	

[a] K_{ow}: *The octanol–water partition coefficient.*
[b] pK_a: *Acidity constant.*
[c] *US National Library of Medicine ChemIDPlus Advanced (http://chem.sis.nlm.nih.gov/chemidplus/), accessed on 10 September 2011.*

extensively in humans and animals to treat microbial infections and also as feed additive to promote growth of livestock animals [16–18]. After administration of an antibiotic, a significant fraction is excreted in the parent form or its metabolite forms along with urine and feces and then reaches the aquatic environment through direct discharge of wastes [19–21] or discharge of effluents from WWTPs due to incomplete removal [22,23]. Moreover, antibiotics associated with biosolid and livestock manure application onto land can also enter into aquatic environment via surface runoff of soil [24]. It has been reported that antibiotics such as ciprofloxacin can influence the bacterial diversity and microbial dynamics in the aquatic ecosystem [25,26]. Antibiotic resistance has become an increasing concern with reports of high antibiotic resistance frequencies and detection of antibiotic resistance genes in aquatic environments [2,3,27–29].

1.2 Nonsteroidal Anti-Inflammatory Drugs

NSAIDs are the important parts of analgesic drugs. NSAIDs are used to relieve pain and also to suppress inflammation in a way similar to steroids but without their side effects [30]. NSAIDs act by inhibiting either reversibly or irreversibly one or both of the two isoforms of the cyclooxygenase enzyme (COX-1 and COX-2), which catalyze the synthesis of different prostaglandins from arachidonic acid [31]. NSAIDs are acidic compounds with variable hydrophobicity. NSAIDs have molecular structures with acid group; hence, they show a high hydrophilic property. These compounds have high water solubilities. The pK_a values vary from 2.8 for salicylic acid to 4.91 for ibuprofen (Table 2). The $\log K_{ow}$ values range from 1.19 for acetylsalicylic acid to 6.02 for meclofenamic acid (Table 2). Paracetamol (or acetaminophen) is a widely used over-the-counter analgesic and antipyretic. Though acetaminophen is used to treat inflammatory pain, it is not generally classified as an NSAID because it exhibits only weak anti-inflammatory activity. But this drug is often included together with NSAIDs in environmental studies.

1.3 Lipid Regulators

Lipid regulators are substances used to lower levels of triglycerides and low-density lipoproteins and increase levels of high-density lipoproteins in the blood. There are two types of blood lipid-lowering agents, namely, statins and fibrates; the latter have been more often targeted analytically in the aquatic environment than the former. Lipid regulators such as clofibric acid, gemfibrozil, and bezafibrate have similar properties as NSAIDs, since they also have acid group in their molecular structure (Table 2).

TABLE 2 Physical Chemical Properties of Pharmaceuticals NSAIDs, Lipid Regulators, Antiepileptics, and Beta-Blockers

Class	Compound	CAS No.	MW	Formula	Solubility (in water, mg/L)	Log K_{ow}[a]	pK_a[b]	Solid–Water Distribution		
								Matrices	K_d *(L/kg)*	K_{oc} *(L/kg)*
NSAIDs	Ibuprofen	15687-27-1	206.28	$C_{13}H_{18}O_2$	21	3.97 [32]	4.91 [32]	Digested sludge	10–60.3 [33]	66.1–1318 [33]
	Diclofenac	15307-86-5	296.15	$C_{14}H_{11}Cl_2NO_2$	2.37	4.15 [32]	4.51 [32]	Digested sludge	18.2–151.4 [33]	158.5–2630 [33]
	Mefenamic acid	61-68-7	241.29	$C_{15}H_{15}NO_2$	20	4.29 [34]	3.90 [34]	Sewage sludge	18916.98 [35]	461.0 [35]
	Naproxen	22204-53-1	230.26	$C_{14}H_{14}O_3$	15.9	4.15 [32]	3.18 [32]	Digested sludge	10.72–51.3 [33]	100–1000 [33]
	Ketoprofen	22071-15-4	254.28	$C_{16}H_{14}O_3$	51	4.45 [32]	3.12 [32]	Soil	1.26–8.24 [36]	229–341 [36]
	Fenoprofen	29679-58-1	242.27	$C_{15}H_{14}O_3$	NA					
	Salicylic acid	69-72-7	138.12	$C_7H_6O_3$	2000	2.26 [37]	2.8 [37]	Soil	0.3–67.9 [37]	404
	Acetylsalicylic acid	50-78-2	180.16	$C_9H_8O_4$	3300	1.19 [35]	3.5 [35]	Sewage sludge	2.22 [35]	10 [35]
	Meclofenamic acid	644-62-2	296.15	$C_{14}H_{11}Cl_2NO_2$	30	6.02	3.70 [38]	Sludge	109 [39]	
	Tolfenamic acid	13710-19-5	261.71	$C_{14}H_{12}ClNO_2$	0.78	5.17	4.30 [40]			
	Indomethacin	53-86-1	357.79	$C_{19}H_{16}ClNO_4$	0.937	4.27	4.50	Sludge	214 [39]	691.83 [39]
	Acetaminophen	103-90-2	151.16	$C_8H_9NO_2$	14,000	0.46 [41]	9.38 [41]			

Continued

TABLE 2 Physical Chemical Properties of Pharmaceuticals NSAIDs, Lipid Regulators, Antiepileptics, and Beta-Blockers—Cont'd

Class	Compound	CAS No.	MW	Formula	Solubility (in water, mg/L)	Log K_{ow}	pK_a	Solid–Water Distribution		
								Matrices	K_d *(L/kg)*	K_{oc} *(L/kg)*
Lipid regulators	Clofibric acid	882-09-7	214.65	$C_{10}H_{11}ClO_3$	582.5	2.88 [42]	3.2 [42]	Agricultural soils	200.73 [43]	14,125 [43]
	Gemfibrozil	25812-30-0	250.33	$C_{15}H_{22}O_3$	27.8	4.77 [44]	4.7 [44]			
	Bezafibrate	41859-67-0	361.82	$C_{19}H_{20}ClNO_4$	16	3.61 [32]	4.25 [32]			
	Fenofibrate	49562-28-9	360.83	$C_{20}H_{21}ClO_4$	12		5.19			
Antiepileptics	Carbamazepine	298-46-4	236.27	$C_{15}H_{12}N_2O$	17.66	13.9 [32]	2.45 [32]	Sewage sludge	25.52 [35]	3870 [35]
	Diazepam	439-14-5	284.74	$C_{16}H_{13}ClN_2O$	50	2.82		Sludge		
β-Blockers	Metoprolol	37350-58-6	267.36	$C_{15}H_{25}NO_3$	16,900	9.7 [32]	1.69 [32]	Primary sludge/ second sludge	44 ± 26/ 21 ± 8 [45]	125 ± 75/ 62 ± 23 [45]
	Propranolol	525-66-6	259.34	$C_{16}H_{21}NO_2$	70	2.6	9.49	Low organic carbon and high clay content/high organic carbon and low clay content	16.3 ± 1.4/ 199 ± 9.6 [46]	4405 ± 378/ 2803 ± 135 [46]
	Atenolol	29122-68-7	266.34	$C_{14}H_{22}N_2O_3$	13,500	9.2 [32]	1.71 [32]	Sewage sludge	0.21 [35]	148 [35]

[a] K_{ow}: *The octanol–water partition coefficient.*
[b] pK_a: *Acidity constant.*

1.4 Psychoactive Drugs

Antidepressants and antipsychotics and antiepileptics belong to psychoactive drugs that are used to treat psychiatric diseases. Antidepressants are often used for conditions such as anxiety disorders, obsessive compulsive disorder, eating disorders, chronic pain, and some hormone-mediated disorders. Antidepressants include many different classes such as selective serotonin reuptake inhibitors, norepinephrine reuptake inhibitors, noradrenergic and specific serotonergic antidepressants, and tricyclic antidepressants. Antipsychotics are psychiatric medications primarily used to manage psychosis, particularly in schizophrenia and bipolar disorder, and are increasingly being used in the management of nonpsychotic disorders. Common antipsychotics include butyrophenones, phenothiazines, and thioxanthenes. Antiepileptic drugs act on the central nerve system by decreasing the overall neuronal activity. Carbamazepine is the primary drug used for the treatment of partial seizures. The antiepileptic carbamazepine was detected most frequently in wastewaters and aquatic environments due to its persistence and mobility.

1.5 Beta-Blockers

Beta-blockers, or β-adrenergic receptor antagonists, are drugs that act on the blood vessels, preventing vasodilatation and reducing the speed and force of heart contractions. Propanolol is an example of a nonselective β-adrenergic antagonist that has equal affinity for β1 and β2 receptors. Substances such as metoprolol and atenolol are examples of selective β1 antagonists as a result of their greater affinity for β1 receptors. They are used in the treatment of high blood pressure and to treat patients after heart attack to prevent further attacks. Most beta-blockers are basic compounds with variable hydrophobicity (Table 2).

1.6 Steroid Hormones

Steroid hormones can be classified into estrogens, androgens, progestogens, glucocorticoids, and mineralocorticoids (Table 3). Natural steroids mainly originated from the excretion (feces and urine) of human, livestock, and aquaculture. Some natural and synthetic steroids have also been used as pharmaceuticals in human daily life for many reasons, such as contraception and human therapy, and as growth promoters in livestock production to prevent and treat diseases, promote growth, and improve productivity [56,57]. Steroid hormones are a class of extremely active biological compounds and produce intensive effects at low doses. These steroids and their metabolites may pose high risks to aquatic organisms at very low environmental concentrations as they are constantly released into the environment [58–61].

TABLE 3 Physiochemical Properties of Steroids Estrogens, Glucocorticoids, Progestogens, and Androgens

								Solid–Water Distribution		
Class	**Compound**	**CAS No.**	**MW**	**Formula**	**S (mg/L)[a]**	**Log K_{ow}[b]**	**pK_a**	***Matrix***	**K_d *(L/kg)***	**K_{oc} *(L/kg)*[c]**
Androgens	Androsta-1,4-diene-3,17-dione	897-06-3	284.39	$C_{19}H_{24}O_2$	102	2.54				525
	4-Androstene-3,17-dione	63-05-8	286.41	$C_{19}H_{26}O_2$	66.0	2.76		Drummer silty clay loam	142 [47]	692
								Freshwater sediment	19.3 [47]	
	Androsterone	53-41-8	290.44	$C_{19}H_{30}O_2$	31.9	3.07				562
	17α-Boldenone	27833-18-7	286.40	$C_{19}H_{26}O_2$	NA	NA[d]				NA
	17β-Boldenone	846-48-0	286.40	$C_{19}H_{26}O_2$	117	3.05				251
	5α-Dihydrotestosterone	521-18-6	290.44	$C_{19}H_{30}O_2$	42.0	3.07				468
	Epiandrosterone	481-29-8	290.44	$C_{19}H_{30}O_2$	31.9	3.07				562
	4-Hydroxy-androst-4-ene-17-dione	566-48-3	302.41	$C_{19}H_{26}O_3$	NA	2.66				NA
	Methyl testosterone	58-18-4	302.46	$C_{20}H_{30}O_2$	51.9	3.72		Sand	4.6 [48]	372
								Sandy loam	1.2 [48]	
								Clay	49.4–256.4 [48]	
								Clay loam	119.7 [48]	
	19-Nortestotserone	434-22-0	274.41	$C_{18}H_{26}O_2$	323	2.82				145

Testosterone	58-22-0	288.43	$C_{19}H_{28}O_2$	67.8	3.27	Conventionally tilled soil column	5.04–11.39 [49]	355
						No-tilled soil column	5.00–13.52 [49]	
						Drummer silty clay loam	42.7 [47]	
						Freshwater sediment	4.57 [47]	
Testosterone-16,16,17-d_3 (IS)	77546-39-5	291.44	$C_{19}H_{25}D_3O_2$					
17α-Trenbolone	80657-17-6	270.37	$C_{18}H_{22}O_2$	NA	NA	Ultic hapludalfs	2.2 [50]	589
						Lamellic Udipsamments	5.3 [50]	
						Udollic Epiaqualfs	6.3 [50]	
						Typic Endoaquolls	17.0 [50]	
						Mollic gleysol	41.1 [50]	
17β-Trenbolone	10161-33-8	270.37	$C_{18}H_{22}O_2$	NA	NA	Ultic Hapludalfs	4.7 [50]	1202
						Lamellic Udipsamments	10.6 [50]	
						Udollic Epiaqualfs	14.5 [50]	
						Typic Endoaquolls	32.6 [50]	
						Mollic Gleysol	73.5 [50]	
Stanozolol	10418-03-8	328.49	$C_{21}H_{32}N_2O$	1.41	4.42			2291
Stanozolol-d_3 (IS)	88247-87-4	331.51	$C_{21}H_{29}D_3N_2O$					

Continued

TABLE 3 Physiochemical Properties of Steroids Estrogens, Glucocorticoids, Progestogens, and Androgens—Cont'd

								Solid–Water Distribution		
Class	**Compound**	**CAS No.**	**MW**	**Formula**	**S (mg/L)**	**Log K_{ow}**	**pK_a**	***Matrix***	**K_d *(L/kg)***	**K_{oc} *(L/kg)***
Estrogens	Estrone-2,4,16,16-d_4 (IS[e])	53866-34-5	274.39	$C_{18}H_{18}D_4O_2$						
	Estrone	53-16-7	270.37	$C_{18}H_{22}O_2$	147	3.43	10.5 [51] ; 10.77 [52]	Roseworthy Campus soil	26 [53]	1047
								Roseworthy farm soil	26 [53]	
								Turretfield soil	54 [53]	
								Waite campus soil	108 [53]	
								Drummer silty clay loam	48.1 [47]	
								Freshwater sediment	3.40 [47]	
	17β-Estradiol-2,4,16,16-d_4 (IS)	66789-03-5	276.41	$C_{18}H_{20}D_4O_2$						
	17β-Estradiol	50-28-2	272.39	$C_{18}H_{24}O_2$	82.0	3.94	10.71 [52]	Loamy sand	35.2 [54]	794
								A light sandy loam soil	23.6–29.6 [54]	
								Slurry separates	372–723 [54]	
								Conventionally tilled soil column	13.25–19.12 [49]	

								No-tilled soil column	9.56–21.90 [49]	
								Roseworthy Campus soil	55 [53]	
								Roseworthy farm soil	31 [53]	
								Terretfield soil	50 [53]	
								Waite campus soil	123 [53]	
								Drummer silty clay loam	83.2 [47]	
								Freshwater sediment	3.56 [47]	
	17α-Ethinylestradiol	57-63-6	296.41	$C_{20}H_{24}O_2$	116	4.12	10.5 [55]	Roseworthy Campus soil	77 [53]	501
								Roseworthy farm soil	62 [53]	
								Terretfield soil	78 [53]	
								Waite campus soil	122 [53]	
	Diethylstilbestrol	56-53-1	268.35	$C_{18}H_{20}O_2$	3.3	5.64				11,482
Glucocorticoids	Cortisol	50-23-7	362.46	$C_{21}H_{30}O_5$	220	1.62				24
	Cortisol-d_2 (IS)	79037-25-5	364.47	$C_{21}H_{28}D_2O_5$						
	Cortisone	53-06-5	360.44	$C_{21}H_{28}O_5$	297	1.81				20
	Dexamethasone	50-02-2	392.46	$C_{22}H_{29}FO_5$	75.1	1.72				37
	Prednisolone	50-24-8	360.44	$C_{21}H_{28}O_5$	221	1.40				25
	Prednisone	53-03-2	358.43	$C_{21}H_{26}O_5$	312	1.59				20

Continued

TABLE 3 Physiochemical Properties of Steroids Estrogens, Glucocorticoids, Progestogens, and Androgens—Cont'd

								Solid–Water Distribution		
Class	**Compound**	**CAS No.**	**MW**	**Formula**	**S (mg/L)**	**Log K_{ow}**	**pK_a**	***Matrix***	**K_d *(L/kg)***	**K_{oc} *(L/kg)***
Progestogens	Ethinyl testosterone	434-03-7	312.45	$C_{21}H_{28}O_2$	74.2	3.44				269
	Medroxyprogesterone	520-85-4	344.54	$C_{22}H_{32}O_3$	22.2	3.50				692
	19-Norethindrone	68-22-4	298.42	$C_{20}H_{26}O_2$	118	3.99				224
	Norgestrel	6533-00-2	312.45	$C_{21}H_{28}O_2$	35.8	3.48				427
	Progesterone	57-83-0	314.47	$C_{21}H_{30}O_2$	5.0	3.67				2884
	Progesterone-d_9 (IS)	15775-74-3	323.52	$C_{21}H_{21}D_9O_2$						

[a] *Solubility, calculated based on EPI Suite from the US EPA.*
[b] K_{ow} *octanol–water partition coefficient, calculated based on EPI Suite from the US EPA.*
[c] K_{oc}, *the organic carbon partition coefficient, calculated based on EPI Suite from the US EPA.*
[d] *Not available.*
[e] *IS, internal standard.*

2 FATE IN THE AQUATIC ENVIRONMENT

Before discharge of wastewater, pharmaceuticals may be removed through microbial degradation or sorption onto sludge in conventional WWTPs. The aqueous removal rates for various pharmaceuticals vary in a WWTP and also vary among different WWTPs. High aqueous removal rates have been reported for some pharmaceuticals such as antibiotics, while very low removal rates were found for some other pharmaceuticals such as carbamazepine. Incomplete removal has often been reported for conventional WWTPs; therefore, advanced treatment technologies such as ozonation, UV oxidation, and membranes may be applied as tertiary treatment to improve their removal.

Once pharmaceuticals enter into aquatic environments, partitioning (sorption and desorption) and degradation (abiotic and biological degradation) occur. Abiotic degradation mainly includes hydrolysis and photolysis, while biological degradation involves different levels of organisms such as bacteria, fungi, and algae. The fate of pharmaceuticals in the environment is dependent on a range of factors such as their physiochemical properties and on processes such as partitioning to sediments and degradation in the environment. Environmental factors such as climate, pH value, redox condition, and water and sediment components also affect the fate and behavior of those pharmaceuticals in the environment.

2.1 Partitioning

Partitioning is an important process in determining the fate of pharmaceuticals in the aquatic environment. Sorption and desorption are responsible for the partitioning of a pharmaceutical between water phase and sediment phase and also affect the bioavailability of the compound to aquatic biota. Since most pharmaceuticals are weak acids, weak bases, or zwitterions, sorption not only is due to hydrophobic interactions but also is driven by other binding processes such as cation exchange, cation bridging, surface complexation, and hydrogen bonding. The sorption of a pharmaceutical onto particles or sediments may cause a loss in detectability and bioactivity due to irreversible bonding. The sorption process is influenced by the amount and nature of suspended solid particles in the water phase and water and sediment properties such as pH value and organic matter content. The solubility or $\log K_{ow}$ is not sufficient for assessment of sorption behavior of pharmaceuticals as they contain polar groups.

Antibiotics have various classes with different physiochemical properties and chemical structures, resulting in different sorption behaviors. For example, the sorption coefficients (K_d) for sulfonamides range from 0.6 to 7.4 L/kg in soils/sediments and 28.6 to 110 L/kg in sludge, while the K_d values for tetracyclines in soils/sediments were reported to be 417–2386 L/kg and for fluoroquinolones 260–5612 L/kg (Table 1). The sorption capability of various antibiotics basically followed the following order: fluoroquinolones $\sim$ tetracyclines $>$ macrolides $>$ sulfonamides. Tetracyclines

and fluoroquinolones can interact strongly with clay, natural organic matter, and metal oxides by cation exchange, surface complexation/cation, bridging hydrophobic partitioning, and electron donor–acceptor interactions [62–66], showing strong tendency to partition into sediment phase in the aquatic environment.

NSAIDs and lipid regulators and antiepileptic drugs have relatively low K_d and K_{oc} values due to their high hydrophilicity (Table 2). The determined K_d values for acidic pharmaceuticals (e.g., ibuprofen, diclofenac, and naproxen) range from below one to several hundred liters/kilograms and the K_{oc} values from 100 to several thousand liters/kilograms in river sediment [67], suggesting that they are mainly partitioned into water phase in the aquatic environment. The K_d and K_{oc} values of clofibric acid in sediments were reported to be 0.3 and 26 L/kg, respectively [68]. The K_d and K_{oc} values for carbamazepine, diazepam, and oxazepam onto two river sediments were 1.3–3 and 83–192 L/kg, respectively [68]. The sorption coefficients K_d and K_{oc} values for nine beta-blockers determined on two river sediments were 0.51–12 and 1.71–2.66 L/kg, respectively [69]. In fact, high water solubilities and moderate K_{ow} values for this group of pharmaceuticals can reflect to a certain degree their low tendency of partitioning into aquatic sediments.

Among the steroid classes, estrogens, androgens, and progestogens have higher hydrophobicities (K_{ow}) and sorption coefficients (K_{oc}) (Table 3) than glucocorticoids, suggesting that estrogens, androgens, and progestogens have a higher tendency to partition into sediment. However, their sorption to sediment is relatively moderate when compared to some antibiotics such as tetracyclines with very high sorption coefficients. Clearly, sorption could play a significant role in the fate of pharmaceuticals in the aquatic environment.

2.2 Abiotic Degradation

2.2.1 Photolysis

Some pharmaceuticals are sensitive to sunlight and can be decomposed or transformed in surface water (Tables 4–6). Photolysis of a chemical is influenced by such factors as chemical structure, water pH, water depth, dissolved organic matter (DOM) and inorganic ions, and climate.

Some antibiotics (e.g., quinolones, tetracyclines, sulfonamides, tylosin, and nitrofuran antibiotics) are light-sensitive (Table 4) [82]. Sulfonamides (e.g., sulfacetamide, sulfathiazole, sulfamethoxazole, and sulfadiazine) can undergo photocatalytic degradation [70,74]. Tetracyclines are also susceptible to photolysis, with a reported half-time of 3 h for tetracycline and its two identified transformation products [86]. The photolysis of tetracyclines was influenced by water hardness (calcium concentration, magnesium concentration, and pH) [82,87]. Photolysis was found responsible for about 70% of oxytetracycline degradation in freshwater and seawater, while hydrolysis was

TABLE 4 The Fate of Antibiotics in the Aquatic Environment

	Photolysis			Hydrolysis			Biodegradation				
Compound	*Matrix*	K	*Half-time*	*Matrix*	K	*Half-time*	*Matrix*	K	*Half-time*	Note	References
Sulfonamides											
Sulfacetamide	Water, UV kmax 366 nm with TiO_2 catalyst, 21 °C	0.0132 min^{-1}					Water		No degradation		[70]
							Adapted aerated reactors		0.6 days		[71]
Sulfachlorpyridazine							Broiler feces		65% degraded in 8 days		[15]
							Sandy loam, clay loam		2.8–3.5 days		[72]
							Liquid pig manure		127 days		[72]
				pH 2, 5, 7, 9, 22 °C		No degradation in 21 days					[73]
Sulfadiazine	Water, UV kmax 366 nm with TiO_2 catalyst, 21 ± 2 °C	0.0130 min^{-1}					Water		No degradation		[70]
	Seawater		26% degraded after 21 days								[74]

Continued

TABLE 4 The Fate of Antibiotics in the Aquatic Environment—Cont'd

	Photolysis			Hydrolysis			Biodegradation				
Compound	*Matrix*	K	*Half-time*	*Matrix*	K	*Half-time*	*Matrix*	K	*Half-time*	Note	References
	Underwater light		Stable								[74]
							Adapted aerated reactors		1.6 days		[71]
							Sediment		90 days		[75]
Sulfadoxine							Adapted aerated reactors		0.6 day		[71]
Sulfadimethoxine	Seawater		18% degraded after 21 days								[74]
	Underwater light		Stable								[74]
				pH 2, 5, 7, 9, 22 °C		No degradation in 21 days					[73]
							Marine sediment		20% degraded after 180 days		[76]
							Sewage sludge		52% degraded after 14 days		[77]
							Sewage sludge		>99% degraded after 14 days		[78]
							Adapted aerated reactors		4.1 days		[71]

Sulfaguanidine					
Sulfamethazine			Sewage sludge	23% degraded after 14 days	[77]
			Adapted aerated reactors	1.5 days	[71]
Sulfamethoxazole	Water, UV kmax 366 nm with TiO_2 catalyst, 21 ± 2 °C	0.0301 min^{-1}	Water	No degradation	[70]
			OECD 301 D	No degradation after 40 days	[15]
			Water	Nonbiodegradable	[79]
			Sewage sludge	59% degraded after 14 days	[77]
			Sewage sludge	>99% degraded after 14 days	[78]
Sulfameter			Adapted aerated reactors	0.4 day	[71]
Sulfamonomethoxine			Sewage sludge	>99% degraded after 14 days	[78]
Sulfanilamide			Adapted aerated reactors	3.8 days	[71]
Sulfapyridine			Adapted aerated reactors	1.5 days	[71]

Continued

TABLE 4 The Fate of Antibiotics in the Aquatic Environment—Cont'd

	Photolysis			Hydrolysis			Biodegradation				
Compound	*Matrix*	K	*Half-time*	*Matrix*	K	*Half-time*	*Matrix*	K	*Half-time*	Note	References
Sulfaquinoxaline											
Sulfisoxazole											
Sulfathiazole	Water, UV kmax 366 nm with TiO_2 catalyst, 21 ± 2 °C	0.0175 min^{-1}					Water		No degradation		[70]
				pH 2, 5, 7, 9, 22 °C		No degradation in 21 days					[73]
Diaminopyrimidines											
Ormetoprim											
Trimethoprim							Sediment		90 days		[75]
	Seawater		No degradation								[74]
				pH 2, 5, 7, 9, 22 °C		No degradation in 21 days					[73]
							Sewage sludge		27% degraded after 14 days		[77]

Tetracyclines								
Chlortetracycline			pH 7, 22 °C	505 h^{-1}	16 h		Epimerization was the dominant initial transformation process	[73]
						Soil and chicken manure (4 °C)	No degradation after 30 days	[80]
						Soil and chicken manure (20 °C)	44% Removed after 30 days	[80]
						Sandy loam, sandy	20–42 days	[81]
Doxycycline								
Methacycline								
Oxytetracycline			pH 7, 22 °C	206 h^{-1}	41 h			[73]
						Sediment	Very persistent	[75]
	Deionized water, light	16 days	Deionized water, light		59 days			[82]
	Freshwater, light	5 days	Freshwater, light		51 days			[82]
	Seawater, light	4 days	Seawater, light		51 days			[82]
	Seawater	<9 days						[74]

Continued

TABLE 4 The Fate of Antibiotics in the Aquatic Environment—Cont'd

	Photolysis			Hydrolysis			Biodegradation				
Compound	*Matrix*	K	*Half-time*	*Matrix*	K	*Half-time*	*Matrix*	K	*Half-time*	Note	References
							Sandy loam, clay loam		16–18 days		[72]
							Liquid pig manure		79 days		[72]
							Aerobic surface water		42–46 days		[83]
Tetracycline							Water		Nonbiodegradable		[79]
							Pig slurry (solid)		55–105 days		[84]
							Pig liquid manure (ventilated, nonventilated)		4.5–9 days	Including abiotic degradation	[85]
							Water (ventilated, nonventilated)		15–30 days	Including abiotic degradation	[85]
				pH 7, 22 °C	59.6 h^{-1}	116 h				Epimerization was the dominant initial transformation process	[73]
	30 °C water		3 h								[86]
	pH 4.75–9.5		0.08–<69 S^{-1}								[87]
	pH 6.4–8.1, $\log C_{Ca2+} = -2$ to -3		12–23 S^{-1}								[87]

Quinolones							
Oxolinic acid (OA)					Sediment	Very persistent	[75]
	Deionized water, light	66 days	Deionized water, darkness	Persistent			[82]
	Freshwater, light	Persistent	Freshwater, darkness	Persistent			[82]
	Seawater, light	99 days	Seawater, darkness	Persistent			[82]
	Seawater	<21 days					[74]
	Underwater light	Stable					[74]
Flumequine (FLU)	Deionized water, light	Persistent	Deionized water, darkness	108 days			[82]
	Freshwater, light	Persistent	Freshwater, darkness	Persistent			[82]
	Seawater, light	Persistent	Seawater, darkness	121 days			[82]
Fluoroquinolones							
Ciprofloxacin					Closed bottle test (CBT) (OECD 301 D)	No biodegradation in 40 days	[88]
	Pure water, simulated solar irradiation	22.9 ± 7.1 min					[89]
	Freshwater, simulated solar irradiation	19.3 ± 0.7 min					[89]

Continued

TABLE 4 The Fate of Antibiotics in the Aquatic Environment—Cont'd

	Photolysis			Hydrolysis			Biodegradation				
Compound	*Matrix*	K	*Half-time*	*Matrix*	K	*Half-time*	*Matrix*	K	*Half-time*	Note	References
	Seawater, simulated solar irradiation		26.0 ± 7.2 min								[89]
			2.9 h								[90]
	With fine particulate organic matter		7.4 h								[90]
	pH 5, 7, 9		0.77 h,0.15 h, 0.38 h								[91]
Danofloxacin	Pure water, simulated solar irradiation		23.9 ± 3.3 min								[89]
	Freshwater, simulated solar irradiation		20.4 ± 4.1 min								[89]
	Seawater, simulated solar irradiation		22.5 ± 5.1 min								[89]
							Three different soil types		87–143 days		[15]
Difloxacin	Pure water, simulated solar irradiation		31.1 ± 2.6 min								[89]
	Freshwater, simulated solar irradiation		84.0 ± 0.54 min								[89]
	Seawater, simulated solar irradiation		52.8 ± 11.2 min								[89]

Enrofloxacin	Pure water, simulated solar irradiation	15.6 ± 2.6 min					[89]
	Freshwater, simulated solar irradiation	48.7 ± 4.2 min					[89]
	Seawater, simulated solar irradiation	29.2 ± 4.7 min					[89]
Ofloxacin						No biodegradation in 40 days	[88]
Sarafloxacin					Sediment	Very persistent	[75]
	Pure water, simulated solar irradiation	29.8 ± 7.0					[89]
	Freshwater, simulated solar irradiation	30.9 ± 12.4					[89]
	Seawater, simulated solar irradiation	30.8 ± 8.4					[89]
					Soil (sandy loam, loam, silty loam)	0.5–0.5% degraded after 80 days	[92]
Macrolides							
Clarithromycin	UV–vis irradiation	1.25 h	Water in the presence of iron (III)	2.67 days			[93]

Continued

TABLE 4 The Fate of Antibiotics in the Aquatic Environment—Cont'd

	Photolysis			Hydrolysis			Biodegradation				
Compound	*Matrix*	K	*Half-time*	*Matrix*	K	*Half-time*	*Matrix*	K	*Half-time*	Note	References
Erythromycin							Water		Nonbiodegradable		[79]
							Manure storage in the dark at 20C, anaerobic conditions		41 days		[94]
Oleandomycin							Soil		23 days		[18]
Roxithromycin							Manure storage in the dark at 20 °C, anaerobic conditions		130 days		[94]
	UV–vis irradiation		1.63 h	Water in the presence of iron(III)		1.99 days					[93]
Tylosin							Soil and manure slurries		3.3–8.1 days		[83]
				pH 7, 22 °C		No degradation in 21 days					[73]
							Pig slurry		2.1 days		[18]
							Soil		95–97 days		[72]
							Aerobic surface water		9.5–40 days		[83]
							Sandy loam, sandy		40–86 days		[81]

	Pond water, sterilized pond water, and ultrapure water	200 days			Pond water, sterilized pond water, and ultrapure water	Degraded 6% after 180 days	[95]
Polyether ionophores							
Salinomycin					Manure storage in the dark at 20 °C, anaerobic conditions	5.1 days	[94]
Monensin					Manure (aerobic)	60–70% degraded after 70 days	[18]
Polypeptides							
Bacitracin					Animal wastewater and manure	Easy dissipation	[9]
					Soil and chicken manure (20 °C)	22.5 days	[80]
					Soil and chicken manure (30 °C)	12 days	[80]
Lincosamides							
Lincomycin			pH 7, 22 °C	No degradation in 21 days			[73]
Chloramphenicol derivatives							
Florfenicol					Sediment	4.5 days	[75]
	Deionized water, light	Persistent	Deionized water, darkness	Persistent			[82]

Continued

TABLE 4 The Fate of Antibiotics in the Aquatic Environment—Cont'd

Compound	Photolysis			Hydrolysis			Biodegradation				
	Matrix	K	*Half-time*	*Matrix*	K	*Half-time*	*Matrix*	K	*Half-time*	Note	References
	Freshwater, light		Persistent	Freshwater, darkness		Persistent					[82]
	Seawater, light		Persistent	Seawater, darkness		Persistent					[82]
							Simulated field conditions, including various pipe materials and conditions of hard or soft and chlorinated or nonchlorinated		Stable		[96]
Chloramphenicol							Sediment (aerobic)		<12 days		[15]
							Sediment (anaerobic)		<4 days		[15]
β-Lactams											
Ampicillin							Water		48% biodegradable		[79]
Ceftiofur				pH 5		100 days	Clay loam soil		22.2 days		[15]
				pH 7		8 days	Sandy soil		49.0 days		[15]
				pH 9		4.2 days	Silty clay loam soil		41.4 days		[15]

TABLE 5 Fate of NSAIDs, Lipid Regulators, Antiepileptics, and Beta-Blockers in the Environment

	Photolysis			Hydrolysis		Biodegradation				
Compound	*Matrix*	K	*Half-time*	*Matrix*	K $t_{1/2}$	*Matrix*	K	*Half-time*	Note	References
NSAIDs										
Ibuprofen	Sunlight irradiation in river water	$0.002\ h^{-1}$	324 h							[97]
						Sewage treatment		Inherently biodegradable		[79]
						Diluted waste activated sludge in aerobic conditions		4 days		[98]
	Water: both the solar simulator and under natural sunlight	$0.0025 \pm 0.001\ h^{-1}$	277 h							[99]
						Full-scale conventional activated sludge	$21–35\ Lg_{ss}^{-1}\ d^{-1}$			[100]
						Sewage in pilot-scale membrane bioreactor	$9–22\ Lg_{ss}^{-1}\ d^{-1}$			[100]
						Water–sediment system		<6–10 days		[68]
	Natural sunlight in water: September 2006/October 2007	$0.0012\ h^{-1}$/ $0.00027\ h^{-1}$	600 h/9900 h			River water: September 2006/October 2007	$015\ h^{-1}$/ $0.0018\ h^{-1}$	4500 h/4800 h		[67]

Continued

TABLE 5 Fate of NSAIDs, Lipid Regulators, Antiepileptics, and Beta-Blockers in the Environment—Cont'd

	Photolysis			Hydrolysis		Biodegradation				
Compound	*Matrix*	K	*Half-time*	*Matrix*	K $t_{1/2}$	*Matrix*	K	*Half-time*	Note	References
Diclofenac						Inoculated with diluted waste activated sludge		Biodegraded after 50 days		[98]
	Exposed in small quartz vials to natural sunlight in lake water	$1.31 \pm 0.03\ h^{-1}$	0.53 h							[101]
	Sunlight exposed lake water	$0.8\ h^{-1}$	0.9 h							[101]
	Sunlight irradiation in STP effluents		5 days							[102]
	Sunlight in water		39 min							[103]
Mefenamic acid	Noon sunlight in water		33 h							[87]
	Sunlight in lake water		27 ± 6.6 days							[34]
				Buffer solutions	>38 h					[104]
				In plasma	7.28 h					[104]
	UV-treated pure water	$0.002\ s^{-1}$								[105]
	Biological treated water	$0.0014\ s^{-1}$								[105]

	Natural sunlight in water: September 2006/October 2007	0.0089 h^{-1}/ 0.0073 h^{-1}	78 h/97 h	River water: September 2006/October 2007	0.00031 h^{-1}/ 0.011 h^{-1}	2500 h/300 h	[67]
Naproxen	Summer sunlight in river water		42 min				[103]
				Soil		2 days	
	Sunlight in lake water		9.6 ± 0.5 days				[34]
				Inoculated with diluted waste activated sludge		80% biodegraded after 50 days	[98]
				Full-scale conventional activated sludge	1.0–1.9 Lg_{ss}^{-1} d^{-1}		[100]
				Sewage in pilot-scale membrane bioreactor	0.4–0.8 Lg_{ss}^{-1} d^{-1}		[100]
				Sewage treatment		Nonbiodegradable	[79]
Ketoprofen	Sunlight irradiation in river water	17.63 h^{-1}	0.04 h				[97]
				Inoculated with diluted waste activated sludge; aerobic batch biodegradation		>99% biodegraded after 50 days	[98]
	Xenon arc lamp in river water		4.1 h				[103]

Continued

TABLE 5 Fate of NSAIDs, Lipid Regulators, Antiepileptics, and Beta-Blockers in the Environment—Cont'd

	Photolysis			Hydrolysis		Biodegradation				
Compound	*Matrix*	K	*Half-time*	*Matrix*	K $t_{1/2}$	*Matrix*	K	*Half-time*	Note	References
	UV-treated pure water	0.025 s^{-1}								[105]
	Biological treated water	0.02 s^{-1}								[105]
Fenoprofen	UV-treated pure water	0.0046 s^{-1}								[105]
	Biological treated water	0.003 s^{-1}								[105]
						Full-scale conventional activated sludge	10–14 Lg_{ss}^{-1} d^{-1}			[100]
						Sewage in pilot-scale membrane bioreactor	3.3–5.9 Lg_{ss}^{-1} d^{-1}			[100]
Salicylic acid	UV light with ZnO catalyst in double-distilled water	0.0421–0.1188 s^{-1}								[106]
	UV light with TiO_2 catalyst in distilled water	0.01716 s^{-1}								[107]
	UV radiation/ hydrogen peroxide/oxygen in aqueous solution	0.058 ± 0.014 s^{-1}								[108]

Acetylsalicylic acid				Sewage treatment		Readily biodegradable	[79]
Indomethacin	UV treatment in pure water	$0.0032\ s^{-1}$					[105]
	Biological treated water	$0.0026\ s^{-1}$					[105]
				Full-scale conventional activated sludge	≤ 0.3 ($Lg_{ss}^{-1}\ d^{-1}$)		[100]
				Sewage in pilot-scale membrane bioreactor	≤ 0.21 ($Lg_{ss}^{-1}\ d^{-1}$)		[100]
	Natural sunlight in water: September 2006/October 2007	$0.044\ h^{-1}$/ $0.034\ h^{-1}$	16 h/21 h	River water: September 2006/October 2007	$0.0016\ h^{-1}$/ $0.0018\ h^{-1}$	430 h/410 h	[67]
Acetaminophen	UV-treated pure water	$0.0018\ s^{-1}$					[105]
	Biological treated water	$0.0013\ s^{-1}$					[105]
				Aerobic batch Biodegradation: inoculated with diluted waste activated sludge		4 days	[98]
				Water–sediment system		3.1 ± 0.2 days	[68]

Continued

TABLE 5 Fate of NSAIDs, Lipid Regulators, Antiepileptics, and Beta-Blockers in the Environment—Cont'd

	Photolysis			Hydrolysis		Biodegradation				
Compound	*Matrix*	K	*Half-time*	*Matrix*	K $t_{1/2}$	*Matrix*	K	*Half-time*	Note	References
	Natural sunlight in water: September 2006/October 2007	0.013 h^{-1}/0.02 h^{-1}	56 h/35 h			River water: September 2006/October 2007	0.014 h^{-1}/ 0.00051 h^{-1}	50 h/1400 h		[67]
Lipid regulators										
Clofibric acid	Sunlight irradiation experiments: STP effluents		About 100 days							[102]
						Full-scale conventional activated sludge	0.3–0.8 Lg_{ss}^{-1} d^{-1}			[100]
						Sewage in pilot-scale membrane bioreactor	0.1–0.23 Lg_{ss}^{-1} d^{-1}			[100]
	UV-sunlight in water		50 h							[103]
						Water–sediment system		119 days		[68]
Gemfibrozil	Xenon arc lamp used in river water		15 h							[103]
						Aerobic batch Biodegradation: inoculated with diluted waste activated sludge		>99% biodegraded after 50 days		[98]

	Sunlight in lake water		119.5 ± 15.6–288.8 ±61.3 days			[34]
				Full-scale conventional activated sludge	6.4–9.6 $L g_{ss}^{-1} d^{-1}$	[100]
				Sewage in pilot-scale membrane bioreactor	0.5–1.8 $L g_{ss}^{-1} d^{-1}$	[100]
Bezafibrate				Full-scale conventional activated sludge	2.1–3.0 $L g_{ss}^{-1} d^{-1}$	[100]
				Sewage in pilot-scale membrane bioreactor	3.4–4.5 $L g_{ss}^{-1} d^{-1}$	[100]
Fenofibrate				Full-scale conventional activated sludge	7.2–10.8 $L g_{ss}^{-1} d^{-1}$	[100]
				Sewage in pilot-scale membrane bioreactor	0.4–1.7 $L g_{ss}^{-1} d^{-1}$	[100]
Antiepileptics						
Carbamazepine	Sunlight irradiation for STP effluents		About 100 days			[102]
				Water–sediment system	328 days	[68]
	Solar UV irradiation in surface water	$5.7 \times 10^{-3} h^{-1}$	121.6 days			[109]

Continued

TABLE 5 Fate of NSAIDs, Lipid Regulators, Antiepileptics, and Beta-Blockers in the Environment—Cont'd

	Photolysis			Hydrolysis		Biodegradation				
Compound	*Matrix*	K	*Half-time*	*Matrix*	K $t_{1/2}$	*Matrix*	K	*Half-time*	Note	References
	Natural sunlight in water: September 2006/October 2007	0.0082 h^{-1}/ 0.00034 h^{-1}	84 h/2100 h			River water: September 2006/October 2007	0.0006 h^{-1}/ 0.00023 h^{-1}	3000 h/5600 h		[67]
	Sunlight in river water	0.010 h^{-1}	67.4 h							[97]
Diazepam	Simulated sunlight in water		200 h							[110]
						Water–sediment system		311 ± 25 days		[68]
β-Blockers										
Metoprolol	UV-treated pure water	0.0026 s^{-1}								[105]
	Biological treated water	0.0013 s^{-1}								[105]
	Xenon arc lamp irradiation for pure water		630 h							[111]
	UV radiation for STP effluents		20.48 h							[112]

Propranolol	Sunlight irradiation for STP effluents		16.8 days				[102]
	Xenon arc lamp used for river water		1.1 min				[103]
	UV-treated pure water	0.0026 s^{-1}					[105]
	Biological treated water	0.0013 s^{-1}					[105]
	Xenon arc lamp irradiation for pure water		16 h				[111]
	Natural sunlight in water: September 2006/ October 2007	0.12 h^{-1}/0.084 h^{-1}	6 h/8.3 h	River water: September 2006/October 2007	0.006 h^{-1}/ 0.0012 h^{-1}	120 h/620 h	[67]
Atenolol	Xenon arc lamp irradiation in pure water		350 h				[111]
	UV radiation in STP water		Stable during 50 h				[112]
	Natural sunlight in water: September 2006/October 2007	0.009 h^{-1}/ 0.0099 h^{-1}	77 h/730h	River water: September 2006/October 2007	0.0034 h^{-1}/ 0.00044 h^{-1}	340 h/2900 h	[67]

TABLE 6 Comparisons of Photolysis and Biodegradation of Steroids in Different Matrixes

		Photolysis			Biodegradation			
Class	Compound	*Matrix*	K	*Half-time*	*Matrix*	K	*Half-time*	References
Androgens	Androsta-1,4-diene-3,17-dione							
	Androsterone				Activated sludge	0.71 h^{-1}	1.0 h	[113]
	4-Androstene-3,17-dione				Activated sludge	0.93 h^{-1}	0.7 h	[113]
	17α-Boldenone							
	17β-Boldenone							
	5α-Dihydrotestosterone							
	Epiandrosterone				Activated sludge	0.64 h^{-1}	1.1 h	[113]
	4-Hydroxy-androst-4-ene-17-dione							
	Methyl testosterone				Activated sludge	0.67 h^{-1}	1.0 h	[113]
					Aerobic sediment	0.51 d^{-1}	3.8 days	[114]
					Sulfate-reducing sediment	0.53 d^{-1}	5.3 days	[114]
					Methanogenic sediment	0.69 d^{-1}	5.1 days	[114]

					Nitrate-reducing sediment	$0.004\ d^{-1}$		[114]
					Iron (III)-reducing sediment	$0.007\ d^{-1}$		[114]
	19-Nortestosterone				Activated sludge	$0.9\ h^{-1}$	0.8 h	[113]
	Stanozolol				Activated sludge	$0.21\ h^{-1}$	3.3 h	[113]
	Testosterones				Activated sludge	$1.24\ h^{-1}$	0.6 h	[113]
					Aerobic; glucose	$0.12\ h^{-1}$	5.78 h	[115]
					Anaerobic; glucose	$0.026–0.181\ h^{-1}$	3.83–27.1 h	[115]
		Lake	$0.196\ h^{-1}$	3.54 h				[116]
		River	$0.191\ h^{-1}$	3.63 h				[116]
		Weir	$0.179\ h^{-1}$	3.87 h				[116]
	17α-Trenbolone							
	17β-Trenbolone				Activated sludge	$1.03\ h^{-1}$	0.7 h	[113]
Estrogens	Diethylstilbestrol							
	17α-Estradiol				Dairy lagoon wastewater	$0.006–0.0172\ h^{-1}$		[117]
	17β-Estradiol				Dairy lagoon wastewater	$0.0143–0.0675\ h^{-1}$		[117]

Continued

TABLE 6 Comparisons of Photolysis and Biodegradation of Steroids in Different Matrixes—Cont'd

		Photolysis			Biodegradation			
Class	Compound	*Matrix*	K	*Half-time*	*Matrix*	K	*Half-time*	References
					Seawater		1–9 days	[118]
					Aerobic; glucose	0.025 h^{-1}	26.9 h	[115]
					River water		0.2–8.7 days	[119]
					Bed sediment		0.11–0.66 days	[119]
					Cultures established from lake water and sediments		6.3–21.0 days	[120]
					Water–sediment, groundwater-aquifer material		No degradation under anaerobic condition	[121]
	Estrone				Dairy lagoon wastewater	0.0068–0.0249 h^{-1}		[117]
		Milli-Q water	0.0132–0.0144 min^{-1}	48.13–52.50 min				[122]
		Distilled water	−0.452–0.010 h^{-1}					[123]
		Ottawa River	−0.361–0.018 h^{-1}					[123]
		Lake Cromwell	−0.087 h^{-1}					[123]

	Raw sewage	$-0.065\ h^{-1}$			[123]
	Raisin River	-0.105–$0.004\ h^{-1}$			[123]
			River water	0.1–10.6 days	[119]
			Bed sediment	0.42–14.3 days	[119]
17α-Ethinylestradiol	Distilled water	-0.038–$0.004\ h^{-1}$			[123]
	Ottawa River	-0.030–$0.013\ h^{-1}$			[123]
	Lake Cromwell	$-0.021\ h^{-1}$			[123]
	Raisin River	-0.015–$0.007\ h^{-1}$			[123]
	Pure water	$0.030\ min^{-1}$			[124]
	Mix water	$0.018\ min^{-1}$			[124]
	Synthetic wastewater	$0.011\ min^{-1}$			[124]
	Real wastewater	$0.008\ min^{-1}$			[124]
			Seawater	3–5 days	[118]
			Cultures established from lake water and sediments	No anaerobic degradation over 3 years	[120]

Continued

TABLE 6 Comparisons of Photolysis and Biodegradation of Steroids in Different Matrixes—Cont'd

		Photolysis			Biodegradation			
Class	Compound	*Matrix*	K	*Half-time*	*Matrix*	K	*Half-time*	References
					Water–sediment, groundwater-aquifer material		No degradation under anaerobic condition, EE2 decreased from 1 to 0.62 μg/g within 70 days in the aquifer material	[121]
Glucocorticoids	Cortisol							
	Cortisone							
	Dexamethasone							
	Prednisolone							
	Prednisone							
Progestogens	Ethinyl testosterone							
	Medroxyprogesterone							
	19-Norethindrone				Activated sludge	0.57 h^{-1}	1.2 h	[113]
	Norgestrel				Activated sludge	0.47 h^{-1}	1.5 h	[113]

	Pure water	$0.0174\ min^{-1}$				[124]
	Mix water	$0.0176\ min^{-1}$				[124]
	Synthetic wastewater	$0.0078\ min^{-1}$				[124]
	Real wastewater	$0.0072\ min^{-1}$				[124]
Progesterone			Activated sludge	$0.69\ h^{-1}$	1.0 h	[113]
			Aerobic; glucose	$0.137\ h^{-1}$	5.06 h	[115]

responsible for about 20% of its degradation following a 14-day exposure at a temperature of +8 °C [82].

Fluoroquinolones are degradable by UV light despite their insensitivity to hydrolysis and temperature [9,90,91]. The photolysis of tylosin and its photo deactivation in surface water have been described [95,125]. Werner et al. [125] reported that tylosin is unique among the macrolides for both its absorption of light within the solar spectrum and the rapid, efficient *cis/trans* photoisomerization.

NSAIDs such as diclofenac, mefenamic acid, and naproxen are photodegradable under sunlight [30] (Table 5). Cholesterol-lowering drug atorvastatin can also be photodegraded in natural water, but the extent of photochemical reactions depends on some variables such as DOM and latitude [126]. Two beta-blockers atenolol and propranolol and an antiepileptic carbamazepine were found to be photodegradable with half-lives of up to 730, 8.3, and 2100 h, respectively[67]. *In situ* photodegradation experiments in a small stream in Germany for 10 pharmaceuticals belonging to various therapeutic classes (e.g., analgesics, beta-blockers, and lipid-lowering agents) show that elimination by photolysis is of minor importance for most drugs in rivers [127]. Only under optimal river conditions photolysis contributes up to 50% to the total elimination for a highly photolabile drug diclofenac [127].

Antiepileptic drug carbamazepine is capable of photolyzing and undergoes photochemical transformation in distilled water and river waters with a half-life of up to 907 sunlight hours [109]. Nitrate and humic acid have opposite effects on its degradation, the latter inhibiting and the former promoting.

Steroids such as estrone (E1), 17β-estradiol (E2), EE2, and norgestrel undergo a rapid photodegradation in natural waters with their half-lives of a few minutes to several hours (Table 6) [116,123,128,129]. E1 and EE2 were found to be degraded rapidly with half-lives of 48–123 min and <1.5 days, respectively [122]. But a simulated natural light laboratory study by Jürgen et al. [119] indicated that both E2 and EE2 were photodegraded in river waters with half-lives of at least 10 days under 12 h of bright sunshine per day. Under the presence of natural water constituents such as nitrate, iron, and humic acid, the photodegradation rate could increase significantly [130]. Photolysis contributes partly to the losses of these pharmaceuticals in the aquatic environment.

2.2.2 Hydrolysis

Hydrolysis is another important process for some pharmaceuticals in the aquatic environment (Tables 4–6). But not all pharmaceuticals can be hydrolyzed in water. For example, steroids and acidic drugs cannot undergo hydrolysis.

For antibiotics, hydrolysis is a significant process for their fate in the aquatic environment. For sulfonamides, an acidic pH solution is most favorable to hydrolysis, followed by neutral and alkaline solutions [131]. A rise in

solution temperature increases the hydrolysis of sulfonamides, but degradation is still low. On acidic hydrolysis, the sulfonamide bond breaks to produce sulfanilic acid and the appropriate amino derivatives as the common degradation products [7]. However, some sulfonamides (e.g., sulfachlorpyridazine, sulfadimethoxine, and sulfathiazole) are recalcitrant to hydrolysis at lower temperatures (7, 22, and 35 °C; pH 2, 5, 7, and 9) and require high concentrations of strong acids or bases [73]. Thus, under typical environmental conditions, sulfonamides are hydrolytically stable with a long half-life.

Tetracyclines are sensitive to hydrolysis [73,82]. The hydrolysis of oxytetracycline, chlortetracycline, and tetracycline is influenced by such factors as temperature and pH value [73]. However, fluoroquinolones are insensitive to hydrolysis [9]. Pouliquen et al. [82] reported that oxolinic acid and flumequine were not hydrolyzed in three types of water (deionized water, freshwater, and seawater).

Many macrolides are weak bases and unstable in acid [9]. Hydrolysis was observed at pH 2 and 11 for tylosin, but not at 5, 7, or 9 [73]. Hydrolysis rates for macrolides in the presence of iron (III) were low with their half-lives calculated to be 1.99 and 2.67 days for roxithromycin and clarithromycin, respectively [93].

Florfenicol is not degradable by hydrolysis or photolysis [82]. Trimethoprim and lincomycin cannot be degraded by hydrolysis either [73]. But for β-lactams, β-lactam ring is easily cleaved in acidic and basic media [9].

2.3 Biological Degradation

Microbial degradation is another important process for pharmaceuticals in the aquatic environment and can result in their partial or complete transformation. Although the literature data on biodegradation of pharmaceuticals in water–sediment systems are limited, microbial processes play a certain role in the dissipation of majority of pharmaceuticals from reported data in various media such as sewage treatment plants, soils, surface water, and sediments (Tables 4–6). In addition to its inherent chemical structure, environmental conditions such as redox potential are the crucial factors influencing the biodegradation of a pharmaceutical in the environment.

Antibiotics, such as oxytetracycline, trimethoprim, oxolinic acid, sarafloxacin, erythromycin, and florfenicol, are quite persistent in the aquatic environment from their wide occurrence in soil, sediment, and water [15,70,75,79,92,96]. However, sulfonamides are found to be degraded in sewage sludge, especially in adapted aerated bioreactors [71,78,132]. Microbial degradation in estuarine and coastal waters was determined for sulfamethoxazole with its half-lives of 85–100 days [133]. Laboratory studies in a simple shake flask system simulating the conditions in surface waters showed variable aerobic degradation of the antibiotics olaquindox, metronidazole, tylosin, and oxytetracycline with their half-lives of 4–8 days, 14–104 days, 9.5–40

days, and 42–46 days, respectively [83]. Half-lives of >100 days were found for trimethoprim under the same test conditions [134]. Addition of sediment (1 g/L) increased the biodegradation potential, but the biodegradation was significantly slower in tests conducted in absence of oxygen. Some antibiotics such as tylosin, salinomycin, and bacitracin are also easily dissipated in animal wastewater and manure [18,80,94,95]. Tetracyclines and quinolones show slow biodegradability [75,80,84]. Strong binding of these antibiotics to soil/sediment components delays their biodegradation and explains their recalcitrance in the environment [75].

Acidic drugs such as NSAIDs are biodegradable in aquatic environments (Table 5). Ibuprofen is degradable in water–sediment systems with its half-lives of 6–10 days, while carbamazepine is quite resistant to biodegradation with its half-lives up to 328 days [68]. A bench-scale biodegradation study showed effective biodegradation for five acidic pharmaceuticals diclofenac, bezafibrate, ibuprofen, naproxen, and gemfibrozil with the half-lives of 2.5–18.6 days with moving sediment (aerobic conditions), but no removal for clofibric acid [135]. In the same study with flat sediment (anaerobic or anoxic conditions), no or limited degradation was observed for these acidic pharmaceuticals. Under aerobic conditions, biofilms of river sediment have a remarkable and common activity for degradation of diclofenac and ibuprofen [136,137]. Biotransformation of beta-blockers in surface water–sediment systems exhibited a low to high persistence with 50% disappearance ranging from 0.13–3 days (pindolol and atenolol) to >30 days (sotalol and propranolol) [69]. Benotti and Brownawell [133] measured microbial degradation rates of 19 pharmaceuticals in estuarine and coastal waters samples and found that antipyrine, carbamazepine, cotinine, sulfamethoxazole, and trimethoprim were the most refractory with the half-lives of 35 to >100 days, while acetaminophen, caffeine, diltiazem, fluoxetine, nicotine, and nifedipine were mostly labile across all treatments with half-lives of 3.5–13 days.

Microbial process also plays an important role in the degradation of steroids. Majority of the studies in the literature focus on estrogens, less on other classes of steroids. Most natural steroids such as E1 and E2 can be degraded by microorganism within several hours or days (Table 6). But some synthetic steroids such as EE2 can be more persistent in the aquatic environment, especially under anaerobic conditions [121,138]. There are some recent in-depth studies on biodegradation of estrogens and androgens in aquatic environments [113,117,118,139,140]. Diverse E2-degrading bacteria have been isolated and identified, and Li et al. [140] found *Stenotrophomonas maltophilia* strain ZL1 was able to convert E1 to amino acid tyrosine through ring cleavage on a saturated ring of the E1 molecule and then utilize tyrosine in protein biosynthesis. In addition to bacteria and fungi, some microalgae can also transform steroids (e.g., EE2) in water [141]. Biodegradation rate can be influenced by factors such as temperature, nutrients, and redox [121,138]. Aerobic conditions are more conducible to the biodegradation of estrogens than anaerobic

conditions. There is evidence that once estrogens are deposited into bed sediments, estrogen residues are likely to persist under anaerobic conditions [121,142]. Less biodegradation information is available for the other classes of steroids in the aquatic environment. Aerobic sludge tests showed rapid biodegradation of most androgens and progestogens with their half-lives of 0.6–3.3 h [113]. Under aerobic conditions, natural steroids such as testosterone dissipated with a similar half-life to E2 and EE2 [143]. Homklin et al. [114] investigated biotransformation of 17α-methyltestosterone (MT) in fish pond sediment under different electron acceptor conditions and showed that MT was biotransformed under aerobic and sulfate-reducing conditions with a half-life of 3.8 and 5.3 days, respectively, with complete disappearance of androgenic activity. However, under methanogenic condition, MT was found to biotransform but the androgenic activity continued to persist even after 45 days of incubation. Three MT-degrading bacteria were also isolated from the fish pond sediment and showed the capability of degrading MT to the products with no androgenic potency [144].

3 OCCURRENCE IN THE AQUATIC ENVIRONMENT

3.1 Antibiotics

Various antibiotics have been frequently detected in surface water with the concentrations ranging from not detected to several micrograms/liters (Table 7). Among the reported antibiotics, sulfamethoxazole, oxytetracycline, ciprofloxacin, norfloxacin, ofloxacin, clarithromycin, and erythromycin-H_2O are frequently detected in the aqueous phase of rivers in the world with concentrations up to several micrograms/liters. It should be noted that more antibiotics with high concentrations are found in rivers, which receive urban wastewater discharges and animal wastes [155,156,163]. However, fluoroquinolones and tetracyclines in water are often found at much lower concentration levels (mostly nanograms/liters) or not detected [20,78,146–148,153], which is mainly due to their strong sorption onto river sediments. Zhou et al. [147] reported relatively low concentrations of sulfonamides and macrolides in the sediments of three large Chinese rivers (Yellow River, Liao River, and Hai River), but the fluoroquinolones and tetracyclines were detected at much higher concentrations in the same river sediments.

3.2 Nonsteroidal Anti-Inflammatory Drugs

NSAIDs have been widely reported in surface water of many countries. The reported concentrations for ibuprofen, diclofenac, mefenamic acid, naproxen, ketoprofen, fenoprofen, salicylic acid, acetylsalicylic acid, meclofenamic acid, tolfenamic acid, and indomethacin are usually at a range from several nanograms/liters to highest several micrograms/liters in surface water

TABLE 7 Occurrence of Antibiotics in the Aquatic Environment

Compound	Country	Location	Surface Water (ng/L)			Sediment (μg/kg)			References
			Range	*Median*	*Mean*	*Range*	*Median*	*Mean*	
Sulfonamides									
Sulfachlorpyridazine	United States	139 stream sites	ND						[145]
	United States	Cache la Poudre River	30		30	1.9–3.32		2.7	[146]
Sulfadiazine	China	Yellow River				ND–22	ND		[147]
	China	Hai River				ND–1.18	ND		[147]
	China	Liao River				ND–11	ND		[147]
	China	Pearl River				ND–83.9	3.16		[115]
	China	Pearl River	ND–26.9						[148]
	China	Victoria Harbour	ND						[149]
	China	Pearl River	ND–336						[149]
	China	Streams with livestock farms	4.57–214			ND			[150]
Sulfadimethoxine	Japan	Nationwide survey	ND–6.4	ND	0.45				[151]
	United States	139 stream sites	Up to 60						[145]
	United States	Cache la Poudre River	10–40		20	1.7–6.8		3.8	[146]
	Vietnam	Urban drainage	ND						[152]
	Vietnam	Mekong River	ND						[152]
	Japan	Tamagawa River	ND						[152]

Sulfamerazine	Japan	Nationwide survey	ND-0.03	ND	0.002			[151]
	United States	139 stream sites	ND					[145]
	United States	Cache la Poudre River	10–60		20	2.3–6.8	4.8	[146]
	Japan	Tamagawa River	ND					[152]
Sulfamethazine	China	Yellow River				ND		[147]
	China	Hai River				ND–5.67	ND	[147]
	China	Liao River				ND		[147]
	China	Pearl River				ND–248	19.7	[115]
	China	Pearl River	ND–446					[148]
	United States	Cache la Poudre River	20		20	1–13.7	4.7	[146]
	United States	139 stream sites	Up to 220					[145]
	Japan	Nationwide survey	ND–62.9	ND	2.55			[151]
	Germany	River waters and drainages	ND	ND				[20]
	Vietnam	Urban drainage	58–328	103	119			[152]
	Vietnam	Mekong River	15–28	19	20.3			[152]
	Japan	Tamagawa River	ND					[152]
	France	Seine River	<10					[153]
	China	Victoria Harbour	ND					[149]
	China	Pearl River	ND–323					[149]
	China	Streams with livestock farms	63.8–101			4.16–5.34		[150]

Continued

TABLE 7 Occurrence of Antibiotics in the Aquatic Environment—Cont'd

			Surface Water (ng/L)			Sediment (μg/kg)			
Compound	**Country**	**Location**	*Range*	*Median*	*Mean*	*Range*	*Median*	*Mean*	**References**
Sulfamethoxazole	China	Yellow River				ND			[147]
	China	Hai River				ND			[147]
	China	Liao River				ND–< LOQ	ND		[147]
	China	Pearl River				ND–< LOQ	ND		[115]
	China	Pearl River	ND–616						[148]
	China	Streams with livestock farms	3.58–11.9			ND			[150]
	United States	Cache la Poudre River	40–320		110	1.2–1.9		1.6	[146]
	United States	139 stream sites	Up to 1900						[145]
	Germany	Water slides in Westphalia	40–200						[154]
	Japan	Nationwide survey	ND–33.9	1.1	4.85				[151]
	Spain	Llobregat River	0.2–1500						[155]
	Spain	Llobregat River	30–11,920		1110				[156]
	United Kingdom	Downstream of WWTPs	<50						[157]

	Sweden	Hoje River	ND–10				[158]
	Italian	Po river	1.83–2.39		2.1		[159]
	Italian	River Arno	1.79–11.4		5.3		[159]
	Germany	River waters and drainages	ND–480	30			[20]
	France	Seine River	Up to 121				[153]
	Vietnam	Urban drainage	37–360	153	179		[152]
	Vietnam	Mekong River	20–33	22	26.3		[152]
	Japan	Tamagawa River	4–23	18.5	7		[152]
	Australia	Six river systems	ND–2000	8			[152]
	China	Victoria Harbour	ND				[149]
	China	Yellow River	<LOQ–56				[43]
	France	Arc River	ND				[160]
	China	Pearl River	ND–193				[149]
Sulfapyridine	China	Yellow River				ND	[147]
	China	Hai River				ND	[147]
	China	Liao River				ND	[147]
	China	Pearl River				ND–< LOQ	[115]
	China	Pearl River	ND–74.6				[148]

Continued

TABLE 7 Occurrence of Antibiotics in the Aquatic Environment—Cont'd

Compound	Country	Location	Surface Water (ng/L)			Sediment (μg/kg)			References
			Range	*Median*	*Mean*	*Range*	*Median*	*Mean*	
	Japan	Nationwide survey	ND–144	1.95	15.2				[151]
	Vietnam	Urban drainage	ND						[152]
	Vietnam	Mekong River	ND						[152]
	Japan	Tamagawa River	21–132	108	41.9				[152]
Sulfathiazole	Japan	Nationwide survey	ND–0.02	ND	0.0005				[151]
	United States	139 stream sites	Up to 130						[145]
	United States	Cache la Poudre River	10–30		10	1.3–5.4		3.3	[146]
	Vietnam	Urban drainage	ND						[152]
	Vietnam	Mekong River	ND						[152]
	Japan	Tamagawa River	ND						[152]
	Australia	Six river systems	ND–40	ND					[152]
Diaminopyrimidines									
Trimethoprim	China	Yellow River				ND–< LOQ	ND		[147]

	China	Hai River				ND–5.63	ND	[147]
	China	Liao River				ND–9.84	0.93	[147]
	China	Pearl River	ND–605					[148]
	China	Streams with livestock farms	6.22–19.2			ND–1.77		[150]
	Germany	Water slides in Westphalia	6–70 ng/L					[154]
	United States	139 stream sites	Up to 710					[145]
	Japan	Nationwide survey	ND–36	0.02	2.50			[151]
	Spain	Llobregat River	ND–35.6					[155]
	Spain	Llobregat River	20–470		140			[156]
	United Kingdom	Downstream of WWTPs	<10–42	<10	12			[157]
	United Kingdom	Estuaries	<4–569	<4				[161]
	Sweden	Hoje River	<1–20					[158]
	Germany	River waters and drainages	ND–200	ND				[20]
	France	Seine River	ND–45					[153]
	Vietnam	Urban drainage	15–46	28	29.9			[152]
	Vietnam	Mekong River	7–19	17.5	15.3			[152]

Continued

TABLE 7 Occurrence of Antibiotics in the Aquatic Environment—Cont'd

			Surface Water (ng/L)			Sediment (μg/kg)			
Compound	Country	Location	*Range*	*Median*	*Mean*	*Range*	*Median*	*Mean*	References
	Japan	Tamagawa River	19–54	29.5	13.7				[152]
	Australia	Six river systems	ND–150	3					[152]
Tetracyclines									
Chlortetracycline	United States	139 stream sites	Up to 690						[145]
	China	Yellow River				ND			[147]
	China	Hai River				ND–10.9	ND		[147]
	China	Liao River				ND–32.5	ND		[147]
	China	Streams with livestock farms	ND-98.2			315–1010			[150]
	United States	Cache la Poudre River	10–210		80	1.1–30.8		10.8	[146]
	Germany	River waters and drainages	ND	ND					[20]
	Australia	Six river systems	ND–600	3					
Doxycycline	China	Yellow River				ND			[147]
	China	Hai River				ND–7.0	ND		[147]
	China	Liao River				ND–2.8	ND		[147]
	China	Streams with livestock farms	ND–12.6			35.8–444			[150]

	United States	Cache la Poudre River	10–50		30	2.2–38.9	15.7	[146]
	United States	139 stream sites	ND					[145]
	Germany	River waters and drainages	ND	ND				[20]
	Australia	Six river systems	ND–400	ND				[162]
Oxytetracycline	China	Yellow River				ND–184	ND	[147]
	China	Hai River				ND–422	2.52	[147]
	China	Liao River				ND–652	2.34	[147]
	China	Pearl River				ND–196	7.15	[115]
	China	Streams with livestock farms	33–60			497–214		[150]
	Japan	Streams with livestock farms	2–6800					[163]
	United States	Cache la Poudre River	10–1210		180	2.4–56.1	14.8	[146]
	United States	139 stream sites	Up to 340					[145]
	Italian	Po river	<1.19–1.82		1.1			[159]
	Italian	River Arno	<1.19		<1.19			[159]
	Germany	River waters and drainages	ND	ND				[20]
	Australia	Six river systems	ND–100	ND				[162]
	France	Arc River	ND–650					[160]

Continued

TABLE 7 Occurrence of Antibiotics in the Aquatic Environment—Cont'd

			Surface Water (ng/L)			Sediment (μg/kg)			
Compound	**Country**	**Location**	*Range*	*Median*	*Mean*	*Range*	*Median*	*Mean*	**References**
Tetracycline	China	Yellow River				ND–18	ND		[147]
	China	Hai River				1.06–135	2.0		[147]
	China	Liao River				ND–4.82	ND		[147]
	China	Pearl River				ND–72.6	4.05		[115]
	China	Streams with livestock farms	ND–8.73			13.7–56.3			[150]
	United States	Cache la Poudre River	10–30		20	1.1–102.7	17.9		[146]
	United States	139 stream sites	Up to 110						[145]
	Germany	River waters and drainages	ND	ND					[20]
	Australia	Six river systems	ND–80	ND					[162]
Fluoroquinolones									
Ciprofloxacin	United States	139 stream sites	Up to 30						[145]
	China	Yellow River				ND–32.8	ND		[147]
	China	Hai River				2.05–1290	16.0		[147]
	China	Liao River				ND–28.7	ND		[147]
	China	Streams with livestock farms	ND–8.91			8.72–20.5			[150]
	China	Pearl River				ND–197	21.8		[115]

	Germany	Water slides in Westphalia	Up to 13 ng/L				[154]
	Italian	Po river	1.32–16		8.8		[159]
	Italian	River Arno	<1.8–37.5		19		[159]
	Australia	Six river systems	ND–1300	ND			[162]
	France	Arc River	ND–9660				[160]
Danofloxacin	France	Seine River	ND–19				[153]
Difloxacin	France	Seine River	<10				[153]
Enrofloxacin	China	Yellow River				ND	[147]
	China	Hai River				ND–2.34 ND	[147]
	China	Liao River				ND	[147]
	China	Streams with livestock farms	ND–2.45			21.3–137	[150]
	France	Seine River	<10				[153]
	Australia	Six river systems	ND–300	ND			[162]
Fleroxacin	China	Streams with livestock farms	ND–4.48			ND	[150]
Lomefloxacin	China	Yellow River				ND	[147]
	China	Hai River				ND–298 1.67	[147]
	China	Liao River				ND–5.82 ND	[147]
	China	Streams with livestock farms	ND			ND–2.78	[150]

Continued

TABLE 7 Occurrence of Antibiotics in the Aquatic Environment—Cont'd

			Surface Water (ng/L)			Sediment (μg/kg)			
Compound	Country	Location	*Range*	*Median*	*Mean*	*Range*	*Median*	*Mean*	References
	Italian	Po river	<0.31		<0.31				[159]
	Italian	River Arno	<0.31		<0.31				[159]
	France	Seine River	<10						[153]
Marbofloxacin	China	Streams with livestock farms	ND–3.46			ND			[150]
Norfloxacin	United States	139 stream sites	Up to 120						[145]
	France	Seine River	ND–163						[153]
	China	Yellow River				ND–114	8.34		[147]
	China	Hai River				ND–5770	32.0		[147]
	China	Liao River				ND–176	3.32		[147]
	China	Streams with livestock farms	ND–14.8			19.9–27.6			[150]
	China	Pearl River				ND–1120	88.0		[115]
	China	Pearl River	ND–174						[148]
	Australia	Six river systems	ND–1150	30					[162]
	China	Victoria Harbour	ND–28.1						[149]
	China	Pearl River	ND–251						[149]
	China	Yellow River	<LOQ–300						[43]

Ofloxacin	China	Yellow River			ND–123	3.07	[147]
	China	Hai River			ND–653	10.3	[147]
	China	Liao River			Up to 50.5	65.3	[147]
	China	Streams with livestock farms	ND–14.5		17.7–235		[150]
	China	Pearl River			ND–1560	156	[115]
	Spain	Llobregat River	<LOD–488.4				[155]
	Spain	Llobregat River	190–8770	2110			[156]
	Italian	Po river	0.65–18.06	10.9			[159]
	Italian	River Arno	<1.4–10.88	5			[159]
	France	Seine River	ND–55				[153]
	China	Victoria Harbour	ND–16.4				[149]
	China	Pearl River	ND–108				[149]
	China	Yellow River	<LOQ–264				[43]
Pefloxacin	China	Streams with livestock farms	ND		4.45–20.5		[150]
Sarafloxacin	United States	139 stream sites	ND				[145]
	France	Seine River	<10				[153]
Carbadox	United States	139 stream sites	ND				[145]
Macrolides							
Azithromycin	Japan	Nationwide survey	ND–44.5	0.0005	1.94		[151]
	Spain	Llobregat River	<MDL				[156]

Continued

TABLE 7 Occurrence of Antibiotics in the Aquatic Environment—Cont'd

			Surface Water (ng/L)			Sediment (μg/kg)			
Compound	**Country**	**Location**	*Range*	*Median*	*Mean*	*Range*	*Median*	*Mean*	**References**
	Vietnam	Urban drainage	ND						[152]
	Vietnam	Mekong River	ND						[152]
	Japan	Tamagawa River	43–448	153	89.1				[152]
	France	Arc River	ND			ND–265			[160]
Clarithromycin	Japan	Nationwide survey	ND–233	1	16.1				[151]
	Italian	Po river	0.89–2.19		1.7				[159]
	Italian	River Arno	6.7–44.76		25.4				[159]
	Vietnam	Urban drainage	ND						[152]
	Vietnam	Mekong River	ND						[152]
	Germany	River waters and drainages	ND–260	ND					[20]
	France	Arc River	ND–1560			ND–3.82			[160]
	Japan	Tamagawa River	55–254	168	71.5				[152]
Erythromycin	Japan	Nationwide survey	ND–27.8	0.01	2.55				[151]
	Spain	Llobregat River	ND–362.5						[155]
	Spain	Llobregat River	10–70		30				[156]
	Italian	Po river	0.78–4.62		2.9				[159]

	Italian	River Arno	2.88–8.12		5.4			[159]
	Australia	Six river systems	Not quantified					[162]
Erythromycin-H_2O	China	Yellow River				ND–49.8	1.28	[147]
	China	Hai River				ND–67.7	<LOQ	[147]
	China	Liao River				ND–40.3	3.61	[147]
	China	Pearl River				ND–385	24.4	[115]
	China	Pearl River	ND–2070					[148]
	United States	Cache la Poudre River	20–450		120	1.3–25.6	10	[146]
	Germany	Water slides in Westphalia	Up to 200 ng/L					[154]
	Japan	Nationwide survey	ND–128	1.1	8.13			[151]
	United States	139 stream sites	Up to 1700					[145]
	Italian	Po river	1.66–5.31		3.7			[159]
	Italian	River Arno	9.68–30.52		17.9			[159]
	Germany	River waters and drainages	ND–1700	150				[20]
	Vietnam	Urban drainage	29–41	35.6	36.5			[152]
	Vietnam	Mekong River	9–12	10.5	10.5			[152]
	Japan	Tamagawa River	21–120	78	32.9			[152]

Continued

TABLE 7 Occurrence of Antibiotics in the Aquatic Environment—Cont'd

			Surface Water (ng/L)			Sediment (μg/kg)			
Compound	Country	Location	*Range*	*Median*	*Mean*	*Range*	*Median*	*Mean*	References
	Australia	Six river systems	Not quantified						[162]
	China	Victoria Harbour	ND–5.2						[149]
	China	Pearl River	ND–636						[149]
	China	Yellow River	<LOQ–102						[43]
Oleandomycin	Australia	Six river systems	ND–20	ND					[162]
Roxithromycin	China	Yellow River				ND–6.8	ND		[147]
	China	Hai River				ND–11.7	2.29		[147]
	China	Liao River				ND–29.6	5.51		[147,164]
	China	Pearl River				ND–133	24.7		[115]
	China	Pearl River	ND–2260						[148]
	United States	Cache la Poudre River	ND			1.1–5.9		2.1	[146]
	United States	139 stream sites	Up to 180						[145]
	Germany	River waters and drainages	ND–560	ND					[20]
	Vietnam	Urban drainage	ND						[152]
	Vietnam	Mekong River	ND						[152]

	Japan	Tamagawa River	13–43	28	11.7				[152]
	Australia	Six river systems	ND–350	9					[162]
	China	Victoria Harbour	ND–30.6						[149]
	China	Pearl River	ND–169						[149]
	China	Yellow River	<LOQ–95						[43]
Tylosin	Germany	Water slides in Westphalia	90 ng/L						[154]
	China	Streams with livestock farms	ND–5.55			ND			[150]
	United States	139 stream sites	Up to 280						[145]
	Italian	Po river	<0.77		<0.77				[159]
	Italian	River Arno	<0.77		<0.77				[159]
	United States	Cache la Poudre River	50			1.1–9.3		3	[146]
	Australia	Six river systems	ND–60	1					[162]
Ionophores									
Salinomycin	Australia	Six river systems	ND–150	ND					[162]
Monensin	Australia	Six river systems	ND–150	2					[162]

Continued

TABLE 7 Occurrence of Antibiotics in the Aquatic Environment—Cont'd

Compound	Country	Location	Surface Water (ng/L)			Sediment (μg/kg)			References
			Range	*Median*	*Mean*	*Range*	*Median*	*Mean*	
Polypeptides									
Bacitracin	Australia	Six river systems	ND						[162]
Lincosamides									
Lincomycin	United States	139 stream sites	Up to 730						[145]
	Italian	Po river	3.72–7.47		5.7				[159]
	Italian	River Arno	5.34–10.92		8.1				[159]
	Australia	Six river systems	ND–50	1					[162]
Clindamycin	Germany	Water slides in Westphalia	3–90 ng/L						[154]
	Australia	Six river systems	ND–10	1					[162]
Chloramphenicol derivatives									
Chloramphenicol	Germany	River waters and drainages	ND–60	ND					[20]
	China	Victoria Harbour	ND						[149]
	China	Pearl River	ND–266						[149]

β-Lactams						
Amoxicillin	Italian	Po river	<2.08		<2.08	[159]
	Italian	River Arno	3.57–9.91		5.7	[159]
	Australia	Six river systems	ND–200	ND		[162]
	China	Victoria Harbour	ND			[149]
	China	Pearl River	ND			[149]
Cloxacillin	Germany	River waters and drainages	ND	ND		[20]
	Australia	Six river systems	ND			[162]

(Table 8). Most of the studies focused on the receiving waters where WWTP effluents are discharged. For example, Ashton et al. [157] reported the concentrations for ibuprofen, diclofenac, and mefenamic acid in the streams with effluent discharges and found higher concentrations in the downstream than in the upstream of effluent outfalls. In the United States, the maximum concentrations for ibuprofen in streams are about 1000 ng/L [145], while this is much lower in larger rivers such as Mississippi River [175]. Similarly, higher concentrations for NSAIDs are usually found in small streams than in large rivers in other places of the world, because small streams receive sewage effluents with lower dilution.

In China, wastewater treatment rates of domestic sewage are still not high in many cities, especially in nondeveloped cities. And direct discharge of domestic sewage in rural area in China is also a common practice. High concentrations of these detected pharmaceuticals in the Pearl River, Yellow River, Hai River, and Liao River were found more frequently at those sites located in metropolitan areas, lower reaches, or river confluences [181,182].

NSAIDs are highly hydrophilic in water with acid group; hence, they are seldom found in sediments. Vazquez-Roig et al. [169] reported the <LOQ values for ibuprofen, diclofenac, and clofibric acid in sediments from Mediterranean coastal wetland.

3.3 Lipid Regulators

Several lipid regulators, clofibric acid and gemfibrozil, have similar properties as NSAIDs with acid group and display higher hydrophilicity. They have been detected in surface waters from Canada, China, Europe, Japan, and the United States [165–168,170,175,180–182]. Bezafibrate and fenofibrate are also found in Canada, Europe, and Japan [168,170,174,178,180] but are seldom reported in China. Similar to NSAIDs, human discharge after domestic use is the main source for lipid regulators into the aquatic environment.

3.4 Psychoactive Drugs

Carbamazepine is the most commonly used antiepileptic drug, and it has been frequently detected in sewage effluents and surface waters [164,167,169–172, 175,178,181,183]. It can be seen that the concentrations reported in rivers vary from not detected to several micrograms/liters (Table 8). In German surface waters, carbamazepine was detected at median value of 250 ng/L and maximum value of 1100 ng/L [167]. In the United Kingdom, the concentration of carbamazepine at the downstream of WWTP effluent outfall is higher than that in the upstream [164]. In the US river surface water, the concentrations of carbamazepine ranged from not detected (ND) to several hundred

TABLE 8 Concentrations of Pharmaceuticals NSAIDs, Lipid Regulators, Antiepileptics, and Beta-Blockers in Surface Waters and Sediments in Different Regions of the World

			Surface Water (ng/L)			Sediment (μg/kg)			
Compound	Country	Location	*Range*	*Median*	*Mean*	*Range*	*Median*	*Mean*	References
NSAIDs									
Ibuprofen	United Kingdom	Surface waters of the lower River Tyne	144–2370	304	613				[165]
	United Kingdom	Upstream of effluent outfall	<20–1555	181	432				[157]
	United Kingdom	Downstream of effluent outfall	<20–5044	826	1105				[157]
	United Kingdom	River Taff in South Wales	<0.3–100	1–33					[166]
	United Kingdom	River Ely in South Wales	<0.3–93	10–36					[166]
	Germany	Rivers and streams	Up to 530	70					[167]
	Germany	River Elbe and its tributaries	<2–146	5	12				[168]
	Spain	Mediterranean coastal wetland	Up to 59		16.3	<LOQ		<LOQ	[169]
	Spain	Ebro river basin (river waters downstream WWTPs)	<LOQ–289	65	97				[170]
	Spain	The Henares–Jarama–Tajo River system	ND–2784	253.9	578.6				[171]

Continued

TABLE 8 Concentrations of Pharmaceuticals NSAIDs, Lipid Regulators, Antiepileptics, and Beta-Blockers in Surface Waters and Sediments in Different Regions of the World—Cont'd

			Surface Water (ng/L)			Sediment (μg/kg)			
Compound	Country	Location	*Range*	*Median*	*Mean*	*Range*	*Median*	*Mean*	References
	Italy	Po river	Up to 17.4	13.0					[172]
	Switzerland	Lakes and rivers from the North Sea	ND–7.8	4.3	4.6				[173]
	Finland	Rivers received WWTP effluents	<0.5–14	5.5	6.2				[174]
	United States	139 streams in America	1000	200					[145]
	United States	Mississippi river waters in New Orleans, Louisiana, United States	0–34						[175]
	United States	Mississippi River in Louisiana	ND						[176]
	United States	Santa Ana River	64–250	151					[177]
	Canada	Otonabee River			ND				[178]
	Canada	Little River			8				[178]
	Canada	Detroit River			ND				[178]
	Korea	Mankyung River, South Korea	ND–414	160	208				[179]
	Japan	Tone River basin	<LOQ (30)						[180]

	China	Liuxi River of Pearl River system	1–11.3	2.45	4.96			[181]
	China	Zhujiang River of Pearl River system	1.9–31.1	15.6	14.6			[181]
	China	Shijing River of Pearl River system	62.8–685	193.5	264.9			[181]
	China	Yellow River (wet/dry)	ND–416	12.4/6.4	40.8/11.3			[182]
	China	Hai River (wet/dry)	ND–127	83.5/49.0	75.2/54.2			[182]
	China	Liao River (wet/dry)	ND–246	2.9/27.9	7.1/61.9			[182]
Diclofenac	United Kingdom	Surface waters of the lower River Tyne	<8					[165]
	United Kingdom	Upstream of effluent outfall	<20					[157]
	United Kingdom	Downstream of effluent outfall	0–568	47	156			[157]
	United Kingdom	River Taff in South Wales	<0.5–85	<0.5–21				[166]
	United Kingdom	River Ely in South Wales	<0.5–261	<0.5–41				[166]
	Germany	Rivers and streams	Up to 1200	150				[167]
	Germany	River Elbe and its tributaries	<1–69	7	11.3			[168]
	Spain	Mediterranean coastal wetland	Up to 16.9		2.9	<LOQ	<LOQ	[169]
	Spain	Ebro river basin (river waters downstream WWTPs)	<5–50	7	14.1			[170]

Continued

TABLE 8 Concentrations of Pharmaceuticals NSAIDs, Lipid Regulators, Antiepileptics, and Beta-Blockers in Surface Waters and Sediments in Different Regions of the World—Cont'd

			Surface Water (ng/L)			Sediment (μg/kg)			
Compound	Country	Location	*Range*	*Median*	*Mean*	*Range*	*Median*	*Mean*	References
	Spain	The Henares–Jarama–Tajo River system	ND–156	18.7	32.8				[171]
	Finland	Rivers received WWTP effluents	<0.5–35	2	6.8				[174]
	Japan	Main stream of Tone River	Up to 3.3	1.7					[180]
	Japan	Tributaries of Tone River	Up to 3.3	2.6					[180]
	China	Liuxi River of Pearl River system	9.1–11.0	10.1	10.1				[181]
	China	Zhujiang River of Pearl River system	1.6–32.7	7.9	11.6				[181]
	China	Shijing River of Pearl River system	16.6–150	61.7	77.5				[181]
	China	Yellow River (wet/dry)	ND–136	5.7/7.2	15.4/8.6				[182]
	China	Hai River (wet/dry)	ND–46.4	23.4/ND	25.2/4.6				[182]
	China	Liao River (wet/dry)	ND–717	9.9/21.9	52.4/32.1				[182]
Mefenamic acid	United Kingdom	Surface waters of the lower River Tyne	<20						[165]
	United Kingdom	Upstream of effluent outfall	<50						[157]
	United Kingdom	Downstream of effluent outfall	<50–366	62	120				[157]

	United Kingdom	River Taff in South Wales	<0.3–169	1–20		[166]
	United Kingdom	River Ely in South Wales	<0.3–33	1–9		[166]
	Spain	Ebro river basin (river waters downstream WWTPs)	2–8	3	3.9	[170]
	Korea	Mankyung River, South Korea	ND–326	89	118	[179]
	Japan	Tone River	<LOQ			[180]
	China	Liuxi River of Pearl River system	ND			[181]
	China	Zhujiang River of Pearl River system	4.7–7.0	5.1	5.4	[181]
	China	Shijing River of Pearl River system	5.6–24.6	13.5	13.9	[181]
	China	Yellow River	ND			[182]
	China	Hai River	ND–3.4	ND	<LOQ	[182]
	China	Liao River	ND			[182]
Naproxen	United Kingdom	River Taff in South Wales	<0.3–146	1–53		[166]
	United Kingdom	River Ely in South Wales	<0.3–113	5–43		[166]
	Germany	Rivers and streams	Up to 390	70		[167]
	Germany	River Elbe and its tributaries	<1–32	1	3	[168]

Continued

TABLE 8 Concentrations of Pharmaceuticals NSAIDs, Lipid Regulators, Antiepileptics, and Beta-Blockers in Surface Waters and Sediments in Different Regions of the World—Cont'd

			Surface Water (ng/L)			Sediment (μg/kg)			
Compound	Country	Location	*Range*	*Median*	*Mean*	*Range*	*Median*	*Mean*	References
	Spain	Ebro river basin (river waters downstream WWTPs)	<20–247	27	55				[170]
	Spain	The Henares–Jarama–Tajo River system	ND–640.4	60.9	112.2				[171]
	Finland	Rivers received WWTP effluents	<2.5–45	<2.5	9.5				[174]
	United States	Mississippi river waters in New Orleans, Louisiana, United States	0–135.2	□					[175]
	United States	Santa Ana River	0–21	9					[177]
	Canada	Otonabee River			ND				[178]
	Canada	Little River			73				[178]
	Canada	Detroit River			ND				[178]
	China	Liuxi River of Pearl River system	ND						[181]
	China	Zhujiang River of Pearl River system	5.0–5.4	5.2	5.2				[181]
	China	Shijing River of Pearl River system	6.6–125	35.4	42.3				[181]

	China	Yellow River (wet/dry)	ND–18.0	6.0/4.9	6.1/4.6	[182]
	China	Hai River	ND			[182]
	China	Liao River (wet/dry)	ND–40.7	ND	<LOQ/5.7	[182]
Ketoprofen	United Kingdom	River Taff in South Wales	<0.5–14	2–3		[166]
	United Kingdom	River Ely in South Wales	<0.5–12	1–3		[166]
	Germany	Rivers and streams	Up to 120	ND		[167]
	Spain	Ebro river basin (river waters downstream WWTPs)	<70			[170]
	Spain	The Henares–Jarama–Tajo River system	ND–991	123	244	[171]
	Finland	Rivers received WWTP effluents	<2.5–23	<2.5	6	[174]
	United States	Santa Ana River	ND			[177]
	Canada	Otonabee River			ND	[178]
	Canada	Little River			ND	[178]
	Canada	Detroit River			ND	[178]
	Japan	Main stream of Tone River	Up to 24	24		[180]
	Japan	Tributaries of Tone River	<LOQ			[180]
	China	Pearl River system	ND			

Continued

TABLE 8 Concentrations of Pharmaceuticals NSAIDs, Lipid Regulators, Antiepileptics, and Beta-Blockers in Surface Waters and Sediments in Different Regions of the World—Cont'd

			Surface Water (ng/L)			Sediment (μg/kg)			
Compound	**Country**	**Location**	*Range*	*Median*	*Mean*	*Range*	*Median*	*Mean*	**References**
Fenoprofen	Germany	Rivers and streams	ND						[167]
	Germany	River Elbe and its tributaries	<2–54	3	5				[168]
	Canada	Otonabee River			ND				[178]
	Canada	Little River			132				[178]
	Canada	Detroit River			ND				[178]
	China	Pearl River system	ND						
Salicylic acid	United Kingdom	River Taff in South Wales	<0.3–302	4–47					[166]
	United Kingdom	River Ely in South Wales	<0.3–234	15–48					[166]
	Germany	Rivers and streams	Up to 4100	250					[167]
	Spain	The Henares–Jarama–Tajo River system	ND–63.1	0.7	11.4				[171]
	China	Yellow River (wet/dry)	ND–121	14.7/35.7	15.6/47.1				[182]
	China	Hai River (wet/dry)	9.5–43.8	26.4/24.9	27.7/26.3				[182]
	China	Liao River (wet/dry)	17.7–295	55.6/52.4	79.2/60.7				[182]

Acetylsalicylic acid	Germany	Rivers and streams	Up to 340	ND				[167]
Meclofenamic acid	Germany	Rivers and streams	ND					[167]
Tolfenamic acid	Germany	Rivers and streams	ND					[167]
Indomethacin	Germany	Rivers and streams	Up to 200	40				[167]
	Germany	River Elbe and its tributaries	<5–60	<5	8			[168]
	Canada	Otonabee River			ND			[178]
	Canada	Little River			18			[178]
	Canada	Detroit River			ND			[178]
	Korea	Mankyung River, South Korea	ND–33.5	18	14.4			[179]
	Japan	Main stream of Tone River	Up to 16	16				[180]
	Japan	Tributaries of Tone River	Up to 8.7	6.3				[180]
Acetaminophen	Spain	Mediterranean coastal wetland	Up to 112.2		7.4	Up to 15.1	2.4	[169]
	Spain	The Henares–Jarama–Tajo River system	ND–202.4	<19.8	<19.8			[171]
	United States	139 Streams in America	10,000	110				[145]
	United States	Mississippi river waters in New Orleans, Louisiana, United States	24.7–65.2	□				[175]

Continued

TABLE 8 Concentrations of Pharmaceuticals NSAIDs, Lipid Regulators, Antiepileptics, and Beta-Blockers in Surface Waters and Sediments in Different Regions of the World—Cont'd

			Surface Water (ng/L)			Sediment (μg/kg)			
Compound	Country	Location	*Range*	*Median*	*Mean*	*Range*	*Median*	*Mean*	References
	United States	Tennessee River	ND–2.24						[183]
	Japan	Main stream of Tone River	Up to 52	22					[180]
	Japan	Tributaries of Tone River	Up to 110	18					[180]
Lipid regulators									
Clofibric acid	United Kingdom	Surface waters of the lower River Tyne	<20						[165]
	United Kingdom	River Taff in South Wales	<0.3–164	<0.3–73					[166]
	United Kingdom	River Ely in South Wales	<0.3–6	<0.3–2					[166]
	Germany	Rivers and streams	Up to 550	66					[167]
	Germany	River Elbe and its tributaries	<1–22	4.5	5.5				[168]
	Spain	Mediterranean coastal wetland	Up to 18.4		0.5	<LOQ		<LOQ	[169]
	Spain	Ebro river basin (river waters downstream WWTPs)	<3–3	<3	<3				[170]
	United States	Mississippi river waters in New Orleans, Louisiana, United States	3.2–26.7	□					[175]

	Canada	Otonabee River			ND	[178]
	Canada	Little River			3	[178]
	Canada	Detroit River			ND	[178]
	Japan	Main stream of Tone River	Up to 7.0	4.1		[180]
	Japan	Tributaries of Tone River	Up to 21	4.2		[180]
	China	Liuxi River of Pearl River system	4.6–7.3	5.0	5.5	[181]
	China	Zhujiang River of Pearl River system	0.1–18.3	9.4	9.0	[181]
	China	Shijing River of Pearl River system	2.7–16.8	6.9	8.0	[181]
	China	Yellow River (wet/dry)	ND–6.4	4.1/3.4	<LOQ	[182]
	China	Hai River (wet/dry)	ND–21.8	ND/9.6	<LOQ/8.0	[182]
	China	Liao River (wet/dry)	ND–82.8	ND/19.2	<LOQ/ 19.2	[182]
Gemfibrozil	Germany	Rivers and streams	Up to 280	45		[167]
	Germany	River Elbe and its tributaries	<2–27	2.5	4.4	[168]
	Spain	Ebro river basin (river waters downstream WWTPs)	5–497	20	90.6	[170]
	United States	139 streams in America	790	48		[145]
	United States	Santa Ana River	16–37	18		[177]
	Canada	Otonabee River			ND	[178]

Continued

TABLE 8 Concentrations of Pharmaceuticals NSAIDs, Lipid Regulators, Antiepileptics, and Beta-Blockers in Surface Waters and Sediments in Different Regions of the World—Cont'd

			Surface Water (ng/L)			Sediment (μg/kg)			
Compound	Country	Location	*Range*	*Median*	*Mean*	*Range*	*Median*	*Mean*	References
	Canada	Little River			34				[178]
	Canada	Detroit River			2				[178]
	China	Liuxi River of Pearl River system	ND						[181]
	China	Zhujiang River of Pearl River system	4.3–7.7	5.6	5.8				[181]
	China	Shijing River of Pearl River system	6.2–19.8	14.9	13.9				[181]
	China	Yellow River (wet/dry)	ND–18.0	6.0/4.9	6.1/4.6				[182]
	China	Hai River	ND						[182]
	China	Liao River (wet/dry)	ND–40.7	ND	<LOQ/5.7				[182]
Bezafibrate	Germany	Rivers and streams	Up to 3100	350					[167]
	Germany	River Elbe and its tributaries	<50–88	<50	<50				[168]
	Spain	Ebro river basin (river waters downstream WWTPs)	4–37	7	13.1				[170]

	Spain	The Henares–Jarama–Tajo River system	ND–46	<37.5	<37.5			[171]
	Italy	Po river	Up to 2.7	1.9				[172]
	Finland	Rivers received WWTP effluents	<1–4.5	<1	1.9			[174]
	Canada	Otonabee River			ND			[178]
	Canada	Little River			137			[178]
	Canada	Detroit River			ND			[178]
	Japan	Main stream of Tone River	Up to 77	16				[180]
	Japan	Tributaries of Tone River	Up to 170	35				[180]
Fenofibrate	Germany	Rivers and streams	ND	ND				[167]
	Spain	Mediterranean coastal wetland	Up to 21.4		4.1	Up to 16.1	1.0	[169]
Antiepileptics								
Carbamazepine	Germany	Rivers and streams	Up to 1100	250				[167]
	United Kingdom	Upstream of WWTP of WWTP effluent outfall	Up to 46–67					[164]
	United Kingdom	Downstream of WWTP effluent outfall	Up to 167–334					[164]
	Spain	Mediterranean coastal wetland	Up to 38.8		5.5	Up to 1.7	0.9	[169]

Continued

TABLE 8 Concentrations of Pharmaceuticals NSAIDs, Lipid Regulators, Antiepileptics, and Beta-Blockers in Surface Waters and Sediments in Different Regions of the World—Cont'd

			Surface Water (ng/L)			Sediment (μg/kg)			
Compound	Country	Location	*Range*	*Median*	*Mean*	*Range*	*Median*	*Mean*	References
	Spain	Ebro river basin (river waters downstream WWTPs)	11–90	56	51.3				[170]
	Spain	The Henares–Jarama–Tajo River system	ND–104	9.4	28.4				[171]
	Italy	Po river	Up to 34.2	23.1					[172]
	United States	Mississippi river waters in New Orleans, Louisiana, United States	42.9–113.7	□					[175]
	United States	Santa Ana River	ND						[177]
	United States	Tennessee River	4.03–5.62	4.49	4.63				[183]
	Korea	Mankyung River, South Korea	ND–595	103	180				[179]
	Japan	Main stream of Tone River	Up to 12	4.5					[180]
	Japan	Tributaries of Tone River	Up to 15	5.6					[180]
	Canada	Otonabee River			2				[178]
	Canada	Little River			80				[178]
	Canada	Detroit River			4				[178]

	China	Liuxi River of Pearl River system	8.5–17.9	10.5	11.5			[181]
	China	Zhujiang River of Pearl River system	3.6–25.5	13.5	14.1			[181]
	China	Shijing River of Pearl River system	18.9–43.1	25.3	27.3			[181]
Diazepam	Germany	Rivers and streams	ND					[167]
	Spain	Mediterranean coastal wetland	Up to 8.6		1.6	Up to 1.2	0.3	[169]
	Italy	Po river	ND					[172]
β-Blockers								
Metoprolol	Germany	Rivers and streams	Up to 2200	45				[167]
	Spain	Mediterranean coastal wetland	Up to 39.3		3.2	<LOD	<LOD	[169]
	Spain	The Henares–Jarama–Tajo River system	ND–26	2	5.3			[171]
	Japan	Tone River	<LOQ					[180]
Propranolol	Germany	Rivers and streams	Up to 590	12				[167]
	Spain	Mediterranean coastal wetland	Up to 16.6		1.6	Up to 2.1	0.1	[169]
	Spain	Ebro river basin (river waters downstream WWTPs)	<7–63	<7	15.4			[170]
	Spain	The Henares–Jarama–Tajo River system	ND–7.3	1.5	2.1			[171]
	Korea	Mankyung River, South Korea	ND–40.1	<LOQ	12.5			[179]
	Japan	Tone River	<LOQ					[180]

Continued

TABLE 8 Concentrations of Pharmaceuticals NSAIDs, Lipid Regulators, Antiepileptics, and Beta-Blockers in Surface Waters and Sediments in Different Regions of the World—Cont'd

			Surface Water (ng/L)			Sediment (μg/kg)			
Compound	Country	Location	*Range*	*Median*	*Mean*	*Range*	*Median*	*Mean*	References
Atenolol	Spain	Ebro river basin (river waters downstream WWTPs)	160–465	241	285				[170]
	Spain	The Henares–Jarama–Tajo River system	ND–334.3	44.5	72.7				[171]
	Italy	Po river	ND						[172]
	Korea	Mankyung River, South Korea	ND–690	<LOQ	170				[179]
	Japan	Main stream of Tone River	Up to 46	3.8					[180]
	Japan	Tributaries of Tone River	Up to 39	11					[180]

nanograms/liters [175,177,183]. In China, the maximum concentration can be found up to tens of nanograms/liters in domestic sewage polluted tributaries [181]. In addition to commonly detected antiepileptic drug carbamazepine, Alonso et al. [184] detected other psychoactive drugs including three antidepressants fluoxetine, citalopram, and venlafaxine and three anxiolytics nordiazepam, oxazepam, and 7-aminoflunitrazepam in the rivers of Madrid metropolitan area in Spain.

3.5 Beta-Blockers

The beta-blockers metoprolol, propranolol, and atenolol were detected in river surface waters of Germany, Italy, Japan, Korea, and Spain [167, 169–172,179,180]. The concentrations for these drugs ranged from ND to several thousand nanograms/liters (Table 8).

3.6 Steroid Hormones

Estrogens, androgens, progestogens, and glucocorticoids have been reported in the aquatic environment, especially from wastewaters of WWTPs, surface waters, and, to a lesser extent, sediments. The concentration ranges of different steroids are summarized in Table 9. Based on the information available, steroids are detected in aquatic environments with their concentrations ranging from <LOQ to hundred nanograms/liters in aqueous phase and from <LOQ to dozens of nanograms/grams in sediment phase. Estrogens in aquatic environments have been widely studied in Brazil, Canada, China, France, Japan, South Korea, the United Kingdom, and the United States [185,188,189,191–194,196]. Occurrence of androgens, progestogens, and glucocorticoids in the aquatic environment has received an increasing attention [188,191,192]. Recently, sensitive methods have been developed to monitor different classes of steroid hormones in the aquatic environment and reported the detection of androsta-1,4-diene-3,17-dione, 4-androstene-3,17-dione, epiandrosterone, estrone, 17α-ethinylestradiol, and norgestrel in surface waters near WWTPs or livestock farms with relatively high concentration levels and detection frequencies[185–187]. Apparently, the concentrations of these steroids detected near pollution sources such as WWTPs or livestock farms are much higher than those detected in surface waters far away from the pollution sources.

4 SUMMARY

Pharmaceuticals have been widely detected at trace levels (mostly nanogram/liter to microgram/liter range) in various aquatic environments. In general, we have acquired a quite good knowledge of the contamination levels of some therapeutic classes of pharmaceuticals in the aquatic environment through

TABLE 9 Comparisons of Range, Median, and Mean Concentrations of Steroids in Aqueous and Sediment Phases in Different Countries

Compound	Country	Location	Surface water (ng/L)			Sediment (ng/g)			References
			Range	*Median*	*Mean*	*Range*	*Median*	*Mean*	
Androgens									
Androsta-1,4-diene-3,17-dione	China	Swine wastewater receiving stream in Guangxi	10–109	79.7	66.23	1–3	1.9	1.97	[185,186]
	China	WWTP upstream in Guangdong	0–8.2						[187]
	China	WWTP receiving stream in Guangdong	0–17.9						[187]
4-Androstene-3,17-dione	China	Swine wastewater receiving stream in Guangxi	10.7–52.4	13.2	25.43	1.4–3.2	2.4	2.33	[185,186]
	China	WWTP upstream in Guangdong	0–8.1						[187]
	China	WWTP receiving stream in Guangdong	0–8.6						[187]
	France	Surface water in Rhône-Alpes	1.6–1.8						[188]
	Japan	Surface water in Koyama River basin	0.28–0.46						[189]

Androsterone	China	Swine wastewater receiving stream in Guangxi	0–59	59	59	4	4	4	[185,186]
	China	WWTP upstream in Guangdong							[187]
	China	WWTP receiving stream in Guangdong							[187]
17α-Boldenone	China	Swine wastewater receiving stream in Guangxi	5.5–16.2	10.85	10.85				[185,186]
	China	WWTP upstream in Guangdong							[187]
	China	WWTP receiving stream in Guangdong							[187]
17β-Boldenone	China	Swine wastewater receiving stream in Guangxi	5.3–18.4	11.85	11.85				[185,186]
	China	WWTP upstream in Guangdong	0.4						[187]
	China	WWTP receiving stream in Guangdong	1.5						[187]

Continued

TABLE 9 Comparisons of Range, Median, and Mean Concentrations of Steroids in Aqueous and Sediment Phases in Different Countries—Cont'd

			Surface water (ng/L)			Sediment (ng/g)			
Compound	**Country**	**Location**	*Range*	*Median*	*Mean*	*Range*	*Median*	*Mean*	**References**
5α-Dihydrotestosterone	China	Swine wastewater receiving stream in Guangxi			0				[185,186]
	China	WWTP upstream in Guangdong	38.6						[187]
	China	WWTP receiving stream in Guangdong	55.3						[187]
Epiandrosterone	China	Swine wastewater receiving stream in Guangxi	394	394	394	3.5–17.3	10.4	10.4	[185,186]
	China	WWTP upstream in Guangdong	0						[187]
	China	WWTP receiving stream in Guangdong	27.6						[187]

4-Hydroxy-androst-4-ene-17-dione	China	Swine wastewater receiving stream in Guangxi	13.7–66.7	40.2	40.2		[185,186]
	China	WWTP upstream in Guangdong					[187]
	China	WWTP receiving stream in Guangdong					[187]
Methyl testosterone	China	Swine wastewater receiving stream in Guangxi	0–5.6	5.6	5.6		[185,186]
	China	WWTP upstream in Guangdong					[187]
	China	WWTP receiving stream in Guangdong					[187]
	Europe	Danube river	<0.30				[190]
19-Nortestosterone	China	Swine wastewater receiving stream in Guangxi			0		[185,186]
	China	WWTP upstream in Guangdong					[187]
	China	WWTP receiving stream in Guangdong					[187]
	Europe	Danube river	<0.92				

Continued

TABLE 9 Comparisons of Range, Median, and Mean Concentrations of Steroids in Aqueous and Sediment Phases in Different Countries—Cont'd

			Surface water (ng/L)			Sediment (ng/g)			
Compound	**Country**	**Location**	*Range*	*Median*	*Mean*	*Range*	*Median*	*Mean*	**References**
Testosterone	China	Swine wastewater receiving stream in Guangxi	2.6–14.7	3.9	7.067	<LOQ			[185,186]
	China	WWTP upstream in Guangdong							[187]
	China	WWTP receiving stream in Guangdong	1.2						[187]
	France	Surface water in Rhône-Alpes	2.8–3.4						[188]
	South Korea	River water in Seoul	<0.5						[191]
17α-Trenbolone	China	Swine wastewater receiving stream in Guangxi	27.6	27.6	27.6				[185,186]
	China	WWTP upstream in Guangdong							[187]
	China	WWTP receiving stream in Guangdong							[187]

17β-Trenbolone	China	Swine wastewater receiving stream in Guangxi			0	<LOQ	[185,186]
	China	WWTP upstream in Guangdong					[187]
	China	WWTP receiving stream in Guangdong					[187]
Stanozolol	China	Swine wastewater receiving stream in Guangxi	0.8–1.6	1.2	1.2		[185,186]
	China	WWTP upstream in Guangdong					[187]
	China	WWTP receiving stream in Guangdong					[187]
Estrogens							
17β-Estradiol	China	Swine wastewater receiving stream in Guangxi	44.3	44.3	44.3		[185,186]
	China	WWTP upstream in Guangdong					[187]

Continued

TABLE 9 Comparisons of Range, Median, and Mean Concentrations of Steroids in Aqueous and Sediment Phases in Different Countries—Cont'd

			Surface water (ng/L)			Sediment (ng/g)			
Compound	Country	Location	*Range*	*Median*	*Mean*	*Range*	*Median*	*Mean*	References
	China	WWTP receiving stream in Guangdong							[187]
	Canada	Mille Îles and St. Lawrence Rivers in Montreal	8–9						[192]
	United States	Stream water in Pennsylvania	0.09–5.04						[193]
	South Korea	River water in Seoul	<0.5						[191]
	United Kingdom	Sediment in Ditchling, Kingston, Ringmer, Lewes				<0.03–1.2			[194]
	Japan	Surface sediment in Tokyo Bay				<0.07–0.59			[195]
Estrone	China	Swine wastewater receiving stream in Guangxi	17.4–174	20.7	70.7				[185,186]
	China	WWTP upstream in Guangdong	6						[187]
	China	WWTP receiving stream in Guangdong	13.3						[187]
	France	Surface water in Rhône-Alpes	0.3						[188]

	United States	Stream water in Pennsylvania	0.66–2.62				[193]
	South Korea	River water in Seoul	0.2–4.2		1.6		[191]
	United Kingdom	Sediment in Ditchling, Kingston, Ringmer, Lewes				0.4–3.3	[194]
	Japan	Surface sediment in Tokyo Bay				0.05–0.36	[195]
17α-Ethinylestradiol	China	Swine wastewater receiving stream in Guangxi	254–338	296	296		[185,186]
	China	WWTP upstream in Guangdong					[187]
	China	WWTP receiving stream in Guangdong					[187]
	South Korea	River water in Seoul	<1.0				[191]
	United Kingdom	Sediment in Ditchling, Kingston, Ringmer, Lewes				<0.04	[194]
Diethylstilbestrol	China	Swine wastewater receiving stream in Guangxi			0	<LOQ	[185,186]
	China	WWTP upstream in Guangdong					[187]
	China	WWTP receiving stream in Guangdong					[187]

Continued

TABLE 9 Comparisons of Range, Median, and Mean Concentrations of Steroids in Aqueous and Sediment Phases in Different Countries—Cont'd

			Surface water (ng/L)			Sediment (ng/g)			
Compound	Country	Location	*Range*	*Median*	*Mean*	*Range*	*Median*	*Mean*	References
Estriol	Brazil	River in Rio de Janeiro	1–7.27		3.68				[196]
	United States	Stream water in Pennsylvania	0.33–19.70						[193]
Glucocorticoids									
Cortisol	China	Swine wastewater receiving stream in Guangxi	167	167	167				[185,186]
	China	WWTP upstream in Guangdong							[187]
	China	WWTP receiving stream in Guangdong							[187]
	Europe	Danube river	<0.17–2.67						[190]
Cortisone	China	Swine wastewater receiving stream in Guangxi	17.8	17.8	17.8				[185,186]
	China	WWTP upstream in Guangdong	0.6						[187]
	China	WWTP receiving stream in Guangdong	1.9						[187]

Dexamethasone	China	Swine wastewater receiving stream in Guangxi	37.8	37.8	37.8				[185,186]
	China	WWTP upstream in Guangdong							[187]
	China	WWTP receiving stream in Guangdong							[187]
	Europe	Danube river	<0.07						[190]
Prednisolone	China	Swine wastewater receiving stream in Guangxi	7.3–22.6	14.95	14.95	<LOQ–1.6	1.6	1.6	[185,186]
	China	WWTP upstream in Guangdong							[187]
	China	WWTP receiving stream in Guangdong							[187]
	Europe	Danube river	<0.28						[190]
Progestogens									
Medroxyprogesterone	China	Swine wastewater receiving stream in Guangxi	14.4	14.4	14.4	<LOQ–1.1	1.1	1.1	[185,186]
	China	WWTP upstream in Guangdong							[187]
	China	WWTP receiving stream in Guangdong							[187]

Continued

TABLE 9 Comparisons of Range, Median, and Mean Concentrations of Steroids in Aqueous and Sediment Phases in Different Countries—Cont'd

			Surface water (ng/L)			Sediment (ng/g)			
Compound	**Country**	**Location**	*Range*	*Median*	*Mean*	*Range*	*Median*	*Mean*	**References**
Norgestrel	China	Swine wastewater receiving stream in Guangxi	14–465	16.6	165.2	<LOQ			[185]
	China	WWTP upstream in Guangdong	3.7						[187]
	China	WWTP receiving stream in Guangdong	22.2						[187]
Progesterone	China	Swine wastewater receiving stream in Guangxi	2.3–30.5	17.8	16.87	1.6–13.6	2.3	5.83	[185,186]
	China	WWTP upstream in Guangdong	0.5						[187]
	China	WWTP receiving stream in Guangdong	2.5						[187]
	Europe	Danube river	<0.37						[190]
	France	Surface water in Rhône-Alpes	1.7–3.5						[188]

	Canada	Mille Îles River in Montreal	3		[192]
	Japan	Surface water in Koyama River basin	0.06–0.09		[189]
	Brazil	River in Rio de Janeiro	0.51–47.2	9.35	[196]
	United States	Stream water in Pennsylvania	7.35–11.81		[193]
	South Korea	River water in Seoul	<0.5		[191]
19-Norethindrone	China	Swine wastewater receiving stream in Guangxi			[185,186]
	China	WWTP upstream in Guangdong			[187]
	China	WWTP receiving stream in Guangdong			[187]
	France	Surface water in Rhône-Alpes	2.7–2.8		[188]

the research in the last decade. But there is a need to gain more knowledge and deep understanding of the fate and occurrence of pharmaceuticals and their transformation products in the environment in order to properly assess the potential risks posed by these pharmaceutical residues to the ecosystems.

Although a lot of monitoring studies have been carried out in some countries around the world, more classes of pharmaceuticals (e.g., psychoactive drugs, antihistamine drugs, cytotoxic drugs, and illicit drugs) should be included in the future monitoring work. Monitoring data on steroids and antisteroids, especially androgens, progestogens, and corticoids, is still very limited. Steroid pharmaceuticals are very potent in terms of their potential adverse effects on aquatic organisms. Attention should also be given to the transformation products of various pharmaceuticals. Furthermore, it is necessary to simultaneously monitor the occurrence of pharmaceuticals and their transformation products in both surface water and sediment since we often have more data for surface water.

A systematic research is still needed to understand better the fate and behavior of various pharmaceuticals in the environment. Currently, it is hard to compare the fate data available in the literature as the fate information for pharmaceuticals is limited and fragmented. More dissipation studies under natural and simulated environmental conditions (water–sediment systems) should be performed in the future. Moreover, there is a lack of information on pathways for degradation and transformation of some classes of pharmaceuticals such as steroids. Few pharmaceutical-degrading microbes have been isolated from the natural aquatic environment.

Most of pharmaceuticals are polar compounds with various functional groups, which results in different environmental behaviors to nonpolar compounds. Under environmental conditions, pharmaceutical molecules can be neutral, cationic, anionic, or zwitterionic, which makes their environmental behavior more complex. Interaction between polar pharmaceuticals and environmental components such as DOM, various minerals, and ions remains to be further explored. In addition, bioavailability of pharmaceuticals to aquatic organisms and their bioaccumulation potential in both water and sediment phases are an interesting topic and remain to be investigated.

REFERENCES

[1] A.W. Garrison, J.D. Pope, F.R. Allen, in: C.H. Keith (Ed.), Identification and Analysis of Organic Pollutants in Water, Ann Arbor Science, Michigan, USA, 1976, pp. 517–566.

[2] K.A. Kidd, P.J. Blanchfield, K.H. Mills, V.P. Palace, R.E. Evans, J.M. Lazorchak, R.W. Flick, Proc. Natl. Acad. Sci. U. S. A. 104 (21) (2007) 8897–8901.

[3] H.-C. Su, G.-G. Ying, R. Tao, R.-Q. Zhang, J.-L. Zhao, Y.-S. Liu, Environ. Pollut. 169 (2012) 42–49.

[4] R. Tao, G.-G. Ying, H.-C. Su, H.-W. Zhou, J.P.S. Sidhu, Environ. Pollut. 158 (6) (2010) 2101–2109.

[5] V.K. Balakrishnan, K.A. Terry, J. Toito, J. Chromatogr. A 1131 (1–2) (2006) 1–10.

[6] Z.M. Qiang, C. Adams, Water Res. 38 (12) (2004) 2874–2890.
[7] P. Sukul, M. Spiteller, Rev. Environ. Contam. Toxicol. 187 (2004) 67–101.
[8] A.L. Batt, D.D. Snow, D.S. Aga, Chemosphere 64 (11) (2006) 1963–1971.
[9] S. Thiele-Bruhn, J. Plant Nutr. Soil Sci. 166 (2) (2003) 145–167.
[10] J. Tolls, Environ. Sci. Technol. 35 (17) (2001) 3397–3406.
[11] Y. Pico, V. Andreu, Anal. Bioanal. Chem. 387 (4) (2007) 1287–1299.
[12] N. Watanabe, B.A. Bergamaschi, K.A. Loftin, M.T. Meyer, T. Harter, Environ. Sci. Technol. 44 (17) (2010) 6591–6600.
[13] M. Hansen, K.A. Krogh, A. Brandt, J.H. Christensen, B. Halling-Sorensen, Environ. Pollut. 157 (2) (2009) 474–480.
[14] Elanco Products Company, NADA technical report, Indianapolis, IN, NADA 95-735, 1989.
[15] A.B.A. Boxall, L.A. Fogg, P.A. Blackwell, P. Kay, E.J. Pemberton, A. Croxford, Rev. Environ. Contam. Toxicol. 180 (2004) 1–91.
[16] G.L. Cromwell, Anim. Biotechnol. 13 (1) (2002) 7–27.
[17] K. Kummerer, Chemosphere 75 (4) (2009) 417–434.
[18] A.K. Sarmah, M.T. Meyer, A.B.A. Boxall, Chemosphere 65 (5) (2006) 725–759.
[19] A.B.A. Boxall, D.W. Kolpin, B. Halling-Sorensen, J. Tolls, Environ. Sci. Technol. 37 (15) (2003) 286A–294A.
[20] R. Hirsch, T. Ternes, K. Haberer, K.L. Kratz, Sci. Total Environ. 225 (1–2) (1999) 109–118.
[21] K. Kumar, S. Gupta, Y. Chander, A.K. Singh, in: L.S. Donald (Ed.), Advances in Agronomy, vol. 87, Academic Press, Salt Lake City, UT, USA, 2005, pp. 1–54.
[22] K.G. Karthikeyan, M.T. Meyer, Sci. Total Environ. 361 (1–3) (2006) 196–207.
[23] N. Le-Minh, S.J. Khan, J.E. Drewes, R.M. Stuetz, Water Res. 44 (15) (2010) 4295–4323.
[24] J.G. Davis, C.C. Truman, S.C. Kim, J.C. Ascough, K. Carlson, J. Environ. Qual. 35 (6) (2006) 2250–2260.
[25] A.L. Cordova-Kreylos, K.M. Scow, ISME J. 1 (7) (2007) 585–595.
[26] J. Naeslund, J.E. Hedman, C. Agestrand, Aquat. Toxicol. 90 (3) (2008) 223–227.
[27] A. Pruden, R. Pei, H. Storteboom, K.H. Carlson, Environ. Sci. Technol. 40 (23) (2006) 7445–7450.
[28] T. Zhang, M. Zhang, X. Zhang, H.H. Fang, Environ. Sci. Technol. 43 (10) (2009) 3455–3460.
[29] N. Wu, M. Qiao, B. Zhang, W.-D. Cheng, Y.-G. Zhu, Environ. Sci. Technol. 44 (18) (2010) 6933–6939.
[30] S.C. Monteiro, A.B.A. Boxall, Rev. Environ. Contam. Toxicol. 202 (2010) 53–154.
[31] J.R. Vane, R.M. Botting, Int. J. Tissue React.: Exp. Clin. Aspects 20 (1) (1998) 3–15.
[32] N.M. Vieno, H. Harkki, T. Tuhkanen, L. Kronberg, Environ. Sci. Technol. 41 (14) (2007) 5077–5084.
[33] M. Carballa, G. Fink, F. Omil, J.M. Lema, T. Ternes, Water Res. 42 (1–2) (2008) 287–295.
[34] L. Araujo, N. Villa, N. Camargo, M. Bustos, T. Garcia, A. de Jesus Prieto, Environ. Chem. Lett. 9 (1) (2011) 13–18.
[35] O.A.H. Jones, N. Voulvoulis, J.N. Lester, Water Res. 36 (20) (2002) 5013–5022.
[36] J. Xu, L. Wu, A.C. Chang, Chemosphere 77 (10) (2009) 1299–1305.
[37] I.G. Dubus, E. Barriuso, R. Calvet, Chemosphere 45 (6–7) (2001) 767–774.
[38] Y. Ruckebusch, P.L. Toutain, Vet. Res. Commun. 7 (1–4) (1983) 359–368.
[39] L. Barron, M. Purcell, J. Havel, K. Thomas, J. Tobin, B. Paull, Occurrence and fate of pharmaceuticals and personal care products within sewage sludge and sludge-enriched soils

(2005-FS-30-M1) STRIVE report, Environmental Protection Agency: Johnstown Castle, Co. Wexford, Ireland, 2009.
[40] P.J. Pentikäinen, A. Penttilä, P.J. Neuvonen, R.G. Khalifah, C.E. Hignite, Eur. J. Drug Metab. Pharmacokinet. 7 (4) (1982) 259–267.
[41] O. Lorphensri, J. Intravijit, D.A. Sabatini, T.C.G. Kibbey, K. Osathaphan, C. Saiwan, Water Res. 40 (7) (2006) 1481–1491.
[42] T. Scheytt, P. Mersmann, R. Lindstadt, T. Heberer, Water Air Soil Pollut. 165 (1–4) (2005) 3–11.
[43] W. Xu, G. Zhang, S. Zou, Z. Ling, G. Wang, W. Yan, Water Environ. Res. 81 (3) (2009) 248–254.
[44] G.G. Ying, R.S. Kookana, D.W. Kolpin, J. Environ. Monit. 11 (8) (2009) 1498–1505.
[45] T.A. Ternes, N. Herrmann, M. Bonerz, T. Knacker, H. Siegrist, A. Joss, Water Res. 38 (19) (2004) 4075–4084.
[46] P. Drillia, K. Stamatelatou, G. Lyberatos, Chemosphere 60 (8) (2005) 1034–1044.
[47] B.S. Das, L.S. Lee, P.S.C. Rao, R.P. Hultgren, Environ. Sci. Technol. 38 (5) (2004) 1460–1470.
[48] S.K. Ong, P. Chotisukarn, T. Limpiyakorn, Water Air Soil Pollut. 223 (7) (2012) 3869–3875.
[49] H.A. Sangsupan, D.E. Radcliffe, P.G. Hartel, M.B. Jenkins, W.K. Vencill, M.L. Cabrera, J. Environ. Qual. 35 (6) (2006) 2261–2272.
[50] B. Khan, X.L. Qiao, L.S. Lee, Environ. Sci. Technol. 43 (23) (2009) 8827–8833.
[51] L.D. Nghiem, A.I. Schafer, T.D. Waite, Desalination 147 (1–3) (2002) 269–274.
[52] K.M. Lewis, R.D. Archer, Steroids 34 (5) (1979) 485–499.
[53] G.G. Ying, R.S. Kookana, Environ. Toxicol. Chem. 24 (10) (2005) 2640–2645.
[54] M.G.M. Amin, S.O. Petersen, M. Laegdsmand, J. Environ. Qual. 41 (1) (2012) 179–187.
[55] L. Joseph, J. Heo, Y.G. Park, J.R.V. Flora, Y. Yoon, Desalination 281 (2011) 68–74.
[56] T.J. Runnalls, L. Margiotta-Casaluci, S. Kugathas, J.P. Sumpter, Hum. Ecol. Risk Assess. 16 (6) (2010) 1318–1338.
[57] G.G. Ying, R.S. Kookana, Y.J. Ru, Environ. Int. 28 (6) (2002) 545–551.
[58] T.A. Hanselman, D.A. Graetz, A.C. Wilkie, Environ. Sci. Technol. 37 (24) (2003) 5471–5478.
[59] E.P. Kolodziej, T. Harter, D.L. Sedlak, Environ. Sci. Technol. 38 (23) (2004) 6377–6384.
[60] Z.H. Liu, Y. Kanjo, S. Mizutani, Sci. Total Environ. 407 (18) (2009) 4975–4985.
[61] R.J. Williams, V.D.J. Keller, A.C. Johnson, A.R. Young, M.G.R. Holmes, C. Wells, M. Gross-Sorokin, R. Benstead, Environ. Toxicol. Chem. 28 (1) (2009) 220–230.
[62] L. Aristilde, G. Sposito, Environ. Toxicol. Chem. 29 (1) (2010) 90–98.
[63] L. Aristilde, C. Marichal, J. Miehe-Brendle, B. Lanson, L. Charlet, Environ. Sci. Technol. 44 (20) (2010) 7839–7845.
[64] W. Kong, C. Li, J.M. Dolhi, S. Li, J. He, M. Qiao, Chemosphere 87 (5) (2012) 542–548.
[65] Z.G. Pei, X.Q. Shan, J.J. Kong, B. Wen, G. Owens, Environ. Sci. Technol. 44 (3) (2010) 915–920.
[66] W. Yan, S. Hu, C. Jing, J. Colloid Interface Sci. 372 (2012) 141–147.
[67] H. Yamamoto, Y. Nakamura, S. Moriguchi, Y. Honda, I. Tamura, Y. Hirata, A. Hayashi, J. Sekizawa, Water Res. 43 (2) (2009) 351–362.
[68] D. Löffler, J. Römbke, M. Meller, T.A. Ternes, Environ. Sci. Technol. 39 (14) (2005) 5209–5218.
[69] M. Ramil, T. El Aref, G. Fink, M. Scheurer, T.A. Ternes, Environ. Sci. Technol. 44 (3) (2010) 962–970.

[70] W. Baran, J. Sochacka, W. Wardas, Chemosphere 65 (8) (2006) 1295–1299.
[71] F. Ingerslev, B. Halling-Sorensen, Environ. Toxicol. Chem. 19 (10) (2000) 2467–2473.
[72] P.A. Blackwell, A.B.A. Boxall, P. Kay, H. Noble, J. Agric. Food Chem. 53 (6) (2005) 2192–2201.
[73] K.A. Loftin, C.D. Adams, M.T. Meyer, R. Surampalli, J. Environ. Qual. 37 (2) (2008) 378–386.
[74] B.T. Lunestad, O.B. Samuelsen, S. Fjelde, A. Ervik, Aquaculture 134 (3–4) (1995) 217–225.
[75] H. Hektoen, J.A. Berge, V. Hormazabal, M. Yndestad, Aquaculture 133 (3–4) (1995) 175–184.
[76] O.B. Samuelsen, B.T. Lunestad, A. Ervik, S. Fjelde, Aquaculture 126 (3–4) (1994) 283–290.
[77] T.-H. Yu, A.Y.-C. Lin, S.C. Panchangam, P.-K.A. Hong, P.-Y. Yang, C.-F. Lin, Chemosphere 84 (9) (2011) 1216–1222.
[78] S.-F. Yang, C.-F. Lin, C.-J. Wu, K.-K. Ng, A.Y.-C. Lin, P.-K.A. Hong, Water Res. 46 (4) (2012) 1301–1308.
[79] M.L. Richardson, J.M. Bowron, J. Pharm. Pharmacol. 37 (1) (1985) 1–12.
[80] J. Gavalchin, S.E. Katz, J. AOAC Int. 77 (2) (1994) 481–485.
[81] B. Halling-Sorensen, A.M. Jacobsen, J. Jensen, G. Sengelov, E. Vaclavik, F. Ingerslev, Environ. Toxicol. Chem. 24 (4) (2005) 802–810.
[82] H. Pouliquen, R. Delpepee, M. Larhantec-Verdier, M.L. Morvan, H. Le Bris, Aquaculture 262 (1) (2007) 23–28.
[83] F. Ingerslev, B. Halling-Sorensen, Ecotoxicol. Environ. Saf. 48 (3) (2001) 311–320.
[84] C. Winckler, A. Grafe, J. Soils Sedim. 1 (2001) 66–70.
[85] M. Kuhne, D. Ihnen, G. Moller, O. Agthe, J. Vet. Med. A-Physiol. Pathol. Clin. Med. 47 (6) (2000) 379–384.
[86] H. Oka, Y. Ikai, N. Kawamura, M. Yamada, K. Harada, S. Ito, M. Suzuki, J. Agric. Food Chem. 37 (1) (1989) 226–231.
[87] J.J. Werner, W.A. Arnold, K. McNeill, Environ. Sci. Technol. 40 (23) (2006) 7236–7241.
[88] K. Kummerer, A. Al-Ahmad, V. Mersch-Sundermann, Chemosphere 40 (7) (2000) 701–710.
[89] L.K. Ge, J.W. Chen, X.X. Wei, S.Y. Zhang, X.L. Qiao, X.Y. Cai, Q. Xie, Environ. Sci. Technol. 44 (7) (2010) 2400–2405.
[90] J.B. Belden, J.D. Maul, M.J. Lydy, Chemosphere 66 (8) (2007) 1390–1395.
[91] T.G. Vasconcelos, D.M. Henriques, A. Konig, A.F. Martins, K. Kummerer, Chemosphere 76 (4) (2009) 487–493.
[92] J.R. Marengo, R.A. Kok, K. Obrien, R.R. Velagaleti, J.M. Stamm, Environ. Toxicol. Chem. 16 (3) (1997) 462–471.
[93] D. Vione, J. Feitosa-Felizzola, C. Minero, S. Chiron, Water Res. 43 (7) (2009) 1959–1967.
[94] M. Schlusener, M.A. von Arb, K. Bester, Arch. Environ. Contam. Toxicol. 51 (1) (2006) 21–28.
[95] D. Hu, J.R. Coats, Environ. Toxicol. Chem. 26 (5) (2007) 884–889.
[96] J.M. Hayes, J. Eichman, T. Katz, R. Gilewicz, J. AOAC Int. 86 (1) (2003) 22–29.
[97] V. Matamoros, A. Duhec, J. Albaiges, J.M. Bayona, Water Air Soil Pollut. 196 (1–4) (2009) 161–168.
[98] J.T. Yu, E.J. Bouwer, M. Coelhan, Agric. Water Manage. 86 (1) (2006) 72–80.
[99] L.E. Jacobs, R.L. Fimmen, Y.P. Chin, H.E. Mash, L.K. Weavers, Water Res. 45 (15) (2011) 4449–4458.

[100] A. Joss, S. Zabczynski, A. Göbel, B. Hoffmann, D. Löffler, C.S. McArdell, T.A. Ternes, A. Thomsen, H. Siegrist, Water Res. 40 (8) (2006) 1686–1696.
[101] H.R. Buser, T. Poiger, M.D. Müller, Environ. Sci. Technol. 32 (22) (1998) 3449–3456.
[102] R. Andreozzi, M. Raffaele, P. Nicklas, Chemosphere 50 (10) (2003) 1319–1330.
[103] J.L. Packer, J.J. Werner, D.E. Latch, K. McNeill, W.A. Arnold, Aquat. Sci.-Res. Across Bound. 65 (4) (2003) 342–351.
[104] J. Jilani, G. Pillai, M. Salem, N. Najib, Drug Dev. Ind. Pharm. 23 (3) (1997) 319–323.
[105] I. Kim, N. Yamashita, H. Tanaka, Chemosphere 77 (4) (2009) 518–525.
[106] A. Nageswara Rao, B. Sivasankar, V. Sadasivam, J. Hazard. Mater. 166 (2) (2009) 1357–1361.
[107] W. Guo, X. Liu, P. Huo, X. Gao, D. Wu, Z. Lu, Y. Yan, Appl. Surf. Sci. 258 (18) (2012) 6891–6896.
[108] C.K. Scheck, F.H. Frimmel, Water Res. 29 (10) (1995) 2346–2352.
[109] R. Andreozzi, R. Marotta, G. Pinto, A. Pollio, Water Res. 36 (11) (2002) 2869–2877.
[110] C.E. West, S.J. Rowland, Environ. Sci. Technol. 46 (9) (2012) 4749–4756.
[111] Q.T. Liu, H.E. Williams, Environ. Sci. Technol. 41 (3) (2007) 803–810.
[112] A. Piram, A. Salvador, C. Verne, B. Herbreteau, R. Faure, Chemosphere 73 (8) (2008) 1265–1271.
[113] H. Chang, Y. Wan, S. Wu, Z. Fan, J. Hu, Water Res. 45 (2) (2011) 732–740.
[114] S. Homklin, S.K. Ong, T. Limpiyakorn, Chemosphere 82 (10) (2011) 1401–1407.
[115] Y.Y. Yang, T. Borch, R.B. Young, L.D. Goodridge, J.G. Davis, J. Environ. Qual. 39 (4) (2010) 1153–1160.
[116] E. Vulliet, M. Falletta, P. Marote, T. Lomberget, J.-O. Païssé, M.-F. Grenier-Loustalot, Sci. Total Environ. 408 (17) (2010) 3554–3559.
[117] W. Zheng, X. Li, S.R. Yates, S.A. Bradford, Environ. Sci. Technol. 46 (10) (2012) 5471–5478.
[118] B.J. Robinson, J. Hellou, Sci. Total Environ. 407 (21) (2009) 5713–5718.
[119] M.D. Jürgens, K.I.E. Holthaus, A.C. Johnson, J.J.L. Smith, M. Hetheridge, R.J. Williams, Environ. Toxicol. Chem. 21 (3) (2002) 480–488.
[120] C.P. Czajka, K.L. Londry, Sci. Total Environ. 367 (2–3) (2006) 932–941.
[121] G.G. Ying, R.S. Kookana, P. Dillon, Water Res. 37 (15) (2003) 3785–3791.
[122] R.R. Chowdhury, P. Charpentier, M.B. Ray, Ind. Eng. Chem. Res. 49 (15) (2010) 6923–6930.
[123] S.K. Atkinson, V.L. Marlatt, L.E. Kimpe, D.R.S. Lean, V.L. Trudeau, J.M. Blais, Arch. Environ. Contam. Toxicol. 60 (1) (2011) 1–7.
[124] D. Nasuhoglu, D. Berk, V. Yargeau, Chem. Eng. J. 185–186 (2012) 52–60.
[125] J.J. Werner, M. Chintapalli, R.A. Lundeen, K.H. Wammer, W.A. Arnold, K. McNeill, J. Agric. Food Chem. 55 (17) (2007) 7062–7068.
[126] B. Razavi, S. Ben Abdelmelek, W.H. Song, K.E. O'Shea, W.J. Cooper, Water Res. 45 (2) (2011) 625–631.
[127] U. Kunkel, M. Radke, Water Res. 46 (17) (2012) 5551–5565.
[128] A.Y.C. Lin, M. Reinhard, Environ. Toxicol. Chem. 24 (6) (2005) 1303–1309.
[129] Y.G. Zuo, K. Zhang, Y.W. Deng, Chemosphere 63 (9) (2006) 1583–1590.
[130] C.P. Silva, M. Otero, V. Esteves, Environ. Pollut. 165 (2012) 38–58.
[131] A. Bialk-Bielinska, S. Stolte, M. Matzke, A. Fabianska, J. Maszkowska, M. Kolodziejska, B. Liberek, P. Stepnowski, J. Kumirska, J. Hazard. Mater. 221 (2012) 264–274.
[132] T.H. Yu, A.Y.C. Lin, S.K. Lateef, C.F. Lin, P.Y. Yang, Chemosphere 77 (2) (2009) 175–181.

[133] M.J. Benotti, B.J. Brownawell, Environ. Pollut. 157 (3) (2009) 994–1002.
[134] F. Ingerslev, L. Torang, M.L. Loke, B. Halling-Sorensen, N. Nyholm, Chemosphere 44 (4) (2001) 865–872.
[135] U. Kunkel, M. Radke, Environ. Sci. Technol. 42 (19) (2008) 7273–7279.
[136] J. Groning, C. Held, C. Garten, U. Claussnitzer, S.R. Kaschabek, M. Schlomann, Chemosphere 69 (4) (2007) 509–516.
[137] M. Winkler, J.R. Lawrence, T.R. Neu, Water Res. 35 (13) (2001) 3197–3205.
[138] G.G. Ying, S. Toze, J. Hanna, X.Y. Yu, P. Dillon, R.S. Kookan, Water Res. 42 (4–5) (2008) 1133–1141.
[139] P.M. Bradley, L.B. Barber, F.H. Chapelle, J.L. Gray, D.W. Kolpin, P.B. McMahon, Environ. Sci. Technol. 43 (6) (2009) 1902–1910.
[140] Z.T. Li, R. Nandakumar, N. Madayiputhiya, X. Li, Environ. Sci. Technol. 46 (11) (2012) 5947–5955.
[141] M. Della Greca, G. Pinto, P. Pistillo, A. Pollio, L. Previtera, F. Temussi, Chemosphere 70 (11) (2008) 2047–2053.
[142] R.L. Gomes, M.D. Scrimshaw, E. Cartmell, J.N. Lester, Environ. Monit. Assess. 175 (1–4) (2011) 431–441.
[143] L.S. Lee, T.J. Strock, A.K. Sarmah, P.S.C. Rao, Environ. Sci. Technol. 37 (18) (2003) 4098–4105.
[144] S. Homklin, S.K. Ong, T. Limpiyakorn, J. Hazard. Mater. 221 (2012) 35–44.
[145] D.W. Kolpin, E.T. Furlong, M.T. Meyer, E.M. Thurman, S.D. Zaugg, L.B. Barber, H.T. Buxton, Environ. Sci. Technol. 36 (6) (2002) 1202–1211.
[146] S.C. Kim, K. Carlson, Environ. Sci. Technol. 41 (1) (2007) 50–57.
[147] L.-J. Zhou, G.-G. Ying, J.-L. Zhao, J.-F. Yang, L. Wang, B. Yang, S. Liu, Environ. Pollut. 159 (7) (2011) 1877–1885.
[148] J.-F. Yang, G.-G. Ying, J.-L. Zhao, R. Tao, H.-C. Su, Y.-S. Liu, J. Environ. Sci. Health B-Pesticides Food Contam. Agric. Wastes 46 (3) (2011) 272–280.
[149] W.H. Xu, G. Zhang, S.C. Zou, X.D. Li, Y.C. Liu, Environ. Pollut. 145 (3) (2007) 672–679.
[150] L.-J. Zhou, G.-G. Ying, S. Liu, R.-Q. Zhang, H.-J. Lai, Z.-F. Chen, C.-G. Pan, Sci. Total Environ. 444 (2013) 183–195.
[151] A. Murata, H. Takada, K. Mutoh, H. Hosoda, A. Harada, N. Nakada, Sci. Total Environ. 409 (24) (2011) 5305–5312.
[152] S. Managaki, A. Murata, H. Takada, B.C. Tuyen, N.H. Chiem, Environ. Sci. Technol. 41 (23) (2007) 8004–8010.
[153] F. Tamtam, F. Mercier, B. Le Bot, J. Eurin, Q.T. Dinh, M. Clement, M. Chevreuil, Sci. Total Environ. 393 (1) (2008) 84–95.
[154] T. Christian, R.J. Schneider, H.A. Farber, D. Skutlarek, M.T. Meyer, H.E. Goldbach, Acta Hydrochim. Hydrobiol. 31 (1) (2003) 36–44.
[155] V. Osorio, R. Marce, S. Perez, A. Ginebreda, J.L. Cortina, D. Barcelo, Sci. Total Environ. 440 (2012) 3–13.
[156] A. Ginebreda, I. Muñoz, M.L. de Alda, R. Brix, J. López-Doval, D. Barceló, Environ. Int. 36 (2) (2010) 153–162.
[157] D. Ashton, M. Hilton, K.V. Thomas, Sci. Total Environ. 333 (1–3) (2004) 167–184.
[158] D. Bendz, N.A. Paxeus, T.R. Ginn, F.J. Loge, J. Hazard. Mater. 122 (3) (2005) 195–204.
[159] E. Zuccato, S. Castiglioni, R. Bagnati, M. Melis, R. Fanelli, J. Hazard. Mater. 179 (1–3) (2010) 1042–1048.
[160] J. Feitosa-Felizzola, S. Chiron, J. Hydrol. 364 (1–2) (2009) 50–57.
[161] K.V. Thomas, M.J. Hilton, Mar. Pollut. Bull. 49 (5–6) (2004) 436–444.

[162] A.J. Watkinson, E.J. Murby, S.D. Costanzo, Water Res. 41 (2007) 4164–4176.
[163] Y. Matsui, T. Ozu, T. Inoue, T. Matsushita, Desalination 226 (1–3) (2008) 215–221.
[164] J.L. Zhou, Z.L. Zhang, E. Banks, D. Grover, J.Q. Jiang, J. Hazard. Mater. 166 (2–3) (2009) 655–661.
[165] P.H. Roberts, K.V. Thomas, Sci. Total Environ. 356 (1–3) (2006) 143–153.
[166] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Water Res. 42 (13) (2008) 3498–3518.
[167] T.A. Ternes, Water Res. 32 (11) (1998) 3245–3260.
[168] S. Wiegel, A. Aulinger, R. Brockmeyer, H. Harms, J. Löffler, H. Reincke, R. Schmidt, B. Stachel, W. von Tümpling, A. Wanke, Chemosphere 57 (2) (2004) 107–126.
[169] P. Vazquez-Roig, V. Andreu, C. Blasco, Y. Picó, Sci. Total Environ. 440 (2012) 24–32.
[170] M. Gros, M. Petrovic, D. Barcelo, Environ. Toxicol. Chem. 26 (8) (2007) 1553–1562.
[171] C. Fernández, M. González-Doncel, J. Pro, G. Carbonell, J.V. Tarazona, Sci. Total Environ. 408 (3) (2010) 543–551.
[172] E. Zuccato, S. Castiglioni, R. Fanelli, J. Hazard. Mater. 122 (3) (2005) 205–209.
[173] H.R. Buser, T. Poiger, M.D. Muller, Environ. Sci. Technol. 33 (15) (1999) 2529–2535.
[174] N. Lindqvist, T. Tuhkanen, L. Kronberg, Water Res. 39 (11) (2005) 2219–2228.
[175] S.Y. Zhang, Q.A. Zhang, S. Darisaw, O. Ehie, G.D. Wang, Chemosphere 66 (6) (2007) 1057–1069.
[176] G.R. Boyd, H. Reemtsma, D.A. Grimm, S. Mitra, Sci. Total Environ. 311 (1–3) (2003) 135–149.
[177] B. Gross, J. Montgomery-Brown, A. Naumann, M. Reinhard, Environ. Toxicol. Chem. 23 (9) (2004) 2074–2083.
[178] C.D. Metcalfe, X.S. Miao, B.G. Koenig, J. Struger, Environ. Toxicol. Chem. 22 (12) (2003) 2881–2889.
[179] J.W. Kim, H.S. Jang, J.G. Kim, H. Ishibashi, M. Hirano, K. Nasu, N. Ichikawa, Y. Takao, R. Shinohara, K. Arizono, J. Health Sci. 55 (2) (2009) 249–258.
[180] N. Nakada, K. Komori, Y. Suzuki, C. Konishi, I. Houwa, H. Tanaka, Water Sci. Technol. 56 (12) (2007) 133–140.
[181] J.L. Zhao, G.G. Ying, Y.S. Liu, F. Chen, J.F. Yang, L. Wang, X.B. Yang, J.L. Stauber, M.S. Warne, Environ. Toxicol. Chem. 29 (6) (2010) 1377–1384.
[182] L. Wang, G.G. Ying, J.L. Zhao, X.B. Yang, F. Chen, R. Tao, S. Liu, L.J. Zhou, Sci. Total Environ. 408 (16) (2010) 3139–3147.
[183] J.M. Conley, S.J. Symes, S.A. Kindelberger, S.A. Richards, J. Chromatogr. A 1185 (2) (2008) 206–215.
[184] S.G. Alonso, M. Catala, R.R. Maroto, J.L.R. Gil, A.G. de Miguel, Y. Valcarcel, Environ. Int. 36 (2) (2010) 195–201.
[185] S. Liu, G.-G. Ying, L.-J. Zhou, R.-Q. Zhang, Z.-F. Chen, H.-J. Lai, Water Res. 46 (12) (2012) 3754–3768.
[186] S. Liu, G.-G. Ying, R.-Q. Zhang, L.-J. Zhou, H.-J. Lai, Z.-F. Chen, Environ. Pollut. 170 (2012) 190–201.
[187] S. Liu, G.-G. Ying, J.-L. Zhao, F. Chen, B. Yang, L.-J. Zhou, J. Chromatogr. A 1218 (10) (2011) 1367–1378.
[188] E. Vulliet, L. Wiest, R. Baudot, M.F. Grenier-Loustalot, J. Chromatogr. A 1210 (1) (2008) 84–91.
[189] H. Chang, S.M. Wu, J.Y. Hu, M. Asami, S. Kunikane, J. Chromatogr. A 1195 (1–2) (2008) 44–51.
[190] A. Tolgyesi, Z. Verebey, V.K. Sharma, L. Kovacsics, J. Fekete, Chemosphere 78 (8) (2010) 972–979.

[191] Y. Yoon, J. Ryu, J. Oh, B.-G. Choi, S.A. Snyder, Sci. Total Environ. 408 (3) (2010) 636–643.
[192] L. Viglino, K. Aboulfadl, M. Prevost, S. Sauve, Talanta 76 (5) (2008) 1088–1096.
[193] M. Velicu, R. Suri, Environ. Monit. Assess. 154 (1–4) (2009) 349–359.
[194] P. Labadie, E.M. Hill, J. Chromatogr. A 1141 (2) (2007) 174–181.
[195] T. Isobe, S. Serizawa, T. Horiguchi, Y. Shibata, S. Managaki, H. Takada, M. Morita, H. Shiraishi, Environ. Pollut. 144 (2) (2006) 632–638.
[196] M. Kuster, D.A. Azevedo, M.J.L. de Alda, F.R.A. Neto, D. Barcelo, Environ. Int. 35 (7) (2009) 997–1003.

Chapter 15

Fate and Occurrence of PhACs in the Terrestrial Environment

María Jesús García-Galán*, Silvia Díaz-Cruz† and Damia Barcelo*,†
*Catalan Institute for Water Research (ICRA), Girona, Spain
†Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

Chapter Outline

1 INTRODUCTION

The quotidian use of pharmaceuticals to treat or prevent illnesses in both human and veterinary medicine has traditionally led to the misconception about their innocuousness. However, the relevant advances and general improvement of different analytic methodologies during the last decades have demonstrated the widespread occurrence of these compounds in basically all the environmental compartments, leading to an arising social and scientific awareness. As a direct consequence of the increase of the global population and the life hope, especially in Western developed countries, sales and consumption of these

Comprehensive Analytical Chemistry, Vol. 62. http://dx.doi.org/10.1016/B978-0-444-62657-8.00015-X

substances has increased in the last decades worldwide. The consequent increase in the food demand has also led to a bigger number of intensive cattle farming activities (usually known as confined animal feeding operations or CAFOs), which also contribute to a higher use of pharmaceuticals. Nowadays, up to 3000 different pharmaceuticals are consumed regularly in EU, some of them in quantities comparable to those of some pesticides [1]. Worldwide, estimations agree on sales over 1000 billion dollars in the last decade.

All types of pharmaceuticals have been detected in environmental compartments, including antibiotics, analgesics, anti-inflammatories, lipid regulators, β-blockers, antiepileptics, contraceptives, steroids, and related hormones [2]. However, currently, the number of publications devoted to their occurrence in the terrestrial environment is more limited than those focused in the aquatic environment (Figure 1A). This is due, in the first place, to the vulnerability of water ecosystems to anthropogenic pollution. In a society exerting an increasing pressure on natural resources, the increasing demand of quality drinkable water makes a sustainable water resource management essential, requiring protection of water resources from those persistent or toxic anthropogenic compounds. To this respect, science plays a relevant role in researching and publishing new data on occurrence and fate of these compounds. On the other hand, the higher complexity of solid matrices makes their pretreatment and chemical analysis usually more laborious. Besides, special dedication to the occurrence of antibiotics in solid matrices can be observed too (Figure 1B), [3–6] and only a few studies have dealt with the analysis of pharmaceuticals other than antibiotics [7–9]. The most feasible reason for this is that the potential environmental impact of pharmaceuticals, their metabolites, and/or transformation products is yet unknown in most cases, whereas the adverse effects derived from the environmental presence of antibiotics, mainly the development of antibiotic resistance in bacteria, are a well-documented fact nowadays and a threat that has been recognized by, among others, the World Health Organization [10]. Their role is up-to-dately superlative in modern agriculture and livestock, a fact that is reflected in their high consumption rates.

Human and veterinary drugs are continuously being released into the environment, in urban ecosystems, mainly due to excretion, disposal of unused or expired products, and manufacturing activities. The efficiency to completely eliminate these substances during conventional biological treatment in wastewater treatment plants has not yet been demonstrated, and so water effluents and sludge are usually considered as the main entrance sources for these substances onto the environment. In rural environments, excretion from medicated animals could be considered as the main entrance pathway of pharmaceuticals onto the natural media. Excreta from grazing animals are directly released on topsoils, whereas manure application as fertilizer on agricultural soils is the main entrance pathway for residues from CAFOs (Figure 2).

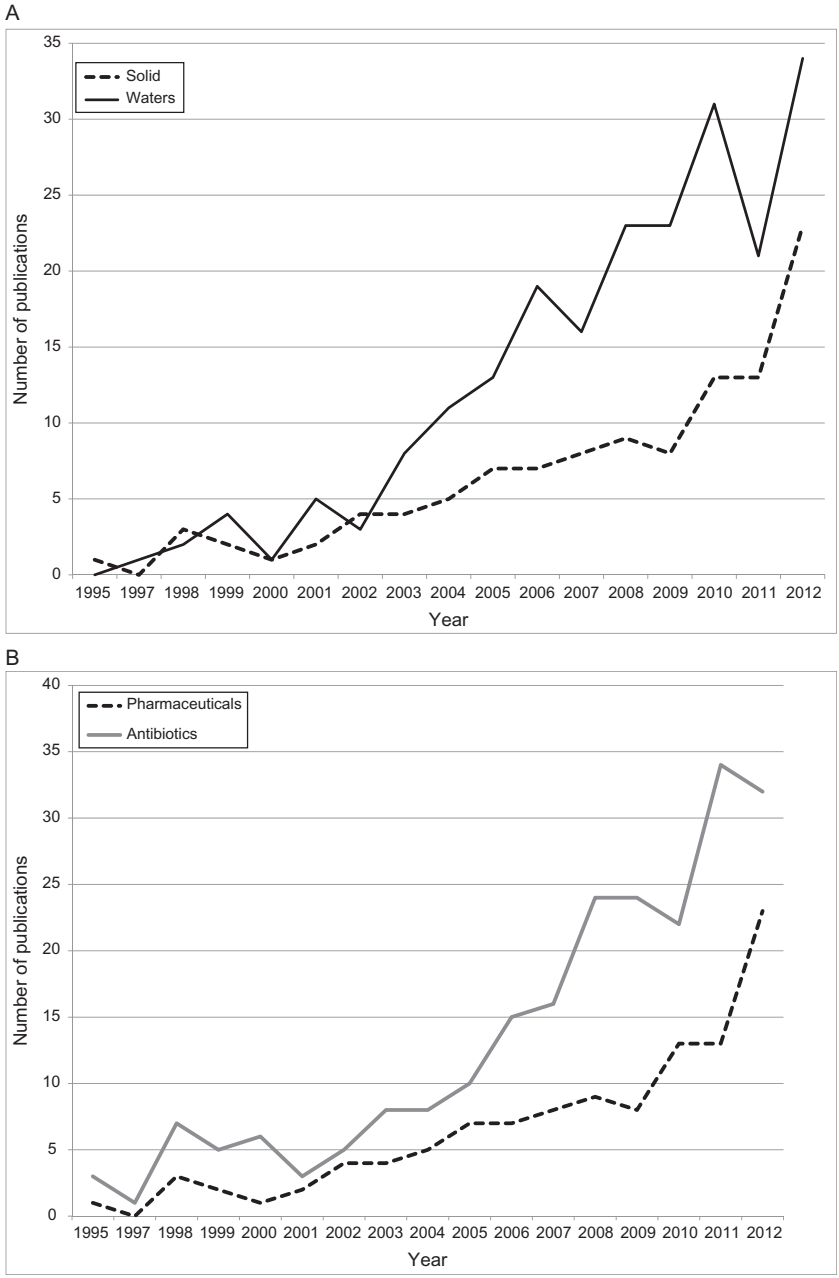

FIGURE 1 Number of scientific publications on the occurrence of pharmaceuticals in soils and environmental waters during the last 17 years (A) and number of scientific publications devoted to the presence of antibiotics in soils (B). *Source: Scopus. Date: 1- 3-2013. Search criteria: 1. Occurrence + pharmaceuticals + surface waters or groundwaters or wastewaters, 2. occurrence + pharmaceuticals + soils, 3. occurrence + antibiotics + soil.*

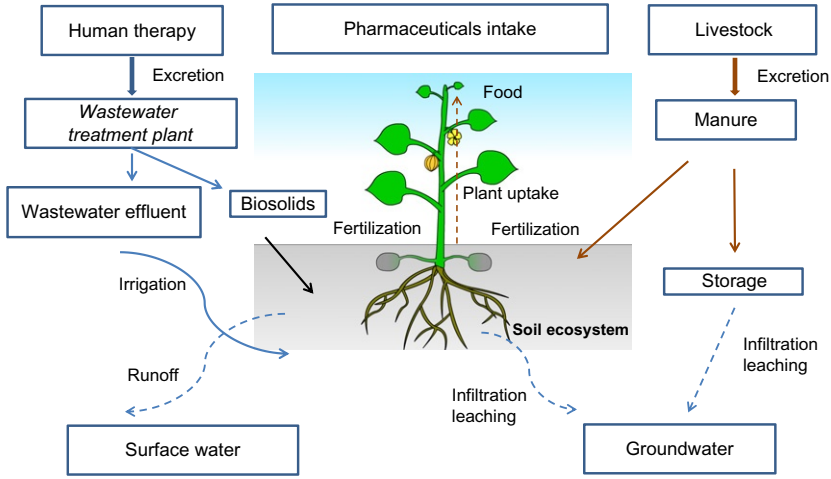

FIGURE 2 Main entrance pathways and potential fate of pharmaceuticals in the soil ecosystem.

The main discussion of this chapter will be focused in the fate and degradation pathway of pharmaceuticals released into the terrestrial environment. The different factors influencing their behavior once released on the environment, such as physicochemical properties of the compounds or intrinsic characteristics of the solid matrix, will be considered. Adverse effects on their environmental presence will also be reviewed.

2 OCCURRENCE OF PHARMACEUTICALS IN SOILS

2.1 Main Entrance Pathways

2.1.1 Manure Fertilization in Soil Systems

Veterinary medicines are administered to animals to treat disease and protect their health. After administration, they are metabolized to different extents depending on the animal species and the substance itself; therefore, a mixture of the parent compound and metabolites is excreted in the urine and feces. For animals on pasture, the excreta will be released directly to soil, whereas for intensively reared animals, the main route of entry will be through slurry and manure spreading. Manure is regarded as a very valuable fertilizer, as it contains essential nutrients for plant growth (NH_4 and NO_3^-, phosphorous, and potassium), and its application on crop lands is widely extended. Veterinary pharmaceuticals have been detected in manure from medicated animals at different concentrations in several studies, which are summarized in Table 1.

TABLE 1 Pharmaceutical Concentrations (mg kg^{-1}) Detected in Animal Manure

		Chicken/Turkey	Pig	Cows	Undetermined	References
Fluoroquinolones	Fleroxacin	3.11	2.23	2.22	–	[11]
	Norfloxacin	4.68	2.09	1.84	–	
	Ciprofloxacin	3.78	2.01	3.44	–	[11]
		–	–	–	0.1–4.3	[12]
	Lomefloxacin	1.47	2.02	1.9	–	[11]
	Danofloxacin	0.72	0.91	1.23	–	
	Enrofloxacin	4.65	2.09	6.79	–	[11]
		2.8–8.3	0.13–0.75	–	–	
	Difloxacin	1.4	1.13	2.63	–	[11]
	Ofloxacin	–	–	–	0.23–15.7	[12]
	Pefloxacin	–	–	–	3.3–24.7	[12]
Sulfonamides	Sulfaguanidine	0.1	0.09	0.1	–	[13]
	Sulfamethoxazole	0.78	0.51	–	–	[11]
		–	–	–	0.23–5.7	[12]
	Sulfadiazine	0.15	0.21	–	–	[11]
		51–91	–	–	–	[13]
		3.5–11.3	–	–	–	[14]
	Sulfanilamide	0.09	0.04	–	–	[11]
	Sulfamerazine	0.23	0.14	0.09	–	[11]
	Sulfamethazine	0.43, 20	0.21	0.14	–	[11]
		7.2	–	–	–	[14]

Continued

TABLE 1 Pharmaceutical Concentrations (mg kg^{-1}) Detected in Animal Manure—Cont'd

		Chicken/Turkey	Pig	Cows	Undetermined	References
	Sulfamonomethoxine	0.3	0.2	0.06	–	[11]
	Sulfachloropyridazine	0.46	0.82	0.36	–	[11]
		–	–	–	0.21–2.76	[12]
	Sulfadoxine	–	–	–	0.1–32.7	[12]
Tetracyclines	Oxytetracycline	1.55	2.69	1.24	–	[11]
			0.21–29		–	[13]
	Chlortetracycline	–	–	–	0.08–183.5	[12]
		1.09	1.15	2.22	–	[11]
		–	–	–	0.14–26.8	[12]
		1.7	0.1–46	–	–	[13]
		1	–	–	–	[14]
		4.9–1435	–	–	–	[15]
	Methacycline	0.59	0.64	0.44	–	[11]
	Doxycycline	3.39	0.79	0.68	–	[11]
	Tetracycline	–	0.36–23	–	–	[13]
		–	–	–	0.11–43.5	[12]
		14.1–41.2	–	–	–	[14]
		6.6–349.3	–	–	–	[15]
Other	Lincomycin	0.12–3.8	–	–	–	[12]
	Trimethoprim	n.d.–17	–	–	–	[13]

Factors such as the year season or the temperature can influence the concentration to expect in these residues. For instance, cold seasons could imply a higher application of veterinary pharmaceuticals due to disease treatment, as well as higher temperatures could lead to higher degradation rates in manure storage [12]. Galvalchin and Katz [16] showed that degradation for a variety of antibiotics in manure, including chlortetracycline (CTC) and tylosin (TYL), was slower at colder temperatures. In their study, degradation at 4 °C yielded a reduction of approximately 30% of initial levels on average, whereas degradation at 30 °C was approximately 85% of initial levels. Similar results were obtained for other two antibiotics, sulfadiazine (SDZ) and difloxacin (DIF), and their corresponding main metabolites, in manure storage at different temperatures and moisture levels [17]. In this study, dilution of manure and storage at higher temperatures for long periods enhanced the rate of reactions for parent drugs and metabolites alike, in which concentrations seemed to increase and decrease proportionally. For instance, the concentration of SDZ increased due to the deconjugation of the acetylated metabolite, N^4-acetylsulfadiazine (AcSDZ), which decreased proportionally. On the contrary, the concentration of the hydroxysulfadiazine (SDZ-OH) increased after 40 days, indicating the metabolism of the parent compound.

Due the color and viscosity, manure may act as a sunlight filter, restricting the entry of sunlight, and so photodegradation of pharmaceuticals contained in this matrix is very limited. In addition, the particulate and suspended matter in manure might shield the organic pollutants from light, as well as the compounds may adsorb to these particles being less vulnerable to sunlight [18]. Sukul et al. [19] estimated a half-life ($t_{1/2}$) value of SDZ in manure under solar irradiation of 158 h (see Table 2) and observed the formation of the SO_2^- extrusion moiety, aminopyrimidine, and 5-SDZ-OH as phototransformation products, which were not found initially in the manure. However, as we will see in Section 3, intrinsic characteristics of the pharmaceuticals and manure themselves will determine the fate of pharmaceuticals in this matrix.

Before its direct application on soils, leaching and runoff from manure stockpiles can also contribute to the presence of pharmaceuticals in soils and also in surface waters and groundwaters. The amount of water losses from the stockpile and the weather conditions, which are obviously linked, are the main factors influencing pharmaceutical losses during storage [28]. In the study by Lamshöft et al. [17], the data indicated that the 96–99% of the amounts of SDZ and DIF and their respective metabolites detected in manure remained in the extractable form after 150 days of storage, implying their potential risk of translocation once applied on soils [17]. However, *in situ* biotic and abiotic degradations during storage could be considered more relevant mechanisms for pharmaceutical losses than successive rainfall events and cumulative leaching or runoff.

TABLE 2 Half-Lives ($t_{1/2}$) for the Most Frequently Found Pharmaceuticals in Soils, Manure, and Biosolids

		Matrix	$t_{1/2}$ (Days)	Comments	References
Nonsteroidal anti-inflammatories	Naproxen	Biosolids	10.2–17.7[a]	OC: 330 Moisture: 53.1%	[20]
		Soil	4.4–15.3[a]	Clay/OC: 11%/72.5 Moisture: 16.3%	
		Biosolid-amended soil	10.3–19.9[a]		
		Soil	3.5–11.2[a]	Clay/OC: 56.3/24.1 Moisture: 21%	
		Biosolid-amended soil	15.1–29.3[a]		
		Soil	3.1–4[a]	Clay/OC: 28%/17.4 Moisture: 14.3%	
		Biosolid-amended soil	13–36.2[a]		
		Soil	6.9–14.3[a]	Clay/OC: 15%/20.9 Moisture: 17.8%	
		Biosolid-amended soil	9.9–10.9[a]		
		Soil	5.68–7.56	Clay content: 3.6% Organic matter: 0.6%	[21]
			38.50[b]		
			5.78	Clay content: 12.5% Organic matter: 1.93%	
			16.82	Clay content: 42.5% Organic matter: 2.46%	
			14.29	Clay content: 18.1% Organic matter: 5.45%	
			17.4	Clay/OC: 4%/0.16% Clay/OC: 25%/0.33%	[22]
			69.3		

	Ibuprofen	Soil	0.91–1.89 31.22[b]	Clay content: 3.6% Organic matter: 0.6%	[21]
			2.36	Clay content: 12.5% Organic matter: 1.93%	
			5.83	Clay content: 42.5% Organic matter: 2.46%	
			6.09	Clay content: 18.1% Organic matter: 5.45%	
			10.4 49.9[b]	Clay/OC: 4%/0.16%	[22]
			15.2	Clay/OC: 25%/0.33%	
			12	Biosolid-amended	[23]
	Diclofenac	Soil	<5		[24]
			3.07–4.31 70[b]	Clay content: 3.6% Organic matter: 0.6%	[21]
			3.47	Clay content: 12.5% Organic matter: 1.93%	
			20.44	Clay content: 42.5% Organic matter: 2.46%	
			8.47	Clay content: 18.1% Organic matter: 5.45%	
			4.8 29.6	Clay/OC: 4%/0.16% Clay/OC: 25%/0.33%	[22]

Continued

TABLE 2 Half-Lives ($t_{1/2}$) for the Most Frequently Found Pharmaceuticals in Soils, Manure, and Biosolids—Cont'd

		Matrix	$t_{1/2}$ (Days)	Comments	References
		Soil	1–1.8	Clay content: 9.2% Organic matter: 3.7%	[25]
			1.8	Clay content: 32.8% Organic matter: 3.7%	
			3.8	Clay content: 3.7% Organic matter: 1.5%	
Lipid regulators	Clofibric acid	Soil	18.48–36.09	Clay content: 3.6% Organic matter: 0.6%	[21]
			46.51[b]		
			4.52	Clay content: 12.5% Organic matter: 1.93%	
			13.15	Clay content: 42.5% Organic matter: 2.46%	
			11	Clay content: 18.1% Organic matter: 5.45%	
			18.48–36.09	Clay content: 3.6% Organic matter: 0.6%	
	Gemfibrozil	Biosolid-amended soil	20		[23]
			75–231		[26]
Psychiatric drugs	Carbamazepine	Biosolid-amended soil	75–495		[26]
			60[a]		[20]
			46		[23]
	Fluoxetine	Biosolid-amended soil	120–1000		[26]
		Biosolid	>1000 >60		[20]

Antibiotics	Azithromycin	Biosolid-amended soil	71		[23]
			360–770		[26]
	Ciprofloxacin	Biosolid-amended soil	120–2310		[26]
	Norfloxacin	Biosolid-amended soil	289	Biosolid-amended	[23]
			120–1155		[26]
	Ofloxacin	Biosolid-amended soil	198		[23]
			360–1386		[26]
	Enrofloxacin		<152		[24]
	Tetracycline	Biosolid	138	Dark anaerobic conditions	[27]
			57	Dark aerobic conditions	
			53	Light aerobic conditions	
		Biosolid-amended	120–578		[26]
		Pig manure	55–105		
	4-Epitetracycline	Soil	120–630		[26]
	Doxycycline	Biosolid	115	Dark anaerobic conditions	[27]
			63	Dark aerobic conditions	
			69	Light aerobic conditions	
		Biosolid-amended soil	120–533		[26]
	Oxytetracycline		<103		[24]
	Clindamycin	Biosolid	86	Dark anaerobic conditions	[27]
			58	Dark aerobic conditions	
			63	Light aerobic conditions	

Continued

TABLE 2 Half-Lives ($t_{1/2}$) for the Most Frequently Found Pharmaceuticals in Soils, Manure, and Biosolids—Cont'd

		Matrix	$t_{1/2}$ (Days)	Comments	References
	Clarithromycin	Biosolid	10	Dark anaerobic conditions	[27]
			11	Dark aerobic conditions	
			12	Light aerobic conditions	
	Erythromycin	Biosolid	30	Dark anaerobic conditions	[27]
			20	Dark aerobic conditions	
			21	Light aerobic conditions	
	Sulfamethoxazole	Soil	11.4	Clay/OC: 4%/0.16%	[22]
			18.3[b]		
			9	Clay/OC: 25%/0.33%	
			15.3[b]		
			58.7[c]	Clay/OC: 4%/0.16%	
	Sulfadiazine		<103		[24]
	Trimethoprim		<103		[24]
		Soil	26.1[b]	Clay/OC: 4%/0.16%	[22]
	Amoxicillin		<1		[24]
Others	Levamisole		<103		[24]
	Tylosin		<103		[24]
	Thiabendazole	Soil	30		[26]
	Miconazole	Soil	360–1386		[26]
	Diphenhydramine	Soil	72–1000		[26]

[a] *Values obtained in assays carried out with pharmaceutical mixtures.*
[b] *Values obtained in anaerobic conditions.*
[c] *Results obtained in sterile conditions.*

2.1.2 Biosolids Fertilization in Soil Systems

During wastewater treatment, the disappearance of organic pollutants does not mean a complete mineralization, or the decrease in concentration does not necessarily indicate photodegradation or biodegradation. Sorption to solid particles is a relevant elimination pathway, depending on the physicochemical nature of the compound [2]. Some compounds such as fluoroquinolones or tetracyclines (TCs) are eliminated by more than 50% due to sorption to sewage sludge [18,29]: for instance, ofloxacin, ciprofloxacin (CPF), and norfloxacin (NFX) are usually detected at the highest concentrations in this matrix.

Similarly to the application of manure as fertilizer in cropland, application of treated sewage sludge (meeting regulations for pathogens, nutrients, and metals) as soil amendment is also a very common practice in agriculture, gardening, or landscaping, as they constitute a relevant source of nutrients and organic matter (OM) for crop growth. In 2006, estimations for the United States were of 8 million dry tons of biosolids per year, of which 50% were applied in agricultural land [30]. In Europe, estimations were of 2.4 million dry tons per year, of which 37% were applied as organic amendments. In some countries, there are legal restrictions regarding maximum amounts of biosolids applied during a given period of time. For instance, in Ontario (Canada), the maximum amount of municipal biosolids that can be applied per 5 years is 22 Mg dw ha^{-1} [31]. This application, therefore, could also represent a different entrance pathway for pharmaceuticals to soils and eventually to natural waters. Several publications have confirmed the presence of a wide variety of therapeutic groups in sewage sludge or biosolids destined for land application, especially those with high distribution coefficient (K_D) values, in some cases reporting levels in mg kg^{-1} level [30,32].

Before land application, biosolids are also stored in tanks for days to months. Chenxi et al. [27] studied the fate of different pharmaceuticals, including different types of antibiotics and the psychiatric drug carbamazepine (CBZ) among others, during biosolid storage under field conditions. Similarly to the results obtained for manure and mentioned in the previous section, it was observed that aeration aided to accelerate the degradation of most of these compounds, whereas photodegradation had a negligible effect. CBZ and CPF were the two compounds for which no dissipation was observed. Likewise, dissipation rates for TCs were very slow, due mainly to their strong adsorption tendency.

2.1.3 Irrigation of Agricultural Land with Wastewaters

Especially in countries with water scarcity issues, reuse of water resources is a highly valued alternative. Treated and untreated wastewaters have been reclaimed and reused for irrigation of agricultural land and other terrains such as golf courses, parks, or recreation areas, especially in arid and semiarid

regions. In 2006, an estimated 9.8 million m^3 day^{-1} of treated municipal wastewater (approximately 7–8% of the total generated) was reused in the United States [33]. In developed countries, soil infiltration is used as a tertiary treatment for wastewater effluents and also a way of reusing water as resources become scarcer [34–36]. In less-developed countries, the use of untreated wastewater to irrigate soils is a common practice that has been carried out in some cases for more than a century, as in Mezquital Valley, located 60 km north from Mexico City. This could be considered as one of the most extreme cases, as around 70% of the raw wastewater produced in the capital is discharged without prior treatment and used for irrigation (flood irrigation) in this agricultural valley of around 900 km^2, a practice that started in 1912. This application has led to the development of a flourishing agricultural area with a yearly production many times fold larger over the national average production [37]. There are other areas that have been extensively investigated also in Mexico and China [38–40].

3 FATE OF PHARMACEUTICALS IN AGRICULTURAL SOILS

Once on the topsoil, both physicochemical properties of the drugs (stereochemical structure, redox potential, water solubility, K_D, K_{OW}, etc.) and intrinsic characteristics of the soils (clay and natural OM (NOM) content, moisture content, pH, etc.) will determine its mobility and potential for leaching or, on the contrary, their tendency to adsorb to solid particles [2]. There are other external factors, which also affect their mobility such as timing of the manure or biosolids applied or weather conditions and temporary humidity of the soil [28,41,42]. On the other hand, degradation should also be regarded as one of the main removal mechanisms in the soil ecosystems.

3.1 Mobility of Pharmaceuticals in Soil Systems: Leaching and Runoff

Drug concentrations decreasing with depth are indicative of mobilization through soil profile. Mobility of pharmaceuticals within soil systems and their potential to reach of groundwaters and surface waters can occur by both leaching and runoff. Agricultural practices can influence the extent of runoff, and it has been demonstrated that it is markedly reduced in arable land by soil cultivation and tillage practices [41,43]. Surface sealing by manure can also hinder infiltration and increase runoff for different pharmaceuticals such as sulfonamides [43–46]. Soil macropores are also responsible for the rapid movement of pharmaceuticals to tile drainage systems and surface water, while smaller macropores appear to have a much less significant effect [47]. Preferential flows due to desiccation cracks or worm channels can also influence the mobility. For instance, Lapen et al. [48,49] observed how, in soils with high content of vertical macroporosity, one single application of

biosolids was sufficient for pharmaceuticals to reach groundwaters and subsurface drainage systems. Therefore, soil tillage prior to manure slurry application could reduce significantly the mobilization of drugs [50]. On the other hand, it should be considered that most of the pharmaceutical losses in leachates and runoff happen during the nongrowing season. After manure application, water percolation and runoff are usually the first set of events. Lower losses during the growing season are a result of less water percolation and runoff due to plant transpiration [41].

3.1.1 Influence of Soil Structure, Clay and OM Content

Leaching of organic contaminants in soils will depend primarily on the equilibrium that is formed between the pollutants in soil solution and adsorbed to the solid components of the soil. This depends partly on the presence of available binding sites in the soil. In clays, for instance, there may be ionic binding sites where strong adsorption of organic compounds in ionic form can occur by van der Waals attractions; the compounds can migrate between sheets in the clay structure and be retained there or in binding sites on the edges of those sheets. Some pharmaceuticals also contain planar aromatic structures, which are favorable for intercalation into the layers of clay minerals; TCs, for instance, are able to form complexes with double cations, such as calcium or magnesium, which can be present in the soil solution or within the clay laminar structure [2]. For instance, the fluoroquinolone clindamycin was found adsorbed to montmorillonite by a cation-exchange mechanism under pH favoring cationic form [51]. Another example is oxytetracycline (OTC), which, despite its low K_{OW}, sorbs strongly in soil, with K_D values between 417 in sand soil and 1026 in sandy loam soil and no significant desorption. The process is assumed to be related to ionic binding with metal complexes formed between soil, metal ion, and OTC, and therefore, some authors consider that it poses very little risk to contaminate groundwater or surface water [52,53]. Kurwadkar et al. [54] showed that experimental K_D for two sulfonamides, sulfamethazine (SMZ) and sulfathiazole, were higher in soils with a higher content of clay (16% vs. 5.2%), although the pH of the soil was also a determinant.

The components of NOM usually have hydroxy and carboxylic acid functionalities, which also function as binding sites. Therefore, higher contents in clay and OM imply more potential binding sites for retention of the analytes and reduced leaching.

Manure composition and its characteristics will influence the fate of pharmaceuticals in soils. For instance, the presence of dissolved OM (DOM) in liquid manure showed increased mobility for TC antibiotics, which typically present a very strong adsorption potential to solid particles [55]. On the contrary, Sukul and Spiteller [56] observed a noticeable increase in the K_D values for SDZ in a laboratory experiment when manure was applied to different

types of soils, in which a weak interaction with the binding sites has been registered so far, K_D values increasing from 0.1–24.3 to 6.9–40.2.

The different procedures to apply biosolids on agricultural soils can also play a role in the sorption–leaching of different organic contaminants. Topp et al. [57] investigated the behavior of different pharmaceuticals contained in manure, which was applied following two different approaches: subsurface injection and broadcast application followed by incorporation. They observed that with the second approach, pharmaceuticals (such as CBZ, the β-blocker agent atenolol, the lipid regulator gemfibrozil, the analgesic naproxen (NPX), or the antibiotic sulfamethoxazole (SMX)) were mobilized more easily in surface runoff and showed that injection of biosolid slurry below the soil surface could effectively eliminate surface runoff of pharmaceuticals.

On the other hand, untreated wastewater contains large amounts of dissolved and suspended solids, which may encourage the organic micropollutants to remain in solution or attach to suspended particles. For this reason, even pharmaceuticals with high K_D values can leach to some extent [40], indicating that dissolved and suspended OM contained in the irrigation water could retain part of those relatively water-insoluble compounds. It has also been observed that successive irrigation events lead to increasing amounts of pharmaceuticals in leachates, especially for acidic drugs. Saturation of binding sites in the soil particles or leaching of analytes previously incorporated to the soil could explain this event.

3.1.2 pH Influence

The sorption behavior of pharmaceuticals can also be highly dependent on the chemical nature of the compounds and the aromatic groups in the molecule and so be strictly linked to pH, which could change the theoretical sorption behavior of a given soil profile or compound. The aqueous solubilities of the acidic pharmaceuticals are usually sensitive to the pH values in soils, such that increased solubility at higher pH values may encourage leaching to occur. After irrigation with reclaimed wastewaters, weakly acidic pharmaceuticals usually increase their mobility, not due to complexation with DOM, as explained in Section 3.1.1, but due to the increase of the soil solution pH [58].

Therefore, hydrophobic sorption is probably involved for acid pH due to the prevalence of nonionized forms, whereas surface sorption could be the main mechanism for higher pH. Lin and Gan [22] observed that even in soils with high clay content, sorption was negligible for the anti-inflammatories ibuprofen (IBF) and diclofenac (DCF). Basic pH in the studied soil (8.7–9.2) makes the carboxyl moieties (COOH) in both drugs dissociated and negatively charged, showing a low affinity for the negatively charged sites on the clay particles. A similar result was obtained for NPX by Chefetz et al. [58]. Similarly, organic carbon content (OC) was found to be the more dominant parameter in the sorption of SMZ, but K_D values were found to

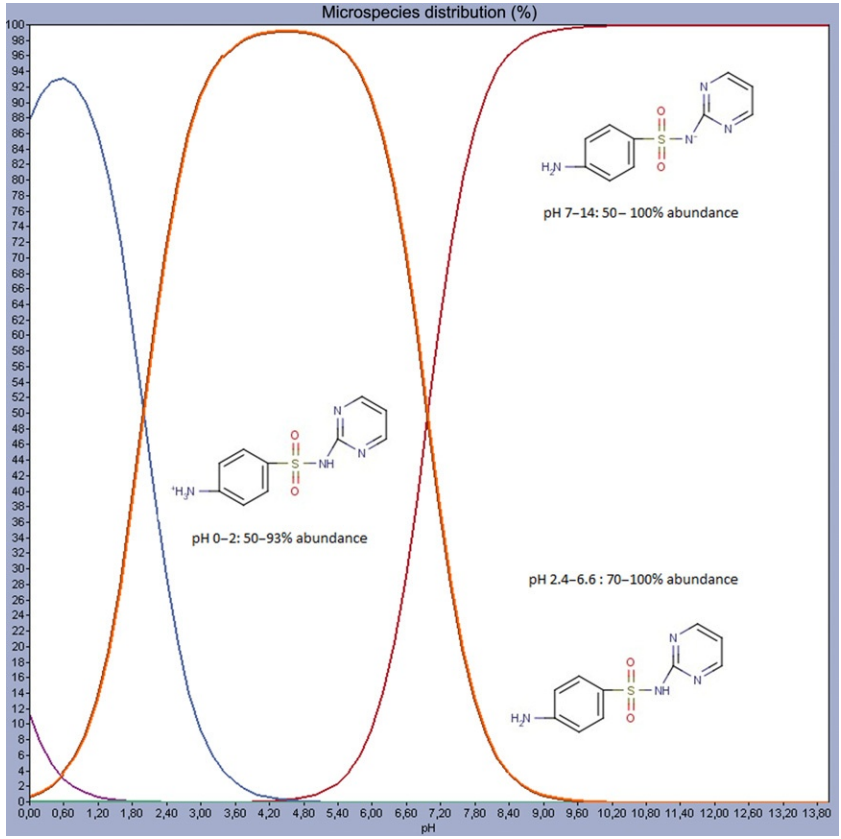

FIGURE 3 Speciation curve of sulfadiazine in function of pH and major microspecies formed.

decrease by more than 50% at pH 9 [59]. As an example, Figure 3 shows the speciation curve of SDZ, indicating the major microspecies in function of the pH. As can be observed, sulfonamides at high pHs are in the neutral or anionic form, which are less soluble and show a higher sorption to soils [54]. On the contrary, K_D values for OTC and TYL increased with increasing soil pH.

3.1.3 In situ *Abiotic and Biotic Degradations*

Evidence from the literature suggests that degradation is most likely occurring in the soil system. Bacteria and fungi are the two groups of organisms that are best able to degrade organic compounds in the soil ecosystem, although a higher number of studies have been devoted to microbial degradation. Seasonal and spatial variations could influence these organism populations and biodegradative activity in solid matrices, and therefore, different soils may have different indigenous communities [22]. The bioavailability of pharmaceuticals in

soils, that is, that they can be desorbed from the solid phase and freely dissolved in the water soil, eases the access of degradative organisms to these compounds. The NOM and clay content can inhibit degradation due to an increased adsorption and reduced bioavailability of the compound; NOM can also be assimilated as an alternative nutrient source by the degradative microorganisms; Xu et al. [21] recently demonstrated that degradation rates were inversely correlated to clay and NOM content of the soil for IBF, NPX, and specially DCF. DCF is one of the most commonly used nonsteroidal anti-inflammatory for humans and animals and frequently detected in wastewaters worldwide. Its rapid mineralization without a lag phase in different types of soils (from sandy to clay–loamy) has been demonstrated in different studies [21,25], proving that DCF was readily biodegradable in agricultural soils. Aerobic microorganisms played a very important role in its degradation; as heat sterilization stopped this mineralization, there was no removal for 84 days; the degradation rate was 23-fold faster in nonsterile soil. Similar results were obtained for IBF and NPX in the same study, with degradation rates in nonsterile soils 3- and 35-fold faster, respectively, than in anaerobic conditions. Relatively fast degradation of erythromycin (ERY) in soil has also been reported, with $t_{1/2}$ of 20 days [60]. Table 2 shows the degradation rates estimated for commonly used pharmaceuticals in different studies considering mainly microbial activity, oxygen status in soil, soil type, and compound characteristics.

The adsorption tendency is therefore negatively correlated to the degradation rate of different drugs. Pharmaceuticals with high K_D values, such as fluoroquinolone and TC antibiotics and the antidepressants fluoxetine (FXT) or CBZ, are less bioavailable to soil microorganisms and therefore show slower degradation rates [23,26]. The study of TC concentrations in a manure-amended soil has shown only a slight decline over a 6-month period, and accumulation was found with repeated manure fertilization [14,15]. Fate studies of SDZ have demonstrated that this antibiotic is retained, together with its hydroxylated metabolite and can persist in soil for several years [61]. Fluoroquinolones are one of the antibiotic families with higher persistence in soils. Their high sorption to OM in biosolids makes that they can be detected in soils after 21 months of fertilization [62].

The addition of liquid biosolids and manure as organic amendments could imply new microbial populations entering the soils ecosystem, which could also increase the rate of mineralization for different pharmaceuticals susceptible to microbial degradation [63]. Warm temperatures and moisture content can also contribute to a higher microbial activity and faster degradation rates, as well as soils with lighter textures. Gottschall et al. [23] observed that after 1 year of biosolids application in an agricultural soil, concentrations of different pharmaceuticals generally declined over time exponentially; only CBZ (30 ng g^{-1}) and 2-hydroxy ibuprofen (174 ng g^{-1}), a metabolite of IBF, were detected. Topp et al. [63] also reported a fast dissipation of NPX in three soils with a $t_{1/2}$ value of only 2 days, which was attributed microbial degradation.

However, different authors have demonstrated that the addition of biosolids to soils can also reduce the degradative activity in the soil systems. Monteiro and Boxall [20] observed how the introduction of the biosolid significantly decreased the degradation rate of NPX in soils with different clay contents (from 11% to 56%) and pHs (from 4 to 8). These data conflict with the data from Topp et al.'s study and other studies, which demonstrated that NPX was removed very quickly via runoff from a field amended with biosolids [29]. The main difference among these outcomes is that in these latter studies, the biosolids were aerobically digested, whereas Monteiro and Boxall worked with anaerobically digested biosolids; differences in biosolid microbial communities could explain these differences. On the other hand, not only amendment with biosolids does increase microbial activity but also the soil content of OC and dissolved organic carbon concentrations, which can affect degradation of compounds in the opposite way. The increase of OC in the soil would increase the sorption of different pharmaceuticals, limiting their bioavailability and biodegradation, and similarly, an increase in DOM in the soil pore water could also reduce availability for degradation [64]. Other compounds such as CBZ, DCF, and FXT were unaffected with the addition of biosolids [20,58,65]. Last of all, inhibitory or toxic effect from the pharmaceuticals contained in the applied manure or biosolids to existing microorganisms should also be considered [66]. For instance, Monteiro and Boxall also observed that the degradation rate of NPX was slower when working with different pharmaceutical mixtures, which could be attributed to the toxic effect of any of the drugs against the microbial community [20,67]. Photodegradation should also be considered as abiotic removal mechanism, although different references indicate that its effects are minor compared to those of biodegradation [68].

The formation of nonextractable residues should also be considered to explain reductions in pharmaceutical concentrations in both manure or biosolids and soils, limiting their mobility and bioavailability. For the antifungal clotrimazole, these nonextractable residues reduce considerably its detectable concentration [69], and their formation for IBF and the anxiolytic diazepam has also been demonstrated [70], pointing out to these nonextractable residues as potential sinks of pharmaceuticals in soils. The formation of nonextractable residues for sulfonamides, despite their low sorption coefficients and interaction with clays and humic substances, has also been reported and attributed to covalent cross coupling to soil OM [71]. The aromatic amine common to all sulfonamides represents a moiety likely to engage in covalent bond formation with NOM in soils. The abundant phenolic moieties contained in NOM, including numerous substituted phenols, serve as substrates for phenol oxidases and manganese oxides present in soils; these enzymes will function as important mediators to incorporate the aromatic amine of the sulfonamide into NOM, oxidizing the phenolic compounds and yielding phenoxy radicals that cross couple with the amine. In addition to diminishing sulfonamide

transport to surface waters and groundwaters, chemical incorporation into NOM may also reduce their biological activity. The free anilinic nitrogen of the sulfonamide is required for the antimicrobial to block folate synthesis, inhibiting the growth of susceptible microorganisms; the covalent linkage formed via the anilinic nitrogen would be expected to eliminate the bioactivity of these compounds.

4 OCCURRENCE OF PHARMACEUTICALS IN AGRICULTURAL SOILS

Two main entrance pathways have been considered in the previous section: fertilization with manure or biosolids and irrigation with raw or reclaimed wastewaters. Occurrence of different pharmaceuticals is therefore expected, especially for those compounds with lower tendencies to biodegradation and high K_D.

No studies were found in the literature on the degradation of CBZ in soils. Its resistance to degradation, already demonstrated during wastewater treatment, could explain the higher concentrations detected in soils, which have been irrigated with raw or reclaimed wastewaters or fertilized for longer periods of time [9,21], being one of the pharmaceuticals that are most commonly detected in soils [36] as it seems to persist in different types of soils. It has been demonstrated that for this drug, NOM content of soil was positively correlated to the detected concentrations, showing a greater retardation of CBZ in these NOM-rich soils, whereas there was no correlation between CBZ and clay contents [40,58]. Furthermore, its persistence allows time for migration through the soil before degradation occurs, and so it can reach groundwater bodies, whereas other drugs with similar solubilities and K_D values are eliminated before [35]. CBZ has been detected in runoff samples from agricultural fields amended with biosolids [29] and even in runoff events 266 days after the application [49]. In semiarid solids, with poor NOM content, the mobility of CBZ and also DFC, which is also considered a slow-mobile compound, could increase considerably and become a serious threat to groundwaters [58]. On the contrary, NPX has exhibited high mobility and very low sorption tendencies in different types of soil. Nevertheless, opposite to CBZ, both DCF and NPX have been degraded during wastewater treatment and would be more likely degraded during their transition through soil, having a much lower potential to reach the groundwater.

Different pharmaceuticals have been detected in agricultural soils irrigated with black waters. In Mexico, Gibson et al. [40] detected CBZ in both A and B horizons of soils with different contents of clay and OM (Phaeozem and Leptosols), at concentrations between 2.6 and 7.5 $\mu g\ kg^{-1}$. The only acidic pharmaceuticals recorded in the A horizon of soils irrigated with wastewater were NPX (0.27–0.61 $\mu g\ kg^{-1}$) and IBF (<LOD to 0.10 $\mu g\ kg^{-1}$), which were present in the raw wastewater at high concentrations (13.6 and 1.4 $\mu g\ L^{-1}$,

respectively). CBZ was present in the A horizon of all soils (from 2.6 to 7.5 μg kg^{-1}). Concentrations in the soil samples from each field generally decreased down the profiles.

ERY, CBZ, FXT, and the antihistamine diphenhydramine were the four pharmaceuticals most frequently detected in wastewater-irrigated soils from Colorado (the United States) at typical concentrations between 0.02 and 15 mg kg^{-1} [36]. Concentrations up to 212 mg kg^{-1} were also found for OTC. IBF, DCF, clofibric acid, and NPX were also detected in golf courses irrigated with reclaimed wastewaters at levels ranging from 0.55 to 9.08 ng g^{-1} [9].

4.1 Occurrence of Antibiotics

As mentioned in the introduction of this chapter, data on the occurrence of antibiotics in soils are much more abundant than for other pharmaceuticals, due mainly to the potential spread of antibiotic resistance genes in different microbial communities and so the environmental risk associated. For instance, TCs show high sorption coefficients compared to other antibiotics, indicating not only the strongest sorption tendency but also the potential accumulation and persistence in solid matrices. As shown in Table 3, TCs have been detected frequently and at the highest concentrations in different agricultural soils fertilized with manure. Hamscher et al. [15] detected TC and CTC in soil samples amended with manure at concentrations between 86.2 μg kg^{-1} in the first 10 cm and 171.7 μg kg^{-1} in the 20–30 cm layer. This apparent increase in the concentration with depth was attributed to the release of bound residues of 4-epitetracycline, a metabolite of this antibiotic that was transferred from the liquid manure into the soil and not to TC translocation. As expected, neither TC nor CTC was detected in deeper soil layers or in soil water or groundwaters located 80–200 cm deep. Furthermore, the authors observed accumulation of TC with successive manure fertilization, corroborating the high retention and persistence of these antibiotics in upper layers of the soil (see Figure 4B). Storage of manure during 6 and 12 months did not seem to decrease the amount of TC that was finally applied to the soil (Figure 4B). Blackwell et al. [53] obtained similar results of OXT and TYL.

On the contrary, due to their lower K_D and high polarity and solubility, sulfonamides are the family of antibiotics that have a greater potential for leaching to groundwater, and several studies have demonstrated their occurrence in aquifers at concentrations up to μg L^{-1} level [74–77]. Because of their low K_D values, sulfonamides are considered to be very mobile and weakly retained in soil and therefore highly bioavailable and generally non-bioaccumulative. Nevertheless, despite their high potential to leach and run off and that some authors have considered sorption to soils of sulfonamides negligible [22], sulfonamides have been detected in soils together with TCs and macrolides [14] in the top 0–30 cm soil. Regarding SMX, one of the most

TABLE 3 Pharmaceutical Concentrations ($\mu g\ kg^{-1}$) Detected in Agricultural Soils

		Soil Properties					
Therapeutic Family	**Pharmaceutical**	*% Clay*	*% OM*	*pH*	**Concentration ($\mu g\ kg^{-1}$)**	**Comments**	**References**
Nonsteroidal anti-inflammatory	Salicylic acid	24–28	1.1–2.5	7.8–8	1.6–4.5	Wastewater-irrigated	[38]
		25–32	1.2–4.3	7.2–7.5	4.6–9.1		
		26–34	2.0–3.0	7.4–7.6	4.7–10.7		
	Ibuprofen	48	3.1	5.88	0.25	90 years of irrigation Raw wastewater	[39]
		44.7	2.9	6.7	<LOD		
		–	7–7.3	6.6–6.7	<LOD	10 years of irrigation	[40]
		–	3.7–6.3	6.7–7.4	0.10–0.33		
		–	0.6–4.5	6.6–7.7	<LOD–0.10	90 years of irrigation	
		–	1.1–5.5	6.9–7.3	<LOD–0.10		
		–	3.3	6.7–8.2	0.098–0.190	Groundwater irrigation	[72]
	2-Hydroxy ibuprofen				174		[23]
	Naproxen	–	3.3	6.7–8.2	0.15–0.22	Groundwater irrigation	[72]
		48	3.1	5.88	0.55	Wastewater-irrigated	[39]
		44.7	2.9	6.7	0.73		
		–	7–7.3	6.6–6.7	<LOD–0.27	10 years of irrigation	[40]
		–	3.7–6.3	6.7–7.4	0.33–0.52		
		–	0.6–4.5	6.6–7.7	0.35–0.43	90 years of irrigation	
		–	1.1–5.5	6.9–7.3	0.48–0.61		
		–	3.3	6.7–8.2	0.2–0.46	Groundwater irrigation	[72]

	Ketoprofen	48	3.1	5.88	<LOD	Wastewater-irrigated	[39]
		44.7	2.9	6.7	<LOD		
	Diclofenac	48	3.1	5.88	<LOD		
		44.7	2.9	6.7			
		–	7–7.3	6.6–6.7		10 years of irrigation	[40]
		–	3.7–6.3	6.7–7.4			
		–	0.6–4.5	6.6–7.7		90 years of irrigation	
		–	1.1–5.5	6.9–7.3			
		–	3.3	6.7–8.2	0.009–0.09	Groundwater irrigation	[72]
Lipid regulator	Clofibric acid	48	3.1	5.88	<LOD	Wastewater-irrigated	[39]
		44.7	2.9	6.7	<LOD		
	Gemfibrozil	48	3.1	5.88	<LOD		
		44.7	2.9	6.7	<LOD		
Psychiatric drugs	Carbamazepine	48	3.1	5.88	6.48	Wastewater-irrigated	[39]
		44.7	2.9	6.7	5.14		
		–	7–7.3	6.6–6.7	2.6–4.8	10 years of irrigation	[40]
		–	3.7–6.3	6.7–7.4	3.2–5.1		
		–	0.6–4.5	6.6–7.7	3.5–6.1	90 years of irrigation	
		–	1.1–5.5	6.9–7.3	4.9–7.5		
		–	3.3	6.7–8.2	0.04–0.26	Groundwater irrigation	[72]
		–	–	–	30	Biosolid-amended	[23]

Continued

TABLE 3 Pharmaceutical Concentrations (μg kg^{-1}) Detected in Agricultural Soils—Cont'd

		Soil Properties					
Therapeutic Family	**Pharmaceutical**	*% Clay*	*% OM*	*pH*	**Concentration (μg kg^{-1})**	**Comments**	**References**
Antihistamine	Diphenhydramine	–	–	–	1.1	Biosolid-amended	[73]
β-blocker agent	Metoprolol	–	–	–	0.32	Groundwater irrigation	[72]
Antibiotics							
Tetracyclines	Oxytetracycline	30–30.7	2.2–3.7	7.8–8	5.8–6.2	Wastewater-irrigated	[38]
		24–28	1.1–2.5	7.2–7.5	5.8–7.5		
		25–32	1.2–4.3	7.4–7.6	54.5–212		
		–	–	–	124–2683	Manure-amended	[12]
	Tetracycline	24–28	1.1–2.5	7.2–7.5	2.8–6.9	Wastewater-irrigated	[38]
		25–32	1.2–4.3	7.4–7.6	12.8–19.9		
		–	–	–	2.5–105		[12]
		2.4	1.8	4.5	2.3–50.1	Manure-amended	[15]
		2.4	1.8	4.5	35–295		[14]
	Chlortetracycline	11.3	–	6.1	0.6–15.5	Loamy soil Sandy soil	[51]
		5.2	–	5.6	0.6–11.7		
		–	–	–	33.1–1079	Manure-amended	[12]
		2.4	1.8	4.5	1.7–59.9	Manure-amended	[15]
		2.4	1.8	4.5	4.1–39		[14]

Macrolide	Tylosin	11.3	–	6.1	6.4–57.4	Loamy soil Sandy soil	[51]
		5.2	–	5.6	1.8–21.3		
Sulfonamides	Sulfamethoxazole	–	–	–	0.03–0.9	Manure-amended	[12]
	Sulfadoxine	–	–	–	1.2–9.1		
	Sulfachloropyridazine	–	–	–	0.18–2.5		
	Sulfamethazine	2.4	1.8	4.5	2		[14]
Fluoroquinolone	Ofloxacin	–	–	–	0.6–1.6		[12]
	Pefloxacin	–	–	–	n.d.		
	Ciprofloxacin	–	–	–	0.8–30.1		
Lincosamide	Lincomycin	–	–	–	1.1–11.7		
Others	Trimethoprim	30–30.7	2.2–3.7	7.8–8	<LOQ	Wastewater-irrigated	[38]
		24–28	1.1–2.5	7.2–7.5	1.6–3.3		
		25–32	1.2–4.3	7.4–7.6	<LOQ		
		–	–	–	0.64		[73]
	Chloramphenicol	–	–	–	0.1–11	Manure-amended	[12]

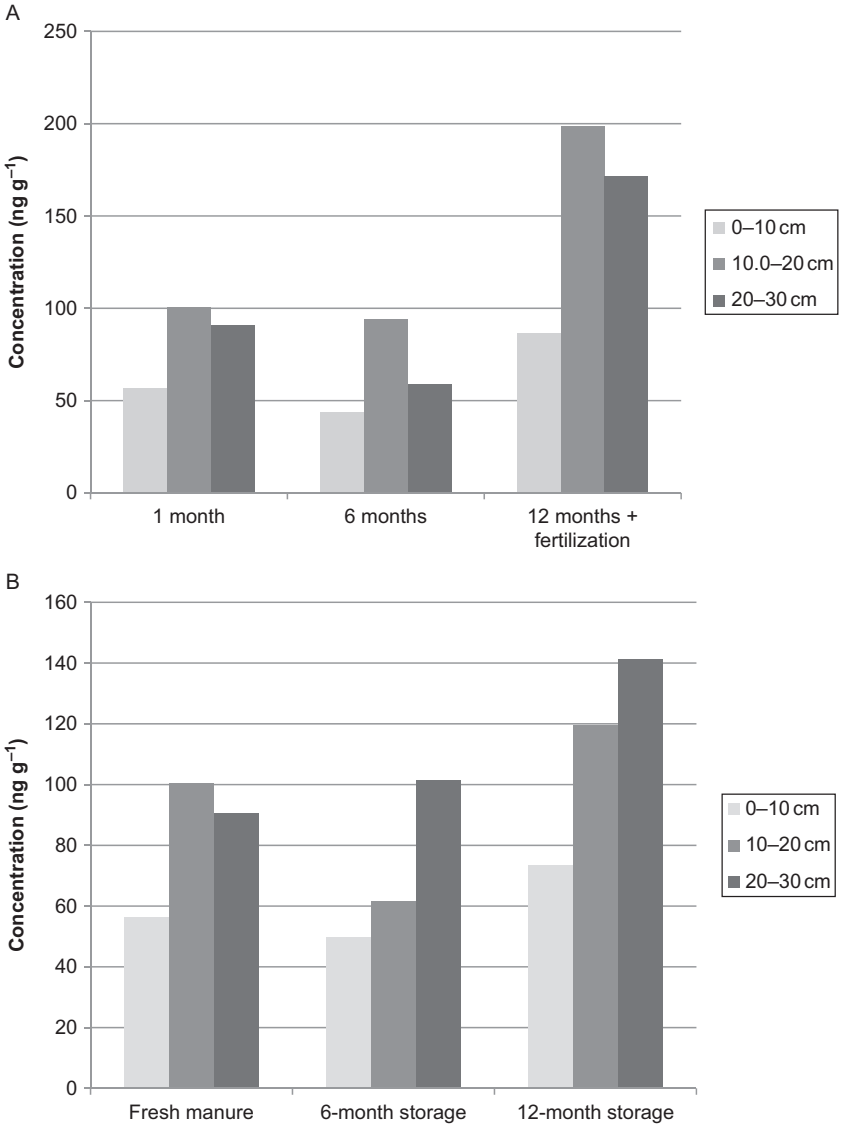

FIGURE 4 (A) Tetracycline (TC) occurrence in the upper layers of an agricultural soil after fertilization with manure during two consecutive years. (B) TC concentrations in soil after application of fresh and stored manure based on [14,15].

commonly found antibiotics in wastewater effluents, it has been demonstrated that it is moderately sorbed in soils, [78,79] and different sorption mechanisms have been proposed such as water bridging interaction between neutral SMX molecular and the clay surface, complexation of exchangeable cations through N or the SO_2^- group, and sorption to natural OM.

5 ENVIRONMENTAL RISKS

5.1 Ecotoxicity of Pharmaceuticals in Soils

It is unknown whether the existence of pharmaceuticals at the concentrations detected in soils poses a risk to human or environmental health. Due to the lack of legislation regarding environmental presence of pharmaceuticals in any environmental matrix, similarly to the environmental risk assessment guidelines established by the European Medicines Agency, the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products proposed that the total concentration in soil should not exceed 100 $\mu g\ kg^{-1}$ (http://vich.eudra.org/htm/guidelines.htm), for ecotoxicity tests with veterinary medical products at this level registered in the United States showed no effects on earthworms, microbes, or plants.

Antibiotic resistance is the best-documented environmental impact derived of the presence of pharmaceuticals (antibiotics) in the environment. Moreover, their presence can promote the formation of resistance and even cross-resistance and multiple drug resistance in microorganisms. Resistant genes have been isolated in both water and solid matrices. It has been demonstrated that the antibiotic-induced tolerance development of microbial communities is more pronounced if soils are amended with nutrients such as pig slurry [80]. Thiele-Bruhn and Beck [67] concluded that certain antibiotics, such as sulfonamides or TCs, could induce a temporary selective pressure on soil microorganisms even at environmentally relevant concentrations, as they observed effective solution concentrations (EC_{10}) ranging from 0.2 to 160 $ng\ g^{-1}$. Hammesfahr et al. [81] studied the impact of manure contaminated with SDZ in the soil microbial community by monitoring changes in nitrification and mineralization patterns, among others. Effects of SDZ on soil microorganisms were significant although the bioavailable SDZ fraction rapidly declined. Manure storage did not seem to decrease the bioactivity of SDZ. However, Halling-Sørensen et al. [82] observed how antimicrobial activity of different antibiotics, including SDZ, TYL, OTC, CPF, or streptomycin (ST), lost their antimicrobial potency in interstitial soil water with time in both aerobic and anaerobic conditions. With the exception of ST and CPF, the antimicrobial agents generally lost a considerable amount of their antimicrobial potency. On the contrary, OTC, ST, and specially CPF seemed to remain active despite decreasing its concentration with time; the formation of degradation products maintaining part of the parent bioactivity could explain these results. Despite of this reduction, resistance genes in bacteria populations from agricultural soils have been reported in different studies [83].

Effects on other terrestrial organisms such as macroinvertebrates and plants have also been investigated in different works. Emergence of seedlings, plant elongation and biomass, earthworm mortality, and soil microbial enzymatic activities are usually selected as toxicological end points for soil organisms [84]. For instance, OTC was innocuous to the earthworm *Eisenia foetida* even at concentrations up to 100 $mg\ kg^{-1}$, which could be attributed to the

high K_D value and low bioavailability of the antibiotic; it should not be forgotten that the risk posed by pharmaceuticals in soils is directly linked to their bioavailability. This result is in agreement with observations on other soil invertebrates including collembola, enchytraeids, and annelida, with lowest observed effect concentration values >5000 mg kg^{-1} in soil [85]. However, this antibiotic did affect soil microbial enzymatic activities, which could be recovered with manure addition, and also inhibited plant growth if manure was added. As mentioned in Section 3.1.3, the addition of liquid manure could maintain certain pollutants in solution and more bioavailable.

Although the presence of micropollutants in biosolids and manure destined for land application is known, the effects of these compounds on terrestrial ecosystems have been only barely investigated. The majority of available ecotoxicity data deal solely with aquatic organisms [86]. To perform a proper risk assessment for soil-dwelling organisms, half-life data for pharmaceuticals are indispensable. As shown in Table 2, $t_{1/2}$ values can be up to 518 days, suggesting that chronic toxicity should be studied more thoroughly over acute lab assays.

Another area of concern would be the potential for bioaccumulation and biomagnifications of microcontaminants throughout the food chain (from plants or earthworms to birds, fish, and mammals).

Metabolites are usually more polar and soluble than parent compounds and may show a lower tendency to adsorb to soil particles. Therefore, once on the soil, these metabolites could deconjugate, transforming back into the original compound [87,88]. Förster et al. [89] demonstrated that this happened with AcSDZ, which was introduced into soil via manure application and transformed into SDZ and SDZ-OH. The concentrations of both compounds in residual fractions increased with time, and both could persist in soils for several years.

5.2 Risk of Groundwaters Contamination

The connection between agriculture and groundwater quality has become a highly relevant issue from a scientific and management point of view. The regular application of organic amendments such as manure or biosolids, together with the use of raw water for irrigation in agricultural land, has compromised the quality of this water resource, as infiltration of rainwater or irrigation return flows are the main recharge sources that aquifers feed on. Groundwater bodies should be considered as especially vulnerable water reservoirs because, despite presenting a bigger inertia to quality changes, once contaminated, the effects can hardly ever be reverted. Soils could be considered as the first protective shield from pollution for groundwater, providing inertia to quality changes and a slowed propagation of the contaminants. Biofilm systems within soils are capable of removing pharmaceuticals in irrigation water as it percolates through the soil profiles, as it has been

demonstrated recently in wastewater treatments based on slow sand filtration or soil aquifer treatment [66]. However, biofilms, fungi, and other soil degradative organisms could also be negatively affected by the drugs present in solution, eliminating this first protective barrier. Furthermore, as discussed in section 3.1, leaching of dissolved pharmaceuticals in soils is a complex mechanism governed by many variables. There are several studies that reflect the occurrence of a big diversity of pharmaceuticals in groundwaters. For instance, Ternes et al. [35] studied the fate of different pharmaceuticals in groundwaters located beneath arable land that had been irrigated with treated wastewaters for more than 45 years; CBZ and SMX were detected in groundwaters at concentrations up to $\mu g\ L^{-1}$ level, but no acidic pharmaceuticals, which were likely sorbed or transformed while passing the topsoil layer. Chen et al. [38] detected salicylic acid and different sulfonamides in groundwaters, which were abstracted and used for crop irrigation. Due to repeated application of irrigation waters, this compound was also detected in the irrigated soils, despite its high solubility and low K_{OW}.

5.3 Uptake of Pharmaceuticals in Soil Solution by Plants

Some authors have also considered the possibility of absorption by the plant root systems of the different pharmaceuticals present in the soil water after irrigation with contaminated waters or after fertilization with manure or biosolids. Consumption of crops by humans or forages by livestock grown in contaminated agricultural land could be a route of human exposure to micropollutants that has not been fully evaluated. Therefore, these agricultural practices may pose potential risks to the environment and public health due to the introduction of different organic pollutants. Irrigation water quality criteria have usually focused on parameters of nutrients, inorganic compounds, and pathogens and have not considered that a high number of pharmaceuticals and other organic contaminants are not fully eliminated during wastewater treatment. Estimations by Boxall et al. [24] indicate that foodborne exposure may be much more significant than drinking water. Experimental uptake investigation with carrots or lettuce leaves in sandy soils spiked at $1\ mg\ kg^{-1}$ showed that these vegetables were not affected by the presence of sulfonamides, but levamisole and trimethoprim were taken up by lettuces, and enrofloxacin and trimethoprim also were taken up by carrots [24]. Atenolol, sulfamerazine, and trimethoprim were detected in tomatoes, NFX in carrots, and CPF in carrot and sweet corn [90]. Dolliver et al. demonstrated that SMZ was taken up by crops, with concentrations in plant tissue ranging from 0.1 to $1.2\ mg\ kg^{-1}$ dry weight. After 45 days of growth, this concentration represented less than 0.1% of the amount applied to soil in manure [91]. In different studies, SDM was toxic for millet, peas, and corn [92]. CTC was detected in tissue of green onions, cabbage, and corn from a CTC-treated soil. In contrast, none of these plants took up TYL, despite higher concentrations

being applied to the soil and the lower K_D of TYL compared to CTC. This was attributed to the larger molecular weight of TYL, which is almost double the mass of CTC, making it unlikely that TYL was taken up by plants either in mass flow (in the transpiration stream) or as active uptake [93].

However, all these studies suggested that exposure of consumers to pharmaceuticals in soils via plants consumption is likely to be considerably below the acceptable daily intake and that the risk to human health is low. Despite these different reports of uptake of pharmaceuticals by various plant species [24,94], it should be taken into account that soils used during these laboratory studies were spiked with drug levels much higher than those usually detected in biosolids, manure, or soils, which may account for the uptake. Under environmental or normal farming conditions, this uptake would be unlikely, evidencing the nonrepresentativeness of these studies under laboratory conditions or performed in greenhouses [23,90,95]. Furthermore, as a preventive measure, current mandated regulations regarding biosolid applications specify a 1-year offset between fertilization and crop harvest, allowing potential degradation, formation of soil-bound residues, and loss by leaching of the pollutants, limiting their availability for subsequent uptake from the soil into crops.

6 CONCLUSIONS

The occurrence of a great variety of pharmaceuticals in soil has been demonstrated in several works. However, the fate of these compounds in the soil system is complex and seems to depend in many different variables, starting from storage conditions in manure or biosolids stockpiles. Once on the topsoil, diverse and intricate processes can be involved in the sorption mechanism of pharmaceuticals in solid matrices. Higher or lower mobility of pharmaceuticals greatly depends on their water solubility and polarity and their tendency to adsorb to solid matrices, but other factors regarding the receiving matrix can also be a determinant, such as the nature of the soil or sediment (OC, texture, particulate matter of the receiving water body, etc.). Many pharmaceuticals can dissipate quickly after being applied to soil, like NPX, yet others, such as fluoroquinolones and TCs, are very persistent. Soils irrigated with contaminated waters have shown the presence of pharmaceuticals previously detected in surface waters. Some of these are not degraded or are retained in the rich OM layer of the soil and seeped into the deeper horizons, reaching potentially groundwaters. On the other hand, it is not the total but the bioaccessible concentration of pharmaceuticals in soils that will determine their biodegradability and also their toxicity against soil organisms. It should be taken into consideration that pharmaceuticals are present in the environment as mixtures with other drugs, including metabolites and transformation products, and never as single compounds, so current risk assessment procedures based on single compounds may underestimate environmental impacts.

ABBREVIATIONS

AcSDZ	N^4-acetylsulfadiazine
CAFO	confined animal feeding operation
CBZ	carbamazepine
CPF	ciprofloxacin
CTC	chlortetracycline
DCF	diclofenac
DIF	difloxacin
ERY	erythromycin
FXT	fluoxetine
IBF	ibuprofen
K_D	distribution coefficient
K_{OW}	octanol/water partition coefficient
NFX	norfloxacin
NPX	naproxen
OC	organic carbon content
OM	organic matter
OTC	oxytetracycline
SDZ	sulfadiazine
SDZ-OH	hydroxysulfadiazine
SMX	sulfamethoxazole
$t_{1/2}$	half-life

REFERENCES

[1] T.A. Ternes, A. Joss (Eds.), Human Pharmaceuticals, Hormones and Fragrances: The Challenge of Micropollutants in Urban Water Management, IWA Publishing, Cornwall, UK, 2006.

[2] K. Kümmerer, J. Environ. Manag. 90 (8) (2009) 2354–2366.

[3] M.J. García-Galán, S. Díaz-Cruz, D. Barceló, J. Chromatogr. A 1275 (2013) 32–40.

[4] J.W. Kwon, Bull. Environ. Contam. Toxicol. 87 (1) (2011) 40–44.

[5] C. Zarfl, J. Klasmeier, M. Matthies, Chemosphere 77 (6) (2009) 720–726.

[6] S.C. Kim, K. Carlson, Environ. Sci. Technol. 41 (1) (2007) 50–57.

[7] P. Vazquez-Roig, R. Segarra, C. Blasco, V. Andreu, Y. Picó, J. Chromatogr. A 1217 (16) (2010) 2471–2483.

[8] C. Wu, J.D. Witter, A.L. Spongberg, K.P. Czajkowski, Water Res. 43 (14) (2009) 3407–3416.

[9] J. Xu, L. Wu, W. Chen, A.C. Chang, J. Chromatogr. A 1202 (2) (2008) 189–195.

[10] M.J. García-Galán, M.S. Díaz-Cruz, D. Barceló, The Llobregat: a story of a polluted Mediterranean river, Handbook Environ. Chem. 21 (2012) 167–192. Springer.

[11] L. Zhou, Y. Hua Dong, H. Wang, Sci. Total Environ. 408 (5) (2010) 1069–1075.

[12] X. Hu, Q. Zhou, Y. Luo, Environ. Pollut. 158 (9) (2010) 2992–2998.

[13] E. Martínez-Carballo, C. González-Barreiro, S. Sharf, O. Gans, Environ. Pollut. 148 (2007) 570–579.

[14] G. Hamscher, H.T. Pawelzick, H. Hoper, H. Nau, Environ. Toxicol. Chem. 24 (4) (2005) 861–868.
[15] G. Hamscher, S. Sczesny, H. Höper, H. Nau, Anal. Chem. 74 (7) (2002) 1509–1518.
[16] J. Galvalchin, S.E. Katz, J. Assoc. Off. Anal. Chem. 77 (1994) 481–485.
[17] M. Lamshöft, P. Sukul, S. Zühlke, M. Spiteller, Sci. Total Environ. 408 (2010) 1563–1568.
[18] S. Thiele-Bruhn, J. Plant Nutr. Soil Sci. 166 (2) (2003) 145–167.
[19] P. Sukul, M. Lamshöft, S. Zühlke, M. Spiteller, Chemosphere 71 (4) (2008) 717–725.
[20] S.C. Monteiro, A.B.A. Boxall, Environ. Toxicol. Chem. 28 (12) (2009) 2546–2554.
[21] J. Xu, L. Wu, A.C. Chang, Chemosphere 77 (10) (2009) 1299–1305.
[22] K. Lin, J. Gan, Chemosphere 83 (3) (2011) 240–246.
[23] N. Gottschall, E. Topp, C. Metcalfe, M. Edwards, M. Payne, S. Kleywegt, P. Russell, D.R. Lapen, Chemosphere 87 (2) (2012) 194–203.
[24] A.B.A. Boxall, P. Johnson, E.J. Smith, C.J. Sinclair, E. Stutt, L.S. Levy, J. Agric. Food Chem. 54 (6) (2006) 2288–2297.
[25] A.J. Al-Rajab, L. Sabourin, D.R. Lapen, E. Topp, Sci. Total Environ. 409 (1) (2010) 78–82.
[26] E. Walters, K. McClellan, R.U. Halden, Water Res. 44 (20) (2010) 6011–6020.
[27] W. Chenxi, A.L. Spongberg, J.D. Witter, Chemosphere 73 (4) (2008) 511–518.
[28] H.A.S. Dolliver, S.C. Gupta, J. Environ. Qual. 37 (3) (2008) 1238–1244.
[29] A.B.A. Boxall, P. Blackwell, R. Cavallo, P. Kay, J. Tolls, Toxicol. Lett. 131 (1–2) (2002) 19–28.
[30] C.A. Kinney, E.T. Furlong, S.D. Zaugg, M.R. Burkhardt, S.L. Werner, J.D. Cahill, G.R. Jorgensen, Environ. Sci. Technol. 40 (2006) 7207–7215.
[31] M. Edwards, E. Topp, C.D. Metcalfe, H. Li, N. Gottschall, P. Bolton, W. Curnoe, M. Payne, A. Beck, S. Kleywegt, D.R. Lapen, Sci. Total Environ. 407 (14) (2009) 4220–4230.
[32] M.S. Díaz-Cruz, M.J. García-Galán, P. Guerra, A. Jelic, C. Postigo, E. Eljarrat, M. Farré, M.J. López de Alda, M. Petrovic, D. Barceló, Trends Anal. Chem. 28 (11) (2009) 1263–1275.
[33] G.W. Miller, Desalination 187 (2006) 65–75.
[34] O. Borgman, B. Chefetz, Water Res. 47 (10) (2013) 3431–3443.
[35] T.A. Ternes, M. Bonerz, N. Herrmann, B. Teiser, H.R. Andersen, Chemosphere 66 (5) (2007) 894–904.
[36] C.A. Kinney, E.T. Furlong, S.L. Werner, J.D. Cahill, Environ. Toxicol. Chem. 25 (2) (2006) 317–326.
[37] J. Siemens, G. Huschek, C. Siebe, M. Kaupenjohann, Water Res. 42 (8–9) (2008) 2124–2134.
[38] F. Chen, G.-G. Ying, L.-X. Kong, L. Wang, J.-L. Zhao, L.-J. Zhou, L.-J. Zhang, Environ. Pollut. 159 (6) (2011) 1490–1498.
[39] J.C. Durán-Alvarez, E. Becerril-Bravo, V.S. Castro, B. Jiménez, R. Gibson, Talanta 78 (3) (2009) 1159–1166.
[40] R. Gibson, J.C. Durán-Álvarez, K.L. Estrada, A. Chávez, B. Jiménez Cisneros, Chemosphere 81 (11) (2011) 1437–1445.
[41] H. Dolliver, S. Gupta, J. Environ. Qual. 37 (3) (2008) 1227–1237.
[42] A.K. Sarmah, M.T. Meyer, A.B.A. Boxall, Chemosphere 65 (5) (2006) 725–759.
[43] R. Kreuzig, S. Höltge, J. Brunotte, N. Berenzen, J. Wogram, R. Schulz, Environ. Toxicol. Chem. 24 (2005) 777–781.
[44] E.R. Campagnolo, K.R. Johnson, A. Karpati, C.S. Rubin, D.W. Kolpin, M.T. Meyer, J.E. Esteban, R.W. Currier, K. Smith, K.M. Thu, M. McGeehin, Sci. Total Environ. 299 (1–3) (2002) 89–95.

[45] M. De Liguoro, C. Poltronieri, F. Capolongo, C. Montesissa, Chemosphere 68 (4) (2007) 671–676.
[46] K. Stoob, H.P. Singer, S.R. Mueller, R.P. Schwarzenbach, C.H. Stamm, Environ. Sci. Technol. 41 (21) (2007) 7349–7355.
[47] P. Kay, P.A. Blackwell, A.B.A. Boxall, Environ. Toxicol. Chem. 23 (5) (2004) 1136–1144.
[48] D.R. Lapen, E. Topp, M. Edwards, L. Sabourin, W. Curnoe, N. Gottschall, P. Bolton, S. Rahman, B. Ball-Coelho, M. Payne, S. Kleywegt, N. McLaughlin, J. Environ. Qual. 37 (3) (2008) 925–936.
[49] D.R. Lapen, E. Topp, C.D. Metcalfe, H. Li, M. Edwards, N. Gottschall, P. Bolton, W. Curnoe, M. Payne, A. Beck, Sci. Total Environ. 399 (1–3) (2008) 50–65.
[50] P. Kay, P.A. Blackwell, A.B.A. Boxall, Chemosphere 59 (7) (2005) 951–959.
[51] A.M. Jacobsen, B. Halling-Sorensen, F. Ingerslev, S.H. Hansen, J. Chromatogr. A 1038 (1–2) (2004) 157–170.
[52] P.A. Blackwell, P. Kay, A.B.A. Boxall, Chemosphere 67 (2) (2007) 292–299.
[53] P.A. Blackwell, P. Kay, R. Ashauer, A.B.A. Boxall, Chemosphere 75 (1) (2009) 13–19.
[54] S. Kurwadkar, C. Adams, M. Meyer, D. Kolpin, J. Agric. Food Chem. 55 (2007) 1370–1376.
[55] D.S. Aga, R. Goldfish, P. Kulshrestha, Analyst 128 (2003) 658–662.
[56] P. Sukul, M. Spiteller, Rev. Environ. Contam. Toxicol. 187 (2006) 67–101.
[57] E. Topp, S.C. Monteiro, A. Beck, B.B. Coelho, A.B.A. Boxall, P.W. Duenk, S. Kleywegt, D.R. Lapen, M. Payne, L. Sabourin, H. Li, C.D. Metcalfe, Sci. Total Environ. 396 (1) (2008) 52–59.
[58] B. Chefetz, T. Mualem, J. Ben-Ari, Chemosphere 73 (8) (2008) 1335–1343.
[59] W. Lertpaitoonpan, S.K. Ong, T.B. Moorman, Chemosphere 76 (4) (2009) 558–564.
[60] M.P. Schlüsener, K. Bester, Environ. Pollut. 143 (2006) 565–571.
[61] K. Schauss, A. Focks, H. Heuer, A. Kotzerke, H. Schmitt, S.r. Thiele-Bruhn, K. Smalla, B.-M. Wilke, M. Matthies, W. Amelung, J.r. Klasmeier, M. Schloter, Trends Anal. Chem. 28 (5) (2009) 612–618.
[62] E.M. Golet, I. Xifra, H. Siegrist, A.C. Alder, W. Giger, Environ. Sci. Technol. 37 (15) (2003) 3243–3249.
[63] E. Topp, J.G. Hendel, D.R. Lapen, R. Chapman, Environ. Toxicol. Chem. 27 (10) (2008) 2005–2010.
[64] A.B.A. Boxall, C.D. Brown, K.L. Barrett, Pest Manag. Sci. 58 (7) (2002) 637–648.
[65] C. Redshaw, M. Cooke, H. Talbot, S. McGrath, S. Rowland, J. Soils Sediments 8 (4) (2008) 217–230.
[66] K.M. Onesios, E.J. Bouwer, Water Res. 46 (7) (2012) 2365–2375.
[67] S. Thiele-Bruhn, I.-C. Beck, Chemosphere 59 (4) (2005) 457–465.
[68] A.L. Boreen, W.A. Arnold, K. McNeill, Aquat. Sci. Res. Across Boundaries 65 (4) (2003) 320–341.
[69] L. Sabourin, A.J. Al-Rajab, R. Chapman, D.R. Lapen, E. Topp, Environ. Toxicol. Chem. 30 (3) (2011) 582–587.
[70] O. Richter, C. Kullmer, R. Kreuzig, Clean – Soil, Air, Water 35 (5) (2007) 495–503.
[71] H.M. Bialk, A.J. Simpson, J.A. Pedersen, Environ. Sci. Technol. 39 (12) (2005) 4463–4473.
[72] A. Azzouz, E. Ballesteros, Sci. Total Environ. 419 (2012) 208–215.
[73] C.A. Kinney, E.T. Furlong, D.W. Kolpin, M.R. Burkhardt, S.D. Zaugg, S.L. Werner, J.P. Bossio, M.J. Benotti, Environ. Sci. Technol. 42 (6) (2008) 1863–1870.
[74] M.J. Garcia-Galan, T. Garrido, J. Fraile, A. Ginebreda, M.S. Diaz-Cruz, D. Barcelo, Anal. Bioanal. Chem. 399 (2) (2010) 795–806.

[75] M.J. García-Galán, T. Garrido, J. Fraile, A. Ginebreda, M.S. Díaz-Cruz, D. Barceló, J. Hydrol. 383 (2010) 93–101.
[76] A.L. Batt, D.D. Snow, D.S. Aga, Chemosphere 64 (11) (2006) 1963–1971.
[77] P.A. Blackwell, H.C.H. Lutzhoft, H.P. Ma, B. Halling-Sorensen, A.B.A. Boxall, P. Kay, J. Chromatogr. A 1045 (1–2) (2004) 111–117.
[78] P. Drillia, K. Stamatelatou, G. Lyberatos, Chemosphere 60 (8) (2005) 1034–1044.
[79] L. Yu, G. Fink, T. Wintgens, T. Melin, T.A. Ternes, Water Res. 43 (4) (2009) 951–960.
[80] H. Schmitt, H. Haapakangas, P. van Beelen, Soil Biol. Biochem. 37 (10) (2005) 1882–1892.
[81] U. Hammesfahr, A. Kotzerke, M. Lamshöft, B.-M. Wilke, E. Kandeler, S. Thiele-Bruhn, Eur. J. Soil Biol. 47 (1) (2011) 61–68.
[82] B. Halling-Sørensen, G. Sengelov, F. Ingerslev, L.B. Jensen, Arch. Environ. Contam. Toxicol. 44 (1) (2003) 7–16.
[83] K.G. Byrne, W. Gaze, E.M.H. Wellington, P. Kay, P. Blackwell, A. Boxall, in: European Symposium on Environmental Biotechnology, ESEB 2004, pp. 615–617.
[84] S. Boleas, C. Alonso, J. Pro, C. Fernández, G. Carbonell, J.V. Tarazona, J. Hazard. Mater. 122 (3) (2005) 233–241.
[85] A.J. Baguer, J. Jensen, P.H. Krogh, Chemosphere 40 (7) (2000) 751–757.
[86] M. Jesús García-Galán, M. Silvia Díaz-Cruz, D. Damià Barceló, Trends Anal. Chem. 28 (6) (2009) 804–819.
[87] M.J. García-Galán, T. Frömel, J. Müller, M. Peschka, T. Knepper, S. Díaz-Cruz, D. Barceló, Anal. Bioanal. Chem. 402 (9) (2012) 2885–2896.
[88] A. Göbel, C.S. McArdell, A. Joss, H. Siegrist, W. Giger, Sci. Total Environ. 372 (2–3) (2007) 361–371.
[89] M. Förster, V. Laabs, M. Lamshöft, J. Groeneweg, S. Zühlke, M. Spiteller, M. Krauss, M. Kaupenjohann, W. Amelung, Environ. Sci. Technol. 43 (6) (2009) 1824–1830.
[90] L. Sabourin, P. Duenk, S. Bonte-Gelok, M. Payne, D.R. Lapen, E. Topp, Sci. Total Environ. 431 (2012) 233–236.
[91] H. Dolliver, K. Kumar, S. Gupta, J. Environ. Qual. 36 (4) (2007) 1224–1230.
[92] L. Migliore, G. Brambilla, S. Cozzolino, L. Gaudio, Agric. Ecosyst. Environ. 52 (2–3) (1995) 103–110.
[93] K. Kumar, S.C. Gupta, S.K. Baidoo, Y. Chander, C.J. Rosen, J. Environ. Qual. 34 (6) (2005) 2082–2085.
[94] C. Wu, A.L. Spongberg, J.D. Witter, M. Fang, K.P. Czajkowski, Environ. Sci. Technol. 44 (16) (2010) 6157–6161.
[95] P.K. Jjemba, Agric. Ecosyst. Environ. 93 (1/3) (2002) 267–278.

Chapter 16

Methods for Elucidation of Transformation Pathways: Identification of Intermediate Products, Chiral, and Isotope-Ratio Mass Spectrometry Analysis

Bozo Zonja*, Jaume Aceña*, Sandra Pérez* and Damià Barceló*,†

*Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

†Catalan Institute for Water Research (ICRA), Girona, Spain

Chapter Outline

Comprehensive Analytical Chemistry, Vol. 62. http://dx.doi.org/10.1016/B978-0-444-62657-8.00016-1

1 INTRODUCTION

The main concern regarding the occurrence of pharmaceuticals in the environment, originating from treatment of humans, animal farming, and aquaculture, resides in their inherent pharmacological activity and their continuous discharge into the aquatic environment [1,2]. Currently, there is public and scientific concern about the presence of drug residues in the environment; however, less attention has been paid to their fate, behavior, degradation, and even their transformation [3,4]. With respect to the transformation processes of drugs, metabolism in the human body commonly produces one or more metabolites that, for the sake of excretability, exhibit higher polarity than the parent compound. Depending on the pharmaceutical, metabolites can make up a large fraction in the mass balance of an administered drug upon excretion, and on the other hand, some drugs are for less amenable to metabolic clearance but are excreted unchanged [5,6]. Then, human pharmaceuticals enter wastewater treatment plants (WWTPs) where biodegradation processes can bring about mineralization or more frequently partial degradation, leading to structurally related compounds referred to as transformation products (TPs) [7]. Once in the aquatic environment, drugs, their metabolites, and their TPs formed in the WWTPs can be transported and distributed in rivers and streams and be subject to further degradation by both abiotic and biotic processes. Various processes may be involved in the degradation of pharmaceuticals, promoting a complex mixture of parent compounds and TPs [8,9]. Abiotic transformation processes such as direct and indirect photolyses are of great importance in describing the aquatic fate of pharmaceuticals [10]. Photolytic reactions are often complex pathways and lead to a multitude of reaction products. They can play a more prominent role than biodegradation for the elimination of those pharmaceuticals in the environment that have survived the biological treatment in WWTP. In order to evaluate the degradability of pharmaceuticals through these different processes, several approaches have been used such as quantification of TPs in environmental samples, determination of the change of the enantiomeric fraction, and evaluation of the change in the isotopic ratio. A few years ago, some researchers started applying the first approach including the identification and quantification of TPs for the assessment of the degradability of pharmaceuticals [11]. In contrast, the determination of changes in the enantiomeric fraction taking place during the biodegradation of pharmaceuticals is still in its infancy [7]. This approach takes advantage of the fact that the enantiomers of racemic drugs interact differently with metabolizing enzymes. Preferred biological conversion of one enantiomer results in enrichment of the other enantiomer. Employing suitable chiral separation techniques eventually allows determining the shift in the enantiomeric ratio [7]. The third approach that relies upon the evaluation of the change in the isotopic fraction has been used rarely.

This chapter describes in detail the three approaches for the evaluation of the degradability of pharmaceuticals in the aquatic environment. It also reviews the analytical techniques that are available for their successful application.

2 ADVANCES IN SEPARATION AND DETECTION TECHNIQUES FOR EVALUATION OF THE DEGRADABILITY OF PHARMACEUTICAL COMPOUNDS

2.1 New Liquid Chromatography Separation Modes

Apart from the advances in mass spectrometry (MS) techniques that have had a significant impact on the field of pharmaceuticals in environmental studies, new columns for chromatography also have made a significant contribution. Pharmaceutical compounds, being more polar and less volatile, are typically better retained on reversed-phase (RP) columns. However, some modifications in the stationary phase of the column made possible for different range of compounds to be better retained than in C18 columns. Other advances in chromatography columns are monolithic columns, which can be a very good alternative to particle-packed columns in terms of separation efficiency [12–14]. Since they typically have small-sized skeletons and wide through pores, much higher separation efficiency can be achieved than in the case of particle-packed columns at a similar pressure drop [15]. One advantage of these columns is that they can work at high flow rates (up to 10 mL/min) in conventional column lengths (4.6 mm ID) without generating high back pressures. [13]. On one hand, technological advances in sorbent materials gave better performance in terms of efficiency and fast liquid chromatography (LC) using RP columns packed with sub-2 μm particles [16]. These ultrahigh-pressure LC columns contribute to faster analysis in many standard applications. However, for research on TPs of pharmaceuticals, there is a chance that the TPs are too polar to be retained on RP columns. This issue can be solved using hydrophilic interaction chromatography (HILIC) columns. HILIC mode of separation relies on polar stationary phases (such as silica gel or aminopropyl HILIC columns) and aqueous–organic mobile phases rich in organic solvents (usually methanol, acetonitrile, or their mixtures) in which water is introduced to play the role of a stronger eluting solvent [13,17]. When more than 1% of water is used in the mobile phases, the layer of water adsorbed on the polar stationary phase is usually thick enough to allow for liquid–liquid partitioning between the bulk mobile phase and the adsorbed aqueous layer. HILIC retention is controlled by a combination of partition and other interactions such as ion exchange, H bonding, and dipole–dipole affecting the selectivity of the separation [18,19]. One advantage of this is that in HILIC mode, analytes elute in a reverse order as

compared to RP chromatography and ion-pairing agents needed for retention in RP columns are not necessary, thereby making it easier to couple to MS. On the other hand, high percentage of organic solvent (acetonitrile) enhances ionization and thus increases sensitivity [13]. HILIC proved to be efficient in the determination of the photolysis products of the antiviral zanamivir which was better retained than in RP-LC and it was possible to separate zanamivir from an isobaric TP [20].

2.1.1 Chiral Columns

Many chiral stationary phases (CSPs) have been developed, but only a few dominate the market (e.g., polymer-based, Pirkle type, protein-bonded, and macrocyclic-based) [7]. Although Pirkle-type CSPs are more selective and well characterized, polysaccharide derivatives (one of the polymer-based classes) are currently the most popular chiral selectors for enantioseparation of various compounds due to their versatility, durability, and loading capacity [21]. They are effective under not only normal-phase conditions but also RP conditions using the appropriate mobile phases [22]. Protein-bonded CSPs have become popular due to the character of the chiral selector that can be changed by a simple modification in the mobile-phase composition (e.g., the nature and the concentration of uncharged modifier or pH), allowing a wide range of enantiomers to be separated [23,24]. However, they are not very efficient and generally give broad sample peaks with fewer than 3500 theoretical plates [25]. The more common protein-bonded phases include bovine serum albumin, human serum albumin, α-1-acid glycoprotein, ovomucoid, and α-chymotrypsin. The macrocyclic-based CSPs (primarily vancomycin, teicoplanin, and ristocetin A) are commonly used for chiral separations in HPLC [7]. Whereas the macrocyclic glycopeptide and aromatic-derivatized cyclodextrins are highly effective in the normal-phase mode, some linear derivatized carbohydrate CSPs have been conditioned to work in RP mode. Most of the chiral recognition elements incorporated into the CSPs are nontarget specific in nature, making reliable prediction of the separability and order of elution of a pair of enantiomers unfeasible. Molecular imprinted polymers (MIPs) offer the opportunity to modify CSPs with predefined chiral recognition properties by using the analytes of interest as binding site-forming templates [26]. However, the chromatographic use of MIP-type CSPs has been hampered by the difficulties associated with engineering suitable chromatographic formats and the inherent mass-transfer characteristics of imprinted polymers. The field of chiral chromatography is constantly developing with truly new technologies entering the market to bring solutions for enantiomeric separation [27]. However, the use of nanotechnology to structure chiral cavities has disappeared [27] but the use of zirconia as a versatile substrate for CSP development has emerged [28].

2.2 MS Analyzers

Considering these different approaches (identification of TPs and enantiomeric/isotope ratio (IR) changes), advances of MS analyzers have considerably helped in the elucidation of TPs, whereas the chromatographic column advances enabled separating enantiomers.

One of the major advantages of using MS for identification of TPs is the complementary natures of the available mass analyzers (ion trap (IT), time of flight (TOF), orbitrap, and the hybrids quadrupole linear IT (QLIT) and quadrupole time of flight (QTOF)); each of them is characterized by resolution (resolving power), mass accuracy/precision, mass range, sensitivity, selectivity, linear/dynamic range, acquisition modes, acquisition speed, size, and price [29].

Good starting points in the elucidation of unknown structures have been the ITMS instruments. ITMS is a simple analyzer that uses three electrodes to trap ions in a small volume. Its relatively low cost and the possibility to trap and accumulate ions (hence increase signal-to-noise ratio) are advantageous. But, what really made it useful is its MS^n capability, which is particularly attractive for structural elucidation since it gives the opportunity to propose fragmentation pathways. The latter are important since in many cases, an MS^2 (e.g., produced from a triple quadrupole (QqQ)) does not give a straightforward answer. However, small trapping volume, limited capacity for ion storage, and overfilling of the IT result in deterioration in the mass spectrum and loss of the dynamic response range due to space charging. This gave rise to linear IT instruments (QLIT (QTRAP®)), which have a larger ion storage capacity and a higher trapping efficiency [29,30].

Further improvements in selectivity were achieved by the use of high-resolution mass analyzers including TOF and Orbitrap-MS. TOF instruments measure the m/z-dependent time it takes for ions of different mass-to-charge ratios to move from the entrance of the analyzer, where they have been orthogonally accelerated in a pulsed fashion, to the detector [31]. Even more powerful in terms of confirmatory analysis are hybrid QTOF–MS systems that allow MS^2 experiments to be performed to provide fragmentation patterns together with accurate mass measurements of product ions [29].

An alternative to TOF analyzers is the Orbitrap analyzer, which is currently in five different configurations. In terms of hardware scheme, the two more simple models Exactive and Q Exactive are comparable to TOF and QTOF (without and with the quadrupole mass filter, respectively). Both Exactives include as key component a collision cell and an Orbitrap mass analyzer. While Q Exactive is limited to MS^2, other models are hybrid instruments with the LTQ IT as first mass analyzer offer MS^n capabilities. All orbitraps provide outstanding mass accuracy, mass resolution, and reliable high sensitivity.

Fourier transform ion cyclotron resonance MS is also a type of high-resolution mass analyzer for determining the mass-to-charge ratio (m/z) of ions based on the cyclotron frequency of the ions in a fixed magnetic field. It has one of the most sensitive ion detection methods with resolving power more than 750,000 full width at half maximum and mass accuracy of <1 ppm [32]; however, due to its cost, it is not currently used for the identification of TPs in environmental matrices.

2.3 Isotope Ratio Mass Spectrometry

Taking into account that pharmaceuticals are typically moderately polar to polar compounds and that only water can be used as eluant in LC–IRMS, it comes as no surprise that little work has been made to this field. Granted, more research has been made replacing liquid with gas chromatography with the required intermedium step of derivatization. When considering modifying the structure of a molecule (in many cases addition of protecting groups), there are several issues to have to be kept in mind like introducing another isotope source that has to be controlled and taking into consideration the possible dilution effect. Derivatization reactions should also be monitored to be complete in order to avoid kinetic isotope fractionation in the structure of the target analyte. Some of the reactions used for derivatization are [33] (a) silylation, trialkylsilyl protection groups applicable to many functional target groups (–OH, $–NH_2$, and –COOH); (b) acetylation, suitable for derivatization of hydroxyl and amino groups; and (c) methylation, only one extraneous carbon atom is introduced.

As already mentioned, the alternative to GC (with laborious and challenging derivatization before analysis) is LC–IRMS, with the first commercially available instrument launched in 2004. In LC–IRMS, following the chromatographic separation, target analytes are converted to carbon dioxide by wet chemical combustion with concentrated sodium peroxodisulfate ($Na_2S_2O_8$) in the presence of phosphoric acid [33–35]. The acidification supports the formation of CO_2 and the high ionic strength enhances transfer of the CO_2 into the gas phase, which occurs through an exchange membrane into a helium counterflow leading to the IRMS [33,36]. To date, only carbon isotopes can be measured in LC–IRMS. Therefore, the eluant cannot be an organic solvent nor can contain organic modifiers since the CO_2 peak corresponding to the target analyte would be masked by the baseline noise. This practically leaves only water to be used as eluant (together with inorganic modifiers). This is an advantage over other MS-based LC where most inorganic modifiers and some organic cannot be used. In contrast to classical gradient elution in LC–MS that is achieved mixing different proportions of water–organic solvent, separation in LC–IRMS is done with water temperature gradient. The problem with this combination eluant and gradient

is column packaging, which can lead to column bleed. However, it was reported that column bleed had no effect on the precision and accuracy of $\partial^{13}C$ values [37].

3 DETERMINATION OF TPs IN THE AQUATIC ENVIRONMENT FOR EVALUATION OF THE DEGRADABILITY

3.1 Generation, Identification of TPs, and Its Subsequent Detection in the Environment

The typical scheme for the present approach is the generation of the TP in laboratory settings, followed by identification of the TP with an array of analytical techniques and ultimately the detection of the TPs in the aquatic environment. In order to generate the TPs for further identification, different approaches are used usually including medium to high concentrations of the parent compound, different native or simulated environmental matrices, and, most importantly, various systems employing well-defined experimental conditions [29]. Several detection techniques can be employed for the identification of the TPs; however, MS is the workhorse for that purpose because when coupled to chromatographic systems, it allows for separation of the compounds of interest and provides valuable structural information, and with the advances in software packages, the instrument does not require expert knowledge. Using this technique, the interpretation of mass spectra of the parent compound and the resulting TPs enables in many cases to propose plausible chemical structures of the TPs. For this purpose, the first step is to determine the molecular mass of the compound from the mass spectrum, followed by comparison of the fragmentation patterns of the parent compound and the TP acquiring their MS^2 and MS^n spectra; this helps to identify functional groups by loss of neutral molecules present in the TP molecule and to predict which parts of the molecule underwent modification in the degradation of the parent compound and which parts remained unchanged [11]. Other complementary approaches can be used including derivatization of functional groups, H/D exchange experiments, or even increasing the separation in the chromatograph or mass spectrometer with enhanced resolution. For instance, accurate mass measurements in instruments such as QTOF–MS and Orbitrap-MS have been proved to be valuable for an unambiguous identification of postulated structures of TPs providing high confidence in assigning elemental compositions of molecular ions and fragment ions [38].

Once the TPs are identified, their detection in environmental samples along with the parent compound can help to assess the degradation of pharmaceuticals. To this end, there is a need for developing sensitive and selective analytical methods including the TPs expected to be present at low concentrations in the environment. One of the main limitations for including TPs in

environmental monitoring is the high price of standards, if available at all. One alternative to obtain reference compounds is via bio/chemical synthesis. For instance, Pérez and Barceló [52] biosynthesized the 4-hydroxy metabolite of diclofenac (DCF) and aceclofenac with recombinant human CYP450 2C9, for their further analysis in samples from WWTPs. In a subsequent work, the same authors Osorio et al. [46] developed a new method based on solid-phase extraction and QLIT–MS for the detection of the two hydroxyl metabolites and two proposed TPs (nitro-DCF and nitroso-DCF) of DCF (identified in reactors amended with mixed liquors from WWTP). While nitroso-DCF was absent in influent samples, it was detected in all 30 corresponding effluent samples, proving that DCF was converted to this TP in the WWTPs (Table 1). In lab studies, evaluating the degradability of triclosan in WWTP, Lindström et al. [51] identified a biotransformation product of triclosan termed methyl triclosan (5-chloro-2-(2,4-dichlorophenoxy)anisole). They developed a method using GC–MS and detected the methylated compound along with triclosan in samples from WWTPs, in lakes, and in river water from Switzerland, showing that microbial degradation occurred in the aquatic environment [51,53]. The two compounds were emitted into surface waters and they were also detected using GC–MS in whitefish (*Coregonus* sp.) and roach (*Rutilus rutilus*) at levels of up to 35 ng/g on a wet weight basis and up to 365 ng/g on a lipid basis [54].

Other studies are dealing with microbial degradation in other types of matrices like soil. Schulz et al. [47] identified the chemical structures of 12 TPs of the iodinated contrast medium iopromide with LC–QLIT–MS in water/soil batch experiments; four of them had already been described in a previous publication where the identification of four TPs was performed in a batch reactor amended with mixed liquor from WWTP using IT–MS [52]. In order to have the standards of the TPs, Schultz et al. generated them under laboratory conditions and then isolated them via semipreparative HPLC. All proposed TPs were detected in municipal effluents from German WWTPs with LC–QLIT–MS at maximum concentrations up to 3.7 ± 0.9 μg/L for TP 819 (Table 1). The microbial degradation of sulfamethoxazole (SMX) in groundwater samples was evaluated under denitrifying conditions, which can produce nitrogen species such as nitric oxide and nitrite [50]. The authors identified two TPs of SMX with MS and nuclear magnetic resonance. Once the standards of the two TPs, desamino-SMX and 4-nitro-SMX, had been chemically synthesized, a method for their analysis in groundwater samples was developed. In this study, they found concentration levels of SMX and its TPs in the nanogram/liter range, which indicated that degradation of SMX was occurring under nitrate-reducing conditions (Table 1).

Apart from microbial degradation, phototransformation of pharmaceuticals in water matrices has been reported. In order to evaluate the photodegradability of the antiviral oseltamivir and its human metabolite oseltamivir ester,

TABLE 1 Detection of Environmental Transformation Products in Environmental Samples

Compound	No. of TP Quantified	Process	Where Are Detected?	MS–MS/HRMS	References
Acetaminophen	1	Biotransformation	Wastewater	LC–QTOF–MS/MS	[39]
Amoxicillin	1	Biotransformation	Surface water, wastewater	LC–QTOF–MS/MS	[40]
	1	Biotransformation	Wastewater	LC–QTOF–MS/MS	[39]
Azithromycin	1	Biotransformation	Wastewater	LC–QTOF–MS/MS	[39]
Carbamazepine	4	Biotransformation	Surface water	LC–QqQ-MS/MS	[16]
	1	Biotransformation	Surface water	LC–LTQ-Orbitrap	[41]
	1	Biotransformation	Surface water, wastewater	LC–QqQ-MS/MS	[42]
	1	Biotransformation	Surface water, wastewater	LC–QqQLIT–MS/MS	[43]
	2	Biotransformation	Wastewater	LC–QTOF–MS/MS	[39]
	3	Biotransformation	Wastewater	LC–Q-MS/MS	[44]
Diazepam	2	Biotransformation	Surface water	LC–QqQ-MS/MS	[16]
Diazinon	1	Biotransformation	Wastewater	LC–QTOF–MS/MS	[39]
Diclofenac	1	Biotransformation	Surface water	LC–QqQ-MS/MS	[16]
	2	Biotransformation	Surface water, wastewater	LC–QqQ-MS/MS	[45]
	4	Biotransformation	Wastewater	LC–QTRAP-MS/MS	[46]
Enalapril	1	Biotransformation	Surface water	LC–QqQ-MS/MS	[16]

Continued

TABLE 1 Detection of Environmental Transformation Products in Environmental Samples—Cont'd

Compound	No. of TP Quantified	Process	Where Are Detected?	MS–MS/HRMS	References
Erythromycin	1	Biotransformation	Surface water	LC–QqQ-MS/MS	[16]
	2	Biotransformation	Wastewater	LC–QTOF–MS/MS	[39]
Iodinated contrast media	6	Phototransformation	Surface water, wastewater, drinking water	LC–QTRAP-MS/MS	[29,59]
	12	Biotransformation	Wastewater	LC–QTRAP-MS/MS	[47]
Oseltamivir	2	Phototransformation	Surface water	LC–QqQ-MS/MS	[48]
Penicillin G	5	Biotransformation	River water, wastewater	LC–Q-MS/MS	[49]
Ranitidine	1	Biotransformation	Surface water	LC–QqQ-MS/MS	[16]
Sulfamethoxazole	2	Biotransformation	Spring water samples	LC–QqQ-MS/MS	[50]
	1	Biotransformation	Surface water	LC–QqQ-MS/MS	[16]
Triclosan	1	Biotransformation	Surface water, wastewater	GC–MS (magnetic sector)	[51]

Gonçalves et al. [48] exposed the two test compounds to simulated solar radiation in a Suntest apparatus. They identified five photoproducts in the aqueous solutions and then developed an analytical method for the quantification of oseltamivir, its ester metabolite, and their TPs in surface water samples. With the developed method, oseltamivir and two photoproducts, referred to as TP330 and TP312, were detected at low nanogram/liter concentration ranges in samples from the Ebro river (Northeast Spain), showing that photodegradation was one of the processes involved in the degradation of oseltamivir in surface waters (Table 1).

3.2 Suspect Analysis

The approach applied in the aforementioned studies is time-consuming: once all TPs are identified in controlled laboratory experiments with advanced MS, they have to be chemically or biologically synthesized or separated by semi-preparative HPLC, and sometimes these studies are limited to one or very few selected compounds. Then, a new analytical method based on MS has to be developed and applied to the analysis of the pharmaceutical and its TPs in environmental samples. However, suspect analysis with high-resolution MS (HRMS) techniques can provide a more comprehensive picture of the overall contamination of samples with pharmaceuticals and their TPs using sensitive detection. The key toward successful suspect analysis resides on the one hand in the application of HRMS for unequivocal determination of ion masses and isotope envelopes, while on the other hand, automated data mining of the full-scan MS datasets, including comparison with compound databases or spectral libraries, is essential for delivering meaningful results in a timely manner. For instance, Kern et al. [55] generated a list of TPs based on computer-aided prediction of microbial metabolism and also incorporated TPs reported in the scientific literature. The search for the TPs including pharmaceuticals and other relevant environmental compounds was based on extracting the accurate mass from the total ion chromatogram with a very narrow extraction window, using the compound database. Checking the plausibility of the retention time and interpretation of mass spectra help to evaluate the tentative detection of the target analyte. Without standards of TPs, they were able to detect 19 TPs to be present in the seven Swiss surface waters investigated. Gómez-Ramos et al. [39] developed a method for the analysis of TPs of organic contaminants in wastewater samples. The approach comprised automatic screening with a database containing accurate mass and fragmentation pathways, identification of possible TPs, and confirmation by MS/MS analysis. In this study, eight TPs of pharmaceuticals were detected and identified and three of them were quantified by analytical standards. The approach using HRMS is faster than the approach proposed in this section and ultimate confirmation and quantification with authentic standards (Figure 1).

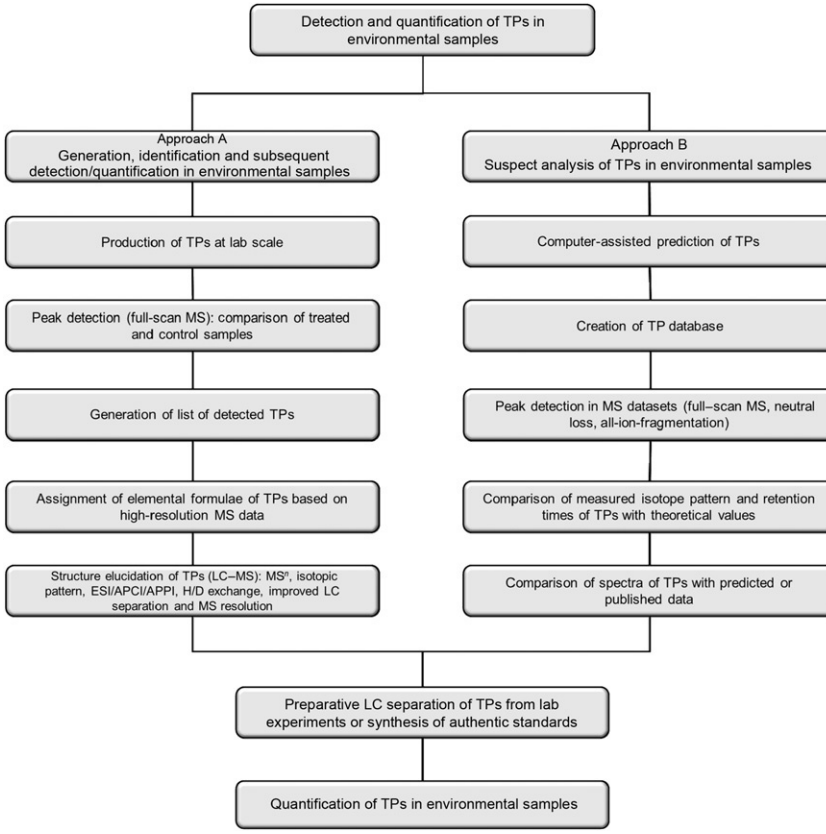

FIGURE 1 Comparison of the two approximations for detection and quantification of TPs in environmental samples.

4 CHANGES IN THE ENANTIOMERIC FRACTION

4.1 Chiral Separations

In recent years, the field of chiral separation has developed mainly following the development of different chromatographic columns that can be used for the chiral analysis. On one hand, chiral methodologies are majorly used and investigated in for pharmacokinetic studies in biological matrices. On the other hand, pesticides are preferred target compounds for reasons, among others, of high abundancy in the environment. Chiral separations of pharmaceuticals in environmental samples, though scarce, do exist. However, studies concerning evaluation of degradability through changes in the enantiomeric fraction are even harder to find.

The first method developed for the enantiomeric separation of several illicit drugs [56] was analysis of structurally related amphetamines (amphetamine, methamphetamine, 4-methylenedioxymethamphetamine (MDMA),

3,4-methylenedioxyamphetamine (MDA), and 3,4-methylenedioxy-*N*-ethylamphetamine(MDEA)), ephedrines (ephedrine, pseudoephedrine, and norephedrine), and venlafaxine in wastewater by means of chiral chromatography coupled with tandem MS. In this study, analytes were extracted with Oasis HLB, which resulted in very good recoveries accounting for >70%. CHIRALPAK® CBH, the column used, has a cellobiohydrolase as chiral selector immobilized on spherical 5 μ silica particles [56]. The column is used in RP mode. Retention and enantioselectivity are regulated by changes of pH, buffer concentration, and organic modifier. Resolution of enantiomers of chiral drugs tested was higher than 1. The method quantification limits in wastewater samples were at low ppt levels and varied from 2.25 to 11.75 ng/L. Finally, the method was successfully applied for the analysis of raw and treated wastewater samples collected from four different WWTPs. What they found was that common occurrence of 1R,2S (−)-ephedrine, 1S,2S (+)-pseudoephedrine, and venlafaxine in both raw and treated wastewater samples. Amphetamine, methamphetamine, MDMA, and MDEA were also detected in several wastewater samples. The influence of wastewater treatment processes on the enantiomeric composition of chiral drugs was also noted and it was hypothesized that enantioselective processes occurred during treatment, although no further studies were conducted. This was thoroughly studied in more detail when the study was done in seven wastewater treatment plants (WWTP) over a period of nine months to address seasonal variance [57]. The target compounds were amphetamine, methamphetamine, ephedrine, pseudoephedrine, MDA, MDMA, and venlafaxine and the study was extended to atenolol. A chiral-CBH column was used for the chiral separation. The results showed that the extent of stereoselectivity depended on several parameters like type of chiral drug (high stereoselectivity was recorded for atenolol and MDMA), treatment technology used (activated sludge showed higher stereoselectivity than trickling filters), and season (higher stereoselectivity was observed in the aqueous environment over the spring/summer time). Further, study was extending to river water samples and using high-resolution QTOF–MS and included amphetamine, methamphetamine, MDA, MDMA, venlafaxine, fluoxetine, atenolol, metoprolol, and propranolol [58]. The method developed was applied to environmental matrices (sewage and river) testing two different columns, the previously used CBH and Chirobiotic V. Although the authors stated that the advantage of using this high-resolution method LC–MS resides in the possibility to be used to monitor breakdown products and be used for nontarget screening in conjunction with routine targeted quantification, unfortunately, they did not extend the study to do it.

One example provided by Barclay et al. [60] who developed an a LC–MS/MS method for the chiral separation of metoprolol, and two of its main metabolites, hydroxymetoprolol (α-OH-Met) and deaminated metoprolol (COOH-Met), in environmental water samples has been developed. The basic target compounds,

metoprolol and α-OH-Met, and the acidic metabolite (COOH-Met) were extracted from water samples by a SPE method employing Oasis HLB cartridges and tested on four different types of CSPs: Chiralcel OD-H, Chirobiotic V, Chiral AGP, and Chiral CBH. Since the chemistry of column is important and influences drastically the separation of enantiomers, a single column was not able to accommodate the separation of all isomers so that the enantiomers of metoprolol and the four stereoisomers of α-OH-Met were separated using Chiral CBH, while the enantiomers of COOH-Met were separated employing Chiral AGP. The method was finally applied for the chiral analysis of the analytes in real treated wastewater. Although the enantiomers and diastereoisomers of α-OH-Met were detected and analyzed in the samples, unfortunately, the authors did not go into depth to explain why they detected changes in the enantiomeric fraction.

4.2 Examples of Evaluation of Degradability

In order to assess the importance of in-stream attenuation of pharmaceuticals in the rivers, Fono et al. [61] compared the decreasing concentrations of four pharmaceuticals (gemfibrozil, ibuprofen (IBP), metoprolol, and naproxen) but addressed only for metoprolol a change of the enantiomeric fraction (EF, $EF = E1/(E1 + E2)$) [61]. The concentration of the four analytes was determined prior to derivatization with GC–MS/MS and decreased by 75–90% as the water traveled downstream. Metoprolol, which is used as racemic drug, was employed as a tracer for the assessment of biodegradation. In the previous studies assessing the changes of EF of metoprolol in the WWTP, MacLeod et al. [62] showed that no shift in the EF was observed through the WWTP. The EF for metoprolol was determined in the river and it decreased with distance downstream from 0.44 at the first sampling point to 0.31 at the last sampling point. As a result, the change in the EF had been brought by abiotic processes should not affect the EF of metoprolol. Therefore, the changes in the EF of metoprolol gave strong evidence that biodegradation occurred in the river [61].

On the other hand, MacLeod et al. [62] reported that raw sewage was found to be enriched with R(−)-fluoxetine, but after treatment, the enantiomeric ratio of fluoxetine's enantiomers changed and led to an enrichment of the S(−)-enantiomer, which is more potent and toxic to certain organisms.

A slightly different approach was used in a study by Borges et al. [63] where they tried to evaluate if some of the strains of endophytic fungi were able to biotransform the chiral drug IBP into its metabolites—2-hydroxyibuprofen (2-OHIBP) and carboxyibuprofen (COOH-IBP). Separation of IBP and the stereoisomers of its main metabolites was achieved by the use of a CHIRALPAK AS-H column and the mobile-phase hexane–isopropanol–trifluoroacetic acid (95:5:0.1, v/v). Among the six fungi studied, only the strains *Nigrospora sphaerica* (SS67) and *Chaetomium globosum* (VR10) biotransformed IBP

enantioselectively, with greater formation of the metabolite (+)-(S)-2-OH-IBP. Formation of the COOH-IBP stereoisomers, which involves hydroxylation at C3, and further oxidation to form the carboxyl group were not observed.

5 ISOTOPIC FRACTION OF PHARMACEUTICALS IN THE ENVIRONMENT

Information on pharmaceuticals' isotope patterns in general is very limited but the knowledge on how the pattern changes in the environment is practically non-existent. Some of the important issues regarding this will be discussed briefly in this section. Several studies dealt with pharmaceutical isotopic composition of tablets rather than active pharmaceutical ingredients (APIs). In these studies, the bulk isotopic effect of the tablet was investigated in order to determine if, for example, it would be possible to determine the difference between different tablets of different manufacturers [64]. On the other hand, some studies tried to see whether the pure API compounds of different manufacturers could have different isotopic ratio [65,66]. However, Kujawinski et al. [66] went one step further in their study of sulfonamide-containing pharmaceuticals by high-temperature LC–IRMS. In this study, they found that a temperature gradient in combination with phosphate buffer achieved baseline separation.

6 CONCLUSIONS

This book chapter highlights the need and benefit of the application of several approaches for the evaluation of the degradability of pharmaceuticals. The first approach focuses on the quantification of TPs in environmental samples; however, only few works dealt with this approach because they encompass long and tedious tasks. Some multiresidue analysis using MRM methods were applied for the (semi-)quantitative analysis of the TPs in environmental water samples. The main issue of these studies is that they are limited to one or very few selected compounds previously identified. Nowadays, HRMS techniques provide high-throughput and sensitive detection of TPs. New screening methods were developed using QTOF or Orbitrap-MS technologies and provided analysis of known, suspect, and unknown compounds. Although the HRMS allow for an unquestionable identification and detection of TPs (qualitative analysis) in real samples, authentic standards are still required for their accurate quantification. While chiral separation of pharmaceuticals has already reached a certain level of maturity and method development is, although time-consuming, achievable, the approach based on the determination of isotopic ratios is still in early stages and considerable efforts still have to be made before it can be considered a routine technique. Nonetheless, both approaches hold great promise and are expected to become increasingly employed in evaluating the degradation of pharmaceuticals.

ACKNOWLEDGMENTS

This work has been supported by the Spanish Ministry of Science and Innovation (project Consolider-Ingenio 2010 Scarce CSD2009-00065). Bozo Zonja acknowledges the Marie Curie Actions ITN CSI:Environment PITN-GA-2010-264329 for the early-stage researcher contract and funding. Sandra Perez acknowledges the contract from the Ramón y Cajal Program of the Spanish Ministry of Economy and Competitiveness. Prof. Damia 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). This work reflects only the authors' views and the European Community is not liable for any use that may be made of the information contained therein.

REFERENCES

[1] D. Barceló, Trends Anal. Chem. 26 (2007) 454.
[2] T.A. Ternes, Water Res. 32 (1998) 3245.
[3] H.F. Schröder, W. Gebhardt, M. Thevis, Anal. Bioanal. Chem. 398 (2010) 1207.
[4] D. Calamari, E. Zuccato, S. Castiglioni, R. Bagnati, R. Fanelli, Environ. Sci. Technol. 37 (2003) 1241.
[5] R.A. Upton, J.N. Buskin, R.L. Williams, N.H.G. Holford, S. Riegelman, J. Pharm. Sci. 69 (1980) 1254.
[6] B. Kasprzyk-Hordern, R.M. Dinsdale, A.J. Guwy, Water Res. 42 (2008) 3498.
[7] S. Pérez, D. Barceló, Trends Anal. Chem. 27 (2008) 836.
[8] M. Farré, S. Pérez, L. Kantiani, D. Barceló, Trends Anal. Chem. 27 (2008) 991.
[9] D.E. Helbling, J. Hollender, H.-P.E. Kohler, H. Singer, K. Fenner, Environ. Sci. Technol. 44 (2010) 6621.
[10] C. Tixier, H.P. Singer, S. Oellers, S.R. Müller, Environ. Sci. Technol. 37 (2003) 1061.
[11] S. Pérez, D. Barceló, Trends Anal. Chem. 26 (2007) 494.
[12] G. Guiochon, J. Chromatogr. A 1168 (2007) 101.
[13] O. Núñez, H. Gallart-Ayala, C.P.B. Martins, P. Lucci, J. Chromatogr. A 1228 (2012) 298.
[14] O. Núñez, K. Nakanishi, N. Tanaka, J. Chromatogr. A 1191 (2008) 231.
[15] B. Buszewski, T. Ligor, LC–GC Eur. 15 (2002) 92.
[16] R. López-Serna, M. Petrović, D. Barceló, J. Chromatogr. A 1252 (2012) 115.
[17] M. Petrovic, M. Farré, M.L. de Alda, S. Perez, C. Postigo, M. Köck, J. Radjenovic, M. Gros, D. Barcelo, J. Chromatogr. A 1217 (2010) 4004.
[18] P. Hemström, K. Irgum, J. Sep. Sci. 29 (2006) 1784.
[19] T. Ikegami, K. Tomomatsu, H. Takubo, K. Horie, N. Tanaka, J. Chromatogr. A 1184 (2008) 474.
[20] B. Zonja, C. Gonçalves, S. Pérez, A. Delgado, M. Petrovic, M.F. Alpendurada, D. Barceló, J. Hazard. Mater. (2013). http://dx.doi.org/10.1016/j.jhazmat.2013.10.008.
[21] J. Chen, W.A. Korfmacher, Y. Hsieh, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 820 (2005) 1.
[22] K. Tachibana, A. Ohnishi, J. Chromatogr. A 906 (2001) 127.
[23] J. Hermansson, A. Grahn, J. Chromatogr. A 694 (1995) 57.
[24] J.S. Munro, T.A. Walker, J. Chromatogr. A 913 (2001) 275.
[25] R.C. Williams, J.F. Edwards, M.J. Potter, J. Liq. Chromatogr. 16 (1993) 171.
[26] N.M. Maier, W. Lindner, Anal. Bioanal. Chem. 389 (2007) 377.

[27] T.E. Beesley, LC–GC North. Am. 24 (2006) 28.
[28] T.J. Ward, B.A. Baker, Anal. Chem. 80 (2008) 4363.
[29] B. Zonja, J. Aceña, A. Jelic, M. Petrovic, S. Pérez, D. Barceló, Wiley Books, accepted.
[30] S. Pérez, M. Petrovic, D. Barceló, Handb. Environ. Chem. 2 (2009) 43.
[31] S. Pérez, P. Eichhorn, D. Barceló, Anal. Chem. 79 (2007) 8293.
[32] M.W. Lam, C.J. Young, S.A. Mabury, Environ. Sci. Technol. 39 (2005) 513.
[33] M. Elsner, M.A. Jochmann, T.B. Hofstetter, D. Hunkeler, A. Bernstein, T.C. Schmidt, A. Schimmelmann, Anal. Bioanal. Chem. 403 (2012) 2471.
[34] J.P. Godin, J.S.O. McCullagh, Rapid Commun. Mass Spectrom. 25 (2011) 3019.
[35] R.U. Meckenstock, B. Morasch, R. Warthmann, B. Schink, E. Annweiler, W. Michaelis, H.H. Richnow, Environ. Microbiol. 1 (1999) 409.
[36] M. Krummen, A.W. Hilkert, D. Juchelka, A. Duhr, H.J. Schlüter, R. Pesch, Rapid Commun. Mass Spectrom. 18 (2004) 2260.
[37] L. Zhang, D.M. Kujawinski, M.A. Jochmann, T.C. Schmidt, Rapid Commun. Mass Spectrom. 25 (2011) 2971.
[38] P. Eichhorn, S. Pérez, D. Barceló, Compr. Anal. Chem. 58 (2012) 217.
[39] M.d.M. Gómez-Ramos, A. Pérez-Parada, J.F. García-Reyes, A.R. Fernández-Alba, A. Agüera, J. Chromatogr. A 1218 (2011) 8002.
[40] A. Pérez-Parada, A. Agüera, M.d.M. Gómez-Ramos, J.F. García-Reyes, H. Heinzen, A. R. Fernández-Alba, Rapid Commun. Mass Spectrom. 25 (2011) 731.
[41] C.M. de Jongh, P.J.F. Kooij, P. de Voogt, T.L. ter Laak, Sci. Total. Environ. 427–428 (2012) 70.
[42] K.H. Langford, K.V. Thomas, Environ. Int. 35 (2009) 766.
[43] M.J.M. Bueno, M.J. Gomez, S. Herrera, M.D. Hernando, A. Agüera, A.R. Fernández-Alba, Environ. Pollut. 164 (2012) 267.
[44] M. Leclercq, O. Mathieu, E. Gomez, C. Casellas, H. Fenet, D. Hillaire-Buys, Arch. Environ. Contam. Toxicol. 56 (2009) 408.
[45] K. Langford, K.V. Thomas, J. Environ. Monit. 13 (2011) 416.
[46] V. Osorio, M. Zonja, M. Imbert, J.L. Abad, S. Pérez, D. Barceló, J. Chromatogr. A (2013) (submitted).
[47] M. Schulz, D. Löffler, M. Wagner, T.A. Ternes, Environ. Sci. Technol. 42 (2008) 7207.
[48] C. Gonçalves, S. Pérez, V. Osorio, M. Petrovic, M.F. Alpendurada, D. Barceló, Environ. Sci. Technol. 45 (2011) 4307.
[49] D. Li, M. Yang, J. Hu, Y. Zhang, H. Chang, F. Jin, Water Res. 42 (2008) 307.
[50] K. Nödler, T. Licha, M. Barbieri, S. Pérez, Water Res. 46 (2012) 2131.
[51] A. Lindström, I.J. Buerge, T. Poiger, P.A. Bergqvist, M.D. Müller, H.R. Buser, Environ. Sci. Technol. 36 (2002) 2322.
[52] S. Pérez, D. Barceló, Anal. Chem. 80 (2008) 8135.
[53] D.C. McAvoy, B. Schatowitz, M. Jacob, A. Hauk, W.S. Eckhoff, Environ. Toxicol. Chem. 21 (2002) 1323.
[54] M.E. Balmer, T. Poiger, C. Droz, K. Romanin, P.A. Bergqvist, M.D. Müller, H.R. Buser, Environ. Sci. Technol. 38 (2004) 390.
[55] S. Kern, K. Fenner, H.P. Singer, R.P. Schwarzenbach, J. Hollender, Environ. Sci. Technol. 43 (2009) 7039.
[56] B. Kasprzyk-Hordern, V.V.R. Kondakal, D.R. Baker, J. Chromatogr. A 1217 (2010) 4575.
[57] B. Kasprzyk-Hordern, D.R. Baker, Environ. Sci. Technol. 46 (2012) 1681.
[58] J.P. Bagnall, S.E. Evans, M.T. Wort, A.T. Lubben, B. Kasprzyk-Hordern, J. Chromatogr. A 1249 (2012) 115.

[59] B. Zonja, S. Pérez, M. Petrovic, D. Barceló (2013) in preparation.
[60] V.K.H. Barclay, N.L. Tyrefors, I.M. Johansson, C.E. Pettersson, J. Chromatogr. A 1269 (2012) 208.
[61] L.J. Fono, E.P. Kolodziej, D.L. Sedlak, Environ. Sci. Technol. 40 (2006) 7257.
[62] S.L. MacLeod, P. Sudhir, C.S. Wong, J. Chromatogr. A 1170 (2007) 23.
[63] K.B. Borges, A.R.M. De Oliveira, T. Barth, V.A.P. Jabor, M.T. Pupo, P.S. Bonato, Anal. Bioanal. Chem. 399 (2011) 915.
[64] R. Santamaria-Fernandez, R. Hearn, J.C. Wolff, Sci. Justice 49 (2009) 102.
[65] J.P. Jasper, B.J. Westenberger, J.A. Spencer, L.F. Buhse, M. Nasr, J. Pharm. Biomed. Anal. 35 (2004) 21.
[66] A.M. Wokovich, J.A. Spencer, B.J. Westenberger, L.F. Buhse, J.P. Jasper, J. Pharm. Biomed. Anal. 38 (2005) 781.
[67] D.M. Kujawinski, L. Zhang, T.C. Schmidt, M.A. Jochmann, Anal. Chem. 84 (2012) 7656.

Chapter 17

Deciphering Emerging Toxicological Effects of Pharmaceuticals on Aquatic Organisms by Using *Daphnia magna* and *Danio rerio* as Model Organisms

Demetrio Raldua[*,‡], Bruno Campos[*], Carlos Barata[*], Benjamin Piña[*], Natàlia García-Reyero[†] and Patrick J. Babin[‡]

[*]*Department of Environmental Chemistry, Institute of Environmental Assessment and Water Research (IDAEA-CSIC), Barcelona, Spain*

[†]*Institute for Genomics, Biocomputing & Biotechnology, Mississippi State University, Starkville, Mississippi, USA*

[‡]*Maladies Rares: Génétique et Métabolisme (MRGM), University of Bordeaux, Talence, France*

Chapter Outline

Comprehensive Analytical Chemistry, Vol. 62. http://dx.doi.org/10.1016/B978-0-444-62657-8.00017-3

1 INTRODUCTION

Almost 500 years ago, Paracelsus wrote, "*Dosis facit venenum*" (the dose makes the poison). This relationship between dose and response (effect) is still one of the most fundamental concepts of toxicology. However, modern toxicology has supplemented Paracelsus' famous statement to read "*Dosis et tempus fiunt (faciunt) venenum*" (dose and time together make the poison) [1]. It serves as a good starting point for the discussion on pharmaceuticals as emerging pollutants in aquatic ecosystems. It reminds us that our goal should not be to achieve zero concentration in our water bodies, a situation that is neither feasible nor economically efficient, but to discuss and move toward acceptable quality standard for our water resources.

There is increasing evidence that the presence of pharmaceuticals in aquatic ecosystems may have detrimental effects on aquatic biota. Many efforts are currently underway to elucidate the impact of this group of chemicals of emerging concern (CECs) on wildlife using omics technologies coupled with physiological and morphological end points. These approaches are expected to help identify any potential health effects on aquatic organisms leading to a more accurate assessment of contaminant effects on aquatic health. The focus of this chapter is not to present a review on the ecotoxicity of pharmaceuticals, as there are many excellent recent reviews already available [2–6]. Here, we intend to present original and previously reported data on toxicological effects and mechanisms of action of different groups of pharmaceuticals on *Daphnia magna* and *Danio rerio*, two powerful model organisms for aquatic nonvertebrate and vertebrate species, respectively.

2 DECIPHERING ADVERSE EFFECTS OF PHARMACEUTICALS IN NONVERTEBRATE SPECIES

2.1 Introduction

In the last decade, substantial research has been conducted to evaluate the toxic effects of pharmaceuticals across nonvertebrate species. Most studies, however, are limited to conventional toxicity assays performed at high doses, and few of them have studied mechanism of action of pharmaceuticals. For example, from over 300 articles found in the database SCOPUS containing the word "pharmaceutical," "invertebrate or daphnia or chironomus or algae or worm," and "toxicity," only 62 articles studied effects at low

concentrations and 39 of them addressed modes of action. This means that we are still lacking information on the mechanism of toxic action of pharmaceuticals at environmentally relevant levels in nonvertebrate biota. Pharmaceuticals have been designed to act on specific targets in mammalian cells. However, both the mode of action and the physiological consequences of the exposure to pharmaceuticals in aquatic nonvertebrates species are largely unknown. Furthermore, the high specificity of pharmaceuticals to mammals precludes unexpected mechanisms of action to nonvertebrates that may or not may have measurable detrimental effects using conventional tests. An example of such effects is illustrated by Heckman et al. [7,8], who studied the effects ibuprofen on *Daphnia magna* reproduction. Ibuprofen had the same molecular initiating event in *Daphnia magna* than in humans interrupting eicosanoid metabolism but in daphnia eicosanoid metabolism appeared to disrupt signal transduction affecting juvenile hormone metabolism, oogenesis, and reproduction. Campos et al. [9] analyzed the effects of selective serotonin reuptake inhibitors (SSRIs) on *Daphnia magna* and reported that they acted in a similar way than in humans. The SSRI exposure increased serotonin activity, but in doing so, it altered oxygen consumption and reproduction increasing offspring production and aerobic metabolism. As a result and in an unexpected way, *Daphnia magna* females exposed to SSRIs increased their fitness performance in terms of reproduction but at the expense of being more sensitive to low oxygen levels. The latter detrimental effect, however, was only detected exposing individuals to low-oxygen conditions and SSRIs. In the amphipod *Echinogammarus marinus*, Guler and Fort [10] reported that the SSRI fluoxetine had a similar effect in acanthocephalan parasites, which are known to alter the swimming behavior in their amphipod hosts through changes in serotonergic activity resulting in increased predation. In zebra mussel, environmentally relevant concentrations of fluoxetine, in the range of nanogram/liter, induced spawning of spermatozoa and oocytes, an effect that is mediated by serotonin [11]. Thus, the aforementioned examples support the hypothesis that at environmentally realistic low environmental doses, pharmaceuticals may have the same molecular mode of action than in mammals but they may produce detrimental effects that are very difficult to predict using conventional assays.

Other important mechanism of action that pharmaceuticals may have on nonvertebrate organisms is the so-called chemosensitization [12,13]. This is the term used to describe the chemical inhibition of multidrug transmembrane transporters that allow cells to efflux out toxic chemicals and/or metabolites. Therefore chemosensitization may be an important mechanism to explain synergistic toxicity of chemical mixtures [12]. The multidrug resistance (MDR) system is conspicuous to all cells, and it was first described in cancer cells that became resistant to treatment with cytostatic drugs by having overexpressed levels of such transporters. The MDR system includes many transporters from the ATP-binding cassette (ABC) superfamily, being the P-gp (ABCB) and the multidrug resistance proteins (MRP and ABCC) having greater environmental

relevance [14]. The P-gp and MRP transporters have been well characterized in several nonvertebrate organisms such as mussels and sea urchins, and their inhibition by pollutants and pharmaceutical substances is well known [15–17]. There is, however, little evidence on the toxicological effects of chemosensitization [13]. There is therefore an urgent need to study the potential adverse effects of low doses of pharmaceuticals on nonvertebrate species and to design relevant assays to study the ecological consequences of such effects. One of the best-suited strategies is to use model ecotoxicological organisms to study potential modes of action of pharmaceuticals and their toxic or detrimental effects.

Here, we describe two case studies that analyze the effects of pharmaceuticals using the model ecotoxicogenomic organism *Daphnia magna*.

2.2 Case Study 1: Identification of Metabolic Pathways in *Daphnia magna* Exposed to SSRIs Using Transcriptional and Physiological Responses

Daphnia magna gravid females were exposed to the SSRIs fluoxetine (10 μg/L) and fluvoxamine (40 μg/L) for 10 days, and the effects on their transcriptome, reproduction, carbohydrates levels, and oxygen consumption rate were assessed. Details of the experimental setup, reproduction, and biochemical and respirometry assays are provided in Campos et al. [9]. Transcriptome studies were performed per quadruplicate using the mRNA of a single female per replicate and a custom *Daphnia magna* 15,000-probe array (GPL13761) purchased from Agilent (Palo Alto, CA, United States) [12]. Procedures for RNA extraction, microarray hybridization, and gene analysis are provided elsewhere [18]. In addition, we also include results obtained by using immunofluorescence on *Daphnia magna* brains with antiserotonin antibodies.

Hierarchical clustering analysis performed with the 1200 differentially expressed genes relative to control treatments (Figure 1; $p < 0.05$, ANOVA test) demonstrated clearly different profiles for SSRI-treated *Daphnia magna* and controls. A further analysis of the different clusters identified 250 unique up- and downregulated genes that had homology with known genes from *Drosophila melanogaster*. The functionality and location in the metabolic path of *Drosophila melanogaster* of the aforementioned genes were studied using several databases for functional enrichment: Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). Many of these genes belonged to the metabolism of nucleotides, serotonin, and lipids and to the Krebs cycle that regulates the oxidative metabolism of carbohydrates (Figure 2). We have also developed the methodology to analyze specifically the impact of SSRIs on the serotonergic neurons in the brain of *Daphnia magna* by performing whole-mount immunofluorescence antiserotonin on dissected brains, with Figure 3 showing the preliminary results in control animals. Biochemical and respirometry assays of *Daphnia magna* females exposed to the studied SSRIs indicated that exposure to these compounds increased oxygen

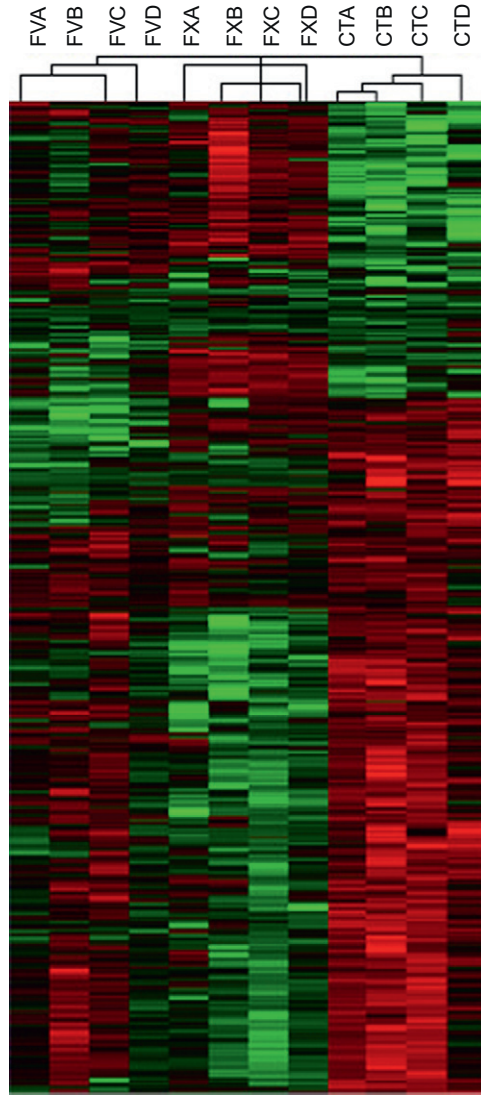

FIGURE 1 Hierarchical clustering of differentially expressed genes in adult *Daphnia magna* exposed to fluoxetine and fluvoxamine. Microarray analysis was performed on four replicate pools of adult *Daphnia magna* control (CTA–CTD) or those exposed to fluoxetine (FXA–FXD) or fluvoxamine (FVA–FVD). Each line corresponds to a gene. Upregulated and downregulated genes are depicted in red and green, respectively, and unchanged ones in black.

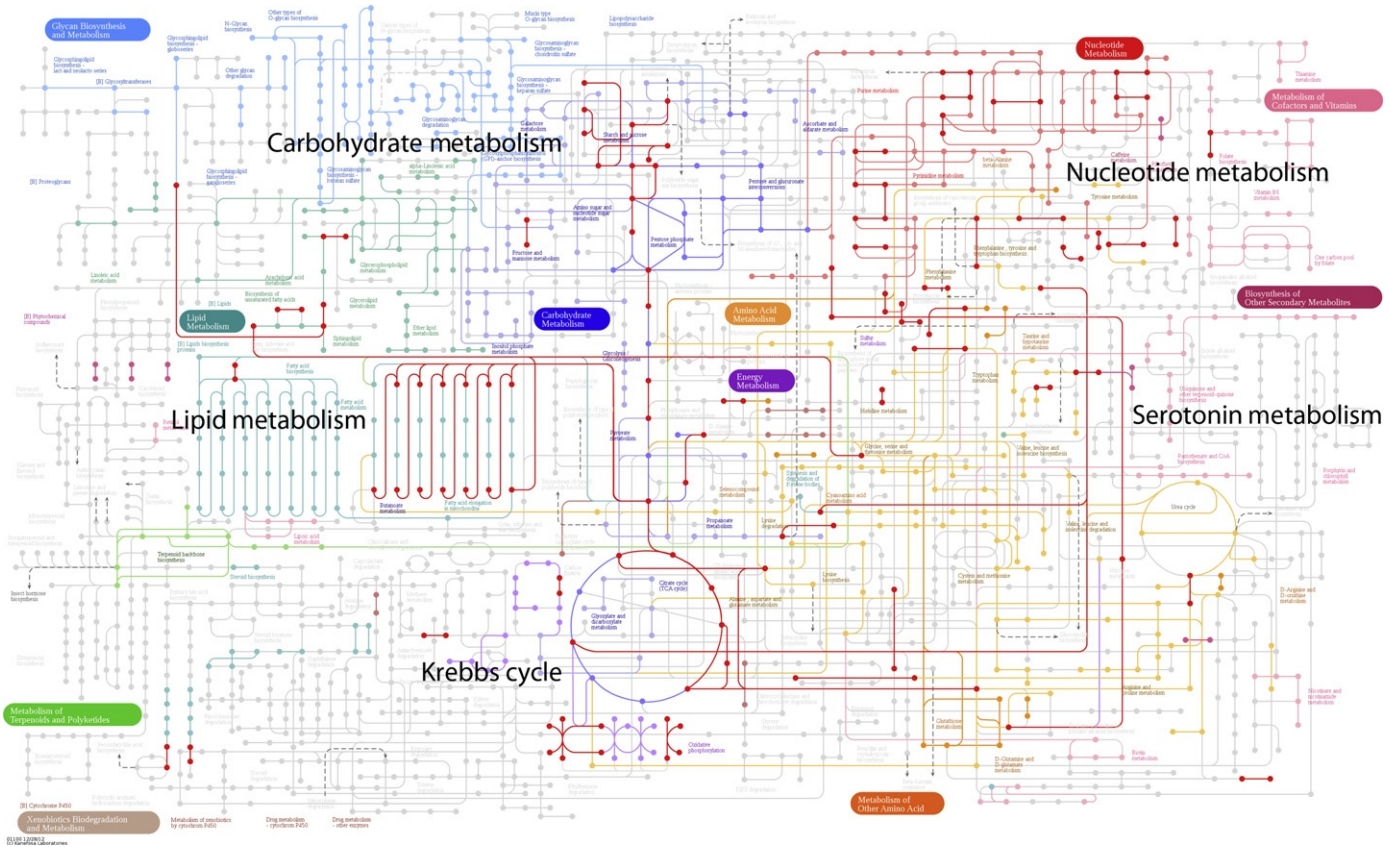

FIGURE 2 KEGG results showing the main metabolic pathways involved in the response to the SSRIs.

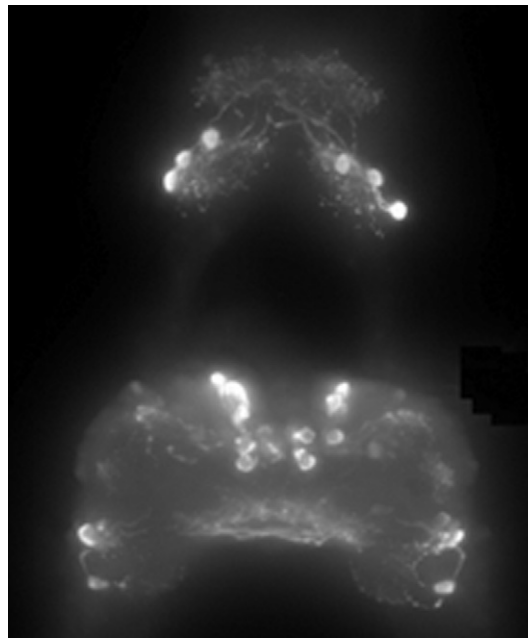

FIGURE 3 Fluorescent microscopy images of immunocytochemical assays performed on *Daphnia magna* dissected whole brains with antiserotonin antibody.

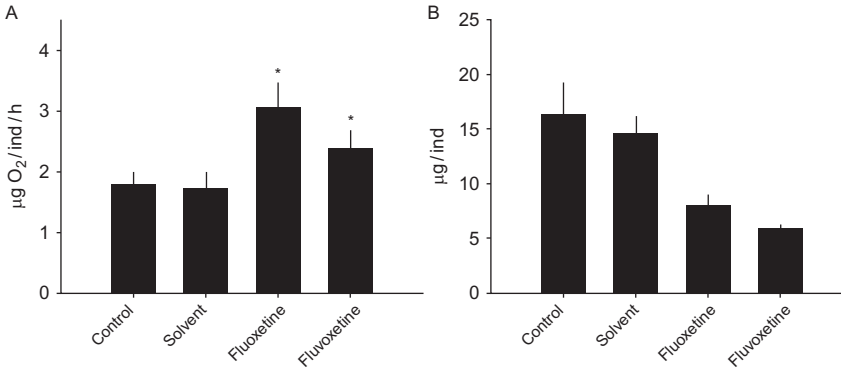

FIGURE 4 Oxygen consumption rates and carbohydrate levels in *Daphnia magna* females exposed to the selected SSRIs. Oxygen consumption rates (A) and carbohydrate levels (B) of adult females exposed to the selected SSRIs. Asterisks indicated significant ($p < 0.05$) differences from control treatments following ANOVA and Dunnett's tests.

consumption rates due to oxidative metabolism (Figure 4A) and the catabolism of carbohydrates decreasing their levels (Figure 4B). Reproduction and survival assays indicated that females exposed to SSRIs had increased number of offspring (Figure 5A) but had lower survival under anoxia (Figure 5B). These

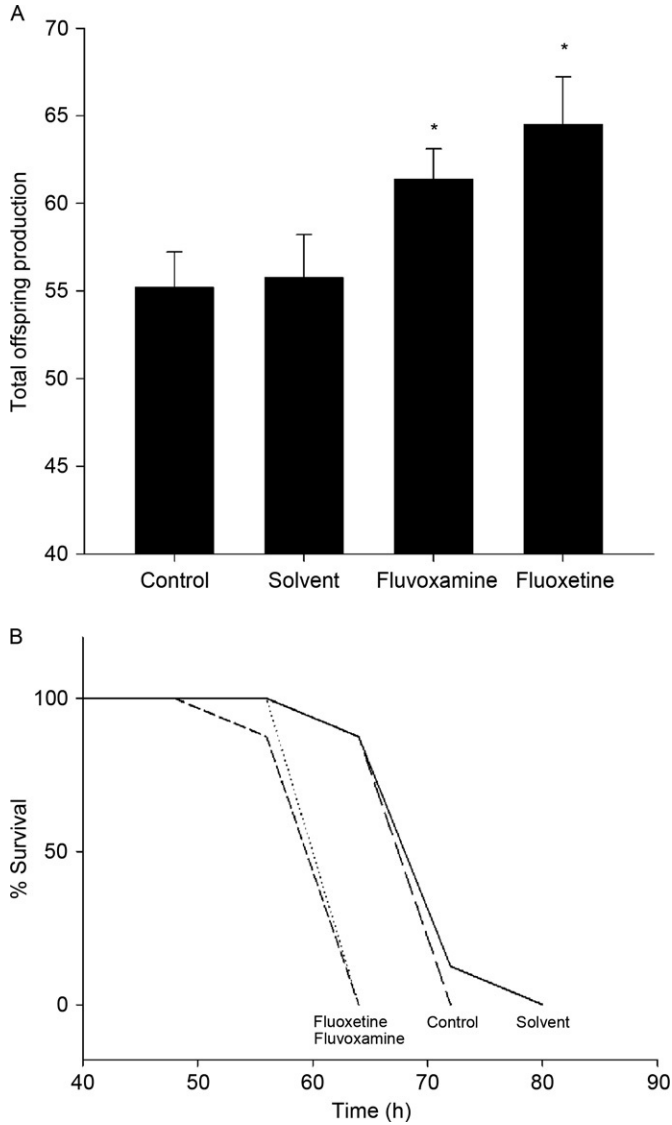

FIGURE 5 Total offspring and survivorship of *Daphnia magna* females exposed to SSRIs. Total offspring (A) and survivorship under anoxic conditions (B) of *Daphnia magna* females exposed to SSRIs in three consecutive clutches. Asterisks indicated significant ($p < 0.05$) differences from control treatments following ANOVA and Dunnett's tests.

results, thus, support the hypothesis that SSRIs disrupted serotonin activity deregulating carbohydrate and aerobic oxidative metabolism making animals to catalyze carbohydrate reserves more efficiently but at a cost of being more susceptible to anoxic conditions. The control of reproduction in *Daphnia magna* is poorly known, but in other crustaceans, serotonin and/or other bioamines

modulate neuropeptide hormones like the crustacean hyperglycemic hormone family that regulate terminal signaling hormones like ecdysone or juvenile hormones, which directly control molting and reproduction processes [19].

2.3 Case Study 2: Multidrug Resistance Mechanisms in *Daphnia magna* and Their Role in Tolerance to Single and Mixture Combinations of Toxicants

Studies of the cellular mechanisms of tolerance of organisms to pollution are a key issue in aquatic environmental risk assessment. The multidrug resistance (MDR) mechanisms based on the activity of ABC transporters represent an essential cellular defense of marine and freshwater organisms against environmental toxicants. Here, we report for the first time data on the expression of potentially MDR-related ABC transporters in the water flea *Daphnia magna*.

Using reported genomic sequences of *Daphnia pulex* [20], we cloned partial cDNAs of an *ABCB*1 ortholog (P-glycoprotein, P-gp) and of the MDR-associated protein (MRP) gene orthologs *ABCC1–3* like *ABCC4* and *ABCC5* and found constitutive expression of the respective transcripts in *Daphnia magna* eggs, embryos, neonates, and juveniles with quantitative real-time PCR (qPCR). The RNA extraction and qPCR methods are described elsewhere [21]. Putative efflux-transporting activity of MDR transporter was analyzed by using fluorescent dyes known to be specific substrates of P-gp and MRP pumps (rhodamine dyes for P-gp transporters and calcein (CA) for both P-gp and MRP), and specific mammalian inhibitors of these transporters were evaluated (reversin 205 for P-gp, MK571 for MRP, and cyclosporine A for both P-gp and MRP) [22]. Toxicity bioassays with cytotoxic drugs known to be substrates of ABCB1 (mitoxantrone) and ABCC (chlorambucil) applied singly and in combination with toxic and model chemosensitizers (reversin 203, MK571, cyclosporine A) were performed to elucidate the tolerance role of ABCB and ABCC efflux transporters. Toxicity responses were performed using standardized 48 h acute *Daphnia magna* test and modeled with the aid of the nonlinear Hill regression model. Binary mixtures were selected to include distinct equitoxic mixture ratios, whose constituents were dosed according to their LC50 less than 0.2 toxic units (TU), respectively, and different mixture effect levels. The design both allowed to account for potential interactions of toxic components across mixture ratios and effect levels and permitted confrontation of observed and predicted responses following the CA and IA conceptual models [23].

Binary mixture combinations followed a fixed ratio design, in which exposure levels were selected to include constant equitoxic (LC_{50}) mixture ratios and from 5 to 10 different mixture effect levels. Joint toxicity predicted according to the concentration addition and independent action model was conducted following established procedures [23].

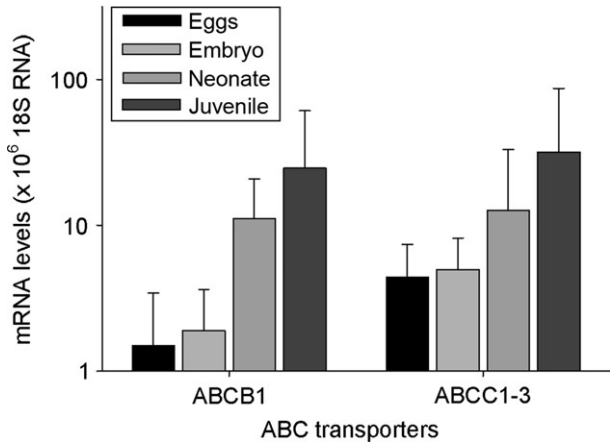

FIGURE 6 Time-course expression of ABCB1 and ABCC1–3 transcripts in *Daphnia magna*. Relative levels of putative ABCB1 and ABCC1–3 transporters depicted as relative copies of 18S ribosomal, mRNA abundance (mean ± SD, $n = 9$) in *Daphnia magna* eggs, embryos, neonates, and juveniles of 4 days.

Results reported in Figure 6 evidenced the existence of transcripts of ABC transporters in embryos and juvenile stages of *Daphnia magna*. In particular, those of ABCB1 and ABCC1–3 had low mRNA levels in eggs increasing in embryos and juveniles. Interestingly, ABCB1 and ABCC1–3 genes encode P-gp and MRP1–3 transporters that are directly involved in detoxifying pollutants [15]. This means that *Daphnia magna* juveniles expressed those transporters constitutively. Exposure of *Daphnia magna* individuals to fluorescent dyes that are also substrates of MDR transporters showed that the gut was the organ having greatest transporter activity (Figure 7A, C). Note that thoracic appendixes also had transporter activity although it was only evident in *ex vivo* assays performed with freshly killed organisms. By killing the organisms, we prevent the dye from being uptaken actively into the gut, and hence, it was possible to view secondary organs having MDR transporter activity. Functional transporter assays with rhodamine B and calcein (CA) (Figure 8) evidenced a concentration-dependent uptake of dye across the studied transporter inhibitors, which indicates that these type of pumps are active in *Daphnia magna* juveniles and able to efflux out the studies' dyes. Such behavior has also been observed in other organism such as mussels, sea urchins, and worms [13,24].

All the four tested compounds were acutely toxic in single exposures (Figure 9), and in all the four binary mixtures performed between the tested chemosensitizers and the MDR toxic substrates mitoxantrone and chlorambucil, joint toxicities were greater than expected by additivity (Figure 10). These results indicate that MDR transporter activity is present in *Daphnia magna* juveniles and that it plays a significant role in its tolerance to environmental contaminants.

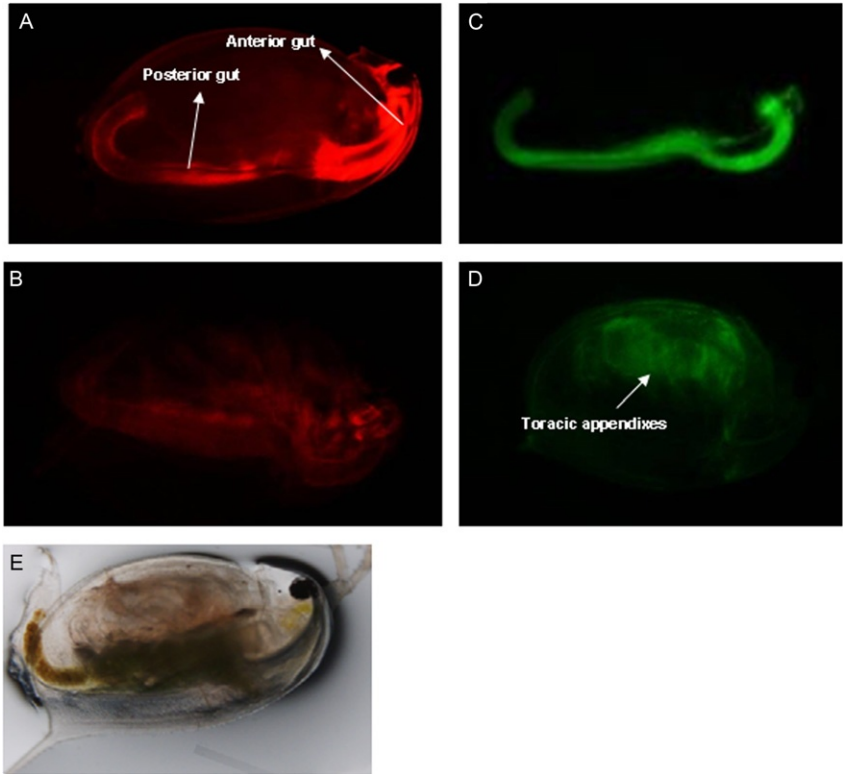

FIGURE 7 Activity of the MDR transporters in the gut and thoracic appendixes of *Daphnia magna*. Fluorescent microscopy images of *Daphnia magna* juveniles exposed to rhodamine B (A, B) and calcein AM (C, D). Images were taken after 1.5 h for *in vivo* (A, C) and 2 h for *ex vivo* (B, D) incubation periods with 5 μM rhodamine B or 0.5 μM calcein AM. Images of a juvenile individual taken with the bright file are also included (E).

3 ANALYSIS OF THE TOXICITY OF PHARMACEUTICALS IN AQUATIC VERTEBRATE SPECIES BY USING THE ZEBRAFISH MODEL

3.1 Introduction

Zebrafish (*Danio rerio*) is a vertebrate model organism widely used for developmental biology, drug discovery, evaluation of toxicological side effects of drugs, and ecotoxicology [25]. Their husbandry costs are much lower than those of mice or other mammals [26]. A single pair of adults breeds once a week, generating 100–200 offspring per brood. Their *ex utero* development and optical clarity during embryogenesis and early larval stages allow the *in vivo* observation of early developmental processes and organogenesis under a stereomicroscope. These functional and morphological

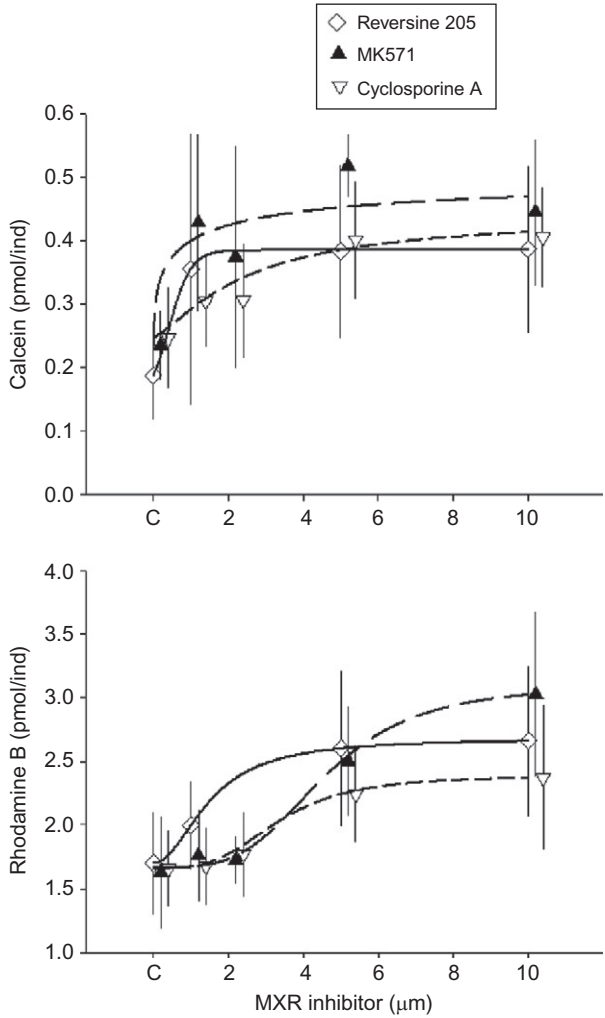

FIGURE 8 MDR transporters are active in *Daphnia magna* juveniles. Effect of transported MXR inhibitors on the accumulation of calcein AM (A) and rhodamine B (B) (pmol/individual) in *Daphnia magna* juveniles. Symbols and error bars are mean ± SD ($n = 10$).

changes may be observed both *in vivo*, by using transgenic lines, vital dyes, and fluorescent tracers, and in whole-mount fixed specimens by using antibodies, riboprobes, and fluorescent markers [27]. Zebrafish embryos grow rapidly, with the basic vertebrate body plan laid out within 24 h postfertilization (hpf). At this stage, the embryo length is around 1.9 mm, so several embryos fit easily inside a single well of a 384-well plate [28]. The majority of organs, including the central and peripheral nervous system (PNS), cardiovascular system, gastrointestinal system, and kidneys, can be studied at 5 days postfertilization (dpf), when the larva is still only 3–4 mm in length, allowing the

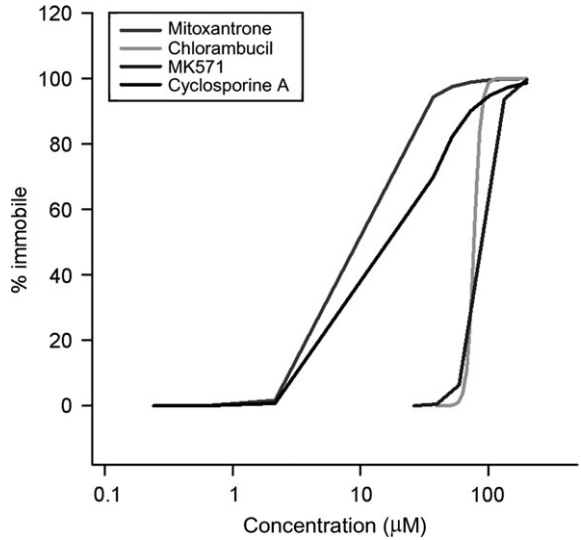

FIGURE 9 Percentage of immobile *Daphnia magna* juveniles after 48 h of single exposure to the studied compounds. Each symbol corresponds to a single replicate. Responses have been fitted to the Hill regression model. Horizontal axis is depicted in log scale.

screenings in 96-well plate format [26]. Thus, zebrafish represents a unique vertebrate model for high-throughput chemical screening, making them useful for toxicological evaluation [28]. It is important to note that zebrafish assays done at the initial endotrophic nutritional period (0–120 hpf) are considered non-animal-based assays by the Directive 2010/63/EU that actualizes the previous Directive 86/609/EEC. Zebrafish model is also a suitable model for the analysis of the toxic mechanisms and effects of emerging pollutants, including pharmaceuticals. Whereas single-cell bioassays can cover only a limited number of end points, the analysis of toxic effects in zebrafish embryos provides a holistic approach, as it includes multiple aspects of the physiology, development, and functionality of complex organic systems. A variety of assays are available for assessing toxicity on the cardiovascular, gastrointestinal, renal, nervous, thyroid, digestive, or skeletal systems [26,29]. In this chapter, we describe, as a proof of concept, the toxicological effects and potential mechanism of action of clofibrate, a normolipidemic drug commonly found as emerging pollutant in many aquatic ecosystems, by using the zebrafish model.

3.2 Case of Study 3: Clofibrate, a Normolipidemic Drug, Induces Embryonic Malabsorption Syndrome in Zebrafish Embryos

3.2.1 Introduction

Fibrates are a group of normolipidemic drugs, widely used in the treatment of coronary heart disease, resulting in a substantial decrease in plasma triglycerides, a moderate decrease in low-density lipoprotein (LDL) cholesterol, and

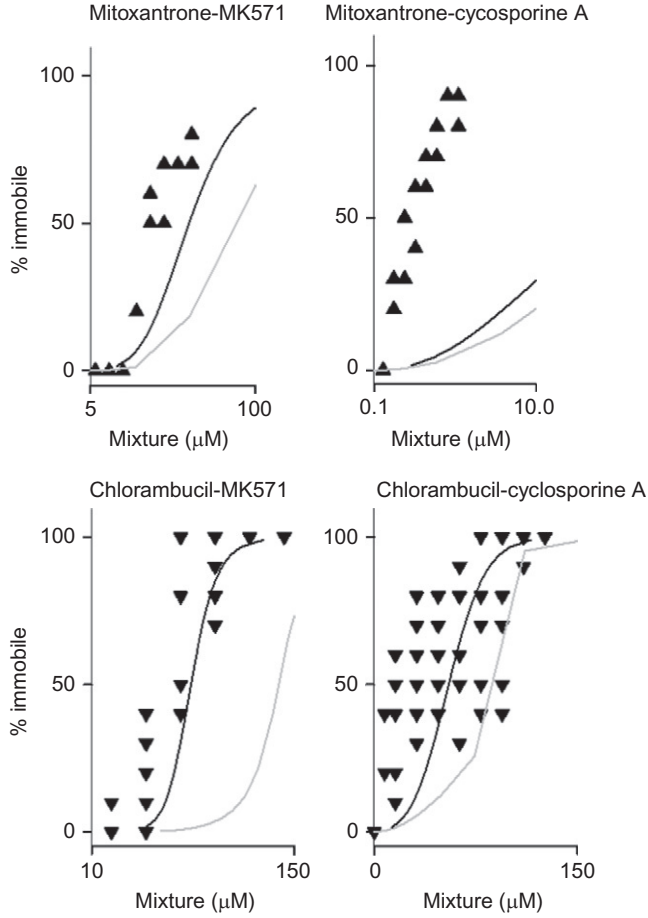

FIGURE 10 Percentage of immobile *Daphnia magna* juveniles after 48 h of exposures to equitoxic binary mixtures of cytotoxic and chemosensitizer drugs. Percentage of immobile *Daphnia magna* juveniles after 48 h of exposures to equitoxic binary mixtures of the cytotoxic drugs mitoxantrone and chlorambucil with the chemosensitizers MK571 and cyclosporine A. Concentration–response curves predicted by the CA (black lines) and IA (gray lines) concepts are also depicted. Horizontal axis is depicted in log scale.

an increase in high-density lipoprotein (HDL) cholesterol concentrations. [30,31]. In some vertebrate species, fibrates also induce a pronounced hepatic peroxisome proliferation by activating peroxisome proliferator-activated receptor-alpha (PPARα) [32], leading to a modulation in the expression of target genes, for example, *acox1* or *apoa1*, involved in the lipid and lipoprotein metabolism [30,33–36]. The magnitude of this response varies considerably among species, for example, rodents are more susceptible to peroxisome proliferation than other species, such as rabbits, nonhuman primates, and humans. PPARs have been characterized in various fish species, including zebrafish

(*Danio rerio*) [37–39], and fibrates induce peroxisome proliferation and peroxisomal beta-oxidation enzymes in both fish hepatocytes *in vitro* [40–42] and exposed adult fish [43–45].

Due to their high consumption, fibrates, together with their metabolites, are continuously released into sewage waters, mainly through excreta, disposal of unused or expired drugs, and directly from pharmaceutical discharges [46]. As a result, fibrates are among the most frequently reported pharmaceuticals in wastewater and surface water [2,47,48]. Thus, aquatic organisms are particularly important targets exposed over their whole lifetime. As the development and survival of fish embryos and eleutheroembryos (yolk sac larvae) depend, at least partly, on the mobilization of yolk lipid constituents, the presence of blood lipid regulators in surface water may disrupt the endotrophic and endo–exothrophic nutritional phases in fish development. Therefore, the aim of this study was to describe the developmental toxicity of a model fibrate, the clofibrate, in zebrafish, by studying the effects of this chemical on the morphology, organ system morphogenesis, and expression pattern of selected target genes involved in lipid transport and metabolism. Our results showed that clofibrate induced an embryonic malabsorption syndrome (EMS), characterized by a considerable decrease in the utilization of endogenous reserves from the yolk sac and highly reproducible morphogenetic and behavioral consequences. These effects were independent of clofibrate's ability to activate the PPARα pathway, seemed not to be due to a pretranslational regulation of transcript level of selected lipid genes in the yolk syncytial layer (YSL), and may be related to an inhibition of constitutive cell secretion by fibrates in the YSL.

3.2.2 *Effects of Clofibrate on Mortality and Hatching Time*

Initial dose–response studies [49] were conducted to determine the developmental toxicity of clofibrate with a nominal range of 0.1–5 mg/L (Figure 11A). No significant mortality was observed in embryos exposed to concentrations of clofibrate under 0.5 mg/L. All the embryos exposed to the highest concentration (5 mg/L) died during gastrulation. The 96 h postfertilization (hpf)-LC50 for clofibrate was 0.89 mg/L (95% CI: 0.79–1.05 mg/L).

Hatching was found to be the most sensitive end point analyzed, with a clear delay in hatching time for embryos treated with clofibrate (Figure 11B). The time course in the control groups showed an onset of hatching at 48 hpf, and over 98% of the embryos had hatched by 72 hpf. The percentage hatching at 52 hpf in embryos treated with the lowest concentration tested, 0.1 mg/L clofibrate, was half that of the controls. By 96 hpf, this percentage had only reached 75% and 23% in embryos treated with 0.75 and 1 mg/L, respectively.

A clofibrate concentration of 0.75 mg/L was selected for most of the further phenotypic analyses, as it induced a strong, reproducible phenotype with

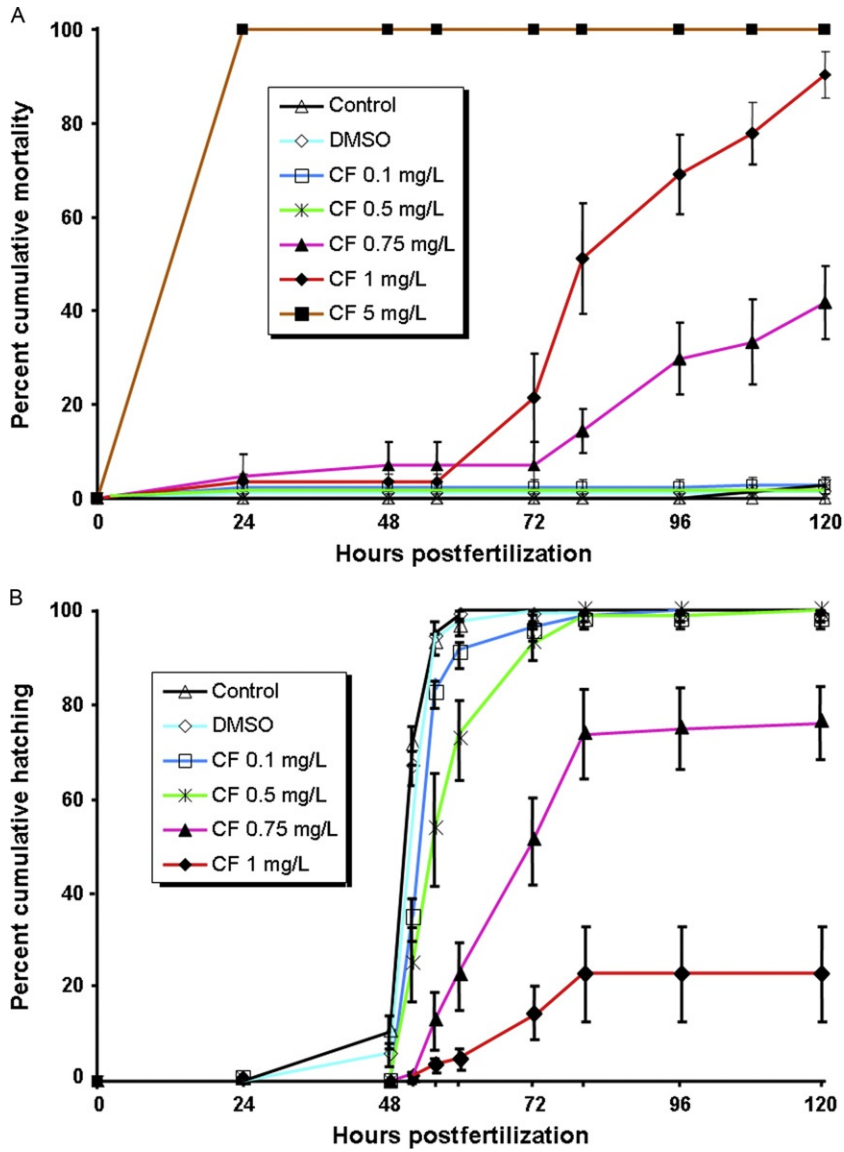

FIGURE 11 Cumulative percentage of mortality and hatching of zebrafish embryos/eleutheroembryos exposed to increasing concentrations of clofibrate. (A) Mortality. (B) Hatching. Values are shown as mean ± SEM of seven replicate experiments, starting with 12 embryos per condition and time. (C) Control animals (open triangle); DMSO, vehicle (0.0025% DMSO)-treated animals (open diamond); 0.1 mg/L clofibrate (CF) (open square); 0.5 mg/L clofibrate (star); 0.75 mg/L clofibrate (closed triangle); 1 mg/L clofibrate (closed diamond); and 5 mg/L clofibrate (closed square). *Reprinted from [49] with permission from Elsevier.*

low-percentage mortality at 72 hpf. However, some structures of the central nervous system (CNS) are not developed in zebrafish before the 5 dpf. Thus, the analysis of the potential neurotoxicity of clofibrate was performed at this developmental stage, using a concentration of 0.6 mg/L.

3.2.3 Clofibrate Induces an Embryonic Malabsorption Syndrome (EMS) by a PPARα-Independent Mechanism

3.2.3.1 Clofibrate Induces EMS

Zebrafish eleutheroembryos treated with 0.5–1 mg/L clofibrate were small and consumed very little yolk (Figures 12–14). By 72 hpf, morphometric analyses demonstrated a significantly shorter body length, in comparison to controls (Figure 12A). The decrease in growth was allometric, with a higher relative decrease in the tail length compared to total length (Figure 12B). The stunted growth induced by clofibrate was associated with EMS, as shown by a strong decrease in yolk sac resorption (Figure 12C). Yolk consumption, estimated by measuring the ratio between eleutheroembryo body and yolk sac areas, was significantly lower in those animals treated with nominal concentrations of 0.75–1 mg/L clofibrate than in controls. There was a strong negative correlation between the width of the yolk sac on a dorsal view and the total length of the larva, and these values differed according to the treatment group (Figure 12D).

The effect of clofibrate on the yolk consumption was paralleled by a decrease in the transfer of neutral lipids between the yolk sac and the embryo/eleutheroembryo. During teleost fish embryogenesis, the formation of the YSL enables the resorption of the yolk reserves and development up to the larval stage [50–52]. Neutral lipid stain oil red O (ORO) was recently proposed for monitoring endotrophic lipid consumption during zebrafish embryonic and larval stages [53]. While yolk sac, head, heart, swim bladder, and vasculature were stained with ORO in control eleutheroembryos (Figure 13A, B, and D), eleutheroembryos treated with clofibrate exhibited strong ORO staining in the yolk sac, but minimal staining in the head, heart, swim bladder, and vasculature (Figure 13C, E, and F). One explanation for the decrease in ORO staining in the clofibrate-exposed eleutheroembryos may be the impairment of normal development of the vascular system. For instance, in *cloche* mutant, lacking blood cells and vascular system, there is no ORO staining in the head, heart, or vasculature [53]. Red blood cell movement was assessed on the posterior cardinal vein, dorsal aorta, and intersegmental vessels of the clofibrate-treated eleutheroembryos. Although the blood flow was reduced with respect to the controls, possibly due to abnormal heart morphogenesis and pericardial edema of the clofibrate-treated animals, there was blood circulation in all of them. Overall, these data support the view that the EMS induced by clofibrate was due to their inability to deliver yolk sac nutrients and hormones to the circulatory system. From a nutritional point of view,

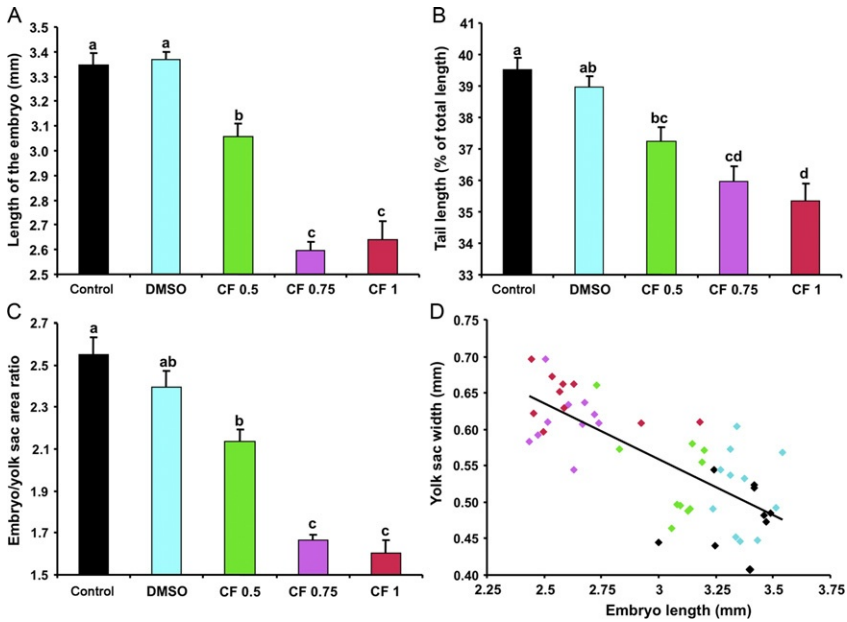

FIGURE 12 Clofibrate induces an embryonic malabsorption syndrome (EMS) in zebrafish. (A) Total body length. (B) Tail length, indicated as a percentage of total body length. (C) Ratio between embryo/eleutheroembryo and yolk sac areas. Animals were exposed to 0.75 mg/L clofibrate from 2 hpf to 3 dpf and the parameters measured at that time. Control, embryos in egg water; DMSO, vehicle (0.0025% DMSO)-treated embryos in egg water; CF 0.5, 0.5 mg/L clofibrate; CF 0.75, 0.75 mg/L clofibrate; and CF 1, 1 mg/L clofibrate. Values are shown as mean ± SEM, $n = 10$. Significance of differences between groups was determined by one-way ANOVA followed by the post hoc Tukey–Kramer multiple comparison test (A, B). When the assumption of equal variances was not verified, the significance of differences between groups was determined by Kruskal–Wallis test followed by the post hoc Dunn's multiple comparison test (C). Groups that are not significantly different at $p < 0.05$ are denoted by the same letter. (D) Relationship between the width of the yolk sac and the total length of the larvae. Control in egg water (black diamond), DMSO vehicle in egg water (blue diamond), 0.5 mg/L clofibrate (green diamond), 0.75 mg/L clofibrate (purple diamond), and 1 mg/L clofibrate (red diamond). The linear regression was $y = -0.1531x + 1.0185$, $r^2 = 0.5544$, slope was significantly different from zero at $p < 0.001$, and correlation between the two variables was significant, with nonparametric Spearman $r = -0.7188$, p (two-tailed) < 0.0001. *Reprinted from [49] with permission from Elsevier.*

zebrafish embryos and eleutheroembryos live in a closed system, as shown by the inverse relationship between yolk sac size and the total length of the eleutheroembryos. As demonstrated in mammals, including humans, embryonic growth and fetal size are ultimately determined by the interplay of the supply of nutrients to the fetus by the uteroplacental unit and the fetal endocrine/paracrine status [54–57]. In teleost fish, the YSL may regulate nutrient composition and supply from the yolk sac to the embryo and eleutheroembryo and supply hormonal

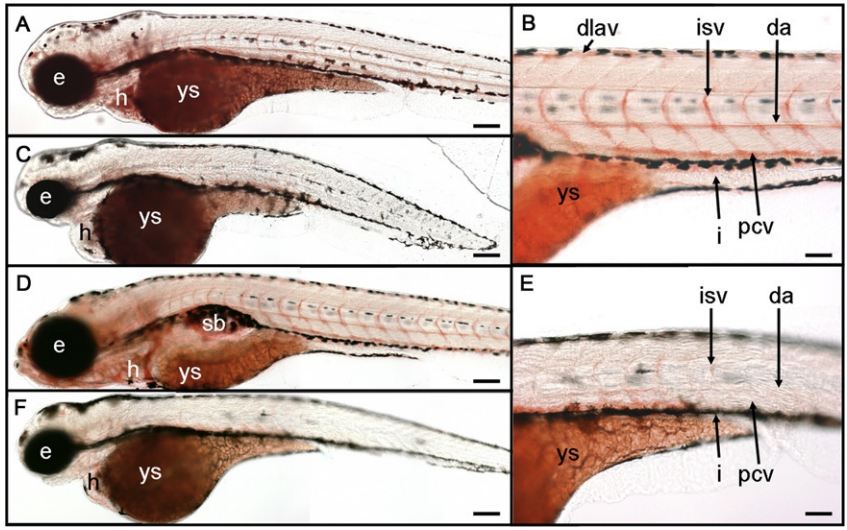

FIGURE 13 Clofibrate impairs endotrophic lipid consumption in zebrafish eleutheroembryos. (A–E) Whole-mount ORO staining of representative eleutheroembryos is shown in ventral view with the anterior part to the left. Enlargement at the trunk level is shown in (B, E). Control eleutheroembryo at 3 dpf (A) and 4 dpf (D, B). 0.75 mg/L clofibrate-treated eleutheroembryo at 3 dpf (C). 0.5 mg/L clofibrate-treated eleutheroembryo at 4 dpf (F, E). Abbreviations: e, eye; da, dorsal aorta; dlav, dorsal longitudinal anastomotic vessel; h, heart; i, intestine; isv, intersegmental vessel; pcv, posterior cardinal vein; sb, swim bladder; yes, yolk sac. Scale bar, 100 µm in (A, C, D, F) and 50 µm in (B, E). *Reprinted from [49] with permission from Elsevier.*

signals, via hormones stored in the yolk during oogenesis, which may affect later stages of organogenesis and metabolism. In addition, an adverse embryonic and larval environment is likely to have profound, long-term effects on the developing organism that may not be reflected in growth retardation.

The effect of clofibrate on the utilization of yolk reserves was reversible and, therefore, was not associated with a permanent disruption of specific biological processes occurring at earlier developmental stages. Thus, animals exposed to 0.75 mg/L clofibrate from around 2 hpf to 3 dpf, then incubated in new containers with egg water only for two additional days, had smaller yolk sacs and larger bodies than eleutheroembryos continuously exposed to the drug for 5 days (Figure 14J and insert), but yolk sac volume was still larger than in control animals (Figure 14I). Resorption of the yolk sac was more pronounced in these animals by 7 dpf (Figure 14L), as all reserves had been consumed in both the control and the larvae initially exposed to clofibrate for 3 days (Figure 14K, L).

3.2.3.2 EMS is a Specific Effect of Clofibrate

The gross morphology of zebrafish eleutheroembryos treated with chemicals inducing a global delay in development has some similarities with the EMS,

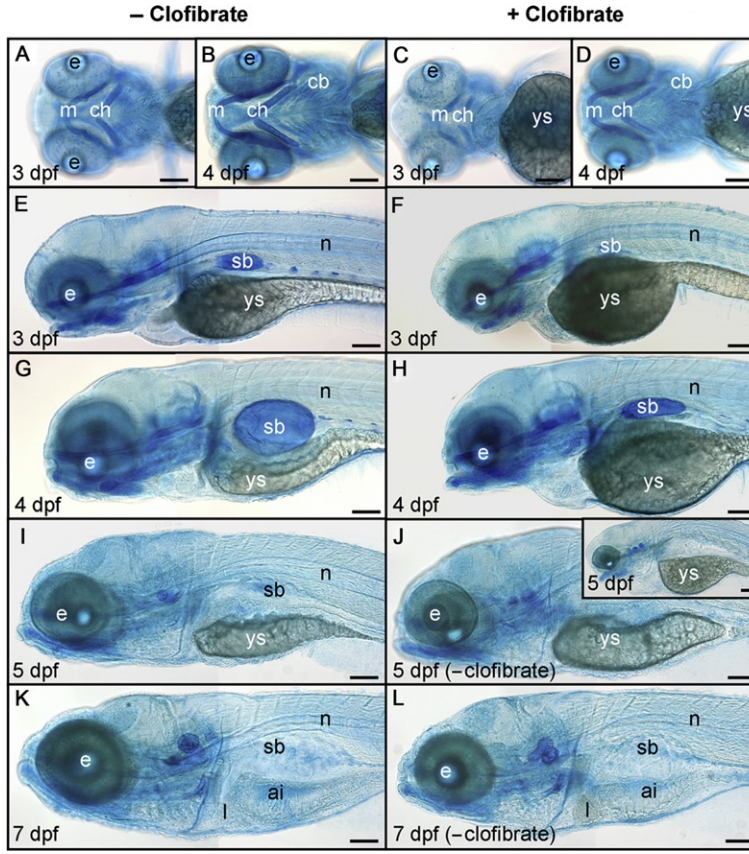

FIGURE 14 Pharyngeal skeleton and reversibility of the clofibrate-induced malabsorption syndrome. (A–D) Whole-mount Alcian blue staining of head skeletons of representative eleutheroembryos is shown in ventral view with the anterior part to the left. Control eleutheroembryo at 3 dpf (A) and 4 dpf (B). 0.75 mg/L clofibrate-treated eleutheroembryo at 3 dpf (C) and 4 dpf (D). (E–L) The reversibility of the malabsorption syndrome induced by clofibrate is demonstrated with representative eleutheroembryos shown in lateral view with the anterior part to the left. Control eleutheroembryo at 3 dpf (E), 4 dpf (G), 5 dpf (I), and 7 dpf (K). 0.75 mg/L clofibrate-treated eleutheroembryo at 3 dpf (F), 4 days (H), and 5 dpf (J, insert). Five-day eleutheroembryo, exposed to 0.75 mg/L clofibrate from 2 hpf to 3 dpf, then incubated with egg water for 2 days (J). Seven-day larva, exposed to 0.75 mg/L clofibrate from 2 hpf to 3 dpf, then incubated with egg water only for 4 days (L). Abbreviations: ai, anterior intestine; cb, ceratobranchial arches; ch, ceratohyal; e, eye; l, liver; n, notochord; m, Meckel's cartilage; sb, swim bladder; ys, yolk sac. Scale bar, 100 μm. *Reprinted from [49] with permission from Elsevier.*

as smaller size of the embryos and a bigger volume of the yolk sac than in age-matched controls. Thus, to demonstrate the specificity of the effect of clofibrate on the induction of EMS, it is necessary to discard the possibility that clofibrate induces a global delay in development by using some

developmental stage markers. Alcian blue staining (Figure 14) of the craniofacial structures demonstrated that clofibrate decreased the size of the head, without a clear delay in morphogenesis of the skeletal structures. Ventral views of the pharyngeal cartilages in control (Figure 14A, B) and clofibrate-exposed (Figure 14C, D) eleutheroembryos at 3 (Figure 14A, C) and 4 days postfertilization (dpf) (Figure 14B, D) showed a similar basic skeletal pattern. Thus, while the ceratohyal angle was significantly smaller in control eleutheroembryos at 4 dpf ($71.92° \pm 1.26°$) than at 3 dpf ($102.66° \pm 2.05°$), this developmental stage marker was even smaller in clofibrate-exposed eleutheroembryos ($62.40° \pm 1.37°$) than in age-matched controls. Jaw movement started by 3 dpf in control and clofibrate-treated animals, indicating their functionality in both conditions. These data demonstrated that clofibrate did not induce a global delay in development in the exposed embryos. Nevertheless, it should be noted that swim bladder inflation and gut morphogenesis, both under mechanical pressure from the yolk ball, were delayed by about 24 h in animals exposed to 0.5 mg/L (Figure 13B, E) or 0.75 mg/L (Figure 14E, G, and H) clofibrate.

3.2.3.3 Induction of EMS by Clofibrate is Not Mediated by the PPARα Pathway

Teleost fish YSL is an extraembryonic structure devoted to the degradation and transfer to the embryo and early larva of the yolk reserves contained in the yolk sac [50–52,57]. The large microsomal triglyceride transfer protein (MTP) subunit is required for the assembly and secretion of apolipoprotein (apo) B-containing lipoproteins, and *mtp* is expressed in the YSL, liver, and intestine of developing zebrafish [58]. Targeted knockdown of *mtp* expression, using an antisense morpholino oligonucleotide approach, led to loss of *mtp* expression and ORO lipid staining in the vasculature, heart, and head structures [53]. The *mtp* morphants were smaller in size than age-matched control eleutheroembryos, consumed little yolk, and thus phenocopied the EMS induced by clofibrate. Although whole-mount *in situ* hybridization may be considered a semiquantitative technique, the YSL *mtp* transcript hybridization signal was unaffected by clofibrate treatment (Figure 15E, F).

It has been demonstrated, mostly in mammals, that clofibrate is nongenotoxic PPARα agonist regulating the expression of target genes via the PPARα pathway. However, to our knowledge, there were no data on the effect of clofibrate on gene expression levels during teleost fish development. Clofibrate, like other peroxisome proliferators, such as di(2-ethylhexyl)phthalate (DEHP), has been shown to increase the expression of genes involved in fatty acid beta-oxidation, as well as those encoding peroxisomal acyl-CoA oxidase 1 (*acox1*), and decrease the encoding proteins involved in lipid mobilization and transport, such as apolipoprotein A-I (apoA1) and MTP

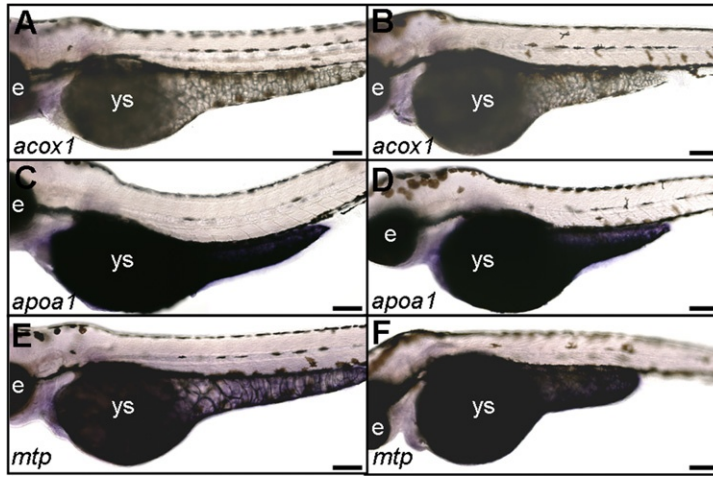

FIGURE 15 Effect of clofibrate on *acox1*, *apoa1*, and *mtp* transcript expression levels in zebrafish eleutheroembryo, evaluated by whole-mount *in situ* hybridization. (A, B) *acox1*. (C, D) *apoa1*. (E, F) *mtp*. Control (A, C, E) and 0.75 mg/L clofibrate-treated (B, D, F) eleutheroembryos, at 3 dpf. Eleutheroembryos were hybridized with sense (data not shown) or antisense (A–F) digoxigenin-labeled riboprobes. The hybridization signal is colored dark blue to purple. The dark brown color in the yolk sac (ys) (A, B) corresponds to an optical effect plus pigmentation and is not a hybridization signal. No staining signal was observed in larvae hybridized with the control sense probes (data not shown). Representative eleutheroembryos are shown in lateral view with the anterior part to the left. Other abbreviation: e, eye. Scale bar, 100 µm. *Reprinted from [49] with permission from Elsevier.*

[34,45,59,60]. Although the presence of PPARα in 7 dpf zebrafish larvae had been described [61], to our knowledge, there were no data available concerning the expression of this gene in earlier stages of development. The two genes selected in these experiments for evaluating the potential up- and downregulation of gene transcription via the PPARα pathway were *apoa1* and *acox1*, as the first is strongly expressed in the YSL [57], while the second is not [62]. Although 0.75 mg/L clofibrate induced a strong EMS phenotype, this concentration in the micromolar range had no effect on the transcript hybridization signal of *acox1* (Figure 15A, B) and *apoa1* (Figure 15C, D). However, fibrates are PPAR ligands at millimolar range [63], and 500–1000 µM clofibrate is commonly used to induce *acox1* mRNA [64] or PPARα immunolabeling [42]. Thus, the apparent absence of regulation of *acox1* and *apoa1* expression levels at micromolar concentrations may be explained by the 1000 times lower concentration used in our work. The fact that there was no apparent regulation by clofibrate in both *acox1* and *apoa1* expressions, classic examples of genes regulated via the PPARα pathway, coupled with the absence of phenotypical effects after exposure to DEHP (Figure 16A, I), a well-known PPARα activator structurally unrelated

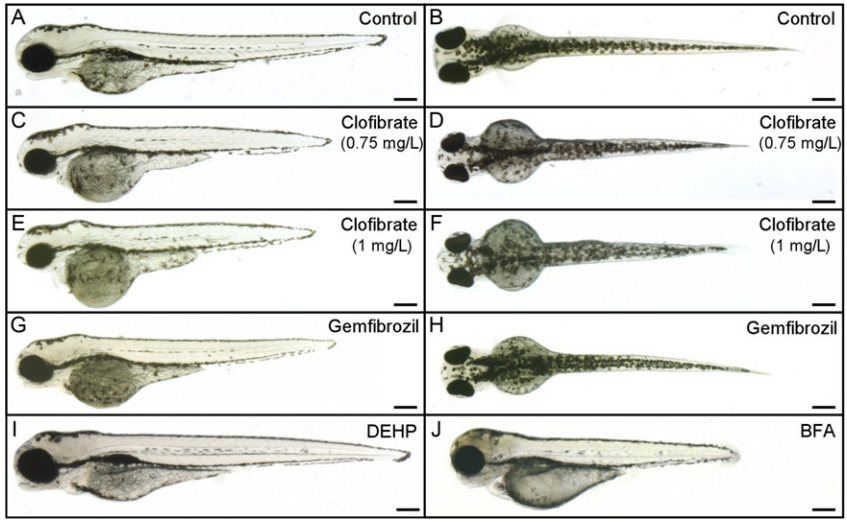

FIGURE 16 Gemfibrozil or BFA, but not DEHP, reproduces the clofibrate-induced EMS. (A, B) Control eleutheroembryo. (C, D) 0.75 mg/L clofibrate-treated eleutheroembryo. (E, F) 1 mg/L clofibrate-treated eleutheroembryo. (G, H) 5 mg/L gemfibrozil-treated eleutheroembryo. (I) 100 mg/L DEHP-treated eleutheroembryo. (J) 1 mg/L BFA-treated eleutheroembryo. Representative 3-day-old live and anesthetized eleutheroembryos are shown in lateral view (A, C, E, G, I, J) or in dorsal view (B, D, F, H) with the anterior part to the left. Scale bar, 200 μm. *Reprinted from [49] with permission from Elsevier.*

to clofibrate, suggested that clofibrate induced EMS via a PPARα-independent mechanism. In contrast to humans, where fibrates act through direct interaction with the nuclear receptor exhibiting the highest affinity to PPARα, several lines of evidence suggest that this is not the case in fish when *in vivo* exposure is carried out [65]. Thus, in goldfish exposed to 1.50 mg/L gemfibrozil, no effects on the levels of hepatic PPARα mRNA were observed [66]. Likewise, no alterations in the expression of PPARα mRNA were observed in fathead minnow after waterborne exposure to 106 mg/L bezafibrate or 1–108 mg/L clofibric acid for 14 days [67]. A similar result was observed in adult male zebrafish exposed to bezafibrate in food, in which no significant alterations in the expression of PPARα mRNA in liver and testis were observed in any of the sampling points during 21 days of exposure [65].

Taken together, these data suggest that the impairment of yolk sac resorption under clofibrate was not mediated at pretranslational level. Nevertheless, another compound with chemical structure similar to clofibrate, the gemfibrozil (5 mg/L; Figure 16G, H), induced a similar EMS phenotype to those induced by clofibrate, increasing the risk of an additive effect on the fish embryos of the cocktail of pollutants present in many surface waters.

3.2.3.4 Induction of EMS by Clofibrate May be Due to an Inhibition of the YSL Secretory Pathway

The YSL is a highly lipoprotein-secreting tissue [50–52], able to express several genes encoding secreted proteins, including apolipoproteins [57,68]. As EMS may be caused by inhibition of the YSL secretory pathway, we investigated whether the effects of clofibrate could be phenocopied by BFA, an inhibitor of Arf1 guanine nucleotide exchange factors known to disrupt COPI function [69] and, therefore, the transport of secretory proteins, for example, apoB [70]. BFA induced a slowdown in yolk resorption (Figure 16), suggesting that EMS was at least partly due to an inhibition of YSL constitutive secretion. Clofibrate has been reported to inhibit membrane trafficking to the Golgi complex and induces its retrograde movement to the endoplasmic reticulum (ER), in a similar way to BFA [71]. In the presence of clofibrate, the forward transport of newly synthesized secretory proteins from the ER to the Golgi was dramatically inhibited. These effects appear to be PPARα-independent, as other PPAR stimulators (DEHP and WY-14643) did not alter the Golgi complex or induce retrograde trafficking [71]. Clofibrate and BFA decreased apoB secretion in HepG2 cells [70,72]. Long-term treatment of rats with clofibrate and fenofibrate caused hypertrophy of the Golgi complex and dilation of the ER in thyroid cells [73], suggesting a disruption of the secretory pathway. Our data suggest that micromolar concentrations of clofibrate may affect the YSL secretome via a similar mechanism, which would explain the inhibition of yolk absorption and lipid transfer and its reversibility in fibrate-free water. However, animals treated with BFA, contrary to clofibrate or gemfibrozil, had normal-sized heads but smaller bodies, associated with a strong inhibition of yolk resorption. This was probably related to a differential sensitivity of these cell types to membrane trafficking in the secretory pathway between ER and Golgi apparatus, as coatomer subunits of the zebrafish COPI complex are supplied maternally and there is an increased demand for coatomer function in some tissues, for example, the notochord [74]. Furthermore, a knockdown of *sec23b*, a gene encoding an integral component of the ER-derived COPII complex, produced a phenotype similar to the *crusher* mutation involving *sec23a*, with growth defects, smaller heads, and slower yolk resorption [75]. In humans, defects in SARA2, a COPII complex component involved in the etiology of chylomicron retention disease and Anderson's disease, result in lipid malabsorption [76,77]. The similar overlapping EMS phenotype obtained after inhibition of the secretory pathway by a knockdown of (lipo)protein trafficking between the ER and Golgi apparatus or following clofibrate embryo exposure supports the hypothesis that the YSL secretory pathway is impaired by this drug. Secretory COPII coat components are essential for craniofacial chondrocyte maturation [75]. Sec23b morphants show complete loss of the ventral pharyngeal skeleton, while the COPI loss-of-function phenotype, produced by injecting *copa* morpholino, results in a

smaller, but normally patterned and differentiated, craniofacial skeleton [75]. Our data revealed that clofibrate treatment resulted in smaller, but well-formed, pharyngeal skeleton, closely resembling the untreated-type pattern. All these data indicate that clofibrate may induce a decrease in yolk sac resorption by inhibiting YSL constitutive cell secretion, presumably by disrupting COPI function.

3.2.4 Clofibrate Induces Organ Toxicity

3.2.4.1 Effects of Clofibrate on the Zebrafish Cardiovascular System Development

Eleutheroembryos exposed to 0.75–1 mg/L clofibrate developed pericardial edema starting by 3–4 dpf (Figure 17), and a high percentage of the eleutheroembryos exposed to 1 mg/L also developed yolk sac edema by 4–5 dpf (data not shown). There was a good match between the onset and severity of the edemas and the mortality in both experimental groups (data not shown). In addition to the pericardial edema, 3 dpf eleutheroembryos exposed to 0.75–1 mg/L clofibrate showed clear effects on heart morphology and contractility by comparison to controls. In clofibrate-treated eleutheroembryos, the atrium was distinctly posterior to the ventricle (Figure 17B), contrary to the looping of the control heart, where the two chambers were side by side (Figure 17A). Moreover, both chambers were elongated in comparison to controls. In addition to morphological changes, clofibrate produced functional changes, with reduced contractility of both chambers. By 3 dpf, the common cardinal vein (CCV) was connected to the sinus venosus of the heart in control eleutheroembryos, with blood fully confined within the

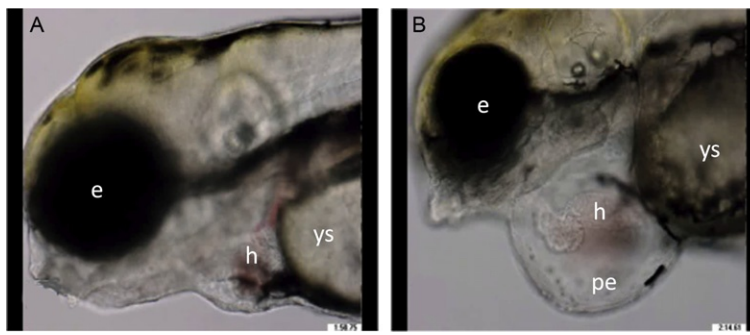

FIGURE 17 Clofibrate impairs the morphogenesis, inducing pericardial edema. (A) Control eleutheroembryo at 3 dpf. (B) 0.75 mg/L clofibrate-treated eleutheroembryo at 3 dpf. Representative eleutheroembryos are shown at the head/heart level in lateral view with the anterior part to the left. Other abbreviations: e, eye; h, heart; pe, pericardial edema; ys, yolk sac.

CCV. However, the remodeling of the CCV was not completed in eleutheroembryos treated with 0.75 mg/L clofibrate, and the sinus venosus was still connected to the visceral pericardium at the ventral edge of the border between the yolk sac and pericardium. There were also differences in the outflow tract of the heart. By 3.5 dpf, the position of bulbus arteriosus was at the level of the ceratobranchial III, connecting with the ventral aorta. However, from 3.5 dpf onward, the outflow tract of the heart in eleutheroembryos treated with clofibrate was clearly more cranial, at the level of the ceratobranchial I, indicating a failure in the elongation of the ventral aorta (Figure 18A, B).

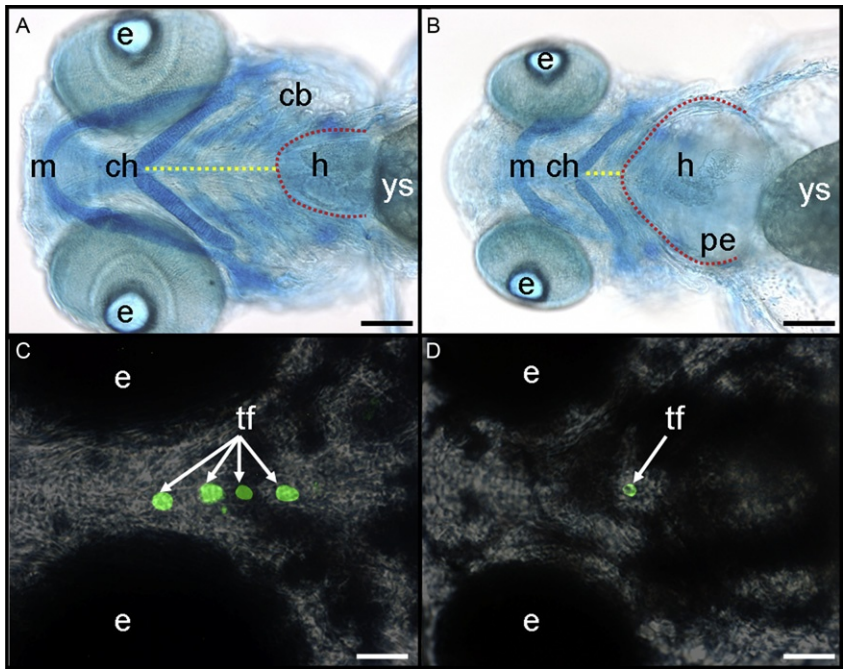

FIGURE 18 Clofibrate induces thyroid abnormalities during early development of zebrafish. (A, B) Whole-mount Alcian blue staining of head skeletons of 5 dpf control (A) and 0.75 mg/L clofibrate-treated (B) eleutheroembryos. The anterior limit of the pericardial cavity and the position of the ventral aorta between this limit and ceratohyal (ch) are highlighted by a red and a yellow dotted line, respectively. (C, D) Whole-mount T4 immunofluorescence staining superposed on bright-field illumination of 5 dpf control (C) and 0.75 mg/L clofibrate-treated eleutheroembryos (D). White arrowheads indicate T4-immunostained thyroid follicles (tf). Representative eleutheroembryos are shown in ventral view with the anterior part on the left. Other abbreviations: cb, ceratobranchial arches; e, eye; m, Meckel's cartilage; pe, pericardial edema; ys, yolk sac. Scale bar, 100 μm in (A, B) and 55 μm in (C, D). *Reprinted from [49] with permission from Elsevier.*

3.2.4.2 Effects of Clofibrate on the Thyroid Gland Morphogenesis

In zebrafish, differentiation of the first thyroid follicle takes place early in embryonic development, and thyroid follicular tissue then grows along the ventral pharyngeal midline [78]. Two phases are distinguishable in thyroid morphogenesis. After induction and evagination from the floor of the primitive pharynx, the thyroid primordium initially adopts a position close to the cardiac outflow tract. In the second phase, dependent on ventral aorta development [79], the primordium expands along the anteroposterior (AP) axis into a strand of follicular tissue, still restricted to the midline [80]. In genetic backgrounds with altered pharyngeal vessel architecture, the first phase of relocation is not disrupted, and the thyroid starts to develop from a normally induced midline primordium. However, during subsequent growth, the thyroid fails to elongate from its initial position at the outflow tract along the AP axis [79]. Instead, it often expands laterally, in an irregular fashion, always adjacent to, or embedded in, vascular endothelial tissue. Correspondingly, follicles do not align along the pharyngeal midline during larval growth and form an irregular group around the cardiac outflow tract.

To evaluate whether the impairment of ventral aorta development found in clofibrate-exposed eleutheroembryos disrupted thyroid gland morphogenesis, we used an antithyroxine (T4) antibody to label thyroid follicles on whole-mount eleutheroembryos (Figure 18C, D). T4 immunostaining of a single thyroid follicle was detected at 3 dpf, in both control and clofibrate-treated eleutheroembryos, at the outflow tract of the heart, ventral to the basibranchial cartilage at the level of the ceratobranchial I (data not shown), demonstrating that there was neither disruption nor delay in the induction, evagination, and first-phase location of the thyroid gland. By 5 dpf, control eleutheroembryos had three to five follicles, oriented longitudinally along the ventral aorta (Figure 18C). However, clofibrate-treated eleutheroembryos still had only a single T4-immunoreactive follicle, located around the outflow tract of the heart (Figure 18D), and a large pericardial edema and a very short ventral aorta, in comparison to controls (Figure 18A, B). These results indicate that clofibrate induces a disruption in the second phase of relocation, phenocopying the genetic backgrounds with altered development of the ventral aorta. T4 immunoreactivity in this group was ventral to the basibranchial cartilage, between the ceratohyal and the ceratobranchial I, at the heart outflow tract. Summarizing, these data demonstrated that the budding off of the thyroid gland primordia and its first morphogenetic relocation phase were not altered by clofibrate treatment, while the disruption of ventral aorta elongation due to the small size of the head precluded alignment of additional thyroid follicles along the AP axis in the hypopharyngeal area, thus drastically impairing thyroid gland morphogenesis. It remains to be determined to which extend thyroid abnormalities may be reversible on removing the drug from the water.

3.2.4.3 Effects of Clofibrate on the Nervous System Development

Eleutheroembryos exposed to 0.6–1 mg/L clofibrate exhibited lethargic behavior at 5 dpf. Moreover, the touch-evoked escape response performed at 72 hpf was clearly disrupted in those clofibrate-treated eleutheroembryos. Impairment in the motor behavior has been found in zebrafish eleutheroembryos after exposure to environmental pollutants and drugs with neurotoxic effect on different components of the motor system. For instance, ethanol, cadmium, caffeine, nicotine, and sodium benzoate have been reported to induce different abnormalities in the stereotypic pattern of the axonal projections of the spinal motor neurons [81]. Moreover, caffeine and sodium benzoate induce also different defects on the neuromuscular junctions (NMJs) [82,83]. In order to identify components of the motor systems impaired by clofibrate, we first analyzed the pattern of axonal projections of spinal primary (PMNs) and secondary (SMNs) motor neurons. Whole-mount immunofluorescence, by using specific antisynaptotagmin 2 (znp1) and antineurolin (zn8) antibodies, was performed on control and clofibrate-treated 5 dpf zebrafish eleutheroembryos. An abnormal pattern in the axonal projections of the PMNs, but not of the SMNs, was found in the clofibrate-treated eleutheroembryos (Figure 19). Because the stereotypic pattern of the SMNs axogenesis can be also used to determine accurately the developmental stage of the embryos, the similar pattern of the SMN axonal projection observed between control and clofibrate-treated eleutheroembryos is consistent with the results of the pharyngeal cartilage development, supporting that most of the clofibrate effects are specific and not the consequence of a global delay in development. The observed decrease in the intensity of the labeling of the SMNs (Figure 19C, D) suggests a reduction in the number of SMNs sending their axons to innervate the fast and slow muscle fibers of the trunk or, alternatively, the specific downregulation in the expression of neurolin in the SMNs of the treated animals.

We analyzed also the NMJ pattern in control and treated eleutheroembryos, using whole-mount acetylcholinesterase (AChE) staining (Figure 20). AChE staining in 3 dpf eleutheroembryos was located both at the myoseptal junctions and at the end-plate regions, running orthogonally across fast muscle fibers, innervated by both primary and secondary motor neurons [84]. Nevertheless, the density of synaptic contacts along the fiber was clearly higher in clofibrate-exposed eleutheroembryos and tended to be more round-shaped NMJs per single muscle fiber than the controls. This phenomenon was associated with an apparent disorganization and less striation of muscular fibers. In mammals and chickens, the change from neonatal to adult muscle innervation patterns implies that some initial neuromuscular synapses had been removed. In chicks, the elimination of synaptic contacts begins *in ovo* and continues after hatching [84]. Nevertheless, it has been demonstrated that zebrafish PMNs establish NMJs almost exclusively on appropriate

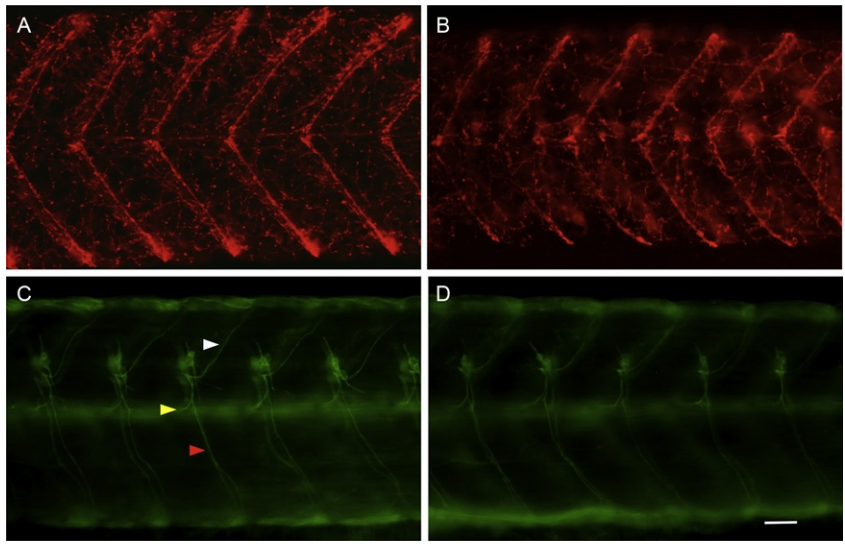

FIGURE 19 Clofibrate impairs the stereotypic pattern of the axonal projections of primary motor neurons, but not of secondary motor neurons. Primary motor neuron (A, B) and secondary motor neurons (C, D) axonal projections. Control (A, C) or exposed to 0.6 mg/L clofibrate (B, D) 5 dpf eleutheroembryos were labeled with znp1 (A, B) or zn8 (C, D), respectively. By 5 dpf, secondary motor axons of control eleutheroembryos (C) have completed migration along the common path and extend along their cell-type-specific paths into the dorsal (white arrowhead), medial (yellow arrowhead), and ventral myotome (red arrowhead), respectively, a pattern that is well preserved in clofibrate-treated animals (D). Representative eleutheroembryos are shown at trunk level in lateral view with the anterior part to the left. Scale bar, 40 μm.

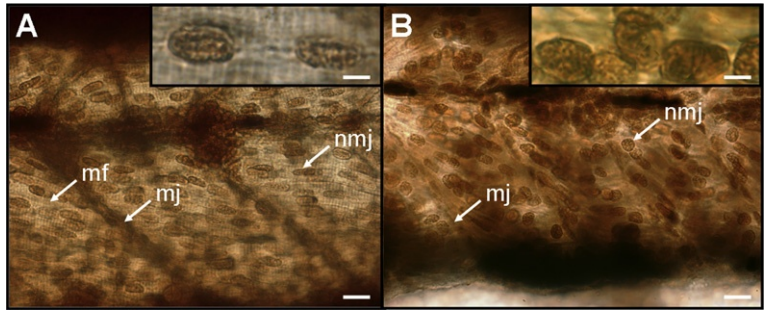

FIGURE 20 Comparison of the neuromuscular junction (NMJ) pattern in control and clofibrate-treated eleutheroembryos, using whole-mount acetylcholinesterase staining. (A) Control eleutheroembryos at 3 dpf. (B) 0.75 mg/L clofibrate-treated eleutheroembryos at 3 dpf. AChE staining labeling to identify NMJ. Morphology of the NMJs in control (A, insert) and clofibrate-exposed (B, insert) eleutheroembryos. Representative eleutheroembryos are shown at trunk level in lateral view with the anterior part to the left. Other abbreviations: muscular fiber (mf) and myoseptal junctions (mj). Scale bar, 25 μm in (A, B) and 10 μm in (A, B, inserts). *Reprinted from [49] with permission from Elsevier.*

muscle fibers, rather than by overproduction and selective elimination of inappropriate branches [85]. As a result, the difference in the number of NMJs per muscle fiber found between the control and clofibrate-exposed eleutheroembryos did not reflect differences in the developmental stage of the neuromuscular synapses.

Myelin disruption impairs the motor behavior in zebrafish eleutheroembryos and larvae [86]. Thus, impaired escape response has been found in erbb3$^{st14/48}$ and erbb3$^{st48/48}$ zebrafish mutants, characterized by a reduction in myelin basic protein (MBP) expression in the PNS but apparently normal expression in the CNS [87]. MBP is a major constituent of the myelin sheath of oligodendrocytes and Schwann cells in the vertebrate nervous system, and because of that, MBP is commonly used as a marker of these two cell types. We performed a double whole-mount immunofluorescence with the antibodies 3A10 and anti-MBP, for labeling the neurofilaments of the axons and the myelin sheath, respectively. Consistently, with the decrease in the intensity observed with the SMNs marker antineurolin, clofibrate induced a decrease in the intensity of the axonal tracts corresponding with the caudal-like spinal SMNs labeled with the 3A10 antibody (Figure 21). Moreover, the axons of these caudal-like motor neurons were covered by a myelin sheath in control (Figure 21), but not in clofibrate-treated eleutheroembryos (Figure 21). The deleterious effect of clofibrate on the myelination was not restricted to the Schwann cells ensheathing the axons of the spinal motor neurons. Thus, although no clear differences were evident in the development of the posterior lateral line (PLL) axons between control and clofibrate-treated eleutheroembryos, there was a strong decrease in the MBP immunofluorescence in those animals treated with clofibrate, reflecting problems in the myelination in these sensory axons (Figure 21). No clear differences were found in the labeling with 3A10 of the longitudinal fascicle and the posterior commissure at the midbrain/hindbrain level. However, clofibrate induced a clear decrease in the MBP immunofluorescence, suggesting an impact on the oligodendrocytes unsheathing these axons (Figure 21). Thus, these results demonstrated that clofibrate inhibits myelination in both PNS and CNS.

Studies over the past five decades have evaluated the effects of nutrition on CNS development in experimental animals and humans. The results reveal that a reduction in the supply of energy and/or several essential nutrients during the first stages of life has profound effects on the structural and functional development of the nervous system [88]. Thus, it is unclear if the described effects of clofibrate on the nervous system development are specific or, on the contrary, are only indirect consequences of the EMS on the neurogenesis. Early in the study of the effects of malnutrition on brain development, the cerebellum was recognized as an area that is particularly sensitive to the effects of early malnutrition [89–91]. Existing data indicated that Purkinje cells are one of the cerebellar cell types more sensitive to protein malnutrition [92]. Thus, malnutrition imposed postnatally modifies the number and the

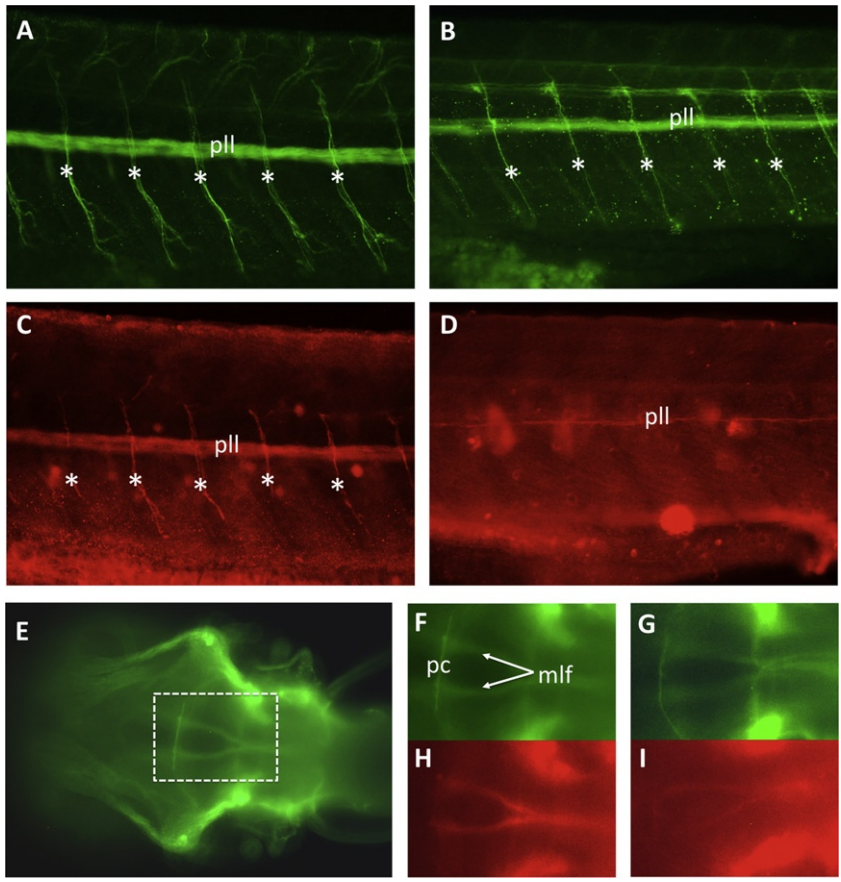

FIGURE 21 Clofibrate impairs myelination in both PNS and CNS. (A–D) Myelin sheath enveloping axons at the trunk level in control (A, C) or exposed to 0.6 mg/L clofibrate (B, D) 5 dpf zebrafish eleutheroembryos. Representative eleutheroembryos are shown at trunk level in lateral view with the anterior part to the left and were labeled by using whole-mount double immunofluorescence with 3A10, labeling axons of sensory (axons of the posterior lateral line in A and B) and motor neurons (asterisks in A and B), and MBP, a marker of Schwann cells in the PNS (on the axons of the posterior lateral line (PLL) and spinal motor neurons in C), and oligodendrocytes in the CNS (on axons of the medial longitudinal fascicle (mlf) in H) and antibodies. At the trunk level, eleutheroembryos treated with clofibrate (B) exhibited a moderate decrease in the intensity of the labeling of the axonal projections of spinal motor neurons (white asterisks in A and B) with respect to the controls (A). Moreover, while the control eleutheroembryos exhibited a myelin sheath enveloping most of the axons of these spinal motor neurons projecting ventrally (white asterisks in C), the treatment with clofibrate induced a total abolition of the myelination of these axons (D). Although clofibrate (B) had no clear effect on the PLL axons (A vs. B), animals exposed to this compounds exhibited a strong reduction in the myelination of this sensory nerve of the PNS (C vs. D). (E–I) Dorsal view of the head anterior part to the left of 5 dpf eleutheroembryos after double immunofluorescence with 3A10 (E, F, G) and MBP (H, I) antibodies. 3A10 antibody labeled axons of the mlf and the posterior commissure (pc; dashed box in E); no clear differences were observed between control (E, F) and 0.6 mg/L CF-treated eleutheroembryos (G). However, clofibrate induced a clear decrease in MBP immunofluorescence (I) by comparison with control (H).

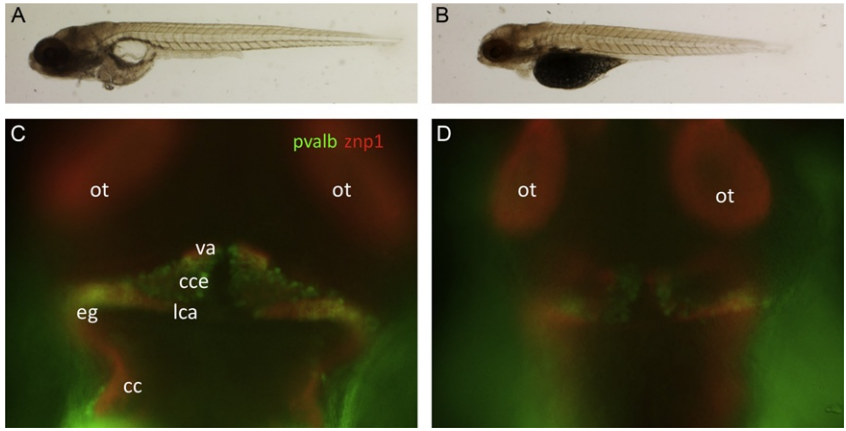

FIGURE 22 Clofibrate disrupts the development of the cerebellum. Control (A, C) or 0.6 mg/L clofibrate-exposed (B, D) eleutheroembryos were costained with antiparvalbumin (pvalb) (green) and znp1 (red) antibodies (C, D). By 5 dpf, control zebrafish eleutheroembryos (A, C) have developed the main structures of the adult cerebellum, including the valvula cerebelli (va), corpus cerebelli (cce), lobus caudalis cerebelli (lca), eminentia granularis (eg), and the crista cerebellaris (cc). Differentiated Purkinje cells are located mainly in the cce and va (B). In contrast, clofibrate-treated eleutheroembryos (B, D) exhibited an impairment in the organization of the different cerebellar regions and a decrease in the number of differentiated Purkinje cells (D). Other abbreviations: ot, optic tectum. Representative 5 dpf eleutheroembryos are shown in lateral view with the anterior part to the left (A, B) or in dorsal view of the head, anterior part to the top (C, D).

electrophysiological properties of Purkinje cells within the cerebellum [93,94]. We then analyzed the effect of clofibrate on development of the Purkinje cells in zebrafish eleutheroembryos, using whole-mount immunofluorescence with an antiparvalbumin antibody labeling specifically this cell type in the zebrafish cerebellum. Most of the clofibrate-treated eleutheroembryos exhibited a disruption in the development of the Purkinje cell layer (Figure 22). Although it has been demonstrated that cerebellum is involved in motor behavior in zebrafish larvae [95,96], the functional significance of our findings is not clear. The fact that the severity of the effect on the cerebellum was closely related with the severity of the EMS (data not shown) supports the hypothesis that, at least, a part of the neurotoxic effects found in clofibrate-exposed animals may be related with the induction of the EMS.

3.2.5 Conclusions

Our data provide evidence of the developmental toxicity of micromolar concentrations of clofibrate, a human blood lipid regulator pharmaceutical, in zebrafish. This molecule was previously considered to be weakly toxic in mammals, on the basis of acute mortality tests at the adult stage. Other

fibrates, like gemfibrozil, bezafibrate, and fenofibrate, whose presence in surface water has frequently been reported [2], and chlorophenoxy herbicides, including 2,4-D (2,4-dichlorophenoxyacetic acid) and MCPA (4-chloro-2-methylphenoxyacetic acid), have similar chemical structures to clofibrate and also have lipid-lowering effects in rats [84]. Gemfibrozil has the potential to be taken up from water and bioconcentrated in goldfish plasma to a large extent and to induce a potential endocrine disruption [97]. Although the environmental relevant concentrations of clofibrate are in several order of magnitude under the low-observed effect concentration in zebrafish, the similar phenotype induced by gemfibrozil, another compound structurally related with clofibrate, emphasizes the need to analyze the potential additive effects of all the structurally related compounds presents in the water and their potential bioconcentration, to gain a fuller picture of the potential impact of these drugs and pollutants, in terms of developmental toxicity, on aquatic species or even in humans. EMS induced by clofibrate in a zebrafish model is also useful for studying the morphogenetic consequences of impaired nutrient availability during the early stages of vertebrate development.

ACKNOWLEDGMENTS

Research on the effects of pharmaceuticals on *Daphnia magna*, conducted by C. B., B. C., B. P., and N. G. R., was supported by the Spanish MICINN grant CTM2011-30471-C02-01 to C. B. This work was also partly funded by the US Army Environmental Quality Research Program (including BAA 11–4838).

Research of the effects of clofibrate on zebrafish embryos, conducted by D. R. and P. J. B., was partially supported by the Agence Nationale de la Recherche (ANR-2010BLAN 1126 01/LIGENAX project), the Association Française contre les Myopathies (AFM-NMM2 2012 14879), and the Conseil Régional d'Aquitaine (CRA-200881301031/TOD project) to P. J. B. The monoclonal antibodies znp1, zn8, developed by Trevarow as well as the monoclonal antibody 3A10, developed by Jessell, Dodd and Morton, were obtained from the Developmental Studies Hybridoma Bank developed under the auspices of the NICHD and maintained by The University of Iowa, Department of Biology, Iowa City, IA 52242.

ABBREVIATIONS

ABC	ATP-binding cassette
AChE	acetylcholinesterase
AP	anteroposterior
apo	apolipoprotein
BFA	brefeldin A
CCV	common cardinal vein
CNS	central nervous system
DEHP	di(2-ethylhexyl)phthalate
DMSO	dimethyl sulfoxide
dpf	days postfertilization

EMS	embryonic malabsorption syndrome
ER	endoplasmic reticulum
hpf	hours postfertilization
MBP	myelin basic protein
MDR	multidrug resistance
MRP	multidrug resistance protein
Mtp	large microsomal triglyceride transfer protein subunit
NMJ	neuromuscular junction
ORO	oil red O
P-gp	permeability glycoprotein or P-glycoprotein
PLL	posterior lateral line
PMNs	primary motor neurons
PNS	peripheral nervous system
PPAR	peroxisome proliferator-activated receptor
SMNs	secondary motor neurons
SSRIs	selective serotonin reuptake inhibitors
T4	thyroxine
YSL	yolk syncytial layer

REFERENCES

[1] K.K. Rozman, J. Doull, Toxicology 144 (2000) 169–178.
[2] K. Fent, A.A. Weston, D. Caminada, Aquat. Toxicol. 76 (2006) 122–159.
[3] J. Corcoran, M.J. Winter, C.R. Tyler, Crit. Rev. Toxicol. 40 (2010) 287–304.
[4] K. Kümmerer, Annu. Rev. Environ. Resour. 35 (2010) 57–75.
[5] J.M. Brausch, G.M. Rand, Chemosphere 82 (2011) 1518–1532.
[6] S. Kar, K. Roy, Expert Opin. Drug Saf. 11 (2012) 235–274.
[7] L.H. Heckmann, A. Callaghan, H.L. Hooper, R. Connon, T.H. Hutchinson, S.J. Maund, R.M. Sibly, Toxicol. Lett. 172 (2007) 137–145.
[8] L.H. Heckmann, R.M. Sibly, R. Connon, H.L. Hooper, T.H. Hutchinson, S.J. Maund, C.J. Hill, A. Bouetard, A. Callaghan, Genome Biol. 9 (2008) R40.
[9] B. Campos, B. Pina, C. Barata, Environ. Sci. Technol. 46 (2012) 2943–2950.
[10] Y. Guler, A.T. Ford, Aquat. Toxicol. 99 (2010) 397–404.
[11] R. Lazzara, M. Blázquez, C. Porte, C. Barata, Aquat. Toxicol. 106–107 (2012) 123–130.
[12] T. Smital, B. Kurelec, Environ. Toxicol. Chem. 16 (1997) 2164–2170.
[13] T. Smital, T. Luckenbach, R. Sauerborn, A.M. Hamdoun, R.L. Vega, D. Epel, Mutat. Res. 552 (2004) 101–117.
[14] E.M. Leslie, R.G. Deeley, S.P.C. Cole, Toxicol. Appl. Pharmacol. 204 (2005) 216–237.
[15] T. Luckenbach, D. Epel, Am. J. Physiol.-Regul. Integr. Comp. Physiol. 294 (2008) R1919–R1929.
[16] I. Bošnjak, K.R. Uhlinger, W. Heim, T. Smital, J. Franekić-Čolić, K. Coale, D. Epel, A. Hamdoun, Environ. Sci. Technol. 43 (2009) 8374–8380.
[17] L.E. Shipp, A. Hamdoun, Dev. Dyn. 241 (2012) 1111–1124.
[18] N. Garcia-Reyero, B.L. Escalon, P.R. Loh, J.G. Laird, A.J. Kennedy, B. Berger, E.J. Perkins, Environ. Sci. Technol. 46 (2012) 42–50.
[19] G.A. LeBlanc, Ecotoxicology 16 (2007) 61–81.

[20] A. Sturm, P. Cunningham, M. Dean, BMC Genomics 10 (2009) 170.
[21] A. Navarro, S. Weißbach, M. Faria, C. Barata, B. Piña, T. Luckenbach, Aquat. Toxicol. 122–123 (2012) 144–152.
[22] M. Faria, A. Navarro, T. Luckenbach, B. Piña, C. Barata, Aquat. Toxicol. 101 (2011) 78–87.
[23] R. Altenburger, M. Nendza, G. Schüürmann, Environ. Toxicol. Chem. 22 (2003) 1900–1925.
[24] D. Epel, T. Luckenbach, C.N. Stevenson, L.A. MacManus-Spencer, A. Hamdoun, T. Smital, Environ. Sci. Technol. 42 (2008) 3914–3920.
[25] D.R. Love, F.B. Pichler, A. Dodd, B.R. Copp, D.R. Greenwood, Curr. Opin. Biotechnol. 15 (2004) 564–571.
[26] P. Goldsmith, Curr. Opin. Pharmacol. 4 (2004) 504–512.
[27] P. McGrath, C.Q. Li, Drug Discov. Today 13 (2008) 394–401.
[28] L. Zon, R. Peterson, Nat. Rev. Drug Discov. 4 (2005) 35–44.
[29] S. Berghmans, P. Butler, P. Goldsmith, G. Waldron, I. Gardner, Z. Golder, F.M. Richards, G. Kimber, A. Roach, W. Alderton, A. Fleming, J. Pharmacol. Toxicol. Methods 58 (2008) 59–68.
[30] O. Barbier, I. Torra, Y. Duguay, C. Blanquart, J.C. Fruchard, C. Glineur, B. Staels, Arterioscler. Thromb. Vasc. Biol. 22 (2002) 717–726.
[31] J.M. Backes, C.A. Gibson, J.F. Ruisinger, P.M. Moriarty, Pharmacotherapy 27 (2007) 412–424.
[32] S.S. Lee, T. Pineau, J. Drago, E.J. Lee, J.W. Owens, D.L. Kroetz, P.M. Fernandez-Salguero, H. Westphal, F.J. Gonzalez, Mol. Cell. Biol. 15 (1995) 3012–3022.
[33] K. Schoonjans, B. Staels, J. Auwerx, J. Lipid Res. 37 (1996) 907–925.
[34] J.A. Kramer, E.A. Blomme, R.T. Bunch, J.C. Davila, C.J. Jackson, P.F. Jones, K.L. Kolaja, S.W. Curtiss, Toxicol. Pathol. 31 (2003) 417–431.
[35] J.P. Despres, I. Lemieux, S.J. Robins, Drugs 64 (2004) 2177–2198.
[36] H. Duez, B. Lefebvre, P. Poulain, I.P. Torra, F. Percevault, G. Luc, J.M. Peters, F.J. Gonzalez, R. Gineste, S. Helleboid, V. Dzavik, J.C. Fruchart, C. Fievet, P. Lefebvre, B. Staels, Arterioscler. Thromb. Vasc. Biol. 25 (2005) 585–591.
[37] M. Robinson-Rechavi, O. Marchand, H. Escriva, P. Bardet, D. Zelus, S. Hughes, V. Laudet, Genome Res. 11 (2001) 781–788.
[38] E. Boukouvala, E. Antonopoulo, L. Favre-Krey, A. Diez, J.M. Bautista, M. Leaver, D.R. Tocher, G. Krey, Lipids 39 (2004) 1085–1092.
[39] C. Batista-Pinto, P. Rodrigues, E. Rocha, A. Lobo-da-Cunha, Biochim. Biophys. Acta 1731 (2005) 88–94.
[40] M. Donohue, L. Baldwin, D. Leonard, P. Kostecki, E. Calabrese, Ecotoxicol. Environ. Saf. 26 (1993) 127–132.
[41] B. Ruyter, Ø. Andersen, A. Dehli, A. Östlund, T. Gjøen, M. Thomassen, Biochim. Biophys. Acta 1348 (1997) 331–338.
[42] A. Ibabe, A. Herrero, M. Cajaraville, Toxicol. In Vitro 19 (2005) 725–735.
[43] L. Scarano, E. Calabrese, P. Kostecki, L. Baldwin, D. Leonard, Ecotoxicol. Environ. Saf. 29 (1993) 13–19.
[44] Z. Du, L. Demizieux, P. Degrace, J. Gresti, B. Moindrot, Y. Liu, L.X. Tian, J.M. Cao, P. Clouet, Lipids 39 (2004) 849–855.
[45] A.B. Venkatachalam, S.P. Lall, E.M. Denovan-Wright, J.M. Wright, BMC Evol. Biol. 12 (2012) 112.
[46] C.G. Daughton, T. Ternes, Environ. Health Perspect. 107 (1999) 907–938.
[47] C.D. Metcalfe, B.G. Koenig, D.T. Bennie, M. Servos, T.A. Ternes, R. Hirsch, Environ. Toxicol. Chem. 22 (2003) 2872–2880.

[48] A. Togola, H. Budzinski, Anal. Bioanal. Chem. 388 (2007) 627–635.
[49] D. Raldúa, M. André, P.J. Babin, Toxicol. Appl. Pharmacol. 228 (2008) 301–314.
[50] G. Poupard, M. André, M. Durliat, C. Ballagny, G. Bœuf, P.J. Babin, Cell Tissue Res. 300 (2000) 251–261.
[51] J.M. Vernier, M.F. Sire, Biol. Cell 29 (1977) 45–53.
[52] C. Walzer, N. Schönenberger, Cell Tissue Res. 196 (1979) 75–93.
[53] A. Schlegel, D.Y. Stainier, Biochemistry 45 (2006) 15179–15187.
[54] P.D. Gluckman, Acta Paediatr. 423 (1997) 153–157.
[55] R. Simmons, Trends Endocrinol. Metab. 16 (2005) 390–394.
[56] A.L. Fowden, J.W. Ward, F.P.B. Wooding, A.J. Forhead, M. Constancia, J. Physiol. 572 (2006) 5–15.
[57] P.J. Babin, C. Thisse, M. Durliat, M. André, M.A. Akimenko, B. Thisse, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 8622–8627.
[58] E. Marza, C. Barthe, M. André, L. Villeneuve, C. Helou, P.J. Babin, Dev. Dyn. 232 (2005) 506–518.
[59] V.A. Baker, H.M. Harries, J.F. Waring, C.M. Duggan, H.A. Ni, R.A. Jolly, L.W. Yoon, A.T. De Souza, J.E. Schmid, R.H. Brown, R.G. Ulrich, J.C. Rockett Environ, Health Perspect. 112 (2004) 428–438.
[60] C. Ameen, U. Edvardsson, A. Ljungberg, L. Asp, P. Akerblad, A. Tuned, S.O. Olofsson, D. Linden, J. Oscarsson, J. Biol. Chem. 280 (2005) 1224–1229.
[61] A. Ibabe, E. Bilbao, M. Cajaraville, Histochem. Cell Biol. 123 (2005) 75–87.
[62] S. Morais, A. Knoll-Gellida, M. André, C. Barthe, P.J. Babin, Physiol. Genomics 28 (2007) 239–252.
[63] D. Bishop-Bailey, D. Wray, Prostaglandins Other Lipid Mediat. 71 (2003) 1–22.
[64] D.R. Bell, C.R. Elcombe, Biochim. Biophys. Acta 1090 (1991) 211–215.
[65] Y.M. Velasco-Santamaría, B. Korsgaard, S.S. Madsen, P. Bjerregaard, Aquat. Toxicol. 105 (2011) 107–118.
[66] C. Mimeault, V.L. Trudeau, T.W. Moon, Toxicology 228 (2006) 140–150.
[67] A. Weston, D. Caminada, H. Galicia, K. Fent, Environ. Toxicol. Chem. 28 (2009) 2648–2655.
[68] P.S. Crosier, A. Bardsley, J.A. Horsfield, A.K. Krassowska, E.R. Lavallie, L.A. Collins-Racie, J.H. Postlethwait, Y.L. Yan, J.M. McCoy, K.E. Crosier, Dev. Dyn. 222 (2001) 637–644.
[69] A. Togawa, N. Morinag, M. Ogasawara, J. Moss, M. Vaughan, J. Biol. Chem. 274 (1999) 12308–12315.
[70] N. Sakata, T.E. Phillips, J.L. Dixon, J. Lipid Res. 42 (2001) 1947–1958.
[71] P. de Figueiredo, W.J. Brown, Cell Biol. Toxicol. 15 (1999) 311–323.
[72] S.E. Hahn, D.M. Goldberg, Biochem. Pharmacol. 43 (1992) 625–633.
[73] S.C. Price, D. Chescoe, R.H. Hinton, Biochem. Soc. Trans. 12 (1988) 1034–1035.
[74] P. Coutinho, M.J. Parson, K.A. Thomas, E. Hirst, L. Saúde, I. Campos, P.H. Williams, D.L. Stemple, Dev. Cell 7 (2004) 547–558.
[75] M.R. Lang, L.A. Lapierre, M. Frotscher, J.R. Goldenring, E.W. Knapik, Nat. Genet. 38 (2006) 1198–1203.
[76] B. Jones, E.L. Jones, S.A. Bonney, H.N. Patel, A.R. Mensenkamp, S. Eichenbaum-Voline, M. Rudling, U. Myrdal, G. Annesi, S. Naik, N. Meadows, A. Quattrone, S.A. Islam, R.P. Naoumova, B. Angelin, R. Infante, E. Levy, C.C. Roy, P.S. Freemont, J. Scott, C.C. Shoulders, Nat. Genet. 34 (2003) 29–31.
[77] C.C. Shoulders, D.J. Stephens, B. Jones, Curr. Opin. Lipidol. 15 (2004) 191–197.

[78] B. Alt, S. Reibe, N. Fitosa, O. Elsalani, T. Wendl, K. Rohr, Dev. Dyn. 235 (2006) 1872–1883.
[79] B. Alt, O.A. Elsalani, P. Schrumpf, N. Haufs, N.D. Lawson, G.C. Schwabe, S. Mundlos, A. Grüters, H. Krude, K. Rohr, Development 133 (2006) 3797–3804.
[80] T. Wendl, K. Lun, M. Mione, J. Favor, M. Brand, S.W. Wilson, K. Rohr, Development 129 (2002) 3751–3760.
[81] D. Raldua, C. Barata, M. Casado, M. Faria, J.M. Navas, A. Olivares, E. Oliveira, S. Pelayo, B. Thienpont, B. Piña, In: D. Barceló (Ed.), The Handbook of Environmental Chemistry, Springer, Berlin, Heidelberg, 2012, pp. 395–414.
[82] H.J. Tsay, Y.H. Wang, W.L. Chen, M.Y. Huang, Y.H. Chen, Neurotoxicol. Teratol. 29 (2007) 562–569.
[83] Y.H. Chen, Y.H. Huang, C.C. Wen, Y.H. Wang, W.L. Chen, L.C. Chen, H.J. Tsay, Neurotoxicol. Teratol. 30 (2008) 440–447.
[84] M.R. Bennett, A.G. Pettigrew, J. Physiol. 241 (1974) 515–545.
[85] D.W.C. Liu, M. Westerfield, J. Neurosci. 10 (1990) 3947–3959.
[86] H.M. Pogoda, N. Sternheim, D.A. Lyons, B. Diamond, T.A. Hawkins, I.G. Woods, D.H. Bhatt, C. Franzini-Armstrong, C. Dominguez, N. Arana, J. Jacobs, R. Nix, J.R. Fetcho, W.S. Talbot, Dev. Biol. 298 (2006) 118–131.
[87] D.A. Lyons, H.M. Pogoda, M.G. Voas, I.G. Woods, B. Diamond, R. Nix, N. Arana, J. Jacobs, W.S. Talbot, Curr. Biol. 15 (2005) 513–524.
[88] R. Uauy, D.R. Hoffman, P. Peirano, D.G. Birch, E.E. Birch, Lipids 36 (2001) 885–895.
[89] B.P.F. Adlard, J. Dobbing, J.L. Smart, Biol. Neonate 23 (1973) 95–108.
[90] H.P. Chase, W.F.B. Lindsey, D. O'Brien, Nature (London) 221 (1969) 554–555.
[91] T.J. Sobotka, M.P. Cook, R.E. Brodie, Brain Res. 65 (1974) 443–458.
[92] S.L. Manocha, Z. Olkowski, Histochem. J. 5 (1973) 105–118.
[93] J. Clos, C. Favrae, M. Selme Matrat, J. Legrand, Brain Res. 123 (1976) 13–26.
[94] S.K. Sharma, U. Nayar, M.C. Maheshwari, B. Singh, J. Neurol. Sci. 78 (1987) 261–272.
[95] M. Aizenberg, E.M. Schuman, J. Neurosci. 31 (2011) 8708–8712.
[96] M.B. Ahrens, J.M. Li, M.B. Orger, D.N. Robson, A.F. Schier, F. Engert, R. Portugues, Nature 485 (2012) 471–477.
[97] C. Mimeault, A.J. Woodhouse, X.S. Miao, C.D. Metcalfe, T.W. Moon, V.L. Trudeau, Aquat. Toxicol. 73 (2005) 44–54.

Chapter 18

The Effect of PhACs on Biological Communities in Rivers: Field Studies

Lorenzo Proia* and Victoria Osorio†

*Catalan Institute for Water Research (ICRA), Girona, Spain

†Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

Chapter Outline

1 INTRODUCTION

Pharmaceutically active compounds (PhACs) constitute a relevant group of products due to their extensive and increasing use in human and veterinary medicine. These compounds cover a wide array of chemical formulations with different physicochemical properties and multiple biological targets and are therefore often classified according to their therapeutic purposes (i.e., analgesics, antibiotics, and β-blockers). These chemicals are designed to have specific mode of action on target organisms and many of them for some persistence [1]. After human and animal consumption, PhACs are excreted as both metabolized and parental forms. Excretion, agricultural runoff, and industrial or direct flush are the main pathways of PhACs and their

Comprehensive Analytical Chemistry, Vol. 62. http://dx.doi.org/10.1016/B978-0-444-62657-8.00018-5

metabolites, of entry in municipal sewage and wastewater treatment plants (WWTPs) [2,3]. Owing to the diverse operating conditions that are applied in the WWTPs and the different physicochemical properties of PhACs (i.e., polarity, water solubility, sorption to solids, and biodegradability) [4], widely varying removals have been reported for these compounds [5]. Consequently, PhACs have been detected in wastewater effluents (WWE), surface waters, and groundwaters, at levels in the ng L^{-1} up to the μg L^{-1} range [6–8]. Since the main emission source of PhACs into freshwater ecosystems is WWE, the increasing levels of PhACs determined in the aquatic environment can be directly related to the growing consumption [9]. As well as consumption and removal rates in WWTPs, the concentration of PhACs in freshwater systems is also determined by the degree of metabolism occurring in patient body [10], by the compound partitions into the water column/sediments, and by other processes such as the photo- and biodegradation [9]. Despite the natural attenuation of PhACs in the water bodies, the continuous progress on the development of new and more sensitive analytical techniques for trace analysis [11,12] has permitted the detection and quantification of PhACs, metabolites, and transformation products in environmental water and even in drinking waters at even lower concentrations [13–16]. As a consequence, the reports on occurrence and fate of PhACs in the aquatic environment, evidencing their continuous release into water bodies, have increased exponentially over the last 30 years (i.e., [1,2]).

Unlike many other chemicals released into the environment, PhACs are intrinsically bioactive compounds, designed to target physiological functions on humans or livestock [9]. This high reactivity with biological systems can be conserved and amplified across different domains, affecting, therefore, wildlife species at any trophic level of the ecosystems. For example, a recent study on zebra fish demonstrated that this species possesses orthologs to 86% of human gene drug targets [17]. If compared with humans, the biological target systems in wild species may play a different physiological role, thus resulting even more sensitive to the effects of a specific compound [9]. An example to this statement is the particular case of the anti-inflammatory diclofenac, which leads to the collapse of local populations of Asian vultures due to renal failure after direct ingestion [18]. Furthermore, the continuous and worldwide increase of PhACs consumption and the consequent input into the environment expose the organisms to mixtures of these substances along their entire lifetime and subsequent generations as well, enhancing the potential biological responses at ecosystem level.

Since freshwater running systems are the primary receptors of discharging WWE, the aquatic ecosystem is expected to be affected by the chronic presence of PhACs. Owing to this fact, the current studies of PhACs in the aquatic environment have focused their interest on two major topics: (i) the description of occurrence and fate and (ii) the investigation of the effects on single species of aquatic organisms [19]. The latter is an approach

based on short-term laboratory exposures that estimates the toxicity of a single compound on single species by measuring the responses of physiological or population-based parameters (i.e., mortality, growth, reproduction, mobility, and metabolism) of the target organism [20]. Over the last decade, several authors reviewed the increasing number of studies assessing the acute toxicity of a wide spectrum of PhACs, on aquatic organisms at different trophic levels [1,21,22]. However, this approach does not reflect the real ecosystem situation, where mixtures of different compounds co-occur and many additional factors and stressors can be present [23] affecting differently any species of the ecosystem community. Advances in the ecological relevance of this kind of ecotoxicological tests have been reached by (i) developing standard procedures to assess toxicity of mixtures of compounds on single species [24,25] and (ii) extrapolation of effects across different levels of biological organization. The latter is achieved by the application of the species sensitivity distribution (SSD) concept, which allows the combination of results from several single-species tests [26,27]. Even though, these approaches may pose the risk of missing the hazardous effects within ecosystems, due to the interactions occurring at the scale of biological communities that cannot be reflected at a lower-tier level [20]. As a matter of fact, the response of organisms in a single-species test might differ from the response of the same organisms in a whole community. For example, Franz et al. [28] studied the acute toxicity of triclosan on algal species suspended, attached, or in periphyton communities and reported differences of three orders of magnitude in the sensitivity to the chemical [28]. Due to this fact, the use of natural microbial communities, directly collected from the river and exposed under controlled conditions to single or mixtures of PhACs, has improved the ecological relevance of laboratory toxicity tests. In particular, the use of fluvial microbial attached communities (epilithic biofilms) allowed the description of direct and indirect effects of multiple stressors on both target and nontarget organisms [29], thus increasing the ecological relevance of the conclusions about the risk associated with chemical pollutants in freshwaters. Several studies assessed responses of natural biofilm communities to PhACs under laboratory-controlled settings [30,31]. The results obtained from these controlled experiments are useful to establish causal relationships between exposure to one or more compounds and responses at community level. These relationships have allowed formulating hypotheses about the upper-scale consequences of PhACs' acute and chronic effects. To summarize, Figure 1 describes the general relationships within the different experimental approaches treated in this introduction to the chapter, in terms of ecological relevance, reproducibility, and establishment of causality in the assessment of the effects of chemical pollutants on river ecosystems.

Despite these improvements, data regarding the effects on the aquatic ecosystems, resulting from chronic exposure of biological communities to PhACs in field situations, are almost lacking, and further investigation is required in

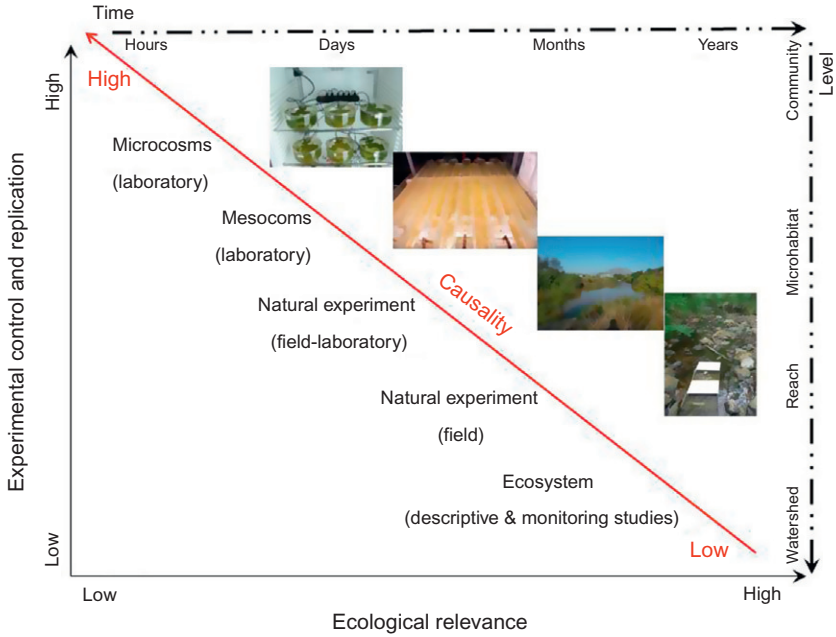

FIGURE 1 Relationship between ecological relevance, experimental control, and replication in fluvial ecology and ecotoxicology experimental approaches. *Modified from Clements and Newman [33].*

order to enhance the ecological relevance of the observed relationships. This chapter aims to review the current literature on field studies assessing the effects of PhACs on fluvial biological communities, highlighting the importance of this approach in the assessment of the risk associated to the presence of these compounds in fresh running waters. The particular case study of the Llobregat river will be presented as strongly PhAC-polluted rivers, where most of the field studies reported in the chapter have been performed.

2 ECOLOGY AND ECOTOXICOLOGY OF BIOLOGICAL COMMUNITIES IN RIVERS

The importance of the use of biological communities in the risk assessment of PhACs' presence in river ecosystems has been mentioned in the introduction of this chapter and will be deeper developed in this section. Community is defined as a group of populations that overlap in time and space, interacting at structural and functional level in both horizontal (within the same trophic level) and vertical (within different trophic levels) directions. The study of community ecology seeks to describe the patterns of the organization and

functioning of communities and to explain the processes that regulate these patterns [32]. In particular, the quantification of the relative importance of biotic and abiotic factors that drive the spatial and temporal variation of the community structure and function is the main concern in the community ecology research [33]. In rivers affected by WWE inputs, the presence of chemical mixtures may be considered as one additional abiotic factor that can modify the relations within fluvial biological communities, either directly or indirectly. The ecotoxicology seeks to investigate the magnitude of the disturbance, generated by the input of potential toxic compounds in the environment, through the measurements of structural and functional responses of the exposed community. These responses of biological communities may be induced directly or indirectly by the toxic compound. Direct effects that normally affect target organisms and are expressed as changes in community structure (as a result of replacement of sensitive species for tolerant ones), alteration of growth rates, alterations of metabolism and physiology, and, ultimately, increase in the death rate are possible. Indirect effects normally affect nontarget organisms via unexpected changes in other trophic levels (e.g., primary producers, consumers, and predators) and are exerted through trophic links [20]. Figure 2 resumes and schematizes the expected direct and indirect effects (and eventual recovery) of toxic compounds on structure and function of simplified community. Geiszinger et al. [20] highlighted the relevance of the community approach to link chemical occurrence to biological responses in pollution assessment. They described how toxic effects at different trophic levels may be transferred both up and down through a complex structured community. For example, a toxic effect on primary producers (normally the lowest trophic level) can lead indirectly to an effect on consumers and predators (at a higher trophic level). In similar way, a toxic effect on consumers or predators may lead to increased primary producer biomass therefore producing relevant effects in the ecosystem functioning [20].

In this framework, the following sections aim to review the current literature about the potential effects of PhACs on the community commonly located at the bottom and the top of river food chain (i.e., microbial and fish communities).

3 EFFECTS ON BIOLOGICAL COMMUNITIES

In this section, an exhaustive review of the current literature about the effects of PhACs on biological communities, assessed trough field studies, will be presented. In general, the most relevant number of studies was performed by investigating the effects on microbial assemblages (particularly focusing on antibiotics) and fish (particularly focusing on endocrine disruptors and hormones). For this reason, this section has been divided in the two following subsections.

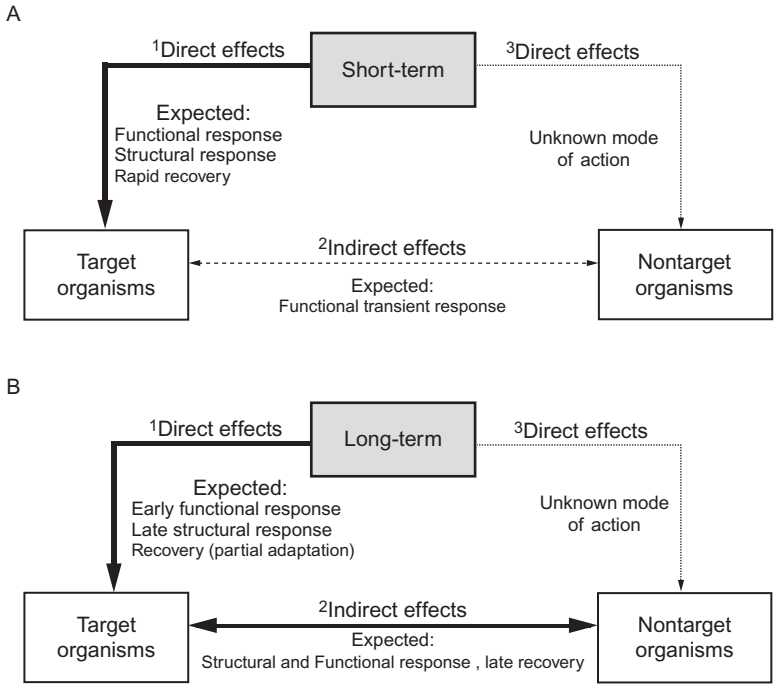

FIGURE 2 Schematic representation of the expected direct and indirect effects of sublethal concentrations of toxic compound/s on target and nontarget organisms of biological communities in case of (A) short-term and (B) long-term exposure. In the case of short-term exposure (A), direct effects of target organisms ([1]) are expected to be the most important ones. In particular, rapid effect and recovery of functional response would be expected. Some structural response could also occur, as well as some direct effect on nontarget organisms ([3]) due to unknown mode of action. These effects can also generate an indirect effect on target organisms. The magnitude of these effects is expected to be less relevant than direct ones. Indirect effects ([2]) are expected to be transient and mainly on function. In the case of long-term exposure (B), direct ([1]) and indirect ([2]) effects are expected to occur. In particular, target organisms would respond quickly in terms of function and later at structural level ([1]). The recovery of these effects is expected to be partial, depending on the magnitude of the response. For example, exposure to some bactericide could result in an initial negative effect on some function sustained by bacteria (i.e., extracellular enzymatic activity). Nevertheless, if exposure persisted, some resistant species are expected to be selected. This selection will result in shift of community composition (structural response). The structural response may therefore restore previous functional levels resulting in a general recovery of functional parameters. Nevertheless, the structural response (shift in community composition) could not be considered recovered until the original community will not be restored or even an adapted community will establish. The occurrence of indirect effects ([2]) is expected to be delayed with respect to direct ones. Thus, the structural and functional response of nontarget organisms will occur after the target organisms responded. The magnitude and delay of indirect effects depend on the interaction with the target organisms directly affected and on physiology, metabolism, and life cycle of the nontarget organism. The recovery of these indirect effects mainly depends on resilience potential of nontarget organisms and the magnitude and the duration of the direct effect observed that generated it. Direct effects on nontarget organisms ([3]) could also occur in consequence of some unknown mode of action. These effects can also generate an indirect effect on target organisms. The magnitude of these effects is expected to be less relevant than direct ones. *Figure has been modified from Proia et al. [65].*

3.1 Effects on Microbial Communities

Microbial communities are among the most studied assemblages in the assessment of the effects of chronic PhACs' presence in river waters. In particular, antibiotics have been widely studied in the last years, since they can act as an ecological factor in the environment that could potentially affect microbial communities [34]. The effects of antibiotics include phylogenetic structure alteration, resistance expansion, and ecological function disturbance in the microecosystem. In the last decade, an increasing number of studies investigated the relationship between antibiotic input and bacterial resistance expansion in freshwaters. In fact, antibiotic resistance represents a growing global health concern due to the overuse and misuse of antibiotics [35]. Numerous studies have detected changes of microbial community structure upon addition of antibiotics in water environment. The most common change detected in these studies is the development of antibiotic-resistant organisms. Ash et al. [36] isolated antibiotic-resistant bacteria in freshwater samples from 16 US rivers and measured the prevalence of organisms resistant to β-lactam and non-β-lactam antibiotics. Another study quantified the occurrence of antibiotic-resistant genes (ARGs) in water and sediment samples collected from a 72 km stretch of the Haihe River in China [37]. The sulfonamide-resistance genes *sul1* and *sul2* were detected at relatively high concentrations in all samples, and the statistical analysis confirmed a positive correlation between the relative abundance of these ARGs and the total concentration of sulfamethoxazole, sulfadiazine, and sulfachloropyridazine. These results suggest that sulfonamides exerted selective pressure for these ARGs [37]. Similar results were found in the study of water and sediments from three water supply reservoirs subjected to a wide pollution gradient. The results showed significant correlation between the presence of ARG-conferring resistance to macrolides and the composition of bacterial communities, suggesting that antibiotic pollution might play a role in the conformation of bacterial communities in reservoir [35].

Antibiotic-resistant organisms mainly enter into water environments from human and animal sources and are able to spread their genes into water-indigenous microbes, which also contain resistance genes [38]. In fact, several studies detected resistant bacteria downstream WWE. For example, Costanzo et al. [39] observed bacteria resistance against six antibiotics in both WWE and receiving surface waters downstream from a sewage discharge. Another study, performed to investigate bacterial community characteristics under long-term antibiotic selection pressures, analyzed water samples from the upstream and the downstream sections of two rivers individually receiving the treated penicillin G and oxytetracycline production farm wastewater [40]. In this work, there were estimated antibiotic resistance ratios of bacterial communities in water samples by culture-based analysis. The results revealed antibiotic resistance to 80 mg mL^{-1} of tested antibiotics in the majority of

bacterial colonies (~55% and 70%) in both downstream rivers and aerobic WWE, while the resistance ratios were less than 10% and 5% for both upstream stretches, respectively. The ability of antibiotic-resistant organisms to spread their genes in the aquatic environment has been confirmed by the detection of resistant bacteria even in drinking waters [41]. The results of this study evidenced the presence of the vancomycin-resistant (*vanA*) and the β-lactam-resistant (*ampC*) genes not only in wastewater biofilms and surface waters but also in drinking water bacterial biofilm communities. The authors concluded about possible mechanism of gene transfer from wastewater bacteria to autochthonous drinking water community [41]. Additionally, the co-occurrence of other stressors could modulate bacterial resistance in polluted aquatic environments. Wright et al. [42] reported an increase of resistance to both metals and antibiotics in stream communities sampled from different microhabitats along a metal pollution gradient when compared with assemblages from reference sites. The authors concluded that metal contamination directly selects for metal-tolerant bacteria while coselecting for antibiotic-resistant bacteria. In parallel, they assessed the transport of antibiotic and metal resistance through a stream network and over time. During a period of 3 months, they monitored the antibiotic and metal resistance patterns in bacteria collected from multiple stream microhabitats: water column, sediment, biofilm, and digestive tracts of *Corbicula fluminea* (Asiatic clam). The results showed that bacteria from biofilm and sediment were the most resistant and bacteria from Corbicula were the least tolerant, respectively. The relevancy of these differences was stressed, since it would be useful to identify reservoirs of resistance and to predict the transfer and transport mechanisms of these genes in metal-contaminated streams [42].

Moreover, effects of antibiotics on ecological functions have also been described, including nitrogen transformation, methanogenesis, and sulfate reduction [34]. Despite numerous studies have described potential effects of antibiotics on microbial communities, the causal relationship between antibiotic input and resistance expansion is still under debate, with evidences either supporting or declining the contribution of antibiotics on alteration of antibiotic resistance [34]. Nevertheless, the evidences presented by the studies reported in this section highlight the potential effect of antibiotic pollution and the presence of ARGs in bacterial communities of freshwater systems, which prompts the fundamental question about potential effects on bacteria-related ecosystem services supplied by these ecosystems [35].

Even though the study of the relation between antibiotics occurrence and bacterial resistance in aquatic environments is largely the most investigated topic in this research field, some works also assessed the effects of other PhACs on different microbial assemblages. In a recent study, the influence of PhACs on stream microbial activity was assessed [43]. Particularly, the occurrence of PhACs in a US headwater stream was measured, and the

changes in sediment respiration and nutrient uptake in response to PhACs were estimated using both *in vitro* and *in situ* techniques. The results of this work showed differences between *in vitro* and *in situ* responses of microbial communities' respiration to the tested PhACs, suggesting that the influence of the PhAC exposure history in stream potentially yielded microbial adaptation and tolerance. Thus, this work evidenced certain effects of PhACs on the activity of microbial communities of stream sediment [43]. Using similar approach, Rosi-Marshall and collaborators [44] measured *in situ* responses of stream biofilms to six common PhACs (caffeine, cimetidine, ciprofloxacin, diphenhydramine, metformin, ranitidine, and a mixture of each one) by deploying PhAC-diffusing substrates in three streams of Indiana, Maryland, and New York (United States). The results of this work clearly demonstrated the effects of the studied PhACs on stream biofilm communities, in each stream and season analyzed. Specifically, algal biomass, measured as chlorophyll *a* concentration, was significantly reduced by the mixture of PhACs, and biofilm respiration was significantly suppressed by caffeine (53%), cimetidine (51%), ciprofloxacin (91%), diphenhydramine (63%), and the mixture (40%). Moreover, gross primary production (GPP) of biofilms was also significantly suppressed by diphenhydramine (99%) and the mixed treatment (88%) in one stream. Pyrosequencing of 16S rRNA genes, used to examine the effects on biofilms at the three sites, revealed that diphenhydramine exposure significantly altered bacterial community composition and resulted in significant relative increases of *Pseudomonas* sp. and decreases of *Flavobacterium* sp. In conclusion, this study indicates that benthic microbial communities in streams are affected by some PhACs, alone or in combination, and that the responses may be common despite the fact that the composition of the biofilm communities may vary among sites and seasons [44].

3.2 Effects on Fish

Fish populations and communities are also among the most studied assemblages in the assessment of the effects of chronic PhACs' presence in river waters. Various effects of PhACs have been documented in fish, but these effects have largely been confined to exposures in the laboratory, often at comparatively high concentrations, and few studies have been undertaken in the field [9]. Laboratory studies have demonstrated the biological effects on fish to be often in accordance with known effects of PhACs in mammals. The observed effect concentrations are usually of one order of magnitude higher than surface waters levels [9]. Despite this, some groups of PhACs are capable to induce biological effects even in the low ng L^{-1} range. In particular, endocrine disruptors and estrogenic active compounds are widely detected in surface waters and may be bioaccumulated in biological matrices (fish tissues), inducing severe biological responses such as alteration of sexual cycles in wild species.

The concept of "endocrine disruption/modulation," referring to those chemicals present in the environment and showing the same or antagonist actions than steroid hormones, was described decades ago [45]. Furthermore, estrogenic effects of discharging WWE on fish were demonstrated in field studies at the end of 1990s [46,47]. In particular, Jobling et al. [47] reported the high incidence of intersexuality in wild populations of riverine fish (roach, *Rutilus rutilus*) throughout the UK rivers. This study demonstrated the widespread sexual disruption in wild populations of fish and related the reproductive disturbances observed with exposure to hormonally active substances discharged into British rivers from WWTPs [47]. Another study, published in the same period, reported the induction of the vitellogenin (VTG) synthesis (a protein synthesized by females during oocyte maturation and, when expressed in males, a biomarker of estrogenic exposure) in caged male trout, placed at various distances downstream of the effluent entry points into five rivers in England [46]. These evidences were confirmed by Desbrow et al. [48] that attributed the feminization of male fishes observed downstream of some wastewater outfalls to the presence of estrogenic substances such as natural estrogens [estrone or 17β-estradiol], the synthetic estrogen used in birth-control pills [17β-ethynylestradiol], or weaker estrogen mimics such as nonylphenol in the water. Using similar approach, Larsson et al. [49] investigated estrogenicity of the WWE by introducing juvenile rainbow trout (*Oncorhynchus mykiss*) in cages downstream of the WWTP. The results of this study showed levels of 17α-ethinyloestradiol exceeding by 45 times the estrogenic levels to fish and reported that all the estrogens detected in WWE were also present in the fish bile. Moreover, VTG was measured in the fish plasma at levels up to 1.5 mg mL^{-1}, thus confirming that the endocrine system of fish exposed to WWE is affected by the widely used synthetic estrogens [49].

More recently, an integrated assessment approach, combining biological and chemical methods, was conducted to investigate the estrogenic potential of WWE and their receiving waters (11 Irish rivers) in male brown trout (*Salmo trutta*). In this work, the induction of VTG in the blood plasma of male fish samples from 8 out of the 11 sampling sites studied was detected [50]. Similarly, Barber et al. [51] investigated the presence of several contaminants (with differing behaviors and biological effects) and their effect on fish (largemouth bass and carp) in a highly sewage-impacted section of the Chicago River (North Shore Channel, Chicago, Illinois). The majority of males in this study exhibited VTG induction, a physiological response related with exposure to the estrogenic compounds detected in river water.

Another recent study assessed whether populations of native walleye (*Sander vitreus*) in the Upper Mississippi River (Minnesota, United States) experienced altered genetic diversity correlated with exposure to estrogenic endocrine active compounds [52]. The results of this work evidenced 4–6 times greater concentrations of VTG in plasma of the individuals sampled

in impacted sites compared to reference ones, thus confirming the acute exposure to estrogenic compounds. However, genetic differences observed among populations were only consistent with geographic distance. In addition to that, no degradation of reproductive organs in individual walleye or alteration in genetic diversity of populations was detected [52].

Vethaak et al. [53] carried out an extensive monitoring on the occurrence of estrogenic compounds in surface water (and other matrices) and on the associated effects on fish in the Netherlands. In this work, almost all studied xenoestrogens in the aquatic environment were detected at low levels, and effects on fish varied depending on the species and the site studied. In the particular case of a small river highly impacted by WWE discharge, there were determined high concentrations of plasma VTG and an increased prevalence of ovotestes in wild male bream. The results of *in vitro* and *in vivo* bioassays, both *in situ* and in the laboratory, confirmed hormones, such as 17α-ethynylestradiol, as one of the main inducers of these effects [53].

Despite the increasing number of studies evidencing the feminization of wild male fish, there is a lack of information about the impact of chronic exposures on the sustainability of wild populations, and the linking between endocrine disruption and reproductive impairment of real fish populations remains, with few exceptions, an open challenge [54].

To get further insight on that issue, Kidd et al. [55] assessed the chronic exposure (7 years) of fathead minnow (*Pimephales promelas*) to low concentrations (5–6 ng L^{-1}) of 17α-ethynylestradiol (EE2), at the Experimental Lakes Area (NW Ontario, Canada). In this study, yet initially, the levels of VTG detected in male fathead minnow were observed to increase up to three orders of magnitude (compared to reference and preaddition samples) after only 7 weeks of estrogen addition. This response was observed in each of the 3 years of EE2 additions. Moreover, testicular tissues of all of the males collected during the first spring after addition displayed delayed spermatogenesis, widespread fibrosis, and malformations of the tubules. This arrested testicular development continued in the following 2 years of addition, and several males captured in the first spring after addition had ovatestes with the presence of primary-stage oocytes. In addition, female fathead minnow also showed early and significant increase of VTG production followed by delayed ovarian development in response to EE2 addition. Moreover, each year subsequent to the start of the addition, there were an increasing number of fish ovaries with atretic follicles, rarely observed in reference and preaddition fish. Finally, the study of population size and structure showed the population collapse because of a loss of young of the year, after the second season of EE2 addition. This reproductive failure was also observed in the third season of amendments and continued for an additional 2 years after the EE2 addition had ceased, causing a near extinction of this species. This study definitively confirmed that the continued inputs of natural and synthetic

estrogens and estrogen mimics to the aquatic environment observed in freshwaters could decrease the reproductive success and sustainability of fish populations impacting the sustainability of wild fish communities [55].

Despite the most clear evidence for the relation between real exposure to PhACs and adverse effect in wild fish is for EE2 [9], bioaccumulation in wild fish tissues has been reported for other classes of PhACs (i.e., antidepressant, antihistaminic, and antihypertensive). Particularly, Ramirez et al. [56] developed a method for the screening of 23 PhACs with different physicochemical properties in fish tissues and detected the antidepressants diphenhydramine, diltiazem, carbamazepine, and norfluoxetine at concentrations ranging between 0.11 and 5.14 ng g^{-1} in all the samples collected from two streams of Texas (United States). The same author reported results of a national pilot study aiming to assess the bioaccumulation of PhACs in fish sampled from five highly sewage-impacted rivers, covering broad geographical areas of the United States [57]. This study confirmed that the antidepressants norfluoxetine, sertraline, diphenhydramine, diltiazem, and carbamazepine bioaccumulated (at ng g^{-1} concentrations) in fish fillet composites and liver tissue. The investigation highlighted that the amount of PhACs bioaccumulated in fish tissue, as well as concentrations and frequency of detection, was lower in those fish samples collected from sampling sites receiving WWE treated with advanced processes after secondary treatment. In view of these observations, the authors suggested that the occurrence of PhACs in fish tissues was subjected to the degree of wastewater treatment applied [57]. A similar study also reported the occurrence of 8 antidepressants (out of the 10 measured) in the brain tissues of white sucker (*Catostomus commersonii*) individuals, sampled in two US streams downstream the WWE discharge point [58]. On the basis of these findings, WWE were pointed out as a source of antidepressants to stream ecosystems, and the selective antidepressant uptake into brain tissue was suggested to occur via exposure of fish to water and/or bed sediment [58].

Environmental effects of PhACs are consistent with high-affinity interactions with conserved targets in affected wildlife species rather than with a general toxic effect. For that reason, the targets conserved along the evolution of species are associated with an increased risk [17]. On the basis of this hypothesis, Guannarson et al. [17] predicted orthologs for 1318 human drug targets in 16 species and analyzed the conservation of different functional categories of targets. Orthologs for 86% of the drug targets were determined in zebra fish, while only 61% and 35% were conserved in daphnia and green algae, respectively. In view of these results, considerations related to evolutionary conservation of human drug targets in aquatic organisms were proposed to be included in aquatic environmental risk assessments for human drugs. As global consumption of PhACs rises, the increasing contamination of surface waters and groundwaters with these biologically active drugs is an inevitable consequence that poses a greater potential for adverse effects

in aquatic wildlife. In view of the continuous population growth and the subsequent ever-increasing discharge of sewage waste on the aquatic systems, these effects are expected to become even more pronounced.

4 THE LLOBREGAT RIVER BASIN: A RELEVANT STUDY CASE

4.1 Study Site

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 watershed is densely populated, with more than 3 million inhabitants living therein. Together with its two main tributaries, the Cardener river and the Anoia river, it is subjected to a heavy anthropogenic pressure (Figure 3). 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

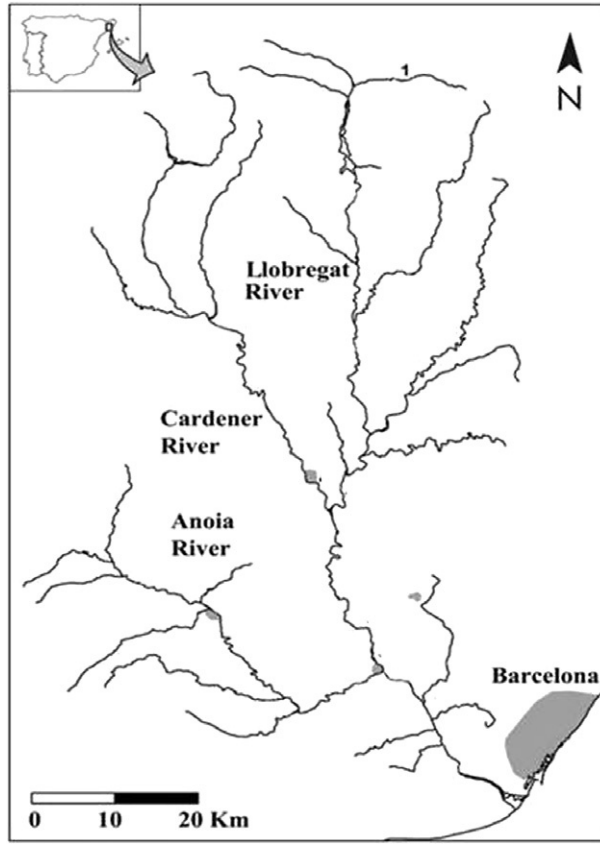

FIGURE 3 The map of the Llobregat river network.

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 basin receives urban and industrial wastewater discharges (137×10^6 m^3 per year, 92% coming from WWTPs) from more than 30 WWTPs (Figure 3), as well as surface runoff from agricultural areas that cannot be efficiently diluted by its natural flow (0.68–6.5 m^3 s^{-1} basal flow). Therefore, this typical Mediterranean river turns into an illustrative example of overexploited river, with high flow variability and heavy chemical contamination being caused by a mixture of natural and human-driven components. As a consequence of these heavy anthropogenic pressures, a huge number of priority and emerging pollutants have been detected in Llobregat surface waters [59]. In fact, this river has been included as a study case of several Spanish and European projects investigating the cause–effect relations of key pollutants on the river biodiversity (i.e., MODELKEY, KEYBIOEFFECTS, VIECO, and SCARCE). Therefore, the Llobregat is probably the river basin where the highest number of studies assessing the effects of PhACs on biological communities has been performed, applying different approaches: from field studies to laboratory-field experiments. For all these reasons, the Llobregat river basin has been selected as the study case of this chapter, and an exhaustive review of the current literature available is reported in following sections.

4.2 Effects on Fluvial Biofilms

Fluvial biofilms are complex microbial benthic communities composed of autotrophic and heterotrophic organisms [60], which act as an interface between the water and the riverbed by interacting and responding rapidly to changes in environmental conditions [61]. Biofilms play a fundamental role in the trophic web and in the biogeochemical cycles within aquatic ecosystems [62,63]. The short life cycle of biofilm microorganisms and the trophic interactions among the microbiota (algae, bacteria, fungi, and protozoa) allow for the detection both of short- and long-term and of direct and indirect effects on the community [64]. Biofilms integrate a variety of responses to chemical stressors, and the effects of dissolved and particulate materials can be expressed as variations of community structure and function. River biofilms can therefore be useful in determining the effects of bioactive compounds (such as PhACs) on freshwater ecosystems [61]. For these reasons, these communities have been widely used to assess potential toxicity of several PhACs in controlled exposures to single compounds [30,31].

Moreover, fluvial biofilms have been used to assess effects of pesticides, metals, and industrial discharge in field studies [65–67]. In the Llobregat river basin, Ricart et al. [68] examined the presence of pesticides and their effects on benthic biological communities. The results of this study revealed, through multivariate analyses, a potential relationship between triazine-type herbicides

and the distribution of the diatom community, as well as certain effects of organophosphates and phenylureas on both structural and functional parameters of the biofilm community [68]. Similarly, Muñoz et al. [69] explored potential relationships between the presence of PhACs and the structural composition of benthic diatom community structure. This investigation reported the characteristics of diatom communities of a perturbed fluvial system with a general decrease of species richness downstream and prevalent abundance of the most tolerant species to organic and chemical pollution. Nevertheless, the statistical analysis tool applied did not confirm any significant correlation between diatom community composition and PhACs variables [69].

By using similar statistical approach, Proia et al. [29] investigated the effects of PhACs and pesticides detected in Llobregat river, on fluvial biofilms by means of translocation experiments performed under controlled conditions. This study detected 57 PhACs in surface river water and revealed structural and functional responses on fluvial biofilms translocated from less to more polluted sites. In particular, autotrophic biomass, measured as chlorophyll *a* density, and extracellular peptidase activity increased, while extracellular phosphatase activity and phosphorus uptake capacity of fluvial biofilm decreased in response to translocation. The multivariate analysis demonstrated that analgesics and anti-inflammatories significantly affected biofilm responses. Particularly, three compounds of this therapeutic class explained a significant percentage of the variance in biofilm responses to translocation: diclofenac, paracetamol, and ibuprofen. Ibuprofen and paracetamol were associated with negative effects on photosynthesis and with the decrease of the green algae–cyanobacteria ratio, while diclofenac was associated with phosphatase activity. This work concluded that, although causality between PhACs occurrence and biological responses cannot be established by this experimental approach, potential effects of real mixtures of these emerging compounds on biofilms structure and function may be reflected in important alterations of river ecosystem [29].

Another recently published study used similar experimental approach and focused on the effects of antibiotics on biofilm bacterial communities in the Llobregat river [70]. This study detected 16 antibiotic compounds in surface river water and revealed structural differences in the bacterial communities of fluvial biofilms grown in waters with different antibiotics concentrations. In particular, the Actinobacteria group was shown to be more abundant in fluvial biofilm growth at higher antibiotics concentrations, and canonical correspondence analysis confirmed this trend. Nine days after switching the biofilms to more polluted waters, the bacterial community structure changed, confirming a general increase of the Actinobacteria abundances at higher antibiotic concentrations. Furthermore, results of this study also showed significant functional responses of fluvial biofilm bacterial communities to the switch from less to more polluted waters. Particularly, the switched biofilms showed changes in the extracellular enzymatic activities and a significant

increase in bacterial mortality; both responses were statistically correlated with the antibiotic concentrations in surface water. In conclusion, the magnitude of the bacterial community responses observed in this study was associated with the local levels of antibiotics detected, therefore demonstrating that the continuous entrance of these compounds in running waters may cause significant structural and functional changes in microbial attached communities. As these communities dominate the metabolism of most river ecosystems and are a major component for the uptake, storage, and cycling of nutrients, the authors suggested that these changes may have consequences in terms of loss of biodiversity and alteration of biogeochemical cycles at ecosystem level. The study finally remarked that although polluted rivers are affected by many co-occurring factors, the presence of antibiotics in urban areas must be considered as a relevant risk factor for bacterial biofilm communities in freshwater ecosystems [70].

Finally, another study performed by the authors of this chapter [71] evaluates the effects of flow changes on the concentration of PhACs and explored the relationships among these two factors and biofilm communities' responses in Llobregat river. The results of this study showed transient dilution of PhACs and reduced biofilm growth rate and extracellular peptidase activity after a flash flood event. Moreover, an increase of the bacterial mortality in biofilms translocated from less to more polluted site was confirmed in this investigation. The conclusions of the work suggest that flood events may alter the relationship between PhAC concentrations and biofilm responses, implying significant changes in the chemical and biological status of the river.

4.3 Effects on Macroinvertebrate Communities

Two important studies were carried out to assess the effects of PhACs detected in the Llobregat river waters on macroinvertebrate community: the first one [69] performed by monitoring approach and the second one [72] using translocation of collected communities and multibiomarker approach.

Within the framework of the assessment of PhACs effects on biological communities, Muñoz et al. [69] aimed to analyze the occurrence of PhACs in the Llobregat river water and to find potential relationships between the presence of PhACs and the structural composition (changes in abundance and biomass) of the invertebrates community. Communities of invertebrates observed in this study were those typical of a perturbed fluvial system, with a general decrease of species richness downstream and prevalent abundance of the species more tolerant to organic and chemical pollution. Regarding individual compounds, a significant correlation was observed between invertebrate abundance and the concentrations of indomethacin and propranolol. A significant correlation was observed with temperature, as well. Invertebrate biomass also showed a significant correlation with the concentrations of ibuprofen, atenolol, and propranolol. The redundancy analysis (RDA) determined

that the concentrations of some anti-inflammatories and the β-blocker propranolol, as well as temperature, explained 71% of the taxonomic variance in invertebrate density. Furthermore, the results of two different multivariate analyses confirmed that sites with higher concentrations of anti-inflammatories and β-blockers and higher temperatures were characterized by a greater abundance and biomass of *Chironomus* spp. and *Tubifex tubifex*. The authors highlighted that the correlational findings also could be the result of cumulative or synergistic effects caused by several stressors that co-occur in the system since PhACs are present in the aquatic environment as a mixture with other pollutants. However, conclusions of the study revealed a potential relationship between the concentrations of a number of PhACs and the abundance and biomass of several key benthic invertebrates [69].

The objectives of the second study were to link the presence of PhACs in the aquatic system to the observed responses of different invertebrate species [72]. The investigation addressed whether the use of short- and long-term responses of field-collected and transplanted macroinvertebrate species would possibly assess detrimental effects of PhACs and other pollutants in the field, by using a multibiomarker approach and multiple species. Particularly, there were assessed and compared individual and biochemical responses of two abundant benthic macroinvertebrates caddis fly larvae (of the species *H. exocellata* and the amphipod *Echinogammarus longisetosus*) and the laboratory test species *D. magna* that were transplanted along three sites of Llobregat river following a pollution gradient. Relevant individual and biochemical responses were observed in field-collected, transplanted, and laboratory species. In particular, the three species showed a clear impact across the studied pollution gradient, which was indicated by higher levels of feeding inhibition and of mortality toward lower reaches. Interestingly, the presence of enhanced levels of detoxification proteins and enzyme activities measured in this study is a common phenomenon in other species adapted to live in polluted environments. Nevertheless, the field-collected and transplanted organisms showed similar levels of tissue oxidative damage (i.e., lipid peroxidation), evidencing that deployments were long enough to detect detrimental effects of pollution in the studied species. In conclusion, the authors underlined that measured levels of pesticides, salinity, and ammonia had greater effects on the target species, rather than PhACs. Nevertheless, they finally remarked that the set of biomarkers deployed did not include biochemical responses targeted to evaluate specific effects of PhACs, and therefore, future research should be focused on developing specific biomarkers of PhACs effects in more aquatic species [72].

4.4 Effects on Fish

Several studies have investigated the effects of endocrine disruptors on fish communities of Llobregat river. Some of them assessing the specific effects

of PhACs demonstrated PhACs as the cause of endocrine dysfunction in fish species (i.e., EE2). As mentioned in the Section 3.2 of this chapter, the presence of the female-specific yolk protein precursor VTG in blood and liver from male fish is widely used as an indicator of endocrine disruption. García-Reyero et al. [73] studied the induction of VTG mRNA in liver from several species of fish, both maintained in fish tanks or captured in the wild. The results of this study showed increased levels of VTG mRNA in males of a population of common carp (*Cyprinus carpio*) from the Anoia river, a tributary of the Llobregat river, well known for its high levels of estrogenic contaminants [74–76]. Although the authors mainly indicated the alkylphenols as the most probable responsible of the induction of this transcript VTG mRNA in carps, some additional effect of PhACs detected in such a sewage-impacted river could not be discarded. Nevertheless, it was highlighted that the use of mRNA quantitation techniques for analysis of feral and cultured fish of different species would allow to detect and control more precisely the noxious effects of contaminants on the local fauna exposed to them [73]. Another study performed in the same river also reported feral carp exhibiting gross gonad abnormalities, altered plasmatic and hepatic VTG content, and depleted plasma sex steroids [75]. This study suggested that these carps had probably been exposed to estrogenic compounds throughout their lives and concluded that while VTG increase is a transient character, the development of gross abnormalities is a permanent feature in cyprinids [77,78] that usually confirms exposure to estrogenic compounds during sensitive early stages of development [75].

A subsequent study aiming the assessment of combined effect xenoestrogens (and POPs) on the reproduction of female carp was published a few years later [79]. Adult female carps (*C. carpio*) were collected from three sites of the Cardener river (a tributary of the Llobregat): one upstream and two downstream the SWE input. Results of this study did not show any significant variation of plasmatic and liver VTG content nor sex hormone alterations in downstream carps. However, the authors suggested that the apparent lack of estrogenic disturbances and effects could be due either to level of xenoestrogens, being not high enough to cause estrogenicity, or to the combined presence of xenobiotic and antagonistic effects of other compounds, which would mask any clear response. They finally concluded that in this moderately polluted environment, biological fluctuations seem to override any possible xenobiotic effects [79].

As a matter of fact, a more comprehensive study investigating the entire reproductive cycle (from the beginning of winter to the end of summer) of carp was carried out in order to confirm estrogenicity in this area and to correlate the chemical data and observed biological effects [76]. In this work, the influents, effluents, and sludge from four WWTPs, water, sediment, and feral carps from the Anoia and Cardener rivers (upstream and downstream of SWE discharge) were collected and systematically analyzed over a 7-month period

in order to study the temporal variation of endocrine disruptors and their effects on carp. The main objective was to study (*in situ*) the effects of WWE on the reproductive physiology of feral carp using (i) plasma VTG concentration as a biomarker of exposure to estrogenic compounds and (ii) indices of fish sexual disruption in both male and female carps. The VTG levels fluctuated among sites and sampling periods and were observed to increase downstream of the WWE discharges, if compared with those upstream the river. Moreover, a correlation between VTG concentrations in plasma of male carp and levels of some estrogenic compounds in river water (estriol and estrone) was observed. The author suggested that changes detected at a biochemical (VTG increase) or histological (gonad abnormalities) level would have consequences on population or community because of the reduced fecundity and viability. However, they concluded that some contribution of other estrogens (natural and/or synthetic) could not be discarded since the levels present in receiving waters (although below our detection limits) could be enough to somehow contribute to the estrogenicity observed in fish [76].

More recently, Figuerola et al. [80] studied the population of Iberian red fin barbel population (*Barbus haasi*) in a small tributary of the Llobregat river (Vallvidrera creek), in order to determine the effects of habitat and water quality at population (size–age structure, fish density, and biomass) and individual level (body condition). An intensive and extensive sampling of fish was carried out along a pronounced pollution gradient determined by WWE inputs. Although this study did not assess directly the potential effects of PhACs on fish community, the results revealed that water quality was the most significant environmental driver for this fish population. In particular, fish density decreases and fish length increases in those sites exposed to WWE. This investigation concluded that the chronic exposure to the effluents from a WWTP was responsible for the deleterious effects detected on a population of *B. haasi* in Vallvidrera creek [80].

REFERENCES

[1] K. Fent, A. Weston, D. Caminada, Aquat. Toxicol. 76 (2006) 122–159.
[2] D.M. Whitacre (Ed.), Reviews of Environmental Contamination and Toxicology, vol. 202, Springer Science, New York, 2010.
[3] S.K. Khetan, T.J. Collins, Chem. Rev. 107 (2007) 2319–2364.
[4] A. Jelic, M. Gros, A. Ginebreda, R. Cespedes-Sánchez, F. Ventura, M. Petrovic, D. Barceló, Water Res. 45 (3) (2011) 1165–1176.
[5] K.M. Onesios, J.T. Yu, E.J. Bower, Biodegradation 20 (2009) 441–466.
[6] K.K. Barnes, D.W. Kolpin, E.T. Furlong, S.D. Zaugg, M.T. Meyer, L.B. Barber, Sci. Total Environ. 402 (2008) 192–200.
[7] M. Gros, M. Petrovic, D. Barceló, Environ. Chem. 26 (8) (2007) 1553–1562.
[8] V. Osorio, S. Pérez, A. Ginebreda, D. Barceló, Environ. Sci. Pollut. Res. 440 (2012) 3–13.
[9] J. Corcoran, M.J. Winter, C.R. Tyler, Crit. Rev. Toxicol. 40 (4) (2010) 287–304.

[10] M. Winker, F. Tettenborn, D. Faika, H. Gulyas, R. Otterpohl, Water Res. 42 (2008) 3633–3640.
[11] W.W. Buchberger, J. Chromatogr. A 1218 (2011) 603.
[12] M. Farré, L. Kantiani, M. Petrovic, S. Perez, D. Barceló, J. Chromatogr. A 1259 (2012) 86–99.
[13] R. López-Serna, M. Petrovíc, D. Barceló, J. Chromatogr. A 1252 (2012) 115–129.
[14] S. Huntscha, H.P. Singer, C.S. McArdell, C.E. Frank, J. Hollender, J. Chromatogr. A 1268 (2012) 74–83.
[15] M.J. Benotti, R.A. Trenholm, B.J. Vanderford, J.C. Holady, B.D. Standford, S.A. Snyder, Environ. Sci. Technol. 43 (2009) 597–603.
[16] M. Huerta-Fontela, M.T. Galceran, F. Ventura, Water Res. 45 (2011) 1432–1442.
[17] L. Guannarson, A. Jauhiaien, E. Kristiansson, O. Nerman, D.G.J. Larsson, Environ. Sci. Technol. 42 (2008) 5807–5813.
[18] J.L. Oaks, M. Gilbert, M.Z. Virani, R.T. Watson, C.U. Meteyer, B.A. Rideout, H.L. Shivaprasad, S. Ahmed, M.J.I. Chaudhry, M. Arshad, S. Mahmood, A. Ali, A.A. Khan, Nature 427 (6975) (2004) 630–633.
[19] E.J. Rosi-Marshall, T.V. Royer, Ecosystems 15 (2012) 867–880.
[20] A. Geiszinger, C. Bonnineau, L. Faggiano, H. Guasch, J.C. López-Doval, L. Proia, M. Ricart, F. Ricciardi, A. Romaní, S. Rotter, I. Muñoz, M. Schmitt-Jansen, S. Sabater, Trends Anal. Chem. 28 (5) (2009) 619–626.
[21] M. Farré, S. Pérez, L. Kantiani, D. Barceló, Trends Anal. Chem. 27 (11) (2008) 991–1007.
[22] P.K. Jjemba, Ecotoxicol. Environ. Saf. 63 (2006) 113–130.
[23] A. Ginebreda, I. Muñoz, M. López de Alda, R. Brix, J. López-Doval, D. Barceló, Environ. Int. 36 (2010) 153–162.
[24] M. Cleuvers, Toxicol. Lett. 142 (2003) 185–194.
[25] S.M. Richards, C.J. Wilson, D.J. Johnson, D.M. Castle, M. Lam, S.A. Mabury, P.K. Sibely, K. Solomon, Environ. Toxicol. Chem. 23 (4) (2004) 1035–1042.
[26] L. Posthuma, T.P. Traas, G.W. Suter II. (Eds.), Species-Sensitivity Distributions in Ecotoxicology, Lewis, Boca Raton, FL, 2002.
[27] P.J. van der Brink, Environ. Sci. Technol. 42 (2008) 8999–9004.
[28] S. Franz, R. Altenburgerm, H. Heilmeier, M. Schmitt-Jansen, Aquat. Toxicol. 90 (2008) 102–108.
[29] L. Proia, V. Osorio, S. Soley, M. Köck-Schulmeyer, S. Pérez, D. Barceló, A.M. Romaní, S. Sabater, Environ. Pollut. 178 (2013) 220–228.
[30] C. Bonnineau, H. Guasch, L. Proia, M. Ricart, A. Geiszinger, A.M. Romaní, S. Sabater, Aquat. Toxicol. 96 (2010) 225–233.
[31] J.R. Lawrence, G.D.W. Swerhone, L.I. Wassenaar, T.R. Neu, Can. J. Microbiol. 51 (2005) 655–669.
[32] P.W. Price, C.N. Slobadchikoff, W.S. Gaud (Eds.), A New Ecology: Novel Approaches to Interactive Systems, John Wiley & Sons, New York, NY, 1984.
[33] W.H. Clements, M.C. Newman, Community Ecotoxicology, Wiley, Chichester, 2002.
[34] C. Ding, J. He, Appl. Microbiol. Biotechnol. 87 (2010) 925–941.
[35] B. Huerta, E. Marti, M. Gros, P. López, M. Pompêo, J. Armengol, D. Barceló, J.L. Balcázar, S. Rodríguez-Mozaz, R. Marcé, Sci. Total Environ. 456–457 (2013) 161–170.
[36] R.J. Ash, B. Mauck, M. Morgan, Emerg. Infect. Dis. 8 (7) (2002) 713–716.
[37] Y. Luo, D. Mao, M. Rysz, Q. Zhou, H. Zhang, L. Xu, P.J.J. Alvarez, Environ. Sci. Technol. 44 (2010) 7220–7225.
[38] F. Baquero, J.L. Martínez, R. Cantó, Curr. Opin. Biotechnol. 19 (2008) 260–265.
[39] S.D. Costanzo, J. Murby, J. Bates, Mar. Pollut. Bull. 51 (2005) 218–223.

[40] D. Li, R. Qi, M. Yang, Y. Zhang, T. Yu, Water Res. 45 (2011) 6063–6073.
[41] T. Schwartz, W. Kohnen, B. Jansen, U. Obst, FEMS Microbiol. Ecol. 43 (2003) 325–335.
[42] M.S. Wright, G.L. Peltier, R. Stepanauskas, J.V. McArthur, FEMS Microbiol. Ecol. 58 (2006) 293–302.
[43] A.R. Bunch, M.J. Bernot, Ecotoxicology 20 (2011) 97–109.
[44] E.J. Rosi-Marshall, D.W. Kincaid, H.A. BEchtold, T.V. Royer, M. Rojas, J.J. Kelly, Ecol. Appl. 23 (3) (2013) 583–593.
[45] T. Colborn, F.S. vom Saal, A.M. Soto, Environ. Health Perspect. 101 (1993) 378–384.
[46] J.E. Harries, D.A. Sheahan, S. Jobling, P. Matthiessen, P. Neall, J.P. Sumpter, T. Tylor, N. Zaman, Environ. Toxicol. Chem. 16 (3) (1997) 534–542.
[47] S. Jobling, M. Nolan, C.R. Tyler, G. Brighty, J.P. Sumpter, Environ. Sci. Technol. 32 (1998) 2498–2506.
[48] C. Desbrow, E.J. Routledge, G.C. Brighty, J.P. Sumpter, M. Waldock, Environ. Sci. Technol. 32 (11) (1998) 1550–1558.
[49] D.G.J. Larsson, M. Adolfsson-Erici, J. Parkkonen, M. Pettersson, A.H. Berg, P.E. Olsson, L. Förlin, Aquat. Toxicol. 45 (1999) 91–97.
[50] M.A. Kelly, A.M. Reid, K.M. Quinn-Hosey, A.M. Fogarty, J.J. Roche, C.A. Brougham, Ecotoxicol. Environ. Saf. 73 (2010) 1658–1665.
[51] L.B. Barber, G.K. Brown, T.G. Nettesheim, E.W. Murphy, S.E. Bartell, H.L. Schoenfuss, Sci. Total Environ. 409 (2011) 4720–4728.
[52] L.M. Miller, S.E. Bartell, H.L. Schoenfuss, Arch. Environ. Contam. Toxicol. 62 (2012) 657–671.
[53] A.D. Vethaak, J. Lahr, S. Marca Schrap, A.C. Belfroid, G.B.J. Rijs, A. Gerritsen, J. de Boer, A.S. Bulder, G.C.M. Grinwis, R.V. Kuiper, J. Legler, T.A.J. Murk, W. Peijnenburg, H.J.M. Verhaar, P. de Voogt, Chemosphere 59 (2005) 511–524.
[54] L.J. Mills, C. Chichester, Sci. Total Environ. 343 (2005) 1–34.
[55] K.A. Kidd, P.J. Blanchfield, K.H. Mills, V.P. Palace, R.E. Evans, J.M. Lazorchak, R.W. Flick, PNAS 104 (21) (2007) 8897–8901.
[56] A.J. Ramirez, M.A. Mottaleb, B.W. Brooks, C.K. Chambliss, Anal. Chem. 79 (2007) 3155–3163.
[57] A.J. Ramirez, R.A. Brain, S. Usenko, M.A. Mottaleb, J.G. O'Donnel, L.L. Stahl, J.B. Wathen, B.D. Snyder, J.L. Pitt, P. Perez-Hurtado, L.L. Dobbins, B.W. Brooks, C.K. Chambliss, Environ. Toxicol. Chem. 28 (12) (2009) 2587–2597.
[58] M.M. Schultz, E.T. Furlong, D.W. Koplin, S.L. Werner, H.L. Schoenfuss, L.B. Barber, V.S. Blazer, D.O. Norris, A.M. Vajda, Environ. Sci. Technol. 44 (2010) 1918–1925.
[59] S. Sabater, A. Ginebreda, D. Barceló (Eds.), The Llobregat: The Story of a Polluted Mediterranean River, The Handbook of Environmental Chemistry, vol. 21, Springer Verlag, Berlin, Heidelberg, 2012.
[60] S. Dürr, J.C. Thomason (Eds.), Biofouling, Wiley-Blackwell, Oxford, 2010.
[61] S. Sabater, H. Guasch, M. Ricart, A.M. Romaní, G. Vidal, C. Klünder, M. Schmitt-Jansen, Anal. Bioanal. Chem. 387 (2007) 1425–1434.
[62] T.J. Battin, L.A. Kaplan, J.D. Newbold, C.M.E. Hansen, Nature 426 (6965) (2003) 439–442.
[63] T.E. Fored (Ed.), Aquatic Microbiology: An Ecological Approach, Blackwell Scientific Publications, Oxford, 1993.
[64] L. Proia, F. Cassiò, C. Pascoal, A. Tlili, A.M. Romaní, in: H. Guasch, A. Ginebreda, A. Geiszinger (Eds.), Emerging and Priority Pollutants in Rivers: Bringing Science into River Management Plans, The Handbook of Environmental Chemistry, Springer Verlag, Berlin, Heidelberg, 2012.

[65] S. Morin, A. Cordonier, I. Lavoie, A. Arini, S. Blanco, T.T. Duong, E. Tornés, B. Bonet, N. Corcoll, L. Faggiano, M. Laviale, F. Pérès, E. Becares, M. Coste, A. Feurtet-Mazel, C. Fortin, H. Guasch, S. Sabater, in: H. Guasch, A. Ginebreda, A. Geiszinger (Eds.), Emerging and Priority Pollutants in Rivers: Bringing Science into River Management Plans, The Handbook of Environmental Chemistry, Springer Verlag, Berlin, Heidelberg, 2012.
[66] A. Tlili, A. Bérard, J. Rouliera, B. Volata, B. Montuelle, Aquat. Toxicol. 98 (2010) 165–177.
[67] S.M. Victoria, N. Gómez, Sci. Total Environ. 408 (2010) 2696–2705.
[68] M. Ricart, H. Guasch, D. Barceló, R. Brix, M.H. Conceição, A. Geiszinger, M.J. López de Alda, J.C. López-Doval, I. Muñoz, C. Postigo, A.M. Romaní, M. Villagrasa, S. Sabater, J. Hydrol. 383 (2010) 52–61.
[69] I. Muñoz, J.C. López-Doval, M. Ricart, M. Villagrasa, R. Brix, A. Geiszinger, A. Ginebreda, H. Guasch, M.J. López de Alda, A.M. Romaní, S. Sabater, D. Barceló, Environ. Toxicol. Chem. 28 (12) (2009) 2706–2714.
[70] L. Proia, G. Lupini, V. Osorio, S. Pérez, D. Barceló, T. Schwartz, S. Amalfitano, S. Fazi, A.M. Romaní, S. Sabater, Chemosphere 92 (9) (2013) 1126–1135.http://dx.doi.org/10.1016/j.chemosphere.2013.01.063.
[71] V. Osorio, L. Proia, M. Ricart, S. Pérez, A. Ginebreda, J.L. Cortina, S. Sabater, D. Barceló. Sci. Total Environ., submitted for publication.
[72] J. Damásio, D. Barceló, R. Brix, C. Postigo, M. Gros, M. Petrovic, S. Sabater, H. Guasch, M. Lopez de Alda, C. Barata, Chemosphere 85 (2011) 1548–1554.
[73] N. García-Reyero, D. Raldúa, L. Quirós, G. Llaveria, J. Cerdà, D. Barceló, J.O. Grimalt, B. Piña, Anal. Bioanal. Chem. 378 (2004) 670–675.
[74] N. García-Reyero, E. Grau, M. Castillo, M.J. López de Alda, D. Barceló, B. Piña, Environ. Toxicol. Chem. 20 (6) (2001) 1152–1158.
[75] M. Solé, D. Raldúa, F. Piferrer, D. Barceló, C. Porte, Comp. Biochem. Physiol. C: Toxicol. Pharmacol. 136 (2003) 145–156.
[76] M. Petrovic, M. Solé, M.J. López de Alda, D. Barceló, Environ. Toxicol. Chem. 21 (10) (2002) 2146–2156.
[77] S. Gimeno, H. Komen, A.G.M. Gerritsen, T. Bowmer, Aquat. Toxicol. 43 (1998) 77–92.
[78] T.P. Rodgers-Gray, S. Jobling, C. Kelly, S. Morris, G. Brighty, M.J. Waldock, J.P. Sumpter, C.R. Tyler, Environ. Sci. Technol. 34 (2001) 1521–1528.
[79] M. Solé, D. Raldúa, D. Barceló, C. Porte, Ecotoxicol. Environ. Saf. 56 (2003) 373–380.
[80] B. Figuerola, A. Maceda-Veiga, A. De Sosto, Hydrobiologia 694 (2012) 75–86.

Chapter 19

Antibiotic Resistance in the Aquatic Environment

Elisabet Marti and Jose Luis Balcázar
Catalan Institute for Water Research (ICRA), Girona, Spain

Chapter Outline

1 INTRODUCTION

Following the discovery and introduction of penicillin in the 1940s, antibiotics have been widely used in human and veterinary medicine to prevent or treat infections as well as growth promoters in animal husbandry. However, overuse and misuse of these antimicrobial drugs have led to the emergence of antibiotic-resistant bacteria. The emergence of this phenomenon has revealed multiple and complex mechanisms by which antibiotic resistance arises and spreads among bacteria of the same species or even among different species. There are several resistance mechanisms; the most important among them are (i) exclusion of the antibiotic by the cell membrane, (ii) intracellular modification and/or deactivation of the antibiotic, (iii) reduction in sensitivity of the cellular target, (iv) extrusion from the cell, and (v) intracellular sequestration [1]. Susceptible bacteria may become resistant to antibiotics through mutation and selection or by acquiring from other bacteria the genetic information that encodes resistance. The last event may be acquired through horizontal gene transfer (HGT; Figure 1), which is largely, although not exclusively, responsible for the development of

Comprehensive Analytical Chemistry, Vol. 62. http://dx.doi.org/10.1016/B978-0-444-62657-8.00019-7

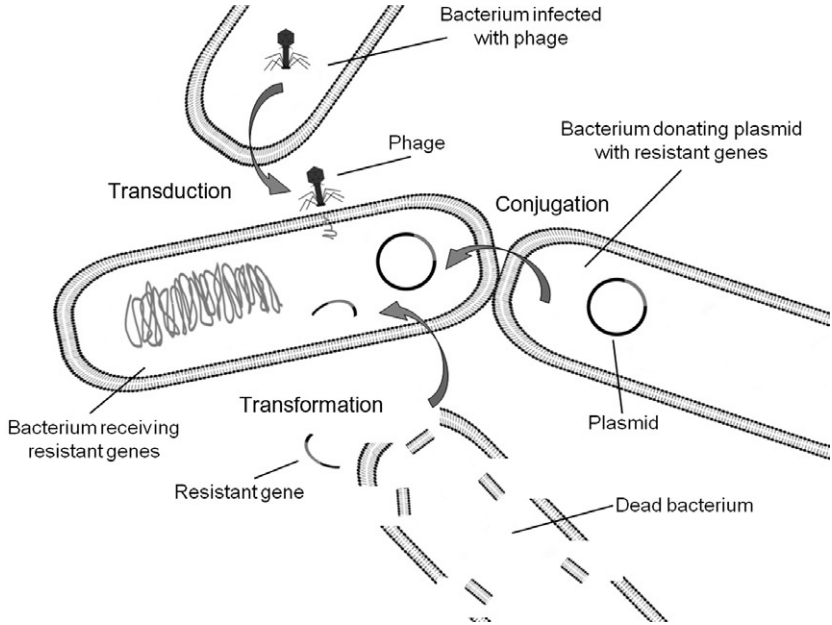

FIGURE 1 Mechanisms of HGT in bacteria. Exogenous nucleic acids can be obtained via cell–cell contact with other bacteria (conjugation), directly from the environment as naked DNA (transformation) or via phages (transduction). *Adapted from Taylor et al. [1].*

antibiotic-resistant bacteria through various processes such as conjugation, transduction, and transformation [2,3].

Although antibiotic resistance is a major and growing human health concern, it has been largely overlooked in environmental settings. This is probably due to the fact that antibiotics in nonclinical settings are generally found at very low concentrations. However, recent studies suggest that selection of resistant bacteria can occur at extremely low antibiotic concentrations, similar to those concentrations that can be found in some aquatic and soil environments [4,5]. Moreover, anthropogenic activities such as sewage treatment, animal husbandry, agriculture, and aquaculture practices may contribute to the spread of antibiotic resistance due to the release of residual antibiotic compounds, antibiotic-resistant bacteria, and antibiotic resistance genes (ARGs) into the environment. Given this, aquatic environments provide ideal settings for the horizontal exchange of mobile genetic elements encoding antibiotic resistance.

Because conventional characterization of antibiotic-resistant bacteria has depended on culture-based techniques, our understanding of antibiotic resistance mechanisms has been restricted to those that can be cultured. However, the advent of high-throughput technologies, such as DNA microarrays and next-generation sequencing, opens the door for comprehensive studies on

antibiotic resistance in bacterial communities. These approaches permit the elucidation of the molecular mechanisms of antibiotic resistance and how bacterial communities respond to the presence of antibiotics.

This chapter aims to describe the current knowledge on the emergence and spread of antibiotic resistance in the aquatic environment, with special emphasis on the role of ARGs, including the diversity and prevalence of these genes in aquatic bacteria (aquatic antibiotic resistome). Understanding sources and mechanisms of antibiotic resistance is critical for developing effective strategies for reducing their impact on the public and environmental health.

2 ACQUISITION OF ARGs IN AQUATIC ECOSYSTEMS AND DEVELOPMENT OF ANTIBIOTIC RESISTANCE

Gene acquisition by HGT results from the successful transfer of genetic material followed by vertical inheritance of the transferred genetic material throughout the generations. The presence of certain physical barriers to transfer, as well as different selective forces over the transferred genes, may explain observed differences in the type of genes involved in HGT [6]. Horizontal transfer may be mediated by mobile genetic elements such as plasmids, genomic islands, transposons, integrons, and insertion sequences (ISs), which are involved in bacterial acquisition and recombination of foreign DNA. Bacteriophages may also be considered as mobile genetic elements, as they play a crucial role in mobilizing genetic elements that facilitate genome rearrangements, gene duplications and deletions, and capture of new genes [7].

Different environmental compartments, such as water and sediment, might have a significant role in driving ARG transfer, ecology, and evolution (Table 1). It has been estimated that the majority of bacteria in natural aquatic ecosystems are organized in biofilms [8], which are complex assemblages of microorganisms that are embedded in a matrix of extracellular polymeric substances. The biofilm matrix facilitates structural organization and protects the microbial community, and it plays a critical role to the spread of antibiotic resistance due to the high cell density, the close proximity, increased genetic competence, and accumulated mobile genetic elements [9]. Recent studies have shown that ARGs in aquatic systems may migrate rapidly to biofilms, where they may persist longer than in adjacent waters [10,11]. These studies thus suggest that biofilms are important reservoirs for ARGs in the environment.

Aquatic sediments also represent an important environmental matrix within which HGT can occur because of the ability of these sediments to retain antimicrobials [1]. Moreover, microbial communities inhabiting aquatic sediments are composed of complex and highly diverse assemblages of prokaryotic and eukaryotic organisms. Several studies have shown the presence

TABLE 1 ARGs Found in Bacteria from Aquatic Environments

Antibiotics	ARG	Host	Source
Aminoglycosides	*aadA1*	*Aeromonas* spp., *Escherichia coli*, *Vibrio* spp.	Wastewater effluents
	aadA2	*Aeromonas* spp., *E. coli*	Wastewater effluents
	aadA5	*E. coli*, *Vibrio* spp.	Wastewater effluents
	aadA13	*E. coli*	Wastewater effluents
	aadB	*E. coli*	Wastewater effluents
	aac(6′)-Ib-cr	*Aeromonas* spp., *E. coli*, metagenome	River, wastewater effluents
	sat1 and *sat2*	*Aeromonas* spp., *E. coli*	Wastewater effluents
β-Lactams	*ampC*	*E. coli*	River, wastewater effluents
	$bla_{\text{CTX-M}}$	*E. coli*	River, wastewater effluents
	bla_{GES}	*Aeromonas* spp.	River
	bla_{IMP}	*Pseudomonas* spp.	Wastewater effluents
	bla_{KPC}	*Klebsiella pneumoniae*, *Acinetobacter* spp.	Wastewater effluents
	$bla_{\text{NDM-1}}$	*Pseudomonas* spp., *Achromobacter* spp., *Citrobacter* spp., *Aeromonas* spp.	Seepage water, tap water
	bla_{OXA}	*Acinetobacter* spp., *Aeromonas* spp.	River, wastewater effluents
	bla_{PER}	*Aeromonas* spp.	River
	bla_{SHV}	*Aeromonas* spp.	River
	bla_{TLA}	*Aeromonas* spp.	River
	bla_{TEM}	*E. coli*, *Citrobacter* spp.,	River, wastewater effluents
	bla_{VEB}	*Aeromonas* spp.	River
	bla_{VIM}	*Acinetobacter* spp., *Pseudomonas* spp.	Wastewater effluents
Chloramphenicol and florfenicol	*catB3*	*Aeromonas* spp.	River, wastewater effluents
	catII	*Aeromonas* spp., metagenome	Surface water samples, wastewater effluents
	floR	*Pseudomonas* spp., metagenome	Fish farms

TABLE 1 ARGs Found in Bacteria from Aquatic Environments—Cont'd

Antibiotics	ARG	Host	Source
Fluoroquinolones	*qnrB*	*E. coli, Citrobacter* spp.,	Wastewater effluents
	qnrD	Metagenome	Farm water
	qnrS	*Aeromonas* spp.	River, lake
	qnrVC	*Aeromonas* spp., *Vibrio* spp.	Wastewater effluents
	qepA	Metagenome	River, farm water
	oqxA	Metagenome	Farm water
	oqxB	Metagenome	Farm water
	aac(6′)-Ib-cr	*Aeromonas* spp., *E. coli*, metagenome	River, wastewater effluents
Macrolides	*ermB*	*Enterococcus* spp., metagenome	Farm water, wastewater effluents
	mefA	*Enterococcus* spp.,	Wastewater effluents
	mphA–mrx–mphR	*Aeromonas* spp.	Wastewater effluents
Rifamycin	*arr-3*	*Aeromonas* spp., metagenome	River, wastewater effluents
Sulfonamide and trimethoprim	*dfrA1*	*Aeromonas* spp., *E. coli*	Wastewater effluents
	dfrA12	*E. coli*	Wastewater effluents
	dfrA13	*E. coli*	Wastewater effluents
	sulI	*Aeromonas* spp., *E. coli*, metagenome	Wastewater effluents
	sulII	*Aeromonas* spp., *E. coli*, metagenome	Wastewater effluents
Tetracycline	*tetA*	Several species	Farm water, wastewater effluents
	tetB	Several species	Marine and freshwater environments
	tetM	Several species	Marine and freshwater environments
	tetO	Metagenome	Biofilm of water supplies, wastewater effluents
	tetQ	Metagenome	Biofilm of water supplies, wastewater effluents
	tetW	Metagenome	Biofilm of water supplies, wastewater effluents

of ARGs in aquatic sediments, particularly those sediments exposed to anthropogenic activities [12,13]. In fact, a recent study has demonstrated that mobile genetic elements, such as class 1 integrons and ISCR elements, were highly overrepresented in river sediments exposed to antibiotics, suggesting that aquatic sediments are important reservoirs for the acquisition and transfer of ARGs [14].

Alongside biofilms and sediments, aquatic organisms may be important intermediates in the development and spread of ARGs. Jiang et al. [15] detected high levels of ARGs, including extended-spectrum β-lactamases (ESBLs) and plasmid-mediated quinolone resistance (PMQR) determinants, in bacterial strains isolated from fish intestinal samples in China. Moreover, chitin, a polymer of β-1,4 linked *N*-acetylglucosamine present in the exoskeleton of many crustaceans and mollusks, has been shown to induce competence for natural transformation, which could mediate the acquisition of ARGs during the growth of *Vibrio cholera* on crab shell fragment immersed in seawater [16]. Additionally, several environmental factors may affect HGT and thereby delineate exchange community boundaries. Among them, it has been demonstrated that temperature is an important factor that controls plasmid transfer rates [17].

2.1 Bacterial Plasmids

Plasmids are extrachromosomal, circular DNA molecules that replicate independently of chromosomal DNA. They are known to carry a considerable variety of genes, including those that confer antibiotic resistance and those that provide virulence determinants and enhance the capacity to repair DNA damage [3]. The probability and rate of plasmid transfer from a donor to a recipient strain are influenced by plasmid-borne genes, which determine the type of transfer mechanism (self-transmissible or mobilizable) and the host range of autonomous plasmid replication [18].

Several studies have shown that genes encoding ESBLs may be carried on plasmids. ESBLs are a group of enzymes that confer resistance to penicillins, cephalosporins, monobactams, and oxyimino-cephalosporins. Since their initial description, more than 400 different ESBLs have been identified (http://www.lahey.org/studies/), most of them belonging to the SHV, TEM, and CTX-M families. Although ESBLs have been detected mostly in members of the *Enterobacteriaceae* family, they have also been found in other bacterial genera, such as *Acinetobacter*, *Aeromonas*, and *Pseudomonas*. For instance, ESBLs have been reported to occur in *Aeromonas* species, which are common inhabitants of aquatic environments, with $bla_{\text{PER-1}}$ identified in *Aeromonas media* from Switzerland [19] and $bla_{\text{VEB-1a}}$, $bla_{\text{SHV-12}}$, $bla_{\text{PER-1}}$, $bla_{\text{PER-6}}$, $bla_{\text{TLA-2}}$, and $bla_{\text{GES-7}}$ in *Aeromonas* strains from the Seine River [20], suggesting that those species may potentially act as reservoirs of ARGs.

It has also been demonstrated that genes encoding quinolone resistance may be present in plasmids. A number of PMQR determinants have been described, including the *qnr* genes encoding pentapeptide repeat proteins, which block the action of ciprofloxacin on bacterial DNA gyrase and topoisomerase IV, the *aac*(6′)-*lb-cr* aminoglycoside acetyltransferase that confers reduced susceptibility to ciprofloxacin by N-acetylation of its piperazinyl amine, and the quinolone efflux pump QepA [21]. PMQR determinants, in the same way as ESBLs, have been detected not only in members of the *Enterobacteriaceae* family but also in other bacterial strains isolated from aquatic environments. Within each *qnr* family, alleles differ in one or more amino acid (Figure 2). These genes have been found on plasmids of different sizes and incompatibility groups, indicating that the spread of multiple plasmids has been responsible for the dissemination of this resistance worldwide. However, the immediate genetic environment of each gene type is similar enough to suggest a limited number of acquisition events followed by transposition, recombination, replicon fusion and resolution, and deletion and insertion of DNA to generate the diversity of plasmid structures that have been described to date [21]. These genes have also been found on the chromosomes of both Gram-positive and Gram-negative bacteria. Poirel et al. [22] reported that the *qnrA* gene located on plasmids and found in quinolone-resistant clinical isolates of *Enterobacteriaceae* is derived from the chromosome of *Shewanella algae*. Moreover, the progenitor of *qnrS*-like genes was found in *Vibrio splendidus*, and genes similar to *qnr* with 40–67% amino acid identity were found in the chromosomes of some bacterial species belonging to the *Vibrionaceae* family, such as *Vibrio vulnificus*, *Vibrio parahaemolyticus*, and *Photobacterium profundum* [23]. The *qnrS2* gene has been identified from a mobilizable IncQ-related plasmid isolated from an activated sludge bacterial community of a wastewater treatment plant (WWTP) in Germany [24]. Similarly, a recent study has demonstrated a high copy number for the *qnrS* gene in human and veterinary hospital wastewater samples using culture-independent methods [25]. Besides these *qnr* genes, the *aac*(*6'*)-*lb-cr* gene has also been identified in aquatic species. Picão et al. [26] detected two PMQR determinants, *qnrS2* and *aac*(6′)-*lb-cr*, along with four different antimicrobial resistance markers, on a single plasmid from *Aeromonas allosaccharophila*. Collectively, these findings suggest that the *qnr* genes in circulation could have originated in the chromosomes of environmental organisms, which might have been fostered due to intensive quinolone pressure [27].

Plasmids can carry several ARGs. In fact, Marti and Balcázar [28] have recently identified a plasmid, which confers multidrug resistance in an environmental *Aeromonas* species. This plasmid encoded resistance to macrolides (*mphA–mrx–mphR*), sulfonamides (*sul1*), and quinolones (*qnrS2*). A class 1 integron was also identified, which included four integrated resistance gene cassettes, namely, *aac*(6′)-*Ib-cr*, $bla_{\text{OXA-1}}$, *catB3*, and *arr-3*, encoding

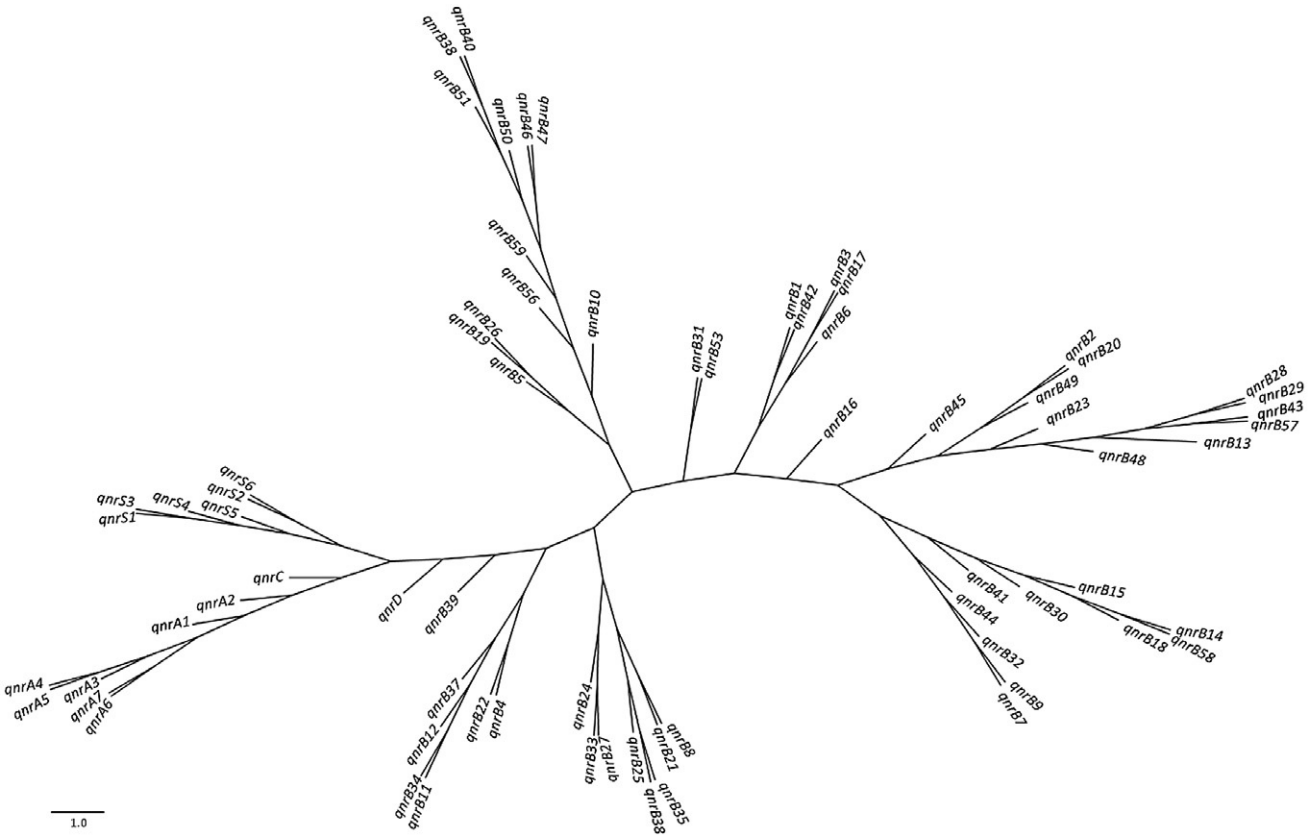

FIGURE 2 Diversity of *qnr* genes. The *qnr* genes encode pentapeptide repeat proteins that block the action of quinolones on bacterial DNA gyrase and topoisomerase IV, resulting in low-level quinolone resistance. Note that the scale bar represents 1.0 substitution per site.

resistance to aminoglycosides and quinolones, β-lactams, chloramphenicol, and rifampicin, respectively.

2.2 Genomic Islands

Genomic islands are a collection of large, potentially mobile regions of DNA that are acquired via HGT and contribute to the rapid bacterial evolution and adaptation. They encode complex biological functions including some involved in metabolism, pathogenesis, and symbiosis [29]. In general, genomic islands are areas of the genome that are present in some bacterial strains, which are usually flanked by direct repeat sequences, associated with tRNA genes [30]. They often harbor functional or cryptic genes encoding factors that are involved in genetic mobility, such as integrases, transposases, IS elements, bacteriophage genes, and origins of replication [29,30]. One of the first genomic islands containing an ARG cluster was identified in *Salmonella enterica*, which was named *Salmonella* genomic island 1 (SGI1). Meunier et al. [31] identified a SGI1 antibiotic resistance gene cluster, with evidence of two integron structures, in an isolate of *S. enterica* serotype Paratyphi B from a tropical fish in Singapore. The first integron carried the *aadA2* gene conferring resistance to streptomycin and spectinomycin and a truncated *sul1* resistance gene, whereas the second integron contained the β-lactamase gene $bla_{\text{PSE-1}}$ and a complete *sul1* gene. Moreover, Doublet et al. [32] identified a variant SGI1 antibiotic resistance gene cluster in a multidrug-resistant strain of *S. enterica* serovar Albany isolated from food fish from Thailand.

2.3 Transposons

Transposons are essentially jumping gene systems that incorporate a resistance gene within the element [3]. There are different types of transposons, which can be distinguished by structure, genetic relatedness, and mechanism of transposition [3]. Most bacterial transposons correspond to composite or noncomposite forms. Composite transposons have two IS elements of the same type bracketing one or more genes. In noncomposite forms, the transposition and nontransposition genes are clustered and flanked by terminal IR sequences [33]. Picão et al. [19] identified the $bla_{\text{PER-1}}$ gene as part of a Tn*1213* composite transposon in an environmental *A. media* isolate from Switzerland. The truncated or complete transposon Tn*1721* containing the *tetA* gene, which confers resistance to tetracycline, has been detected on plasmids in *Aeromonas salmonicida* from different geographic locations [34,35].

Some transposons contribute to the spread of ARGs as part of class 1 integrons. These transposons include the Tn*3* family, the Tn*5053* family, and Tn*402*-like transposons. Transposons belonging to the Tn*3* family have been found in a diverse range of Gram-negative and Gram-positive bacteria. For instance, Tn*21*-like transposon, which belongs to a subgroup of the Tn*3* family, has been detected in a multidrug-resistant *Aeromonas hydrophila* isolate,

which showed a macrolide inactivation gene cluster *mphA–mrx–mphR* adjacent to a class 1 integron [36]. Scotta et al. [37] demonstrated the presence of $bla_{\text{VIM-13}}$ associated with a Tn*1721*-class 1 integron structure in several metallo-β-lactamase-producing isolates from hospital sewage.

2.4 Integrons

Integrons are genetic systems that allow bacteria to capture and express gene cassettes and they can be found as part of plasmids, chromosomes, and transposons. Integrons are formed by an *intI* gene, encoding an integrase that is a site-specific recombinase, an attachment site (*attI*), and one or two strong promoters (P) that drive the expression of inserted gene cassettes [38]. Gene cassettes can be inserted one after the other into the integron insertion site [3], producing the formation of long arrangements of ARGs that can be transferred simultaneously among bacterial populations [39]. This mobile genetic element can be usually found in clinical bacterial strains, possibly because most of the cassettes identified are associated with antibiotic resistance. However, in the last years, several studies have been performed in order to determine the occurrence of integrons in bacteria from aquatic environments.

There are three main classes of integron structures, depending of their integrase, but most resistance integrons conform to a structure known as a class 1 integron [3]. Moura et al. [38] detected genes encoding integrases belonging to classes 1 and 2 integrons among *Enterobacteriaceae* and *Aeromonas* spp., in influents and effluents of a WWTP. These integrons harbored different gene cassettes conferring resistance to penicillins, fluoroquinolones, and chloramphenicol, among others. Another recent investigation [40] has demonstrated that suspended aggregates of bacteria in natural aquatic systems (the so-called flocs) contained class 1 integrons with clinically important ARGs.

Industrial activities have also been shown specifically to contribute to the increase of mobile genetic elements. Wright et al. [41] quantified class 1 integrase (*intI1*) gene abundance in total community DNA extracted from contaminated and reference riverine and estuarine microhabitats and in metal- or antibiotic-amended freshwater microcosms. Results showed that the *intI1* gene was more abundant in all contaminant-exposed bacterial communities, indicating that relative gene transfer potential is higher in these communities [41]. Additionally, Rosewarne et al. [42] demonstrated that the abundance of *intI1* was increased as a result of ecosystem perturbation, indicated by a strong positive correlation with heavy metals such as zinc, mercury, lead, and copper. Moreover, the abundance of *intI1* at sites located downstream from treated sewage outputs was associated with the percentage contribution of the discharge to the basal flow rate [42]. All these studies show that integrons make an important contribution to the dissemination of ARGs.

2.5 Bacteriophages as vectors for ARGs

Transfer of antibiotic-resistant genes among bacteria through mobile genetic elements has been widely studied and demonstrated. However, it took several decades to understand the contribution of bacteriophages in the transfer of genetic material. Bacteriophages, also known as phages, are the viruses that infect prokaryotic cells (bacteria) with the aim of replicating themselves due to their lack of needed components that cells have to reproduce. Once the phages inject their genetic material into host cell, it can immediately use the host's cellular machinery to replicate and release newly produced viruses, or it can integrate into bacterial genome as a prophage, which is in a latent state until some physiological conditions induce it to replicate. These two different life cycles are called lytic and lysogenic and are carried out by virulent and temperate phages, respectively.

One of the consequences of phages replication is the DNA transfer from one bacterium to another, called transduction. There are two different types of transduction: generalized and specialized. Generalized transduction is performed by either temperate or virulent phages, and it occurs when bacterial genome is packaged into new phage particles capable of infecting another bacterium that can incorporate the donor's DNA by genetic recombination. In contrast, specialized transduction is exclusive for temperate phages and it takes place when the excision of prophage includes a flanking region of the bacterial host genome. In this case, since the prophages have sequence-specific integration sites, the number of genes that can be transferred is limited but they can be moved frequently, increasing their transfer efficiency [43].

Numerous experiments have confirmed the *in vitro* transduction of antibiotic resistance determinants among *Pseudomonas aeruginosa* by phages F116 or G101 [44], among *Actinobacillus actinomycetemcomitans* by temperate bacteriophages Aaϕ23 [45], among *S. enterica* serovar *Typhimurium* by P22-like phages [46], and more recently among a group A streptococci by Φm46.1 [47]. In addition, some studies have demonstrated that bacterial 16S rRNA genes from *Alpha-*, *Beta-*, and *Gammaproteobacteria*, *Actinomycetes*, and *Firmicutes* were transduced by phages isolated from a WWTP [48], indicating that transduction also occurs in the environment. Other studies have detected β-lactamase genes in phages carried by sewage [49,50], showing undoubtedly the contribution of phages in the spread of ARGs.

Previous studies based on microscopic enumerations determined that virus particles represent one of the most abundant entities in nature [51,52]. Moreover, recent advances in metagenomics have confirmed that the viral metagenomes are dominated by phage, estimated as being about 10^{31} phages in the biosphere [53]. This large abundance and the fact that they can occur in any place where their hosts are found and move between environments make the phages one of the most efficient vehicles for moving genetic material between bacterial cells [49]. Nevertheless, if phage host ranges are not

confined to a single bacterial species, as proposed by Hendrix et al. [54], phages would be able to move genetic material over phylogenetically unrelated species. Additionally, it is well known that all groups of phages are more persistent in the environment than their bacterial hosts [43]. Taken together, these previous observations demonstrate that the phages play an important role in the transfer of ARGs in natural habitats.

3 CONCLUSIONS

The high efficiency of these mobile genetic elements transferring antibiotic resistance determinants among phylogenetically distant bacteria from different environments makes difficult the distinction between naturally occurring resistance and the resistance promoted by antibiotics released from anthropogenic sources. However, the European Council concluded in 1998 that there was a relationship between the consumption of antimicrobials and the prevalence of antibiotic-resistant bacteria. Moreover, it was also established that the dissemination of resistant bacteria can occur in both hospital and environmental settings [55]. Since then, both the European Union and the United States launched many projects of antibiotic resistance surveillance to assess the public health risk associated to this phenomenon. Nevertheless, the recent advances in metagenomics showed that little is known about the antibiotic resistome of the vast majority of environmental bacteria [56]. Given that antibiotics are the main weapon we have against infectious diseases, further research of environmental reservoirs is needed to better understand mechanisms of antibiotic resistance.

ACKNOWLEDGMENTS

This work was supported by the Fundación Eugenio Rodríguez Pascual, Spain. J. L. B. acknowledges the Ramon y Cajal research fellowship (RYC-2011-08154) from the Spanish Ministry of Economy and Competitiveness.

REFERENCES

[1] N.G.H. Taylor, D.W. Verner-Jeffreys, C. Baker-Austin, Trends Ecol. Evol. 26 (2011) 278–284.
[2] M. Barlow, Methods Mol. Biol. 532 (2009) 397–411.
[3] P.M. Bennett, Br. J. Pharmacol. 153 (2008) S347–S357.
[4] E. Gullberg, S. Cao, O.G. Berg, C. Ilbäck, L. Sandegren, D. Hughes, D.I. Andersson, PLoS Pathog. 7 (2011) e1002158.
[5] J.L. Martínez, Science 321 (2008) 365–367.
[6] L. Boto, Proc. R. Soc. B 277 (2010) 819–827.
[7] R.W. Jackson, B. Vinatzer, D.L. Arnold, S. Dorus, J. Murillo, Mob. Genet. Elem. 1 (2011) 55–65.
[8] R.M. Donlan, J.W. Costerton, Clin. Microbiol. Rev. 15 (2002) 167–193.
[9] C.A. Fux, J.W. Costerton, P.S. Stewart, P. Stoodley, Trends Microbiol. 13 (2005) 34–40.

[10] C.A. Engemann, P.L. Keen, C.W. Knapp, K.J. Hall, D.W. Graham, Environ. Sci. Technol. 42 (2008) 5131–5136.
[11] W. Zhang, B.S.M. Sturm, C.W. Knapp, D.W. Graham, Environ. Sci. Technol. 43 (2009) 7643–7650.
[12] A. Pruden, R. Pei, H. Storteboom, K.H. Carlson, Environ. Sci. Technol. 40 (2006) 7445–7450.
[13] D.E. Cummings, K.F. Archer, D.J. Arriola, P.A. Baker, K.G. Faucett, J.B. Laroya, K.L. Pfeil, C.R. Ryan, K.R.U. Ryan, D.E. Zuill, Environ. Sci. Technol. 45 (2011) 447–454.
[14] E. Kristiansson, J. Fick, A. Janzon, R. Grabic, C. Rutgersson, B. Weijdegard, H. Söderström, D.G.J. Larsson, PLoS One 6 (2011) e17038.
[15] H.X. Jiang, D. Tang, Y.H. Liu, X.H. Zhang, Z.L. Zeng, L. Xu, P.M. Hawkey, J. Antimicrob. Chemother. 67 (2012) 2350–2353.
[16] K.L. Meibom, M. Blokesch, N.A. Dolganov, C.Y. Wu, G.K. Schoolnik, Science 310 (2005) 1824–1827.
[17] A. Richaume, J.S. Angle, M.J. Sadowsky, Appl. Environ. Microbiol. 55 (1989) 1730–1734.
[18] A. Hoffmann, T. Thimm, M. Dröge, E.R.B. Moore, J.C. Munch, C.C. Tebbe, Appl. Environ. Microbiol. 64 (1998) 2652–2659.
[19] R.C. Picão, L. Poirel, A. Demarta, O. Petrini, A.R. Corvaglia, P. Nordmann, Antimicrob. Agents Chemother. 52 (2008) 3461–3462.
[20] D. Girlich, L. Poirel, P. Nordmann, Antimicrob. Agents Chemother. 55 (2011) 1256–1261.
[21] J. Strahilevitz, G.A. Jacoby, D.C. Hooper, A. Robicsek, Clin. Microbiol. Rev. 22 (2009) 664–689.
[22] L. Poirel, J.M. Rodriguez-Martinez, H. Mammeri, A. Liard, P. Nordmann, Antimicrob. Agents Chemother. 49 (2005) 3523–3525.
[23] L. Poirel, A. Liard, J.M. Rodriguez-Martinez, P. Nordmann, J. Antimicrob. Chemother. 56 (2005) 1118–1121.
[24] G. Bönemann, M. Stiens, A. Pühler, A. Schlüter, Antimicrob. Agents Chemother. 50 (2006) 3075–3080.
[25] E. Marti, J.L. Balcázar, Appl. Environ. Microbiol. 79 (2013) 1743–1745.
[26] R.C. Picão, L. Poirel, A. Demarta, C.S. Ferreira Silva, A.R. Corvaglia, O. Petrini, P. Nordmann, J. Antimicrob. Chemother. 62 (2008) 948–950.
[27] A. Robicsek, G.J. Jacoby, D.C. Hooper, Lancet Infect. Dis. 6 (2006) 629–640.
[28] E. Marti, J.L. Balcázar, Clin. Microbiol. Infect. 18 (2012) E366–E368.
[29] J. Hacker, J.B. Kaper, Annu. Rev. Microbiol. 54 (2000) 641–679.
[30] O. Gal-Mor, B.B. Finlay, Cell. Microbiol. 8 (2006) 1707–1719.
[31] D. Meunier, D. Boyd, M.R. Mulvey, S. Baucheron, C. Mammina, A. Nastasi, E. Chaslus-Dancla, A. Cloeckaert, Emerg. Infect. Dis. 8 (2002) 430–433.
[32] B. Doublet, R. Lailler, D. Meunier, A. Brisabois, D. Boyd, M.R. Mulvey, E. Chaslus-Dancla, A. Cloeckaert, Emerg. Infect. Dis. 9 (2002) 585–591.
[33] M.R. Schaefer, K. Kahn, J. Bacteriol. 180 (1998) 6059–6063.
[34] H. Sørum, T.M. L'Abée-Lund, A. Solberg, A. Wold, Antimicrob. Agents Chemother. 47 (2003) 1285–1290.
[35] G. Rhodes, G. Huys, J. Swings, P. Mcgann, M. Hiney, P. Smith, R.W. Pickup, Appl. Environ. Microbiol. 66 (2000) 3883–3890.
[36] T.L. Poole, T.R. Callaway, K.M. Bischoff, C.E. Warnes, D.J. Nisbet, J. Antimicrob. Chemother. 57 (2006) 31–38.
[37] C. Scotta, C. Juan, G. Cabot, A. Oliver, J. Lalucat, A. Bennasar, S. Albertí, Antimicrob. Agents Chemother. 55 (2011) 5376–5379.

[38] A. Moura, C. Pereira, I. Henriques, A. Correia, Res. Microbiol. 163 (2012) 92–100.
[39] J.L. Martínez, Environ. Pollut. 157 (2009) 2893–2902.
[40] C.N. Drudge, A.V.C. Elliott, J.M. Plach, L.J. Ejim, G.D. Wright, I.G. Droppo, L.A. Warrena, Appl. Environ. Microbiol. 78 (2012) 4367–4372.
[41] M.S. Wright, C. Baker-Austin, A.H. Lindell, R. Stepanauskas, H.W. Stokes, J.V. McArthur, ISME J. 2 (2008) 417–428.
[42] C.P. Rosewarne, V. Pettigrove, H.W. Stokes, Y.M. Parsons, FEMS Microbiol. Ecol. 72 (2010) 35–46.
[43] M. Muniesa, L. Imamovic, J. Jofre, Microb. Biotechnol. 4 (2011) 725–734.
[44] J. Blahová, K. Králiková, V. Krcméry Sr., N. Bartoníková, Eur. J. Clin. Microbiol. Infect. Dis. 18 (1993) 152–154.
[45] K. Willi, H. Sandmeier, E.M. Kulik, J. Meyer, Cell. Mol. Life Sci. 53 (1997) 904–910.
[46] H. Schmieger, P. Schicklmaier, FEMS Microbiol. Lett. 170 (1999) 251–256.
[47] M.C. Di Luca, S. D'Ercole, D. Petrelli, M. Prenna, S. Ripa, L.A. Vitali, Antimicrob. Agents Chemother. 54 (2010) 4464–4466.
[48] A. Del Casale, P.V. Flanagan, M.J. Larkin, C.C.R. Allen, L.A. Kulakov, FEMS Microbiol. Ecol. 76 (2011) 100–108.
[49] M. Muniesa, A. García, E. Miró, B. Mirelis, G. Prats, J. Jofre, F. Navarro, Emerg. Infect. Dis. 10 (2004) 1134–1137.
[50] M. Colomer-Lluch, J. Jofre, M. Muniesa, PLoS One 6 (2011) e17549.
[51] O. Bergh, K.Y. Børsheim, G. Bratbak, M. Heldal, Nature 340 (1989) 467–468.
[52] R. Danovaro, A. Dell'Anno, A. Trucco, M. Serresi, S. Vanucci, Appl. Environ. Microbiol. 67 (2001) 1384–1987.
[53] E.A. Dinsdale, R.A. Edwards, D. Hall, F. Angly, M. Breitbart, J.M. Brulc, M. Furlan, C. Desnues, M. Haynes, L. Li, L. McDaniel, M.A. Moran, K.E. Nelson, C. Nilsson, R. Olson, J. Paul, B.R. Brito, Y. Ruan, B.K. Swan, R. Stevens, D.L. Valentine, R.V. Thurber, L. Wegley, B.A. White, F. Rohwer, Nature 452 (2008) 629–632.
[54] R.W. Hendrix, M.C. Smith, R.N. Burns, M.E. Ford, G.F. Hatfull, Proc. Natl. Acad. Sci. 96 (1999) 2192–2197.
[55] V.T. Rosdahl, K.B. Pedersen (Eds.), Report from the Invitational EU Conference on the Microbial Threat: The Copenhagen Recommendations, Copenhagen, 1998.
[56] H.K. Allen, J. Danato, H.H. Wang, K.A. Cloud-Hansen, J. Davies, J. Handelsman, Nat. Rev. Microbiol. 8 (2010) 251–259.

Chapter 20

Reporting and Evaluating Ecotoxicity Data for Environmental Risk Assessment: How Can Current Practices Be Improved?

Marlene Ågerstrand, Magnus Breitholtz and Christina Rudén
Department of Applied Environmental Science, Stockholm University, Stockholm, Sweden

Chapter Outline

1 INTRODUCTION

In 2006, the European Medicines Agency (EMA) decided that all new marketing authorization applications for human pharmaceuticals should be accompanied by an environmental risk assessment [1]. This legislation puts responsibility on the pharmaceutical industry to generate or gather the

Comprehensive Analytical Chemistry, Vol. 62. http://dx.doi.org/10.1016/B978-0-444-62657-8.00020-3

required data and also for performing the risk assessments. The regulatory agency is then responsible for reviewing the risk assessment.

In this chapter, we will discuss how environmental risk assessment of pharmaceuticals is currently done and how it can be improved focusing on the generation, reporting, and use of ecotoxicity data for the purpose of preventing risks in the environment.

2 ENVIRONMENTAL RISK ASSESSMENT OF PHARMACEUTICALS: THE EMA RULES

The environmental risk assessment process for pharmaceutical substances for human use is divided into two phases with the latter consisting of two tiers.

In phase I—the exposure assessment—emissions are estimated using models based on estimations such as sales data, providing a predicted environmental concentration (PEC). If the PEC value is equal to or above the legal cutoff value of 0.01 μg/L or if the pharmaceutical is known to have reproductive effects on vertebrates or invertebrates at concentrations below 0.01 μg/L, phase II should be performed. In addition, lipophilic substances with a log $K_{OW} > 4.5$ should be screened for persistence, bioaccumulation, and toxicity according to the European Commission Technical Guidance Document [1].

In phase II tier A, physicochemical properties, environmental fate, and effect studies are reviewed and a predicted no-effect concentration (PNEC) is calculated. Data from standard long-term toxicity tests on algae, daphnids, and fish (OECD Guidelines 201, 211, and 210) are recommended, and appropriate assessment factors are applied [1]. Assessment factors are used to compensate for variations in sensitivity within and between species, for transfer from laboratory data to field conditions, and, if acute data are used, for extrapolating short-term data to chronic data. The PNEC is used as a reference concentration below which unacceptable effects in the environment will most likely not occur [2].

In the risk characterization, risk quotients for surface water, groundwater, and microorganisms are calculated by dividing PEC with PNEC. If the PEC/PNEC quotient is above 1, an extended environmental fate and effect analysis, according to tier B in phase II, is needed [1]. Other criteria for entering a tier B evaluation include a PEC/PNEC ratio above 1 for groundwater, PEC/PNEC ratio above 0.1 for microorganisms, potential of substance to bioaccumulate (log $K_{OW} > 3$), potential of substance to partition to sewage sludge (log $K_{OC} > 4$), and substances that are not readily biodegradable (10% of the substance present after 14 days in a standard test for ready biodegradation).

A phase II tier B evaluation may include a refinement of the environmental fate analysis and the PEC value for surface water, an extended effect analysis for sediment-living organisms and microorganisms, and a terrestrial fate and effect analysis, depending on the outcome of tier A.

The general objective of the regulatory risk assessment process is to identify and characterize environmental risks and, on the basis of that, make decisions with the purpose to prevent unacceptable harm to the environment [2]. However, the environmental risk assessment for human pharmaceuticals cannot be used as a part of the decision basis for the authorization of human pharmaceuticals. The reason for this is that the patients' needs for medicines have been considered more important than environmental concerns. For veterinary medicines, the situation is different and the result of the environmental risk assessment is allowed to limit the marketing authorization.

3 EVALUATION OF TEST DATA: THE SCIENTIFIC BASIS OF RISK ASSESSMENT

There are many types of effect studies that can be used to determine a PNEC. What is in fact studied, that is, included endpoints, is one aspect by which tests can differ. Effects can, for instance, be measured on the molecular, cellular, organ, individual, or population level. Tests can also be made on different species. Different species and endpoints will often have different sensitivity in relation to a specific substance, so the choice of what (type of) data to include in the risk assessment can therefore have a major impact on the PEC/PNEC ratio and hence the overall assessment of risk. The general idea is to base the risk assessment on the most sensitive and relevant species and endpoint. A crucial part of the risk assessment is thus to define what data represents in fact the most sensitive and relevant species and endpoint. According to the EMA guidelines, the choice of test species should be "justified." Risk assessors base decisions on inclusion of data on the judged *reliability* and *relevance* of the individual studies [2,3]. The *reliability* evaluation has to do with characterization of the test protocol and sufficient reporting to ensure the study's reproducibility. In REACH [3], reliability is defined as

the inherent quality of a test report or publication relating to preferably standardised methodology and the way the experimental procedure and results are described to give evidence of the clarity and plausibility of the findings. Reliability of data is closely linked to the reliability of the test method used to generate the data.

Basically, this evaluation should answer the question: Has the experiment generated and reported a true and correct result? Sometimes the words "quality" or "validity" are used instead of reliability.

Reliability is closely connected to reproducibility, which is important in all scientific work. Therefore, reliability concerns all type of studies, irrespective of whether it will be used in risk assessment or not. Environmental factors, such as oxygen saturation, salinity, pH, hardness, and temperature, can, for example, have drastic impact on uptake and effects of chemical substances

[4–6], and therefore, these aspects need to be monitored and reported in order to ensure the reproducibility of the study.

The *relevance* evaluation has to do with the appropriateness of the study. In REACH, [3] relevance is defined as

... the extent to which data and tests are appropriate for a particular hazard identification or risk characterisation.

The relevance evaluation should thus answer questions like: Is the tested endpoint relevant for the test species? Does the test exposure scenario exist for the tested substance? How do the tested doses relate to measured or PECs?

4 STANDARD AND NONSTANDARD TEST PROTOCOLS

The reliability and relevance of a study can partly depend on whether a test is performed according to a standard test protocol or a nonstandard method.

Standard test protocols are described and provided by internationally agreed organizations, such as the OECD. A standard protocol defines in detail how a test should be designed, executed, and reported. Nonstandard methods are, in contrast, any other test method, designed case-by-case depending on the research question, and typically developed and performed by academic research groups. Regardless of whether a test is performed according to a standard protocol or not, it should meet some general criteria to demonstrate the reliability and thereby the reproducibility of the test results.

Reproducibility and transparency has been a cornerstone in the development of standard protocols; strict instructions for how to design, perform, and report the standard tests have resulted in tests with high reliability. Recommending the same standard test for all substances also facilitates the work for regulators since it makes comparisons between substances more forthright.

However, the relevance of standard tests can vary depending on the tested substance, selected species, and the included endpoints. There are, for instance, examples of combinations of substance and test where the most sensitive endpoints were not included in the standard approach. One striking example is ethinyl estradiol where nonstandard endpoints provide a PNEC that is up to 200,000 times lower than what would have been the case using a standard test (see Table 1). So, in some instances, a nonstandard test can be more sensitive and thereby contribute additional and significant information to a risk assessment.

In summary, it should be acknowledged that if all aspects have been reported transparently, a nonstandard test can be just as reliable and reproducible as tests performed under strict implementation of a standard protocol and following a standard will not automatically ensure that the test has sufficient relevance for risk assessment purposes.

TABLE 1 Comparison of the Lowest Standard and Nonstandard Effect Values for the Sex Hormone Ethinyl estradiol

	Test Species Endpoint/Standard Test NOEC Value	Test Species Endpoint/Standard Test LOEC Value	Test Species Endpoint/Standard Test EC_{50} Value
Lowest standard test value	*Desmodesmus* spp. (algae) Growth inhibition/OECD 201 0.1 mg/L [7]	*Danio rerio* (fish) Reproduction/OECD 210 0.000003 mg/L [8]	*Daphnia* (crustacean) Reproduction/OECD 211 0.105 mg/L [9]
Lowest nonstandard test value	*Danio rerio* (fish) Fertilization success 0.0000005 mg/L [10]	*Oryzias latipes* (fish) Induced intersex 0.00000003 mg/L [11]	*Danio rerio* (fish) Fertilization success 0.0000011 mg/L [12]
Ratio between standard and nonstandard values	200,000	100	95,455

NOEC, no observed effect concentration; LOEC, lowest observed effect concentration; EC_{50}, lowest identified effect concentration where 50% of the tested population have been found to be affected.

5 REGULATORS PROMOTE THE USE OF STANDARD TESTS (AND GOOD LABORATORY PRACTICE)

The use of standard test protocols is often promoted by regulatory agencies. Table 2 provides an overview of recommendations from a range of risk assessment guidelines, including environmental risk assessment guidelines for human and veterinary pharmaceuticals. Phrases like "preferably," "recommended," "need to be carried out in accordance with," "must be conducted according to," and "can be accepted if the guideline is comparable with those guidelines mentioned" are used to indicate the importance of standard tests. At the same time, it should be acknowledged that several guidance documents also open up for use of nonstandard studies when applicable. However, all guidance documents listed below state that tests "should" or "must" be conducted in compliance with the rules for Good Laboratory Practices (GLPs). If this criterion were to be implemented strict, then data from academic research could not be used since very few, if any, noncommercial laboratories follow GLP.

GLP is a quality control system for laboratories to ensure the consistency, reliability, and reproducibility of chemical safety tests. It was instituted following cases of fraud by chemical manufacturers [17]. GLP has been criticized for not promoting the most appropriate and sensitive state-of-the-art studies and excluding nonstandard studies from risk assessments, thereby altering the outcome of the risk assessment substantially [18,19].

6 AVAILABLE METHODS TO PROMOTE A STRUCTURED APPROACH TO TEST DATA EVALUATION

There are several attempts to make the evaluation process of ecotoxicity (and toxicity) studies more structured, by using either checklists or predefined evaluation criteria. The majority of these methods focus on reliability evaluation and not on relevance. Examples of available data evaluation methods are Refs. [20–26]. Still, to a significant extent, evaluation of studies usually relies on case-by-case assessments based on expert judgment.

A structured evaluation method can promote predictability of the risk assessment process. For instance, both a checklist and predefined criteria will contribute to ensuring that at least a minimum and similar set of aspects are considered in each evaluation. Predefined evaluation criteria may also support increased transparency of the evaluation process since all aspects taken into account are clearly reported. Disadvantages of using predefined evaluation criteria and checklists may be less flexibility and that focus might be limited to the general aspects of a study.

TABLE 2 Overview of Test Recommendations in Risk Assessment Documents

Substance Group	Guidance Document	Recommended Test Methods
Pharmaceuticals	Pharmaceuticals Guidance for Industry Environmental Assessment of Human Drug and Biologics Applications [13]	Section IV. D *Test methods and report formats are provided in the FDA Environmental Assessment Technical Handbook. Equivalent tests, such as those provided by the EPA (40 CFR 796 and 797), the Organization for Economic Co-operation and Development (OECD), or other validated, peer-reviewed methods can be used*
Human pharmaceuticals	Guideline on the environmental risk assessment of medicinal products for human use [1]	Section 5 *Experimental studies should preferably follow the test protocols issued by the European Commission, Organization for Economic Co-operation and Development (OECD) or the International Organization for Standardization (ISO)*
Veterinary pharmaceuticals	Guideline on the environmental impact assessment for veterinary medicinal products phase II [14]	Section 2.5 *The specific test guidelines/protocols recommended in Phase II are those finalized by OECD/ISO*
"New substances"	Technical Guidance Document on Risk Assessment. Part II [2]	Part II, Chapter 3, Section 3.2.1.2 *The tests for new substances need to be carried out in accordance with the EU test guidelines as laid down in Annex V to Directive 67/548 or, if no EU guidelines are available or they are not applicable, following internationally recognized guidelines, preferably those of the OECD*
"Existing substances"	Technical Guidance Document on Risk Assessment. Part II [2]	Part II, Chapter 3, Section 3.2.1.2 *Any new tests carried out for risk assessments under Regulation 793/93 should be conducted according to the testing methods laid down in Annex V to Directive 67/548, or if no EU methods are available or they are not applicable, in accordance with internationally recognized guidelines, preferably those of the OECD (1993b). Greater weight should normally be attached to studies carried out according to current methods (e.g. EU, OECD, or US EPA)*

Continued

TABLE 2 Overview of Test Recommendations in Risk Assessment Documents—Cont'd

Substance Group	Guidance Document	Recommended Test Methods
Biocides	Technical notes for guidance on data requirements for active substances and biocidal products [15]	Chapter 1, Section 1.3 *According to Article 8(8), as a general principle, tests must be conducted according to the methods described in Annex V of Council Directive 67/548/EEC, according to the most recent adaptation to the technical progress. These are based on those recognized and recommended by international bodies in particular OECD. In the event of a method being inappropriate or not described, other methods used should, whenever possible, be internationally recognized and must be justified*
Plant protection products	Guidance Document on Aquatic Ecotoxicology [16]	Section 2.1.5 *Tests conducted in accordance with internationally recognized guidelines (even if not specifically recommended in the Annex II or III) can be accepted if the guideline is comparable with those guidelines mentioned in Annex II or III. Tests with species mentioned in the aforementioned guidelines are in principle acceptable, although not all species are indigenous in Europe*
Industrial chemicals	REACH Guidance documents. European Chemicals Agency [37] (2011)	Part B, Section 4.3.1 *According to REACH, Article 13(3), tests required for generating information on intrinsic properties of substances shall be conducted in accordance with the test methods included in a Commission Regulation or in accordance with other international test methods recognized by the Commission or the Agency as being appropriate* Chapter R.11, Section 1.3.3 *As the aquatic T criterion is based on a NOEC for pelagic organisms, the standardized chronic tests on fish, daphnids and algae are preferred to assess the NOEC*

EU, European Union; ISO, International Organization for Standardization; NOEC, no observed effect concentration; OECD, the Organisation for Economic Co-operation and Development; REACH, Registration, Evaluation, Authorisation and Restriction of Chemical substances (European regulation, EC 1907/2006); US EPA/EPA, United States Environmental Protection Agency.

In 2011, we published a comparison of different reliability evaluation methods [26,27]. The overall aim of the study was to investigate if the reliability of nonstandard ecotoxicity studies could be evaluated in a systematic way in environmental risk assessments of pharmaceutical substances. We investigated the usefulness of four evaluation methods [20–22,25]. As a comparison, we included the OECD reporting criteria for chronic ecotoxicity tests (guidelines no. 201, 210, and 211) that corresponds to the EMA guideline recommendations.

The results of this effort showed that choice of evaluation method matters. The four methods differ in their scope, user friendliness, and how criteria are weighted and summarized. The outcome of the different methods can therefore differ, depending on the evaluators' previous experience and knowledge. All four methods require expert judgment. It is therefore important to be aware of the different methods' strengths and limitations. In our view, it is neither possible nor desirable to develop a method that completely leaves out expert judgment, but we can strive towards a method that promotes transparency and reduces bias and vagueness.

Durda and Preziosi [20] provide the method with the broadest scope and it also had the highest resemblance with the OECD Guidelines. Durda and Preziosi [20], Hobbs et al. [21], and Schneider et al. [25] differ in how evaluation criteria are weighted and summarized but all three methods are functional and understandable. Durda and Preziosi [20] and Schneider et al. [25] both provide useful guidance information to the risk assessors, which enhances the user friendliness. Klimisch et al. [22], which is the method recommended in the REACH guidance document for industrial chemicals, showed low resemblance with the OECD reporting requirements and provided least guidance to the evaluators.

7 CAN WE IMPROVE THE PROCESS FOR DATA EVALUATION?

Since we have identified a discrepancy between the criteria proposed in the four available evaluation methods and the OECD reporting requirements, we developed a new evaluation method [26]. This was done in collaboration with the German Federal Environmental Agency in order to incorporate the regulators' views and experiences in this work. The new evaluation method is based on the criteria and evaluation questions suggested in the four evaluated methods, together with the OECD reporting requirements, the recommendations from the European Chemicals Agency REACH guidance information regarding evaluation of available information (Chapter R.4) [3], and the European Chemicals Bureau's Technical Guidance Document (TGD) (Part II, Chapter 3.2) [2]. The criteria were then complemented with our own suggestions based on experiences from working as regulators, risk assessors, and experimentalists [23,27]. Suggestions for improvements of the criteria

were also received from ecotoxicologists working at Brixham Environmental Laboratories/AstraZeneca and researchers within the Swedish research program MistraPharma.

The suggested criteria differ from previously described methods by including more criteria and by covering both reliability and relevance. Table 3 provides an overview of five evaluation methods [20–22,25,26]. When comparing the methods with OECD reporting requirements, the Ågerstrand criteria show the highest similarity, indicating that the criteria could be a useful tool for risk assessors when evaluating data and researchers performing ecotoxicological experiments. We also suggest that the criteria are used for education purposes and in the peer-reviewed process for scientific papers. The 62 reliability criteria are divided into ten different categories: purpose and endpoint, protocol, test compound, dosing system, test organism, controls, test environment, statistical design, biological effect, and other considerations (Table 4). The 12 relevance criteria are phrased as open-ended questions (Table 5).

To further guide risk assessors, regulators, and researchers towards increased use of peer-reviewed data in risk assessments, we have developed a web-based tool with reporting and evaluation criteria for ecotoxicity and toxicity studies: www.scirap.org (manuscript in preparation). The tool is free of charge, publically available, and will be updated regularly.

8 CAN WE IMPROVE REPORTING OF DATA?

It is not unusual that there is a mismatch between how nonstandard tests are performed and reported and regulators' needs for data to be used in risk assessments. Criticism against peer-reviewed data often concerns experimental design and statistical analyses [23,28].

We have investigated whether nine recently published nonstandard ecotoxicity studies from the peer-reviewed literature fulfilled available reliability criteria [27]. A striking result from this exercise was that in several cases, sufficient information to fulfill the reliability criteria was not reported by the authors of the selected studies. Examples of aspects often omitted are information about the controls, results from statistical evaluations, whether there is a dose–response relationship or not, tested concentrations, and clear description of the test environment. Overall, the evaluation of the nine selected nonstandard tests resulted in a low number of studies with acceptable reliability. Table 6 presents an overview of the results. The nine selected studies were evaluated using the four different methods described in the preceding text; this resulted in 36 evaluations. Only 14 (39%) of these evaluations resulted in acceptable or high reliability. Since there is a lack of transparency, it is often not possible to decide whether the low reliability is due to poor performance and design of the study or due to underreporting.

Reporting problems could be a consequence from journals' desire to publish concise papers. However, an increasing number of journals provide

TABLE 3 Description and Comparison of Five Evaluation Methods for Ecotoxicity and Toxicity Studies

Evaluation Method	Klimisch et al. [22]	Durda and Preziosi [20]	Hobbs et al. [21]	Schneider et al. [25]	Ågerstrand et al. [26,27]
Data types	Toxicity (*in vivo* and *in vitro*) and ecotoxicity (acute and chronic) data	Ecotoxicity data	Ecotoxicity (both acute and chronic) data	Toxicity data (both *in vivo* and *in vitro*)	Ecotoxicity data
Coverage	Reliability	Reliability	Reliability	Reliability and also a few aspects of relevance	Reliability and relevance
No. of criteria	12 (acute ecotoxicity), 14 (chronic ecotoxicity)	40	20	21	62 reliability criteria, 12 relevance criteria
Type of criteria	Recommended	Recommended and mandatory	Recommended, mark between 0 and 10	Recommended and mandatory, mark between 0 and 1	Recommended and mandatory
Additional guidance	No	Yes	No	Yes	No
Information on how to summarize the evaluation	Not stated	Stated	Stated	Stated and calculated automatically	Not stated
Evaluation categories	Reliable without restrictions, reliable with restrictions, not reliable, and not assignable	High, moderate, and low quality and not reliable and not assignable	High, acceptable, and unacceptable quality	Reliable without restrictions, reliable with restrictions, not reliable, and not assignable	No evaluation categories

Continued

TABLE 3 Description and Comparison of Five Evaluation Methods for Ecotoxicity and Toxicity Studies—Cont'd

Evaluation Method	Klimisch et al. [22]	Durda and Preziosi [20]	Hobbs et al. [21]	Schneider et al. [25]	Ågerstrand et al. [26,27]
Additional information	Recommended in the REACH guidance document for industrial chemicals	Based on standards from US EPA, OECD, and ASTM	Based on a method developed for the Australasian ecotoxicity database	The method is called ToxRTool (toxicological data reliability assessment tool)	Based on previous methods and regulatory requirements
No. of OECD criteria that the method matched	14/37	22/37	15/37	14/37	31/37

US EPA, United States Environmental Protection Agency; OECD, Organisation for Economic Co-operation and Development; ASTM, American Society for Testing and Materials.

TABLE 4 The Relevance Criteria Proposed by Ågerstrand et al. [26]

Relevance Criteria
Is the substance tested representative for the substance being risk assessed? If not (e.g., is it a metabolite), how relevant is the tested substance for the risk assessment?
Is the appropriate test species studied?
Are the appropriate life stage(s) studied?
Are the appropriate endpoint(s) studied?
Is the route of exposure relevant for the species?
Does the test exposure scenario exist for the tested substance?
Are the stated tested doses/concentrations appropriate?
How do the tested doses relate to measured or predicted environmental concentrations (if available)?
Is the time of exposure relevant and appropriate for the studied endpoints?
Have other critical parameters influencing the endpoints than exposure time been considered adequately (examples of parameters: pH, temperature, and light conditions)?
Should the measured endpoint be considered to be an adverse effect (e.g., reproduction and developmental effects) or not (e.g., metabolization and gene expressions)?
Are the references reported?

possibilities to include additional data as supplementary electronic information, which means that this should not be a major obstacle for making such information publicly available. Reliability evaluation methods can be used as checklists for authors and reviewers to ensure that all important aspects are reported. A more structured reporting format could ensure the reliability of the test data without limiting the researcher's creativity in the design of a nonstandard study.

In addition, the result from the evaluation showed that the four methods differed at a surprisingly high rate. Using the four methods led to the same evaluation result for two studies only, both summarized as studies with unacceptable reliability. The evaluation result differed from unacceptable reliability to acceptable reliability for five studies and from unacceptable reliability to high reliability for two studies.

Weighting of criteria was part of the difference of the results. Evaluating the nonstandard studies using the method described by Durda and Preziosi [20] resulted in zero studies with acceptable or high reliability, while the method described by Hobbs et al. [21] gave a different result with seven out of nine studies with acceptable reliability. The method by Durda and Preziosi [20] has a mandatory criterion that was not reported by any of the studies:

TABLE 5 The Reliability Criteria Proposed by Ågerstrand et al. [26]

Category	Reliability Criteria	Mandatory Criteria	Optional Criteria
Purpose and endpoint	Purpose of study	–	X
	Description of endpoints	X	–
Protocol	Standard/modified standard (if used)	X	–
Test compound	Identification (e.g., name and CAS number)	X	–
	Physicochemical data (e.g., volatility, stability, solubility, degradability, and adsorption)	–	X
	Source	–	X
	Purity	X	–
	Vehicle (if used)	X	–
	Radiolabeled (if used)	–	X
Dosing system	Tested doses or concentrations	X	–
	Measured doses or concentrations	X	–
	Exposure duration	X	–
	Exposure route	X	–
	Exposure schedule (static, semistatic, flow through system, and others)	X	–
	Method of preparation of stock solutions	–	X
	Time points of observations	X	–
	Analytical method	X	–
Test organism	Scientific name	X	–
	Body weight or length	–	X
	Age/life stage	X	–
	Growth/reproductive condition	–	X
	Gender	X, when relevant and possible	X, for others than given in the mandatory column

TABLE 5 The Reliability Criteria Proposed by Ågerstrand et al. [26]—Cont'd

Category	Reliability Criteria	Mandatory Criteria	Optional Criteria
	Strain, clone	X, for algal tests and *Daphnia*, provided if known for other species	X, for others than given in the mandatory column
	Source	X	–
	Culture handling	X	–
	History of contamination for field-collected species	X	–
Controls	Control described	X	–
	Control media identical to test media in all respect except the treatment variable	X	–
	Control(s) identical to treatments in physical and chemical test condition aspects: light, location, temperature in the room/climate chamber	X	–
	Control and test organism drawn from same population	X	–
	Control mortality/morbidity	X	–
	Positive/negative control (if used)	X	–
	Vehicle control (if used)	X	–
	Known concentrations of vehicle (if used) in treatments and controls	X	–
	Control mortality/morbidity reported for vehicle/positive control (if used)	X	–
	Historical control data	–	X
Test environment	pH	X	–
	Temperature	X	–
	Conductivity	X, for brackish and marine tests	X, for others than given in the mandatory column

Continued

TABLE 5 The Reliability Criteria Proposed by Ågerstrand et al. [26]—Cont'd

Category	Reliability Criteria	Mandatory Criteria	Optional Criteria
	Light intensity and quality (source and homogeneity)	X, for algae and for long-term fish studies	X, for others than given in the mandatory column
	Photo period	X	–
	Hardness of water	–	X
	Dissolved oxygen content	X, but not for algae and aquatic macrophytes	X, for others than given in the mandatory column
	Ammonium/nitrite content in water	X, for frogs and fish	X, for others than given in the mandatory column
	Material and volume on aquarium/container	X	–
	Test medium	X	–
	Feeding protocols (for long-term tests)	X	–
	Food composition	–	X
Statistical design	Sample size per replicates, number of organisms per replicates	X	–
	No. of organisms from each replicates used for statistical analysis (if not all used)	X	–
	Randomized treatments	–	X
	Independence of observations	–	X
	Statistical method used	X	–
	Significance level for NOEC and LOEC data (0.05 or less)	X	–
	Estimate of variability for LC and EC data	X	–
Biological effect	Results reproduced by others	–	X
	Consistent with other findings	–	X

TABLE 5 The Reliability Criteria Proposed by Ågerstrand et al. [26]—Cont'd

Category	Reliability Criteria	Mandatory Criteria	Optional Criteria
	Statistically significant responses noted (e.g., EC_x)		X
	Dose–response reported in figure/text/table	X	
	Each effect concentrations explicitly related to a specific endpoint	–	X
Other considerations	References to support the reliability of the study should be reported	–	X
	Produced according to GLP	–	X
	Availability of raw data	–	X

TABLE 6 Summary of the Reliability Evaluation of Nonstandard Test Data According to Four Different Methods

References	Klimisch et al. [22]	Durda and Preziosi [20]	Hobbs et al. [21]	Schneider et al. [25]
Andreozzi et al. [29]	–	–	–	–
Ferrari et al. [30]	–	–	–	–
Huggett et al. [31]	–	–	+	–
Robinson et al. [32]	+	–	+	–
Schmitt-Jansen et al. [33]	+	–	+	–
Quinn et al. [34]	–	–	+	–
Metcalfe et al. [11]	–	–	+	+
Nentwig [35]	+	–	+	++
Halm et al. [36]	+	–	+	++

–, Unacceptable reliability; +, acceptable reliability; ++, high reliability.

acceptable control mortality/morbidity. Hobbs et al. [21] have a similar criterion but since it is not mandatory, it did not have the same effect on the summarized evaluations.

9 SUMMARY AND CONCLUSIONS

Research and risk assessment are often described as strictly scientific activities, free from subjective values, while risk management is allowed to include also other factors such as costs and feasibility. This holds true if we look at the formal description, but if we look closer at research and risk assessment, the picture is different. Tradition, history, and policy decisions affect how we produce science, the design of our risk assessment models, and what type of data we choose to include. This is important to remember when we evaluate and suggest improvements in the risk assessment process, and a major aim should be to ensure an unbiased and transparent procedure for the benefit of the environment and human health.

In this chapter, we argue that data evaluation is a crucial step in the risk assessment process and that the data inclusion criteria employed will have fundamental effects on the outcome of any risk assessment. Therefore, evaluation methods and data inclusion criteria need, as a minimum, to be transparent, unbiased, and systematic.

The purpose of designing and using structured evaluation methods is to help risk assessors evaluate data in a more structured and transparent way, not to replace the risk assessor, and all the methods that were scrutinized require some degree of expert judgment. It follows from this that two experts evaluating the same study might end up with different results depending on their expertise and previous experiences. However, the idea is not to harmonize all risk assessors, but to promote a systematic approach and increase transparency in the evaluation process.

Current legislations emphasize the use of standard test protocols and GLP. We argue that nonstandard data can contribute important information to a risk assessment. The main advantage of facilitating the use of nonstandard (and non-GLP) data for regulatory risk assessment purposes is that it can include the most sensitive and relevant, state-of-the-art test methods, test species, and endpoints.

However, opening up the process for nonstandard approaches is a two-edged sword. The demand for standardization (and GLP) was a response to dishonest use of data in the past. Therefore, we need a system that allows the use of the most relevant and reliable data and at the same time ensure a transparent and unbiased process.

An important step is to improve the reporting of peer-reviewed studies, so that risk assessors are equipped with sufficient information to judge the studies' reliability and relevance for risk assessment. Our limited investigation

implies that significant improvements can be made in this sense; when the reporting of peer-reviewed ecotoxicity studies was scrutinized, crucial information was often missing. The possibility to allow for an increased use of nonstandard tests in regulatory risk assessment should also be explored.

As we see it, the following development is required:

1. Improved reporting of peer-reviewed studies
2. A regulatory system that allows and can cope with nonstandard data in a transparent and unbiased fashion
3. Data evaluation criteria that are comprehensive and useful and have a high degree of general acceptance
4. A pharmaceutical industry that accepts a general responsibility to show that their products are safe also from an environmental perspective, showing safety not just doing standard tests

To achieve this, actions must be taken by researchers, regulatory agencies, the industry, and risk assessors/regulators.

ACKNOWLEDGMENTS AND COMPETING INTERESTS

The authors are part of the MistraPharma research program 2008–2015 (www.mistrapharma.se). MistraPharma is funded by the Swedish Foundation for Strategic Environmental Research (Mistra). The authors declare that they have no competing interests.

REFERENCES

[1] European Medicines Agency, Committee for Medicinal Products for Human Use (CHMP). Guideline on the Environmental Risk Assessment of Medicinal Products for Human Use, 2006. Ref. EMEA/CRMP/SWP/4447/00.

[2] European Chemicals Bureau, European Commission Technical Guidance Document in Support of Commission Directive 93/67/EEC on Risk Assessment for New Notified Substances and Commission Regulation (EC). No. 1488/94 on Risk Assessment for Existing Substances, Part II, 2003.

[3] European Chemicals Agency, REACH Guidance Documents, 2008.

[4] P.M. Chapman, F. Wang, C. Janssen, G. Persoone, H.E. Allen, Can. J. Fish. Aquat. Sci. 55 (1998) 2221–2243.

[5] J. Gardeström, T. Elfwing, M. Löf, M. Tedengren, J.L. Davenport, J. Davenport, Comp. Biochem. Physiol. A Mol. Integr. Physiol. 148 (2007) 869–875.

[6] E.H.W. Heugens, T. Jager, R. Creyghton, M.H.S. Kraak, A.J. Hendriks, N.M. Van Straalen, W. Admiraal, Environ. Sci. Technol. 37 (2003) 2145–2151.

[7] Schering, n.d. Scherings report A12518. Result published by Bayer, for the Swedish environmental classification system for pharmaceuticals (www.fass.se).

[8] M. Fenske, G. Maack, C. Schäfers, H. Segner, Environ. Toxicol. Chem. 24 (2005) 1088–1098.

[9] B. Halling-Sorensen, S.N. Nielson, P.F. Lanzky, F. Ingerslev, H.C. Holten Lützhoft, S.E. Jorgensen, 1998. Occurrence, fate and effects of pharmaceutical substances in the environment – a review, Chemosphere 36 (2) 357–393.

[10] J.P. Nash, D.E. Kime, L.T.M. Van der Ven, P.W. Wester, F. Brion, G. Maack, P. Stahlschmidt-Allner, C.R. Tyler, Environ. Health Perspect. 112 (2004) 1725–1733.

[11] C.D. Metcalfe, T.L. Metcalfe, Y. Kiparissis, B.G. Koenig, C. Khan, R.J. Hughes, T.R. Croley, R.E. March, T. Potter, Environ. Toxicol. Chem. 20 (2001) 297–308.
[12] H. Segner, J. Navas, C. Schäfers, A. Wenzel, Ecotoxicol. Environ. Saf. 54 (2003) 315–322.
[13] US FDA, Guidance for Industry. Environmental Assessment of Human Drug and Biologics Applications, 1998.
[14] European Medicines Agency, Guideline on the Environmental Impact Assessment for Veterinary Medicinal Products Phase II, 2004.
[15] European Chemicals Bureau, Technical notes for guidance on data requirements for active substances and biocidal products, 2000.
[16] European Commission, Guidance Document on Aquatic Ecotoxicology. Plant Protection Products, 2001.
[17] K. Schneider, Amicus J. (Natural Resources Defence Council) (1983) 14–26.
[18] J.P. Myers, F.S. vom Saal, B.T. Akingbemi, K. Arizono, S. Belcher, T. Colborn, I. Chahoud, D.A. Crain, F. Farabollini, L.J. Guillette, T. Hassold, S. Ho, P.A. Hunt, T. Iguchi, S. Jobling, J. Kanno, H. Laufer, M. Marcus, J.A. McLachlan, A. Nadal, J. Oehlmann, N. Olea, P. Palanza, S. Parmigiani, B.S. Rubin, G. Schoenfelder, C. Sonnenschein, A.M. Soto, C.E. Talsness, J.A. Taylor, L.N. Vandenberg, J.G. Vandenbergh, S. Vogel, C.S. Watson, W.V. Welshons, R.T. Zoeller, Environ. Health Perspect. 117 (2009) 309–315.
[19] T. Tweedale, Environ. Health Perspect. 118 (2010) A194.
[20] J.L. Durda, D.V. Preziosi, Hum. Ecol. Risk. Assess. 6 (2000) 747–765.
[21] D.A. Hobbs, M.S.J. Warne, S.J. Markich, Integr. Environ. Assess. Manag. 1 (2005) 174–180.
[22] H.-J. Klimisch, M. Andreae, U. Tillmann, Regul. Toxicol. Pharmacol. 25 (1997) 1–5.
[23] A. Küster, J. Bachmann, U. Brandt, I. Ebert, S. Hickmann, J. Klein-Goedicke, G. Maack, S. Schmitz, E. Thumm, B. Rechenberg, Regul. Toxicol. Pharmacol. 55 (2009) 276–280.
[24] B.J.W.G. Mensink, C.E. Smith, M.H.M.M. Montforts, Manual for summarizing and evaluating aspects of plant protection products, Bilthoven, The Netherlands 2008. RIVM. Report number 601712006/2010. ISBN/EAN: 978-90-6960-245-5.
[25] K. Schneider, M. Schwarz, I. Burkholder, A. Kopp-Schneider, L. Edler, A. Kinsner-Ovaskainen, T. Hartung, S. Hoffmann, Toxicol. Lett. 189 (2009) 138–144.
[26] M. Ågerstrand, M. Breitholtz, C. Rudén, Environ. Sci. Eur. 23 (2011) 17.
[27] M. Ågerstrand, A. Küster, J. Bachmann, M. Breitholtz, I. Ebert, B. Rechenberg, C. Rudén, Environ. Pollut. 159 (2011) 2487–2492.
[28] R.E. Alcock, B.H. MacGillivray, J.S. Busby, Environ. Int. 37 (2011) 216–225.
[29] R. Andreozzi, V. Caprio, C. Ciniglia, M. de Champdoré, R. Lo Giudice, R. Marotta, E. Zuccato, Environ. Sci. Technol. 38 (2004) 6832–6838.
[30] B. Ferrari, R. Mons, B. Vollat, B. Fraysse, N. Paxēaus, R.L. Giudice, A. Pollio, J. Garric, Environ. Toxicol. Chem. 23 (2004) 1344–1354.
[31] D.B. Huggett, B.W. Brooks, B. Peterson, C.M. Foran, D. Schlenk, Arch. Environ. Contam. Toxicol. 43 (2002) 229–235.
[32] A.A. Robinson, J.B. Belden, M.J. Lydy, Environ. Toxicol. Chem. 24 (2005) 423–430.
[33] M. Schmitt-Jansen, P. Bartels, N. Adler, R. Altenburger, Anal. Bioanal. Chem. 387 (2007) 1389–1396.
[34] B. Quinn, F. Gagné, C. Blaise, Sci. Total Environ. 389 (2008) 306–314.
[35] G. Nentwig, Arch. Environ. Contam. Toxicol. 52 (2007) 163–170.
[36] S. Halm, N. Pounds, S. Maddix, M. Rand-Weaver, J. Sumpter, T. Hutchinson, C. Tyler, Aquat. Toxicol. 60 (2002) 285–299.
[37] European Chemicals Agency (ECHA), REACH Guidance Documents. Helsinki, Finland, 2011.

Chapter 21

Conclusions and Future Research Needs

Mira Petrovic*, Sandra Pérez† and Damia Barcelo*,†
*Catalan Institute for Water Research (ICRA), Girona, Spain
†Water and Soil Quality Research Group, Department of Environmental Chemistry, IDAEA-CSIC, Barcelona, Spain

Chapter Outline

1 INTRODUCTION

Pharmaceuticals are an extraordinarily diverse group of chemicals used in human and veterinary medicine. Many pharmaceuticals are highly bioactive, most are polar, many are optically active, and all, when present in the environment, occur usually at low trace concentrations. They are a class of new, the so-called emerging, contaminants that have raised great concern in the last years. Hundreds of tons of pharmaceuticals are dispensed and consumed annually worldwide. The usage and consumption of pharmaceuticals have been increasing consistently due to the discoveries of new drugs, the expanding population, and the inverting age structure in the general population and due to expiration of patents with resulting availability of less-expensive generics. Recent development and continual improvement of the advanced instruments and analytic methodologies made possible the detection of pharmaceuticals at low levels in different environmental matrices. Traces of pharmaceuticals and their metabolites have been found in all environmental compartments along the water cycle, such as wastewater, surface water and aquatic sediment, biota, soil irrigated with reclaimed water and soil that received biosolids from urban sewage treatment plants, and groundwater, and finally in drinking water. The research in such broad field is very active and results in huge number of papers published every year, divided broadly

Comprehensive Analytical Chemistry, Vol. 62. http://dx.doi.org/10.1016/B978-0-444-62657-8.00021-5

into four main categories: (i) development of the analytic methodology for the detection of pharmaceuticals; (ii) studies on environmental occurrences; (iii) studies on fate, behavior, and treatment; and (iv) studies on ecotoxicology and effects on organisms and ecosystem. However, in spite of that, still, there are many gaps in knowledge and the study of pharmaceuticals as environmental contaminants requires more comprehensive approach.

The objective of this overview and final chapter is to identify some of the priority areas and research gaps pointing the way forward for scientific research in the field.

2 CHEMICAL ANALYSIS

One of the reasons for the increasing concern on pharmaceuticals has certainly been the improvement in analytic techniques during the last decade. Many pharmaceutical compounds that were not possible to detect at low concentrations in environmental samples are nowadays part of the routine analysis, with detection limits below nanogram/liter level. At the same time, with the progresses in analytic instrumentation, extraction techniques have become more simple, fast, and inexpensive, providing the enrichment of analytes of interest from matrices as complicated as wastewater or sewage sludge.

There are, however, few questions that still need to be solved. The main drawback of the conventional analytic approach is preselecting target compounds, usually parent compounds, which is often insufficient to assess the environmental risk posed by pharmaceuticals, since some relevant compounds may not be identified and detected. In the need to monitor pharmaceutical residues in the environment, numerous sensitive, accurate, and reliable analytic methods have been developed for determination of pharmaceuticals in aqueous solutions. In the last years, a gradual shift is observed from the analysis of single class of compounds to multiresidue methods involving analysis of 50–100 compounds belonging to different therapeutic classes. The pharmaceuticals more frequently included in such multiresidue methods are analgesics and anti-inflammatory drugs, antibiotics, lipid regulators, psychiatric drugs, and β-blockers; however, some potentially relevant compounds are not analyzed (see Section 4). The selection of target compounds is usually based on high consumption and ubiquity in both surface and wastewaters as determined by previously published studies. Due to their comprehensive approach, multiresidue analytic methods have become preferred tools since they allow determination of a large number of pharmaceuticals in a single analysis, thus reducing its time and cost. In ideal case, multiclass/multiresidue method should fulfill several criteria:

1. Sample preparation and preconcentration is achieved in a single step although analytes possess different physicochemical properties.
2. Limits of detection and quantification low enough for each analyte.

3. Substance-specific detection.
4. Easy application to various matrices (e.g., natural water, drinking water, and wastewater).

Nevertheless, for reliable and reproducible multiresidue method, a compromise in the selection of experimental conditions is required resulting in the performance of the method not optimal for each single compound, but rather acceptable for the majority. Simultaneous analysis of multiclass compounds with quite different physicochemical characteristics often imposes compromises between the performance parameters, in case of preconcentration (cartridge selection, elution solvent, and pH), chromatographic separation (column and solvent gradient), and MS detection (interface and operational parameters). In practice, achieving acceptable performances for all compounds in the method sometimes results in the elimination of relevant compounds from the list of target compounds simply because they may require specific and more selective analytic approach and cannot be analyzed simultaneously with other compounds.

Another pitfall of current methodologies is that generally very few metabolites and transformation products (TPs) are included into monitoring studies. There are several reasons for that: not all the TPs are commercially available or they are too expensive. The alternative is to use a second analyzer, like time of flight (TOF) or ion trap (IT), to look for "known unknowns," such as possible TPs of the pharmaceuticals degraded by microbial action and/or UV light that can be present in the samples. This obviously requires a second analysis; besides, there is often a lack of sensitivity and, perhaps, also of experience of the laboratory to identify the tentative list of the breakdown products formed. In addition to that, the problems related to conjugated metabolites, like glucuronide and sulfate conjugates that can be deconjugated by microbial action during wastewater treatment processes, need also further and careful investigation. Indeed, since many pharmaceuticals are excreted as conjugates by humans, they may actually increase in concentration after passage through the wastewater treatment plant (WWTP). Thus, the matrix of pharmaceutical compounds exiting the plant may be very different from those entering.

Analysis of solid samples (sewage sludge, sediment, and soil) is still a challenge and methods are available for a limited number of‘ compounds. Of the various solid samples, sewage sludge is one of the more complex. Some of the biggest analytic challenge is that a "complete" analysis of sewage sludge includes overcoming the large negative surface charges and interstitial spaces that provide multiple active sites for charged compounds and the cleanup step for removing the bulk material (e.g., fats, proteins, and surfactants) that are coextracted with the pharmaceuticals. Research efforts involving novel approaches based on highly selective cleanup procedures using molecularly imprinted polymers (MIPs) or integrated sample cleanup and

analysis systems (such as dual LC column systems based on turbo-flow chromatography for cleanup) are explored to isolate new pharmaceutical residues and their TPs from complex sludge samples. Analytic methodology to detect pharmaceuticals in biota (fish, bivalves, and other aquatic organisms) in order to study their bioaccumulation has advanced significantly in the last few years and several methods have been developed [1,2]. Nonetheless, there are still unresolved analytic challenges associated with the complexity of biological matrices, which require exhaustive extraction and purification steps and highly sensitive and selective detection techniques.

Another relevant issue is the quality of data and the performance of interlaboratory tests combined with the use of reference materials. Methodological challenges in the analysis of pharmaceuticals in environmental samples are numerous and each of them can lead to erroneous data and wrong interpretations. Main pitfalls and sources of errors are generally linked to the sampling strategy and representativeness of the samples, preservation of samples, stability of analytes in the sample, stability of standard solutions, possible losses of analytes due to sorption on the glassware and/or filter materials, and matrix effects in MS detection; and each of these should be carefully studied and proper strategies to reduce the errors adopted. Although several interlaboratory exercises have been organized for anti-inflammatory drugs, antibiotics, and steroid hormones in water [3–6], there is still a lack of such studies for other groups of compounds and matrices. In addition and complementary to that, the availability of reference materials to be used by the laboratories performing the monitoring studies is limited and should be increased.

3 WASTEWATER TREATMENT

WWTP using secondary biological sewage treatment plants has brought enormous benefits to society and the environment. Considering the short hydraulic residence time (few hours), the large reduction in the amount of natural and xenobiotic compounds is remarkable. However, municipal WWTPs are basically designed to remove pathogens and organic and inorganic suspended and flocculated matter, but not pharmaceuticals. Four key factors are critical in predicting the impact of each WWTP: (1) the size of the human population connected to the WWTP, (2) the flow through the works, (3) the type of treatment employed, and (4) the available dilution in the receiving water.

Given the variations in “human discharge” and flow into the WWTP typically resulting in the 8–9 a.m. peak flushes, proper sampling strategy is of crucial importance. Poor sampling and analysis of nonrepresentative samples is generally dominant source of error in water quality data, leading to wrong conclusions regarding elimination of certain compounds and efficiency of applied treatment. Therefore, appropriate number of samples, duration of sampling, and frequency are crucial to obtain reliable data. Another key issue is

the hydraulic retention time (HRT). Activated sludge is the most intensive biological treatment in which bacteria are suspended in a tank and vigorously aerated, with HRT varying from typically 5 to 36 h. In some studies, negative values of removal (i.e., difference in the loads between influent and effluent) have been reported. The explanation for this could be found in sampling protocols, not only because they could be inadequate but also because of the nature of disposal of pharmaceuticals. The fact is that the substances arrive in a small number of wastewater packets to the influent of WWTP, in unpredictable amounts and time intervals; thus, the influent loads, especially, are easily systematically underestimated. Therefore, well-planned and performed sampling is the key point in pharmaceutical analysis.

It can be noticed in the literature that large differences are observed when comparing elimination rates for certain pharmaceuticals, like diclofenac, with reported elimination ranging from 0 to >90%. Pharmaceuticals can be eliminated by sorption onto the sludge or through microbial degradation. In many cases, the metabolites formed during biodegradation are more polar than the parent compound. The high polarity combined with the low biodegradability that some pharmaceutical compounds exhibit results in inefficient elimination. Moreover, the negative removal can be explained by the formation of unmeasured products of human metabolism and/or TPs (e.g., glucuronide conjugate, methylates, and glycinates) that passing through the plant converts back to the parent compounds. The efficiency of contaminant removal is strongly dependent on the type of treatment technology (e.g., physicochemical vs. biological treatment) and on the operational parameters of the plant. The factors indicated in the preceding text can contribute to these differences, and another conclusion is that there is a need for an increased understanding of the mechanisms of degradation and elimination of pharmaceuticals in WWTPs at environmentally relevant concentrations.

In order to understand the process taking place in the WWTP and to increase the knowledge on biodegradation of contaminants in WWTP, biodegradation studies of pharmaceuticals under laboratory-controlled conditions simulating WWTPs should be conducted. A few studies have investigated biodegradation pathways in various environmental compartments and reported identities of biotransformation products during primary biodegradation. The identification of degradates in environmental samples is a challenging task because they not only present in very low concentrations but also are mixed with complex matrices that interfere with detection. There is a need to increase our knowledge about the fate of pharmaceuticals during sewage treatment for the implementation of better removal technologies. Future work on WWTP will show to what extent pharmaceuticals can be removed from wastewater and to what extent the implementation of an improved technology is feasible, taking into account other macro- and micropollutants and the broad variety of complex matrices.

Linked to this is the complex issue of modeling of the behavior of pharmaceuticals in the WWTP. The distribution of pharmaceuticals between the various compartments in treatment plants depends on the physicochemical and biological properties that are relevant to each individual pharmaceutical and process, and the information about the influence of operating conditions on pharmaceutical removal is a key point to better understand and improve removal mechanisms. Thus, models simulate many operating conditions, and predicting pharmaceutical removal, sorption and desorption on suspended solids, biotransformation including the formation of metabolites, and chemical transformation processes is a useful tool that may help reduce the release of pharmaceuticals to the environment. Recently, Pomiès et al. [7] presented a critical overview of the models proposed in the literature to describe micropollutant removal in activated sludge processes. They concluded that the models still need to be improved with a more accurate description of the mechanisms involved, including consideration of oxidation–reduction conditions, consideration of not only parent compounds but also metabolites in order to better characterize the fate of contaminants, and delimiting the range of applicable conditions (i.e., SRT, HRT, T, and pH) for the model utilization. One of the main difficulties is to find a compromise between the precision of the model and the accessibility of the model parameters. Furthermore, development of standardized protocols, calibration and validation methods, seems particularly necessary.

This book also summarizes current knowledge on other treatment technologies (Chapters 9–11), such as membrane bioreactors (MBRs) and other membrane treatments (nanofiltration and reverse osmosis), advanced oxidation processes (AOPs), and natural treatments based on bank filtration and artificial recharge and constructed wetlands (Chapters 12 and 13).

The increased use of MBRs with a similar process as the one taking place in secondary treatment seems to be an excellent alternative to improve the biodegradation of pharmaceuticals in the environment to increase their removal rates. However, membrane treatment processes should be optimized by a modification of the membranes (variation of materials and reduction of molecular mass cutoff limits) and/or by modification of the treatment process (inoculation of special microorganisms). The efficiencies of diverse microbial populations in the elimination of selected pharmaceuticals and the optimization of design and operating parameters of a laboratory-scale MBR should be considered as a future research needed in this area. Scale-up from pilot MBR to real-world WWTP should also be investigated in order to assess if the processes and elimination in the pilot pant are still valid in a large-scale plant.

Technologies involving natural attenuation such as bank filtration or artificial groundwater recharge and constructed wetlands can be of help to the removal of pharmaceutical residues from water matrices. WWTPs and drinking water suppliers are deeply interested in such technological developments

in order to improve the quality of the water. The limiting factors are the costs of all these technologies when they need to be implemented at real scale, since they will have a direct cost for the consumer, therefore increasing water prices. So, compromises will always be needed in selecting the most appropriate technology that is cost-effective. One way of doing so is by advanced treatment options and reusing the treated water for different purposes, in accordance with the water quality achieved following the tailor-made treatments. However, in order to achieve higher removal efficiencies for pharmaceuticals as compared to conventional activated sludge of advanced end-of-pipe technologies (e.g., advanced MBRs, AOPs, and/or eco-friendly treatments such as white-rot fungi), we need to improve our understanding of mechanisms involved in the removal and transformation of pharmaceuticals by advanced treatments.

It is also clear that more efforts should also be directed toward reducing the contaminant loads to WWTPs, for instance, by not throwing away unused pharmaceutical into the waste or into the toilet. In their review survey on attitudes and practices to medicine disposal methods around the world, Tong et al. [8] concluded that the most popular methods for medication disposal were in the garbage, toilet, or sink. Liquid medications were more likely to be rinsed down the sink, as opposed to solid tablets and capsules that were more likely deposited in the rubbish bin. The establishment of formalized state-run collection and disposal systems that are cost-effective and easily accessible to the public is of supreme importance. However, many countries do not have standard medication disposal protocols. Another issue is overprescribing of drugs and current practice to prescribe a fixed dose for an amount of time in order to maximize profit instead of patient care (see Chapter 2). Daughton and Ruhoy [9] recommended optimizing drug dose as a major factor in improving the sustainability of health care. Customized dosing and incorporation of consideration of the potential for adverse environmental impacts into the practice of prescribing could improve patient care and public health protection and at the same time reduce the effects of pharmaceuticals in aquatic systems.

4 OCCURRENCE STUDIES

Numerous research publications have reported on the occurrence of pharmaceuticals in the environment; however, the compounds monitored represent only a snapshot of all pharmaceuticals approved for use. Recently, Hughes et al. [10] estimated that fewer than 4% of pharmaceuticals have been analyzed for and detected in freshwaters. Their analysis of all published studies on pharmaceuticals in the environment showed that more than 50% of entries in the database correspond to just 14 compounds belonging to the groups of antibiotics, antiepileptics, cardiovascular drugs, and painkillers. Frequently studied compounds include ibuprofen, sulfamethoxazole, erythromycin,

TABLE 1 Top 20 PhACs in the United States Based on Their Environmental Loads and Aquatic Toxicity

Top 20 Environmentally Loaded PhACs	Top 20 Priority Scores Over Six Aquatic Endpoints
Metformin HCl	Montelukast sodium
Polyethylene glycol	Clopidogrel bisulfate
Amoxicillin trihydrate	Levothyroxine sodium
Cephalexin	Simvastatin
Ranitidine HCl	Ranitidine HCl
Trimethoprim	Telmisartan
Furosemide	Bupropion HCl
Levothyroxine sodium	Trimethoprim
Fluticasone propionate	Nitrofurantoin
Gabapentin	Tramadol HCl
Atenolol	Hydroxyzine pamoate
Hydrochlorothiazide	Acetaminophen
Ciprofloxacin HCl	Amoxicillin trihydrate
Acetaminophen	Hydroxychloroquine sulfate
Clopidogrel bisulfate	Pioglitazone HCl
Levetiracetam	Sulfamethoxazole
Levofloxacin	Irbesartan
Sulfamethoxazole	Furosemide
Fexofenadine HCl	Atenolol
Valacyclovir HCl	Trazodone HCl

Modified from Dong et al. [19].

carbamazepine, fluoxetine, and diclofenac, while some potentially very relevant drugs have not been studied at all. Table 1 lists top 20 compounds based on their environmental loads and aquatic toxicity over six endpoints (algae 96-h EC_{50}, algae chronic value; daphnid 48-h LC_{50}, daphnid chronic value; and fish 96-h LC_{50}, fish chronic value, all calculated using ECOSAR).

For example, there are no data on the occurrence of potentially harmful and widely used montelukast and fluticasone, both used to treat asthma and to relieve symptoms of seasonal allergies; levothyroxine, a synthetic form of

the thyroid hormone thyroxine; and clopidogrel, an antiplatelet agent used to inhibit blood clots in coronary artery disease, peripheral vascular disease, and cerebrovascular disease. Very few studies included pioglitazone, an antihyperglycemic and antidiabetic that was associated with bladder cancer and has been withdrawn in some countries.

Furthermore, the information regarding the occurrence and fate of the metabolites and TPs of pharmaceuticals is also limited, although recent methods and monitoring studies are doing more efforts to include relevant TPs. During complex metabolic processes in the human body and biochemical processes in wastewater treatment, various scenarios of transformation from parent compound to metabolite and derivatives and vice versa can occur. Generally, metabolites tend to increase the water solubility of the parent compound and can be just as active as their parent compounds. Therefore, the occurrence of metabolites and TPs and pathways should be included in the future studies in order to obtain accurate information on removal of pharmaceuticals during treatment and to evaluate their environmental risks.

Another issue is poor spatial distribution of research on pharmaceuticals in the environment showing a heavy bias toward Europe and North America, with little or no data for developing countries. Moreover, most of the current publications on pharmaceutical residues in the water cycle have addressed the contamination of surface waters and wastewaters. Water cycle and pharmaceutical residue analysis should also include all the compartments especially groundwater and the leaching of pharmaceuticals through the soil and to groundwater. Attention should be paid to the distribution of pharmaceuticals between groundwater and surface water in certain parts of the river like alluvial plains and to the quality and environmental impact of such waters for their possible use as drinking water, since many aquifers are used as a source of water supply.

Furthermore, monitoring studies should be aimed to improve evidence of causal links between the occurrence of pharmaceuticals, local ecological status, and climate variability. This includes the design of tailor-made monitoring programs that include measuring of pharmaceutical residues in water, suspended solids, sediments, and biota samples, together with long-term, concurrent hydrometeorological, aquatic morphology, and biological monitoring of reference sites.

5 FATE AND BEHAVIOR

Pharmaceuticals are a special class of contaminants because they can undergo transformations through metabolism before their entrance into aquatic and terrestrial environments. Human and veterinary pharmaceuticals are metabolized in mammals through different enzymatic systems that are designated to chemically transform the foreign compounds to metabolites. Therefore, mixtures of pharmaceuticals reach the aquatic and terrestrial environment as it has been

reported by a large number of studies showing the presence of pharmaceuticals and even their human metabolites. However, little is known about their behavior and fate in these systems. Regarding human pharmaceuticals and their metabolites that are discharged into WWTPs as a first step of their total distribution and transformation in the aquatic environment, they can be removed from the wastewater stream through microbial degradation, photolysis by application of advanced treatment processes, or by sorption onto sludge. Their removal, disappearance, or transformation in sewage treatment systems and also in the aquatic and terrestrial environment depends on different variables from physicochemical properties of pharmaceuticals and their metabolites to the inherent characteristics of the WWTP, soil or water where the compounds end up. In Chapters 14 and 15, respectively, a detailed description of the fate of pharmaceuticals in aquatic and terrestrial environments is reported. In these chapters, it is pointed out that different physicochemical and biochemical processes are affecting the fate of pharmaceuticals in the aquatic and terrestrial environment. However, only the partitioning processes onto sediment–water and sewage–water systems for a few compounds are reported, such as antibiotics, non-steroid anti inflamatory drugs (NSAIDs), lipid regulators, and steroids, and their sorption is not easy to be attributed only to hydrophobic interactions because their molecules bear a lot of polar moieties. Another factor that affects the sorption in soils is the mobility of the pharmaceuticals that greatly depends on their water solubility and the composition of the soil like the organic content. The fate of pharmaceuticals in soils and in natural treatments (see Chapters 12 and 15) is also affected by their bioavailability. For instance, some pharmaceuticals have been detected in different parts of plant crops, because treated wastewater containing pharmaceuticals is used for irrigation and manure or biosolids are still used for fertilization of soils. Antibiotics (ciprofloxacin, chlortetracycline, enrofloxacin, sulfamethoxazole, sulfamerazine, trimethoprim, and tylosin) and the β-blocker atenolol were taken up into plants (Chapter 15). Bioavailability is also a key parameter affecting the partitioning of pharmaceuticals from suspended matter and sediment to organisms that are living in contact with the pharmaceutical-contaminated water. For instance, no more than ten pharmaceuticals have been detected in fish tissues, and bioconcentration was only observed for the anti-inflammatory drug ibuprofen [11]. Conversely, the concentrations of pharmaceuticals are not always increasing because there are some mechanisms of natural and man-made attenuation such as the degradation via biotic or abiotic processes responsible for the attenuation of pharmaceuticals in the environment and sewage treatment processes [12]. The biotic processes are directly related to the bioavailability of pharmaceuticals for the organisms in different environmental and man-made compartments. A few studies have investigated biodegradation pathways in various environmental compartments and in sewage treatment processes, while only few are dealing with biodegradation of pharmaceuticals at lab-scale. Photolysis can be one of the main processes affecting pharmaceutical disappearance in

surface waters where light can enter into the entire water column [13]; in contrast, in the terrestrial environment, it only can affect compounds in the surface of the top soil. Direct and indirect photolyses of pharmaceuticals are influenced by the chemical structure of the pharmaceutical, water pH, water depth, dissolved organic matter and inorganic ions, and climate. Most of the studies already published have little environmental relevance because they have been performed in lab-scale setups. To date, only few studies describe the photolysis in surface waters and under real conditions, including tetracyclines, fluoroquinolones, anti-inflammatory drugs, iodinated contrast media, and steroids, which are prone to photolysis. Only few studies are studying their hydrolysis and it is an important process for pharmaceuticals such as for antibiotics that can be easily hydrolyzed.

In order to evaluate the degradation processes in the environment and in sewage treatment processes, three approaches are proposed in Chapter 16: (a) generation, identification, and detection of TPs in environmental samples; (b) evaluating shifts in the enantiomeric fraction of chiral drugs brought about by stereoselective biotransformation processes; and (c) changes in the isotopic ratio of 13C/12C. The first approach includes degeneration of the TPs of pharmaceuticals, identification, and its subsequent detection in the environment. It is a tedious approach, because it encompasses an interpretation of many data and the need for laboratories equipped with liquid chromatography–high-resolution mass spectrometry and well-trained personnel. However, since new hardware and software packages are launched on the market, a combination of full-scan datasets in mass spectrometry and powerful software, which allows to detect predicted formation of TPs, will facilitate the workflow of the first approach will be easier. Only few examples are reported with the other identification TP approach and a gradual shift to suspect analysis of samples containing parent compounds and TPs can be expected. The second approach evaluates the enantiomeric shifts of the drugs, making profit of the chirality of the drugs; this property has received very little attention in the field of environmental analysis despite the fact that many drugs are marketed as racemates. It is an interesting field for both analytic and environmental chemist because biological effects are usually stereoselective, and to evaluate these effects, advance liquid chromatography techniques and sensitive mass spectrometric analyzers should be used. As for the third approach that for a successful application needs a lot of experience with the liquid chromatography-isotope ratio mass spectrometry (LC–IRMS) which only a few laboratories have and an expensive instrumentation. Therefore, the two latter approaches need expensive analytic features for their implementation as a routine method in the laboratories.

6 TOXICITY AND EFFECTS ON FRESHWATER ECOSYSTEMS

Pharmaceuticals are designed to activate or inhibit the function of specific enzymes or receptors, which induces the desired pharmacological effect and ultimately turns into therapeutic benefit to treated humans and livestock.

However, pollution constituted by pharmaceutical compounds has worried the scientific community because they are continuously entering the environment, they are under no regulations, and they are bioactive compounds. Therefore, questions on their acute and long-term chronic effects on exposed biota have been raised. Establishing cause–ecotoxicological effect relationships of pharmaceuticals in the environment is extremely challenging because of the intrinsic difficulties of field studies (Chapter 17). However, there are some studies reporting worrisome numbers of intersex fish that has been linked to exposure to estrogens and estrogen-like compounds [14]. Other scientists have detected that antidepressants like fluoxetine may disrupt frog maturation [15]. However, one of the well-known effects in the aquatic environment is antibiotic resistance caused by antibiotics (Chapter 19). Antibiotic resistance is a major concern for human health because the selection of resistance strains eventually compromises the efficacy of the antibiotics. Different environmental compartments might have a significant role in the development of antibiotic resistance. For instance, the continued land application of manure containing tetracyclines and other antibiotics can exert selective pressure on soil microbial populations and promote the selection of resistant microbes. Once in the environment, resistant genes are capable of being transferred from bacteria to native soil bacteria through mutation and selection or by acquiring from other bacteria the genetic information. The key questions in antibiotic resistance to be addressed include (a) the role antibiotics play in developing antibiotic resistance and multiple antibiotic-resistant bacterial populations, (b) to determine whether there is a direct relationship between antibiotic residues and antibiotic-resistant bacteria in the environment, and (c) whether continuous exposure to low levels of complex mixtures of antibiotics has negative effects on the quality of water and ecosystem health [16]. It should be added that every month, new papers are published in the scientific literature covering some aspects of the toxic effects of a certain pharmaceutical in the environment on a different organism, vertebrate or invertebrate or aquatic plant. However, the phenomenon of antibiotic resistance is still far from being well understood because its conventional characterization has depended on culture-based techniques; however, new technologies such as DNA microarrays and next-generation sequencing will be useful for the understanding of antibiotic resistance (Chapter 19) and the toxicity of pharmaceuticals in general. Another example, which triggered ample public concern, is the direct correlation between consumption of diclofenac-containing animal carcasses and renal failure in three vulture species in India and Pakistan. Diclofenac had been administered to livestock through feeding [17]. The drugs that are provoking these effects are currently detected in wastewater influents.

Since surface waters are mainly contaminated with pharmaceuticals originating from discharges of WWTPs, the aquatic ecosystem is expected to be affected by their continuous presence. However, toxic effects of

pharmaceuticals have been evaluated on single species of aquatic organisms based on short-term laboratory exposures that generally assess the acute toxicity of a single compound by measuring simple responses such as growth, mortality, or reproduction. This approach does not reflect the real situation because in the environment, mixtures of compounds are present and other factors and stressors can affect differently the ecosystem community (Chapter 18). Moreover, Fent [18] pointed out that little has been done to evaluate effects under (sub)chronic exposure. Current data on acute and chronic toxicity of pharmaceuticals support the conclusion that more target or biomolecule-oriented or mode-of-action-based investigations will allow more relevant insights into effects on survival, growth, and reproduction than traditional standard ecotoxicity testing. Taking into account that exposure to pharmaceuticals, particularly those occurring in surface waters, is in fact of chronic nature, firm conclusions on the long-term hazards or risks of pharmaceuticals for the aquatic ecosystem cannot be drawn.

REFERENCES

[1] B. Huerta, S. Rodríguez-Mozaz, D. Barceló, Anal. Bioanal. Chem. 404 (9) (2012) 2611–2624.
[2] B. Du, P. Perez-Hurtado, B.W. Brooks, C.K. Chambliss, J. Chromatogr. A 1253 (2012) 177–183.
[3] E. Heath, T. Kosjek, M. Farre, J.B. Quintana, L.F. de Alencastro, S. Castiglioni, O. Gans, K. Langford, R. Loos, J. Radjenović, L.M. Rocca, H. Budzinski, D. Tsipi, M. Petrovic, M.D. Barcelo, Talanta 81 (4–5) (2010) 1189–1196.
[4] E. Heath, T. Kosjek, H.R. Andersen, H.C. Holten, M. Lützhøft, M. Adolfson, M. Erici, M. Coquery, R.A. Düring, O. Gans, C. Guignard, P. Karlsson, F. Manciot, Z. Moldovan, D. Patureau, L. Cruceru, F. Sacher, A. Ledin, Environ. Pollut. 158 (3) (2010) 658–662.
[5] M. Farré, M. Petrovic, M. Gros, T. Kosjek, E. Martinez, E. Heath, P. Osvald, R. Loos, K. Le Menach, H. Budzinski, F. De Alencastro, J. Müller, T. Knepper, G. Fink, T.A. Ternes, E. Zuccato, P. Kormali, O. Gans, R. Rodil, J.B. Quintana, F. Pastori, A. Gentili, D. Barceló, Talanta 76 (3) (2008) 580–590.
[6] B. Roig, M. Brogat, S. Mompelat, J. Leveque, A. Cadiere, O. Thomas, Talanta 98 (2012) 157–165.
[7] M. Pomiès, J.-M. Choubert, C. Wisniewski, M. Coquery, Sci. Total Environ. 443 (2013) 733–748.
[8] A.Y.C. Tong, B.M. Peake, R. Braund, Environ. Int. 37 (2011) 292–298.
[9] C.G. Daughton, I.S. Ruhoy, Sci. Total Environ. 443 (2013) 324–337.
[10] S.R. Hughes, P. Kay, L.E. Brown, Environ. Sci. Technol. 47 (2013) 661–677.
[11] M. Lahti, J.-M. Brozinski, A. Jylha, L. Kronberg, A. Oikari, Environ. Toxicol. Chem. 30 (2011) 1403–1411.
[12] S. Pérez, D. Barceló, Anal. Chem. 80 (2008) 8135–8145.
[13] C. Gonçalves, S. Pérez, V. Osorio, M. Petrovic, M.F. Alpendurada, D. Barceló, Environ. Sci. Technol. 45 (2011) 4307–4314.
[14] C.A. Harris, P.B. Hamilton, T.J. Runnalls, V. Vinciotti, A. Henshaw, D. Hodgson, T.S. Coe, S. Jobling, C.R. Tyler, J.P. Sumpter, Environ. Health Perspect. 119 (2011) 306–311.

[15] H.R. Foster, G.A. Burton, N. Basu, E.E. Werner, Environ. Toxicol. Chem. 29 (2010) 2845–2850.
[16] A.K. Sarmah, M.T. Meyer, A.B.A. Boxall, Chemosphere 65 (2006) 725–731.
[17] R. Risebrough, Nature 427 (2004) 596–598.
[18] K. Fent, A.A. Weston, D. Caminada, Aquat. Toxicol. 76 (2006) 122–159.
[19] Z. Dong, D.B. Senn, R.E. Morgan, J.P. Shine, Regul. Toxicol. Pharmacol. 65 (2013) 60–67.

Index

Note: Page numbers followed by "*f*" indicate figures and "*t*" indicate tables.

B

C

D

E

F

G

H

I

K

L

M

N

O

P

T

U

W

Y

Z