The Handbook of Environmental Chemistry 46

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Emerging Contaminants in River Ecosystems

Occurrence and Effects Under Multiple Stress Conditions

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The Handbook of Environmental Chemistry

Founded by Otto Hutzinger

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Aims and Scope

Since 1980, *The Handbook of Environmental Chemistry* has provided sound and solid knowledge about environmental topics from a chemical perspective. Presenting a wide spectrum of viewpoints and approaches, the series now covers topics such as local and global changes of natural environment and climate; anthropogenic impact on the environment; water, air and soil pollution; remediation and waste characterization; environmental contaminants; biogeochemistry; geoecology; chemical reactions and processes; chemical and biological transformations as well as physical transport of chemicals in the environment; or environmental modeling. A particular focus of the series lies on methodological advances in environmental analytical chemistry.

Series Preface

With remarkable vision, Prof. Otto Hutzinger initiated *The Handbook of Environmental Chemistry* in 1980 and became the founding Editor-in-Chief. At that time, environmental chemistry was an emerging field, aiming at a complete description of the Earth's environment, encompassing the physical, chemical, biological, and geological transformations of chemical substances occurring on a local as well as a global scale. Environmental chemistry was intended to provide an account of the impact of man's activities on the natural environment by describing observed changes.

While a considerable amount of knowledge has been accumulated over the last three decades, as reflected in the more than 70 volumes of *The Handbook of Environmental Chemistry,* there are still many scientific and policy challenges ahead due to the complexity and interdisciplinary nature of the field. The series will therefore continue to provide compilations of current knowledge. Contributions are written by leading experts with practical experience in their fields. *The Handbook of Environmental Chemistry* grows with the increases in our scientific understanding, and provides a valuable source not only for scientists but also for environmental managers and decision-makers. Today, the series covers a broad range of environmental topics from a chemical perspective, including methodological advances in environmental analytical chemistry.

In recent years, there has been a growing tendency to include subject matter of societal relevance in the broad view of environmental chemistry. Topics include life cycle analysis, environmental management, sustainable development, and socio-economic, legal and even political problems, among others. While these topics are of great importance for the development and acceptance of *The Handbook of Environmental Chemistry,* the publisher and Editors-in-Chief have decided to keep the handbook essentially a source of information on "hard sciences" with a particular emphasis on chemistry, but also covering biology, geology, hydrology and engineering as applied to environmental sciences.

The volumes of the series are written at an advanced level, addressing the needs of both researchers and graduate students, as well as of people outside the field of "pure" chemistry, including those in industry, business, government, research establishments, and public interest groups. It would be very satisfying to see these volumes used as a basis for graduate courses in environmental chemistry. With its high standards of scientific quality and clarity, *The Handbook of Environmental Chemistry* provides a solid basis from which scientists can share their knowledge on the different aspects of environmental problems, presenting a wide spectrum of viewpoints and approaches.

The Handbook of Environmental Chemistry is available both in print and online via www.springerlink.com/content/110354/. Articles are published online as soon as they have been approved for publication. Authors, Volume Editors and Editors-in-Chief are rewarded by the broad acceptance of *The Handbook of Environmental Chemistry* by the scientific community, from whom suggestions for new topics to the Editors-in-Chief are always very welcome.

Damià Barceló
Andrey G. Kostianoy
Editors-in-Chief

Volume Preface

The emission of emerging contaminants as an environmental problem has raised increasing awareness and widespread consensus that this kind of contamination deserves special attention. Emerging contaminants are used in large quantities in everyday life, such as in human and veterinary pharmaceuticals, personal care products, surfactants, plasticizers, and various industrial additives. Thus, they can cause negative environmental effects even if they are not persistently produced, since their high transformation/removal rates are compensated by their continuous introduction into the environment.

Although emerging contaminants constitute a very trendy research topic, resulting in hundreds of studies and papers published every year, their potential ecological effects in the aquatic environment are still not well understood. This is especially so because of a lack of data regarding their effects resulting from long-term low-dose exposure. Such analysis of chronic toxicity on organisms is essential to obtain a realistic environmental risk assessment. This is especially the case for biologically active compounds such as pharmaceuticals, which are selected for their distinct molecular modes of action. Estimating their potential effects on ecosystems is not straightforward, and today, one key objective for environmental scientists is to establish causal links between their presence and the quality of ecological systems. Emerging contaminants today appear in complex mixtures that differ according to the prevalence of agriculture, industrial activities, or human conurbations, and potential effects depend, among others, on the dilution capacity of the receiving river.

Mediterranean rivers are among those most vulnerable to climate and global change, due to their natural water scarcity, which is often compounded by other stressors such as dams (which prevent the biological migration across river networks), water withdrawal that reduces dilution capacity, and the occurrence of invasive species. Intermittency and low flows associated to water scarcity affect biogeochemical processes and reduce the ability to attenuate organic contaminants by river biota.

This book aims to fill some of the current knowledge gaps, by summarizing the main findings of project Consolider SCARCE. More specifically, it seeks to summarize information on the presence of emerging organic contaminants and their links with chemical and ecological quality of Mediterranean rivers. The book provides an in-depth view of the occurrence of emerging contaminants and their effects under multiple stress conditions. The observations collected in the book are applicable also to other parts of the world with Mediterranean climate (Australia, California, etc.) and to other regions where water scarcity is an issue.

We hope that the book will be of interest and useful for a broad audience of researchers, including environmental chemists, ecologists, environmental engineers and ecotoxicologists, together with other professionals responsible for water management, and decision-makers The book is also aimed for anyone with interest in the environment who wants to gain new insights and reach new perspectives in the field.

Finally, we would like to thank all contributing authors for their effort in preparing this comprehensive compilation of research papers. Special thanks are due to the editorial staff of Springer and especially to Andrea Schlitzberger, who helped us during the process.

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Part I
Sources and Occurrence of Emerging Contaminants in Rivers

Introduction on Emerging Contaminants in Rivers and Their Environmental Risk

Ladislav Mandaric, Mira Celic, Rafael Marcé, and Mira Petrovic

Abstract This chapter gives an introduction on emerging organic contaminants, defined as chemicals that occur in water resources and pose a potential environmental risk, although currently it cannot be clearly defined given the paucity of existing data. The main source of emerging contaminants are discussed, giving special attention to wastewater treatment plants (WWTP) and processes occurring therein that determine their fate and transformation. Fate and behavior in the aquatic environment is briefly outlined, discussing main biotic and abiotic transformations occurring in rivers. Finally, specific classes of emerging organic contaminants, such as pharmaceuticals, personal care products, perfluorinated compounds, polar pesticides, and nanoparticles, are discussed in more details, giving examples of the most relevant compounds and their characteristics and risk indicators.

Keywords Emerging contaminants, Environmental risk, Fate, Sources, Transformation

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1 Introduction and Definitions

The issue of environmental microcontaminants emerged in 1962 when Rachel Carson's "Silent Spring" described the detrimental effects of pesticides on the environment and on human health, making a call to consider unintended or unanticipated consequences of man-made chemicals released into the environment.

The amount of nonpolar hazardous compounds, i.e., persistent organic pollutants (POPs) and heavy metals, released by the industry started to decrease in the 1970s when the legislation forced reduction at source and implementation of efficient wastewater treatment plants (WWTP). The main concerns were related with the persistence of POPs in the environment, their bioaccumulation in human and animal tissues, and their biomagnification in food chains, which lead to significant impacts on both human health and the environment. Selected POPs were defined as priority pollutants, and intensive monitoring and control programs were implemented. To address the global concern, the United Nations signed a treaty in Stockholm, Sweden, in May 2001. Under the treaty, known as the Stockholm Convention, countries agreed to reduce or eliminate the production, use, and/or release of an initial twelve chemical groups, the so-called dirty dozen. Today, the emission of POPs has been reduced drastically by adopting appropriate measures and eliminating the dominant pollution sources.

In the European Union, water pollution is regulated under the Water Framework Directive [1], which established a framework for community action in the field of water policy. The most recent European regulation set Environmental Quality Standards (EQS) for 45 priority substances [2] and established a watch list with 10 additional substances (or groups of compounds) of possible concern that require targeted EU-wide monitoring in order to support the prioritization process in future reviews of the priority substance list.

However, our technological society is using a continuously growing number of chemicals, which currently can be estimated in some hundreds of thousands of compounds (most of them organics) in daily use. Consequently, a wide range of man-made chemicals, designed for use in industry, in agriculture, or as consumer goods, are emitted, as well as many other chemicals unintentionally formed as by-products of industrial processes or of combustion. There is a widespread consensus that this kind of contamination requires legislative intervention.

There are varying definitions for emerging contaminants, as well as discussion on the types of substances that should be included under this category. Norman network (http://www.norman-network.net/) defines “emerging substances” as substances that have been detected in the environment, which are not included in routine monitoring programs at the EU level, and whose fate, behavior, and (eco) toxicological effects are not well understood. On the other hand, “emerging pollutants” are defined as those pollutants not included in routine monitoring programs in the EU, but which may be candidates for future regulation, depending on research on their (eco)toxicity, on their potential health effects and public perception, or on their occurrence in the environment.

Currently the most frequently discussed emerging substances are:

- Algal toxins
- Biocides
- Disinfection by-products
- Drugs of abuse
- Flame retardants
- Industrial chemicals
- Nanomaterials (organic and inorganic)
- Perfluorinated substances
- Personal care products including fragrances, UV filters, antimicrobials, insect repellents, etc.
- Pharmaceuticals used in human and veterinary medicine, including hormones
- Plant protection products
- Plasticizers
- Surfactants
- Trace metals and their compounds
- Microplastics

Although most people make no differentiation between emerging contaminants and emerging pollutants, *contamination* and *pollution* should not be seen as the same, since all pollutants are contaminants, but only those contaminants that can result in adverse biological effects are pollutants. Therefore, to differentiate emerging pollutants from emerging contaminants, the chemical analyses and information on their presence in the environment must be complemented with information on their bioavailability and toxicity.

This chapter deals with emerging organic contaminants, defined as chemicals that occur in water resources and pose a potential environmental risk, although currently it cannot be clearly defined given the paucity of existing data.

2 Main Sources and Routes of Entry of Emerging Contaminants to the Aquatic Environment

The main source of emerging contaminants found in waters is wastewater of urban, industrial, and agricultural origin (Fig. 1). Sewage generated in households includes waste liquid from toilets, baths, showers, kitchens, and sinks, which is disposed of via sewers and treated in wastewater treatment plants. However, municipal WWTPs are basically designed to remove pathogens and suspended or flocculated matter, but not other microcontaminants. Since 1980, health concerns related to microcontaminants have driven the development of new treatment technology (biotic and abiotic membranes, advanced oxidation and reduction, electrochemical treatments, combined processes, etc.). However, despite the range of advanced treatment options available, urban WWTPs typically use secondary biological

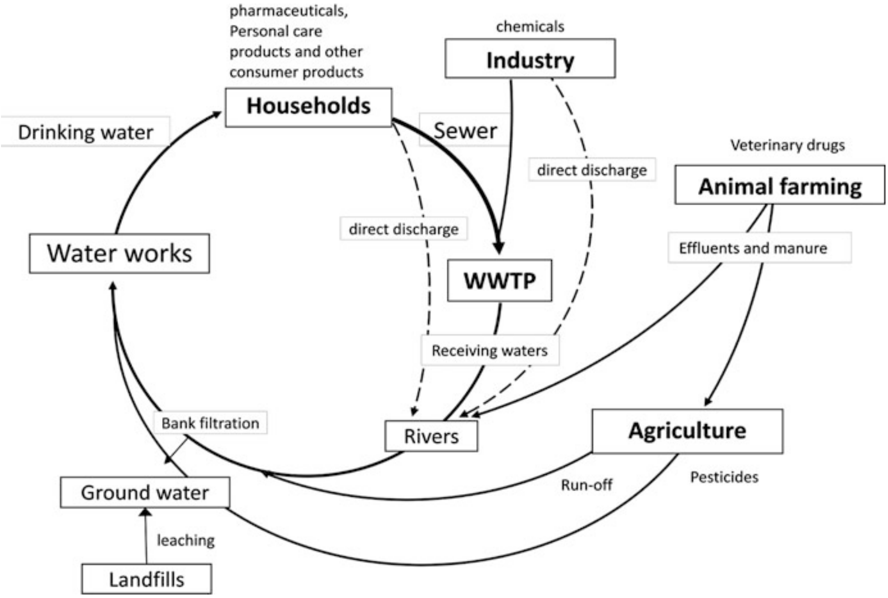

Fig. 1 Emerging contaminants within the water cycle

treatment such as conventional activated sludge, which removes only a fraction of the emerging contaminants. Therefore, WWTP effluents are currently the main route of entry of many emerging contaminants into the aquatic environment. The most concerning contaminants are persisting polar compounds of high solubility in water, which thus are able to penetrate through natural filtration and man-made treatments and present a potential risk in drinking water supply.

Other potential point sources of pollution by emerging contaminants include landfill sites, fish farms, power stations, and oil spillage from pipelines.

Emerging contaminants can also cause diffuse pollution as a result of activities such as farming and forestry. For instance, the leaching from manures applied as fertilizers, the runoff of pesticides used in agriculture and forestry, or the atmospheric deposition of industrial contaminants can all adversely affect the quality of surface and groundwater.

3 Elimination in WWTP and Sewer Systems

For polar microcontaminants like pharmaceuticals, physicochemical removal in WWTPs is of minor importance, and their elimination is largely dependent on microbial degradation in activated sludge tanks [3]. In there, the removal of emerging contaminants is related to four main processes (Fig. 2): photolysis, volatilization to air, sorption to sludge, and biodegradation [5]. Volatilization and sorption consist in a transfer of the contaminant between two phases (dissolved to gas and dissolved to solid, respectively), whereas the term biodegradation includes a myriad of catabolic processes that eventually lead to the partial or total breakdown of contaminant molecules.

Biodegradation is perhaps the most complex process occurring in biological treatments. It is a catabolic process, but the pathways leading to partial or total breakdown of contaminant molecules are not well known. Biodegradation can be achieved at stages of:

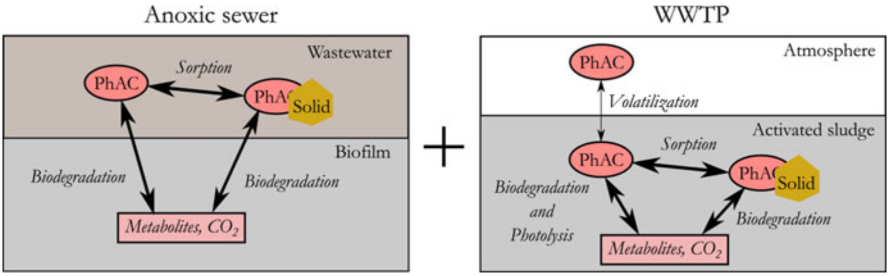

Fig. 2 The processes involved in the fate of pharmaceuticals in WWTPs and associated sewers. The role of anoxic sewers is *highlighted* in the figure because they are the most likely hot spots for removal of pharmaceuticals in sewer networks. The role of volatilization is minor in the plot, following the literature on pharmaceuticals. Modified and expanded from Pomiès et al. [4]

1. *Primary degradation*. Alteration of the chemical structure of a substance resulting in loss of a specific property of that substance.
2. *Environmentally acceptable*. Biodegradation to such an extent as to remove undesirable properties of the compound. This often corresponds to primary biodegradation, but it depends on the circumstances under which the products are discharged into the environment.
3. *Ultimate degradation*. Complete breakdown of a compound to either fully oxidized or reduced simple molecules (such as carbon dioxide/methane, nitrate/ammonium, and water).

Two main mechanisms have been suggested: direct metabolization (i.e., the use of the microcontaminant as a source of carbon and/or energy by the biological community) and co-metabolism. Co-metabolism refers to the fortuitous degradation of a nongrowth substrate (i.e., the pharmaceutical) in the obligate presence of a growth substrate or another transformable compound (e.g., dissolved or particulate organic carbon). Since the amounts of microcontaminant are usually too low to be used as a growth substrate, co-metabolism is supposed to be the main biodegradation pathway in activated sludge. However, given the complexity of the matrix and of the biological communities present, most likely direct metabolism and co-metabolism coexist in biological treatments, at different rates depending on the operational parameters of the facility and the overall quality of the raw water arriving into the WWTP.

Sorption the aqueous compartment and the solid phase of the sludge or mixed liquor in a biological reactor continuously exchange pollutants in both directions (sorption and desorption). The mechanisms that sustain the process of sorption are complex and still not fully understood, and recently the colloidal fraction has been suggested to also play a significant role [6]. Sorption appears to be influenced by the characteristics of both the matrix and the pollutant. This complexity is frequently lumped in a linear formulation that uses a single sorption coefficient [7], also referred as partition coefficient (K_d). Substantial effort has been devoted to the empirical quantification of K_d values for different compounds in particular WWTPs (see [4] for a good compilation of K_d values in activated sludge, including pharmaceuticals), although the generation of K_d values from octanol–water partition coefficient (K_{ow}) values is common practice as well [8]. However, because the K_d of pharmaceuticals depends on sludge characteristics (including pH), using K_{ow} to derive K_d can lead to severe bias. In fact, sorption is known to depend on several mechanisms beyond the hydrophobic interactions summarized by K_{ow}: electrostatic interactions, cationic exchanges, cationic bridges, surface complexation, and hydrogen bridges [9]. However, because sorption depends also on sludge characteristics, K_d values can vary widely among WWTPs, in a way that is nowadays difficult to predict.

Photolysis in WWTP may occur by direct absorption of light by the microcontaminants or through the absorption of light by intermediary compounds [10]. Photolysis is dependent on the intrinsic absorbance properties of the pollutant and the light conditions in the WWTP tanks, basically governed by the presence of suspended solids.

Volatilization of contaminants in WWTPs depends strongly on the physicochemical properties of the pollutant and the operational features of the WWTP. Aeration of activated sludge dramatically affects volatilization that can also take place by atmosphere-biological reactor equilibration following Henry's law. As such, volatilization is only relevant for volatile substances, but we still lack clear guidelines to define volatile and nonvolatile contaminants, mainly because the interactions between the properties of the molecules and the operation of the WWTPs.

3.1 Sewers: From Unreactive Pipes to Hot-Spot Reactors?

The major source of many emerging microcontaminants such as pharmaceuticals or personal care products reaching WWTP facilities is households, where products are consumed by population and eventually released to the sewer networks. Sewers are infrastructures composed by drains, pipes, and pumping stations that transport wastewater to the treatment facilities. There are two main types of sewer networks: gravity networks where water flows unimpeded following elevation gradients and pressure networks composed of completely filled tubes that transport wastewater with the aid of pumping stations [11]. Sewers have been traditionally considered as unreactive transport networks, but recent findings suggest that they are in fact active chemical and biological reactors [12]. There is still few information on the occurrence of microcontaminants in sewers, and in-sewer transformation of contaminants is usually neglected. However, sorption and biodegradation mechanisms are possible in sewers (Fig. 2), although the available information to parameterize those processes is extremely scarce [13,14]. It seems that pressure pipes, which increase the residence time and facilitate the development of anoxic conditions and the establishment of biofilms in pipe walls, are the most biologically active sections of sewer networks.

As far as we know, there is only one study reporting in situ changes in pharmaceutical concentrations along sewers [13]. This study showed that the concentration of most compounds suffered changes below 10% along the sewers, whereas diltiazem, citalopram, clarithromycin, bezafibrate, and amlodipine suffered decreases up to 60%. Even negative removal was observed for sulfamethoxazole and irbesartan, probably due to the conversion of conjugates back to their parent compounds in the sewer. All in all, recent findings show that the transformation of pharmaceuticals begins in sewers, and we anticipate that this topic will be a focus of future research, because the estimates of apparent consumption using inflow loads to WWTPs can be deeply flawed by neglecting transformation in sewers.

4 Fate and Behavior in the Aquatic Environment: Biotic and Abiotic Transformations in Rivers

Once released to the environment, a number of processes govern the fate and transport of contaminants and control their concentrations. Most important are the physical processes of dispersion and dilution and chemical and biological processes such as biodegradation, abiotic oxidation and hydrolysis, photolysis, adsorption/ desorption, and volatilization (Fig. 3). Since the basic concepts of these processes are already defined and discussed in Sect. 3, here we mention only some specificity when occurring in natural aquatic environment.

4.1 Biodegradation

Biodegradability is generally regarded as the most important property for environmental hazard assessment of organic microcontaminants. It is strongly dependent on environmental conditions, such as temperature, redox potential, and the microbial communities present. Also, the degree of bioavailability of a microcontaminant is important, i.e., accessibility of the compound to microorganisms and its uptake

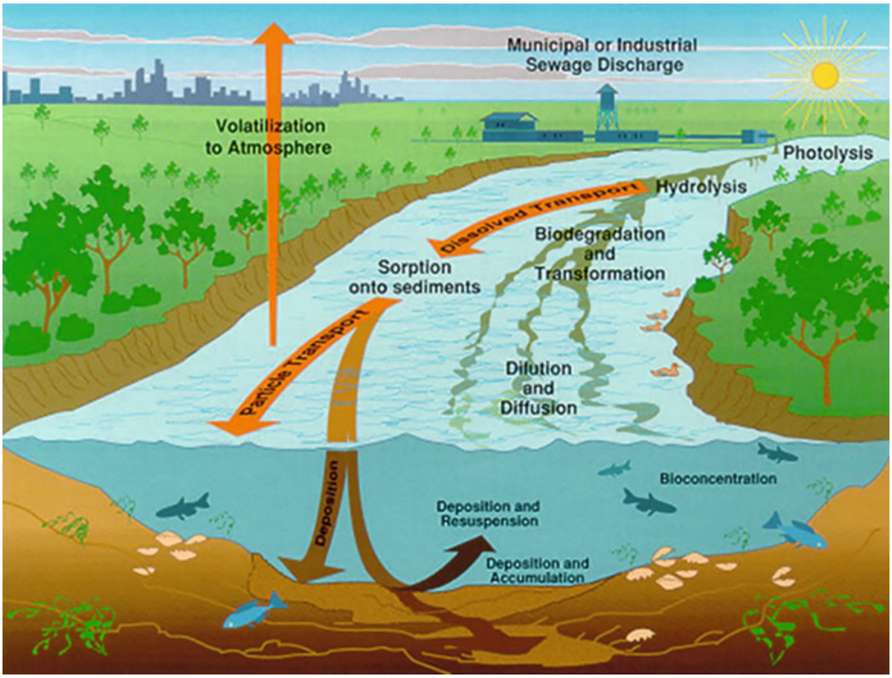

Fig. 3 Fate of contaminants in the aquatic environment

by microbial cells. Dissolved compounds generally are more bioavailable. Other important factors influencing the biodegradation are exposure time to biomass, availability of co-substrates (for compounds degraded co-metabolically), and the fraction of inert matter.

4.2 Photolysis

Direct photolysis in natural water involves the transformation of contaminants resulting from the direct absorption of a photon and should be distinguished from indirect photolysis, a second important abiotic degradation pathway in the environment. Indirect photolysis in natural water involves the transformation of contaminants due to energy transfer from naturally occurring photosensitizers or the transformation of a chemical due to reactions with transient oxidants such as hydroxyl radicals, singlet oxygen, and peroxy radicals.

4.3 Sorption

The interface between water and natural solids (e.g., suspended particulate matter and sediments in rivers) plays an important role in the transport of microcontaminants in river systems. Adsorption depends on both the surface characteristics and the properties of the contaminant. Neutral compounds tend to sorb onto solid organic matter, and cations and anions tend to sorb onto negatively (e.g., clay) and positively (e.g., iron oxide) charged surfaces, respectively. A number of other reactions like complex formations with metal ions, ion exchange, and hydrogen bindings also affect the partition of the organic compound between the solid and the liquid phase. Once the contaminants are sorbed, they can be deposited and eventually become buried in the sediments. However, the buried contaminants can be remobilized, by resuspension of the sediments during flood events.

The relative importance of the abovementioned processes depends on the rates at which they occur under environmental conditions. These rates are, in turn, dependent on the chemical structure and properties of the substance and its distribution in the various compartments of the environment. The most important physical properties of contaminants are water solubility, acidity (pK_a), vapor pressure, Henry's law constant (H), hydrophobicity expressed as the octanol/water partition coefficient (K_{ow}), and the organic carbon/water partition coefficient (K_{oc}).

The degree of ionization of ionizable and polar contaminants (many pharmaceuticals and pesticides), which depends on pH, affects their solubility, transport, sorption, and bioavailability. For an ionizable compound, acidic or basic, which can exist as neutral or dissociated form, the partitioning depends on pH and pK_a of the compound. In addition, the charged groups within the molecules can lead to ionic, ion paring and complexation reactions with the particulate matter and microorganisms, thus contributing to partitioning the contaminants to the solids. For example, microcontaminants having carboxylic acid functionalities with pK_a values much less than 7 (such as some nonsteroidal anti-inflammatory drugs or polar pesticides) are likely to remain in the solution phase, and removal by sorption to settling particles may be limited. For contaminants having functional groups that are prone to photolysis (e.g., conjugated aromatics, nitro-compounds, furans, phenols), a diverse set of photochemical processes are expected, and oxidative losses via reaction with mineral and humic substances also occur in sediments or soils. Photolytic reactions are often complex, involving various competing or parallel pathways and leading to multiple reaction products that may either be more toxic than the parent compound, retain the properties of the parent compound, or lose toxicity [15].

Some of the above physical properties are strongly dependent on environmental conditions. For example, temperature strongly affects vapor pressure, water solubility, and, therefore, Henry's law constant. Temperature may also affect deposition. For example, the distribution of POPs is inversely related to vapor pressure and thus to temperature. Lower temperatures favor greater partitioning from the vapor phase to particles suspended in the atmosphere. The pH is also important in evaluating environmental processing of the compounds, even though they are not subject to hydrolytic reactions. The speciation of the compound will influence its partitioning behavior, as well as its light-absorbing properties. Some compounds have multiple pH-sensitive functional groups (e.g., tetracycline antibiotics have three or four pK_a values), which results in the possibility of protonated/positive, neutral (or zwitterionic), and deprotonated/negative forms of a drug being present depending on the pH of the specific water body.

For the majority of emerging contaminants covered by this chapter (polar compounds such as pharmaceuticals, polar pesticides, personal care products, etc.), the attenuation in the aquatic environment is governed by three main processes: biodegradation, sunlight photolysis, and sorption to bed sediment. Table 1

Table 1 Environmental persistence and partitioning of selected emerging contaminants with relatively high potential ecological risk and high consumption

Compounds	Photolysis	Biodegradation	Sorption
Ciprofloxacin	++	–	+++
Sulfamethoxazole	++	+++	–
Naproxen	+++	+	++
Ibuprofen	+	+++	–
Diclofenac	+++	++	–
Mefenamic acid	+	+	–
Acetaminophen	+	+++	–
Carbamazepine	–	–	–
Propranolol	+++	+	+++
Gemfibrozil	–	–	–
Triclosan	++	++	+++
Methylparaben	–	+++	+

+++ rapid, ++ medium, + slow, – very poor or nonexisting gives an overview of relative contribution of biodegradation, photolysis, and sorption to the attenuation for selected emerging contaminants with high potential to enter the aquatic environment.

5 Main Classes of Emerging Contaminants: Characteristic and Risk Indicators

5.1 Pharmaceuticals and Hormones

Pharmaceuticals are a group of chemical substances that have medicinal properties and encompass all prescription, nonprescription, and over-the-counter therapeutic drugs, in addition to veterinary drugs. They are produced worldwide on a 100,000 t scale, and in a vast array of contaminants of anthropogenic origin reaching our water supplies, pharmaceuticals are among the ones with the most continuous input into the environment. Most modern drugs are small organic compounds with a molecular weight below 500 Da, which are moderately water soluble as well as lipophilic, in order to be bioavailable and biologically active. They are designed to have specific pharmacologic and physiologic effects at low doses, and thus, are inherently potent, and can produce unintended outcomes in wildlife [16]. Their consumption will continue to increase due to the expanding population, inverting age structure, increase of per capita consumption, expanding potential markets, patent expirations, new target age-groups, etc. After the oral, parenteral, or topical administration, pharmaceuticals are excreted via the liver and kidneys as a mixture of parent compounds and metabolites that are usually more polar and hydrophilic than the original drug. After their usage for the intended purpose, a large fraction of these substances is discharged into the wastewater unchanged or in the form of degradation products that are often hardly eliminable in conventional WWTPs. Depending on the efficiency of the treatment and chemical nature of a compound, pharmaceuticals can reach surface and groundwaters.

Pharmaceuticals have been found in treated sewage effluents, surface waters, soil, and tap water. Although the levels are generally low, there is rising concern about potential long-term impacts to both humans and aquatic organisms as a result of the continuous environmental exposure to these compounds. These levels are unable to induce acute effects in humans, i.e., they are far below the recommended prescription dose, but have been found to affect aquatic ecosystems.

Some examples of common pharmaceuticals found in the aquatic environment are shown in Table 2.

Table 2 Pharmaceuticals frequently found in the aquatic environment

Therapeutic class	Representative compounds
Analgesic/anti-inflammatory	Ibuprofen
	Ketoprofen
	Naproxen
	Diclofenac
	Salicylic acid (aspirin metabolite)
	Acetaminophen (Paracetamol)
	Codein
Antibiotics	Sulfamethoxazole
	Ofloxacin
	Ciprofloxacin
	Norfloxacin
	Trimethoprim
	Erythromycin
	Azithromycin
	Clarithromycin
Beta-blockers	Atenolol
	Metoprolol
	Sotalol
	Propanolol
Lipid regulators	Gemfibrozil
	Bezafibrat
	Clofibric acid (metabolite)
Antidepressants	Diazepam
	Citalopram
	Paroxetine
	Fluoxetine
Antiepileptic	Carbamazepine
Gastric protectors	Ranitidine
Diuretics	Hydroclorotiazida
	Furosemide
X-ray contrast media	Iopromida
	Diatrizoate
	Iopamidol
Antidiabetic	Glibenclamide

5.1.1 Natural and Synthetic Hormones

Natural and synthetic steroid hormones are considered as the most potent endocrine-disrupting compounds. The three major naturally occurring estrogens in women are estrone (E1), 17β-estradiol (E2), and estriol (E3); synthetic estrogens, such as 17α-ethinyl estradiol (EE2); and progestogens, such as norethindrone, norgestrel, and levonorgestrel which are widespread in contraceptive formulations and for treatment of certain hormonal disorders and cancers. Both natural and synthetic steroids, in either a conjugated (as glucuronides and sulfates, principally) or an unconjugated form, are excreted in the urine of mammals and enter the aquatic environment via WWTP effluents or untreated discharges. These potent estrogenic compounds have been shown to induce estrogenic responses in fish at low concentrations in water (0.1–1 ng/L) [17–19], and such concentrations are sometimes found at sites downstream of discharges of WWTP effluents and have been associated to impaired reproduction and developmental processes, such as feminization, decreased fertility, or hermaphroditism [20–22].

5.2 Personal Care Products (PCP)

Personal care products include all consumer chemicals typically found in fragrances, lotions, shampoos, cosmetics, sunscreens, soaps, etc.

5.2.1 Fragrances

Synthetic musk fragrances are a group of bioaccumulative and persistent xenobiotics. They are used in a wide range of consumer products such as detergents, cosmetics, and other personal care products. There are four different musk families according to their physicochemical properties: nitro musks (musk ketone, musk ambrette, musk xylene, musk tibetan, and musk moskene), polycyclic musks (galaxolide, tonalide, celestolide, phantolide, cashmeran, and traseolide), macrocyclic musks (ambrettolide, muscone, ethylene brassilate, globalide), and alicyclic musks (romandolide and helvetolide). Polycyclic and nitro musks are not structurally or chemically similar to the natural ones and have a lipophilic nature, causing them to bioaccumulate in sediments, sludge, and biota and biomagnify throughout the food chain [23].

5.2.2 UV Filters

Organic UV filters are found in sunscreen cosmetics and other personal care products as protection against UV radiation. The organic UV filters most commonly found in the aquatic environment are:

- Benzophenones (BP-1, BP-3, BP-4)
- 2-Phenylbenzimidazole-5-sulfonic acid (PBSA)
- 4-Methyl-benzylidene camphor (4-MBC)
- Ethylhexyl methoxycinnamate (EHMC)
- Isoamyl methoxycinnamate (IAMC)
- Octocrylene (OC)

- Octyl dimethyl-*p*-aminobenzoate (OD-PABA)

Most of these compounds are lipophilic (log K_{ow} 4–8) with conjugated aromatic rings and are relatively stable against biotic degradation [24]. It has been reported that the estrogenic activity of most of the commonly used organic UV filters is in the range of other well-characterized estrogenic chemicals such as estradiol [25].

5.2.3 Antimicrobials and Preservatives

Antimicrobials and preservatives are chemicals that kill or prevent the growth of microbes (bacteria, viruses, fungi, or protozoa), and as such, they are commonly added to many consumer products to reduce or prevent microbial contamination. The most commonly used antimicrobials are triclosan and triclocarban, frequently added to soaps and body washes, toothpastes, and other cosmetics. They have received increasing attention because of their pronounced microbial and algal toxicity, suspect estrogenicity, and their potential for fostering antimicrobial resistance [26]. Triclosan is also known to undergo phototransformation in aqueous solution to form toxic 2,8-dichlorodibenzo-*p*-dioxin (2,8-DCDD).

Parabens are another group of compounds with bactericidal and fungicidal properties. They are widely used preservatives in cosmetic and pharmaceutical products, such as shampoos, commercial moisturizers, shaving gels, spray tanning solution, makeup, personal lubricants, topical pharmaceuticals, and toothpaste. There are seven different types of parabens with bactericidal and fungicidal properties currently in use (benzyl, butyl, ethyl, isobutyl, isopropyl, methyl, and propyl). They are also used as food additives. They are becoming increasingly controversial, because in vitro and in vivo tests have shown that parabens have weak estrogenic activity [27]. Preliminary data on environmental concentrations, however, suggest only minimal risk to aquatic organisms, as effect concentrations are generally 1000× higher than what has been observed in surface water.

5.3 Perfluorinated Compounds (PFCs)

PFCs comprise a large group of compounds characterized by a fully fluorinated hydrophobic linear carbon chain attached to one or more hydrophilic heads.

The examples of same PFCs are:

- PFOA or perfluorooctanoic acid, used to make fluoropolymers such as Teflon, among other applications
- PFOS or perfluorooctanesulfonic acid, used in the semiconductor industry and fire-fighting foam mixture
- PFNA or perfluorononanoic acid, used as surfactant in the emulsion polymerization of fluoropolymers, like PFOA
- PFBS or perfluorobutanesulfonic acid, used as a replacement for PFOS

- POSF or perfluorooctanesulfonyl fluoride, used to make PFOS-based compounds
- PFOSA or perfluorooctanesulfonamide

PFCs repel both water and oil and are therefore ideal chemicals for surface treatments. These compounds have been used for many industrial applications including stain repellents (such as Teflon), textile, paints, waxes, polishes, electronics, adhesives, and food packaging [28]. PFCs have been found in the aquatic environment in different compartments, including water at levels of pg/L in lakes, ng/L in rivers, soils and sediments (at levels of ng/g), and biota samples (at levels of μg/kg in fish samples) [29]. They are resistant to breakdown and may be accumulated attached to proteins and biomagnified through the food chain. Among PFCs, PFOA and PFOS are regarded as being the terminal degradation end-products, and these are the chemicals that have frequently been detected in environmental samples and often occur at high concentrations. Studies have shown that PFOA and PFOS have potential to cause liver cancer, affect the lipid metabolism, disturb the immune system, and reduce human fertility. PFCs enter the environment through direct (directly from manufacture wastes or direct application) and indirect sources (due to their decomposition or disposal through the life cycle of products). WWTPs have been also identified as relevant pathway of PFCs releases into the environment.

5.4 Plasticizers

Phthalate acid esters (*PAEs*) are chemical compounds widely used in different industrial applications, mainly as plasticizers for polyvinyl chloride (PVC) resins, adhesives, and cellulose film coatings. They comprise a large group of compounds, being main representatives dimethyl (DMP), diethyl (DEP), dibutyl (DBP), butylbenzyl (BBP), di(2-ethylhexyl) (DEHP), and di-*n*-octyl phthalate(DnOP). DEHP is included on the list of priority pollutants under the EU Water Framework Directive (WFD). Considerable direct (production of plastic materials) and indirect emission, via leaching and volatilization from plastic products after their usage, disposal, and incineration, explains their ubiquity in the environment. Because of this, urban and industrial wastewater treatment plants, landfill areas, and municipal incinerators are the major sources of these contaminants. In all reported studies, DEHP was found to be the predominant PAE, due to its high production (nearly 90% of European plasticizer use) and its physicochemical properties (low solubility and relatively high K_{ow}). PAEs are substances of low acute and chronic toxicity, which generally decreases with increasing alkyl chain length. They are also characterized by their limited bioaccumulation through the aquatic food chain because of their biotransformation that progressively increases with trophic level [30]. However, phthalates are categorized as suspected endocrine disrupters [31]. Endocrine disruptive effects were observed in vivo for DBP, DEHP, BBP, DEP, and DHP with relative potency ranged from 10^{-4} to 10^{-7} (relative to 17β-estradiol) [32].

Bisphenol-A (BPA) is used to make polycarbonate polymers and epoxy resins that are used in the production of various types of food and drink containers, compact disks, electronics, and automobile parts. BPA is released into the environment through WWTP effluents, landfill leachates, via hydrolysis of BPA from plastics, or natural degradation of polycarbonate plastics due to moderate water solubility and low vapor pressure. The potential risks of BPA include reproduction and development effects and neurochemical and behavioral effects [33]. Endocrine disruptive effects were observed in vivo with relative potency from 1.0×10^{-5} to 8.1×10^{-5} (relative to 17β-estradiol) [34].

5.5 Anticorrosive Agents

Benzotriazole (BT) and tolyltriazole (TT) are high production volume chemicals that have been extensively used as corrosion inhibitors. Additionally, they are used as silver polishing agents in dishwasher tablets and powders, as antifreezes, in heating and cooling systems, hydraulic fluids, and vapor phase inhibitors. They are water soluble, are not readily degradable, and have a limited sorption tendency. In WWTP they are only partially removed, and a substantial fraction reaches surface water. Several studies showed that the BT and TT are widely distributed in surface waters. Loos et al. [35] detected BT and TT in 94% and 81% of 122 river water samples distributed over the EU, with mean concentrations of 493 ng/L and 617 ng/L for BT and TT, respectively. Harris et al. [36] showed in vitro that BT possessed clear antiestrogenic properties. This chemical was approximately 100-fold less potent than tamoxifen, which was used as a positive control. Later in vivo studies confirmed that BT had adverse potential on the endocrine system of fish, which may lead to adverse effects, including compromised growth and reproduction, altered development, abnormal behavior, and alterations in hormone biosynthesis [37].

5.6 Surfactants

Surfactants are organic chemicals that reduce surface tension in water and other liquids and exhibit a tendency to form micelles in solvents. Surfactants, used in industrial processes as well as in households, are among the most produced of all organic chemicals. The most familiar uses for surfactants are soaps, laundry detergents, dishwashing liquids, and shampoos. Other important uses are in textile and leather processing, mining flocculates, petroleum recovery (also after oil spills), pesticide adjuvants, additives in lubricants, and additives for emulsion polymerization. There are hundreds of compounds that can be used as surfactants and are usually classified by their ionic behavior in solutions: anionic, cationic, nonionic, or amphoteric. Surfactants are among the most relevant organic pollutants of anthropogenic origin and have very high potential to enter the environment, since their widespread use, primarily in aqueous solutions, leads to later introduction into the environment via wastewater discharges.

The awareness of potential environmental problems has led to a series of legislation measures resulting in a ban and replacement of nonbiodegradable and toxic surfactants. Examples are the replacement of ABS with LAS in the mid-1960s, voluntary replacement of DTDMAC in some European countries, and more recent restriction of use of alkylphenol ethoxylates (APEOs) in household detergents, which resulted in a substantial drop in the levels observed. APEOs are a class of nonionic surfactants that raised great attention and concern. Although parent APEOs are not highly toxic, their environmental acceptability is strongly disputed because of persistent metabolic products (alkylphenols (APs) and carboxylic derivatives (APECs)) generated during wastewater treatment. The estrogenic potential of these compounds is four to six orders of magnitude lower than that of the endogenous 17β-estradiol [38]; however, it has been reported that concentrations found in certain sewage effluents and in receiving rivers are sufficient to induce hormonal response in some fish species [33]. Moreover, NP and short-chain NPEOs are lipophilic compounds with a log K_{ow} of 4,48 (NP) and around 4.2 (NP_1EO, NP_2EO, and NP_3EO), so they partition preferentially to the organic fraction of sediments and show considerable potential to bioaccumulate in freshwater organisms [39]. Because of these findings, APEOs are banned or restricted in the EU.

Additionally, due to their unique surface-active properties, surfactants have the capacity to remobilize nonpolar contaminants, such as polycyclic aromatic hydrocarbons or heavy metals that have accumulated in sediments or WWTP sludges. This ability can be positively exploited, e.g., in the remediation of contaminated soils, but it is undesirable in cases where the surfactants are present in digested sewage sludge used to amend soil.

5.7 Nanomaterials (NMs)

Nanomaterials are small-scale substances and materials that have structural components smaller than 1 μm or (usually less than 100 nm) in at least one dimension [40]. Nanomaterials are divided simply into organic (carbon-based) nanomaterials, such as fullerenes, graphene, single-walled and multiwalled carbon nanotubes, and inorganic nanomaterials, such as titanium dioxide, zinc oxide, and silver. Due to their size, nanomaterials possess unique physical, chemical, and biological properties. In addition, they settle very slowly and, as they remain suspended for long periods in water and air, can be transported over greater distances than larger particles of the same material [41].

The specific properties of nanomaterials raise concerns about adverse effects on biological systems, which, at the cellular level, include structural arrangements that resemble nanomaterials [42, 43]. However, the toxic effects and environmental impact of nanomaterials are not fully understood, and the study of nanomaterials in the aquatic environment is currently a “hot topic.” A number of carefully designed studies have been conducted to understand the fate, transport, stability, and toxicity of nanoparticles in the aquatic environment [44]. Current results suggest that the main risk for the environment is associated to metals and metal oxides, both in particles and as ions, but further investigation of ecotoxicity, including long-term studies, especially of algae and aquatic invertebrates, are needed, together with studies of their action mechanisms at the cellular and the genetic level [45].

5.8 Plant Protection Products (Emerging Pesticides)

Pesticides represent a wide range of chemical compounds used to limit, inhibit, and prevent the growth of harmful animals, insects, invasive plants, weeds, bacteria, and fungi. Despite their recognized importance, pesticides are among the most important types of environmental pollutants, produced and released into the environment in huge quantities. Nearly 20,000 pesticide products have entered the market since registration began in 1947 [46], with an estimated 1–2.5 million tons of active ingredients used each year, predominantly in agriculture [47].

Although pesticides are applied mostly on soils, they have a high potential to reach the aquatic environment, via runoff, agricultural storm-water discharges, and return flows from irrigated fields. Consequently, numerous monitoring studies have documented the occurrence of pesticide residues at trace concentrations (on the order of μg/L and lower) in water around the world. As reported by several authors [48–50], many pesticides have the potential to cause adverse effects on human health and the environment, even at low concentrations, since they are persistent and bioaccumulate in biota. Although various pesticides are currently included in the list of priority substances in the EU regulations (1/3 of priority substances listed in EU WFD are pesticides), many others are still unregulated.

6 Monitoring of Emerging Contaminants in the Aquatic Environment: Chemical Analysis, General Trends, Main Challenges

In the last two decades, numerous analytical methods have been developed for the determination of different classes of emerging contaminants in environmental samples (water, sediment, soil, biota). Generally, the identification and quantification of emerging contaminants at low concentrations in complex environmental matrices requires analytical methods of high sensitivity and selectivity, which typically rely on liquid or gas chromatography (LC or GC) coupled to mass spectrometry (MS). The application of advanced low- or high-resolution MS instruments in environmental analysis has allowed the determination of a broader range of compounds and, thus, a more comprehensive assessment of environmental contaminants.

The preferred analytical approach is based on target analysis of preselected compounds of interest, using tandem MS instruments. Over the years, a gradual shift from class-specific methods to multi-residue methods for simultaneous analysis of a large number of target compounds, belonging to different classes, has occurred. For example, Robles-Molina et al. [51] developed a multi-residue method based on solid-phase extraction and liquid chromatography-time-of-flight mass spectrometry for the simultaneous determination of over 400 priority and emerging pollutants in water and wastewater. On the other hand, Pintado-Herrera et al. [52] used atmospheric pressure gas chromatography-time-of-flight-mass spectrometry (APGC-ToF-MS) for the simultaneous determination of 102 regulated and emerging contaminants in aqueous samples after stir bar sorptive extraction (SBSE). A number of multi-residue methods have been developed for the analysis of multiclass pharmaceuticals, illicit drugs, pesticides, PFC. Just to mention a few, Gros et al. [53] developed a method to determine 81 pharmaceutical residues, covering various therapeutic groups, and some of their main metabolites, in surface and treated waters (influent and effluent wastewaters, river, reservoirs, sea, and drinking water); Baker and Kasprzyk-Hordern [54] defined a multi-residue method for the environmental monitoring of 65 stimulants, opioid and morphine derivatives, benzodiazepines, antidepressants, dissociative anesthetics, drug precursors, human urine indicators, and their metabolites in wastewater and surface water. Masiá et al. [55] developed a method for the analysis of 43 polar pesticides in environmental waters.

However, the advances in analytical instrumentation and analytical capabilities do not provide the answer for many important questions such as the following ones: Which compounds should be monitored? Is it worthy to monitor hundreds of emerging contaminants that analytical chemists are capable of analyzing today? Is chemical analysis of specific compounds sufficient to assess contaminants present in the environment?

The current analytical approach has another drawback. The majority of analytical methods only focus on parent target compounds and rarely include metabolites and transformation products, which sometimes can be more toxic and persistent than the original compounds. One reason for that trend is that the majority of transformation products are not known and many of those that are known are not commercially available or are too expensive. But the main reason is that all relevant contaminants, metabolites, and transformation products that may be encountered in the aquatic environment are impossible to be included in any targeted multi-residue method, making therefore a strong case for the application of nontarget screening protocols using high-resolution mass spectrometry (HR-MS) [56, 57]. In comparison to triple quadrupole mass spectrometers, which operate at unit resolution for specific target analytes, HR-MS instruments such as time of flight (TOF), quadrupole time of flight (QqTOF), and Orbitrap mass spectrometers are capable of acquiring full-scan mass spectra at high resolution for all analytes having, therefore, the unique potential of retrospective data analysis for compounds not included in the first data processing. Accurate mass full-scan MS permits analysis of a large number of compounds and their degradation products that fall outside the scope of target methods. However, general screening for unknown substances is time-consuming and expensive and is often shattered by problems, such as lack of mass spectral libraries. Therefore, the main challenge is to prioritize contaminants and decide on the significance of the chemical data. Effect-related analysis, focused on relevant compounds, nowadays seems to be the most appropriate way to assess and study environmental contamination.

7 Future Challenges

Today, the major challenge is to evaluate the potential ecological effects associated with the presence of emerging contaminants in the aquatic environment and to establish causal links between pollution by emerging contaminants and the quality of ecological systems. Nevertheless, the direct estimation of effects caused by environmental pollutants on ecosystems is not straightforward, since we lack data on the occurrence of many emerging contaminants, on their fate and behavior in the environment, and on their long-term effects on aquatic ecosystems. In addition, the ecotoxicological significance of many emerging contaminants and effects of “chemical cocktails” remains largely unknown, and novel tools are needed to evaluate the risk of emerging contaminants in the environment.

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Contaminants of Emerging Concern in Mediterranean Watersheds

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Abstract The present chapter provides a general perspective on the occurrence, ecotoxicological risk, and prioritization of emerging and classical contaminants in Mediterranean river basins with special focus on the Iberian Peninsula as representative case, in the light of the results recently obtained. Risk assessment and prioritization criteria based on ecotoxicological risk with respect to different trophic levels are explained and applied to the Mediterranean basins studied. This enables to rank contaminants according to their ecotoxic risk and to quantify their joint effect as a mixture on a river site.

Keywords Compound prioritization, Emerging contaminants, Mediterranean rivers, Priority contaminants, Risk assessment, Risk index, Toxic units

Contents

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1 Introduction

Chemical pollution of aquatic ecosystems is one of the major threats to aquatic life biodiversity and human health [1]. Increasing pollution of water with a variety of chemical compounds, with their mostly unknown long-term effects, could easily lead to environmental problems of great magnitude [2]. In the coming century, climate change and a growing imbalance among freshwater supply, consumption, and population will alter the water cycle dramatically [3]. In the European Union, there are more than 100,000 registered chemicals listed by EINECS (the European Inventory of Existing Commercial Chemical Substances), of which many are in everyday use. There are many possible sources of pollutants in the environment such as surface runoff from agricultural and urban areas, through industrial and urban wastewater discharge, atmospheric deposition, accidental spills, etc. Some compounds are not properly eliminated by wastewater treatments and are continuously released into the environment as a part of the effluent. Most of these chemical compounds are present in the environment at low concentrations, but still many of them may possibly be a threat to different biological end points [2]. Given the huge number of chemicals present in the environment and their potential adverse effects, there is a need to prioritize chemicals according to the risk they pose to the ecosystem. Prioritization of chemicals is necessary for optimization monitoring efforts, to provide appropriate and scientifically sound information to legislators and water managers. To provide this information, many environmental risk assessment approaches have been developed [4].

In the European Union, the legislation considering aquatic environment protection is mostly covered by the introduction of the Water Framework Directive (WFD) in year 2000. The aims of WFD are to achieve good ecological and good chemical status of European surface waters. The WFD issued the list of priority and hazardous substances including contaminants which are recognized for their adverse effects, mainly on the basis of persistence, bioaccumulation, and toxicity properties (PBT). In order to achieve good chemical status, the Environmental Quality Standards (EQS) of the priority pollutants must be met [5], that is, the levels of concentrations of these compounds must be below the EQS. Moreover, recently the European Commission issued a proposal for updating the list of priority substances by adding 15 new candidates. Member states are obliged to identify river basin-specific pollutants, i.e., the pollutants of regional and local importance. Moreover, regulated chemicals that are monitored on a regular basis are just a small fraction of numerous chemicals present in the environment [6]. Many emerging contaminants are detected due to improvement of analytical techniques [7, 8]. They might be ecotoxicologically relevant compounds as well, especially when present as the constituents of the complex chemical mixtures in the environment [2]. Therefore, it is necessary to evaluate the risk of emerging contaminants and if necessary to include them into the monitoring and regulation programs [9]). Representative examples of compounds that are considered as emerging contaminants or contaminants of emerging concern are pharmaceuticals and personal care products, polar pesticides, natural toxins, biocides, perfluorinated compounds, and nanomaterials, among others.

One approach for identifying potentially dangerous compounds is screening of the environment for a large set of chemicals together with an assessment of the potential toxicity at the observed concentrations [10].

Generally, the ecotoxicity of a given pollutant is determined by standardized tests, with the use of selected model organisms and toxicity end points, such as acute toxicity or lethality in algae, invertebrates, and fish so that different trophic levels are covered as recommended by WFD [11].

2 Pollution of Mediterranean Rivers

In the SCARCE Consolider project [12], four Iberian river basins were studied as the representatives of Mediterranean region river basins. The samples of water, sediment, and biota were collected at 77 sampling sites for chemical characterization (Fig. 1). Studied river basins are situated in areas of multiple land use types, from natural forests and grasslands to agricultural lands and highly industrialized and urbanized areas. The pollution of rivers is reflecting the land use in the chemical mixtures present in water. Each of the river basins has a different proportion of land use types and the number and concentrations of chemicals detected (Table 1). In general, more than 50 chemicals were detected in each sample. Of the studied groups of chemicals, industrial organic compounds were measured at highest concentrations in majority of samples except in Júcar where pesticides as a group were measured at highest concentrations (Fig. 6). A high number of pharmaceuticals were detected in all four rivers, as well as several hormones, personal care products, and illicit drugs related to urban zones.

In brief, Llobregat was the most contaminated river in terms of number and concentration of organic compounds detected. Several sampling sites in Ebro were highly polluted by a variety of chemicals. Júcar pollution was mainly dominated by pesticides. Guadalquivir was the least contaminated of studied river basins.

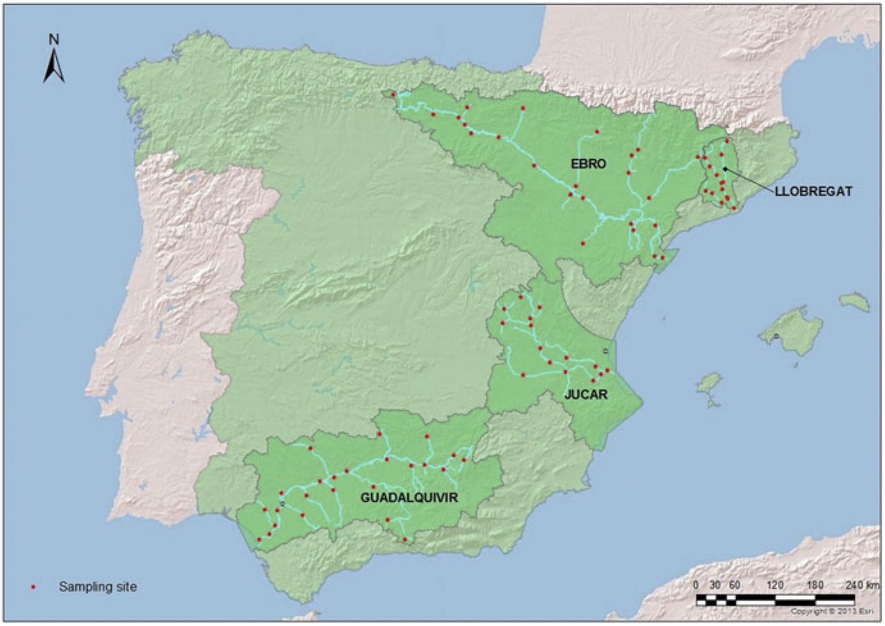

Fig. 1 SCARCE project sampling sites

Table 1 Minimum and maximum number of individual chemicals of each compound group detected in samples analyzed

Number of chemicals detected in sample	Llobregat	Ebro	Júcar	Guadalquivir	Reference
Pesticides	6–11	6–17	6–17	8–15	[13]
IOCs	6–9	5–10	5–9	7–12	[14]
PFCs	0–10	0–8	1–9	3–9	[13]
Pharmaceuticals	10–55	9–60	35–40	9–35	[15]
PCPs	0–10	0–7	3–7	2–8	[14, 16]
Hormones	1–3	0–5	0–3	0–4	[14]
Illicit drugs	0–4	0–4	0–4	0–3	[17]

IOCs industrial organic chemicals, *PFCs* perfluorinated compounds, *PCPs* personal care products

2.1 Llobregat

The Llobregat river basin is located northeast of Spain. The lower part of the basin is highly urbanized and densely populated, so the anthropogenic pressure at that area is the strongest [18] (Fig. 2). Surface runoff and industrial and urban wastewaters discharges are possible sources for pollutants in river water and sediment. Agricultural lands are surrounding the urban zones, so diffuse pollution from agriculture is also present. In spite of the severe pressures it receives, Llobregat is

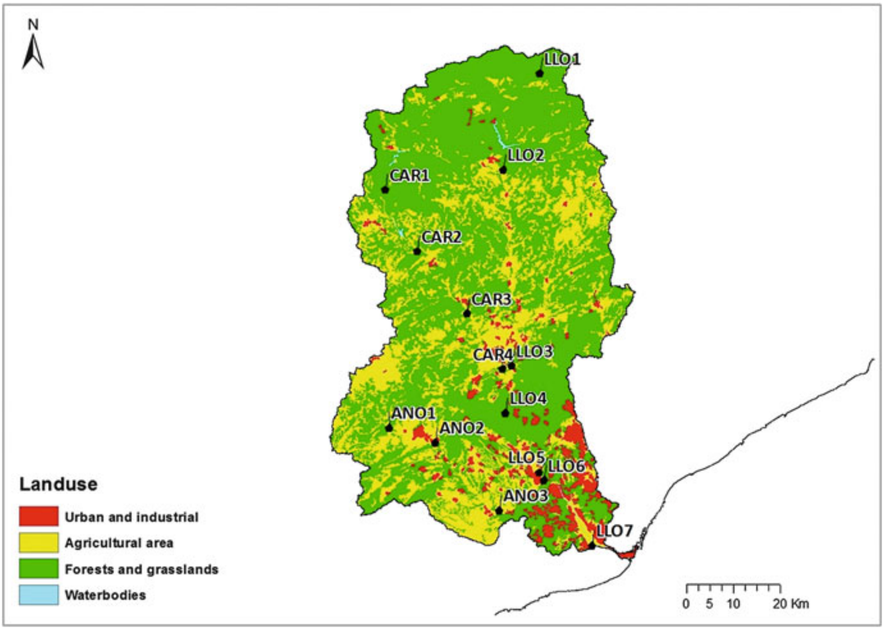

Fig. 2 Land use types in Llobregat river basin and sampling sites of SCARCE project

the drinking water supply for Barcelona and surrounding cities. Due to the Mediterranean climate characteristic for this geographical area, this river is subjected to periodic drought periods which lead to water scarcity and therefore to reduced dilution capacity of the river. In these periods, the risk to the aquatic ecosystem could be increased because of the higher exposure of biological communities to pollutants.

There is a notable increase of pollution downstream (Fig. 3), particularly in the lower part of the basin, close to the mouth of the river (LLO5, LLO6, and LLO7), and in the Anoia tributary (Sites ANO1, ANO2, and ANO3) which is passing through the industrial area of Igualada not far away from Barcelona city. The compound group measured at highest concentration at the majority of sampling sites was industrial organic compounds (IOCs, gray color). Of the compounds belonging to this group, alkylphenols (octylphenol, nonylphenol, and related compounds) and anticorrosion agents as tolyltriazole and 1H-benzotriazoles were the most relevant. The highest concentration of IOCs was measured at site LLOB7 (Fig. 3), the most downstream site in the basin (IOCs = 10.5 μg/L). Pharmaceuticals are the second group in terms of concentration, especially in the lower, urbanized part. Perfluorinated compounds (PFCs) were measured in Anoia tributary with concentration up to 2.8 μg/L which was measured at site ANO2. The most abundant compounds of this group were perfluorobutanoic acid (PFBA) and perfluorooctanesulfonic acid (PFOS), the latter included in the WFD priority list.

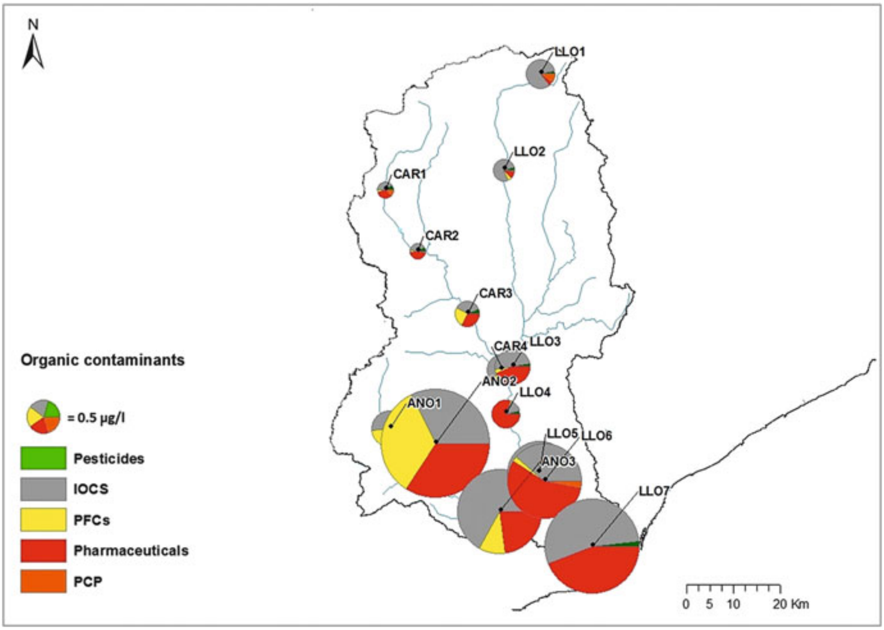

Fig. 3 Pollution of Llobregat river water with organic chemicals

2.2 Ebro

The Ebro river basin is located northeast of the Iberian Peninsula. It generates the delta and the big wetland area (320 km) with a specific biodiversity and a high ecologic value. A large share of land in this basin is devoted to agriculture (Fig. 4). Along the river, there are the fields for cereal and corn cultivation, vineyards, fruit orchards, etc. and for the delta rice production and horticulture. Precipitation decrease, increase of water demand for irrigation in agriculture, reforestation, and several other factors caused the river flow decrease of approximately 40% in the last century. The population is concentrated in several big cities like Pamplona, Zaragoza, and Lleida, mainly, in the northern and central part of the basin. Industrialized areas are surrounding the cities, so the urban types of pressures to water quality are located in several spots, while agricultural pollution potential is present along the basin.

In Ebro, the highest organic contaminant concentration was measured at Zadorra site (Fig. 5; ZAD, approx. 10 µg/L) sampled close to wastewater treatment plant, downstream of Basque city Vitoria. Industrial organic chemicals were the major group of contaminants at almost all sampling sites. The second group corresponded to pharmaceuticals, which included the compounds belonging to different therapeutic classes. The maximum concentration of pharmaceuticals was measured at the aforementioned Zadorra site (Fig. 5). The concentration levels of pesticides are relatively higher at sites in the lower part of the basin and Ebro delta. At sites

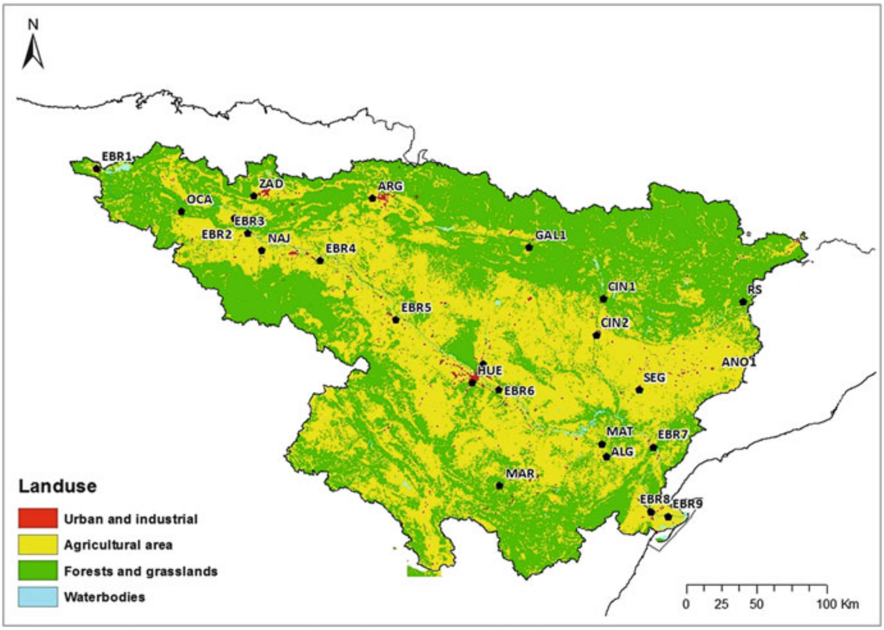

Fig. 4 Land use types in Ebro river basin and sampling sites of SCARCE project

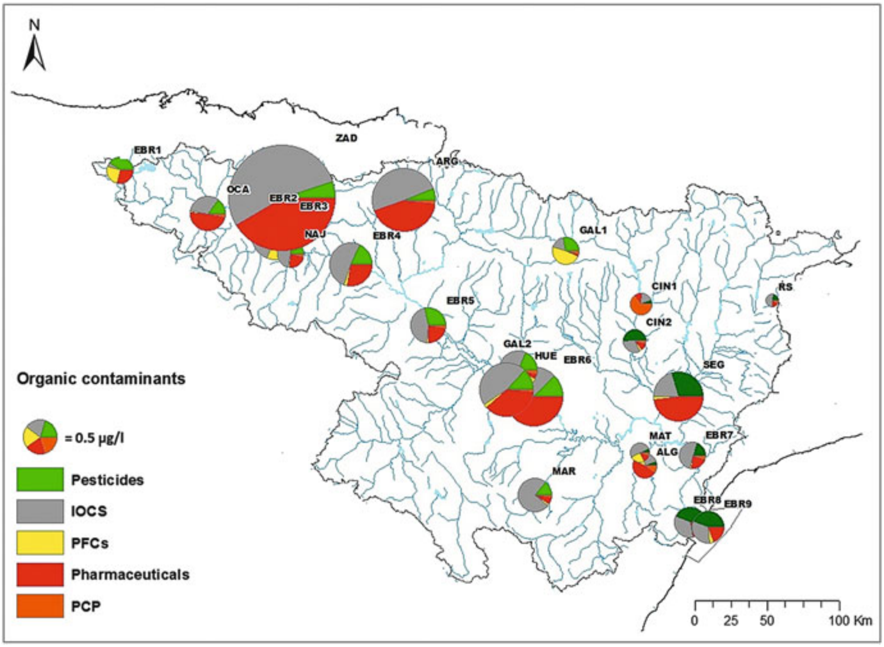

Fig. 5 Pollution of Ebro basin

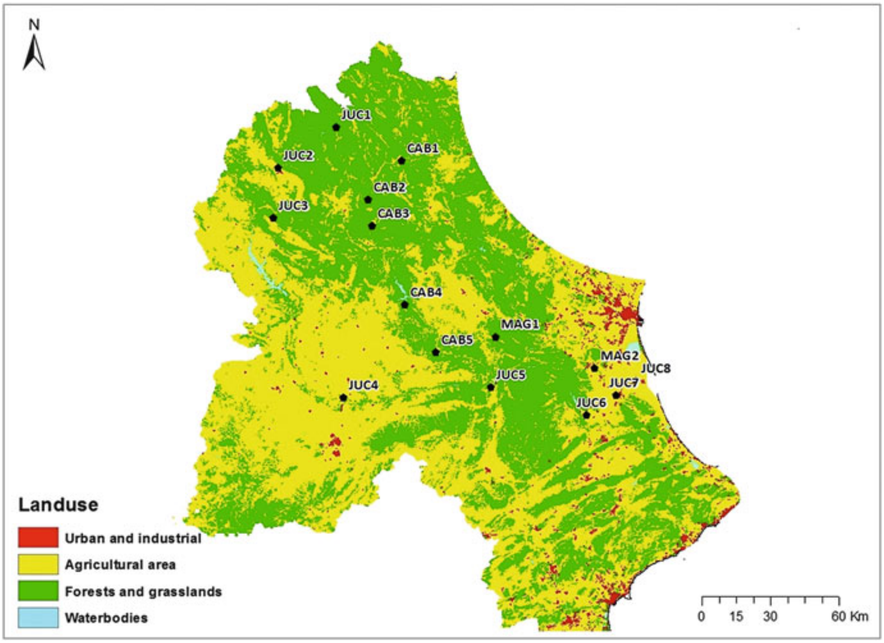

Fig. 6 Land use types in the Júcar river basin and sampling sites of SCARCE project

situated in the delta where agriculture is very developed (Ebro 8 and Ebro 9), pesticides are the major pollutant group measured. Compared to heavily contaminated sites like Zadorra or Arga, the total concentration of organic contaminants is relatively small (approx. 0.5 μg/L).

2.3 Júcar

The Júcar river basin is located east of Spain. It has a population of approximately 2.5 million inhabitants mainly located in the coastal area of the basin. Agricultural lands used for rice production, citrus fruit growth, etc. are located in the lower part of the basin surrounding the urban and industrial zones and in the southeast at the less urbanized zone (Fig. 6). The basin is situated in an area of semiarid climate which is adding to the problem of overexploitation of water for agricultural purposes and other uses resulting in decreasing the flow. The lower part of the basin is impacted by urban, industrial, and agricultural pressures influencing on the water quality.

Pesticides were the main group of pollutants found at majority of the sites of the Júcar basin (Fig. 7). Industrial organic compounds were measured at highest concentration at MAG1 site which is also the most polluted site in the river basin with the concentration of organic contaminants approximately 4 μ/L.

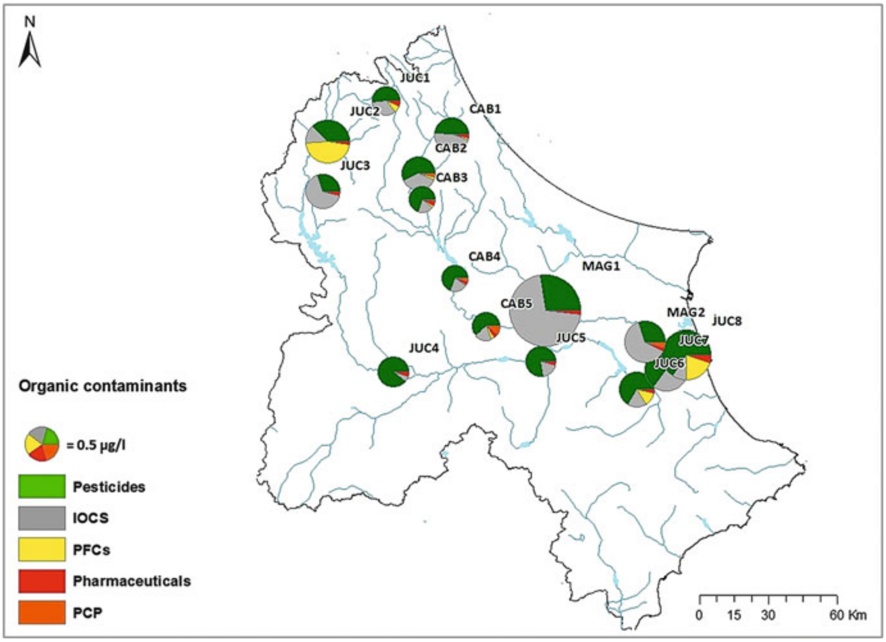

Fig. 7 Pollution of river Júcar with organic pollutants

Comparatively, pharmaceuticals and personal care products were measured at lower concentrations than in Ebro and Llobregat.

2.4 *Guadalquivir*

The Guadalquivir river basin is situated southwest of the Iberian Peninsula. The large proportion of the land is used for agricultural purposes (Fig. 8) with cultures such as olive trees, grapes, sugarcane, corn, etc. Mediterranean fruits and rice are grown in the lowest part of the basin. The delta is an area of high ecological value constituting the Doñana National Park. The Guadalquivir river together with its tributaries represents the main water source of the region serving the population of big cities such as Granada, Córdoba, and Seville. As a consequence of the high population of the cities, the river receives many inputs from the anthropogenic sources that may cause deterioration of water quality, together with the runoff of pesticides and fertilizers from agricultural areas.

Compared to other studied basins, Guadalquivir was the least contaminated. The main pollutants group in Guadalquivir was, like in Ebro and Llobregat, industrial organic compounds. The following group in terms of concentrations was perfluorinated compounds. The pollution was slightly higher in lower and middle part of the basin (Fig. 9).

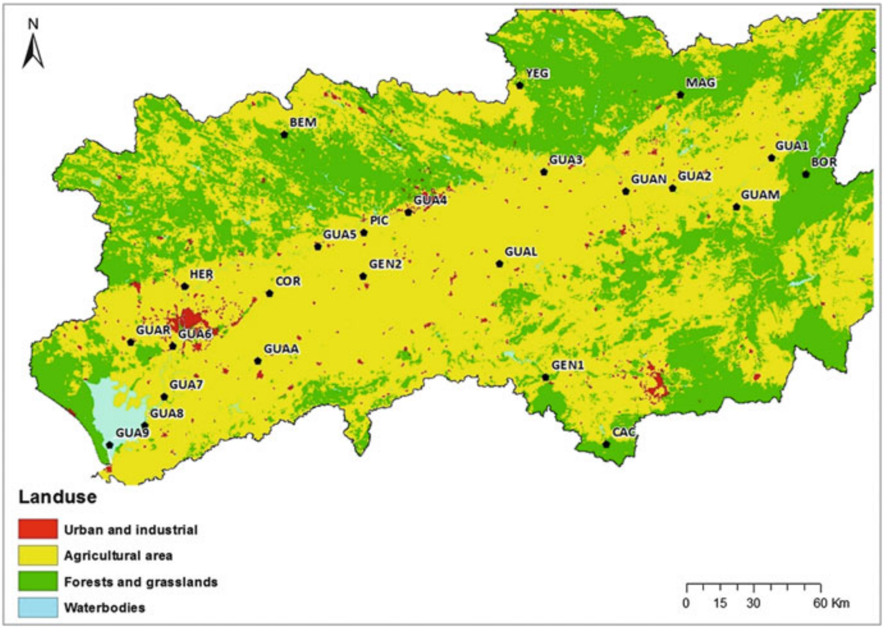

Fig. 8 Land use types of Guadalquivir basin

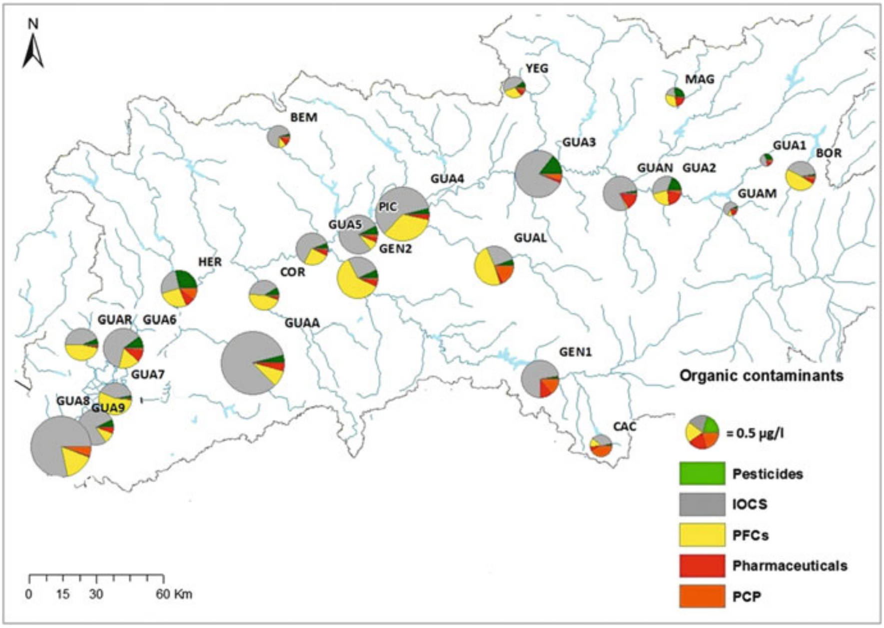

Fig. 9 Organic pollution of Guadalquivir river basin

3 Risk Assessment of Chemical Pollution in Iberian Basins

In order to assess the risk of chemical pollution [19, 20] and to determine the compounds of highest priority in four Iberian river basins [19, 20], the so-called toxic unit (TU) approach [21] was used to determine the ecotoxicological risk of individual compounds detected. Both aspects are developed in the following sections.

3.1 Toxic Units

Toxic unit is defined as the ratio of measured or predicted concentration of the compound and the corresponding toxicity value. In the aforementioned studies, measured concentration of compounds was used (C_i) together with acute toxicity data for three standard test species algae *Pseudokirchneriella subcapitata*, invertebrate *Daphnia magna*, and fish *Pimephales promelas*, representatives of trophic. EC50 values for algae and invertebrates (effect concentration for 50% of individuals at 48 or 96 h exposition time) and LC50 values for fish (lethal concentration for 50% of individuals) were used (Eq. 1). Toxicity data measured in vivo were collected from databases such as ECOTOX [37], Pesticides Properties DataBase [36], PAN Pesticide Database [22], and ECHA [23] or were collected from the literature. Missing data were evaluated by ECOSAR.

$$\mathrm{TU}_{i\ \text{(algae, invertebrates and fish)}} = \log \frac{C_i}{\mathrm{EC50}_i} \tag{1}$$

where TU_i is the toxic unit of the compound i, C_i (μg/L) is the measured concentration in the water phase, and EC50 is the effect concentration for the same compound (EC50 for algae and invertebrates and LC50 (μg/L) for fish, respectively). For convenience we express TUi in log units along the present chapter.

For the sediment toxic unit calculation, bioavailable pore water concentration was estimated following equilibrium partitioning approach [24]. From the bulk sediment concentrations measured, the bioavailable fraction of contaminants in the pore water was predicted by using the partitioning coefficient between sediment and water (K_d) (Eq. 2) as suggested by several authors [25].

$$C_\mathrm{S} = C_\mathrm{PW} \times K_\mathrm{d} \tag{2}$$

K_d is the partitioning coefficient between water and sediment, C_S the bulk sediment concentration, and C_PW the pore water concentration of the contaminant.

Therefore, sediment toxic units were defined as the ratio of the estimated pore water concentration of a contaminant and the water exposure-based toxicity values. Since the organic matter is assumed to be the major binding phase for nonionic organic chemicals in sediments [24], fraction of organic carbon in sediment (f_{oc}) and partitioning coefficient between organic carbon and water (K_{oc}) were used to calculate the pore water concentration (Eq. 3):

$$C_{\mathrm{PW}} = \frac{C_{\mathrm{S}}}{f_{\mathrm{OC}} \times K_{\mathrm{OC}}} \tag{3}$$

3.2 Site-Specific Risk

To assess the risk of chemical pollution at each sampling site, site-specific toxic unit ($\mathrm{TU}_{\mathrm{Site}}$, Eq. 4) was calculated as the sum of TUs of the compounds detected at that site [19, 20]. The concentration addition (CA) concept [26] mixture toxicity model was followed. It is generally used as the first tier approach, especially in the cases when the modes of action of many compounds in the sample are not known. The site-specific risk was expressed as the logarithm of the sum of individual toxic units (Eq. 2):

$$\mathrm{TU}_{\mathrm{SITE}} = \log \sum_{i=1}^{n} \mathrm{TU}_i \tag{4}$$

where TU_i is the toxic unit of each of individual compounds at the site.

Furthermore, the thresholds for effects in ecosystems were set as proposed in the study by Malaj et al. [27]. Acute risk threshold of $\mathrm{TU} \geq -1$, corresponding to 1/10 of the EC50 or LC50 of all three standard test species, was chosen due to the fact that changes in biological communities exposed to this level of pollution were observed [28–30]. On the other hand, different chronic risk thresholds were used for algae, invertebrates, and fish. Value of $\mathrm{TU} \geq -3$ was used for invertebrates based on the field studies; chronic risk thresholds for algae and fish were based on acute to chronic ratio [27]. For algae the acute to chronic factor 5 was used and for fish factor 10 [31–33].

3.3 Acute and Chronic Effect Risk in Iberian Rivers

Based on this risk assessment approach, there was risk of acute effects at 42% of the sampling sites and risk of chronic effects at all sampling sites in river basins studied. Risk was higher in 2010, when there was acute risk threshold exceedance at 42% of sites for invertebrates and 3% for fish. In 2011 there was exceedance at 20% of the sites for invertebrates and no exceedance for algae and fish. Even though Llobregat was the most polluted of this four rivers, the risk was the highest in Ebro (74% of the sites with acute risk) and Júcar (67% of the sites with acute risk), while in Llobregat, there was less than 25% of the sites with acute risk (Fig. 10). The river and campaign showing the highest number of sites with acute risk was the Ebro in

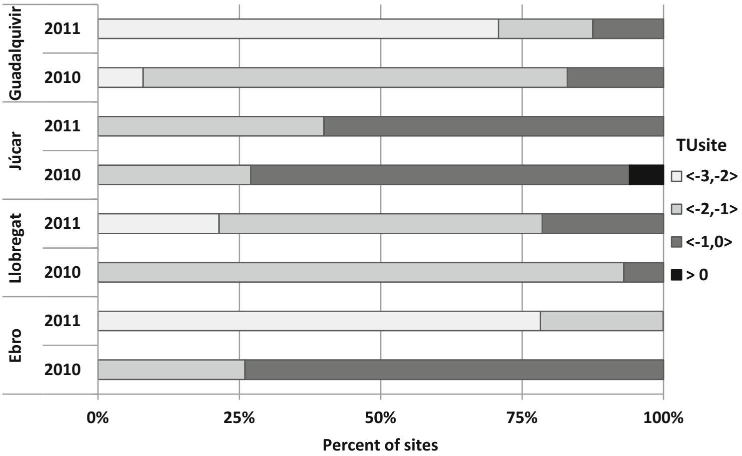

Fig. 10 Percentage of sampling sites with TU_{site} (most sensitive test species) belonging to one of four toxic unit ranges for each of four river basins in 2010 and 2011 in four studied basins. Dark gray shades, toxic units associated with acute effects; light gray shades, toxic units associated with chronic effects

2010 due to high concentration of toxic pesticides. However, they were not present at such high concentrations in 2011, so the acute risk threshold was not exceeded even though many other compounds were detected, indicating the importance of pollutant prioritization for monitoring and regulation purposes. In Guadalquivir, there was the smallest number of sites with acute risk exceedance (Fig. 10). In general, of studied organic chemicals, only pesticides present in water were related to acute risk. However, other groups of compounds (pharmaceuticals, personal care products, industrial organic compounds, etc.) were related to chronic risk threshold exceedance.

4 Prioritization of Pollutants in Iberian Basins

Of all the compounds present in the environment, typically there are only few which are responsible for the majority of the risk for biological communities [26]. Therefore, for the risk mitigation purposes, it is crucial to identify those compounds that are the most important in terms of ecotoxicological risk for each of studied river basins. To cope with this, ranking index [19, 20] was developed to prioritize the compounds on the basis of their ecotoxicological potential and distribution of concentrations along the river basin. The approach is based on toxic unit concept (TU) [21].

Table 2 Ranks of toxic units with corresponding weights

Rank (X)	Range (TU)	Weight (w_x)
1	>0	1
2	<0, −1>	0.5
3	<−1, −2>	0.25
4	<−2, −3>	0.125
5	<−3, −4>	0.0625
6	<−4	0

4.1 Ranking Index

Ranking index (RI) for prioritization was developed based on the previously used prioritization approach by von der Ohe et al. [10]. For calculating the ranking index, toxic units were ranked into six ranges, and to each of them a different weigh is assigned (Table 2).

The ranks are covering the ranges of toxic unit that could be related with acute and chronic effects in ecosystems, and for the lowest rank, TU level of −4 is taken, for which no effects are expected in most of the cases [34, 35]. Rank frequencies f_x are calculated as a percentage of the sites in the river basin where TU of the compound belongs to one rank (Eq. 4):

$$f_x = \frac{n_x}{N_{\text{total}}}\ (\%) \tag{4}$$

where n_x is the number of sites in the river basin with toxic unit level falling in rank x and N_{total} is the total number of sampling sites in river basin. The sum of all rank frequencies is equal to 100% as it covers all the sampling sites. The ranking index of the compound in the studied basin is calculated by the sum of the rank frequencies f_x multiplied by the given rank weights w_x (Eq. 5):

$$\begin{aligned}\text{Ranking index} = \sum_{x=1}^{6} f_x \cdot w_x = {} & (f_1 \times 1) + (f_2 \times 0.5) \\ & + (f_3 \times 0.25) + (f_4 \times 0.125) + (f_5 \times 0.0625) + (f_6 \times 0.0)\end{aligned} \tag{5}$$

The ranking index is scaled from 0 to 100, where 0 signifies that the toxic units of the compound were below the risk threshold of −4 at all the sampling sites. On the contrary, RI of 100 means that the toxic units of the compound were above 0 at all sampling sites. That is, the threshold for acute effect risk, EC50 or LC50 (for algae and invertebrates and fish, respectively), of standard test species concerned is exceeded at all sites.

4.2 Prioritization of Water and Sediment Pollutants

Ranking indexes were calculated related to toxic units of algae, invertebrates, and fish separately to cover the risk of compounds to different trophic levels. The ranking index values then can be used to give the information of the risk of the compound in the studied river basin. In particular, the values of RI give us the idea whether the compound is in high or low toxic units or below toxicity threshold (RI = 0%) in the studied river basin. We considered the RI higher than 12.5%, as the value of classifying the compound as the most important for studied area. This value means the compound was both in high toxic units and frequently exceeded the toxicity threshold of −4. The compounds which were classified as the most important for each of the studied river basins are represented in Table 3 and other compounds that were either in low toxic units at many sampling sites or at high toxic units but only at the few sampling sites in Table 4.

The most important compounds for studied river basins were mainly pesticides and industrial organic compounds. Among them, two pesticides classified as priority pollutants of WFD were ranked the highest. Pesticides as chlorpyrifos and chlorfenvinphos are the highly toxic compounds, designed to be biologically active even at low concentrations. However, when present in the environment, they might cause acute effects on nontarget species. Therefore, if they are frequently found in high toxic units in river basin, we might expect losses of biodiversity in local biological communities.

Compounds for which RI was between 0 and 12.5% are listed in Table 3. Several pharmaceuticals like sertraline, losartan, etc. were found at low TU at many sites in the studied rivers (Table 4). Therefore, their chronic effects could not be excluded. But, for more accurate risk assessment of chronic effects, chronic toxicity data should be used. However, chronic toxicity data for pharmaceuticals and other emerging contaminants is in fact very scarce. Several pesticides were in high TU

Table 3 Most important water pollutants in studied basins according to RI based on toxicity to algae, invertebrates, and fish

	Llobregat			Ebro			Júcar			Guadalquivir		
Compound	**A**	**D**	**F**	**A**	**D**	**F**	**A**	**D**	**F**	**A**	**D**	**F**
Chlorpyrifos		**X**	**X**		**X**			**X**	**X**		**X**	
Chlorfenvinphos					**X**			**X**			**X**	
Dichlofenthion					**X**	**X**		**X**	**X**			
Diazinon		**X**			**X**			**X**			**X**	
Prochloraz							**X**					
Ethion								**X**				
Carbofuran		**X**										
OPs/NPs		**X**									**X**	
Diuron	**X**											

Underlined WFD priority pollutants
A algae, *D Daphnia magna*, *F* fish

Table 4 Other important pollutants for each of the studied basins according to RI in water

	Llobregat			Ebro			Júcar			Guadalquivir		
Compound	A	D	F	A	D	F	A	D	F	A	D	F
Sertraline	X	X		X			X					
Triclosan	X			X			X			X		
Parathion-ethyl					X			X				
Caffeine	X			X			X			X		
Terbutrine	X			X								
Isoproturon	X			X								
Losartan	X			X	X							
Imazalil				X		X	X	X	X			
Tolyltriazole	X	X		X						X		
Simazine	X			X						X		
Atrazine				X			X			X		
Azinphos-ethyl		X			X						X	
Malathion		X	X		X			X	X		X	X
Azinphos-methyl		X			X							
Thiabendazole					X							
Methiocarb		X			X						X	
Venlafaxine	X	X		X	X							
Gemfibrozil			X									X
Pyriproxyfen						X			X			

Underlined WFD priority pollutants
A algae, *D Daphnia magna*, *F* fish

Table 5 Most important sediment pollutants for each of the studied basins according to RI

	Llobregat			Ebro			Júcar			Guadalquivir		
Compound	A	D	F	A	D	F	A	D	F	A	D	F
Chlorpyrifos		X	X		X	X		X	X		X	X
Chlorfenvinphos											X	
Nonylphenol	X	X	X								X	X
Diazinon		X						X			X	
Malathion								X				
Ciprofloxacin										X	X	
Methiocarb								X				

Underlined WTD priority pollutants
A algae, *D Daphnia magna*, *F* fish

but only at few sites in the studied basins; therefore, their ranking index was relatively low. That is, they may pose high risk, but only at specific areas of the basins concerned.

As regards to sediments, pesticides as chlorpyrifos and chlorfenvinphos were the most important pollutants according to the ranking index (Table 5). They were in higher concentrations in sediment; therefore, the ranking index was higher as well (chlorpyrifos max $\mathrm{RI}_{\mathrm{sediment}} = 80\%$; max $\mathrm{RI}_{\mathrm{water}} = 35\%$).

5 Conclusions

The four Mediterranean Iberian river basins studied were found contaminated by a variety of man-made compounds. In general, there were more than 50 compounds detected in each sample, including pesticides, pharmaceuticals, different industrial compounds, personal care products, and other types of contaminants. Llobregat was the most polluted of studied rivers, especially the lower part of the river which is passing through highly industrialized and urban zones. However, the ecotoxicological risk was the highest in Ebro and Júcar due to presence of acutely toxic pesticides in water. There was risk of acute effects posed by organic compounds at altogether 42% of the sampling sites and risk of chronic effects at all the sites. Pesticides were the compounds responsible for acute risk at the four rivers. However, other compounds like industrial organic compounds and emerging contaminants like pharmaceuticals and personal care products were responsible for the chronic risk threshold exceedance as well. A ranking index (RI) was used to order the compounds according to their environmental risk which takes into account both the frequency of occurrence and the ecotoxicological relevance (levels of toxic units) . In the studied river basins, nine compounds were regarded as the most important water pollutants (chlorpyrifos, chlorfenvinphos dichlofenthion, etc.) and seven compounds as the most important sediment pollutants (chlorpyrifos, chlorfenvinphos, nonylphenol, etc.).

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Emerging Organic Contaminants in Aquifers: Sources, Transport, Fate, and Attenuation

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Abstract In this chapter we take a look at the presence of emerging organic compounds (EOCs) in groundwater, with emphasis in sources of pollution, processes affecting the spatial and temporal concentration of these compounds as they move through the aquifer and interact with the soil. Emphasis is placed on the combination of transport mechanisms and physical and biogeochemical processes that combine for natural attenuation and the potential eventual output to the biosphere. Some considerations about risks associated to the presence of such substances are also included.

Keywords Degradation, Emerging compounds, Groundwater, Natural attenuation, Risk assessment, Sources, Transport

Contents

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1 Introduction

Groundwater is a main actor in the water cycle. It is a major source of water supply and the primary source of drinking water in areas having a decentralized supply system. For this reason, it is of utmost importance to characterize its quality and how it evolves as a function of location and time. Groundwater quality is progressively being deteriorated in many parts in the world, affected by either natural or anthropogenic activities or a combination of both. The list of contaminants that could potentially affect groundwater is very large, and so traditionally only a subset of those has been investigated. On the other hand, analytical chemistry has made extraordinary advancements in recent years, so that now it is possible to discriminate even minute concentrations (on the order of ng/L), which were not known to be significant but may result in potentially harmful effects, especially in large exposures [1]. This is the main reason why until very recently, the presence of emerging organic compounds (EOCs) in surface or subsurface water bodies has gone largely unnoticed.

For these reasons, the presence of different kinds of EOCs in rivers, reservoirs, and effluents from wastewater treatment plants (WWTP) has been reported only in the last few years. Reports on the presence of EOCs in aquifers are even more recent, and initially they involved only qualitative descriptions, associated to different compounds being expected to found associated to the interactions between polluted surface water bodies and subsurface reservoirs. An analysis of a number of cases, distributed worldwide, where the presence of EOCs in aquifers has been quantitatively reported, was recently compiled by Kurwadkar and Venkataraman [2]. Several studies of the occurrence of EOCs at the national level are also available, including the works of Jurado et al. [3] emphasizing rural and urban areas in Spain, Meffe and de Bustamante [4] in Italian aquifers, and Stuart et al. [1] in British aquifers. In this latter study, it was found that emerging organic pollutants are found in larger concentrations in surface waters as compared to those from aquifers, an observation that the authors associated to a combination of the higher complexity involved in groundwater sampling and the action of a number of processes taking place in the subsurface that result in the removal of contaminants below EOC detection. However, Stuart et al. [1] observed larger concentrations of some pollutants in groundwater, associated to the existence of multiple sources and entry pathways, and pointing the existence of different degradation rates in the subsurface.

Transport through aquifers has been found as the most efficient way to remove some EOCs from water due to a combination of physical, chemical, and biological processes. While it is true that concentration of EOCs can be naturally attenuated in aquifers, it is also true that such compounds are in most cases recalcitrant, thus implying the difficulty to be degraded even in favorable conditions. The shorter the residence times and the lower the temperatures would combine to little development of microbial population, favoring the persistence of EOCs in groundwater. Large residence times usually imply very large percentage of removal of organic compounds.

Aquifers can also be classified according to their vulnerability to pollution sources; it is reasonable to assume that the aquifers more prone to pollution are the shallow ones conformed by sandy soils or heavily fractured rocks. The least vulnerable ones should then be deep aquifers confined by clayey soils or unweathered rocks. Confining or semiconfining layers are then expected to provide protection of aquifers against EOC pollution.

Persistence of EOCs in groundwater might result in significant, albeit low, concentrations of a number of EOCs reaching either a sensitive surface water body or a pumping well. In the former case, there is a potential for water-controlled ecosystems to become affected, with a cascading impact on flora and fauna, resulting in a loss in ecosystem services; in the case water is used for supply purposes, and combined with the toxicity of some compounds, it may result in human health issues [5, 6]. Even when compounds are partially biodegraded along the groundwater path, the resulting daughter compounds might be potentially more toxic than the parents, and so there is a need to study the complete degradation chain of any given compound as a function of space and time related to the changing conditions in the aquifer (e.g., the transition from aerobic to anaerobic).

Despite according to the European Directive 2006/118/EC, groundwater is the largest body of freshwater in the European Union, to date, legislation on pollution of groundwater by organic compounds is restricted only to pesticides. The environmental quality standards have been established, both for individual substances (0.1 mg/L) and the total sum of detected compounds (0.5 mg/L), here including metabolites (biodegradation subproducts). The remaining EOCs are not yet legislated at the European level, although the regulation of a number of them is expected to be available in the next future. The US EPA published a contaminant candidate list in 2009 that includes three pharmaceuticals and eight hormones. The European Commission has drafted a list of new substance limits including a short list of EOCs.

In this chapter, we take a look at the sources of groundwater pollution by EOCs, with some particular examples that can be used for illustrative purposes; later, we evaluate the main processes controlling the fate of these components during groundwater transport and their eventual output to the biosphere, either naturally or anthropically controlled. The paper emphasizes several aspects regarding monitoring and the quantitative evaluation of sources. Finally, some considerations about risks associated to the presence of EOCs in groundwater are included.

2 Sources of EOCs in Groundwater Bodies

The presence of EOCs in aquifers is directly linked to the different processes involved in the water cycle. In such cycle there converge different environmental compartments and anthropogenic activities that may lead to quality deterioration. The sources for EOCs according to the corresponding pollution routes can be classified as (1) leaks from sewage systems or septic tanks in urban areas; (2) leaks from industrial refuse; (3) reuse or mishandling of effluents from wastewater treatment plants; (4) agricultural and livestock practices; (5) inflows from polluted surface water bodies such as rivers, reservoirs, or wetlands; (6) artificial recharge practices; and (7) landfill leachates from urban or industrial refuse.

These sources of pollution can alternatively be classified as point and diffuse (or nonpoint) sources. The former are discernible and discrete (localized in space) sources, occurring when pollutants are discharged into the aquifer at a given point or a very small area. Examples include sewage or industrial leaks, accidental spills, and landfills. Point sources are generally easier to identify and monitor. Remediation efforts can be best designed to deal with point source pollution, most generally involving the need to eliminate the source. Point sources have a number of relevant characteristics as compared to diffuse sources [7]: higher concentrations at the very local scale, potential affection to a limited number of environmental receptors, and easier to control and remediate once detected.

Nonpoint (or diffuse) source pollution is caused by contamination extended over a wide surface area, and sometimes their origin cannot be easily identified. Irrigation for agriculture is the main source of nonpoint pollution of groundwater, caused by the combination of the application of pesticides, fertilizers, and other agrochemicals with suboptimal irrigation practices [8]. Similarly, runoff from urban areas and leakage from urban wastewater systems are also sources of nonpoint pollution [9]. The compound characteristic of areas affected by nonpoint sources may have a greater impact on groundwater quality, as compared to point sources, due to their areal extension. This extension also calls for difficulty in remediation efforts and the impossibility to isolate or remove the source term.

Sources can also be classified according to their temporality, as continuous or discontinuous. The former include human, industrial and livestock activities, as well as landfill leachates; the latter mainly correspond to agricultural practices. The pollution load may change significantly with time, indicating the need to properly monitoring the variability of concentration in target compounds in order to properly perform mass balance evaluations. Even when the source is continuous, the seasonality or discontinuity of other events, such as water table fluctuations or flow conditions in the vicinities of the source term, produces significant changes in the spatial and temporal distributions of the pollutants.

EOCs can be classified into seven groups according to their use, those being:

- Pesticides: substances intended for preventing, destroying, repelling, or mitigating pests or bad herbs
- Pharmaceuticals: natural or synthetic substances used to treat or prevent diseases, either physiological or mental, of humans or animals
- Industrial products: used in production or for cleaning large industries and machinery
- Drugs of abuse: chemicals that are used for nontherapeutic purposes and can produce changes in perception, mood, and behavior of individuals
- Personal care products (PCPs): used for beautification and in personal hygiene, directly applied, and that should not show significant biochemical activity
- Estrogens: a group of steroid compounds relevant in the estrous cycle
- Recreational substances: including nicotine, caffeine, sweeteners, and other legal substances used on a daily basis by the population

There is clear link between the sources and the type of EOCs that could be expected in a given aquifer recharged by one or a number of sources. It is also important to state that each source might combine more than one of the previously defined groups. We now describe each individual source, combined to the EOCs that are expected to be present in groundwater associated to these sources, and providing a few examples of recent investigations reported in the literature.

2.1 Urban Sewage Systems and Septic Tanks

The actual composition of sewage waters in terms of spatial and temporal distribution of EOCs depends on the actual land uses, for example, regarding the potential presence of households, hospitals, construction sites, landscaping, transportation, commerce, or industrial uses [10], but also associated to the social and demographic distribution.

In aquifers recharged with water coming from leaks in urban sewage systems, all kinds of pharmaceuticals and personal care products can be expected, as well as drugs of abuse, steroids, and substances of daily use. An example of the latter is the presence of amidotrizoic acid (X-ray contrast) and acesulfame (artificial sweetener) during a 6-year monitoring period in a shallow urban groundwater body [11], associated to sewer leaks.

Sewage systems can also be a source of illicit drugs. A full review of the presence of such substances and their metabolites in groundwater (as well as other water bodies) in many places in the world was elaborated by Pal et al. [12]. An analysis of groundwater from the city of Barcelona showed the presence of methadone, cocaine, and MDMA, as well as a number of metabolites, as well as the presence of solar filters [13]. Similarly, septic tanks are another source of groundwater pollution by EOCs. A case study in a karst area in Florida is presented by Katz et al. [14]. Pharmaceuticals were analyzed showing the strong attenuation of most substances but still showing the presence of a number of organic wastewater and pharmaceutical compounds.

2.2 Industrial Activities

There is a wide range of compounds linked to industrial activities that can be released into the environment. The main groups are surfactants, used in a variety of industrial products (cleaners, degreasers, and detergents), as well as bisphenol A (BPA) and phthalates, present in plastics. These compounds have been detected in aquifers located in industrial areas but can also affect urban groundwater and agricultural areas located nearby. Two examples are the works of Latorre et al. [15], who reported the occurrence of nonylphenol and octylphenol in agricultural areas of Catalonia, and Tubau et al. [16], who investigated the occurrence of surfactants in the aquifer underlying the city of Barcelona.

A new area of study is the potential presence of EOCs in aquifers associated to industrial injection activities, such as “fracking” that could eventually leak to groundwater bodies.

2.3 Wastewater Treatment Plants

All types of EOCs could be present in effluents of WWTPs just depending on the source of the influent water. Treated or untreated effluents of WWTPs may be used in different applications (agriculture, artificial recharge, etc.) being potentially the largest source of EOCs in groundwater. Many examples are available in the literature, where authors have reported the presence of pharmaceuticals associated to such activities. An example is Fang et al. [17] reporting the presence of

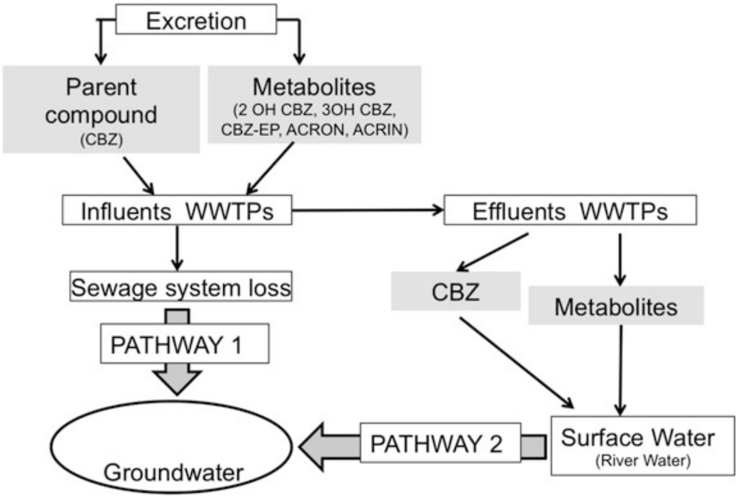


Fig. 1 Pathways for groundwater pollution of carbamazepine (CBZ) and the corresponding metabolites related to the different sources (adapted from [18]). The chemical composition (in terms of magnitude and proportion) reaching the urban aquifer through the two pathways is different, as it has been affected by different processes

gemfibrozil in an aquifer in an irrigated area after land application of effluent from a WWTP.

Actually effluents may eventually reach groundwater by more than one pathway. An example of the possible combination of pathways for groundwater pollution of carbamazepine (CBZ) and a number of metabolites (associated to the different sources) was presented by Jurado et al. [18] (see Fig. 1). For example, water may infiltrate either before or after reaching the WWTP, with very distinctive chemical signatures (mostly in terms of the number of metabolites present in water).

2.4 Agricultural and Livestock Practices

Pesticides are classified into four main classes according to the type of pest control: herbicides, fungicides, insecticides, and bactericides. The use of pesticides is regulated worldwide, and consequently their presence and fate in groundwater, as well as their metabolites, have been widely reported. For this same reason, pesticides are regulated by the EU Directive 2006/118/EC. According to this directive, a reference threshold of 100 ng/L was established as the quality standard for individual pesticides in groundwater.

The presence of pesticides is correlated to the crop type and agricultural practices. For example, the occurrence of specific pesticides has been studied according to specific crops, such as maize (triazine and an anilide), cereal (herbicides), vineyards (triazines, anilides, and an organophosphate herbicide), and citrus (simazine and triazine metabolites). Arráez-Roman et al. [19] found carbamate insecticides from greenhouses. Costanzo et al. [20] focus on one insect repellent DEET.

Antibiotics (veterinary drugs and feed additives) and their bioactive metabolites or degradation products can be introduced in agroecosystems through fertilization and irrigation with antibiotic-polluted manures [21]. When irrigation is performed with reclaimed water, many EOCs can be introduced in the groundwater system simultaneously. An example is provided by Estévez et al. [22] who found pharmaceuticals and pesticides in the groundwater of a volcanic area irrigated by reclaimed water for more than 35 years.

An example of pollution by farming practices is reported by Makris and Snyder [23]. Hansen et al. [24] report the presence of antiparasitic compound (ionophores) in groundwater associated to their increasing application as feed additives in modern livestock production. Several studies report the presence of sulfadimethoxine (a sulfonamide), associated to veterinary practices.

2.5 Polluted Surface Water Bodies: Rivers, Reservoirs, or Wetlands

Rivers and other surface water bodies might be polluted from effluents of WWTPs, industries, and agricultural practices. Therefore, the interaction between surface water bodies and aquifers is a key issue when evaluating the performance of EOCs in subsurface water bodies, particularly in aquifers that are heavily exploited, and where rivers are the main source of recharge. Due to the natural attenuation capacity of aquifers (as a consequence of physical and/or biochemical processes), in general EOC concentrations in surface waters are higher than those in aquifers for a wide variety of pesticides, pharmaceuticals, drugs of abuse, and industrial compounds. The literature shows some exceptions where concentrations in groundwater are comparable to those in rivers. An example can be found in Jurado et al. [3], corresponding to the Llobregat and Besós aquifers (Barcelona, Spain), who studied the presence of different EOCs in surface and subsurface waters, finding quite different water compositions, with a number of EOCs displaying higher concentrations in aquifers as compared to the corresponding rivers (Fig. 2).

Groundwater recharge can be induced by riverbank filtration. This methodology consists of allowing water from a river to infiltrate laterally through the banks of the river. Extraction is usually performed at wells located some distance away from the river. The process may directly yield drinkable water of drinkable quality or be a relatively uncomplicated way of pretreating water for further purification. The increase in quality is produced by a combination of physical filtration (straining) and biological and chemical filtration.

The combination of physical, chemical, and biological processes taking place after riverbank filtration removes most organic pollutants, but it has been

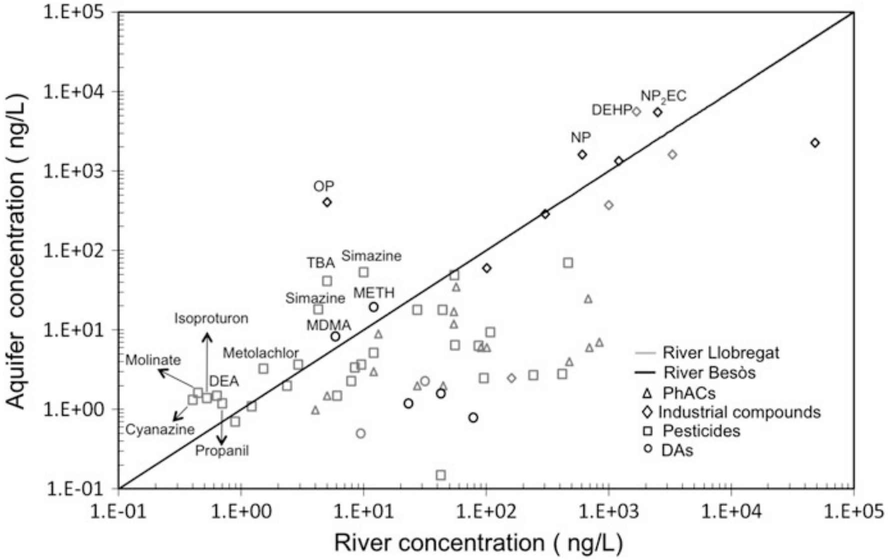


Fig. 2 Concentration of a number of EOCs in the Llobregat and Besós rivers (located in Northeast Spain) and the associated aquifers (adapted from [3])

traditionally considered that some EOCs, such as most pharmaceutical compounds, are not sufficiently removed [25]. The presence of EOCs and the attenuation of some compounds in riverbank filtration facilities are mostly associated to the river flow regime; the actual concentration of EOCs generally increases with a decrease in river flow rate, but on the other hand, it also implies a lower proportion of river water in the recovered groundwater [26].

2.6 *Artificial Recharge Practices*

Managed aquifer recharge (MAR) practices include several methods that aim to recover and enhance groundwater quality and productivity of depleted aquifers. All these methodologies achieve a certain quality improvement by just inducing transport through the porous media. A common example of MAR is the use of excavated surface ponds (SP), where recharge is induced by flooding the excavation with water coming from an available source (e.g., reclaimed water, stormwater, river water), which percolates toward the subsurface. The maximum rate at which water can infiltrate in the subsurface is known as the infiltration capacity (I_c). I_c regulates both the infiltrated volumes and the residence time of water within an SP, both quantities needed for appropriate facilities management.

Transport through aquifers has been found as the most efficient way to remove some EOCs from the solution, as compared to the processes taking place in WWTP, these including chlorination, activated carbon filtration, and flocculation. Many examples are found in the literature for particular compounds such as iodinated X-ray contrast media, personal care products, complexing agents, pharmaceuticals, and sweeteners (e.g., [27, 28]). A comprehensive study involving the injection of treated wastewater via vadose zone injection wells was performed in a field site in Arizona [29]. Thirty-three EOCs were found above detection limits in the injected water; sixteen of them were detected within 150 m of the injection wells, and only six remained above their respective detection limit in monitoring wells located more than 560 m away from the injection area.

Removal efficiency is significantly influenced by redox conditions (e.g., [28, 30]), a topic that will be addressed later. Also, there is increasing evidence of the effect of the hyporheic and the vadose zones in the degradation of EOCs, due to their specific biogeochemical conditions.

2.7 Landfill Leachate

Despite the disposal of waste materials into specialized and centralized locations seems the best option to minimize overall pollution, drainage from landfills produces long-term threats to groundwater. According to the review presented by Ramakrishnan et al. [31], landfill leachates contain a number of EOCs including pharmaceuticals, personal care products, surfactants, plasticizers, fire retardants, pesticides, and nanomaterials.

Groundwater beneath two landfills in Central Oklahoma revealed the presence of variety of emerging contaminants in shallow groundwater immediately downstream of the landfill sites [32]. Peng et al. [33] studied leachate from landfills that eventually discharged into a reservoir.

3 Quantifying EOC Input to the Groundwater System

3.1 Quantifying Infiltration Rates

Often, the estimation of the amounts of contaminant released to the environment is a difficult task. It has a large uncertainty mostly because of the lack of control and the fact that they are due to unintentional or, on the contrary, to deliberate acts.

For instance, leaks from sewage systems are usually evaluated as a fraction of the amount of water circulating in the pipes. It is different to consider the case of combined (designed to transport both stormwater runoff and sewage in the same pipe) or separated. The actual organic matter loads of each individual leak are site specific. Loads might be evaluated as the product of the flow rates times the concentration of each individual EOC.

Composition of leachates from landfills is extremely changeable according to the nature, the deposit time, and the climatic conditions. Leaching volumes are mostly a function of quality construction, as in principle they should be canalized.

Infiltration from excess irrigation practices mostly in agriculture must be obtained by means of water balance at the soil, linked to crop growth.

Finally, infiltration from surface ponds in artificial recharge operations varies in space and time linked to a number of physical, biological, and chemical mechanisms, including (1) microscale reactions leading to a macroscale clogging effect and (2) site-specific factors, such as soil textural and density heterogeneities, chemical variability, and fluctuations in water temperature.

In any case, sources affect quite differently aquifers depending on the capacity of the soil to reduce the impact of pollutants reaching the aquifer. A standard method for assessing groundwater vulnerability to EOCs is the use of simple GIS-based methods (such as DRASTIC). Despite such methods are heavily contested due to their simplicity and the relative representativity of physical parameters in pollutant fate, it is precisely this feature of simplicity the one that makes these methods so popular.

In some cases, it is better to use alternative indices for vulnerability. An example is the "groundwater ubiquity score" (GUS index) [34]. The GUS index is used to assess the ability of a pesticide leaching and classifies leachable pesticides ($GUS > 2.8$), non-leachable ($GUS > 1.8$), and transition values. It has been shown [35] that detection frequencies of EOCs in soils and groundwater significantly correlate with the GUS index.

3.2 Characterizing Water Quality

In general it is not possible to properly quantify the loads of EOCs reaching subsurface water bodies, as it would imply taking a large number of water samples and screening a very large number of compounds. One possible alternative is to characterize directly the chemical signature of existing groundwater by applying multivariate statistical techniques. An example is Nosrati and Van Den Eeckhaut [36], who reported that discriminant analysis technique could be the most effective in distinguishing groundwater clusters.

3.3 Identifying Sources Through Mixing Analysis

The presence of inorganic and organic compounds in groundwater allows the evaluation of mixing ratios, implying a double process of identifying the sources of water and also quantifying the ratios of mixing of these sources. This would allow predicting the presence of unsampled chemicals in the mixture. Once sources are identified, the chemical signature at each sampling point may be considered as a result of mixing of water from sources (conservative process), where the unknowns are the mixing proportions, modified by the presence of reactions in the groundwater (nonconservative) process.

Mixing can be evaluated using the MIX code [37]. This code allows incorporating uncertainty in both recharge sources and observation point measurements. Uncertainty is mainly related to sampling and analytical errors (incorporated in the analysis by means of an assumed a priori standard deviation), as well as to the lack of data and the occurrence of additional processes, such as geochemical reactions. Measurement uncertainty is quantified through covariance matrices.

An example of the application of MIX to the analysis of EOCs can be found in Jurado et al. [13], who identified redox zonation in the Barcelona aquifers, using it as a way to analyze the potential degradation of EOCs associated to redox processes.

4 Transport of EOCs in the Subsurface: Conservative Processes

There are three basic physical mechanisms by which solutes are transported in the subsurface: advection, diffusion, and dispersion.

4.1 Advection

Advection is the process by which contaminants are dragged by the fluid flows as a result of hydraulic gradient. Due to advection, nonreactive solutes are transported at an average speed equal to that of the fluid. For saturated flow conditions, the advective mass flux, $\boldsymbol{J}_\mathrm{a}$, is a vector that represents the mass flowing through a unit area section per unit time, and it is given by

$$\boldsymbol{J}_\mathrm{a} = \boldsymbol{q}c \tag{1}$$

where $\boldsymbol{q} = \boldsymbol{v}\phi$ is Darcy's velocity, $\boldsymbol{v}$ is fluid velocity, ϕ is porosity, and c is the solute concentration in the liquid phase.

4.2 Molecular Diffusion

Diffusion is a process that takes place at the molecular scale. Molecules of solute display an apparently random movement caused by collisions with the molecules of the fluid. The net result is a smoothing of concentrations. The amount of diffusion transfer between two volume elements is directly proportional to the concentration difference between them and inversely to the distance between them. This is represented by Fick's first law as

$$\boldsymbol{J}_{\mathrm{d}} = -\phi D_{\mathrm{d}} \nabla c \tag{2}$$

where $\boldsymbol{J}_{\mathrm{d}}$ is the diffusive mass flux per unit area section and unit time and D_{d} is the diffusion coefficient in the fluid phase.

4.3 Hydrodynamic Dispersion

Hydrodynamic dispersion is associated to the local variations of velocity within a porous media. Such local variations enhance the spread of solutes. The dispersion effect is thus governed by the velocity heterogeneity at all scales, represented by:

1. Water velocity in a given pore is smaller close to the wall and larger in the middle of it, theoretically displaying a parabolic distribution.
2. Average water velocity depends on pore size.
3. Pathways at the local scale are different for each molecule, providing local tortuosity values for each individual molecule.
4. Particle pathway sample areas of varying hydraulic conductivity.

The dispersive mass flux is usually modeled by a Fickian process:

$$\boldsymbol{J}_{\mathrm{h}} = -\phi \boldsymbol{D}_{\mathrm{h}} \nabla c \tag{3}$$

where $\boldsymbol{D}_{\mathrm{h}}$ is the dispersion tensor.

4.4 Conservative Transport Equation

The fundamental equation of solute transport is based on mass conservation, which states that for a given volume, the net increase in mass in the volume equals the variations of mass fluxes plus the variations caused by reactions occurring within the volume. Mass fluxes involve the sum of those corresponding to advection, diffusion, and dispersion. An additional term is included to account for all the reactions that will be considered in the next section. The final expression becomes

$$\phi \frac{\partial c}{\partial t} = -\boldsymbol{q} \cdot \nabla c - \phi (D_{\mathrm{d}} \boldsymbol{I} + \boldsymbol{D}_{\mathrm{h}}) \nabla c + M \tag{4}$$

where $\boldsymbol{I}$ is the identity matrix and M is a generic term to account for nonconservative processes.

5 Fate of EOCs Linked to Biogeochemical Reactions

The concentration of EOCs in aquifers is affected by many processes, such as dilution (reduction in concentration due to an increase in solvent), adsorption (removal from the solution by interaction with a solid surface), biodegradation (concentration decrease by the action of biological agents), and chemical transformation.

A review of pathways and fate of EOCs associated to nonconservative behavior (i.e., subject to geochemical and biological reactions) can be found in Lapworth et al. [7]. In general, it is observed that transport through the aquifer results in strong attenuation of a number of EOCs. For example, Laws et al. [38] report a strong attenuation of a number of target compounds after 60 days of residence time, associated to a combination of nonconservative processes including adsorption and biodegradation.

EOC behavior in groundwater depends on the physicochemical properties of the particular substance polluting the aquifer and also in the redox potential in site. The former tends to control the mobility; the latter controls the degradability [39, 40]. The different processes affecting the fate of EOCs in groundwater are presented next.

5.1 Adsorption

Adsorption is defined as the set of processes by which different atoms, ions, or molecules of a chemical compound dissolved in water are accumulated on the solid-phase surfaces. It may be caused by a number of mechanisms:

- The inner-sphere surface complex: the complex that is obtained when there are no water molecules between the solid surface and the sorbed molecule. It involves ionic and covalent bonds or a combination of both.
- The outer-sphere surface complex: complex obtained when at least one water molecule is placed between solid and sorbed molecule. It involves electrostatic bonds, less stable than ionic and covalent.
- Diffuse-ion swarm: involving ions that are completely dissociated from any functional group, free to move through the solution. It also involves electrostatic bonds.

Adsorption accounts for fixing mechanisms of the compounds to the soil surface. In the case of EOCs, adsorption is controlled by three main properties: the octanol-water partition coefficient (K_{ow}), usually expressed as $\log\text{-}K_{\mathrm{ow}}$, the soil-water partition coefficient (K_{d}), and the solubility in water (S_{w}). Some of these compounds have a hydrophobic behavior ($\log\text{-}K_{\mathrm{ow}} > 4$ and low solubility), with tendency to bioaccumulation and high sorption capacity. Examples of these could be β-blockers (e.g., propranolol) and a few pharmaceuticals (ketoprofen) or illicit drugs (THC). On the other hand, substances with log-$K_{\mathrm{ow}} < 4$ (most pharmaceuticals and drugs of abuse) present a hydrophilic behavior, consequently being more frequently detected in groundwater. Indeed some pharmaceuticals and, in particular, carbamazepine have been used as anthropogenic markers in the aquatic environment (e.g., [41]). The hydrophobic effect sets a trend of water molecules (polar) to exclude nonpolar molecules. This leads the organic matter in the soil (nonpolar) to adsorb the organic compounds (also nonpolar). As EOCs are organic compounds, sorption processes will be done with organic matter presence in the subsurface. Then, K_{d} is transformed to K_{oc} by relating it to the organic matter fraction in the aquifer (f_{oc}).

In an example of riverbank filtration analysis, Henzler et al. [42] emphasized adsorption as a key process influencing transport of a number of EOCs, mostly MTBE and carbamazepine. Ying et al. [40] found strong adsorption of beta-estradiol to the local aquifer material under aerobic conditions.

Different models of adsorption have been used to model observations regarding different pharmaceuticals in aquifers, going from linear to nonlinear. Examples of the latter involve the use of a Langmuir isotherm to model the adsorption of ibuprofen in volcanic soils [43] and of a Freundlich one for octylphenol and triclosan in a number of different soils [44].

5.2 Complexation

Complexation involves the attraction of species to solid surfaces of amorphous aluminosilicates, oxides/metal hydroxides, and organic matter. Although ion exchange is a type of surface complexation, it is usually restricted to the exchange of cations associated with the permanent charge on the surface of a clay mineral. Ion exchange reactions occur primarily due to electrostatic attraction and are characterized by the exchange coefficient, while surface complexation reactions occur by chemical and electrostatic components together. Surface complexation is analogous to aqueous complexation, based on the intimate association of a core molecule surrounded by those of the solute.

An example of complexation influencing transport of EOCs has been reported by Chen et al. [45], who studied the effect of complexation of the antibiotic ciprofloxacin with Fe/Al oxides/hydroxides.

5.3 Redox Reactions

The redox conditions of a contaminant plume constitute an important part of the chemical framework controlling the behavior of the contaminants [46] since redox state significantly affects the rate of biodegradation of the substances. They are arguably the most important reactions affecting EOCs. They correspond to chemical reactions where electrons are exchanged between an acceptor (the compound is reduced) and a donor (oxidized). This type of reactions liberates a significant amount of energy, as there is a large difference between the energy of reactives and products. They are irreversible reactions, mediated by microorganisms that do not participate in the reaction but act as catalysts.

Ordering a reduction-oxidation sequence based on the energy liberated (in descending order), we find: (1) oxygen reduction – aerobic respiration, (2) denitrification, (3) manganese reduction, (4) iron reduction, (5) sulfate reduction, and (6) methanogenic production – fermentation. It is important to stress that redox conditions might be highly transient and variable in space even in the same aquifer. So, the local removal value of a given substance associated to a given particular redox condition is highly heterogeneous [42].

The relevance of aerobic/anaerobic conditions with respect to the fate of EOCs in groundwater can be seen from a number of examples of specific compounds. An example was reported by Meffe et al. [47], who showed that para-toluene-sulfonamide (an industrial product) was degraded under oxic conditions but that it behaved conservatively under anoxic conditions. A similar behavior was reported by Ying et al. [40] for beta-estradiol (an endocrine disrupting compound).

EOC degradation is usually much faster under aerobic conditions compared to anaerobic conditions. These, combined with the long residence time of compounds in aquifers, are potential reasons for the low concentrations of EOCs usually detected in groundwater. Unfortunately, there are few studies that reflect the redox state or the water age; therefore, (1) the analysis of the EOCs should be associated with groundwater studies to understand the observations, and (2) new research on the processes of degradation and transformation is needed to evaluate the behavior of EOCs in groundwater.

In order to illustrate the effect of redox conditions on the fate of EOCs, we will concentrate in one particular compound, that of the sulfonamide bacteriostatic antibiotic sulfamethoxazole (abbreviated SMZ). Due to its low adsorptivity, it is postulated that SMZ reduction is mainly due to microbial activity. However, proper balancing studies are rare, presumably because of missing information regarding its metabolic fate and produced metabolites. In general, the literature pertaining to SMZ elimination in WWTPs and natural aqueous environments is marked by inconsistent results [48]. This is supposedly because elimination depends on various environmental factors such as in situ redox potential, available nutrients, soil characteristics, seasonal temperature, and light variations.

Müller et al. [48] concluded that SMZ was degraded under aerobic conditions in an activated sludge system. When SMZ acted as a co-substrate, the main metabolite found was 3-amino-5-methyl-isoxazole. On the other hand, other authors observed that SMZ was better degraded under anoxic conditions. For example, an experiment of Barbieri et al. [39] demonstrated that SMZ was transformed into 4-nitro-sulfamethoxazole under denitrification conditions (when nitrite was accumulated) and when a labile organic carbon was added. It was also postulated that when nitrite was depleted, 4-nitro-sulfamethoxazole was retransformed to previous SMZ (see Fig. 3). Other experiments observed that SMZ could be fast degraded under iron

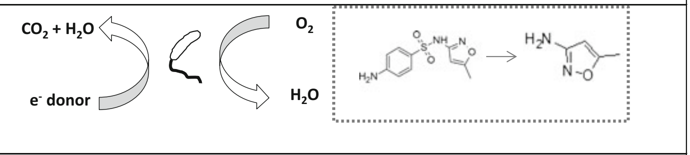
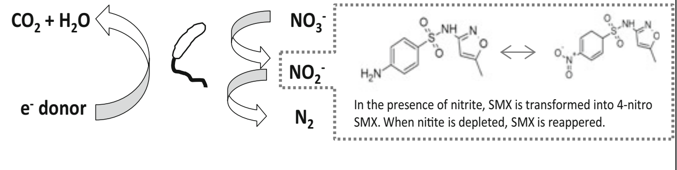
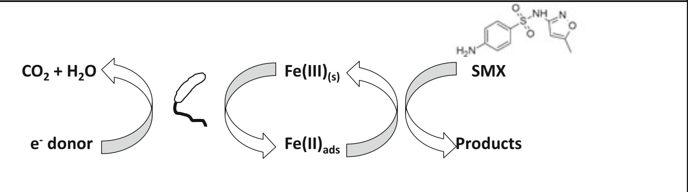
Environmental conditions	Degradation pathway
Aerobic conditions ([48])	
Denitrification (from [39])	 In the presence of nitrite, SMX is transformed into 4-nitro SMX. When nitite is depleted, SMX is reappered.
Iron reduction (from [49])	

Fig. 3 Different pathways observed in SMZ degradation associated to redox conditions

reduction conditions [49]. This transformation was attributed to abiotic reactions between SMZ and Fe (II) generated by microbial reduction of Fe (III) soil minerals. SMZ transformation was initiated by a one-electron reductive cleavage of the N–O bond in the isoxazole ring substituent (Fig. 3).

All these works indicate that different degradation pathways exist for SMZ. They also agree in specifying that SMZ is better degraded by co-metabolism, when degradation of labile organic matter coexists. Depending on environmental (redox) conditions, one or other pathway will occur. We could generalize this behavior to other compounds and conclude that understanding the interactions between the different EOCs and the redox conditions is key to understand the fate of specific compounds.

The impact of redox conditions in natural attenuation of EOCs is thus compound specific. For instance, the natural attenuation of three β-blockers (atenolol, metoprolol, and propranolol) was investigated under denitrifying conditions by Barbieri et al. [50]. Results indicated that atenolol was removed (about 65%) via abiotic and biotic processes, whereas metoprolol and propranolol were not biotransformed. Also, laboratory-scale batch studies with estrogens (17β-estradiol and 17α-ethinyl estradiol) showed that the main process removing these compounds from the system was adsorption onto soil particles [51]. Microcosm experiments were performed to investigate the biodegradation of benzotriazole and some metabolites under aerobic and anaerobic conditions, finding that each specific compound was degraded under different aerobic or anaerobic nitrate-reducing conditions [52].

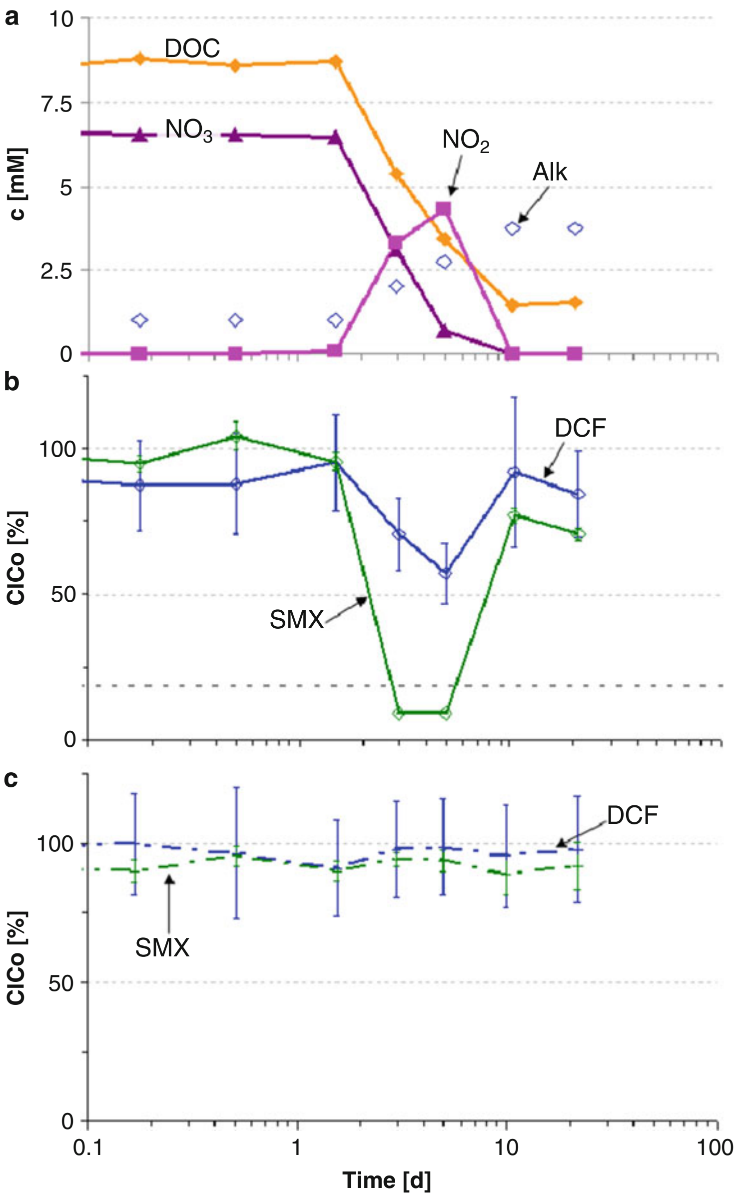


Fig. 4 Batch experiment results for degradation of pharmaceuticals under nitrate reduction conditions (adapted from [39]): (**a**) Concentration of nitrogen compounds versus time in a biotic test; (**b**) evolution with time of the average normalized concentration of diclofenac (DCF) and sulfamethoxazole (SMZ) in the biotic test; (**c**) similar results for an abiotic test

Most of the studies presented so far deal with the fate of individual compounds without considering the effects of transient geochemical variations. An exception was the work of Barbieri et al. [39] who showed in a suite of batch experiments that concentrations of the aromatic amines diclofenac and sulfamethoxazole could be temporarily and reversibly affected by denitrifying conditions in aquifers. As illustrated in Fig. 4, concentrations of these two drugs decreased when nitrite concentrations built up, because they transformed into the respective nitro-derivatives, namely, nitro-diclofenac and nitro-sulfamethoxazole. However, concentrations of the parent compounds increased again when nitrite was reduced toward nitrogen. This observation may extend to other aromatic amines and their nitro-derivatives and should be taken into account in order not to overestimate elimination efficiencies of these compounds in field and laboratory studies.

Water temperature also affects the performance of EOC removal in groundwater. In a study of riverbank filtration, denitrification rates diminished at water temperatures below 10°C [53]. This is associated to temperature strongly influencing the redox conditions in the hyporheic zone, a most relevant place for natural attenuation of EOCs, where oxic conditions prevailed with cold water, whereas a rapid transition to manganese-reducing conditions occurred with warm water [54]. The highest removal rates of micropollutants were observed under experimental conditions combining warm temperatures and oxic conditions. Comparatively, lower removal rates were obtained with cold and oxic conditions, and the lowest removals were achieved with warm water under manganese-reducing conditions.

Natural attenuation of EOCs through biotransformation processes could result in the formation of products more recalcitrant than the parent compounds. This was the case observed during the biotic transformation of the β-blocker atenolol under nitrate-reducing conditions [50]. After 87-day long batch experiments, atenolol was not mineralized and was biotransformed into atenololic acid. This transformation product was neither further degraded nor sorbed onto either minerals or biofilms. This reflects the importance of including such recalcitrant transformation products in monitoring studies [39]. Similarly, in the case of 17β-estradiol, biotic transformation under oxic conditions resulted in a lower estrogenicity of the mixture than under anoxic conditions [51]. The persistence of the different metabolites, defined by their half-lives, is also relevant. As an example, Liu et al. [52] observed that benzotriazole was more persistent in aquifers as compared to its transformation products, regardless of the degradation scenarios being aerobic or anaerobic.

Batch experiments reported so far can be complemented with soil column experiments. Onesios and Bouwer [55] report removal of a large number of pharmaceuticals and personal care products, with only a few showing a recalcitrant behavior. Such experiments allow the introduction of easily degradable primary organic substrates to evaluate the difference in degradation rates with respect to natural conditions. Chen et al. [56] examined the transport of two antibiotics, sulfamethoxazole (SMZ) and ciprofloxacin (CIP), in laboratory columns packed with quartz sand, finding that the former was more mobile than the latter under a combination of varying pH and ionic strength conditions.

5.4 Nonconservative Transport Equation

As mentioned before, there are different physical and biogeochemical processes affecting EOCs, and so they should all be included in the transport equation. Adsorption of organic compounds can be modeled using experimental expressions. Since the concentration of EOCs is usually very low, a linear adsorption isotherm can be invoked. Defining S as the concentration in adsorbed state, and invoking the soil-water partition coefficient, we can write

$$S = K_{\mathrm{oc}} f_{\mathrm{oc}} c \tag{5}$$

where f_{oc} is the fraction of organic carbon in the soil matrix. Alternatives to the linear model can be used by just substituting the concentration c in (5) by a function $g(c)$. Using the linear model, and assuming local equilibrium, it is possible to define a retardation factor

$$R = 1 + \frac{\rho_{\mathrm{b}}}{\phi} K_{\mathrm{oc}} f_{\mathrm{oc}} \tag{6}$$

so that the final transport equation can be written as

$$\phi R \frac{\partial c}{\partial t} = -\boldsymbol{q} \cdot \nabla c - \phi (D_{\mathrm{d}} \boldsymbol{I} + \boldsymbol{D}_{\mathrm{h}}) \nabla c - \lambda(c) \phi R c \tag{7}$$

where R is the retardation coefficient porosity and λ is the biodegradation constant that is a nonlinear function of concentration. In the case that sorption cannot be modeled by a linear function (5), then R is also a function of concentration.

Alternative models could be used where the M in (4) is written in terms of other products or subproducts. In these conditions, the equation can be written in terms of a system of coupled equations (coupled through reactions), where c would then be a vector of concentrations involving all the moving compounds.

5.5 Promoting Site Water Treatment

Enhanced degradation of EOCs in the aquifer can be achieved by promoting biological reactions. This can be done by in situ treatments or else by changing the quality of the water during infiltration. An example of in situ treatment was recently proposed by Ji et al. [57] who found the ferrous ion-activated decomposition of persulfate as an efficient in situ chemical oxidant for remediation of groundwater contaminated by antibiotics. Recent work shows the potential of the use of nanoparticles for in situ remediation of EOCs in the future [58].

As explained before, another possibility is a pretreatment of water before it is actually infiltrated into the aquifer, to enhance biochemical reactions that may potentially lead to co-degradation of otherwise recalcitrant substances. This concept was already proposed by Rauch-Williams et al. [59], who found that the presence of biodegradable organic carbon enhanced the decay of degradable EOCs by promoting soil biomass growth, suggesting that organic carbon may serve as a co-substrate in a co-metabolic transformation of these contaminants. These authors also found that largest removal of EOCs was observed for aerobic conditions. A practical and unique example of application of this concept is the placing of a reactive barrier at the bottom of an infiltration basin. Valhondo et al. [30] showed the effect of such a layer composed by sand from the aquifer itself, combined with vegetable compost and traces of clay and iron oxide dust (to promote sorption). The goal of the compost was to sorb neutral compounds and release dissolved organic carbon, aimed at generating a broad range of redox conditions to promote the transformation of emerging trace organic contaminants (EOCs). The reactive barrier increased removal rates of some drugs such as sulfamethoxazole and trimethoprim, but, on the contrary, there was a decrease in the rates of elimination of compounds that are easily degraded under aerobic conditions, such as ibuprofen and acetaminophen.

6 Monitoring, Sampling, and Analysis

Although great advances have been made in the detection and analysis of trace pollutants during recent decades, due to the continued development and refinement of specific techniques, a wide array of undetected contaminants of emerging environmental concern need to be identified and quantified in various environmental components and biological tissues.

It is noteworthy that most EOCs are usually detected at very low concentrations in the ng/L range, or not detected at all in groundwater, but there are a growing number of individual compound concentrations ranging in the order of μg/L.

Sampling is a very important problem to proper characterization of the chemical signature at a given point. Sampling involves the use of strict protocols to eventually obtain meaningful results that are really representative of the water present in the sampling point. The aim of using protocols is to ensure the representativeness of the samples and preserve them in the most appropriate way for the time elapsed between the sampling and laboratory analysis.

Before the campaign, all the materials and equipment must be prepared and cleaned following the protocols. In this type of sampling, all materials that are reused must be washed prior to each use in order to prevent cross contamination between wells. For instance, the pumps are purged by flowing through it at least 3–4 times the volume of water contained in the pump and pipes.

Groundwater samples must be obtained by pumping until a volume corresponding to at least three times that initially present at the sampling point (e.g., piezometer) has been extracted. Control at the site involves recording a number of parameters easy to measure in the field, those including electric conductivity, pH, temperature, Eh, and dissolved oxygen, critical to check later for major errors in the analysis. Such measurements should be taken using some insulated device to avoid contact with the air, trying to minimize temperature changes and effects of sun and wind. Instruments should be calibrated periodically (at least once a day) by means of standard solutions. Samples must be collected once field parameters have stabilized and stored in a field refrigerator until they can be taken to the laboratory. In general, best practice implies shortening the time between sampling and analysis.

Apart from sampling conditions in a specific well or piezometer, it is important to interpret the observation points in the hydrogeological context. Key issues are the aquifer levels measured, the details in piezometer construction, the local hydraulics of the point (e.g., full-screened piezometer versus multilevel piezometer), the groundwater flow paths affecting that specific point, and the understanding of the main hydrodynamics of the system. All these elements are needed to properly monitor the fate of pollutants and the potential biogeochemical reactions occurring in the aquifer. For instance, sampling two wells located in different groundwater flow paths when studying attenuation can give us a complete wrong understanding of the processes taking place in the subsurface. Furthermore, a good knowledge of the main recharge and discharge patterns will help us explaining the fate of EOCs.

Furthermore, it is also important to understand the degradation paths linked to a specific compound and sampling, when possible, its metabolites. In order to characterize the environmental conditions of the aquifer (i.e., redox conditions), other compounds, such as major and minor ions or different isotopes, can be also used to improve understanding. Complementary, different laboratory experiments under controlled conditions can be carried out to improve knowledge about what is occurring at field scale.

Analysis of EOCs in water samples is done at specialized chemical laboratories. Liquid chromatography-electrospray ionization tandem mass spectrometry and liquid chromatography-electrospray ionization high-resolution mass spectrometry are the methods most widely applied, either directly or after solid-phase extraction. Such methods allow for the simultaneous analysis of EOCs corresponding to different groups (e.g., pesticides, biocides, pharmaceuticals, industrial products, and many of their transformation products).

Limits of quantification (LOQs) are quite small and in most compounds are on the environmentally relevant concentration range of > 0.1 ng/L. One of the advantages of the increase in resolution in the analytical methods combined to the seemingly low degradation rates of some EOCs is their consideration as potentially good compounds to be used in tracer tests. Recently, Hillebrand et al. [60] used caffeine as a tracer in a karst system.

Finally, it is relevant to discuss the potential impact of continuity in the mass of compounds reaching the aquifers. It is thus advisable to properly monitor also the source in terms of water inflow (including periodicity) and the concentration of the different EOCs in the inflow.

7 Receptors and Hazards Associated to Human and Environmental Issues

Receptors include wells, surface water bodies, ecosystems, and natural springs. Wells are the most common devices to obtain water for supply purposes. The presence of EOCs may pose some hazards. In such cases, a multiple-barrier system can be used to decrease the concentration of specific substances before water is supplied (e.g., [61]).

Other receptors are surface water bodies, potentially affecting the related ecosystems. An example was presented by Peng et al. [33], analyzing the discharge to reservoirs through groundwater with input from municipal landfills in South China. In this work, groundwater discharges several pharmaceuticals posing high risk to aquatic organisms (e.g., sulfonamides and macrolides) and algae (antibacterials).

Another natural outflow of groundwater is springs. A spring is any natural situation where water flows from an aquifer to the earth's surface. Analyses of EOCs in springs have not been frequently performed. Some exceptions are the works of Reh et al. [62] in a highly karstified area, where water sampled at the springs has a fast path through the subsurface system, and that of Gibson et al. [63] intended at comparing wastewater with that arriving to a spring having passed through the aquifer after using this same wastewater for irrigation.

Water from springs may be directly intended for bottling, thus the need to assess the presence of EOCs, such as the case of Bono-Blay et al. [64] where a number of pesticides were analyzed for sanitary purposes in a number of springs in Spain.

8 Risk Assessment for Emerging Compounds and Their Metabolites

There is little toxicological information for the majority of the chemicals in use, predominantly with regard to long-term, low-level exposure. Current challenges faced by the environment are often hidden, so that long-term threats or intermittent exposure of ecosystems often lead to a decrease in biodiversity and a loss of important functions and services. In this context, a major problem lies in the identification of future hazardous or potentially dangerous chemicals. An inventory of the available information in terms of persistence, fluxes, toxicity, endocrine disruption potential of both individual compounds, and complex mixtures is lacking. Recently, the concept of biomonitoring tools (e.g., bioassays, biomarkers, microbial community analyses) was suggested [65] as a way to increase confidence in the risk assessment in EOCs. Sensors developed to determine several analytes in parallel are potentially very useful tools in environmental monitoring and screening.

Assessing the impact of water pollutants on human health relies on our ability to accurately assess: (1) the transport and possible reactions between pollutants in a hydrosystem and (2) evaluating the physiological response of humans to such contaminants and the resulting adverse effects on human health [66]. Both these fields contain uncertainties related to lack of characterization data, inadequate conceptual models, and occurrence of natural variability. A proper approach to the problem must inherently include in uncertainty of risk.

Risk analysis should be based on the combination of exposure to a given concentration, combined with toxicological studies to assess whether actual concentrations may or may not result in any hazard for a given organism (animal or vegetal). Diseases can be either caused by accumulation over the years or by acute exposure, i.e., over a very short period of time. Synergetic effects may cause the same pollutant to have different toxicity in different parts of the world. For a given hazardous substance, the potential (risk) of developing a disease increases with concentration and with time of exposure. When several hazardous substances are assumed to coexist, risk can be assumed additive.

The remaining issue is to evaluate the contaminant concentration at any particular point within an environmentally sensitive target over a period of time. Spatial and temporal variability in concentration is due to the ubiquitous heterogeneity in physical and biochemical processes, boundary conditions, and contaminant release patterns.

Uncertainty in the concentration reaching a particular receptor can be reduced by conditioning on measurements of either the dependent variables (e.g., concentrations, groundwater heads, river discharges, etc.) or the parameters themselves (through field or laboratory tests). Once it is decided which components to investigate in more detail, specific methods for optimal experimental design can be used, e.g., for optimal sampling layouts [67]. The concentrations evaluated should be the starting point of ecotoxicological analysis. This opens the door to a huge amount of combinations, since eventually it would be necessary to explore all the combinations of EOCs (and metabolites) with all organisms that could be affected. Additionally, very little is known regarding the potential synergetic effect of a cocktail of EOCs. An example of an area where almost no information exists regarding toxicological effects on aquatic organisms is illicit drugs. The few available studies on this topic were compiled by Pal et al. [12].

9 Summary

In this chapter, we take a look at the presence of emerging organic compounds (EOCs) in groundwater, with emphasis in sources of pollution, processes affecting the spatial and temporal concentration of these compounds as they move through the aquifer and interact with the soil, until they reach a receptor.

Sources are most significant in terms of the compounds that are supplied to the groundwater bodies. Depending on sources, it would be possible to classify the different families of EOCs that could be expected in aquifers. The existence of point and diffuse sources results in significant differences regarding interaction between compounds, monitor, and remediation possibilities.

Quantifying EOC input is difficult and error prone. One way to deal with this problem is to analyze the chemical signature of sources and how they combine by mixing in the subsurface. This can be done by means of statistical multivariate models, potentially incorporating simple geochemical reactions.

Transport of EOCs involves a juxtaposition of processes, involving advection, dispersion, diffusion, dilution, adsorption, complexation, biodegradation, and transformation driven by redox reactions. Degradation of EOCs depends on various environmental factors such as in situ redox potential, available nutrients, soil characteristics, seasonal temperature, and light variations. Combined with the long residence time of compounds in aquifers, it is possible to model the low concentrations of EOCs usually detected in groundwater.

Redox conditions are arguably the most important reactions affecting EOCs. An example is sulfamethoxazole, where it has been found to degrade at different rates and also involving different process related to aerobic degradation as compared to nitrate, iron, or sulfate-reducing conditions. There is a need for future studies along this direction that could combine a large number of target compounds under different redox conditions. This would allow devising efficient methods that would enhance degradation. The impact of potential metabolites should also be included. Natural attenuation of EOCs through biotransformation processes could result in the formation of products more recalcitrant than the parent compounds.

Enhanced degradation of EOCs in the aquifer can be achieved by promoting biological reactions. This can be done by in situ treatments or else by changing the quality of the water during infiltration that can be seen as a water pretreatment. Natural attenuation is also significantly driven by adsorption.

Monitoring and sampling of EOCs in aquifers are a challenging problem presently under development. Most EOCs are present at very low values, so there is a need to improve analytical methods to be able to measure concentration values on the order of ng/L with high accuracy. As with other pollutants, optimal location, number of sampling points, and frequencies are case specific. Sampling should include subsurface water bodies but also sources and receptors: wells, surface water bodies, ecosystems, and natural springs.

The work is completed with the little amount of the toxicological information existing for the majority of the chemicals in use, predominantly with regard to long-term, low-level exposure. Current challenges faced by the environment to specific compounds are often nonexistent. An inventory of the available information in terms of persistence, fluxes, toxicity, endocrine disruption potential of both individual compounds, and complex mixtures is lacking. This opens the need to follow the research in order to finally explore all the combinations of EOCs (and transformation products) with all organisms that could be affected. Additionally, very little is known regarding the potential synergetic effect of a combination of EOCs on individuals.

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Part II

Implications for the Biota and the Ecosystem

Effects of Emerging Contaminants on Biodiversity, Community Structure, and Adaptation of River Biota

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Abstract Most river ecosystems are under the joint effects of co-occurring stressors, and attributing the mechanisms by which multiple stressors interact and produce individual and combined effects can be highly complex. This chapter describes the structural and functional responses of the biological communities (biofilms and macroinvertebrates) to different chemical stressors with a special attention to the presence of emerging compounds that become more frequently in the list of co-occurring stressors in rivers. The suitability of different methods (i.e., toxic units and statistical tools) to determine toxicological risk and to establish potential causality of effects on communities has been discussed using Mediterranean basins from the SCARCE project as case study basins.

Literature review shows that emerging contaminants may produce effects at the community level, by means of changes in the survival ratio and reproduction, but also by species interactions (e.g., changes in behavior of predator and prey). A significant reduction of general biodiversity in algae and invertebrate communities and ecosystem functioning (primary production, use of organic matter, feeding

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rates) has been observed in the studied river basins apparently caused by all stressors operating together. However, the predicted toxic pressure from emerging compounds appears as a determinant factor of the biological responses. The link between biological community responses and chemical stress is complex, and other environmental variables covariate with chemical pollution (e.g., hydrology and nutrient concentrations). To get an appropriate evaluation of the risk related to emerging chemicals in natural conditions, it is necessary considering the role of other environmental perturbations and provides statistical attribution to their potential causation.

Keywords Biofilm, Endocrine disrupter compounds, Macroinvertebrate, Mediterranean basins, Multiple stressors, Pesticides, Pharmaceuticals, Scarce, Toxic units

Contents

1 Introduction

Many ecosystems are under the joint effects of co-occurring stressors. A stressor is defined as any external abiotic or biotic factor that moves a biological system out of its normal operating range [1]. A stressor may therefore cause (negative or positive) effects on individual organisms, communities, and even ecosystem functioning [2]. Water quality of rivers and freshwater systems is affected by all the secondary products that reach the watercourses. These products are a result of diverse human activities and uses within the catchment; therefore, they are systems where the presence of different stressors are common; not surprisingly rivers may be considered as the most altered ecosystems in the world [3]. Changes in land use, removal of riparian vegetation, alterations in channel morphology and connectivity, inputs of nutrients or sediments, contaminants, and pathogens are some of the human-driven stressors that affect river water quality.

In the last decades, one of the most pressing questions in ecosystem conservation has been disentangling the interactive effects of multiple stressors on ecosystems (e.g., [4, 5]). However, attributing the mechanisms by which multiple stressors interact and produce individual and combined effects can be highly complex. A growing body of literature analyzes pairs or more stressors having joint action in systems; still, most of these studies are controlled laboratory experiments of limited applicability to real ecosystems. On the other extreme, field studies that use correlational approaches and historical baselines are useful to infer multiple stressor effects, with many variables co-occurring at the same time. Still, these observations complement laboratory-based observations and are useful to address the effects of stressors that are difficult to manipulate in factorial studies. Further, these studies might help to interpret and predict when and where cumulative stressors interact and affect community responses, and which might be the potential implications on ecosystem functioning and stability. A greater understanding of the combined effects of different pressures in the river should help also to develop comprehensive and more efficient river basin management strategies.

Rivers suffer of a higher decrease in species diversity compared to other aquatic and terrestrial ecosystems [6]. The basic effect of a stressor on the most sensitive species determines the decrease of the overall community diversity, and this usually has consequences on ecosystem functioning [7]. The greater the number of species in an ecosystem, the greater would be the number of functional roles in the functioning of the whole ecosystem [8, 9]. Ecosystems which are richer in species maintain the stability of ecosystem functioning in changing environments [10] because apparently they have more alternative ways to maintain multiple types of ecosystem processes. Although organisms are fundamentally adapted to natural variations in environmental conditions (this allows them tolerating altered situations for a limited time), the combined existence of various unfavorable stressors, either additive or amplified, can cause the elimination of keystone species and consequently affect their roles in ecosystem functioning. Overall, local species loss impacts on many different ecosystem processes and on the resistance of the system to stressors. The specific response will obviously differ based on the stressors and their combination and severity, the local community composition, and the community tolerance to the type of stressor affecting the system. Tilman et al. [11] highlighted that diversity loss has a similar or greater effect than other global environmental changes (e.g., elevated CO2, drought, nutrient addition). Developing an understanding of the potential consequences of environmental change and biodiversity loss is one of the challenges in ecology [12, 13].

1.1 The Relevance of Emerging Contaminants Under Water Scarcity

As for other substances, the effects of emerging pollutants are searched mostly through laboratory standard experiments, testing short time effects, using selected species in toxicology, and usually employing concentration ranges which are not ecologically relevant. In addition, the endpoints used in standard tests are not adequate to reveal effects at the population or community levels. The difficulties of the translation from the laboratory results to the population or community level in the natural systems are obvious and require approaches designed at the community level.

The SCARCE project had as a main goal the assessment of the effects of chemical and environmental stressors on the biota (mostly algae, bacteria, and macroinvertebrates) in Mediterranean rivers, systems which are exemplary of the water scarcity effects. Precisely, this chapter describes the structural and functional responses of the biological communities to different stressors (environmental, priority, and emerging compounds) co-occurring in Mediterranean basins. This chapter is organized as follows. Section 5.2 presents a bibliographic review of the effects of emerging compounds on organisms and populations that may be able to translate into effects in the community. Section 5.3 describes the results in the studied river basins, an overview on how the different communities (biofilms and macroinvertebrates) changed related to the presence of emerging pollutants. Section 5.4 includes the quantification of the toxic stress in SCARCE basins for a risk evaluation.

2 How Emerging Compounds Can Affect Fluvial Community?

Experimental evidences of the effects of emerging pollutants on organisms' traits with significant relevance for community structure have been reported in the last 15 years. Effects have been studied in several freshwater organisms reflecting different trophic levels and taxonomic groups (Tables 1–4, from a revision of 90 studies).

Laboratory tests have shown that emerging contaminants may produce effects on the community structure, by means of changes in the survival ratio and reproduction, but also to species interactions (e.g., changes in behavior of predator and prey). The laboratory experiments have shed light on the behavior of several sorts of contaminants. In the case of nanomaterials (materials synthesized at scales of nanometers), still is lacking information of their effects [126] and the few data compiled suggested that their effective concentrations are above the mg/L (Table 1). For an accurate study of their effects on organisms, it is necessary to improve the toxicological methods [127]. In addition, information on concentrations of these new materials in the environment is still scarce and assessing their real environmental risk is difficult [15, 16].

In the case of pharmaceuticals (PhACs) and endocrine disrupter compounds (EDCs), effective concentrations are in the range of ng/L and μg/L, the range found in the environment [128]. Effects of PhACs in freshwater organisms have been widely studied, and changes on behavior, reproduction, survival, and biomass have been observed in animals, as well as effects on growth rate and photosynthesis in primary producers (Table 2). Psychiatric drugs caused changes in behavior at environmental concentrations in fish and invertebrates (Table 2), and these changes

Table 1 List of references for nanomaterials identified in our search of the Web of Science™ abstracting databases with effects potentially translated at population or community level

Compound	Organisms	Time of exposure	Ef. conc	Endpoint	Description	Reference
Metal nanoparticles	*Ceriodaphnia dubia*	48 h	3 mg/l	Survival	LC50	[14]
	Chlamydomonas reinhardtii	1 h	3.3 μM	Photosynthesis	EC50	[15, 16]
	Chlamydomonas reinhardtii	6 h	10 mg/l	Photosynthesis	Significant impairment	[17]
	Chlamydomonas reinhardtii	48 h	10 mg/l	Growth	Significant impairment	[18]
	Chlorella vulgaris	24 h	1 mg/l	Cell viability	Significant effects	[19]
	Chlorella vulgaris	72 h	38.28 mg/l	Growth	Significant impairment	[20]
	Danio rerio	48 h	1.5 mg/l	Survival	LC50	[21]
	Danio rerio	5 days	10 mg/L	Behavior	Lower motility in embryos and adults	[22]
	Danio rerio	13 weeks	0.1 mg/L	Reproduction	Significant diminution of number of zebrafish eggs	[23]
	Daphnia magna	24 h	22 mg/l	Survival	LC50	[24]
	Daphnia magna	48 h	0.003–7.75 mg/l	Survival	LC50	[25, 26]
	Dunaliella tertiolecta	24 h	1 mg/l	Cell viability	Significant effects	[19]
	Pimephales promelas	48 h	500 mg/l	Survival	LC50	[14]
	Pseudokirchneriella subcapitata	72 h	0.04–5.83 mg/l	Growth	Significant impairment	[27]
	Other invertebrates	48 h	1.65 mg/l	Reproduction	Effects on embryo development	[28]
	Other invertebrates	10 days	75 mg/l	Feeding, growth	LC50, consumption and growth inhibition	[29]

(continued)

Table 1 (continued)

Compound	Organisms	Time of exposure	Ef. conc	Endpoint	Description	Reference
Polymeric materials	*Chlamydomonas reinhardtii*	72 h	1.9–4.8 mg/l	Growth	Significant impairment	[30]
	Daphnia magna	48 h	0.2 mg/l	Reproduction	Mortality of neonates	[31]
	Daphnia magna	48 h	35 mg/l	Survival	Significant reduction	[32]
	Daphnia magna	21 days	5 mg/L	Reproduction	Significant reduction	[33]
	Daphnia magna	21 days	>5 mg/L	Survival	Significant reduction	[33]
	Dunaliella tertiolecta	96 h	0.82 mg/l	Growth	Significant impairment	[34]
	Dunaliella tertiolecta	96 h	10 mg/l	Photosynthesis	Significant inhibition	[34]
	Other invertebrates	48 h	5–5,000 μg/Kg	Survival	Significant reduction	[35]
Quantum dots	Other invertebrates	96 h	2.93 nM	Survival	Significant reduction	[36]

Table 2 List of references for pharmaceutical compounds identified in our search of the Web of Science™ abstracting databases with effects potentially translated at population or community level

Compound	Organisms	Time of exposure	Ef. conc	Endpoint	Description	Reference
Antibiotics	*Ceriodaphnia dubia*	48 h	26 to >100 mg/l	Survival	EC50	[37]
	Chironomus tentans	10 days	14.3 mg/l	Survival	EC50	[38]
	Cyclotella meneghiniana	96 h	0.016–0.026 mg/l	Growth	EC50	[37]
	Danio rerio	10 days	>16 mg/l	Reproduction	LOEC larvae mortality	[37]
	Daphnia magna	48 h	4.6 to >1,000 mg/l	Survival	EC50	[39, 40]
	Daphnia magna	21 days	5.4 to >1,000 mg/l	Reproduction	EC50	[40]
	Hyalella azteca	10 days	1.5 mg/l	Survival	EC50	[38]
	Microcystis aeruginosa	7 days	0.003–0.0034 mg/l	Growth	EC50	[41]
	Pseudokirchneriella subcapitata	72–96 h	0.53 μg/L to >4 mg/l	Growth	EC50	[37, 42, 43]
	Scenedesmus obliquus	24–96 h	2–88.39 mg/l	Growth	Significant diminution	[44, 45]
	Selenastrum capricornutum	3 days	0.133–40 mg/l	Growth	EC50	[41]
Anti-histaminic	*Gammarus fasciatus*	83 days	0.07 μg/l	Recruitment and population growth	Significant diminution	[46]
b-blocker	*Ceriodaphnia dubia*	27 days	0.250 μg/l	Reproduction	Significant diminution	[47]
	Ceriodaphnia dubia	48 h	1.51 mg/l	Survival	EC50	[37]
	Cyclotella meneghiniana	96 h	0.668 mg/l	Growth	EC50	[37]
	Danio rerio	72 h	31 mg/l	Reproduction	Developmental effects on larvae	[48]
	Danio rerio	10 days	2 mg/l	Reproduction	LOEC larvae mortality	[37]

(continued)

Table 2 (continued)

Compound	Organisms	Time of exposure	Ef. conc	Endpoint	Description	Reference
	Daphnia magna	48 h	2.75–313 mg/l	Survival	EC50	[37, 49, 50]
	Daphnia magna	9 days	0.11–6.2 mg/l	Reproduction	Significant reduction of offspring and effects on further generations	[51]
	Daphnia magna	21 days	0.05–0.4 mg/l	Reproduction	Significantly enhanced	[52]
	Daphnia magna	21 days	0.8 mg/l	Reproduction	Significant reduction	[52]
	Daphnia magna	5 generations	1.2 μg/l	Reproduction	Significant alterations in age of first reproduction, number of offspring, and body size of neonates in further generations	[53]
	Desmodesmus subspicatus	72 h	0.7–620 mg/l	Growth	EC50	[49, 50]
	Gambusia holbrooki	96 h	7.36 mg/l	Behavior	Changes in swimming	[54]
	Hyalella azteca	27 days	0.100 μg/l	Reproduction	Significant diminution	[47]
	Lemna minor	72 h	114 to >320 mg/l	Growth	EC50	[49]
	Oryzias latipes	48 h	24.3 to >100 mg/l	Survival	EC50	[47]
	Pimephales promelas	7 days	0.093 mg/l	Growth	Significant reduction	[52]
	Pimephales promelas	21 days	1 mg/l	Reproduction	Significant decrease in number of eggs	[55]
	Pseudokirchneriella subcapitata	96 h	7.4 mg/l	Growth	EC50	[37]
Lipid regulator	*Ceriodaphnia dubia*	48 h	>200 mg/l	Survival	EC50	[37, 39]
	Ceriodaphnia dubia	7 days	2.56 mg/l	Reproduction	LOEC inhibition	[39]
	Cyclotella meneghiniana	96 h	>100 mg/l	Growth	EC50	[37]
	Danio rerio	10 days	70 mg/l	Reproduction	LOEC larvae mortality	[37]

	Daphnia magna	48 h	4.9–240.4 mg/l	Survival	EC50	[37, 39, 49, 56]
	Desmodesmus subspicatus	72 h	115 mg/l	Growth	EC50	[49]
	Lemna minor	*72 h*	*12.5 mg/l*	*Growth*	*EC50*	[49]
	Other invertebrates	*48 h*	*0.74 mg/l*	*Survival*	*LOEC*	[39]
	Pseudokirchneriella subcapitata	*96 h*	*94–150 mg/l*	*Growth*	*EC50*	[37, 39]
NSAIDs	*Ceriodaphnia dubia*	48 h	22.43 mg/l	Survival	EC50	[37, 39]
	Ceriodaphnia dubia	7 days	2 mg/l	Reproduction	LOEC inhibition	[39]
	Chironomus riparius	10 days	166 μg/g	Biomass	Significant increase	[57, 58]
	Chironomus riparius	10 days	1.5 and 159 μg/g	Biomass	Significant decrease	[57, 58]
	Cyclotella meneghiniana	96 h	19.24 mg/l	Growth	EC50	[37]
	Cyprinus carpio	30 days	3 mg/l	Reproduction	Significant increase of larvae and juvenile mortality	[59]
	Danio rerio	72 h	5.3 mg/l	Reproduction	Developmental effects on larvae	[48]
	Danio rerio	6 days	0.010 mg/l	Reproduction	Significant negative effects on hatching and development	[60]
	Danio rerio	6 days	0.001 mg/L	Survival	Significant mortality of larvae	[60]
	Danio rerio	10 days	4–8 mg/l	Reproduction	LOEC larvae mortality	[37]
	Daphnia magna	48 h	18 mg/l	Reproduction	Significant reduction of offspring	[61]
	Daphnia magna	48 h	22.43–625.5 mg/l	Survival	EC50	[37, 39, 49, 62]
	Daphnia magna	48 h	59.8 mg/l	Survival	Significant negative effects	[61]
	Daphnia magna	14 days	20–40 mg/l	Reproduction	Significant inhibition of reproduction	[63]
	Daphnia magna	14 days	80 mg/l	Survival	Significant reduction	[63]

(continued)

Table 2 (continued)

Compound	Organisms	Time of exposure	Ef. conc	Endpoint	Description	Reference
	Daphnia magna	5 generations	0.36 µg/l	Reproduction	Significant alterations in age of first reproduction, number of offspring, and body size of neonates in further generations	[53]
	Desmodesmus subspicatus	72 h	72–320 mg/l	Growth	EC50	[49]
	Lemna minor	72 h	7.5–24.2 mg/l	Growth	EC50	[49]
	Oryzias latipes	9 days	1 mg/l	Behavior	Significant changes in swimming and feeding	[64]
	Oryzias latipes	12 days	0.010 mg/l	Reproduction	Significant increased number of eggs per brood	[65]
	Oryzias latipes	42 days	0.100 mg/l	Reproduction	Increase in number of eggs per day, decreasing number of spawning events	[66]
	Oryzias latipes	90 days	10 mg/l	Reproduction	Significant effects on hatching	[61]
	Pseudokirchneriella subcapitata	96 h	16.3–20 mg/l	Growth	EC50	[37, 39]
	Other invertebrates	48 h	25 mg/l	Survival	LOEC	[39]
	Other invertebrates	72 h	21 days	Growth	LOEC	[67]
	Other invertebrates	72 h	21 days	Reproduction	LOEC hatching success	[67]
	Other invertebrates	72 h	17.1 mg/l	Survival	EC50	[67]
Psychiatric drug	*Carassius auratus*	28 days	0.054 mg/l	Behavior	Negative effect on feeding behavior	[68]
	Ceriodaphnia dubia	48 h	77.7 mg/l	Survival	EC50	[37, 39]
	Ceriodaphnia dubia	7 days	0.100 mg/l	Reproduction	LOEC inhibition	[39]
	Chironomus riparius	28 days	0.16 µg/g	Emergence	EC50	[69]
	Chironomus riparius	28 days	666 mg/kg	Survival	Significant decrease	[70]
	Chironomus tentans	10 days	47.3 mg/l	Survival	EC50	[38]

	Chlorella pyrenoidosa	24 h to 5 days	10–1 mg/l	Chl-a	Significant diminution	[71]
	Chlorella pyrenoidosa	24 h to 5 days	2–5 mg/l	Growth	Significant diminution	[71]
	Chlorella vulgaris	96 h	0.76–10.2 mg/l	Growth	EC50	[72]
	Cyclotella meneghiniana	96 h	31.6 mg/l	Growth	EC50	[37]
	Danio rerio	72 h	86.5 mg/l	Reproduction	Developmental effects on larvae	[48]
	Danio rerio	10 days	25–50 mg/l	Reproduction	LOEC larvae mortality	[37, 39]
	Daphnia magna	48 h	>13.8 mg/l	Survival	EC50	[37, 39]
	Daphnia magna	21 days	0.030 mg/l	Reproduction	Length diminution in third brood	[70]
	Daphnia magna	30 days	0.036 mg/l	Reproduction	Enhanced fertility	[73]
	Daphnia magna	5 generations	0.5 μg/l	Reproduction	Significant alterations in age of first reproduction, number of offspring, and body size of neonates in further generations	[53]
	Desmodesmus subspicatus	72 h	74 mg/l	Growth	EC50	[49]
	Dreissena polymorpha	6 days	0.2 μg/l	Reproduction	Diminution in number of spermatozoa and oocytes	[74]
	Gammarus pulex	1.5 h	10–100 ng/l	Behavior	Significant reduction of movement	[75]
	Hyalella azteca	10 days	9.9 mg/l	Survival	EC50	[38]
	Hyalella azteca	30 days	0.100 mg/l	Reproduction	Significant effects on development of offspring	[70]
	Hybrid striped bass (*Morone saxatilis* × *M. chrysops*)	6 days	0.023 mg/l	Behavior	Impairment of predation	[76]
	Lemna minor	72 h	25.5 mg/l	Growth	EC50	[49]
	Oryzias latipes	9 days	6.15 mg/l	Behavior	Significant changes in swimming and feeding	[64]

(continued)

Table 2 (continued)

Compound	Organisms	Time of exposure	Ef. conc	Endpoint	Description	Reference
	Pseudokirchneriella subcapitata	96 h	0.012 to >100 mg/l	Growth	EC50	[37, 39, 72]
	Scenedesmus acutus	96 h	0.091–3.62 mg/l	Growth	EC50	[72]
	Scenedesmus obliquus	24 h to 5 days	2–1 mg/l	Chl-a	Significant diminution	[71]
	Scenedesmus obliquus	24 h to 5 days	2–5 mg/l	Growth	Significant diminution	[71]
	Scenedesmus quadricauda	96 h	0.212–3.56 mg/l	Growth	EC50	[72]
	Other invertebrates	9 days	2 μg/l	Behavior	Significant stimulation	[77]
	Other invertebrates	9 days	2 μg/l	Development	Significant impairment	[77]
	Other invertebrates	28 days	0.94 and 2.34 mg/kg	Reproduction	Significantly enhanced	[78]
	Other invertebrates	28 days	0.08–0.250 mg/l	Reproduction	Impairment or enhancement	[69, 78]
	Other invertebrates	42 days	0.069 mg/l	Reproduction	Significant diminution	[70]
	Other invertebrates	48 h	0.754 mg/l	Survival	LOEC	[39]

Table 3 List of references for industrial organic compounds identified in our search of the Web of Science™ abstracting databases with effects potentially translated at population or community level

Compound	Organisms	Time of exposure	Ef. conc	Endpoint	Description	Reference
4NP	*Daphnia magna*	48 h	0.144–0.155 mg/l	Survival	LC50	[79, 80]
	Daphnia magna	96 h	0.236 mg/l	Reproduction	Deformities in embryos	[80]
	Daphnia magna	14 days	0.1 mg/l	Reproduction	Reduction of offspring	[81]
	Daphnia magna	21 days	0.46 μM	Reproduction	Abnormal embryos	[82]
	Daphnia magna	21 days	0.08 mg/l	Reproduction	Diminution offspring in first generation	[83]
	Daphnia magna	24 h	0.15–0.038 mg/l	Survival	LC50	[83, 84]
Alkylphenols	*Ceriodaphnia dubia*	48 h	0.21–2.54 mg/l	Survival	LC50	[84, 85]
	Daphnia magna	24 h	0.34–4.22 mg/l	Survival	LC50	[84, 86]
	Oryzias latipes	100 days	0.3 mg/l	Sexual development	Abnormal development of gonads	[87]
	Other fishes	60 days	0.3 mg/l	Reproduction	Histological alteration of gonads	[88]
BPA	*Chironomus riparius*	24 h	6 mg/l	Survival	Significant diminution	[89]
	Chironomus riparius	24 h	6 mg/l	Survival	Significant diminution	[90]
	Chironomus riparius	24 h	6 mg/l	Survival	LC50	[89]
	Chironomus riparius	240 h	11.51 mg/l	Mortality	LC50	[91]
	Chironomus riparius	2 generations	0.078 μg/l	Emergence	Significant effects on second generation	[91]
	Chironomus riparius	Life cycle	0.001 μg/l	Emergence	Delay	[92, 93]
	Chironomus tentans	48 h	5.5 mg/l	Survival	Significant effects	[94]
	Chlorella pyrenoidosa	24 h	50 mg/l	Growth rate	Significant diminution	[71]
	Chlorella pyrenoidosa	24 h	25 mg/l	Chl a content	Significant diminution	[71]

(continued)

Table 3 (continued)

Compound	Organisms	Time of exposure	Ef. conc	Endpoint	Description	Reference
	Danio rerio	24 h	16.75 mg/l	Survival	LC50	[95]
	Danio rerio	72 h	13.81 mg/l	Reproduction	EC50 hatching	[95]
	Danio rerio	120 h	0.01 μM	Behavior	Impairment of learning skills	[96]
	Danio rerio	Full life cycle	6,140 nM	Reproduction	EC50	[91]
	Daphnia magna	24 h	8.57 mg/l	Survival	LC50	[83]
	Gammarus pulex	240 h	1.49 mg/l	Mortality	LC50	[91]
	Gammarus pulex	24 h	8.4 mg/l	Reproduction	Significant effects on sexual behavior	[91]
	Gammarus pulex	24 h	8.4 mg/l	Reproduction	Changes in behavior	[92, 93]
	Hyalella azteca	42 days	1.5 mg/l	Reproduction	Significant reduction offspring/female	[94]
	Marisa cornuarietis	48 h	6.84 mg/l	Survival	Significant effects	[94]
	Marisa cornuarietis	5 months	0.014 μg/l	Reproduction	EC10 number of embryos	[97]
	Oryzias latipes	3 weeks	0.837 mg/l	Reproduction	Histological alteration of gonads	[98]
	Potamopyrgus antipodarum	4 weeks	0.010 mg/l	Reproduction	Increased number of embryos	[99, 100]
	Potamopyrgus antipodarum	8 weeks	1 μg/l	Reproduction	NOEC number of embryos	[97]
	Scenedesmus obliquus	24 h	10 mg/l	Growth rate	Significant diminution	[71]
	Scenedesmus obliquus	24 h	5 mg/l	Chl a content	Significant diminution	[71]
	Other fishes	20 days	0.6 μg/l	Reproduction	Sperm damage	[101]
	Other fishes	Spawning period	1.75 μg/l	Reproduction	Sperm damage	[102]
	Other invertebrates	24 h	20 mg/l	Survival	LC50	[103, 104]

Flame retardant	*Chlorella pyrenoidosa*	24 h	3.13 mg/l	Survival	EC50	[105]
	Chlorella pyrenoidosa	168 h	2.67 mg/l	Chl a content	Significant diminution	[106]
	Danio rerio	96 h	0.1 mg/l	Reproduction	Embryo malformation	[107]
	Danio rerio	30 days	3 μM	Behavior	Lethargy and disorientation	[108]
	Danio rerio	30 days	0.047 μM	Reproduction	Diminution in egg production	[108]
	Danio rerio	Life cycle	0.3 μg/l	Reproduction	Effects on sex ratio and fecundity diminution	[109]
	Daphnia magna	48 h	0.045 mg/l	Reproduction	Significant reduction offspring	[110]
	Daphnia magna	48 h	0.044 μM	Survival	EC50	[111]
	Daphnia magna	Life cycle	50 μM	Reproduction	Significant diminution on organisms fed with algae exposed to 50 uM	[111]
	Lumbriculus variegatus	28 days	2,500 μg/g	Survival	Significant diminution	[112]
	Pseudokirchneriella subcapitata	72 h	7.3 μM	Growth rate	Significant inhibition	[103, 104]
	Other algae	72 h	<1.8–7.3 μM	Growth rate	Significant inhibition	[103, 104]
	Other invertebrates	24 h	2.3–8.3 mg/l	Survival	LC50	[103, 104]
NP	*Ceriodaphnia dubia*	48 h	0.22–0.092 mg/l	Survival	LC50	[84, 85]
	Chironomus riparius	10 days	315–673 μg/g	Survival	LC50	[113]
	Chironomus riparius	28 days	381–385 μg/g	Emergence	EC50	[114]
	Chironomus riparius	48 h	3.94 μM	Survival	LC50	[115]
	Chlorella vulgaris	24 h	1 mg/l	Growth rate	Significant diminution	[116]
	Chlorella vulgaris	24 h	2 mg/l	Photosynthesis	Significant diminution chlorophyll fluorescence	[117]
	Chlorella vulgaris	96 h	2 mg/l	Chl a content	Significant diminution	[117]
	Danio rerio	60 days	0.010 mg/l	Reproduction	Significant effects on sex-ratio	[118]

(continued)

Table 3 (continued)

Compound	Organisms	Time of exposure	Ef. conc	Endpoint	Description	Reference
	Hyalella azteca	96 h	0.021 mg/l	Survival	LC50	[79]
	Lumbriculus variegatus	48 h	6.24 µM	Survival	LC50	[115]
	Lumbriculus variegatus	96 h	0.342 mg/l	Survival	LC50	[79]
	Microcystis aeruginosa	15 min	0.25 mg/l	Photosynthesis	LOEC	[119]
	Oryzias latipes	60 days	0.011 mg/l	Reproduction	Histological alteration of gonads	[120]
	Oryzias latipes	100 days	0.03 mg/l	Sexual development	Abnormal development of gonads	[87]
	Pimephales promelas	96 h	0.128 mg/l	Survival	LC50	[79]
	Pimephales promelas	28 days	0.15 µg/l	Reproduction	Changes in behavior	[121]
	Pimephales promelas	64 days	0.005 mg/l	Reproduction	Changes in behavior	[122]
	Pseudokirchneriella subcapitata	15 min	0.5 mg/l	Photosynthesis	LOEC	[119]
	Scenedesmus obliquus	10 h	0.25 mg/l	Photosynthesis	Significant diminution chlorophyll fluorescence	[123]
	Scenedesmus obliquus	5 days	4 mg/l	Growth rate	Significant diminution	[123]
	Selenastrum capricornutum	24 h	1 mg/l	Photosynthesis	Significant diminution chlorophyll fluorescence	[117]
	Selenastrum capricornutum	96 h	1.4 mg/l	LOEC	Diminution growth rate	[79]
	Selenastrum capricornutum	96 h	0.25 mg/l	Chl a content	Significant diminution	[117]
	Tubifex tubifex	28 days	417–422 µg/g	Reproduction	EC50 number of juveniles	[114]

	Tubifex tubifex	28 days	427–429 μg/g	Reproduction	EC50 number of cocoons	[114]
	Other fishes	96 h	0.209–0.221 mg/l	Survival	LC50	[79]
	Other fishes	96 h	172 μM	Survival	LC50	[124]
	Other invertebrates	50 days	5 mg/l	Survival	Significant diminution	[125]
	Other invertebrates	96 h	0.596–0.774 mg/l	Survival	LC50	[79]
OP	*Ceriodaphnia dubia*	48 h	0.07 mg/l	Survival	LC50	[84]
	Danio rerio	Full life cycle	136 nM	Reproduction	EC50	[91]
	Marisa cornuarietis	5 months	<1 μg/l	Reproduction	NOEC number of embryos	[97]
	Microcystis aeruginosa	15 min	0.1 mg/l	Photosynthesis	LOEC	[119]
	Oryzias latipes	60 days	0.048 mg/l	Reproduction	Histological alteration of gonads	[120]
	Potamopyrgus antipodarum	8 weeks	1 μg/l	Reproduction	NOEC number of embryos	[97]
	Pseudokirchneriella subcapitata	15 min	0.1 mg/l	Photosynthesis	LOEC	[119]
	Scenedesmus obliquus	5 days	4 mg/l	Growth rate	Significant diminution	[123]
	Scenedesmus obliquus	10 h	1 mg/l	Photosynthesis	Significant diminution chlorophyll fluorescence	[123]
TBT	*Lymnaea stagnalis*	3 weeks	0.320 μg/l	Reproduction	Significant effects on hatching	[91]
	Lymnaea stagnalis	3 weeks	0.032 μg/l	Reproduction	Deformities in embryos	[91]
	Marisa cornuarietis	5 months	0.098 μg/l	Reproduction	EC50 number of embryos	[97]
	Potamopyrgus antipodarum	8 weeks	0.115 μg/l	Reproduction	EC50 number of embryos	[97]
TPT	*Marisa cornuarietis*	5 months	0.039 μg/l	Reproduction	EC50 number of embryos	[97]
	Potamopyrgus antipodarum	8 weeks	0.068 μg/l	Reproduction	EC50 number of embryos	[97]

Table 4 List of references for personal care and household products identified in our search of the Web of Science™ abstracting databases with effects potentially translated at population or community level

Compound	Organisms	Time of exposure	Ef. Conc	Endpoint	Description	Reference
Bacteriostatic and bactericide	*Chironomus tentans*	10 days	0.4 mg/l	Survival	EC50	[38]
	Daphnia magna	30 days	0.010 mg/l	Sex ratio	Significant change	[73]
	Oryzias latipes	9 days	0.17 mg/l	Behavior	Significant changes in swimming and feeding	[64]
	Other invertebrates	10 days	0.2 mg/l	Survival	EC50	[38]
Parabens	*Daphnia magna*	48 h	2.1–61 mg/L	Mortality	EC50	[133–135]
	Oryzias latipes	96 h	0.7–63 mg/L	Mortality	EC50	[135]
	Pimephales promelas	48 h	3.3–>160 mg/L	Mortality	EC50	[133]
	Pseudokirchneriella subcapitata	96 h	1.2–80 mg/L	Growth	EC50	[135]
UV filters	*Chironomus riparius*	72 h	4.10–6 M	Reproduction	Hatching time	[136]
	Daphnia magna	21 days	0.08–0.5 mg/l	Reproduction	LOEC length of adult	[99, 100]
	Daphnia magna	48 h	0.3–50 mg/l	Mortality	EC50	[99, 100, 137]
	Oryzias latipes	21–28 days	0.02–0.6 mg/L	Reproduction	Egg production and hatching	[138, 139]
	Pimephales promelas	19–21 days	0.003–5 mg/L	Reproduction	Egg production, gonadal development	[140, 141]
	Other invertebrates	28–56 days	50 mg/Kg	Mortality		[142, 143]
	Other invertebrates	56 days	0.2–50 mg/kg	Reproduction	Number of embryos, malformations	[142, 143]

impaired feeding rate or jeopardize the defense ability against predators. Antibiotics showed more toxicity to prokaryotic and eukaryotic microbial community [41].

Animals exposed to EDCs of industrial origin since long term showed dramatic effects on reproduction (deformities on embryos, reduction of offspring) due to alterations on gonads (e.g., feminization of male individuals), gamete development, or affecting viability of neonates or embryos (Table 3). Alkylphenols, Bisphenol A (BPA), and flame retardants are the most studied substances because of their notable presence in the environment [129]. Effects on behavior due to neurological alterations after exposure have been described in fish and crustaceans (Table 3). In the case of freshwater mollusks, long-term exposures caused effects on reproduction but were not always deleterious. Sieratowicz et al. [99, 100], Duft et al. [97], and Oehlmann et al. [130] found increased production of embryos after chronic exposure to BPA and OP; this enhancement of reproduction could be problematic to the own population (excessive use of energy in reproduction) and to other species (increased interspecific competition). Inhibitions of growth rate, chlorophyll content, and photosynthetic efficiency have also been reported in algae even at short-term exposures.

Personal care and household products can also cause toxicity responses in freshwater organisms at environmental concentrations [131] (Table 4). UV filters cause impairment in reproduction in fish and invertebrates due to effects on gonadal development or impairment of offspring. Triclosan could produce behavioral changes in fish [64] besides lethal and reproductive effects on aquatic organisms [132] (Table 4).

In addition, effects at molecular or cellular level and at concentrations lower than those that cause effects at population level have been described for most of the emerging compounds. However, the expression of these molecular effects at higher organization levels is not always mandatory [144] although they could be early warnings of future damage.

3 Multiple Stressors and Effects on Community Biodiversity in Iberian Rivers

Four river basins in Spain, the Llobregat, Ebro, Júcar, and Guadalquivir, were sampled for chemical and biological data. Within the SCARCE project, more than 200 chemicals were measured in water, sediment, and biota of these rivers. The compounds measured included different types of organic chemicals as pesticides, pharmaceuticals, personal care products, industrial organic compounds, perfluoroalkyl compounds, and heavy metals. Field sampling was combined with field and laboratory experiments to assess the effects of changes in water quality, including priority and emerging compounds, on the biological communities (algae and bacteria, and macroinvertebrates). In this section, we include structural descriptors – species composition or the biomass distribution – to analyze these effects.

3.1 Biofilm Community

Biofilms are consortia of algae, bacteria, and fungi, assembled in a very efficient manner [145, 146]. Biofilms are the first receptors of environmental and chemical stress because of their position in the interface between water and sediments [147]. As such, they colonize all solid river substrata (sediments, rocks, stones), where bacteria are the dominant component especially in light-limited systems, and algae also make a relevant part when light is available. Stream biofilms drive a large part of the ecosystem metabolism and biogeochemical cycling, and this role is shared by the primary producers (algae) and the heterotrophs (bacteria, fungi). As such, biofilms may retain, uptake, and transform nutrients [148], but are also able to accumulate and transfer other organic and inorganic elements such as heavy metals and organic microcontaminants.

Biofilms are sensitive to a wide array of chemical substances, from nutrients to pesticides, and other organic and inorganic pollutants [149, 150]. These elements may reflect their influence on their structure (i.e., their biomass and the taxonomic composition of the biological community), since pollutants may affect the growth rate of particular species as well as the equilibrium between the competing species within the community. Pollutants are also able to affect biofilm functioning, both the primary producers (photosynthesis) and the transformation capacity of dissolved materials (enzymatic activities) [146, 151].

SCARCE has aimed to understand the relevance of co-occurring stressors (including hydrological, land uses, and chemical pollutants) in the structure and functioning of epilithic biofilms. This was made in four Mediterranean basins of the project under two different hydrological periods during the late summer (2010–2011). The two samplings (high waters vs basal waters respectively) allowed different ways of pollutants arrival. During high waters, this is mostly associated to runoff; during basal waters, the preferential arrival is mostly associated to local inputs. These two main ways of entering freshwaters is being received by biofilms which are also dynamic in their growth and composition, associated to different successional phases and thickness. Altogether, the specificity in the response of biofilms to pollutants could be different in each situation [152, 153]. Biofilm response to multiple stressors was explored by means of a variety of endpoints and data treated by means of multivariate methods to uncover joint patterns of ordination. Chlorophyll-a (Chl-a) concentration was used as a surrogate of algal biomass in the biofilm. The effective quantum yield (Yeff), maximum photosynthetic capacity (Ymax), and non-photochemical quenching (NPQ) were used as photosynthetic estimations of the health and proficiency of the primary producers functioning [146]. Yeff and Ymax were respectively used as indicators of photosynthetic efficiency and maximal photosynthetic capacity of the algal community. NPQ was used as an indication of the algal capacity to dissipate the light excess during stress conditions [154]. Alkaline phosphatase activity (APA) provides an estimation of the ability of the biofilm in transforming organic phosphorus into inorganic compounds [155]. The diatom community composition was used as representative of the algal community as a whole [146]. Only the most remarkable results are commented below.

The Chl-a and the bacterial abundance usually increased downstream, but longitudinal changes were sometimes erratic (e.g., Júcar and Ebro rivers). The two were higher during basal flow; biofilm was thicker and more complex during this period. The photosynthetic efficiency was also higher during the low flow period in most of the sites and more erratic during the wet period. The NPQ decreased from upstream to downstream in 2010, but it was heterogeneous in 2011 except in the Guadalquivir. The alkaline phosphatase activity did not show clear patterns according to flow, but values decreased in the more polluted sites.

The ordination of the diatom assemblages was performed by means of a principal component analysis. The first axis of the analysis showed that diatom taxa were arranged in a gradient from the Júcar, Ebro, and Guadalquivir headwaters (right side of this axis) to the taxa common in the downstream sites of the Ebro, Llobregat, and Guadalquivir (left side of the axis). The arrangement was opposing taxa such as *Achnanthidium pyrenaicum*, *Achnanthidium minutissimum*, and *Encyonopsis microcephala* – which are considered to be sensitive to pollution [156] against taxa tolerant to pollution, such as *Eolimna subminuscula, Navicula recens, Nitzschia insconspicua, Nitzschia palea*, and *Nitzschia frustulum*.

We used a multivariate analysis of redundancy (RDA), which is a direct ordination analysis, in order to predict the biofilm responses by means of the patterns of environmental and pollution variables. The data of all four basins were used (Fig. 1). The first axis of the RDA (44.3% of variance) defined a gradient of general pollution, where sites on the right side presented high concentrations of industrial organic compounds (IOCs), herbicides, and pharmaceutical products (PhACs), as well as of dissolved organic carbon (DOC) and dissolved inorganic nitrogen (DIN). The first component evidenced that sites more correlated with pollution were the downstream sites of the Llobregat, Ebro, and Guadalquivir rivers. The Júcar sites and the upstream sites of the Ebro and Guadalquivir were those with lower pollutant loads. Higher levels of pollution co-occurred with tolerant diatom taxa and with biofilms of higher photosynthetic capacity (related with Ymax). The second axis of the general RDA explained a low proportion (3.6%) of the variance and was related with the sampling periods. Sites during low flow were associated with low concentrations of DOC and the presence of herbicides; the biofilms in that period were characterized by high algal biomass and high APA activities. Sites during high flow were characterized by high DOC concentrations and PhACs in waters, while biofilms showed higher bacterial abundance and high rates of NPQ.

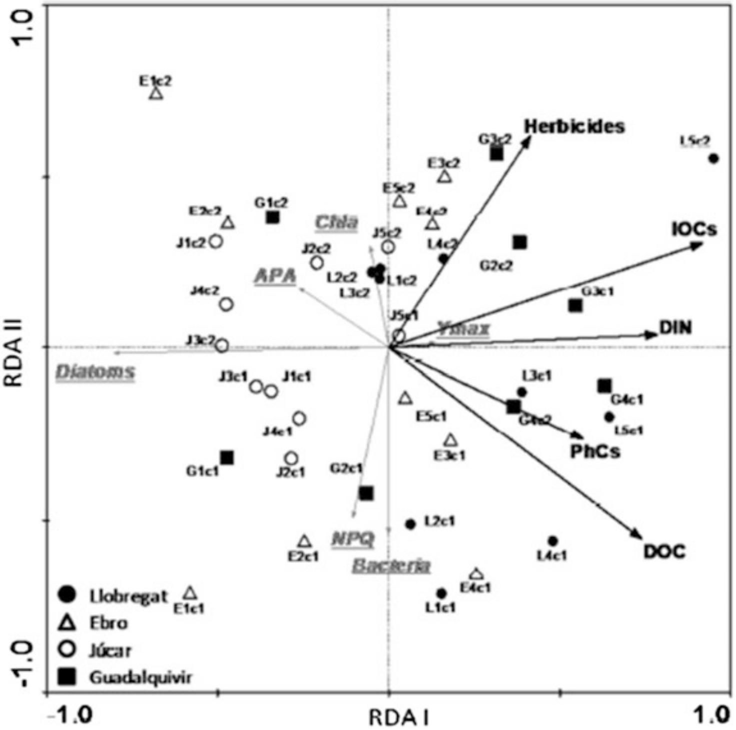

Fig. 1 Redundancy analysis plot of biofilm variables for the Ebro (E), Júcar (J), Guadalquivir (G), and Llobregat (L) River sites and the two sampling campaigns (c1, c2). Significant environmental and chemical variables are represented by *arrows*

The variance partitioning analysis of this RDA outlined that pollutants accounted for the 13.3% and the physical and chemical variables for the 10.3% of the total explained variance. The shared variance between these two groups of variables was 26.7%. This leaves a large fraction of the variance unexplained, probably accounted for other variables such as variations in water flow or land use occupation. IOCs, herbicides, and PhACs were the most significant pollutant variables and DOC and DIN the most significant physical and chemical variables. The NPQ and bacterial density were the biofilm metrics most associated with herbicides, and diatoms community structure was the one associated with DIN concentration and other organic pollutants (Fig. 1).

The interaction between the organic pollutants and nutrients was reflected in the shared percentage of variance accounted by the RDA and is pointed out as the main determining factor of biofilm responses in polluted rivers.

Our analysis revealed that during high water flow periods, biofilms are subject to high shear stress, drag, and abrasion, and show low biomass and a community composition made up of early successional species [157]. Biofilms are thin and with a few, well-adapted species of active metabolism. Accordingly, Chl-a concentrations

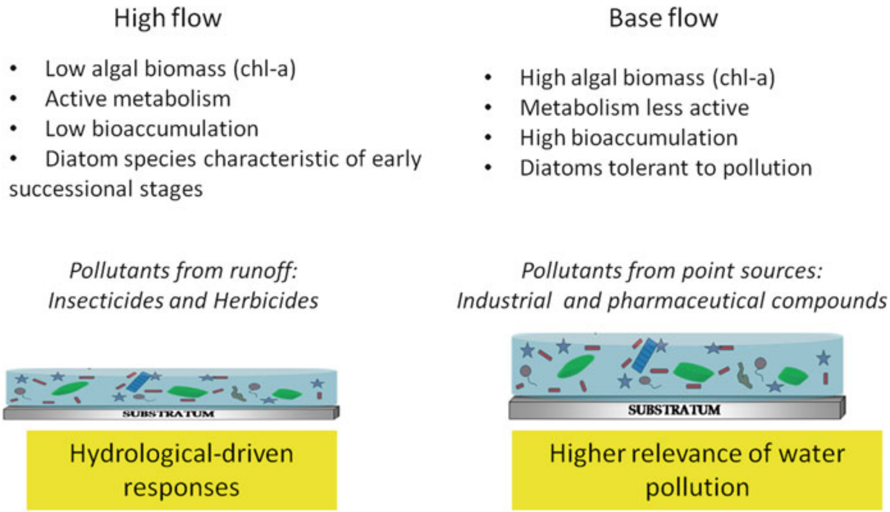

Fig. 2 Summary of potential cause-effects on river biofilms, when submitted to pollutants, nutrients in excess and hydrological stress

were substantially low, and the diatom taxa were characteristic of early successional stages [158]. The higher bacterial abundance in these biofilms could be a result of the lower bacterial-algal competition in the early stages of biofilm development and to the presence of higher DOC concentrations in water [159]. However, during the low water flow period, thicker biofilms were able to develop with high Chl-a and greater thickness. These biofilms included diatom species tolerant to pollution [158], characteristic of later successional stages (Fig. 2).

PhACs, IOCs, and herbicides, together with DOC and DIN, were the most prominent compounds affecting biofilms. The potential relevance of pollutants on biofilms also differed between periods. High concentrations of organic pollutants were coincident with the occurrence of high DIN and DOC concentrations, especially during low flow. The local conditions prevailed; industrial organic compounds increased in a downstream direction, hence reflecting the dominance of point source of pollution sources during base flow [160]. During high flow, these interactions became less tight and the physical forces prevail in the biofilm responses.

Specific biofilm responses in each of the basins were indeed related to the specific land uses and to the water management in each basin. Water flow in the Llobregat and Guadalquivir quickly responded to heavy rains, but the Ebro and Júcar were reacting less apparently. River regulation contributed to these differences: the Ebro and Júcar are heavily regulated rivers, and water is intensively used for irrigation, while the effect of regulation is lower in the Guadalquivir and Llobregat.

3.2 Macroinvertebrate Community

The macroinvertebrate community contains hundreds of species living on the surface of different materials scattered on the river bed (e.g., bedrock, stones, sediments), including organic materials such as wood, leaves, or aquatic vegetation. The invertebrates represent a fundamental link in the river food web between organic matter resources and fish and are actors of essential ecosystem processes, such as the decomposition of organic matter. Their relative long life cycles makes them receptors of regular or intermittent perturbations occurring in the river.

The macroinvertebrate species composition in the sediment of the studied basins (see De Castro-Català et al. [161] for methodological details) was checked against the variation of chemical and environmental variables by applying multivariate statistical techniques (canonical correspondence analysis, CCA, and redundancy analysis, RDA), in similar way that the one applied to biofilms. Results obtained for the first two axes (20.7% of the species data variation) showed how the invertebrate species were distributed in the river sites together with the variables related to water quality. The species on the left side of the first axis (Chironomidae, *Orthocladius* spp., *Thienemannimyia sp.*; Oligochaeta, *Limnodrilus hoffmeisteri*, *Branchiura sowerbyi*, and also Ephemeroptera, *Caenis luctuosa;* and Heteroptera*: Corixa sp., Micronecta sp.*) were the most abundant in sites characterized by a general organic pollution, represented with higher nutrient concentrations (DIN: NO3 and NH4) but also by higher concentrations of compounds of the families of PhACcs and EDCs. The highest concentrations of pharmaceuticals in all of basins corresponded to the family of analgesics and anti-inflammatories, and for the EDCs to the family of azoles, along with alkylphenols, and phosphate flame retardants. These conditions were especially observed for the Llobregat and the Guadalquivir rivers. These species have been described as opportunistic and tolerant to organic pollution. This group of species contrasted with that on the right side of the same axis, represented by species more sensitive to pollution (Hydracarina; Coleoptera, Elmidae; Chironomidae, Tanypodinae, *Nanocladius sp.*) and occurring in samples at the upstream sites. In general, upstream and downstream sites were also characterized by higher and lower richness and diversity respectively (Table 5). In the Júcar, the same pattern regarding diversity changes downstream was observed, but the pollution was mainly driven by pesticides (mainly fungicides). Agriculture represents in that basin the 36% of the land uses, with frequent supply of pesticides, including some components forbidden in the European regulation [162]. A general species impoverishment related to the presence of priority and emerging compounds and general organic pollution has been observed along the main Júcar river but also on different river sections of the Llobregat (middle-downstream part) and the upper-middle section in the Ebro and the Guadalquivir. Previous studies have already reported the influence of PhACs [163, 164] in the benthic invertebrate communities of the Llobregat, and it is now clear that this influence could be extended to other rivers, especially those characterized with urban pollution (Fig. 3).

Table 5 Maximum values of species richness (S) and Shannon diversity (H′) measured in the studied sites

	Max S	Max H′
EBR1	24	3.29
EBR2	10	2.72
EBR3	9	2.97
EBR4	4	1.69
EBR5	5	1.92
GUA1	28	3.74
GUA2	6	1.98
GUA3	11	3.30
GUA4	8	2.48
JUC1	30	3.73
JUC2	8	1.40
JUC4	14	3.24
JUC5	14	2.58
JUC6	2	1.50
JUC7	5	1.88
LLO3	19	3.76
LLO4	7	2.47
LLO5	8	2.40
LLO6	4	1.49
LLO7	4	1.81

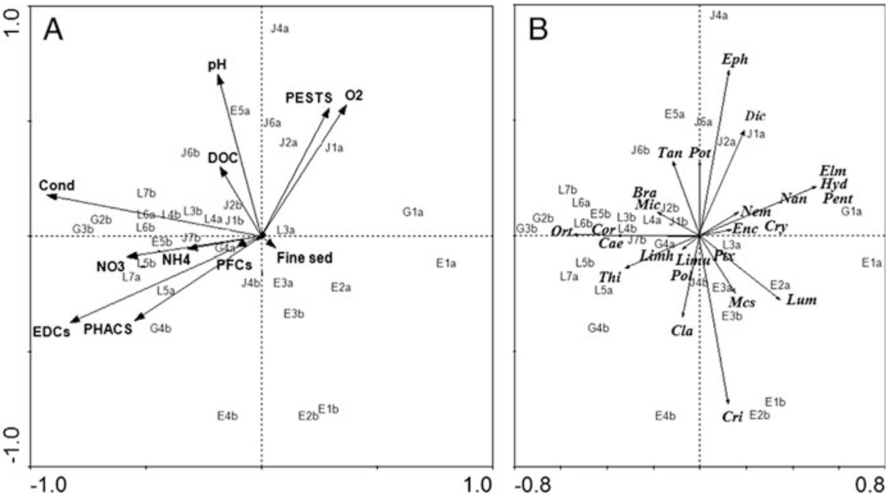

Fig. 3 Ordination plot based on the canonical correspondence analysis (CCA) of the invertebrate density for the Ebro (E), Júcar (J), Guadalquivir (G), and Llobregat (L) River sites and the two sampling campaigns (*a*, *b*). Significant environmental and chemical variables are represented by *arrows*. (**A**) Ordination of sampling sites. (**B**) Ordination of invertebrate species

Pollution favored the dominance of tolerant species versus sensitive species, and this pattern was also expressed in changes in biomass distribution. Ranking the species in terms of biomass (Fig. 4a) shows the greater dominance of one or two most abundant species. This is more evident in sites with lower diversity (EBR4, LLO7, JUC 6), where Chironomidae and Oligochaeta accumulated most of the biomass. Figure 4 (b) shows the average k-dominance curves for the cumulative macroinvertebrate biomass at each site. For the Llobregat, Ebro, and Júcar, the curves revealed differences in the relative species biomass distribution at downstream sites in Llobregat (LLO 6 and 7) and Júcar (JUC6 and 7) and in almost all sites in the Ebro. The most elevated curves have the lowest diversity and highest biomass, suggesting some kind of pollution perturbation at these sites [165].

Another evidence of the effects of pollution in the decline of macroinvertebrate diversity was the in situ response of the postexposure feeding rates in *Daphnia magna* [161]. Water fleas exposed to natural conditions in the river for 24 h were fed with *Chlorella vulgaris* and their grazing efficiency determined [166]. The feeding rates decreased downstream in the Ebro and Llobregat rivers and fluctuated in the Júcar sites. The sites with lower feeding rates (below 50%) were EBR5, LLO7, and JUC2, all of which were characterized by high pollution and low species diversity. Rivetti et al. [167] included the analyses of the cholinesterase activity in *Daphnia magna* in the SCARCE basins and observed that it covaried similarly with the biodiversity of macroinvertebrate communities. Previous studies performed in the Llobregat River already determined a good correlation between the ecological quality of invertebrate communities, *D. magna* feeding rates, and cholinesterase activity [168], suggesting that these two measures are good markers of ecotoxicological quality.

The oxidative stress measuring the activity of the enzyme catalase was evaluated in the previously described sites by the response of the macroinvertebrate *Hydropsyche exocellata* [161], present in most sites of the studied basins. It was found again a tight relationship with PhACs and EDCs. In particular, pharmaceuticals showed a quadratic response, meaning that catalase activity increased until reaching a plateau when concentrations arrived to μg/L level and then decreased. The response to EDCs was linear and positive.

In addition, the reproduction performance of the snail *Physella acuta* was experimentally studied in these sites and contrasted with the EDC concentrations and with the estrogenic activity in the water, expressed as estradiol equivalents (EEQs) [169]. Significant positive correlations were found between the number of eggs per clutch and the sum of all of the EDCs; the highest risk (higher EEQs) was predominantly associated with estrone and alkylphenols. The hormone usually showed low concentrations but its high disruption factor made it in a risky endocrine disrupter; however, the industrial compounds though showed lower disruption potential were present with higher concentrations in the water.

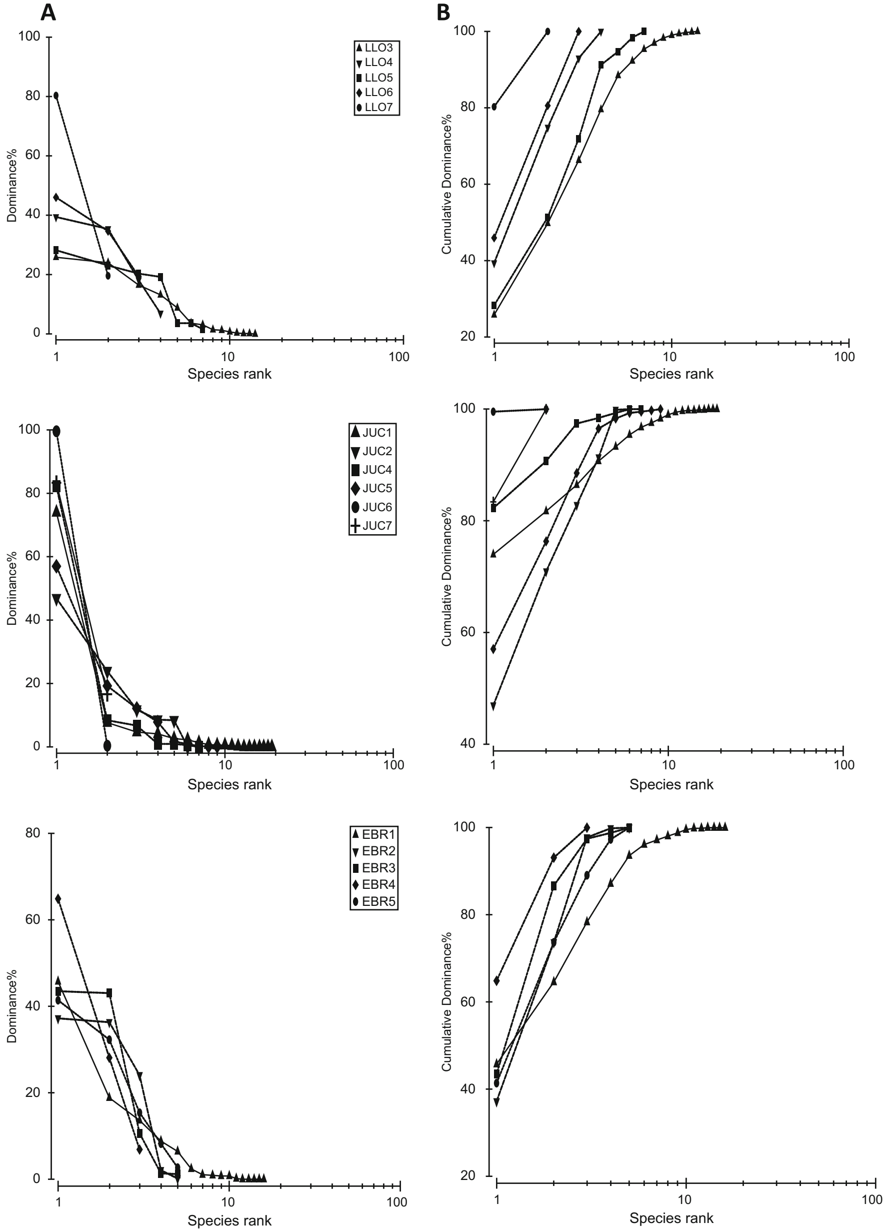

Fig. 4 (**a**) Average-ranked invertebrate biomass curves for each sampling site at Llobregat (L), Júcar (J), and Guadalquivir (G); (**b**) k-dominance (biomass) curves for the same sites and rivers

4 Evaluation of the Toxic Stress in the SCARCE Basins

In environments affected by multiple stressors, it is a challenge to establish the link between the individual stressor and the responses of the biological communities. The complexity of anthropogenic stressors, their mutual interactions, and the variability of natural characteristics at different sites along the river basins make this a very difficult task. However, identification of the most important stressors is crucial for the prevention of further deterioration of aquatic ecosystems [170].

Chemical pollution is one of the most important stressors for aquatic communities [170], and many anthropogenic chemicals are known to cause adverse effects if they enter natural environments. Toxic unit (TU) [171] is the approach commonly used to assess the ecotoxicological risk of individual chemicals, based on their measured concentration in the field and on the laboratory derived acute toxicity threshold for standard test species (Eq. 1). Usually, algae, invertebrates, and fish acute toxicity values (EC50 and LC50, 50% effective or lethal concentration) are used for the TUs calculations, since they are considered as representatives of three trophic levels:

$$\mathrm{TU}_{\mathrm{i}\ (\mathrm{algae},\ \mathit{Daphnia}\ \mathrm{sp.},\ \mathrm{fish})} = \frac{C_{\mathrm{i}}}{\mathrm{EC50}_{\mathrm{i}}} \tag{1}$$

where TU_i is the toxic unit of a compound i, c_i measured concentration (μg/l) of the compound in the water phase, and $\mathrm{EC50}_{\mathrm{i}}$ or $\mathrm{LC50}_{\mathrm{i}}$ (μg/l) effective or lethal concentration for 50% of individuals when exposed to the substance concerned. Since aquatic species in the aquatic environment are exposed not only to single compounds but also to complex mixtures of different chemicals, several mixture toxicity approaches can be used. The most commonly concentration addition (CA) and independent action (IA) are used [171, 172]. Concentration addition assumes a similar mode of action for the components of the mixture. Independent action assumes that the components of mixture affect the same endpoint but on different subsystems [172]. Owing to its calculation simplicity, CA is commonly recommended as a first-tier approach to quantify mixture toxicity [172]. However, neither of these two concepts takes into account the interactions between chemicals in the mixture.

In order to evaluate the ecotoxicological status of the SCARCE rivers, toxic units were calculated for all chemical compounds detected at each sampling sites, and site-specific toxic risk was assessed by using concentration addition (CA) concept, i.e., summing the TUs of individual compounds detected at site (Eq. 2). The site-specific risk was expressed for convenience as logarithm of mixture toxicity for organics and metals separately (Eq. 2):

$$\mathrm{TU}_{\mathrm{site}} = \log \sum_{i=1}^{n} \mathrm{TU}_i \tag{2}$$

where $\mathrm{TU_i}$ is the toxic unit of each of individual compound *i* at the site under study. In order to have a better picture on how the toxic load may affect the aquatic communities, toxic units and hence $\mathrm{TU_{site}}$ are calculated for three trophic levels, i.e., fish, daphnids, and algae, using the corresponding EC50 or LC50.

The site-specific risk ($\mathrm{TU_{site}}$) of those in the four Iberian rivers showed remarkable sensitivity differences with respect to the three test species used. Algae were the most sensitive to heavy metals, whereas invertebrates were responsive to organic chemicals (Fig. 5). Fish were the least sensitive compared to the other test species. Assuming that a situation of high risk occurs if the site-aggregated risk exceeds -1 (log units) ($\mathrm{TU_{site}} \geq -1$), we found that risk was high at all sampling sites, both due to organic chemicals and metals. Risk associated to heavy metal toxicity was higher in the Llobregat and Guadalquivir basins, there was a risk of organic toxicity in Jucar, and risk was almost equally allocated between organic compounds and metals in the Ebro basin (Fig. 5). Prioritization based on toxic units showed that compounds to which the majority of risk could be attributed were pesticides, in particular chlorpyrifos and chlorfenvinphos. Still, the risk from other groups of compounds was not negligible [173, 174]. Such highly toxic risk situation can be likely interpreted as responsible of causing changes in the so affected biological communities.

To capture the relation between the presence of toxic chemicals in the environment and changes in biological communities, several biological indexes can be used. The so-called Species at Risk Index (SPEAR) is a stressor-specific developed index based on aquatic macroinvertebrate traits that is relatively free of other types of influences [175, 176]. It has been successfully applied to detect the decrease of sensitive species with the increase of pesticides risk [177, 178] and organic chemicals risk [175]. In the four rivers studied, organic toxic stress respect Dahnia ($\mathrm{TU_{site}}$ [Daphnia]) and $\mathrm{SPEAR_{organic}}$ for macroinvertebrates were correlated (Spearman rang correlation), thus revealing significant negative correlation, i.e., the decrease of sensitive species at sites with high organic toxic risk (M. Kuzmanovic et al., unpublished data).

5 General Discussion

In this chapter, we have presented the suitability of different methods (toxic units and statistical relationships) to predict the expected effects of chemical water quality and, more specifically, the toxic stress of emerging compounds on the basis of measured concentrations of chemicals in surface waters. The rivers incorporated to SCARCE are under multiple stress conditions; therefore, the link between biological community responses and chemical stress is complex and hardly elucidated. Other environmental variables covariate with chemical pollution

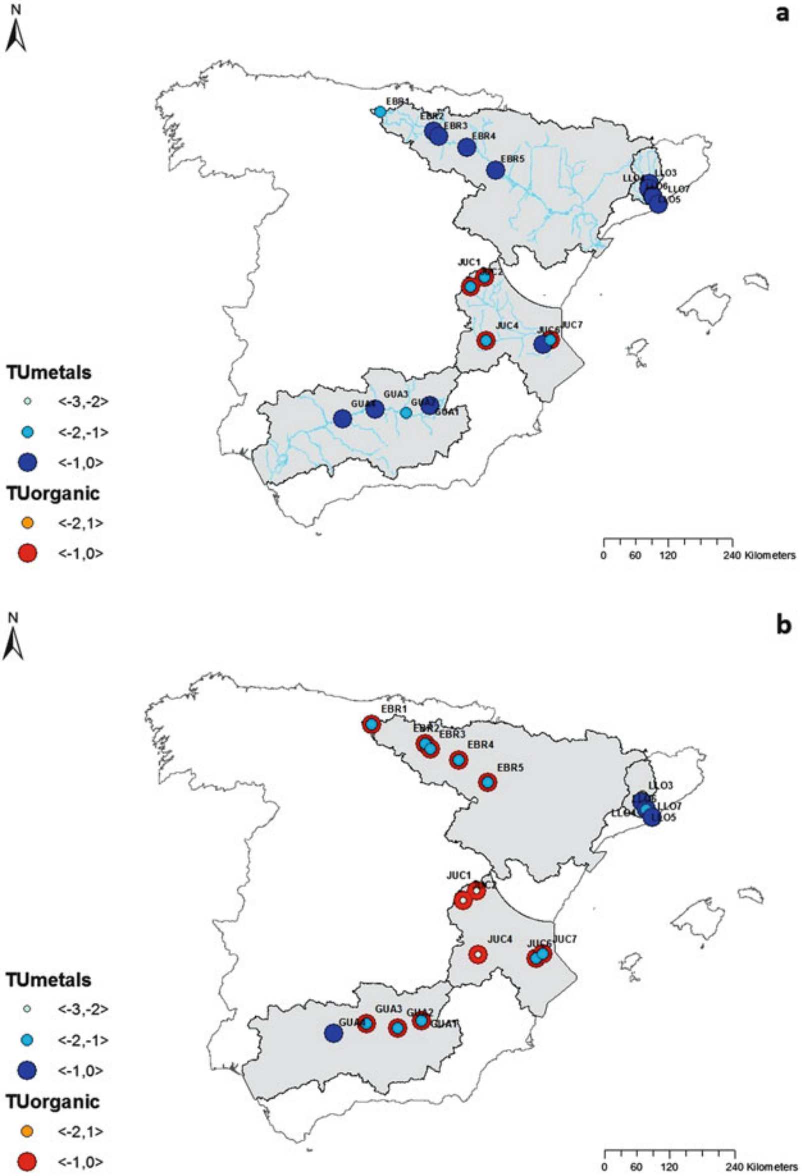

Fig. 5 Site-specific toxic risk expressed as toxic units sum of all individual compounds detected at sampling site for metals (*blue*) and organic compounds (*red*): (**a**) for algae, (**b**) *Daphnia* sp., and (**c**) fish

(e.g., hydrology and nutrient concentrations) and need to be incorporated to provide statistical attribution to their potential causation. We concluded that there is a significant reduction of general biodiversity and ecosystem functioning in the

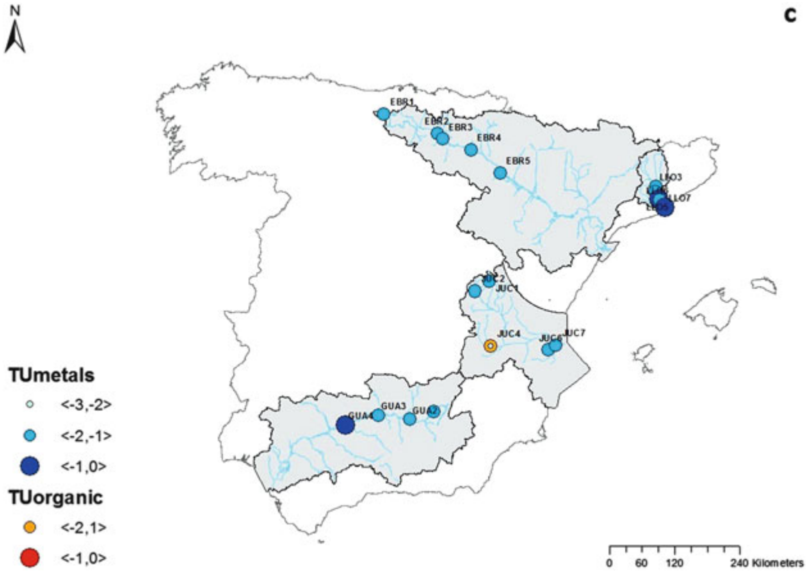

Fig. 5 (continued)

studied systems apparently caused by all stressors operating together. However, the predicted toxic pressure from emerging compounds appears as a determinant factor of the biological responses.

The existing literature shows that different families of emerging compounds have effects on growth, reproduction, or behavior in organisms, potentially translated into effects of ecological importance at population or community level (e.g., individual fitness, population persistence, community resilience). Reduction of primary production or biofilm biomass by herbicides effects can compromise the resource availability for consumers, reducing their feeding rates and affecting their growth and development [179]. In fish, spawning and courtship behaviors are extremely important for reproductive success in many organisms, and these behaviors may be disrupted by exposure to various contaminants (e.g., [180]). Because many emerging compounds enter continuously the environment, organisms may be exposed for extended parts of their life cycle and even for multiple generations to this stress. However, most experiments performed so far have been conducted in laboratory conditions, a few in mesocosms, and very few directly in the field. Moreover, the studies were heavily weighted toward single species in comparison with community-level experiments. This makes hard to draw any general conclusions regarding the ecological level impacts of these compounds.

Studies working with the whole community [161, 149] can only demonstrate statistical causal relationships between the presence of some emerging compounds and changes in species composition and abundance. However, these works provide information about those stressors that can be drivers of ecological change in the river and used as an inventory of stressors in the risk assessment approach [1].

From the TU analysis, it can be concluded that risk was high in almost all the sites analyzed. Prioritization work based on these TU showed that some pesticides (mainly chlorpyrifos and chlorfenvinphos) were the most responsible of organic risk in the studied basins. Pesticides for invertebrate community and herbicides for biofilm were also a significant factor in the multivariant analyses (statistical attribution to likely causation). Higher presence of pesticides was related with lower invertebrate richness and diversity. Herbicides also were associated with lower diatom richness and lower functionality (lower values of NPQ and APA) in biofilms. So the toxic risk associated to pesticides can be likely interpreted as responsible of changes in biological communities. In a recent study [181], chlorpyrifos was one of the compounds with more toxicological risk in the sediments of the SCARCE basins. Heavy metals also represent a high toxicity risk in SCARCE basins [57, 58], but they were not included in the multivariate analyses done with communities because metals were measured as total concentration and not those bioavailable [182]. The risk of PhACs and other organic compounds different from pesticides were not relevant using the TU calculation; however, invertebrate and biofilm communities showed structural changes related to their presence.

Our results not only showed causation with chemical compounds but also indicated that hydrology determines the concentration and type of compounds more common in the river. Low discharges favored point source pollution (IOCs and PhACs) and higher nutrient and organic matter amount. Biofilms responded clearly to these conditions, increasing their biomass and reducing their metabolism; besides the algae composition was dominated by tolerant species. Eutrophication and general organic loading were also relevant variables shaping macroinvertebrate community. The common pattern was a reduction of species richness, diversity, and changes from sensitive to tolerant species. These results confirm that toxicant influences on ecological systems should not be considered without considering other stressors at the same time [183, 184]. Organisms living under unfavorable conditions appeared to be more vulnerable to additional chemical stress [185]. In this sense, the described evidences to provide causality of emerging toxic stress on biological communities in the studied river basins have also to consider the effects of other environmental stressors operating together. The consequences of not considering this issue could evaluate in non-appropriate manner the risk related to emerging chemicals in natural conditions being a crucial basis to identify potential key stressors and priority sites for managers.

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Bioaccumulation of Emerging Contaminants in Aquatic Biota: Patterns of Pharmaceuticals in Mediterranean River Networks

Sara Rodríguez-Mozaz, Belinda Huerta, and Damià Barceló

Abstract The presence of emerging pollutants such as pharmaceuticals (PhACs) in the aquatic environment is well known, but their bioaccumulation in aquatic organisms has only been studied in the last years. It is, therefore, an issue of emerging concern, particularly in Mediterranean regions, where the WWTP effluents may represent a high percentage of some streams' flow (especially under water scarcity), and thus bioaccumulation of pollutants can be favored. In this chapter, studies worldwide about bioaccumulation of PhACs in aquatic organisms are first reviewed, and the species and contaminants of concern are tentatively identified. Results from a recent study performed in different river basins in Spain are presented in this work. In our study, biota samples from different trophic levels – periphyton (basal resource), macroinvertebrates (*Hydropsyche* sp. (collector-filterer insect larvae) and *Dreissena polymorpha* (zebra mussel)), and different species of fish – were collected from four rivers basins in Spain: Ebro, Llobregat, Júcar, and Guadalquivir. A total of 20 sites were sampled for the analysis of up to 61 multi-class pharmaceuticals. Several aspects are addressed and discussed in this chapter such as trends regarding the most prevalent compounds in different river basins and trophic levels and in relation to their physicochemical characteristics. Levels found in aquatic biota were in general in the low ng/g range but occasionally reached values up to 229.8 ng/g of sertraline in periphyton, 93.7 of diclofenac in macroinvertebrates, or 17.9 ng/g of carbamazepine in fish liver. Diclofenac was

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overall the most prevalent compound; it was the only compound detected in all types of biological matrix analyzed. Llobregat was identified as the most polluted river according to the number and levels of pharmaceuticals in biota. Finally, periphyton is proposed as a potential sentinel of exposure to pharmaceuticals in the aquatic environment: they are able to retain higher number of compounds and at higher concentrations than other organisms.

Keywords Aquatic organisms, Bioaccumulation, Emerging pollutants, Pharmaceuticals

Contents

1 Introduction

1.1 Pharmaceuticals in the Environment

Occurrence, fate, and adverse effects of emerging contaminants in the aquatic environment have become a matter of concern in the last two decades [1, 2]. Pharmaceuticals are considered emerging pollutants since their presence in the environment has lately been reported in the scientific literature and information about the risk they pose for the environment and human health is not fully comprehended yet. Pharmaceuticals have been detected in wastewater treatment plant (WWTP) effluents, surface water, seawater, and groundwater all worldwide [3, 4]. They are widely used for both human and veterinary treatment, and therefore input of their input in the environment occurs through direct discharges of WWTP effluents, direct deposition on land of WWTP sludge, and feces from livestock (all containing pharmaceutical residues) or through runoff from the fields to receiving waters [2]. Agriculture and aquaculture also consume large quantities of specific pharmaceutical compounds, such as antibiotics and hormones for growth promotion, therapeutic treatment, or disease prevention [5]. Even though at relatively low levels, pharmaceuticals can be found in the natural ecosystems: their generally swift degradation rates are exceeded by their introduction rates in the aquatic environment, and thus, they are considered pseudo-persistent contaminants [6].

1.2 Bioaccumulation in Biota: Environmental Relevance

As a consequence of the constant input of emerging contaminants into freshwater ecosystems, a variety of aquatic organisms are continuously exposed to these pollutants: from bacterial communities to fish, as well as aquatic plants and insect larvae. One of the main concerns related to the presence of pharmaceutical compounds in the environment is that they are biologically active – they are specifically designed to target specific metabolic and molecular pathways in humans and animals – resulting in unexpected effects in nontarget aquatic organisms [7, 8]. The impact of the contaminants can be evaluated by assessing selected toxicological effects or by measuring the bioaccumulation of the target compounds in the organisms. Bioaccumulation has indeed been proposed as a hazard criterion itself, since some effects may only be recognized in a later phase of life, are multi-generation effects, or manifest only in higher members of a food web [9]. 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 [10]. However, lipid partitioning processes do not sufficiently explain the bioaccumulation observed in the case of pharmaceuticals, but also other tissue components may be playing a role. Therefore, uptake and depuration kinetics of ionized compounds (as it is the case of many pharmaceuticals) by organisms needs to be considered when assessing bioaccumulation potential of these compounds. Other biological factors that can influence the uptake of pollutants by organism considered are their life cycle, reproductive strategy, feeding type, and habitat. To this respect, fish has been commonly studied in monitoring studies because of its ubiquity and its significant role as a carrier of energy from lower to higher trophic levels, being potentially more susceptible to pollutants' bioaccumulation. Other organisms, such as invertebrates and algae, also play a critical role in the natural flow of energy and nutrients in aquatic systems. They also possess the capacity to integrate rapid environmental variations, which validates their position as indicator species of environmental change, so they should be taken into consideration for the monitoring of PhACs.

1.3 Pharmaceuticals in Aquatic Biota

First studies of PhACs in freshwater organisms were published 10 years ago, when different authors reported the presence of psychoactive drugs (low ng/g range), in fish collected in an effluent-dominated stream in Texas, USA [11–13], and in fish collected in a contaminated harbor in Canada [14]. Most of the studies published in freshwater environment so far have been carried out in the USA. Up to seven studies with bioaccumulation data from aquatic ecosystems in the USA are reported in Table 1, followed by Spain (3) and China (2). Other reports have also been published in countries such as Canada, France, Italy, the UK, the Czech Republic, Sweden, Finland, as well as Japan (see Table 1), confirming the increasing interest in this subject in the last few years.

As indicated before, most of the literature available about contamination of aquatic biota by PhACs has focused in fish (14 studies out of the 20 studies reported in Table 1), whereas studies addressing the presence of these compounds in other organisms are rather scarce. Only a few studies have been investigated in aquatic invertebrates (5), mollusks, (1) and periphyton (2), as reported in Table 1. In line with it, literature about contamination of marine aquatic biota by pharmaceuticals has also increased in the past years. Up to 24 studies have been published since 2007, as it has been reported in a recent review [32]. Marine fishes have also been the most researched organism (14 papers) followed by mollusk (12), crustacean (2), and macroalgae (1). In this case, not only wild but also farmed organisms were considered, probably due to human health implications derived of the consumption of seafood.

Many of the studies about bioaccumulation in fish so far have focused on muscle tissue or whole-body concentrations, in particular in the early studies. However, the concentration range of some compounds was higher in fish liver and brain tissues than in muscle or whole body according to various studies [11, 16, 28, 31, 33, 34]. This suggests that bioaccumulation studies should consider the most probable target organ, in particular in the case of pharmaceuticals, where well-studied pharmacokinetics and pharmacodynamics may provide useful information that is not available for other contaminants. Accumulation of PhACs in plasma in particular has acquired a significant importance lately as it can be seen in Table 1, where up to five studies have reported values in plasma from wild fish [17, 19, 31, 35, 36]. This recent interest is based on the read-across hypothesis that stipulates that fish plasma concentration of a particular pharmaceutical is in the same range as the human therapeutic plasma concentration; the compound is assumed to have potential adverse effects in aquatic organisms [37]. This assumption is based on the premise that the main targets (receptors and enzymes) of the drug are similar between humans and fish [38, 39].

Concerning the therapeutic classes of pharmaceuticals, psychoactive drugs are the compounds investigated the utmost. They have been thoroughly studied in freshwater environment and have been detected in up to 18 studies (out of 20 reported in Table 1) in contrast to other compounds such as analgesics/anti-

Table 1 Occurrence of pharmaceuticals in freshwater biota

Location	References	Matrix	PSY	ABs	AHs	β-B	A/A	CCB	LR	AP	T/A
US	[11]	Fish muscle	0.1–1.1								
		Fish liver	0.8–12								
		Fish brain	1.6–15.6								
US	[13]	Fish muscle	0.8–5		0.7–1.3	0.1–0.3					
US	[15]	Fish brain	nd – 6								
US	[16]	Fish muscle	nd – 12		0.1–0.3						
		Fish liver	nd – 600		nd – 8.6	nd – 0.9			11–34		
US	[17]	Fish plasma	<4[a]								
US	[18]	Mollusc homogenate	nd – 79								
US	[19]	Fish plasma	nd – 14[a]		<25						
		Invertebrates	nd – 140		<7		nd – 33				
		Periphyton	1.3		18		<4				
Canada	[14]	Fishhomogenate	nd – 1.1								
Spain	[20]	Fish homogenate	0.6–0.8[b]			BLQ–4[b]	4–9[b]			BLQ	2.6[b]
		Fish liver	18[b]								
Spain	[21]	Invertebrates					nd – 183[b]				
Spain	[22]	Periphyton	nd – 44[b]				nd – 103[b]	nd – 21[b]	nd – 10[b]		
France	[23]	Invertebrates	<2								
Sweden	[24]	Fish muscle	0.39–13								
Finland	[25]	Fish bile					nd – 148[a]				
Germany	[26]	Fish muscle	1.6–3.3		0.04–0.1						
UK	[27]	Invertebrates	<9[b]	<5[b]			BLQ				
CzechRep	[28]	Invertebrates	<5	nd – 85							
China	[29]	Fish muscle	<0.2	<8			<1.2				
		Fish liver	<2	<40			<9				
		Fish brain	<1	<28			<4				
		Fish gills	<0.7	<25			<1.5				

(continued)

Table 1 (continued)

Location	References	Matrix	PSY	ABs	AHs	β-B	A/A	CCB	LR	AP	T/A
China	[30]	Phytoplancton	nd	nd – 8.03^b		nd – 15.1^b	nd – 21.6^b				
		Zooplancton	nd – 2.12^b	nd – 24.2^b		nd – 22.8^b	nd – 23.6^b				
		Zoobenthos	nd – 10.6^b	nd – 132^b		nd – 45.1^b	nd – 129^b				
		Shrimps	nd – 50.6^b	nd – 73.8^b		nd – 1.35^b	nd – 73.8^b				
		Fish	nd – 10.7^b	nd – 61.2^b		nd – 5.27^b	nd – 81.5^b				
Japan	[31]	Fish plasma	$<1^a$				$<23^a$				
		Fish liver	<15				nd				
		Fish brain	<10				nd				

Results expressed in ng/g wet weight, unless otherwise indicated. Data compiled from studies published in literature between 2005 and 2015
PSY psycoactive drugs, *ABs* antibiotics, *AHs* antihistamines, β-*B* beta-blockers, *A/A* analgesics/antiinflammatories, *CCB* calcium channel blockers, *LR* lipid regulators, *AP* Antiplatelet agent, *T/A* to treat asthma, *nd* not detected

[a] ng/mL for bile and plasma analysis

[b] Results expressed in dry weight

inflammatories (detected in 8 monitoring studies) or antihistamines, beta-blockers, and antibiotics (each reported in biota samples of 4 different studies). Antibiotics, in contrast, are the drugs investigated in marine biota due mainly to fish farming activities in coastal areas, which are one of the major sources of antibiotic contamination in the marine environment [32].

2 Case Study

Most of the studies reporting the presence of pharmaceuticals in aquatic biota have been carried out using one type of organism although a few have considered up to three species from different trophic levels both in marine ecosystem [40–42] and in freshwater ecosystem [19, 30, 43]. This type of studies allows to address issues such as fate and distribution of pollutants in the environment and their potential biomagnification in trophic chain as well as to identify best sentinel species to monitor the ecological status of the aquatic environment impacted by anthropogenic pollution. As an example of an integrated approach to monitor the presence of contaminants in different aquatic ecosystems, a comprehensive study was carried out in the frame of the SCARCE project, where, besides water and sediment samples, biota samples were collected in up to 4 different rivers from a Mediterranean area. As a consequence of global change, water scarcity and its quality preservation are becoming important issues in those Mediterranean regions [44]. Therefore, this pioneer study about the distribution and bioaccumulation of emerging pollutants such as pharmaceuticals in aquatic biota can provide valuable insights about the impact of these pollutants in ecosystems heavily impacted by anthropogenic and climate pressures.

2.1 Sampling Campaign

Four Iberian river basins (Fig. 1) were studied as representatives of Mediterranean rivers, which are characterized by low summer flow and large floods in autumn and winter seasons [45]. The four rivers (Ebro, Llobregat, Júcar, and Guadalquivir) are subjected to anthropogenic pressures due to urban, industrial, and agricultural activities, either scattered or combined along the basin. Detailed description of the study area and river basin characteristics can be found elsewhere [44]. Sampling was performed in 5 points in each of the four rivers (to achieve a pollution gradient, from the upper points to the lower points of the rivers). A total of 20 sites were sampled during the summer of 2010 for the analysis of up to 61 multi-class pharmaceuticals in aquatic organisms from different trophic levels: periphyton, insect larvae, mussels, and fish. Samples of epilithic periphyton were obtained from cobbles near the stream shore by gently scraping the rock surface. These samples were kept in a dark cool box and transported to the laboratory, where they were

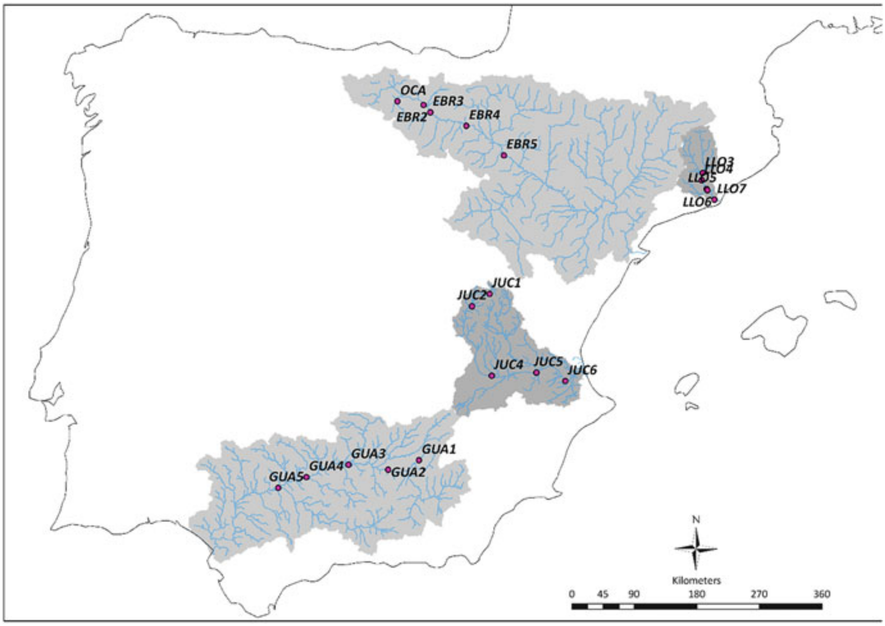

Fig. 1 Sampling points located in the four river basins under study in the Iberian peninsula: *EBR* Ebro, *LLO* Llobregat, *JUC* Júcar, *GUA* Guadalquivir

freeze-dried and kept frozen (−20°C) until analysis. Specimens of *Hydropsyche* at their last larval instars were placed in 50 mL Falcon® tubes, filled with river water, and transported in a dark cool box. At the lab, they were placed in filtered river water during 24 h to allow for gut clearance. A composite sample was then freeze-dried, homogenized with a mortar, and kept at −20°C until analysis. Fish individuals belonging to 11 different species (*Barbus graellsii*, *Micropterus salmoides*, *Cyprinus carpio*, *Salmo trutta*, *Silurus glanis*, *Anguilla anguilla*, *Lepomis gibbosus*, *Gobio gobio*, *Luciobarbus sclateri*, *Alburnus alburnus*, *and Pseudochondrostoma willkommii*) as well as zebra mussels (*Dreissena polymorpha*) were collected in the sampling points. Whole individuals of each class ($n = 3$) were homogenized using a meat grinder, composited into a single sample, freeze-dried, and kept at −20°C until analysis.

2.2 Sample Analysis

For the analysis of pharmaceuticals in biological matrices, appropriate extraction and purification methodologies need to be applied in order to overcome the complexity of the sample matrix, especially rich in undesirable components that could interfere with the analysis [10]. Specific sample preparation techniques were thus applied to each of the biological matrix considered. Periphyton samples were analyzed using the methodology developed by Huerta et al. [22]: 200 mg of freeze-dried periphyton samples were extracted with citric buffer (pH 4)/acetonitrile by pressurized liquid extraction (PLE), and extracts were purified using solid-phase extraction (SPE) with Oasis HLB cartridges. For the analysis of macroinvertebrates, another analytical methodology was applied in which macroinvertebrate samples (100 mg) were extracted with methanol using a sonication probe and further purified in Ostro™ 96-well plate for phospholipid and protein removal [21]. Fish and mussel samples were extracted according to a third methodology [20], where approximately 1 and 0.5 g, respectively, of freeze-dried sample were extracted by pressurized liquid extraction (PLE). Extracts were passed through a preparative column in a gel permeation chromatography system for lipid removal. Purified extracts obtained in each of the three methodologies applied were evaporated to dryness and dissolved in methanol/water (1:9, v/v) for the analysis of PhACs. An appropriate volume of standard mixture containing labeled compounds was added in all biota extracts as internal standard and further analyzed by ultra-rapid liquid chromatography tandem mass spectrometry (UPLC/MS/MS).

3 Results

3.1 Occurrence of Pharmaceuticals in Biota Samples

A total of 18 PhACs and 4 metabolites (out of 61 compounds analyzed) were detected in the different biota samples analyzed: either fish, invertebrates, or periphyton. Table 2 shows the compounds detected in each of the matrix considered. A different accumulation behavior between different trophic levels was observed. Nine compounds (carazolol, carbamazepine, citalopram, clopidogrel, diclofenac, propranolol, salbutamol, and venlafaxine) from five therapeutic families were determined at concentrations higher than method detection limits (MDLs) in fish samples (Table 2). Pharmaceutical levels were lower than 10 ng/g in fish homogenates, which are consistent with the published scientific literature on the topic (see Table 1). No difference in the accumulation among fish species, according to their feeding habits, age, or niche, was observed. The highest level was found in trout liver, where the concentration of carbamazepine was 17.9 ng/g. The most ubiquitous and recurring compound was diclofenac, detected in 9% of the total number of samples. In the case of invertebrates, only four compounds (diclofenac, levamisole, carbamazepine, and citalopram) were found in the mussels collected in Ebro River (zebra mussel is an invasive species in Ebro River, and it is not present in the other rivers). Levels found for these pharmaceuticals (low ng/g range) were lower than those found in fish, except in the case of levamisole (up to 6.6 ng/g). Particularly remarkable is the case of macroinvertebrates, where diclofenac was the sole pharmaceutical detected. Diclofenac was indeed found in

Table 2 Occurrence values for compounds detected in the organisms analyzed as well as water and sediments

	Water (ng/L)	Sediment (ng/g)	Fish (ng/g)	Mussels (ng/g)	Invertebrates (ng/g)	Periphyton (ng/g)	Log K_{ow}	Log D_{ow}
Analgesics/anti-inflammatories								
Diclofenac	9.7–62.1	BLQ – 3.1	nd – 15.4	nd – 0.2	10.2–93.7	nd – 35.1	4	1
Antihelminthics								
Levamisole	0.2–23.8	BLQ – 0.3	nd	nd – 6.6	nd	–	2	2
Antihistaminic								
Desloratadine	0.3–1.9	0.2–2.7	–	–	–	nd – 8.6	4	3
Lipid regulator								
Gemfibrozil	0.3–302.7	0.2–3.8	–	–	nd	nd – 21.5	4	1
Diuretic								
Hydrochlorothiazide	9.2–288.7	BLQ	nd	nd	nd	nd – 36.9	−1	−1
Psychoactive drugs								
Carbamazepine	0.1–28.4	BLQ – 0.4	nd – 17.9[a]	nd – 0.2	nd	nd	3	3
OH-CBZ	–	–	nd	nd	nd	nd – 1.4	2	2
Epoxycarbamazepine	–	–	nd	nd	nd	nd – 1.5	2	2
Acridone	0.2–4.9	0.6–26.3	–	–	nd	nd – 23	4	4
Citalopram	0.3–11.3	1.1–4.6	nd – 1.4	nd – 0.9	nd	nd – 17.2	4	2
Sertraline	0.3–144.9	2.3–238.6	nd	nd	–	nd – 229.8	5	3
Venlafaxine	0.1–62.1	0.1–1.1	nd – 0.6	nd	nd	nd – 4.1	3	2
Antibiotics								
Ciprofloxacin	0.6–20	BLQ – 0.4	–	–	–	nd – 211	2	−1
Ofloxacin	BLQ – 2	BLQ	–	–	–	nd – 105.9	2	−1
Azithromycin	0.8–8.4	BLQ	–	–	–	nd – 58.5	2	−0.1
β-blockers								
Propranolol	0.3–0.9	0.9–2.9	nd – 4.5	nd	nd	nd	3	1
Metoprolol	1.3–102.9	0.2–1.8	nd	nd	nd	nd – 181	2	0.1
Metoprolol acid	–	–	–	–	–	nd – 169.1	−1.24	−0.53

Sotalol	2.3–144.9	0.1–0.6	nd – BLQ	nd	nd	–	0.1	−2
Carazolol	1.3–1.3	0.3–2.9	nd – 3.8	nd	–	–	3	0.2
To treat asthma								
Salbutamol	0.5–3.4	BLQ	nd – 2.6	nd	nd	–	1	−1
Antiplatelet agent								
Clopidogrel	0.1–3.6	nd – 0.9	nd – BLQ	nd	–	nd	4	4

Log K_{ow} and log D_{ow} at pH 7.4 (environmental pH)

Compounds below their limit of detection were set as “nd” in the tables

BLQ: compounds below their limit of quantification

Empty cells mean that the compound was not analyzed in the corresponding matrix

[a] Trout liver

all small macroinvertebrates collected and reached values up to 93.7 ng/g. There are a few studies that have investigated the presence of pharmaceuticals in small macroinvertebrates (see Table 1), and only Huerta et al. [21] have also reported the presence of diclofenac in these organisms, also in Spanish rivers, at a concentration of 12.4 ng/g. In contrast, 15 pharmaceuticals were detected in periphyton samples at concentration ranging between low ng/g and values higher than 100 ng/g (sertraline, ofloxacin, metoprolol, and metoprolol acid). Sertraline was in fact pointed out, along with gemfibrozil and loratadine, as the most concerning pharmaceutical along the catchments based on its predicted ecotoxicity (sertraline was considered of ecotoxicological relevance for algae and daphnia) and its level in water [46]. Out of the 15 pharmaceuticals detected, only diclofenac, gemfibrozil, and venlafaxine had been previously detected in river periphyton [22]. Diclofenac was overall the most prevalent compound; it was the only compound detected in all types of biological matrix analyzed, periphyton, macroinvertebrates, mussels, and fish. The psychoactive drugs citalopram, venlafaxine, and carbamazepine were rather ubiquitous too: venlafaxine was detected in fish and periphyton and carbamazepine in fish and mussel, whereas citalopram was detected in fish, mussels, and periphyton. The rest of the pharmaceuticals were detected just in one biological matrix out of the 4 considered in this study.

3.2 Bioaccumulation Mechanisms

It is well known that factors such as lipid content (more attractive for hydrophobic compounds), individual size (and, therefore, different metabolism), or life stage (exposure time) can affect uptake levels [47, 48]. The main factor used to predict or to explain bioaccumulation of a compound is usually the octanol-water partitioning coefficient (K_{ow}), a measure of hydrophobicity that drives sorption and accumulation. Hydrophobic compounds (especially those poorly metabolized and with high log K_{ow} values) are more likely to present bioaccumulation hazards to aquatic organisms [49]. Accumulation of PhACs in several aquatic organisms does not seem to support that hypothesis, as only 50% of PhACs accumulated in any of the matrices had a log $K_{\mathrm{ow}} \geq 3$ (see Table 2). For those compounds that are ionizable, as it is the case of many PhACs, the log D_{ow} (which is the log of K_{ow} at a defined pH) seems to be a better parameter to apply to predict or explain bioaccumulation at environmental pH. However, when log D_{ow} at environmental pH (7.4) is considered, only 20% of the compounds retained had a log D_{ow} value superior than 3 (Table 2). This suggests that although lipophilicity and hydrophobic interactions are influencing bioaccumulation even in the case of these compounds, possibly other mechanisms are contributing too. Further research is needed to determine if processes such as active transport are inducing the uptake of these compounds. This seems a reasonable assumption, particularly when other compounds that were present in the water at higher concentrations were never detected in the biota, even at log K_{ow} and log $D < 3$, as the case of diazepam and lorazepam found at concentrations of up to 35.5 and 305.6 ng/L, respectively, in water samples from these rivers [46]. In addition, periphyton accumulated the highest number of PhACs and at higher concentration than in other matrices, notwithstanding the low lipid content of this matrix (~20%) [50]. The higher retention capacity of periphyton could be related to the influence of the extracellular polymeric substances (EPSs), which is constituted by high-molecular-weight compounds (mostly composed of polysaccharides and proteins secreted by microorganisms), and that establishes the functional and structural integrity of periphyton. The EPS matrix is considered to determine the physicochemical properties of periphyton, and its capacity to interact with the PhACs could lead to physical sorption instead of their uptake by cells.

3.3 Bioaccumulation and Biomagnification in Trophic Chain

The variety of samples collected in the four different watersheds provided a considerable amount of data, which allow to assess the bioaccumulation and potential biomagnification of these compounds in different trophic levels in the natural environment. Bioaccumulation factors (BAFs) were calculated for those contaminants that were present both in water and biota samples (e.g., diclofenac and azithromycin). Observed BAFs were obtained as the ratio between concentrations of given pollutants in a particular biota sample to those detected in water. Maximal BAF for azithromycin in periphyton and diclofenac in macroinvertebrates achieved values of up to 1,638 and 1,415 L/Kg, respectively (bioaccumulation is considered significant when BAFs > 1,000), but none of the BAFs calculated for pharmaceuticals in fish surpassed the threshold value. In line with it, environmentally derived BAFs calculated for 8 pharmaceuticals in several organisms in a freshwater food web in China were always lower than 1,000 value, although neither azithromycin nor diclofenac was determined in the study [30]. In contrast propranolol, fluoxetine, and desmethylsertraline exceeded that value in the snail *Planorbis* sp. in an effluent-dependent stream in the USA [19]. In this study, the presence of 24 pharmaceuticals (including diclofenac) in biota was investigated, although only a few pharmaceuticals were detected in periphyton and in snails. Finally there is a third study where different biota samples from the trophic chain were also investigated in a WWTP-influenced river in Spain [43]. BAFs were calculated in this study for diclofenac, ibuprofen, gemfibrozil, and venlafaxine in periphyton samples as well as in three macroinvertebrate taxa. Venlafaxine, only found in periphyton samples, was the sole compound whose BAF was higher than 1,000 value.

Among the 61 pharmaceuticals determined in our study, only 9 were analyzed in all matrices, namely, anti-inflammatory diclofenac, diuretic hydrochlorothiazide, psychoactive drug carbamazepine and its metabolites 2-hydroxycarbamazepine (2-hydroxyCBZ) and 10, 11-epoxycarbamazepine (10, 11-epoxyCBZ), citalopram, venlafaxine, and beta-blockers propranolol and metoprolol. Only one compound was detected in macroinvertebrates, whereas five were found in fish and seven in periphyton. As highlighted in the previous section, periphyton was also the biological matrix accumulating these PhACs at the highest concentrations. Periphyton can thus be proposed as a sentinel for monitoring the presence of pharmaceuticals in the aquatic environment as they are able to bioaccumulate higher number of compounds and at higher concentrations than other organisms. Mussels are normally used as a sentinel species in monitoring programs in the marine environment for persistent pollutants because of their sessile nature, wide geographical distribution, and high bioaccumulation capacity [51]. However, there is no mussel population in rivers (except in the case of Ebro River) and consequently, there is a need to identify alternative indicator organisms for pharmaceuticals, as it has been recommended for other pollutants.

Though our study in the four river basins was not designed to study specifically trophic transfer of target pharmaceuticals, the occurrence of these compounds along the biota samples at different trophic position allowed observing some general trends. Based on the results of these nine compounds in all biota matrices analyzed in the four water basins considered, it can be concluded that concentration followed the pattern fish < invertebrates < periphyton. Two main processes may lead to the bioaccumulation of PhACs by aquatic organisms: direct partitioning from the abiotic environment through inhalational exposure (bioconcentration) and dietary sources (trophic transfer) [43]. Higher trophic positions may be differentially affected by trophic transfer [52], and thus biomagnification of pollutants (and hence toxicity) in top predators has long attracted ecological research. Our findings highlight the hypothesis that pharmaceuticals are not biomagnified in the trophic chain. These results are in line with conclusions extracted from the limited number of studies reported in literature. Experiments performed in the lab under controlled conditions showed that very little biomagnification, if any, occurred in the trophic chain in the case of the psychoactive drug carbamazepine [53] and propranolol [54]. Similar results have been observed in the three field studies that considered different organisms in the trophic chain performed in China, Spain, and the USA and discussed above [19, 30, 43].They support the idea that bioaccumulation of PhACs from the lower to the upper trophic levels did not occur in the field.

3.4 Metabolization of Pharmaceuticals in Biota

Uptake of contaminants by aquatic organism is irrevocably associated to degradation/removal, including biodegradation, which highlights the role of the determination of metabolites and degradation products in the study of the uptake/elimination kinetics. In fact, some known transformation products of PhACs were targeted with the analytical methods and actually detected in biota samples, as it is the case of metoprolol acid (human metabolite), but also biodegradation by-products of metoprolol and atenolol [55] were found in periphyton samples at a high concentration of 168 ng/g. In line with these concentrations, metoprolol was also detected in the same periphyton samples. Unfortunately metoprolol acid was not determined in water samples, and therefore it is not possible to distinguish if this metabolite is generated by periphyton degradation of metoprolol or it was uptaken directly from water. Metabolites of psychiatric drug carbamazepine, 2-hydroxyCBZ and 10, 11-epoxyCBZ, were also found in periphyton samples (1.4 and 1.5 ng/g, respectively), whereas carbamazepine was not detected in the same samples. Carbamazepine metabolites had been previously detected in fish [56] and mussels [57] from exposure experiments under controlled conditions in the lab, although this is the first time that they have been detected in wild biota from a river. As in the case of metoprolol acid, the absence of data about water concentration of metabolites does not allow further discussion about their presence in biota samples. However, in the study of Valdes et al. [56], their presence in fish organs was attributed to biotransformation of parental compound by *Jenynsia multidentata*, since carbamazepine metabolites were not detected in exposure solutions. Future field monitoring studies would need to cover not only parent compounds but metabolites in all environmental matrices. Lab experiments under controlled conditions in which target PhACs are spiked are the ideal approach to elucidate both uptake and degradation mechanisms for each particular compound.

Another consideration and advisable future step is the study of the role that the organisms play in the attenuation observed in most of contaminants in river water, i.e., the level of biodegradation taken place in the environment. Evidence of biodegradation of emerging contaminants by river periphyton has been already reported [58]. More research is needed to determine how bioaccumulation and biodegradation are related, i.e., whether bioaccumulation increases bioavailability and therefore increases the chances of biodegradation. Overall, we need to better understand how biotic interactions mediate the bioaccumulation of emerging pollutants [59].

3.5 Occurrence of PhACs in the Four River Basins

Despite being comprehensive, the sampling campaign has some limitations such as the different types of samples collected in each water basin: fish and invertebrates were sampled and analyzed in the 4 rivers, whereas periphyton was only studied in Ebro and Llobregat River. The invasive species zebra mussel was only present in Ebro River. In addition, even though both fish and macroinvertebrate *Hydropsyche* were collected in all water basins, fish species differ from one river to the other. Regardless of the limitations of the sampling campaign, differences in the occurrence of pharmaceutical between the four rivers under study were observed in terms of the average concentration and the number of compounds detected in biota (Table 3). Llobregat River seemed to be the most polluted water basin followed by Ebro, while no clear differences could be established between the less polluted rivers Júcar and Guadalquivir. The results are in agreement with the observations in water as reported by Osorio et al. [46], where Llobregat and Ebro river basins were

Table 3 Occurrence of pharmaceuticals (ng/g) in freshwater biota of the three water basins considered

River basin	Matrix	PSY	ABs	AHs	β-B	A/A	CCB	LR	AP	T/A	AH	DIU
Ebro	Fish homogenate	nd – 0.2	–	–	nd – 4.5	nd – 5.7	–	–	nd	0–2.6	nd	nd
	Mussels	0.85	–	–	nd	nd – 0.2	–	–	nd	nd	2.7–6.6	nd
	Invertebrates	nd	–	–	nd	36.1–36.2	nd	nd	–	nd	nd	nd
	Periphyton	nd	nd	nd	nd	nd	nd	nd	nd	–	–	nd
Llobregat	Fish homogenate	nd – 1.4	–	–	nd	nd – 15.4	–	–	BQL	nd	nd	nd
	Invertebrates	nd	–	–	nd	23.3–67.1	nd	nd	–	nd	nd	nd
	Periphyton	nd – 229.8	nd – 211	nd – 8.6	nd – 181	nd – 35.1	nd	nd – 21.5	nd	–	–	nd – 36.9
Guadalquivir	Fish homogenate	nd	–	–	nd	nd – 4	–	–	nd	nd	nd	nd
	Invertebrates	nd	–	–	nd	10.2–93.7	nd	nd	–	nd	nd	nd
Júcar	Fish homogenate	nd	–	–	nd – BQL	nd – 11.76	–	–	nd	nd	nd	nd
	Invertebrates	nd	–	–	nd	19.5–86.7	nd	nd	–	nd	nd	nd

Compounds below their limit of detection were set as “nd” in the tables
BLQ: compounds below their limit of quantification
Empty cells mean that the compound was not analyzed in the corresponding matrix
PSY psychoactive drugs, *ABs* antibiotics, *AHs* antihistamines, *β-B* beta-blockers, *A/A* analgesics/anti-inflammatories, *CCB* calcium channel blockers, *LR* lipid regulators, *AP* antiplatelet agent, *T/A* to treat asthma, *AH* antihelminthics, *Di* diuretics

reported as displaying the highest ubiquity and concentrations of PhACs in surface waters. Highest concentrations of pollutants were detected in all cases in the organisms' sampled downstream of WWTPs. In the case of Llobregat River, that was extremely evident even if only two fish individuals were sampled at this particular site. Pharmaceutical concentrations in biofilm in the same site were the highest detected in biota for all organisms analyzed and surpassed the 100 ng/g value for psychiatric drug sertraline, antibiotic azithromycin, and beta-blocker metoprolol and its metabolite metoprolol acid. Analgesics and anti-inflammatories were the most prevalent compounds, being found in biota from all rivers. These compounds were also the most concentrated therapeutic group in surface waters [46]. Analgesics and anti-inflammatories were the only ones quantified in Guadalquivir and Júcar, although beta-blockers were also detected in Júcar. In Ebro River, apart from analgesics/anti-inflammatories and beta-blockers, psychoactive drugs, antihelminthics, and drugs to treat asthma were also found (Table 3). The highest diversity of drugs detected in biota was observed in Llobregat River where eight different pharmaceutical classes were found: psychoactive drugs, antibiotics, antihistamines, beta-blockers, analgesics/anti-inflammatories, lipid regulators, antiplatelet agents, and diuretics.

3.6 Impact of Pharmaceuticals in the Environment

Although the correlation between bioaccumulation and measurable effects is still very much unknown, few studies have tried to correlate both in recent years, particularly in the case of PhACs. Some of the effects observed in bioaccumulation experiments after exposure to a compound or a mixture of drugs ranged from behavioral modifications [60, 61] to histopathological changes [62], reproductive alterations, mortality [63], estrogenicity [64], neurotoxicity, and modifications of gene expression and proteomic changes [65–68]. However, these studies used generally greater exposure concentrations than those normally detected in the environment (μg/L to mg/L), and consequently the tissue concentrations were also usually higher. Thus, the detected concentrations of PhACs in the field may not represent an immediate risk for the exposed organisms. Nevertheless, future research should include more studies on this issue to build a robust correlation between the fate and effects of pharmaceuticals in aquatic ecosystems.

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Ecosystem Responses to Emerging Contaminants: Fate and Effects of Pharmaceuticals in a Mediterranean River

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Abstract There is concern about the environmental effects of pharmaceuticals, since these substances have strong biological impacts and are found in an increasing number of sites, especially downstream from wastewater treatment plants (WWTP). Most information existing on the effects of pharmaceutical products is based on simple laboratory assays with single compounds, whereas pharmaceuticals in the environment typically appear in complex mixtures that include secondary metabolites as well as other pollutants. Therefore, real-world situations may contribute to the understanding of the fate and effects of pharmaceuticals in freshwaters. Here we report the effects of pharmaceuticals in the river Segre (Pyrenees, Iberian Peninsula) in a river segment affected by the effluent of a

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WWTP. The removal efficiencies of pharmaceuticals and their metabolites in both the WWTP and the river were analyzed by comparing the inflow and outflow concentrations at the WWTP and along the studied river segment, and their transformations and interactions were modeled. The WWTP had a higher removal efficiency (45%) than the river segment (20%), but the latter was also important. In general, the compounds most efficiently removed in the WWTP were also those more efficiently removed in the river. The removal efficiency in the river was higher during the day than during the night, suggesting that attenuation was driven by either photodegradation or biological transformation by primary producers. The effects of pharmaceuticals were analyzed across different scales, from those on biofilms to functional impairment of the river ecosystem. Laboratory toxicity tests showed that stream biofilms at the most polluted site developed community tolerance to anti-inflammatory drugs. Biofilms in the field also showed altered metabolic profiles and reduced algal diversity. WWTP effluents were able to alter the balance between autotrophic and heterotrophic processes: while ecosystem respiration was subsidized, gross primary production showed some stress effects.

Keywords Biofilm, Metabolism, Pharmaceuticals, River, WWTP

Contents

1 Introduction

Pharmaceutical compounds may reach the aquatic environment via human and animal excretion both as active metabolites and in unmetabolized form (parental forms). They are considered “emerging pollutants” since they are not currently covered by existing water-quality regulations, and their effects on the environment are still poorly understood. As wastewater treatment plants (WWTP) are unable to effectively remove all pharmaceutical compounds, their effluents are an important source into aquatic ecosystems, where pharmaceuticals may reach concentrations from the ng L^{-1} to the low μg L^{-1} level. Even though these concentrations seem not to produce acute toxic effects on aquatic organisms [1], they can lead to long-term sublethal effects by bioaccumulation, as well as by interaction with other stress factors [2–4].

Most information existing on the effects of pharmaceutical products is based on simple laboratory assays with single compounds, whereas pharmaceuticals in the environment typically appear in complex mixtures that include secondary metabolites as well as other pollutants [5, 6]. WWTP effluents are one of the main sources of pharmaceuticals into rivers, but their complex composition, which also includes nutrients, organic matter, and many other potential contaminants, makes it difficult to predict their joint environmental effects. The subsidy-stress framework [7] remarks the fact that contaminants may act either as a subsidy or as a stressor for biological activity. Assimilable contaminants such as dissolved nutrients and organic matter subsidize biological activity, at least up to a threshold, beyond which they can suppress it. On the other hand, toxic contaminants are deleterious to organisms and tend to suppress biological activity. Therefore, because of their mixed composition and the resulting concentrations in river ecosystems, the effect of WWTP effluents can be either a subsidy or a stress for the receiving ecosystem [8]. Furthermore, the potential response to contaminants differs between the different groups of organisms, and ecological interactions add a level of complexity [9]. Thus, the response to pollution can differ from the scale of the river biofilm to the scale of the whole ecosystem. Although there are a number of papers describing the effects of WWTP effluents on ecosystem processes (e.g., [10, 11]), relatively few described the downstream evolution of these effects [12, 13].

Therefore, it is important to gather information on the fate and effects of pharmaceuticals in real-world situations. Here we report some the main findings of a study performed in the river Segre near Puigcerdà, in the Spanish part of the Pyrenees, (Iberian Peninsula) in a segment affected by the effluents of a WWTP. We studied four reaches (one control upstream the effluents of a WWTP and three impacted ones downstream, with a decreasing concentration of pollutants) along a 6-km river segment, as well as the WWTP to gain insight on their effects on the river ecosystem.

2 Materials and Methods

2.1 Study Site

The study was conducted in the Segre River, a tributary to the Ebro River in the Oriental Pyrenees (NE Iberian Peninsula). At the study site (UTM X: 411856 and UTM Y: 4698346, 31 N/ETRS 89), the Segre drains an area of 287 km^2, with a rain-/snow-fed flow regime. It receives the effluent from the WWTP of Puigcerdà (UTM X: 411856 and UTM Y: 4698346, 31 N/ETRS 89) with 30,000 population

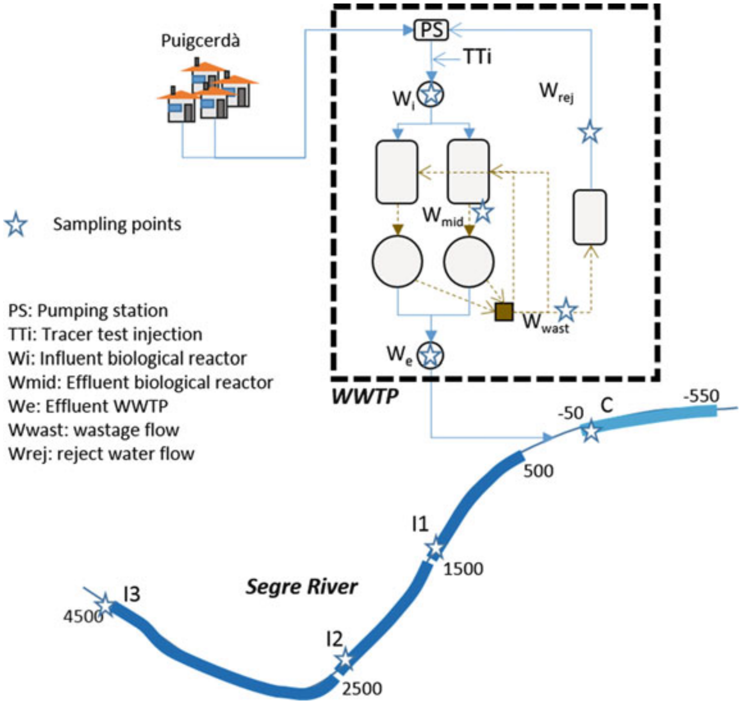

Fig. 1 Study design. The Segre River runs from *right* to *left*

equivalents. The study area is located at an elevation of ca.1100 m a.s.l., and the river runs in riffles and pools through sedimentary and silicate substrata. The average annual precipitation in the region ranges between 700 and 1,000 mm, and the average monthly air temperature ranges from 3 to 18°C. The riparian vegetation of the river segment is well developed and mainly composed by deciduous trees (*Alnus glutinosa*, *Fraxinus excelsior*, and *Salix alba*). The basin is covered in 85% by natural vegetation, 12% by agriculture, and 3% by urban land uses.

We compared a 500-m control reach (C) upstream from the WWTP effluent with three impacted reaches at increasing distances from the WWTP effluent: 500–1,500 m (I1), 1,500–2,500 m (I2), and 2,500–4,500 m (I3) (Fig. 1). Hereafter, we refer to all of them (control plus impacts) as reaches for simplicity and use the term reach only when making overall comparisons between conditions upstream and downstream from the WWTP. Acuña et al. [14] showed that self-purification reduces the total concentration of pharmaceuticals by ca. 37% (average of the considered 75 compounds) along the impact segment during spring.

2.2 Environmental Measurements

Discharge, channel width, and depth were measured with a tape and water velocity with a flow tracker (Handheld-ADV, SonTek, San Diego, USA) at the sampling points located at the end of each river segment (Fig. 1). We measured the water travel time and dilution from the entrance of the WWTP (sampling point Wi in Fig. 1) to each river sampling point using a bromide slug addition. For this addition, 10 kg NaBr dissolved in 80 L of wastewater was added at the entrance of the WWTP. Water samples for Br analysis were collected at each sampling point every 4 h using automatic samplers. The Br experimental data were used to fit a hydraulic model of the integrated system. Specifically, the best model fit was achieved when combining two continuous stirred tank reactors in series for the WWTP with a Lagrangian pollutant transport model for the river.

Above-canopy global radiation (GLR) data were obtained from the meteorological station of the Catalan Meteorological Service (Das, located at ca. 5 km from the studied segment). Radiation reaching the streambed was estimated by filtering the series of data of global radiation by light interception coefficients calculated by the HemiView canopy analysis software (version 2.1, Dynamax, Inc., Houston, USA). Hemispherical photographs of the canopy were taken during the study period (9–10 October 2012) with a high-resolution digital camera (Nikon D70s, NIKON Corporation, Tokyo, Japan) fitted to a 180° fisheye (Fisheye-NIKKOR 8 mm, NIKON Corporation, Tokyo, Japan) and analyzed with HemiView.

Water temperature, conductivity, and pH were measured with handheld probes (WTW multiline 3310, Yellow Springs, USA) in the sampling points at the end of each river segment two times (noon and midnight). Water samples were collected in parallel, filtered through fiberglass filters (Whatman GF/F 0.7 μm, Whatman International Ltd., Maidstone, England), and frozen at −20°C until analysis. The concentration of ammonium was analyzed by ion chromatography using a Dionex ICS-5000 (Dionex Corporation, Sunnyvale, USA). The concentration of phosphate was determined colorimetrically using an AMS Alliance SmartChem 140 spectrophotometer (AMS, Frepillon, France). The concentration of DOC was measured on a Shimadzu TOC-V CSH analyzer (Shimadzu Corporation, Kyoto, Japan). Three water samples (each 2 L) for suspended particulate organic matter (SPOM) were taken at each sampling point and filtered through pre-ashed and pre-weighed Whatman GF/F filters. Filters were frozen for transport, and once in the laboratory, they were dried (70°C, 72 h), weighed, ashed (500°C, 5 h), and reweighed to estimate ash-free dry mass (AFDM).

2.3 Pharmaceutical Sampling and Analysis

At the influent of the biological reactor of the WWTP (Wi), 24 grab samples were collected over 72 h (one sample every 4 h and extra samples in the morning and evening peaks). This sampling strategy was defined after analyzing influent variability through the installation of an online ammonium sensor (ammolyzer from S: CAN, Vienna, Austria). We observed small fluctuations in pollutant concentrations within the sampling regime established. Pharmaceutical concentrations were well correlated with ammonium at the influent of the WWTP. At both the effluent of the WWTP and in the river sampling points of I1, I2, and I3 reaches, we collected 13 samples (one every 4 h). Furthermore, water samples at the control sampling point C were collected every 12 h to determine the background concentrations of the selected pharmaceuticals.

Pharmaceuticals (91 compounds) from several therapeutic classes (NSAIDs, lipid regulators, diuretic, antihypertensive, psychiatric drugs, β-blockers, and antibiotics) were measured in each sampling point, from samples collected in parallel to those for nutrients, filtered through nylon filters (0.2 μm mesh, Whatman, Maidstone, UK), and kept at −20°C until analysis. Analysis of pharmaceuticals was performed following the fully automated online methodology described in detail by López-Serna et al. [15]. Briefly, 5 mL of surface water was loaded on the online chromatographic system (Thermo Scientific EQuan^{TM}, Franklin, MA, US) consisting of two quaternary pumps and two LC columns, one for pre-concentration of the sample and the other for chromatographic separation. The sample was further eluted by means of the mobile phase into the coupled mass spectrometer (TSQ Vantage triple quadrupole; Thermo Scientific, Franklin, MA, USA). Chromatographic separation was achieved using a Thermo Scientific Hypersil Gold^{TM} column (50 × 2.1 mm, 1.9 μm particle size). Target compounds were analyzed under dual negative/positive electro-spray ionization in multiple-reaction monitoring (MRM) mode, monitoring two transitions between the precursor ion and the most abundant fragment ions for each compound. Recoveries of the compounds ranged from 62% to 183% (sulfamethoxazole and ibuprofen, respectively), whereas limits of detection ranged from 0.81 to 7.86 ng L^{-1} (sulfamethoxazole and venlafaxine, respectively).

2.4 Removal Efficiency in the WWTP and the River

A hydraulic model was built using the SIMBA modeling platform which was calibrated using the tracer test data. The hydraulic model was extended with dummy variables for the pharmaceuticals. Inputs to the model were the dynamic flow (at 15-min intervals) and the influent concentrations of pharmaceuticals at the WWTP (Wi) (covering a period of 72 h). Simulations were run to obtain effluent concentration profiles at the effluent of the WWTP (We, Fig. 1). These simulated concentrations (q_0 bounds) describe the behavior of pharmaceuticals without attenuation, and the differences with the measured concentrations correspond to the relative attenuation, which can be positive (if the compound is removed) or negative (if the compound is generated). The same approach was followed for the river, using flows and concentrations at the sampling point I1 as input to the hydraulic model, and running simulations to obtain the concentration profiles at the bottom of the impacted segment (sampling point I3). The instantaneous relative attenuation (IRA) at each sampling time was calculated as the percentage of change between q_0 and b (mean experimental value), for all samples (n) (see Eq. 1). The result of such model-based approach is a daily profile for pharmaceuticals instantaneous relative attenuations (IRA) at the WWTP and at the river. The mean relative attenuation (MRA) is the average of all IRAs:

$$\mathrm{IRA}(n) = \left(\frac{q_0(n) - b(n)}{q_0(n)}\right) \tag{1}$$

$$\mathrm{MRA} = \frac{\sum_1^n IRA(n)}{n} \tag{2}$$

2.5 Benthic Organic Matter and Biofilm

Five Surber nets (0.09 m^2, 0.2 mm mesh size) for benthic organic matter (BOM) were taken at random per reach, the material was frozen for transport, and once in the laboratory, it was dried (70°C, 72 h) and ashed (500°C, 5 h) to calculate ash-free dry mass (AFDM). Chlorophyll-a (*Chl-a*) samples were obtained from the upper exposed part of cobbles. From each cobble a surface of 2–3 cm^2 was scraped with a knife and pooled together to obtain a mixed sampling area of 9–18 cm^2 according to the available biomass. Five replicates were considered in each river reach. Then, samples were immediately frozen (−20°C) until analysis. In the laboratory, Chl-*a* was extracted with 90% v/v acetone overnight at 4°C and quantified spectrophotometrically (Shimadzu UV1800) after filtration (Whatman GF/C 1.2 μm) [16].

2.6 Ecosystem Metabolism

River metabolism was calculated for each study reach (C, I1, I2, and I3) from diel dissolved oxygen (DO) changes by the open-system method with either one or two stations [17, 18] using 10 d of base flow conditions. We chose the best method (single-station or two-station) to estimate ecosystem metabolism in each reach following Reichert et al. [18] by comparing the ratio of flow velocity to reaeration coefficient (v:k) with reach length. Thus, we used the single-station method for segments CR and IR1 and the two-station method for IR2 and IR3. DO and temperature were recorded at 10-min intervals at the upstream and downstream ends of each reach with optical oxygen probes (YSI 6150 connected to YSI 600 OMS, YSI Inc., Yellow Springs, Ohio, USA). Exchange of DO with the atmosphere was estimated using slug additions of mixed tracer solutions [19]. Six solutions of propane-saturated water were prepared in the laboratory by filling hermetic 20-L plastic tanks with 10 L of distilled water and 10 L of 99% pure propane gas (Linde Industrial Gases, Barcelona, Spain). The solutions were prepared few days before the additions and shaken to allow sufficient time for propane to dissolve into the water. A total of three slug additions were performed: the first one covering reach IR3, the second one covering IR1 and IR2, and the third one covering CR. For each slug addition, two of the propane-saturated water solutions were added in situ to 60-L containers filled with a solution of 40 L of stream water with a measured amount of conservative tracer (chloride, as NaCl). Immediately after mixing, the solutions were injected into the stream channel approx. 50 m upstream from the first sampling point to allow for complete lateral mixing. The breakthrough curves of chloride were followed at each sampling point using a handheld conductivity meter (WTW, Germany). Five replicate water samples were collected at the conductivity peak using 60-mL plastic syringes fitted with stopcocks. After adding 30 mL of air to each syringe, these were shaken for 10 min to allow equilibration of the propane gas into the air space. Then, the air space was collected in pre-evacuated 20-mL glass vials, which were stored in the fridge until analysis on a gas chromatograph (Thermo Fisher Scientific, CA, USA). Oxygen exchange (k) with the atmosphere was calculated using the decline in conductivity-corrected propane concentrations between sampling points as described by Jin et al. [19]. Nominal travel time of water (τ, in min) was calculated measuring the time between the peaks of the two breakthrough curves at the upstream and downstream points [35]. Ecosystem respiration (ER) was calculated as the sum of net DO production rate during the dark period and respiration values during the light period, these being calculated as the linear interpolation between the net metabolism rate values of sunrise and sunset of the nights before and after the day of interest. Gross primary production (GPP) was the sum of net metabolism rate during the light period and respiration rates during the light period. NEP was calculated as the sum of GPP and ER and EF as the sum of GPP and ER in absolute values.

3 Results and Discussion

3.1 *Environmental Characteristics in the WWTP-River System*

The tracer breakthrough curves (Fig. 2) showed travel times to be 40 h in the WWTP and 3.3 h in the river. Therefore, the expected effects of the WWTP on water quality are much higher than those of the studied river segment. Furthermore, data from the tracer addition and from the discharge measurements with the flow tracker allowed the identification of dilution along the study river reaches. Discharge, as well as irradiance, increased along the river as a consequence of the

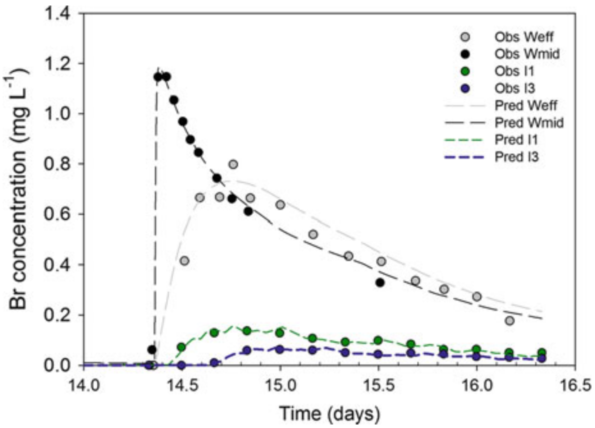

Fig. 2 Observed (Obs) and predicted (Pred) bromide (Br) concentrations in sampling points located in the WWTP (Wmid and Weff) and the river (I1 and I3)

Table 1 Water physicochemical characteristics measured at the downstream end of each river reach (mean ± SD). The * symbol indicates significant difference ($p < 0.05$) in comparison to point C according to generalized least square models. K_{20}, reaeration coefficients corrected with temperature; DOC, dissolved organic carbon; SPOM, suspended particulate organic carbon

	C	I1	I2	I3
Discharge (m^3 s^{-1})	0.29 ± 0.03	0.50 ± 0.17	0.64 ± 0.03*	0.83 ± 0.24*
Velocity (m s^{-1})	0.18 ± 0.06	0.20 ± 0.08	0.38 ± 0.14	0.33 ± 0.08
Depth (m)	0.14 ± 0.02	0.15 ± 0.01	0.19 ± 0.05	0.23 ± 0.01*
Width (m)	11.90 ± 0.85	10.25 ± 2.47	9.45 ± 1.34	10.70 ± 0.42
Below canopy global radiation (MJ m^{-2} d^{-1})	4.62 ± 0.82	9.52 ± 1.69*	11.93 ± 2.12*	14.34 ± 2.54*
Leaf area index	2.52 ± 0.83	1.76 ± 0.55	0.71 ± 0.42*	0.72 ± 0.16*
K_{20} (day^{-1})	32.67	28.79	29.76	34.45
Temperature (°C)	13.58 ± 1.41	13.80 ± 1.10	13.49 ± 0.87	13.60 ± 0.86
pH	8.54 ± 0.39	8.63 ± 0.01	8.55 ± 0.12	8.65 ± 0.25
Conductivity (μS cm^{-1})	180.90 ± 0.85	225.75 ± 13.79*	214.5 ± 2.12*	207.75 ± 7.42*
Ammonium (mg L^{-1})	0.012 ± 0.001	1.92 ± 1.03*	0.90 ± 0.41*	0.37 ± 0.33*
Phosphate (mg L^{-1})	0.039 ± 0.001	0.292 ± 0.111*	0.200 ± 0.020*	0.182 ± 0.004*
DOC (mg L^{-1})	2.54 ± 0.15	3.67 ± 0.41*	3.14 ± 0.34*	2.79 ± 0.16
SPOM (mg L^{-1})	2.90 ± 0.08	4.48 ± 0.51*	3.04 ± 0.08	3.02 ± 0.34

inflow of groundwater (Table 1), but water temperature and pH did not change significantly.

Conductivity increased 25% from sampling points C to I1, while ammonium increased 160-fold (0.01–1.9 mg L^{-1}) and phosphate 7.5-fold (0.04–0.3 mg L^{-1}; Table 1). Even though the effects were not causative of evident signs of eutrophication such as anoxia or algal blooms common in highly polluted rivers [20, 21], the ammonium concentration after the effluent seemed high enough to produce toxic effects on stream invertebrates and to affect litter decomposition rates [22, 23]. These three variables decreased further downstream, but their values remained significantly higher than at sampling point C (Table 1). The decrease in ammonium was a result of attenuation processes and not only of dilution or dispersion, since its load in transport increased from 3.48 mg s^{-1} in point C to 960 mg s^{-1} in point I1 and then decreased to 576 and 307 mg s^{-1} in points I2 and I3, respectively. Ammonium is a highly reactive nutrient that is readily nitrified or taken up by the biota [11]. On the other hand, the WWTP effluents increased the phosphate load from 11.3 mg s^{-1} in point C to 146 mg s^{-1} in point I1, but remained steady further downstream (128 mg s^{-1} and 151 mg s^{-1}). DOC values averaged 2.5 mg L^{-1} in C, increased to 3.7 mg L^{-1} at point I1, and decreased to 2.8 mg L^{-1} at I3. Acuña et al. [14] showed that phosphate attenuation was also related to sorption in sediments, and Merseburger et al. [12] reported a higher decrease in ammonium than in phosphate concentration downstream a WWTP effluent. Similarly, SPOM values averaged 2.9 mg L^{-1} in the point C, increased to 4.5 mg L^{-1} at point I1, and decreased to 3.0 mg L^{-1} at I3. Both DOC and SPOM increased considerably from point C to I1 and then decreased with distance from the WWTP ($R^2 > 0.51$, $P < 0.05$), the decrease approaching pre-disturbance values (Table 1).

Pharmaceutical concentrations followed a pattern similar to that of nutrients. Twenty-two different compounds from seven therapeutic groups were detected in surface water out of the 91 pharmaceuticals and metabolites analyzed. Pharmaceutical concentrations were higher in the impact segment, and the maximum concentration (considering the sum of all therapeutic families) occurred in I1 (values up to 2,300 ng L^{-1}) (Fig. 3). The concentrations of contaminants such as diclofenac at I1 were far from the highest measured concentrations [24, 25], but were comparable to those below WWTP effluents, that generally fall below 100 ng L^{-1} [26]. All identified groups of pharmaceuticals (anti-inflammatories, lipid regulators, diuretics, antihypertensive, psychiatric drugs, and β-blockers), except antibiotics, showed the same pattern of longitudinal decrease of concentrations from I1 to I3 (Fig. 3). This pattern was more marked in the case of the NSAIDs. Among the seven therapeutic groups of pharmaceuticals detected, concentrations of the NSAIDs group were the highest in all sampling sites, up to three times higher than other groups (Fig. 3). High concentrations of anti-inflammatories at I1 (1,120 ng L^{-1}) decreased downstream to 290 ng L^{-1} at I3. Ibuprofen and diclofenac occurred in the impact reaches but not in the control reach and also decreased from I1 to I3. Moreover, ibuprofen metabolites occurred at higher concentrations in the impact reaches than ibuprofen itself. Up to 192.6, 572.6, and 87.7 ng L^{-1} were detected in I1 for Ibuprofen, 1-hydroxy-ibuprofen, and 1-hydroxy-ibuprofen, respectively. The levels of the metabolites also decreased from I1 to I3. Overall, among the identified compounds, none is currently included in the list of priority pollutants by the EU, though diclofenac is in the list of substances subject to review for possible identification as priority or priority hazardous substance by the European Water Framework Directive (Directive 2008/105/EC).

MRAs at the WWTP were positive for all pharmaceuticals and ranged from 3% (diclofenac) to 100% (acetaminophen) (Fig. 4) and were consistent with those reported in previous studies [14]. MRAs for parent compounds were lower in the river than in the WWTP, except for diclofenac (3% at the WWTP and 12% in the river). In the river, MRA was the highest for ibuprofen (61%) and the lowest for

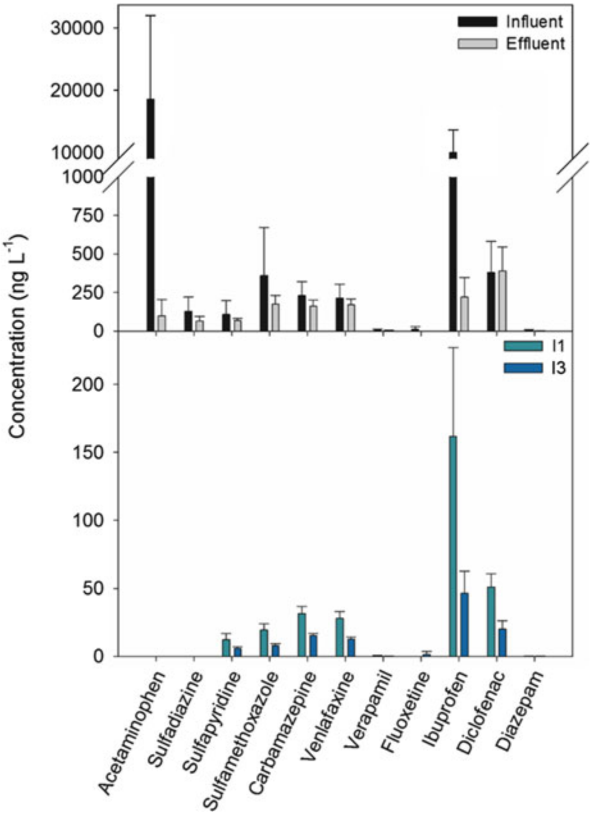

Fig. 3 Concentrations of 11 pharmaceuticals in influent and effluent of the WWTP (*top panel*) and in sampling points at the I1 and I3 river reaches (*low panel*)

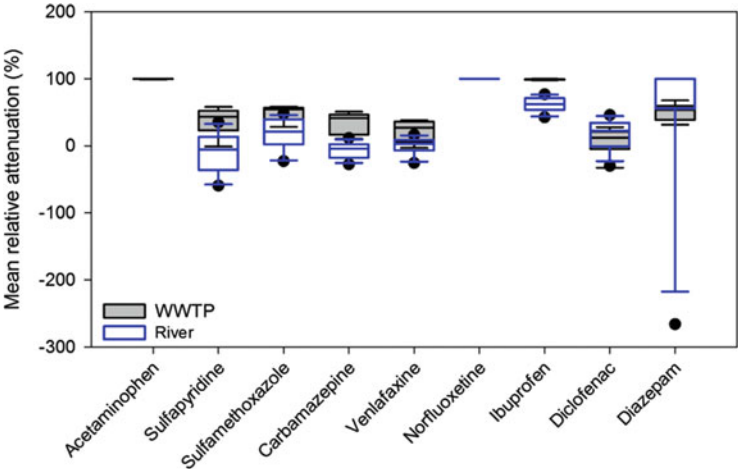

Fig. 4 Mean relative attenuation measured for pharmaceuticals in the WWTP and in the river

sulfapyridine (−9%). More interestingly, our approach allowed the comparison of the relative removal efficiencies of the WWTP and the river within a single experiment, using an integrated tracer addition, analytical methods, and modeling approach. Furthermore, the followed approach allowed the determination of the instantaneous relative attenuations along the diel cycle. For example, light-mediated processes (either photodegradation or biotransformation linked to primary producers) seemed to play a considerable role in the removal efficiencies in the river [27], but not in the WWTP.

3.2 Ecosystem Response to WWTP Pollutants

Organic matter and chlorophyll concentration showed contrasting responses to the WWTP effluent. BOM values averaged 26.9 g AFDM m^{-2} at the C river reach, increased to 139.0 g AFDM m^{-2} at I1, and decreased downstream to 72.8 g AFDM m^{-2} at I3 (Table 2), but values were not statistically significantly different from those at C. Chl-*a* values in the C reach averaged 1.2 μg cm^{-2} and showed a progressive increase downstream up to 9.6 μg cm^{-2} at I3 (linear regression with distance, $R^2 = 0.62$, $P < 0.0001$). BOM was positively correlated with conductivity, ammonium, and phosphate, and Chl-*a* was with discharge and global radiation below canopy ($R^2 > 0.90$ $P < 0.05$). Biofilm variables thus showed little effect of the WWTP.

Metabolic processes followed contrasting longitudinal patterns (Fig. 5). There was an almost threefold increase in ER between C and I1 (from 3.1 to 8.8 g O_2 m^{-2} d^{-1}) and a decrease along the impact segment down to 6.6 g O_2 m^{-2} d^{-1} at I3, a value still two times higher than the control. Overall, ER was significantly higher in the impact than in the control reach, and the decrease downstream from the WWTP was also significant (linear regression with distance, $R^2 = 0.29$ $P = 0.002$). ER was not correlated to DOC or SPOM, but it was to ammonium ($R^2 = 0.99$ $P = 0.001$), phosphate ($R^2 = 0.98$ $P = 0.003$), pharmaceuticals ($R^2 = 0.99$ $P = 0.002$), and to BOM ($R^2 = 0.91$ $P = 0.043$). GPP did not show an obvious response to the WWTP effluents: it averaged 0.5 g O_2 m^{-2} d^{-1} in C and did not differ between C and I1, but then increased significantly to 1.24 in I2 and 2.3 in I3 (Fig. 5) following the increase on the light availability ($R^2 = 0.51$ $P < 0.0001$). All river segments were heterotrophic, net ecosystem metabolism (NEM) averaged 2.6 g O_2 m^{-2} d^{-1} in C, increased to 8.1 g O_2 m^{-2} d^{-1} in C at I1, and decreased downstream to 4.3 g O_2 m^{-2} d^{-1} at IR3.

Table 2 Benthic organic matter and biofilm characteristics in each river reach (mean ± SD). The * symbol indicates significant difference ($p < 0.05$) in comparison to CR site. **BOM**, benthic organic matter; *Chl-a*, chlorophyll-a

	C	I1	I2	I3
BOM (g m^{-2})	26.95 ± 11.99	138.99 ± 202.36	68.56 ± 48.51	72.79 ± 55.85
Chl-*a* (μg cm^{-2})	1.24 ± 0.24	4.20 ± 1.89*	6.16 ± 1.71*	9.61 ± 5.83*

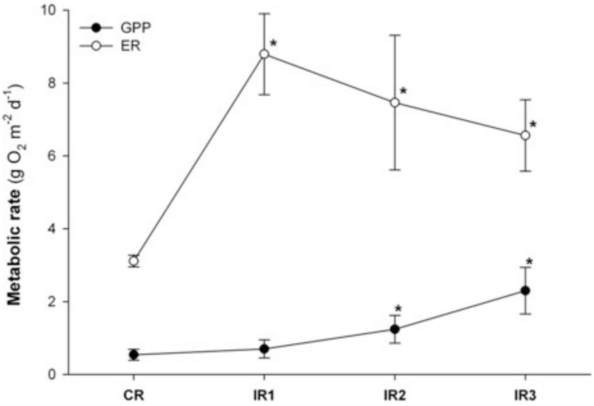

Fig. 5 Daily metabolic rates (mean ± SD) in each river reach. Positive values represent gross primary production (GPP) and negative values ecosystem respiration (ER). The * symbol indicates significant difference ($P < 0.05$) in comparison with the C reach

The *P*/*R* ratio decreased from 0.17 in C to 0.080 in I1 and then increased to 0.18 in I2 and to 0.36 in I3. ER was mostly related to BOM, indicating the likely coupling between both variables along the river, as has been described elsewhere (e.g., [28, 29]). Whatever the direct driver, ER was directly related to human-generated inputs of nutrients and organic matter [30, 31]. GPP was less affected by the WWTP effluent, but showed a downstream increase instead, which suggests light to be the primary driver of this variable in the studied river. Although GPP has often been linked to nutrient status (e.g., [10]), this relationship only holds when irradiance is not a limiting factor, what makes light availability a more important control than nutrients (Artigas et al., 2013). In the case of our study river downstream the WWTP, although GPP was not depressed, values were lower than those expected according to the available irradiance [32], therefore indicating lower light-use efficiency with respect to the control reach. This pattern suggests that GPP was experiencing a stress effect by the chemical pollution from the WWTP, which was in fact also reflected in higher values of the non-photosynthetic quenching (NPQ) downstream the WWTP, which was 54% higher at IR1 than at CR. In this direction, NPQ has been reported to increase in order to protect the photosynthetic apparatus from excess light that cannot be used for photosynthesis [33, 34]. With regard to the mixed effects of the discharge of the WWTP, the likely subsidy effect of the assimilable pollutants on GPP right downstream the WWTP effluent was overridden by the toxic effect of other toxic pollutants such the pharmaceuticals. It was therefore obvious that different trade-offs were achieved for ER and GPP between the subsidy and toxic effects by the discharge of the WWTP effluent. While there was a dominant subsidy effect for ER, there was a dominant toxic effect for GPP. Overall, our results indicate that WWTP effluents tend to decouple river metabolism from upstream systems, as ER rises abruptly and GPP becomes at least partially decoupled from the available light. Therefore, the discharge of WWTP creates an at least local spatial discontinuity that could have important consequences for river ecosystem functioning downstream.

4 Concluding Remarks

In this study, we have investigated the fate and effects of pharmaceuticals derived from a WWTP in a Mediterranean river by combining an intensive field campaign with state-of-the-art analytical techniques and modeling tools. Our results demonstrate that the receiving river significantly contributes to the removal of pharmaceuticals and their metabolites along relatively short distances. Moreover, we have shown that river ecosystem functioning is strongly affected by the WWTP effluent through a combination of subsidy and stress effects. This functional discontinuity was recorded over several kilometers downstream from the WWTP effluent. How much further downstream this effect persists, and the potential recovery of the river ecosystem to the disturbance remains to be investigated. We advocate in favor of considering WWTP and receiving rivers as coupled systems if we aim for an integrated management of rivers in humanized landscapes.

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Fate and Degradation of Emerging Contaminants in Rivers: Review of Existing Models

J. Aldekoa, R. Marcé, and F. Francés

Abstract Nowadays, society and regulatory authorities claim rigorous environmental risk and exposure assessment procedures. Prediction models of emerging contaminants may provide results within these assessment practices. This review gathers models that have been used by scientific researchers in order to predict emerging contaminant concentrations in rivers. A description of PhATE, GREAT-ER, WASP, SWMM, EUSES, QUAL2E, ChemCAN and AQUASIM models is provided. After reviewing more than 40 scientific applications of these emerging contaminant models, PhATE and GREAT-ER result to be the most used tools in the literature. Overall most applications point out the utility and necessity of these models. In any case, uncertainty is always related to any model outcomes. Thus, an analysis of propagated uncertainty in emerging contaminant basic processes is reviewed. Results indicate that the apparent contaminant emission from the population is the most significant issue in terms of propagated uncertainty. All considered factors suggest that there is still potential for further development of emerging contaminant models and that there is still the necessity of complementing the applications with measured data.

Keywords Emerging contaminants, Exposure assessment, Freshwater pollution, GREAT-ER, Models, PhATE

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Contents

Acronyms

API	Active pharmaceutical ingredient
EC	Emerging contaminants
EU	European Union
GIS	Geographic information system
PEC	Predicted environmental concentration
PhRMA	Pharmaceutical Research and Manufacturers of America
PNEC	Predicted no-effect concentration
UK	United Kingdom
US and USA	United States of America
USEPA	US Environmental Protection Agency
WWTP	Wastewater treatment plant

1 Introduction

Representative measured data and model predictions are key elements of environmental exposure assessment of new substances. In general, measuring processes are related with a large amount of devoted time, resources and expenses, in addition to the difficulties in detecting and measuring low ng/L concentrations in surface waters. Mathematical models are needed when and/or where we do not have observations [1]. In time, because we need longer series of data than the observed ones in the past or because we need to predict the future response which is not already observed. And in space, we need mathematical models for locations where we do not have observations. Mathematical models are prediction tools which may interact and complement measured data, resulting on a robust approach to perform risk assessment of emerging contaminants in rivers.

The main inputs of emerging contaminants into rivers are the wastewater treatment plants (WWTPs) or, in case of no treatment, the direct discharge from sewer systems. Humans consume regularly pharmaceuticals, detergents and fragrances that are disposed in the sewer systems and released through WWTPs in surface waters. Therefore, contaminants are partly lost by the human body metabolism (except for detergents), WWTP treatments and in-stream degradation.

Georeferenced exposure assessment models aim at predicting emerging contaminant concentrations in surface waters at catchment scale considering the processes mentioned above. We will call it EC models. GREAT-ER, presented in 1997 by [2] and PhATE, presented in 2004 by [3] are examples of such EC models for European and US rivers, respectively. These models allow the comparison between the predicted environmental concentration (PEC) and the predicted no-effect concentration (PNEC), in order to determine if a contaminant presents a risk to environmental organisms.

Similar large river catchment scale tools are still being developed due to the necessity of evolution of prediction models in the exposure and risk assessment framework. This is the case of the recently developed HydroROUT [4] which is a river-routing model that is being tested in Canada with several down-the-drain contaminants such us carbamazepine and diclofenac.

In any case, before GREAT-ER and PhATE models were introduced in the risk assessment arena, generic steady-state multimedia models were used [5], such as ChemCAN [6], HAZCHEM (ECETOC 1994) and EUSES (EC 1996). These models predict fate, transport and distribution of chemicals in different compartments (e.g. air, water, soil, sediment and biota), but they do not consider explicitly the source of emission, neither the spatial nor temporal variability of environmental elements [2, 7].

Other non-multimedia models but in-stream water quality models, such as Mike 11 (DHI 2003) and QUAL2E (USEPA 1985), have been broadly applied to simulate traditional quality parameters (e.g. biochemical oxygen demand and dissolved oxygen). These models are relatively complex tools that include dispersion and advection processes of chemicals and imply relatively high data requirements. However, they do not explicitly include WWTP simulation components. Hence, they may be used for site-specific risk assessment where lower amount of data than in a full catchment is required [8]. Note that when modelling emerging contaminants, the available information is limited, and, therefore, simpler models are usually more useful than complex ones.

In this water quality framework, PhATE and GREAT-ER are specifically designed models to predict realistic distribution of emerging contaminants in a river catchment, including WWTP components as main input of these chemicals in the environment. These models do not take into account contaminant fate and transport into estuarine or marine environments, i.e. they are mainly designed to simulate rivers and lakes within a river basin.

The purpose of this chapter is to describe and provide some applications of emerging contaminant models or EC models. First PhATE and GREAT-ER models are reported, which are widely used tools and particularly designed to simulate the so-called down-the-drain chemicals. Afterwards, other models that researchers used to predict emerging contaminants in the environment are included. This chapter also aims at gathering the hypotheses, difficulties and conclusions that authors found when they applied the models, in particular the most relevant sources of uncertainty when modelling pollutant fate. In this way, the readers can evaluate the suitability of these tools for particular applications.

2 PhATE Model

The PhATE (acronym of Pharmaceutical Assessment and Transport Evaluation) model was developed by PhRMA PIE Task Force and AMEC Earth and Environmental as a tool to predict concentrations of active pharmaceutical ingredients (APIs) in the environment due to patient use at catchment level. This approach facilitates a deeper understanding of the cumulative impact of human activities and allows the suitability evaluation of existing fate information for pharmaceuticals.

Dr. Paul Anderson, from AMEC Earth and Environmental, was a researcher in the emerging pollutant issue when he began with the development of the screening-level model PhATE. He published and presented the model in 2004, as trademark of the Pharmaceutical Research and Manufacturers of America (PhRMA) [3]. PhRMA is the trade organisation in the USA representing the country's leading biopharmaceutical researchers and biotechnology companies. In 1999, PhRMA established a task force for pharmaceuticals in the environment, whose technical representatives from member companies also worked on the development of PhATE model. PhATE was developed using the commercial integrated development environment Microsoft Visual C++, and model input and output data is stored in Microsoft Access database [3].

PhATE model has two main applications: an exposure assessment application estimating PECs of pharmaceuticals in surface waters and a risk assessment application estimating PNECs concerning impacts of human activities. In rational terms, the first approach to fulfil is the exposure assessment application to complete next with the risk assessment purpose.

Overall, the model estimates environmental concentrations of pharmaceuticals in surface waters using a mass balance approach applied to river segments. The unique included input to release pharmaceuticals in surface waters is wastewater treatment plants; thus, no other pollutant discharge mode (i.e. diffuse sources) can be considered in the model. PhATE manages a geographic information system (GIS) in order to show key inputs and outputs at catchment scale.

Initially, the internal model database was based on 11 catchments of the USA, which were selected to represent most hydrologic regions of the country. Around 14% of the US population lives within the selected catchments [3]. The model database provides the data of hydrological, WWTP and drinking water systems of each catchment area. This hydrological information, such as river segment configuration, flow and velocity rates, is obtained from the Better Assessment Science Integrating Point and Nonpoint Sources (BASINS) database from the US Environmental Protection Agency (USEPA). By 2011 the PhATE model accounted for 12 catchments from the USA, integrating 2,712 river stream segments with a total length of 44,398 km and 1,302 WWTPs [9]. Moreover, Park et al. [10] included in PhATE a Korean catchment of the Han River which runs through the heart of the Seoul Metropolitan Area where approximately 22.5 million people live.

The version 4.0 of PhATE incorporates an additional module to estimate PECs in sludge and biosolids [9]. The module generates PECs in WWTP wastewater effluent, sludge and biosolids, so the concentration distribution can be displayed in a catchment area. It is also capable of estimating PECs in amended soils depending on the type of land application.

2.1 PhATE System Description

In order to run the PhATE model, two main categories of data are required: data about the emerging contaminant compound to be evaluated and data about the catchment where the compound shall be evaluated. The summary of key inputs and parameters for the PhATE model is listed below:

1. Compound use and characteristics
   - Physical and chemical properties
   - Toxicological information
   - Usage per capita
   - In-stream first-order loss
   - Human loss (fractional loss prior to WWTP, e.g. metabolism)
   - WWTP and drinking water system (DWS) removal efficiency for each treatment type
2. Catchment characterisation
   - WWTP or publicly owned treatment works (POTW)
     - Name
     - Location
     - WWTP treatment type
     - Population served (number)
     - Flow rate
     - Next segment distance
   - Dams and reservoirs
     - Name
     - Volume
     - Surface area

    - Length
    - Depth

  - River segments

    - System segment number
    - Segment sequence
    - Mean flow
    - Low flow, defined by the 7Q10 (7-day, consecutive low flow with a 10-year return period)
    - Mean flow velocity
    - Low-flow velocity, corresponding to the 7Q10
    - Length
    - Depth
    - Width

The PhATE model is comprised of two modules: the exposure module and the human health effect module.

On the one hand, the exposure module generates PECs of emerging contaminants in surface waters of a given catchment essentially following the principle of mass conservation. The entry pathways of pollutants into the catchment are the WWTPs, and, therefore, the first step of the model is to define the pollutant release from WWTPs.

WWTP discharge loads are estimated according to four parameters: the compound use per capita, the served population by the WWTP, the potential loss of the compound associated with human use (e.g. metabolism) and the compound removal efficiency in the WWTP.

The model considers that the compound per capita use (kg/year/cap) across the USA is homogeneously distributed so regional differences in consumption habits cannot be introduced. Therefore, the model calculates the pollutant compound mass entering into the WWTPs from the annual sale volume (kg/year) and from the proportion of the number of people served by each wastewater plant. In addition, the model assumes that all pharmaceutical sales are consumed by patients, excreted and treated in WWTPs (i.e. disposal through solid waste has to be previously considered by the user).

The removal efficiency in the WWTP of the pollutant compound depends of both the treatment type and compound characteristics. The model considers seven possible treatment types: no discharge, raw discharge, primary treatment, advanced primary treatment, secondary treatment and advanced treatments I and II. Therefore, the loss in WWTPs includes mechanisms such as biological decay and partitioning to sludge. The model divides the compound mass entering and leaving the WWTP by the WWTP flow rate, in order to provide concentration values in the influent and effluent of the plant. In this way, the mass of compound released from the WWTP reaches river segments.

Second approach of the model is to define the in-stream transport and fate of the pollutant. PhATE takes into account the river division provided as an input data and creates a segment sequence where the compound mass balance shall be applied. Each river segment receives the pollutant mass load from its corresponding WWTP (if any) and from its corresponding upstream segment. In the case of reservoirs, PhATE models series of tanks where full-mix hypothesis is considered and where the compound load passes from one tank to another due to advection.

The pollutant mass is lost via in-stream loss mechanisms, i.e. degradation, and via flow diversions. In-stream degradation processes are simulated with a fixed fraction or with the first-order loss rate constant. This constant represents the sum of first-order loss rate constants related to main degradation processes, e.g. biodegradation, hydrolysis, photolysis, evaporation, sedimentation and so forth.

Once again, compound mass is divided by river flow in order to provide concentration values along the catchment. The model predicts these concentrations under mean flow conditions and low-flow conditions. Low-flow conditions are defined by the 7Q10 flow: the lowest consecutive 7-day average flow with a 10-year return period.

On the other hand, the human health effect module aims at estimating PNECs based on toxicology data in relation to human health affected by drinking water and fish consumption. In order to define PNECs, the PhATE model follows the standard human health risk assessment criteria that takes into account the human acceptable daily intake of a compound and the amount of water and fish that humans are assumed to consume.

As previously mentioned, PhATE v 4.0 includes PEC estimation in sludge and biosolids. With this purpose, the module shall determine the contaminant concentration adsorbed and retained in the WWTP sludge and shall also determine the loss of compound during the sludge treatment and conversion to biosolids. Therefore, the module considers API partitioning to primary and secondary solids, degradation related to aqueous phase and loss during the sludge treatment by aerobic or anaerobic digestion and composting processes (or no treatment at all). PhATE databases provide information about biosolid treatments in about 70% of the WWTPs [9].

PhATE may display results as ‘print results’, ‘graph results’ and ‘map results’. The output data is provided by PECs in WWTP effluents, in river segments and, if considered, in drinking water, in biosolids and in land applications and by PNECs as human health index.

2.2 PhATE Model Applications

PhATE model has been used by different researchers to estimate environmental concentrations of contaminants (mainly pharmaceuticals) in surface waters that come from patient consumption of medicines generally in the USA. Table 1 gathers several studies collected from literature where PhATE model has been applied.

Most of the studies included in Table 1 attempt to corroborate the PhATE model utility comparing MECs (measured environmental concentrations) and

Table 1 Reviewed studies with PhATE model

Reference	Catchment	Environment	Contaminants	Assessment approach	Corroboration method
Hannah et al. [11]	11 catchments in the USA	Surface waters	One pharmaceutical (17a-ethinylestradiol)	Exposure	Evaluation of measured concentrations reported in the literature, simple mass balance calculations and predicted concentrations generated by PhATE
Anderson et al. [3]	11 catchments in the USA	Surface waters	Three surrogate compounds (caffeine, linear alkylbenzene sulfonates and triclosan) and 11 APIs	Exposure	A first-phase comparison of field data with model PECs for the same locations on a point-by-point basis. A second-phase comparison of cumulative probability distributions of field-measured concentrations to the distributions of PECs generated by PhATE
Cunningham et al. [12]	11 catchments in the USA	Surface waters	One pharmaceutical (paroxetine)	Risk	–
Cunningham et al. [9]	12 catchments in the USA	WWTP sludge and biosolids	Two surrogate compounds (triclosan and linear alkylbenzene sulfonates) and 18 APIs	Risk	Comparison of measured concentrations reported in the literature and predicted concentrations generated by PhATE
Park et al. [10]	Han River catchment (Korea)	Surface waters	Four pharmaceuticals (acetaminophen, cimetidine, roxithromycin, chloramphenicol)	Exposure	Comparison of point-by-point model data to field data
Cunningham et al. [13]	11 catchments in the USA	Surface waters	44 APIs	Risk	–
Robinson et al. [14]	Aire and Calder catchments in UK and Merrimack, the Mississippi Headwaters and the Lower Colorado River catchments in USA	Surface waters	One API (propranolol hydrochloride)	Exposure	–

Cunningham et al. [15]	11 catchments in the USA	Surface waters	One API (carbamazepine)	Risk	Comparison of PNECs to both MECs and PECs generated by PhATE
Schwab et al. [16]	11 catchments in the USA	Surface waters	26 APIs	Risk	Comparison of PNECs to both MECs from the published literature and to maximum PECs generated by PhATE
Caldwell et al. [17]	US catchments	Drinking water	Estrogens	Risk	–
Anderson et al. [18]	12 catchments in the USA	Surface waters	Four steroid estrogens (estrone, 17β-estradiol, 17α-ethinyl estradiol and estriol)	Risk	–
Hosseini et al. [19]	Grand River catchment (Canada)	Surface waters	Four pharmaceuticals (ibuprofen, naproxen, carbamazepine and gemfibrozil)	Exposure	Calibration and validation of the model parameters with previously measured concentrations

PECs generated by the model. The model provides PEC results by their probabilistic distribution. If field measurements are limited, the median or 50th percentile may be used to compare PECs with MECs. However, the 90th percentile value of predicted concentrations in low-flow conditions may provide a conservative exposure estimation useful for risk assessment approaches. Some authors found that the 90^{th} percentile estimations are one or two orders of magnitude higher than the average concentrations [11].

Studies included in Table 1 express different accuracy level in the results obtained from PhATE model application. The ratio of the median PEC to the median MEC is favourable for most APIs with high detection frequency: 0.29 to 2.5. Nevertheless, a deviation of more than one order of magnitude may also result for some of these APIs [9]. Most APIs with low detection frequency may be considered consistent because PECs are less or equal to the method reporting limits of the APIs [9, 11]. Simulations of surrogate compounds show that PECs generated by PhATE are generally within an order of magnitude of measured concentrations [3, 10]. In risk assessment results, when PECs are compared to PNECs, small safety factors are concluded (SF = PEC/PNEC), between 3×10^{-8} and 3×10^{-3} [12].

Cunningham et al. [9] included sludge and biosolids in their application. Although the main fraction of the entering compound in the plant is completely transformed in treatments or released through effluent water, another fraction may remain in treatment sludge and therefore end up in biosolids. This fact leads to care about risk exposure of animals, plants and humans that may come into contact with such soils, and prediction of contaminant fate in sludge or biosolids may result of concern. Cunningham et al. [9] proved the utility of the model on predicting contaminant concentrations in biosolid compartment instead of surface water compartment.

Some studies conclude that model parameters need to be adjusted due to the differences between measured and predicted concentrations. Anderson et al. [3] considered the overestimation of in-stream decay constant for triclosan and also considered that untreated water discharges from WWTPs during heavy rainfall events may cause PhATE to underestimate predictions in certain areas. When PhATE overestimated PECs, it was considered that the WWTP removal rate had to be higher and that the excretion rate for same pharmaceutical was too conservative [10]. If reported measured concentrations were higher than the 99th percentile PECs, methodological problems or unusual environmental circumstances were also considered [11].

The studies gathered in Table 1 also found some impediments in the application of PhATE model, most of them related to the necessity of introducing a lot of accurate data into the model. Firstly, researchers find problems defining parameters related to emerging contaminants, such as their in-stream decay constant, removal rate in WWTPs, human metabolism rate and other physicochemical properties such as partition coefficient. These parameters are determined experimentally on the field or laboratory, but this practice is possible just for certain compounds and under certain conditions due to their high cost-effective relation. When experimental data is not published, other models are used to estimate compound parameters, such as EPI Suit and SimpleTreat [9, 10, 12].

The EPI (Estimation Program Interface) Suite is a Windows-based suite of physical/chemical properties and environmental fate estimation programs developed by the EPA's Office of Pollution Prevention Toxics and Syracuse Research Corporation (SRC). When parameters for the PhATE biosolid module are not available, SimpleTreat 3.1 may be useful. SimpleTreat is a multimedia mass balance model, a spreadsheet based on box model with fugacity approach, which predicts the fate of an organic xenobiotic in a WWTP [20]. Its full description can be consulted in the handbook of Struijs [21].

As previously mentioned, the compound degradation in river segments can be simulated by a fixed fraction or by a first-order in-stream loss. The first-order decay constant attempts to simulate real degradation process in water bodies, but, at the same time, it is harder to be estimated [10]. In some occasions, a zero value was adopted for in-stream decay constant due to the absence of experimental data for paroxetine [12]. The PhATE decay constant is the sum of first-order loss rate constants related to main degradation processes. Thus, the PhATE model is not capable of identifying which key environmental processes (e.g. biodegradation, hydrolysis, photolysis, evaporation, sedimentation, etc.) affect significantly to the contaminant attenuation [22].

Some research works found limitations in the development of their study due to the small amount of available flow data. Consequently, it was not possible to simulate low-flow conditions, and the model was run only under mean flow conditions [10].

Considering PhATE model simulation approach and results in application studies, it is clear that the emerging contaminant per capita use is a key input parameter and that concentration predictions are highly dependent to this parameter [11]. Authors express the advantage of including regional and demographic patterns in water and pharmaceutical consumptions to obtain more realistic contaminant distributions [11]. At the same time, WWTP removal efficiency, which directly affects to the contaminant release in the water body, affects to all predictions in the catchment equally, whereas changes in in-stream degradation constant are prominent in river segments far from WWTP effluents where travel times are long enough to allow significant contaminant degradation [3].

Field measurements of emerging contaminant concentrations are essential for model corroboration and for developing suitable model input parameters [11]. Cunningham et al. [9] pointed the necessity of investing in biosolid fate mechanisms, because the aerobic, anaerobic and composting treatments have a direct impact on result accuracy.

Overall, studies conclude that PhATE model has the potential to estimate environmental concentrations in surface waters under a range of streamflows and WWTP inputs, even in wastewater plant biosolids [3, 9, 11]. The model is particularly useful when analytical methods are not sensitive enough to detect the compounds in field [3], and the PhATE model application allows a potential environmental risk assessment as a result of PEC and PNEC comparison [12].

3 GREAT-ER Model

The GREAT-ER (Georeferenced Regional Exposure Assessment Tool for European Rivers) model is an aquatic exposure prediction tool for down-the-drain chemicals within environmental risk assessment schemes and river basin management. This EC model predicts concentrations of chemicals in surface waters under a geographic information system, originally for a set of European catchment areas [23]. GREAT-ER was sponsored by the Environmental Risk Assessment Steering Committee (ERASM) of the Association Internationale de la Savonnerie, de la Détergence et des Produits d'Entretien (AISE) and the Comité Européen des Agents de Surface et Intermédiares Organiques (CESIO), in cooperation with the UK Environment Agency and Yorkshire Water. The project was managed by a task force of ECETOC (European Centre for Ecotoxicology and Toxicology of Chemicals) who developed and validated the model [8].

GREAT-ER is capable of simulating pollutants whose main emission pathways into the environment are point sources, that is, chemicals consumed by humans and released through WWTPs in surface waters [7, 23]. River segments not affected by WWTPs can also be introduced in the river network including boundary conditions.

GREAT-ER is defined as a steady-state deterministic EC model with a stochastic approach (via Monte Carlo simulations) applied on top of this. The deterministic approach simulates emerging contaminant emission, transport in sewers, treatment in WWTPs and chemical transport and transformation in rivers. The model generates statistical distributions of PECs, and the spatial distribution of concentrations is visualised in colour-coded river maps. The GIS tool also allows identifying river segments with PEC values exceeding PNECs. In addition, an overall catchment approach can be calculated with several weighting methods, which consider river segment volume, length or flow increments.

3.1 GREAT-ER System Description

Input data is introduced into GREAT-ER model both through text files and through a specific interface.

On the one hand, the model requires seven text files that represent the catchment characteristics to be implemented:

- River digital network: this file includes river segments with an identification number and geographic coordinates of segments points.
- River network attributes: this file includes hydrogeological properties of each river segment, that is, water flow, speed, segment length and depth.
- Discharge points: WWTP data is included within this file, which includes geographical location, served population, treated flow, treatment type and discharge river segment.

- Lakes: this file identifies the lakes and reservoirs included in the catchment model.
- Images: this file allows the introduction of catchment images into the model.
- Background: a geographical background layer can be also introduced.

River network segmentation should take into account different factors, such as homogeneous environmental conditions, constant flow distributions within each stretch, location of WWTPs and river confluences and bifurcations [7].

On the other hand, the model interface requires the information summarised below in reference to the chemical behaviour and to the simulation process:

1. Chemical data:
    - Identification
    - Physicochemical properties
    - Removal in WWTP according to treatment type
    - In-stream removal
    - Chemical consumption and emission (domestic emission, non-domestic emission or land runoff emission)
2. Simulation data:
    - Boundary concentration conditions
    - Monte Carlo shots
    - Simulation mode

GREAT-ER model facilitates both deterministic and stochastic approaches. The deterministic approach simulates a specific percentile value taken from the frequency distribution of input data and, therefore, generating a unique output. Some authors consider that the use of the 90th percentile value of the low-flow predicted concentrations from GREAT-ER is considered to provide a conservative estimation for risk assessment purposes [11]. Alternatively, the stochastic approach runs probabilistic simulations based on Monte Carlo generation method in order to take into account temporal variability and uncertainty of input data.

The deterministic approach, which is the core of the model, considers three operation modes to simulate chemical transport and fate which represent the complexity grade that the user pretends to assume. The simplest mode is Mode 1, where the following main steps are simulated [24]:

- Estimation of chemical emission into WWTPs: the chemical load (g/s) at the entry of a WWTP is calculated based on chemical market consumption data (kg/inhab/year, which is a unique parameter for the whole catchment) and population served by the plant (inhabitants, which is a particular parameter for each WWTP). The chemical metabolism in the human body is not integrated in the model; thus, the user shall consider this load loss (e.g. excretion rate) when estimating the chemical load into the WWTP.
- Estimation of chemical removal in the WWTPs: the WWTP efficiency is simulated with a removal rate distribution that is applied to the chemical

emission. This rate may be obtained considering the removal of each treatment (primary settler, active sludge treatment and tricking filter) or with a unique and global removal rate, which in any case depends on the chemical. The WWTP removal rate is applied to the chemical emission, and the result represents the chemical release in rivers.

- Simulation of in-stream transport and fate: a mass balance approach is applied through river segments; hence, each river segment or stretch receives flow and quality data from upstream segment, from tributaries (if any) and WWTP load discharge (if any). Moreover, a first-order decay model is used to calculate mass fluxes (g/s). The first-order decay model considers chemical degradation depending on the travel time in the stretch and the natural degradation constant. The resulted mass load in each stretch is combined with the stretch water flow to generate concentration outputs.
- Simulation results: the model outputs are summarised in terms of concentration distributions. Both tabulated values and graphical distribution of PECs are generated, including concentration profiles along particular river stretches. These profiles may illustrate chemical fate from a river's headwater down to its mouth and they can be exported [25]. In order to express the concentration variability, PEC quartiles are provided. The GIS analysis allows identifying the locations within a region where PEC values exceed PNEC values. Moreover, external water quality maps may be overlaid onto the model outputs to compare chemical concentrations with other quality indices.

GREAT-ER Mode 2 includes contaminant degradation in the sewer system calculated with a fixed removal factor. Within this mode, WWTP removal efficiency is calculated with a more complex multimedia model called SimpleBox [26]. The in-stream decay constant is calculated distinguishing degradation, sedimentation and volatilisation processes.

GREAT-ER Mode 3 uses the same models as in Mode 2 for sewer and WWTP systems. But, in addition, in-stream decay constant adds the possibility of biodegradation, hydrolysis and photolysis processes to previous ones.

As happens in the PhATE model, WWTP removal factor can be predicted using the SimpleTreat model, which is currently also used in EUSES [25].

The normal, lognormal or uniform distributions can be introduced for WWTP removal efficiency and chemical consumption data parameters. In addition, mean and 5th percentile values shall be introduced when describing water flow, speed and depth of river segments. These statistical distributions facilitate Monte Carlo simulation. In Monte Carlo simulation, a certain number of 'shots' are generated and randomly select input values from the statistical distributions. For each of these 'shots', the deterministic model is applied with the particular selected conditions and an output value is generated. The ensemble of discrete outputs or results are statistically analysed to provide concentration distributions. This stochastic approach takes into account temporal variability, such as seasonality of river flows, and uncertainty, due to the difficulty to estimate certain parameters of input data [7]. The Monte Carlo approach generally requires about 1,000 ‘shots’ or runs to guarantee a reasonable convergence [10].

3.2 GREAT-ER Model Applications

GREAT-ER model, like PhATE model, may be applied for an exposure assessment study or for a risk assessment study. GREAT-ER is capable of identifying the river segments exceeding a fixed concentration value defined by the user, which can be used to represent compound PNEC calculated by other sources.

Table 2 gathers studies collected from literature that use GREAT-ER model in order to predict concentrations of down-the-drain contaminants. Authors reporting on behalf of ECETOC GREAT-ER team are not included in the table, such as Holt et al. [38], Feijtel et al. [2], Schowanek and Webb [25, 39] and Verdonck et al. [40]. These studies aim at validating GREAT-ER model at different European catchments: four catchments in the UK (Aire, Calder, Don and Went Rivers), one in Italy (Lambro River), one in Belgium (Rupel River) and one in Germany (Rur River). Large monitoring programmes were performed in order to evaluate the model. In some cases, data on dissolved oxygen, biochemical oxygen demand and ammonia was used to calibrate the model [2].

The main purpose of studies in Table 2 is to predict concentrations of emerging contaminants, such as pharmaceuticals and detergent ingredients, in surface waters in Europe. Catchments in Switzerland (Glatt River) [23] and Spain (Llobregat River) [37] have also been constructed. Most authors evaluated the GREAT-ER model comparing predicted concentrations with measured concentrations in monitoring campaigns. Hannah et al. [11] attempted a model evaluation calculating a mass balance of the contaminant in WWTP effluent. The model applications are always performed assuming that the only source of contaminants is the WWTP discharge after human consumption. The healthcare company IMS Health has been used in order to estimate pharmaceutical sales [11, 23]. In general, contaminant degradation fractions are estimated from other literature data or laboratory exercises.

Hydrological data can be introduced setting fix flow values in order to obtain deterministic results or providing mean flow and 5th percentile flow in order to consider flow variability and uncertainty in a stochastic approach. In general, flow data is known in river segments where gauging stations exist; in the rest of river stretches, flow values can be interpolated [23] or other hydrological models can be used to estimate flow conditions [24]. Flow velocities can also be estimated usually assuming uniform flow conditions and through different energy loss equations, such as Strickler [24] or Manning formula [37]. In Aldekoa et al. [37], also the river hydraulic characteristics were estimated using geomorphological regressions with the water flow, slope and drained area. Johnson et al. [24] applied LF2000-WQX water quality model to predict the concentrations of the pharmaceuticals diclofenac and propranolol in rural Tamar (UK) catchment. This model is an extension of the

Table 2 Reviewed studies with GREAT-ER model

Reference	Catchment	Contaminants	Assessment approach	Corroboration method
Hannah et al. [11]	Five catchments in Germany, Italy and the United Kingdom	One pharmaceutical (17a-ethinyl estradiol)	Exposure	Evaluation of measured concentrations reported in the literature, simple mass balance calculations and predicted concentrations generated by GREAT-ER
Johnson et al. [24]	Tamar River catchment (UK)	Two surrogate compounds (chloride and orthophosphate) and two pharmaceuticals (diclofenac and propranolol)	Risk	Comparison of measured concentrations by analytical methods and predicted concentrations generated by GREAT-ER just for surrogate compounds
Alder et al. [23]	Glatt Valley catchment (Switzerland)	Four b-blockers (atenolol, sotalol, metoprolol, propranolol)	Exposure	Comparison of measured concentrations from monitoring sites and predicted concentrations generated by GREAT-ER
Klasmeier and Matthies [27]	River Main catchment (Germany)	One detergent ingredient (LAS), one musk fragrance (HHCB) and one pharmaceutical (diclofenac)	Exposure	Comparison of measured concentrations from monitoring samples and predicted concentrations generated by GREAT-ER
Cunningham et al. [13]	10 catchments in France, Belgium, Germany, Italy, the Netherlands and the United Kingdom	44 APIs	Risk	–
Robinson et al. [14]	Aire and Calder catchments (UK)	One API (propranolol hydrochloride)	Exposure	–
Cunningham et al. [15]	10 catchments in Belgium, France, Germany, Italy, the Netherlands and the United Kingdom	One API (carbamazepine)	Risk	Comparison of PNECs to both MECs and PECs generated by PhATE

(continued)

Table 2 (continued)

Reference	Catchment	Contaminants	Assessment approach	Corroboration method
Price et al. [28]	Aire, Calder, Went and Rother catchments (UK)	One detergent ingredient (LAS)	Exposure	Comparison of measured concentrations reported in literature and predicted concentrations generated by GREAT-ER
Wind et al. [29]	Itter River catchment (Germany)	Two detergent ingredients (boron and LAS) and three household product ingredients (EDTA, NTA and Triclosan)	Exposure	Comparison of measured concentrations from monitoring samples and predicted concentrations generated by GREAT-ER
Johnson et al. [30]	Aire and Calder catchment (UK)	One cytotoxic drug (5-fluorouracil)	Risk	–
Hüffmeyer et al. [31]	Ruhr River catchment (Germany)	One trace metal (zinc)	Exposure	Comparison of measured concentrations from monitoring samples and predicted concentrations generated by GREAT-ER
Schröder et al. [32]	Itter River catchment (Germany)	Two detergent ingredients (boron and LAS)	Exposure	Comparison of measured concentrations from monitoring samples and predicted concentrations generated by GREAT-ER
Schulze and Matthies [33]	Ruhr River catchment (Germany)	Two detergent ingredients (boron and LAS) and two household product ingredients (EDTA and NTA)	Exposure	Comparison of measured concentrations from monitoring samples and predicted concentrations generated by GREAT-ER
Sabaliunas et al. [34]	Aire River catchment (UK)	One household product ingredient (triclosan)	Exposure	–
Price et al. [35]	Two catchments in England	One household product ingredient (triclosan)	Risk	–

(continued)

Table 2 (continued)

Reference	Catchment	Contaminants	Assessment approach	Corroboration method
Sumpter et al. [36]	–	Four endocrine disrupting chemicals (estrone, estradiol, ethinyl estradiol and nonylphenol)	Risk	–
Aldekoa et al. [37]	Llobregat River catchment (Spain)	One pharmaceutical (diclofenac)	Exposure	Comparison of measured concentrations from monitoring samples and predicted concentrations generated by GREAT-ER

Low Flows 2000 [41] software system which is a GIS software that characterise river flows at gauged and ungauged sites, and, in particular, LF2000-WQX tool calls the GREAT-ER module in order to apply its mass balance formula for water quality prediction [24].

The impediments found among literature in GREAT-ER applications are similar to the problems expressed by the authors for PhATE model applications. In general, GREAT-ER is able of predicting contaminant concentrations in surface waters within a factor of 2–3 comparing to measured concentrations [11, 23, 27, 42]. Most authors state that the model has been successfully applied [23, 24, 27, 40]. However, they also find information on pharmaceutical degradation in rivers scarce or inaccurate. They express that the compilation of reliable data sets implies great time-consume, and, therefore, simulations with nondegradable compounds may be assumed [23, 27].

The model considers a uniform spatial distribution of pharmaceutical consumption among the catchment. However, some authors appear the necessity of considering spatial consumption variation through population, due to possible differences in pharmaceutical consumption between residential population and transient or employee population [23] or even due to the possible high pharmaceutical sales in rural areas where older people may predominate [24]. In any case, it is hard to estimate contaminant emission without uncertainties, as well as estimate unused drugs that do not reach wastewater.

The predicted concentrations by GREAT-ER may also be affected by temporal fluctuation of different factors, such as temporally variable emission patterns, WWTP removal efficiency, alteration on in-stream degradation, variable river flows and singular high rain events which may cause a bypass flow of the WWTP (the so-called CSOs: combined sewer overflows). These temporal variations are difficult to define and may induce overestimations or underestimations on predicted contaminant concentrations.

Verdonck et al. [40] concluded that it is possible to simplify the river network without a big loss of accuracy in the results. Their study proved that it was possible to reduce effort regarding to geographical data collection and simulate a partially simplified river network obtaining acceptable predicted concentrations at catchment scale. Nevertheless, major tributaries of the river should be included in the model in order to consider most contaminant potential sources [27].

GREAT-ER model applications conclude that when the contaminant has been investigated in laboratory experiments in order to define with accuracy its behaviour, or when the contaminant is defined as persistent chemicals, the model is capable of estimating distributed average exposure concentrations with enough accuracy. On the contrary, monitoring data in space and time should be used for model calibration purposes, especially to assess point sources and investigate degradation rates under certain spatial and temporal circumstances.

4 Other Models

4.1 WASP Model

The Water Quality Analysis Simulation Program (WASP) is a broadly used water quality model developed by the USEPA and presented by Di Toro et al. [43]. WASP aims at modelling contaminant fate and transport in surface waters within a pollution management framework and with a flexible compartmental approach which allows addressing problems in one, two or three dimensions.

The hydrodynamic module of WASP relies on the continuity equation and the kinematic wave equation in order to simulate one-dimensional water flows, and it is able of performing steady and unsteady flows. The water quality module, in addition to conventional pollution, also simulates toxic pollution involving organic chemicals, metals and sediments. The model simulates advection, dispersion, point and diffuse mass loading and boundary exchange processes in time. The model assumes completely mixed segments, and, therefore, proper segmentation is required as in PhATE and GREAT-ER models. Fate mechanisms in WASP take into account transformation processes as volatilisation, hydrolysis, photolysis and biodegradation [22].

Overall, WASP has been used in studies regarding biochemical oxygen demand, dissolved oxygen dynamics, nutrients and eutrophication, bacterial contamination and organic chemical and heavy metal contamination. The WASP model has occasionally been used as an EC model (i.e. to model emerging contaminants): Rygwelski et al. [44] developed a screening-level model for atrazine pesticide, and Arlos [22] modelled selected antiandrogens and pharmaceuticals.

The aim in Arlos [22] was to predict transport processes and fate mechanisms using the organic toxicant subroutine in WASP. In this study, a model of the portion of river affected by a major WWTP discharge was constructed with ten river segments. As WASP model does not include WWTP components, the major plant effluent was modelled as an extra river segment discharging in the main river.

The model was initially run only with advection and dispersion conditions, and hydro-geometric properties related to advective transport were calibrated with site-specific conditions. The compounds sorbed onto suspended solids were also considered with WASP solid transport module. No transfer and transformation mechanisms were added when the target compounds were accurately enough represented by the model. Otherwise, fate mechanisms (volatilisation, hydrolysis, photolysis and biodegradation) were integrated through a stepwise fitting: processes considered to be the least significant were added first, and according to resulted and measured concentrations, other processes were added. This procedure facilitated corroborating most significant fate mechanisms for each contaminant. After calibration process, the model was subjected to validation with independent data set of measured concentrations. The model results pointed out which fate processes affected notably which contaminants. For example, carbamazepine pharmaceutical appears to be persistent in surface waters, and sorption and biodegradation mechanisms were found to be quite irrelevant, whereas photolysis had greater impact in most target compounds, especially in the antiandrogenic personal care product triclosan ([22]).

4.2 SWMM Model

The Storm Water Management Model (SWMM) was originally developed for the USEPA and first presented by Metcalf et al. [45] as an urban runoff analysis model. It is defined as a hydrology-hydraulic water quality tool used for single-event or long-term simulation of runoff mainly in urban areas, although Jang et al. [46] proved the model suitability in natural watersheds [46]. The model basically generates runoff and (nonpoint) contaminant load in sub-catchments, which are conducted into the sewer system mainly formed of pipes, channels, storages and treatment devices. This process is performed through four modules: runoff, transport, extran and storage/treatment modules. SWMM relies on full Saint-Venant equations to simulate one-dimensional unsteady flow, and in order to simulate solute transport applies a mass balance calculation including chemical decay and complete mix assumption in each system element.

Since the SWMM model release, it has been used widely by different authors throughout the world primarily in sewer system applications. However, particularly Park et al. [10] used SWMM as an EC model to approach exposure assessment of emerging contaminants in a Korean watershed. These authors constructed a model of the Kyungahn Stream (a major branch of the Han River, Korea) to simulate veterinary pharmaceutical concentrations generated by the runoff of livestock farming areas. Kyungahn Stream supplies part of Seoul metropolitan area with drinking water and includes both rural and suburban areas. The model consisted on 11 sub-catchments and 16 conduits which represented the target basin. Hydrogeological data was gathered from literature and experimental practices. The model was run under the runoff and transport modules and afterwards calibrated and validated with independent sampling data. The results showed acceptable performance both for flow rates and contaminant prediction. However, discrepancies for two particular pharmaceuticals (sulfamethoxazole and trimethoprim) were remarkable, mainly due to the contribution of WWTP which was not considered by SWMM. These two contaminants are consumed by humans and used for animals [10]. Consequently, Park et al. [10] performed further modelling for these two pharmaceuticals adding PhATE predictions of WWTP discharges to Kim results. The concentrations predicted by two models were arithmetically added for each of the pharmaceuticals. Therefore, the previously underestimated values for SWMM- and PhATE-independent applications had a better fit with measured data when both results were integrated.

4.3 EUSES Model

The European Union System for the Evaluation of Substances (EUSES) was developed to evaluate risks generated by new and existing chemical substances and biocides to man and the environment (EC 1996). The system is based on the methods and hypotheses of the EU Technical Guidance Documents for risk assessment. EUSES is a successor of USES (Uniform System for the Evaluation of Substances) tool developed by the European Commission to the National Institute of Public Health and the Environment (RIVM, 1994). Since it was released, EUSES has been used regularly for some years in the EU as a risk assessment tool [42].

EUSES structure gathers five modules: input, emission, distribution, exposure, effect and risk characterisation module. The distribution module aims at estimating concentrations of a substance in the relevant environmental compartment (air, surface water, marine water, sediment, soil and groundwater). The distribution module takes into account: partition coefficients, degradation rates, treatment in WWTPs, regional distribution (includes point and diffuse contaminant sources) and local distribution. Local models are used in each compartment, such as SimpleTreat [47] for WWTP modelling, and for regional analysis SimpleBox [26] is used, which is a Mackay level III steady-state multimedia model.

The model requires input data of the substance (physicochemical properties, partition coefficients and degradation and transformation rates) and of the compartments (area, height or depth, residence time and other fractions). In any case, comparing to other georeferenced models, the information required in EUSES is significantly lower.

EUSES is not specially designed for site-specific assessments [48]. Even if model default parameters may be modified according to local or regional conditions, it does not account for spatial or temporal distributed simulations. The model calculates regional PECs (homogeneously distributed regional environmental concentrations) and local PECs (reasonable worst-case concentrations around a local point source), but it is not able of detecting high exposure or risk points in a catchment area.

The model has been used several times in order to predict exposure and risks of emerging contaminants as an EC model [42, 48–52]. Kawamoto et al. [50] simultaneously used EUSES and ChemCAN tools to predict 68 chemicals in Japan. The results showed that the predicted environmental concentrations by the two models agree adequately. PECs were consistent just with the lower range of concentrations measured in the environment. The authors justified the low accuracy in estimating concentrations mainly due to uncertainties of emission rates and degradation parameters. These uncertainties are also manifested in non-multimedia generic models. Wind [42] compared EUSES (nonspatial) and GREAT-ER (georeferenced) model prediction capabilities. Overall, the author concluded that generic models such as EUSES seem less capable to predict local environmental concentrations due to the safety margins included in regional parameters. EUSES tended to generate higher PECs than MECs, whereas GREAT-ER predicted compound distribution accurately enough without underestimating MECs. On the contrary, GREAT-ER georeferenced model requires greater effort in collecting input data [42].

4.4 QUAL2E Model

QUAL2E model (Bowden and Brown 1984) is quasi-dynamic water quality model developed by the USEPA and based on the one-dimensional advection-dispersion mass transport equation. It is not a full dynamic model because it simulates temporal changes in flow conditions along the stream by computing a series of steady-state water surface profiles. The model can calculate waste load allocations and total maximum daily loads in order to study the impacts along the main river channel.

The model estimates 15 element concentrations including traditional water quality components (DO, BOD, temperature, algae, nutrients, etc.) and generic conservative and non-conservative components. These last components may simulate fate and transport of emerging contaminants [8]. Fate mechanisms for non-conservative compounds are simulated using a first-order decay process. However, QUAL2E was not originally designed to predict down-the-drain chemicals, and, thus, the model lacks of WWTP removal simulation component. The entry pathway of pollutants in surface waters (point source load) is represented directly by discharge concentrations. QUAL2E has been mainly used to model traditional water quality components [53–56] and only occasionally used to predict emerging contaminants in rivers [57, 58].

McAvoy et al. [57] predicted the impacts caused by conventional pollutants and by consumer product ingredients (LAS) with QUAL2E in surface waters of Balatuin River (Philippines) that receive untreated wastewater. 17.7 km of river were divided in 12 reaches, and input data for the model (river flow, velocity, dispersion coefficients, wastewater discharge concentrations, LAS loss, etc.) was calculated from field or laboratory measurements. Model output was validated with laboratory and field data. QUAL2E is able to perform uncertainty analysis, and, thus, the robustness of the model was demonstrated using the enhancement QUAL2EUNCAS. The model provided most outputs in good agreement with observed field values for conventional pollutants as well as for LAS pollutants. Therefore, the author concluded the reliability of QUAL2E model to assess fate and risk of consumer product ingredients in surface waters receiving untreated wastewater. Finally, a risk assessment study was approached considering the effects of LAS in the self-purification process of the receiving water and in the aquatic organisms. The result was the predicted exposure concentrations remained below the determined no-effect level for the purification process and below the predicted no-effect concentrations for aquatic organisms [57].

4.5 ChemCAN Model

ChemCAN model was developed by Mackay et al. [6] and supported by Health Canada as a human exposure assessment tool. It estimates average concentrations of chemicals in different environmental media (air, surface water, soil, sediment, groundwater, coastal water, fish and vegetation) in 24 regions of Canada, although the user can define local parameters for new regions. ChemCAN is a steady-state Mackay level III model which predicts chemical fate according to mass balance equations. The degradation of organic compounds is represented by a first-order decay.

ChemCAN has been mainly used to predict chemical fate in Canada regions. In particular, some authors attempted to apply the model to emerging contaminant simulations: Managaki et al. [59] predicted the distribution of the industrial chemical hexabromocyclododecane in the sediment of three Japanese rivers; Mackay et al. [6] predicted chlorobenzene and the detergent ingredient LAS in the southern Ontario; and Webster et al. [52] studied the effects of regional differences in the fate of several industrial chemicals including pesticides, such as atrazine.

4.6 AQUASIM Model

AQUASIM model was developed by the EAWAG Swiss aquatic research institute which allows performing simulations, estimation of model parameters and uncertainty or sensitivity analyses using measured data. The model simulates the spatial configuration as a set of compartments which are connected by advective or diffusive links. Reichert [60] provides further information of the model capabilities.

This model has been used in Switzerland as an EC model to simulate degradation processes of emerging contaminants, such as the pharmaceutical diclofenac, the personal care product triclosan and fluorescent whitening agents [61–64]. In these cases, photodegradation was the main elimination process and, in order to estimate the photochemical rates of the compounds, the GCSOLAR program was used. GCSOLAR is an exposure assessment tool of the USEPA that calculates photolysis rates and half-lives of pollutants in the aquatic environment [65].

5 Uncertainty in Modelling Emerging Pollutants

No matter which model we use, we will always have some degree of uncertainty in our model outcomes. Uncertainty stems from five main sources [66, 67]:

- Intrinsic uncertainty. Uncertainty regarding the predictability (e.g. chaotic or stochastic behaviour) of the system or effect.
- Natural variation. The natural systems change in time and place and so do the parameters of interest. This kind of uncertainty can be quantified estimating the possible ranges and relative probabilities of the unknown quantities.
- Measurement error: Measurement error causes uncertainty about the value of the measured quantity. This is quite important for emerging contaminant modelling, because background concentrations are usually very low (nanogram per litre scale) and the analytical procedures to be applied are often complex due to matrix variability (e.g. samples from the inlet and outlet of WWTPs). Fortunately, most papers dealing with occurrence of emerging contaminants inform about the uncertainty of measurements, which is less than 10% (in terms of standard deviation) in most occasions. This information can then be incorporated on estimation of uncertainty propagation in model exercises.
- Systematic error in the measurements: It results from a bias in the sampling and is more difficult to quantify or even notice. This is also of fundamental importance for micro-pollutants, particularly when a mass balance in an engineered system like a WWTP has to be calculated. For instance, Ort et al. [68] demonstrated that the sampling procedure (particularly sampling frequency) affected the calculated WWTP removal efficiency. This kind of uncertainties are very difficult to deal with, so it is extremely important to know all sampling procedures of the data analysed before using them for model calibration or validation.
- Model uncertainty. Models are always abstractions of the natural or engineered system, so a number of variables and interactions are always left out, and mathematical formulations may not adequately describe the underlying physical or chemical processes. Current state of the art may also have insufficient knowledge about relevant processes, the shapes of the functions or the associated parameter values. Uncertainty of the model parameters can be accounted for in probabilistic models much the same way as natural variation, with careful consideration of the range of possible values and their probabilities, while uncertainty about the model's structure is often very difficult to quantify.

Uncertainty arising from the numerical resolution of the model (e.g. rounding errors) can also be placed in this category.

When constructing a numerical model, many types of uncertainty are typically present, and it is often impossible to distinguish the various sources of uncertainty. In uncertainty assessment studies, those different sources are often assigned to four main uncertainty types: input uncertainty, model structure uncertainty, parameter uncertainty, and model technical uncertainty. There are many methodologies for supporting uncertainty assessment. Refsgaard et al. [66] identified 14 main methods and tools, including among others error propagation equations, inverse modelling, Monte Carlo analysis, multiple model simulation, or sensitivity analysis.

Despite the availability of tools and the conceptual framework to perform precise uncertainty assessments, it is frequent that models for emerging pollutants do not include uncertainty bounds in model outcomes, or only very simplistic and even naive uncertainty assessments are reported. This is frequently the case for complex dynamical models that ask for tens of adjustable parameters and many observational data series to be used as inputs to the model. In those cases, the tools to perform a solid uncertainty analysis can be complex as well (e.g. [69]), sometimes beyond the expertise of scientific teams in charge and the resources of supporting scientific projects.

6 Sources of Uncertainty: An Inventory Across Engineered and Natural Systems

Apart from the ultimate sources of uncertainty presented in the last section, a very pertinent question is where uncertainty accumulates in the continuum of engineered and natural systems that models for emerging pollutants must include in their structure. In this section, we introduce a first answer to this question performing a field data-driven analysis of the uncertainty propagated to modelled contaminant river concentration by the three basic processes included in models for human pharmaceutical fate: pharmaceutical emission by population, contaminant removal in WWTPs and removal in river reaches. We selected human pharmaceuticals for this analysis to avoid the complexity of diffuse pollution associated to other types of emerging pollutants, to keep the underlying model as simple as possible.

We considered a very simple model in which the load of a given pharmaceutical in a river reach is the result of human emission and subsequent removal in engineered and natural systems:

$$\text{Human emission} \times \text{WWTP removal} \times \text{river removal} = \text{load in rivers}$$

This is a linear model with a simple mathematical structure allowing a direct propagation of the uncertainty arising from modelling the three basic steps of contaminant fate. The following is a succinct explanation of the processes involved at each step and how they were modelled.

Human emission. We calculated human emissions for 51 pharmaceuticals using data from 68 samplings in ten Spanish WWTPs compiled by Mira Petrovic and Damià Barceló's team at the Catalan Institute for Water Research (Girona, Spain). We calculated apparent human emissions using the inflow concentration at the WWTPs, the operational discharge and the operational population equivalents for each WWTP. The emissions calculated in this way are in fact apparent emissions lumping a number of processes: actual pharmaceutical consumption per capita, elimination from human bodies and potential transformation processes in sewers during travelling to the WWTP.

Removal in WWTP. We calculated percent removal in WWTP with the information coming from same sampling mentioned above, using the inflow and outflow loads from each WWTP and sampling date. Time-integrated samples were used to calculate the mass balances for the 51 pharmaceuticals.

Removal in river reaches. Removal in rivers was calculated from data in Acuña et al. [70], where they calculated removal rates in four different river reaches across Spain for 21 of the 51 pharmaceuticals we used in the other steps. From this data, we calculated retention in nominal reaches with travel time of 2 h. We preferred to use this source and not a wider range of literature data to work with data from the same climatic region and using databases for pharmaceuticals coming from the same lab. However, this implies that the amount of data for river removal is not comparable with the amount of data used in the other terms, so any comparison should be taken with caution.

Then, we estimated the relative contribution of the different processes to the uncertainty of modelled load in rivers normalising all emissions and removal values and computing the standard deviation of these normalised quantities. Since we normalised emissions and removals and the model is a simple multiplication of terms, uncertainties calculated in this way can be directly compared between the different sources. Of course, we cannot differentiate between fundamental sources of uncertainties within each considered process: therefore, the uncertainties reported here should be considered as an inextricable mix of input and model uncertainty, together with variability between different sites.

The apparent emission ranged between 0.1 and 1,000 mg person^{-1} year^{-1}, and distributions were very contrasting among pharmaceuticals (Fig. 1). Most distributions showed ranges that spanned at least one order of magnitude, and distributions spanning two orders of magnitude were not uncommon. As for retention in WWTP, results showed that this process is quite variable for single compounds, suggesting that the dynamics of retention can vary a lot between different WWTPs (Fig. 2). However, several studies have shown that disentangling the dependence of retention on operational parameters and technology available in WWTP is difficult [71], so we cannot expect huge reductions in uncertainty with further refining of our rough WWTP model, which basically considers a homogeneous removal across WWTPs. Finally, mean retention in rivers showed a similar picture (Fig. 3), with more variability for single pharmaceuticals than among them, also suggesting

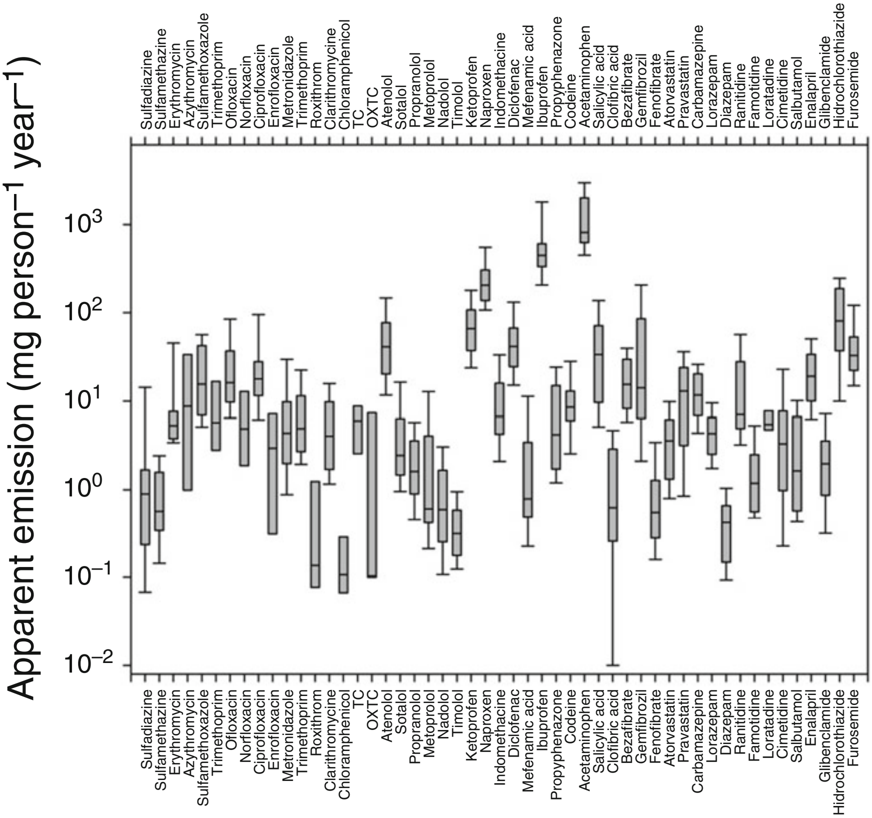

Fig. 1 Box plots for the apparent emission of 51 pharmaceuticals by population calculated from data collected in ten Spanish WWTPs

that further improvements are guaranteed taking into account key ecosystem properties.

The mean uncertainty that is propagated from each process to the final contaminant concentration value for each pharmaceutical shows that the apparent emission from the population is the most important term in terms of propagated uncertainty (116 ± 47%, mean and standard deviation among pharmaceuticals), followed by river removal (81 ± 46%) and removal in WWTP (47 ± 18%) (Fig. 4). However, the differences between river and WWTP removal should be regarded with caution, considering the scarcity of data for river removal. It can be argued that our simplified model misses a lot of components, so most probably the uncertainties are overestimated. However, the same reasoning applies to the three processes considered, and the three processes were modelled with approximately the same degree of model complexity, so the comparison is still valid. From these analyses, it is quite clear that more effort should be applied to collect data and develop better models to decrease the uncertainty associated with the apparent emission of

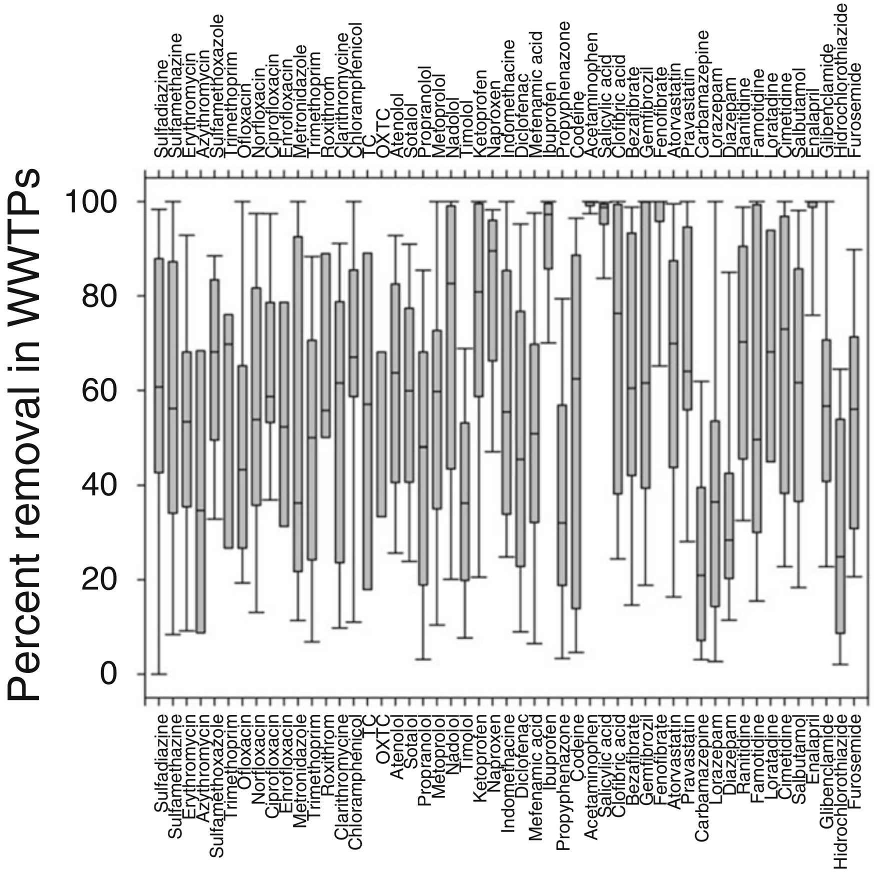

Fig. 2 Box plots for the removal in WWTPs of 51 pharmaceuticals, calculated from data collected in ten Spanish WWTPs

pharmaceuticals. This is reasonable if we consider that emission values are unbounded (a priori they can reach any arbitrary positive value), while retention figures can only move between 0 and 100%.

7 Conclusions

Nowadays, GREAT-ER and PhATE models are the most used in literature as EC models all around the world. They cannot be considered high complex tools, but these models are specifically oriented to the simulation of emerging contaminants at catchment scale.

Contaminant emission is considered a key input for EC models due to the direct and proportional effect in the predicted concentrations. Not surprisingly, this term

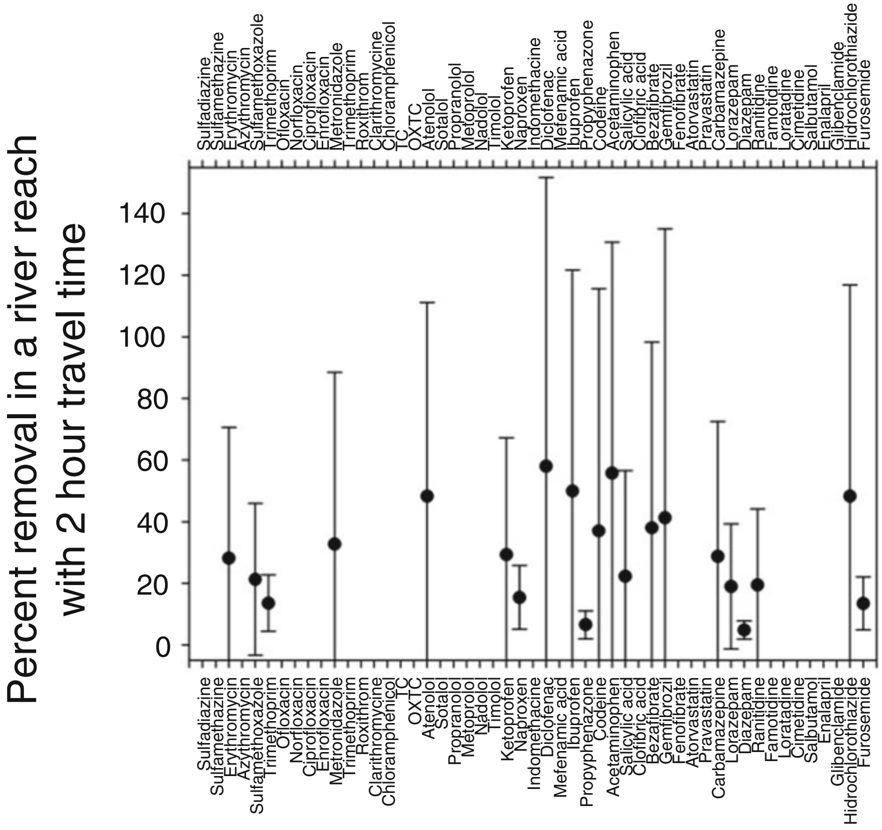

Fig. 3 Mean retention and standard deviation calculated for selected pharmaceuticals in four Spanish rivers, considering a travel time of 2 h

is the one carrying most uncertainty in the predictions. In general, compound sales data is obtained to estimate the chemical consumption per capita that requires the model. However, GREAT-ER and PhATE do not consider the unused fraction that may be released through solid waste and do not reach the wastewater pathway. In addition, the only sources of contaminants in these EC models into surface waters are the WWTPs, that is, point sources. No diffuse sources can be simulated; thus, these models omit leaks or agricultural contamination. GREAT-ER and PhATE models assume a uniform compound consumption among the catchment and no regional differences in per capita use can be applied. In fact, these last two drawbacks are the most usual reason for using other more general contaminant models.

Other parameters that shall be introduced in the EC models are the removal of contaminants in WWTPs and in-stream degradation. These processes depend on the plant treatment type, environmental conditions and on chemical phototransformation, biotransformation, sorption and volatilisation behaviour. In order to

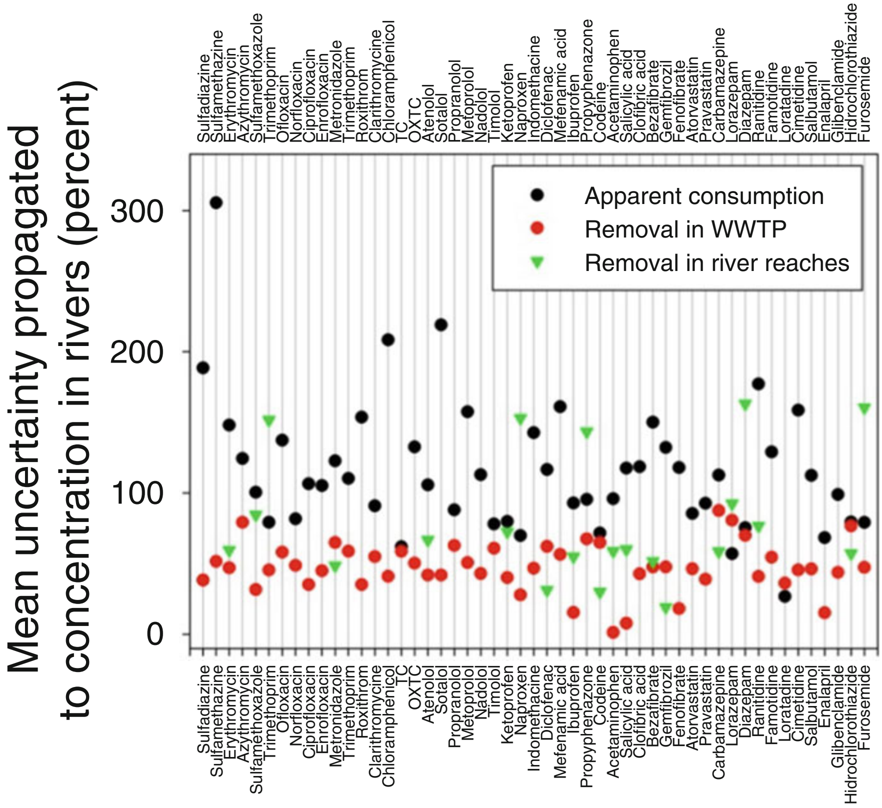

Fig. 4 Mean uncertainties propagated to modelled river concentration values of 51 pharmaceuticals from the three basic processes affecting the fate of pharmaceuticals of human use

quantify these processes, laboratory experiments are performed, but authors do not always agree or obtain decisive results. Therefore, the model remains conditioned by the uncertainty introduced with these parameters.

Hydrological data may also be related to uncertainty and variability. Generally, river flow values are obtained from gauge stations and then treated statistically. The accuracy of the statistical distributions depends on the amount of gauge stations working within the target catchment and their operation time. Moreover, the flow of some rivers modifies significantly with year seasons, and this should be considered when a specific time is being simulated.

These considerations raise the fact that model calculations and measured data can complement one another: the uncertainties remaining in input parameters of EC models can be diminished taking advantage of field observations. The EC model may be subjected to ideally both a calibration and a validation or corroboration process comparing results to measured environmental concentrations (MECs). In order to proceed with a calibration process, measured concentrations in the studied river water and of the simulated contaminant are required. When PECs generated by PhATE or GREAT-ER are compared to measured values in samples collected and analysed by analytical methods, model parameters may be adjusted and calibrated. The model corroboration fulfils when the calibrated model is validated under new measured values. Model corroboration does not imply full verification or validation, due to the difficulty to achieve a model absolutely free of flaws [72]. However, the purpose of a corroboration process is to demonstrate the utility and confidence grade of the model predictions.

When no own field-measured data is available, measured concentrations reported in literature may be used assuming the deviations that this approach might imply. Sometimes, there is very little information and knowledge about the emerging contaminant behaviour and its parameters (e.g. compound degradation constants in WWTPs and in surface waters). In such cases, a screening mode may be applied using conservative input values, including zero degradation constants or WWTP removal. But probably overestimated results will be generated and a conservative representation of contaminant behaviour can be provided ([3, 10, 22]).

Therefore, EC model corroboration implies the selection of contaminants that are frequently detected and measured in the environment, as well as contaminants whose modelling parameters are relatively known. The point-by-point comparison of predicted and measured concentrations facilitates estimating the model prediction error subjected to similar environmental conditions. The more monitoring samples are available, the more effective the corroboration process shall result. In any case, a validation criterion (e.g. a suitable PEC/MEC factor, the Root Mean Square Error or the Nash-Sutcliffe Efficiency coefficient) should be defined to evaluate the model prediction accuracy.

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Part III

Implications for Water Management and Policy

The Emerging Contaminants in the Context of the EU Water Framework Directive

Mario Carere, Stefano Polesello, Robert Kase, and Bernd Manfred Gawlik

Abstract The WFD (Water Framework Directive) requires that good chemical status of surface waterbodies is achieved by all member states of the European Union by 2015. The assessment of the chemical status is based on monitoring of the list of priority substances included in the Annex X of the WFD. In August 2013, the Directive 2013/39/EU has been published and contains a revised list of priority substances for the European aquatic environments and the derivation of environmental quality standards in the water column and biota: 12 new substances were selected through a procedure of prioritisation based on a simplified risk assessment methodology with the use of monitoring and modelling data collected over a period of 4 years. In the list of the 12 new substances, also emerging contaminants and some biocides are included. The Commission is establishing a so-called watch list of substances for which Union-wide monitoring data are to be gathered for the purpose of supporting future prioritisation exercises. For the substances diclofenac, beta-estradiol (E2) and 17-alpha-ethinylestradiol (EE2), the Directive has foreseen a monitoring obligation in order to gather data to facilitate the determination of appropriate measures to address the risk to surface waters posed by those substances. Furthermore, on the basis of the outcome of a study on the risks posed by medicinal products in the environment and of other relevant studies and reports, the Commission shall develop a strategic approach to pollution of water by pharmaceutical substances.

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The European Commission, with the aim of improving the application and implementation of chemical monitoring programmes by the member states, has established in 2010 an expert group, the so-called chemical monitoring and emerging pollutants (CMEP). The group, chaired jointly by Italy and the European Commission's Joint Research Centre, has operated under the umbrella of the CIS (Common Implementation Strategy) of the WFD for the period 2010–2013. CMEP activities were related also to the emerging pollutants including analytical methods, hazard information, levels in the environment and usage patterns. In this context, a pilot exercise on emerging pollutants has been coordinated by the JRC aimed at the feasibility assessment of an experimental monitoring exercise in support to the watch list mechanism in a collaborative design involving EU member state laboratories and about 200 official monitoring stations operated by the member states. In the context of CMEP group, an activity on aquatic effect-based tools has also been launched with the aim to detect effects caused by emerging substances not included in routine monitoring programmes. As a follow-up of the CMEP effect-based tool activity and the science-policy interface, an international project combining effect-based and chemical analytical monitoring for the steroidal oestrogens started in 2014 supporting national and EU monitoring for endocrine disruptors in the aquatic environments.

Keywords Chemical status, Effect-based tools, Emerging substances, Priority substances, Water Framework Directive

Contents

1 Introduction

1.1 WFD and Directive 2013/39/UE

The European Water Framework Directive [1], which was published in the *Official Journal of the European Community* on 22 December 2000, is probably the most significant legislative instrument in the water field to be introduced on an international basis for many years. The Directive aims to achieve and ensure "good ecological and chemical status" of all waterbodies throughout Europe by 2015 through the updating and implementation of management plans at the river basin level. The WFD requires an integrated approach to the monitoring and assessment of the quality of surface waterbodies. The assessment of ecological status takes into account the effects at population and community level, based on the use of specific indices and ecological quality ratios. Good ecological status is defined in terms of the values of the biological quality elements (phytoplankton, macroalgae, angiosperms, benthic invertebrate fauna and fish), the hydrological and morphological conditions and the physico-chemical elements. Good ecological status (or potential) requires that the concentrations of the specific pollutants (also called river basin-specific pollutants) do not exceed the environmental quality standards (EQSs) set at member state level. There is an indicative, not exhaustive, list in Annex VIII of the WFD of possible specific pollutants, which includes a wide range of substances and groups of substances that can often be detected in surface waterbodies. Member states are required to assess whether these or other potential pollutants (including emerging contaminants) are discharged in significant quantities into waterbodies.

The chemical status assessment and classification are based on the compliance with legally binding European environmental quality standards (EQSs) for selected chemical pollutants (priority substances) of EU-wide concern. EQSs for priority substances are set in the Directive 2008/105/EC, recently amended by the Directive 2013/39/EU [2]; the EQSs are designed to protect the environment and human health.

Two types of water column EQSs are proposed for each priority substance:

- The annual average (AA) value or concentration of the substance concerned calculated over a one-year period. The purpose of this standard is to ensure the long-term quality of the aquatic environments.
- The maximum allowable concentration (MAC) of the substance. The purpose of this second standard is mainly to limit short-term pollution peaks.

EQSs have been also derived for biota, using the approach recommended by the technical guidance for deriving environmental quality standards [3] that has been published under the WFD Common Implementation Strategy. At national level, the Directive gives also the possibility to derive EQS for the sediment compartment.

The Directive 2013/39/EU includes a revised (second) list of priority substances and provisions to improve the functioning of the legislation: the number of priority (and group of priority) substances is currently 53. The technical content of the Directive has been elaborated on the basis of 4 years of work by Working Group

(WG) E (now Chemical Aspects) of the Common Implementation Strategy (CIS) of the WFD. This Group is composed of representatives of all member states and several key stakeholders (industrial and environmental associations).

The main features of the Directive are:

- 12 additional priority substances, six of them designated as priority hazardous substances
- Stricter EQS for four existing priority substances and slightly revised EQS for three others
- The designation of two existing priority substances as priority hazardous substances (to be eliminated from all sources of pollution)
- The introduction of biota standards for several substances (a technical guidance for the assessment of biota EQS has been endorsed by the water directors in December 2014 in Rome)
- Provisions to improve the efficiency of monitoring and the clarity of reporting with regard to certain substances behaving as ubiquitous persistent, bioaccumulative and toxic (PBT) substances
- Provision for a watch list mechanism designed to allow targeted EU-wide monitoring of substances of possible concern to support the prioritisation process in future reviews of the priority substances list
- Provisions for a strategy on pharmaceuticals

1.2 The Monitoring Aspects and CMEP Subgroup

The WFD requires three monitoring programmes for the chemical substances:

Surveillance monitoring: to supplement and validate the impacts analysis, to support the efficient and effective design of future monitoring programmes and to assess long-term changes in natural conditions and changes resulting from anthropogenic activity. The monitoring is performed at least once every management cycle (usually every 6 years).

Operational monitoring: to establish the status of those waterbodies identified as being at risk of failing to meet the WFD environmental objectives and to assess any changes in the status resulting from the programmes of measures.

Investigative monitoring: to determine the reasons for exceedances or predicted failure to achieve environmental objectives if the reasons are not already known and to determine the magnitude and impacts of accidental pollution.

The European Commission, with the aim of improving the application and implementation of chemical monitoring programmes by the member states, has established an expert group, the so-called chemical monitoring and emerging pollutants (CMEP) group [4]. This expert group has provided guidance on sampling, monitoring and analysis of chemical substances, including quality assurance and control issues. The group, chaired jointly by Italy and the European Commission's Joint Research Centre, has been composed of experts of all the member states, key stakeholders and the research community and has operated under the umbrella of WG E (now WG “Chemical Aspects”).

CMEP dealt also with topics related to emerging pollutants, including analytical methods, hazard information, levels in the environment and usage patterns. The activity of CMEP focused on the provision of EU-wide information about the levels and occurrences of emerging environmental pollutants which can eventually support the identification of new designated priority substances.

2 Prioritisation

2.1 *Regulative Framework*

Article 16 of the Water Framework Directive (WFD) is the legal basis for the identification and review of the European priority substances that should be reduced or eliminated (priority hazardous substances) from all emissions, discharges, releases and losses in surface waterbodies.

Article 16 of the WFD requires the setting out of a list of priority substances (PS) and priority hazardous substances (PHS) presenting a significant risk to or via the aquatic environment. Substances should be prioritised taking into account (i) risk assessments carried out under existing chemically relevant EU directives and regulations, (ii) targeted risk-based assessments focusing on aquatic ecotoxicity and human toxicity via the aquatic environment, and (iii) simplified risk-based assessments based on intrinsic hazards, widespread environmental contamination, production volumes and use patterns. PHS are defined as substances that are persistent, bioaccumulative and toxic (i.e. PBT) or that give rise to an equivalent level of concern (e.g. endocrine disruptors).

Furthermore, Article 16 of the WFD requires that substances designated as priority substances and priority hazardous substances are reviewed every 4 (but now 6) years. Each review comprises an assessment of existing priority substances and priority hazardous substances and also a review of candidate substances for consideration as new priority substances. The first (and the most recent) review was conducted in 2007–2011. The next is in progress and will have to be completed by 2017.

2.2 *Last Prioritisation Process*

In the years 2007–2011, an overall prioritisation process has been coordinated by the Commission and its Working Group E on priority substances, which took into account the results of a modelling-based approach as well as results of a monitoring-based approach. The principles and the tools used in this last prioritisation process have been explained and discussed in two reports [5, 6].

The starting list of chemicals (SLoC) contained 2,034 substances and was based on substances suggested by EU member states (MS), the European Parliament (EP), stakeholders, research consortiums, international organisations and several EU lists of substances of possible concern such as PBT, potential endocrine disruptors and plant protection products.

The modelling-based approach was necessary to consider also substances that are not included in monitoring programmes such as the emerging contaminants.

The risk scoring in the modelling-based prioritisation exercise was based on the integration of two separated scores provided after a hazard and exposure assessment, plus an additional ranking step based on the PEC/PNEC ratios [7]. The risk scoring for hazard and exposure ranged from 1 to 5, being 1 the highest priority (i.e. highest risk) and 5 the lowest risk. The hazard assessment is based on the PBT (persistence, bioaccumulation and toxicity) approach developed in the REACH Guidance, integrated with a classification on endocrine disruption potential, whereas the exposure assessment is based on production and uses data obtained from the IUCLID and SPIN databases. To rank all substances classified with a score of 1 (78 substances), a PNEC value was estimated using, whenever possible, experimental data and QSAR (quantitative structure-activity relationship) models. The majority of QSARs have been developed for organic substances. The substance was considered only when experimental data was available. PEC values were estimated using both the ECETOC TRA (targeted risk assessment) tool and the long-range transport potential (LRTP) OECD tool. The PEC/PNEC risk ratio was then calculated and a ranked list of the 78 substances produced.

As regards the monitoring-based approach, carried out by INERIS, FR, a two-step data collection has been put into place so as to gather as exhaustively as possible monitoring data of chemical substances in European member states. A database was compiled and developed, gathering $\sim$ 14,600,000 data points of 1,153 substances in three different matrices (water, sediment and biota) from 28 countries (EU member states plus Norway). A tiered algorithm based on risk assessment principles was elaborated to prioritise substances. Based on this methodology, a list of 316 substances for which there were monitoring data from more than three countries in water, sediment and/or biota was selected as candidates for prioritisation. The predicted environmental concentration (PEC) and predicted no-effect concentration (PNEC) were calculated and based on the risk ratio, i.e. PEC/PNEC, the substances were ranked, and a list of 41 substances was produced with another 21 substances considered in relation to human consumption.

In general, substances were only included on the final shortlist of candidate substances if they were prioritised by more than one of the procedures. Considering also the ongoing implementation of reduction measures for some substances, a final shortlist of 20 substances was then recommended by the WG E based on their PEC/PNEC ratios. For 15 substances, EQSs were derived and they were finally proposed as new priority substances [8]. For the other substances, it was concluded that additional information was needed before they could be considered further. After a long discussion at the Council and the Parliament, it was decided that the three pharmaceuticals should be included in the "watch list" for a possible inclusion in the list of the priority substances.

Table 1 Final list of new priority substances in Directive 2013/39/EU

Substance	AA-EQS µg/L (inland surface waters)[a]	EQS biota (µg/kg wet weight)[b]	Uses/emissions	Priority hazardous substances
Dicofol	1.3×10^{-3}	33	Organochlorine acaricide	X
Perfluorooctane sulphonic acid (PFOS)	6.5×10^{-4}	9.1	Largely used products (textiles, carpets, plastics)	X
Quinoxyfen (5,7-dichloro-4-(p-fluorophenoxy) quinoline)	0.15		Fungicide	X
Dioxins and dioxin-like compounds[c]		0,0065 TEQ*	Industrial processes, waste dumps, incomplete combustion	X
Aclonifen	0.12		Herbicide	
Bifenox	0.012		Pesticide—various uses	
Cybutryne	0.0025		Antifouling biocide	
Cypermethrin	8×10^{-5}		Insecticide	
Dichlorvos	6×10^{-4}		Insecticide	
Hexabromocyclododecane (HBCDD)	0.0016	167	Flame retardant	X
Heptachlor and heptachlor epoxide	2×10^{-7}	6.7×10^{-3}	Organochlorine insecticide	X
Terbutryn	0.065		Herbicide	

[a] Inland surface waters encompass rivers and lakes and related artificial or heavily modified waterbodies

[b] Unless otherwise indicated, the biota EQS relates to fish. An alternative biota taxon, or another matrix, may be monitored instead, as long as the EQS applied provides an equivalent level of protection

[c] *PCDD* polychlorinated dibenzo-p-dioxins; *PCDF* polychlorinated dibenzofurans; *PCB-DL* dioxin-like polychlorinated biphenyls; *TEQ* toxic equivalents according to the World Health Organisation 2005 Toxic Equivalence Factors

Table 1 contains the 12 new substances included in the Directive 2013/39/EU.

Pesticides (insecticides, fungicides, herbicides) are the predominant class of compounds confirming that diffuse sources still continue to cause impairment to the achievement of the good status; historical bioaccumulative substances such as dioxins, PCBs and heptachlor continue to represent a risk due to the persistence properties. There are also substances commonly present in surface waterbodies due to their very widespread use (e.g. PFOS) or their use as flame retardants (HBCDD). Furthermore, cybutryne (irgarol) has been included, which is a biocide used in the substitution of tributyltin.

2.3 Recent Development of the Prioritisation Process

The latest revision of the priority list has started immediately after the publication of the Directive 2013/39/EU, and it has been carrying out by a sub-Working Group on prioritisation under the coordination of the Joint Research Centre of the EU Commission. This Working Group is composed of experts from the member states, the European Commission and stakeholders, including representatives of the industrial and pharmaceutical associations.

The work is still in progress and we cannot discuss the outputs of the collaborative work, but we can present the fundamental principles and novelty of the planned approach which should improve the prioritisation procedure.

The bottleneck point of every prioritisation procedure is the choice of the starting list of candidates to be evaluated and ranked. In the current review process, in order to minimise the resources needed to rank very large numbers of substances, a two-way approach is proposed [9] which is based on the procedures used in the last review but which also allows the findings of the last review to be taken into account.

The first process will involve the re-evaluation of selected substances from the last review without the need for their reinclusion in the main monitoring or modelling-based ranking exercises. This process would cover (a) substances which were prioritised but not finally proposed for inclusion in the list in the last review and (b) substances ranked highly in the monitoring and modelling-based ranking exercises in the last review but not taken forward. The re-evaluation would draw on the information previously obtained and any new information that has become available.

The second process involves applying modelling- and monitoring-based ranking methodology (as used in the last review) to identify potential priority substances, but applying it only to:

- Substances that were initially considered in the last review but which were not ranked highly, for which new information has become available
- Other substances (“new substances”) selected on the basis of, e.g. information from other legislation, recent monitoring data from member states, information on river basin-specific pollutants or literature reports regarding risks from emerging pollutants

The principal novelty in the current prioritisation process is that a new collection of data at EU scale will be carried out, based also on the recently launched Information Platform for Chemical Monitoring Data, IPCHEM. In the previous review, monitoring data have to be aggregated to a certain extent to be used in the risk assessment, reducing variability but also giving up the information linked to the variability in concentration, space and time. On the contrary, the current procedure will quantify variability by using it in the risk assessment procedure and implement a scoring system that focuses on situations when a potential risk is identified (i.e. measured concentration is above the threshold of toxicological concern).

All substances identified by the modelling- and monitoring-based processes will then be subject to a final prioritisation step, based on their estimated risk at EU level (screening PEC/screening PNEC). In this way, the substances identified by the two processes will be properly compared before the final list is drawn up.

Relevant data for the future prioritisation exercise will derive from the so-called watch list foreseen by the Directive 2013/39/EU.

3 The Watch List: An Approach for the Detection of Emerging Contaminants

3.1 Regulatory Framework

The monitoring data collected from member states, although significantly improved over the past years, are not always fit for purpose in terms of quality and territorial coverage on European Union. Monitoring data are particularly lacking for many emerging pollutants, which can be defined as pollutants currently not included in routine monitoring programmes at EU level but which could pose a significant risk requiring regulation, depending upon their potential ecotoxicological and toxicological effects and on their levels in the aquatic environment.

The Directive 2013/39/EU mentions that a new mechanism is needed to provide the Commission with targeted high-quality monitoring information on the concentration of substances in the aquatic environment, with a focus on emerging pollutants and substances for which available monitoring data are of insufficient quality for the purpose of risk assessment. The new mechanism should facilitate the gathering of that information across EU river basins and complement monitoring data from programmes under Articles 5 and 8 of Directive 2000/60/EC and other reliable sources. In order to maintain monitoring costs at reasonable levels, the mechanism should focus on a limited number of substances, included temporarily in a watch list, and a limited number of monitoring sites but should provide representative data that are fit for the purpose of the EU prioritisation process. The list should be dynamic and its validity in time should be limited, in order to respond to new information on the potential risks posed by emerging pollutants and to avoid monitoring substances for longer than necessary.

The watch list must have a limited number of such substances and monitoring them EU-wide for up to 4 years. A maximum number of substances or groups of substances shall be included in the first watch list, increasing by one at each update, up to a maximum of 14 substances or groups of substances. For the member states, the number of monitoring stations can be variable (in Italy, e.g. the total number will be 20). Frequent reviews of the list will ensure that substances are not monitored longer than necessary and that substances for which a significant risk at EU level is confirmed are identified as candidate priority substances with as little delay as possible.

3.2 The Pilot Exercise

In order to investigate the technical feasibility of the EU-wide coordinated data collection and assessment that would be required for the substances on a watch list, the European Commission's Joint Research Centre (JRC) proposed under the umbrella of the Common Implementation Strategy of the Water Framework Directive to design a pilot exercise in collaboration with the member states, based on previous exercises of similar character.

The pilot bundled resources in a collaborative design involving EU member state laboratories and some 200 official monitoring stations operated by the member states. The conceptual layout as well encountered challenges that are highlighted and discussed in the perspective to address EU-wide monitoring needs for a growing number of less-investigated substances while resources are limited. The methodologies and criteria of the pilot were discussed in the context of the subgroup CMEP.

In total [10], 219 whole water samples originating from 25 EU member states and 2 other European countries were assessed for contents of acesulfame, glyphosate and its metabolite, AMPA, 1H-benzotriazole and tolyltriazoles, bisphenol A, triclosan and triclocarban, carbamazepine and its metabolite 10,11-dihydro-10,11-dihydroxycarbamazepine, sulfamethoxazole, pentafluoropropionic acid, tris-2-chloropropyl phosphate, methyl tert-butyl ether, silver, boron and chloride ($Cl-$) in water. Furthermore, 23 sediment samples were analysed for decabromodiphenylethane and decabromodiphenyl ether. The underlying analytical methods are carefully documented with regard to their performance characteristics. Obtained results have been assessed statistically and where possible compared to other findings. Although the analysed single samples are insufficient to make any statement on water quality at a specific station over time, the collective data allowed a glance at the pan-European situation as regards the studied compounds. In addition, the need to better share valuable and costly monitoring data by linking existing data collections and databases as shown by the recently launched Information Platform for Chemical Monitoring Data, IPCHEM, is addressed.

3.3 The Pharmaceuticals Included in the Watch List

Three substances with mainly pharmaceutical use have already been selected for inclusion in the first watch list in order to collect sufficient monitoring data for the determination of risk reduction measures: the steroidal oestrogens 17-alpha-ethinylestradiol (EE2), 17-beta-estradiol (E2) and diclofenac.

3.3.1 Steroidal Oestrogens EE2 and E2

Endocrine-active pharmaceuticals such as EE2 can cause adverse effects on aquatic ecosystems at very low levels [11].

The most frequent use of EE2 is as the oestrogen component of combined oral contraceptives, but it is also added to pharmaceutical products such as hormonal replacement therapies and used for the treatment of menopausal and postmenopausal symptoms (especially the vasomotor effects).

In veterinary pharmaceuticals, EE2 is used in livestock to treat reproductive disorders and to control ovulation [12]. The primary female sex hormone E2 is also used in pharmaceuticals, mostly in hormone replacement therapy, but also to treat infertility in women or advanced prostate cancer, as well as to relieve symptoms of breast cancer. Excess E2 and EE2 are excreted via urine in the form of water-soluble conjugates and either enter the aquatic environment directly (veterinary pharmaceuticals) or, due to their incomplete removal, via waste water treatment plants (WWTP) [13, 14]. Besides the natural oestrogens estrone (E1), E2 and estriol (E3), EE2 is the synthetic oestrogen most commonly found in waste water [14].

The freshwater environmental quality standards (AA-EQSs) proposed at European level [8] for EE2 and E2 are 0.035 ng/L and 0.4 ng/L, respectively (Table 2). For some European countries, EE2 concentrations have been predicted by modelling to exceed the above EQS in 2–25% (most likely 12%) of total river length [15]. Even then, the expected concentrations are mostly in the very low sub-ng/L concentration range, which demonstrates the necessity of having sensitive detection methods available.

3.3.2 Diclofenac

Diclofenac is an active pharmaceutical ingredient (non-steroidal anti-inflammatory drug (NSAID), antiphlogistic) used by patients for the treatment of inflammation and pain predominantly via oral and dermal application. Diclofenac is commonly used as human and veterinary pharmaceutical. For example, the consumption data for human pharmaceutical are around 91 t/year in Germany in 2009. An EU-wide high exposure relevance is expected, but the monitoring data were reported by only a few member states in the past, leading to an inclusion in the watch list. The EU Commission suggested in 2012 an EQS of 100 ng/L based on direct fish toxicity. In

Table 2 Proposed EQS [8] and uses of the pharmaceuticals included in Directive 2013/39/EU

	Proposed EQS		Use
	Freshwaters (AA)	Marine waters (AA)	
17-alpha-ethinylestradiol	0.035 ng/L	0.007 ng/L	Synthetic oestrogen (birth control pills)
17 beta-estradiol	0.4 ng/L	0.08 ng/L	Oestrogenic hormone
Diclofenac	0.1 μg/L	0.01 μg/L	Anti-inflammatory drug

the meanwhile, different studies initiated by industry and from agencies were showing controversial effects regarding the fish toxicity of young and subadult trout [16] and other species [17]. Therefore, a revision of the EQS proposal is recommended to address the risk posed by this substance. No monitoring problems for diclofenac are expected at the current EQS level [18] (Table 2).

3.4 Watch List in Progress

The Joint Research Centre (JRC) has been tasked, in the context of the WG chemical aspect activities, to propose seven substances as candidates for the completion of the first watch list and identify analytical methods for their monitoring. The main criteria for inclusion in the initial list of candidate substances were that (i) the substance is suspected of posing a significant risk to, or via, the aquatic environment, meaning there is reliable evidence of hazard and of a possible exposure to aquatic organisms and mammals, but (ii) there is not enough information to assess the EU-wide exposure for the substance, i.e. insufficient monitoring data or data of insufficient quality, nor sufficient modelled exposure data to decide whether to prioritise the substances.

Article 8b of the Directive 2013/39/EU sets out a comprehensive list of information sources to be considered when establishing the watch list. These include:

- The results of the most recent regular review of Annex X to Directive 2000/60/EC provided for in 130 Article 16(4) of that Directive (in particular substances ranked highly but not prioritised because of a paucity of monitoring data)
- Research projects (even though these are likely to be the same research projects assessed in the priority substances review, the results would be considered more frequently for the watch list updates; their reliability should be considered)
- Recommendations from the stakeholders referred to in Article 16(5) of Directive 2000/60/EC (these may include recommendations from the SCHER, MS, the EP, EEA, research programmes, international organisations, European business organisations including SMEs and environmental organisations)
- Member states' characterisation of river basin districts and the results of monitoring programmes, under Articles 5 and 8 of Directive 2000/60/EC, respectively (consideration of river basin-specific pollutants (RBSPs), if there is not already enough evidence from enough MS)
- Information on production volumes, use patterns, intrinsic properties (including, where relevant, particle size), concentrations in the environment and effects, including information gathered in accordance with Directives 98/8/EC, 2001/82/EC and 2001/83/EC and with Regulations (EC) No 145 1907/2006 and (EC) No 1107/2009

For the elaboration of the final watch list, a risk assessment of all the substances must be done by combining the substance-specific hazard data and information on exposure to the substance in or via the aquatic environment. According to the substance's physico-chemical properties, the receptors and compartments at risk must be identified and an assessment done for each route of exposure, including the estimation of specific PEC and PNEC values. In general, the criteria to identify the required assessments followed those specified in the Technical Guidance No. 27 of the Common Implementation Strategy (CIS) of the WFD [3]

4 Strategy on Pharmaceuticals

The Commission is beginning to develop, as required by Directive 2013/39/EU, a strategic approach to the pollution of water by pharmaceuticals.

That strategic approach shall, where appropriate, include proposals enabling, to the extent necessary, the environmental impacts of medicines to be taken into account more effectively in the procedure for placing medicinal products on the market. In the framework of that strategic approach, the Commission shall, where appropriate, by 14 September 2017 propose measures to be taken at Union and/or member state level to address the possible environmental impacts of pharmaceutical substances, with a view to reducing discharges, emissions and losses of such substances into the aquatic environment, taking into account public health needs and the cost-effectiveness of the measures proposed.

This will actually consider and build on the actions already taken by the Commission in this area, taking also into account the study on environmental risks from medicinal products carried out recently for DG SANCO by BIO IS [19] and other relevant information.

The study highlighted some key facts:

- Active pharmaceutical ingredients authorised at national level might vary significantly: about 3,000 authorised in EU, ranging from 850 to 3,000 per country.
- Consumption is very heterogeneous among member states: 50–150 g/capita/year.
- Sensitivity to waste water treatment strongly varies between active pharmaceutical ingredients.
- The active pharmaceutical ingredients detected in the environment include medicinal products put on the market several decades ago as well as new medicines.
- Medicinal products can be bioaccumulated in fat tissues and introduced in the food chain [20].
- Evidence on ecotoxicological impacts exists for certain molecules under specific exposure scenarios.
- Medicinal products in ecosystems are present in mixtures.
- Ecotoxicity of a mixture is almost always higher than the effects of its individual components even if all components are present only in low concentrations that do not provoke significant toxic effects if acting singly on the exposed organisms.

The resulting EU workshop on the development of a strategic approach to pollution of water by pharmaceutical substances was held in Brussels on 11 September 2014 and aimed to facilitate constructive and transparent dialogue to inform the development of the strategic approach. In this workshop, more than 120 participants discussed the environmental risks of medicinal products and the outcome of the study.

Discussions in ten working groups occurred, dealing with three main topics about possible options for (1) development/authorisation/production, (2) consumption/use and (3) relevant disposal. The working groups provided a prioritisation of options regarding:

1. Priority (high, medium, low)
2. Timescale (short, medium, long)
3. Costs of the option (high, medium, low)
4. Benefits of the option (high, medium, low)
5. Mechanism EU hard (H) or soft (S) law, MS hard or soft law (M), voluntary (V)

The group results were presented and discussed in a plenary session and were intended to be used to develop a strategic approach to the pollution of water by pharmaceuticals.

5 Novel Approaches: The Effect-Based Monitoring in the WFD

5.1 The European Technical Report on Aquatic Effect-Based Tools

The classical single-chemical risk assessment approach for the management of chemical pollution of waterbodies has some limitations highlighted by recent European projects (e.g. EDA-Emerge ITN-FP 7 http://www.ufz.de/eda-emerge/) because it is not possible to analyse, detect and quantify all substances that are present in the aquatic environment. We must understand which are the real effects caused by the sum of the chemical substances in the aquatic environment (including emerging pollutants, metabolites and transformation products) and to link the observed effects with cost-effective management objectives. Furthermore, the substances present in the aquatic environment can form mixtures whose effects may not be predictable on the basis of chemical analyses alone.

In the mandate of the mentioned European subgroup CMEP, a specific task was foreseen for the elaboration of a technical report on aquatic effect-based tools. The activity was chaired by Sweden and co-chaired by Italy and progressively involved several member states and stakeholders in an EU-wide drafting group (47 experts).

The report [21] was approved by the CMEP subgroup in Gent, Belgium (October 2012), by the Working Group on Chemical Aspects in Brussels (April 2013) and by the SCG (strategic coordination group of the WFD) in Brussels (October 2013) and endorsed by the Water Director Meeting in Vilnius, Lithuania (December 2013).

The technical report aims at presenting the state of the art of aquatic effect-based monitoring tools and to describe in which way these tools can help EU member states to make more efficient monitoring programmes (including reduction of monitoring costs). The tools described in the report are categorised into three main groups:

1. Bioassays, in vitro and in vivo, which measure the toxicity of environmental samples under defined laboratory conditions, on cellular or individual levels, respectively
2. Biomarkers, i.e. biological responses at the cellular or individual levels, measured in field exposed organisms
3. Ecological indicators, measuring changes observed at higher biological organisation levels, i.e. the population and/or community

In Europe, several of the tools described in the report are already used for both marine and limnic applications [22]. Bioassays are used, for example, to support risk assessment and management of contaminated sediment and to provide decision support for reducing the release of toxic substances into the environment. They are also used in broad screening of different pollutant sources (such as sewage treatment plant effluents). Other applications include alarm systems directly triggering control measures in relation to drinking waters. Effect-based tools support currently the ecotoxicological characterisation and classification of hazardous wastes in the context of the Waste Framework Directive (2008/98/EC).

Some objectives for the use of effect-based tools in a WFD context are mentioned in the report:

- As screening tools, as part of the pressures and impact assessment to aid in the prioritisation of waterbodies
- To establish early warning systems
- To take the effects of chemical mixtures or chemicals that are not analysed into account (e.g. to support investigative monitoring where causes of a decline of specific species are unknown)
- To provide additional support in water and sediment quality assessment, though not as a replacement for conventional chemical and ecological monitoring under the WFD

The optimal approach will frequently involve several effect-based tools as well as chemical analysis, as illustrated by several of the case studies described in the Annex of the report. EDA (effect-directed analysis) methodology has been also included in the report.

5.2 *The International Project on E2 and EE2 in the Context of the WFD*

As a follow-up of the CMEP effect-based tool activity and the science-policy interface [23], an international project combining effect-based and chemical analytical monitoring for the steroidal oestrogens started in 2014 which is supporting national and EU monitoring for endocrine disruptors.

Only a limited number of institutes in Europe currently have the capacity to quantify the steroidal oestrogens EE2 and E2 at their suggested EQS levels. This could cause problems for the EU watch list mechanism, where reliable exposure data are required from across the EU. Complementary methods will help European states to identify and quantify the chemical pressures from oestrogenic watch list substances in conjunction with the best available chemical analytical methods.

In this project, harmonised methods for sample collection, sample extraction and data evaluation, in close collaboration with ISO experts, will be provided. Additionally, recommendations for screening and risk assessment for steroidal oestrogens will be generated, which might be used to support national endocrine disruptor strategies.

Sensitive in vitro bioassays (effect-based methods) are capable of reducing the current monitoring difficulties by measuring the oestrogenic activity of environmental samples as 17-beta-estradiol equivalent (EEQ) values in a cost-efficient way [11]. The applicability of this approach has been demonstrated in various projects carried out during the last decade [24]. The main advantage of in vitro bioassays is their ability to detect the presence of (xeno-)oestrogens and, at the same time, to quantify their activity, making them cost-effective monitoring tools for the detection of the possible presence of E2 and EE2 and/or other oestrogen-receptor-binding substances. In vitro bioassays are able to detect oestrogenic activity in environmental mixtures, which is one of the main challenges highlighted in the context of the European strategy on endocrine disrupting chemicals [25]. For this purpose, 20 surface water and 20 waste water samples across Europe will be collected and analysed. Approximately 20 institutes or agencies from 13 nations will be involved in the project. Eleven national institutes intend to contribute with polluted surface and waste water samples in 2015.

The following detection methods are covered: best possible chemical analysis (Joint Research Centre (JRC), IT and Federal Institute of Hydrology (BfG), DE) and eight effect-based methods: ER-CALUX (BioDetection Systems NL), MELN (INERIS, FR), BG1Luc4E2 + ER-GeneBLAzer (UFZ, DE), Hela 9903 (RECETOX, CZ), yeast oestrogen screen assays (BfG, DE), LIBER assay (JRC, IT) and T47D-Kbluc assay (RWTH Aachen, DE). Some of the effect-based methods applied here are currently going through the OECD validation process or being developed as ISO standards.

This multinational, interdisciplinary and collaborative project aims at:

- Promoting reliable screening methods to support the monitoring of endocrine disrupting activity in waste water and surface water
- Harmonising monitoring options across Europe
- Linking reliable effect-based tools, some of them being developed as ISO standards, with regulatory needs
- Linking effect-based tools with chemical analysis for oestrogen monitoring
- Supporting national and EU monitoring for endocrine disruptors, addressing a regulatory research need which is relevant for all European states

6 Conclusions

The activities described in this chapter carried out jointly by the European Commission, the member states plus other countries (e.g. Switzerland) and the stakeholders have produced a relevant legislative progress for the monitoring, evaluation and management of chemical pollution in the waterbodies based on robust technical/scientific criteria. The current watch list mechanism, the new approach for the prioritisation and the strategy on pharmaceuticals, combined with the novel approaches based on the effects, should better address and manage at European level the complex issue of the presence of emerging contaminants in the aquatic environments. The implementation of these new approaches and the consequent benefits for the management and reduction of the emerging contaminants in the aquatic environments will also depend mostly from the political willingness of the governments of the member states of the European Union.

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