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REVIEW

Pharmaceutical residues in environmental waters and wastewater: current state of knowledge and future research

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Abstract Pollution from pharmaceuticals in the aquatic environment is now recognized as an environmental concern in many countries. This has led to the creation of an extensive area of research, including among others: their chemical identification and quantification; elucidation of transformation pathways when present in wastewater-treatment plants or in environmental matrices; assessment of their potential biological effects; and development and application of advanced treatment processes for their removal and/or mineralization. Pharmaceuticals are a unique category of pollutants, because of their special characteristics, and their behavior and fate cannot be simulated with other chemical organic contaminants. Over the last decade the scientific community has embraced research in this specific field and the outcome has been immense. This was facilitated by advances in chromatographic techniques and relevant biological assays. Despite this, a number of unanswered questions exist and still there is much room for development and work towards a more solid understanding of the actual consequences of the release of pharmaceuticals in the environment. This review tries to present part of the knowledge that is currently available with regard to the occurrence of pharmaceutical residues in aquatic matrices, the progress made during the last several years on identification of such compounds down to trace levels, and of new, previously unidentified, pharmaceuticals such as illicit drugs, metabolites, and photo-products. It also tries to discuss the main recent findings in respect of the capacity of various treatment technologies to remove these contaminants and to highlight some of the adverse effects that may be related to their ubiquitous existence. Finally, socioeconomic measures that may be able to hinder the introduction of such compounds into the environment are briefly discussed.

Keywords Pharmaceuticals · Water/wastewater · Analysis · Removal technologies · Transformation by-products · Adverse effects

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Introduction

There has been an increasing concern in recent years about the occurrence, fate, and adverse effects of pharmaceutical residues in the aquatic environment. Some of the most widely and frequently used drug classes, for example antibiotics, are used in quantities similar to those of pesticides and in some countries some are even sold without the requirement of a prescription. Despite this, pharmaceuticals are not required to undergo scrutiny; they are not tested for low-doses vs. long-term exposure or when present in mixtures. The full extent and consequences of the presence of these compounds in the environment are therefore still largely unknown.

These compounds have been detected in a wide variety of environmental water samples including sewage flows,

surface and groundwater, with concentrations generally ranging from traces to ppb levels. It is, therefore, often thought to be unlikely that pharmaceuticals will have a detrimental effect on the environment, but this is based on tests performed with individual compounds and short-term exposure. The lack of validated analytical methods, non-uniform monitoring data, and the lack of definite information about the fate and effects of these compounds and/or their metabolites and transformation by-products in the aquatic environment makes accurate risk assessments problematic. It is now known that some pharmaceuticals can persist in the environment and, either via the food chain or via drinking water, can make their way back to humans. It is also accepted that some of these compounds are beginning to be associated with adverse developmental effects in aquatic organisms at environmentally relevant concentrations, that are usually believed to be infinitesimal and harmless [1]. It is accepted that knowledge concerning the effects of human exposure to low-dose mixtures of pharmaceuticals or of low-dose pharmaceuticals mixed with other low-dose synthetic pollutants is from extremely limited to absent. However, the little that is known may provoke concern and several serious questions that are related to practices such as wastewater reuse for irrigation, discharge into the sea and other aquatic environments, groundwater replenishment, etc. According to Khetan and Collins [1] and Fatta-Kassinos et al. [2], such practices may be related to concerns about the pharmaceuticals' potential effects on non-target organisms, including plants, animals, and humans.

Even though the study of pharmaceutical residues in the environment is a fairly new topic, a vast amount of literature has already been published. Figure 1 shows the impressive increase of the various studies on the occurrence of these compounds in various environmental water matrices during the last decade.

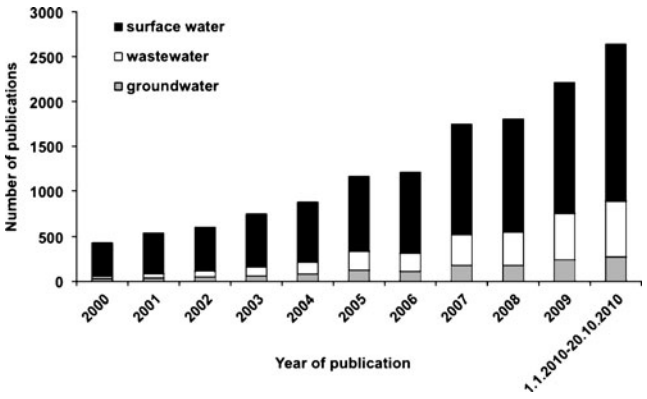

Fig. 1 Publications during the last decade (source: Advanced Research in Science Direct; date, 20.10.2010; subject, all fields; keywords used, occurrence/pharmaceuticals/wastewater-surface water-groundwater)

In this review paper, information on the special characteristics of pharmaceutical compounds, on their occurrence and the various problems related to this, on analytical technological advances and treatment technologies, on the identification of metabolites and transformation by-products, and on socioeconomic measures related to the prevention of drugs entering the environment, is provided, the objective being to try to provide an updated integrated picture of the current status and trends prevailing in the specific field. Furthermore, another objective is to present important questions that still need to be answered and identify some of the urgent research directions that need to be looked at by the scientific community.

Why are pharmaceuticals regarded as "special" micropollutants?

When pharmaceuticals are regarded as pollutants released in the environment, their environmental fate and biological potency can be predicted or assessed on the basis of their special physicochemical and biological characteristics. It is important to emphasize here that these characteristics of pharmaceuticals differentiate them from other industrial chemical compounds. These characteristics include polymorphism, their introduction into the environment after human metabolism, their chemically complex structure, and the fact that they can be ionized and have multiple ionization sites spread throughout the molecule [3]. Relevant processes regarding pharmaceuticals in the environment include sorption to soils and sediments, complexation with metals and organics, chemical oxidation, photolysis, volatilization, and biodegradation [4]. Thus, physicochemical properties, for example octanol/water partition coefficient, dissociation constants, vapor pressure, or Henry's Law constant, may facilitate determination of whether a compound is likely to become concentrated in the aquatic, terrestrial, or atmospheric environment. The chemical composition and structure of drugs determine a vast array of their properties. Drugs may be acidic, basic, or neutral and of a variety of chemical forms e.g. small organic molecules, large polymers such as proteins, carbohydrates, and other compounds with complex chemistry. The partition coefficient of drugs is a common indicator of drug hydrophobicity/lipophilicity, and is routinely used during drug development to predict membrane permeability.

Polymorphism arises when a given molecule has the ability to crystallize in more than one crystalline form. Polymorphic forms may have different physical, chemical, electrical, and thermal properties. Polymorphs usually differ in bioavailability, solubility, dissolution rate, chemical and physical stability, melting point, color, density, and flow

properties. These different physicochemical properties may be related to difficulties in attempts to correlate pharmaceutical experimental results with relationships derived from less complex compounds and may lead to erroneous conclusions. The varying water solubility of the various polymorphic forms of pharmaceuticals should always be taken into account. Furthermore, the solubility should also be considered in relation to the pH of the matrix in which it is present. These issues may affect not only fate or transport but also assessment of environmental effects, because solubility constraints imposed by the particular salt forms may lead to underestimation of potential biological effects, including toxicity.

Active pharmaceutical ingredients may extensively or partly be metabolized by a variety of mechanisms. Pharmaceuticals are generally metabolized to form more polar and water-soluble derivatives that have reduced pharmacological activity compared with the parent compounds and are rapidly excreted. There are also cases where the administered compound is a prodrug, which is first metabolized *in vivo* to the active metabolite and then to less active forms. Studies on parent compounds may not adequately address the chemical, physicochemical, pharmacological, or toxicological differences of these metabolites. Moreover, bio-transformation or photo-transformation of the parent compounds during wastewater treatment must always be taken into account during assessment of fate and effects. Because of the general availability of glucose in biological systems, glucuronide formation is one of the most common mechanisms of drug metabolism. Therefore, administered parent compound may be excreted unchanged, as a glucuronide or sulfate conjugate, as a "major" metabolite, and as a complex mixture of many metabolites. There is evidence that glucuronides, which are the simplest and most common form of conjugated pharmaceutical compounds excreted by humans, are capable of being deconjugated to the parent compound during municipal sewage treatment [5].

The transfer of drugs in the human body is determined by their ability to move across the lipid bilayer of epithelial cell linings. The main properties of a drug affecting its permeation through biological membranes are lipophilicity, hydrogen bonding capacity, charge, and size. The lipophilicity of a drug is the single physicochemical property most used to predict its permeation in biological systems. Behind this property lies a net of intermolecular interactions such as hydrogen bonding and dipole effects [6]. Thus, although lipophilicity is a property ascribed to the drug, it is highly dependent on the choice of environment [7].

The heteroatom content and multifunctional composition of pharmaceuticals make them, among other things, polar, ionizable molecules, and affected by solution pH. More specifically, the octanol/water distribution coefficient (D_{ow}) and the octanol/water partition coefficient (K_{ow}) must be carefully evaluated with regard to multiple ionization sites. When modeled, sorption to organic matter in the solid state should account for the fact that an active pharmaceutical ingredient may assume charge states that may lead to more complex ionic, ion pairing, or complexation mechanisms. The degree of ionization of the drug substance at a particular pH will affect its availability to biological organisms, its chemical and physical activity, and its ultimate environmental fate. For example, an ionized molecule will generally have greater water solubility and will be less likely than its non-ionized form to partition to lipid-like substances. Ionic charge will also affect the potential of a molecule to participate in environmental ion-exchange processes that are ubiquitous in soil and sludge systems. Knowledge of the pK_a can assist experimenters in their design of appropriate sorption and ecotoxicity studies and in accurately interpreting the results from these studies [3].

The octanol/water distribution coefficient (D_{ow}) has long been used in environmental assessments to estimate other properties, for example water solubility, soil-sediment adsorption coefficients, and bioconcentration factors for aquatic life. The *n*-octanol/water distribution coefficient indicates the tendency of an organic chemical to partition into lipids or fats, to sorb to particulates such as soils, sediments, biomass, and sludge, and to distribute among the various environmental compartments. It can also be used to predict the bioconcentration potential in aquatic and terrestrial organisms. However, in most cases, these relationships were derived from and applied, mainly, to neutral industrial chemicals and pesticides. They do not seem to be that applicable to pharmaceuticals, which are multifunctional organic compounds that are ionized in the aquatic environment at environmentally relevant pH. Usually the un-ionized species will be the predominant species to partition into octanol from water, with the ionized species remaining in the aqueous phase. Therefore, D_{ow} should be corrected for the ionization of the compound so that only the concentration of the un-ionized species is considered. Use of the corrected value for an ionizable compound will result in values that represent only the un-ionized species and overestimate the hydrophobicity of the compounds, and hence their potential bioaccumulation potential. Also, many ecotoxicity models use log K_{ow} which may over-predict toxicity for ionizable compounds [3].

The biosolids/water distribution coefficient, $K_{biomass}$ or K_p is the ratio of the concentration of a chemical in two phases, biosolids and water, when the solid phase is biomass and the phases are in equilibrium with each other and the test chemical is a dilute solution in both phases. The ability to estimate the sorption of a pharmaceutical to solids in various media is critical to understanding its environmental fate. Hence, great care must be taken in applying environmental

fate models derived from neutral hydrophobic compounds to ionizable, hydrophilic pharmaceuticals. It is important to also note that different mechanisms are involved in sorption of pharmaceuticals to soil, including ion exchange, surface adsorption to minerals, formation of complexes with metals, hydrogen bonding, association with organic matter, etc. Because most pharmaceuticals are ionizable, pH is a crucial factor for their sorption to soil. Because soil organic matter is negatively charged it is expected that sorption of more basic compounds will be stronger, because at soil pH, most often pharmaceuticals are present in their cationic form.

Drugs are not a homogeneous group of compounds. They vary widely, in molecular weight, structure, and are complex molecules with different functionalities, developed and used for a specific biological activity. Most of them are polar compounds. The molecular weights of the chemical molecules range typically from 200 to 500/1000 Da [8].

Progress in analysis of pharmaceuticals in aqueous matrices

Pharmaceuticals are one of the most important new classes of environmental pollutants. Their occurrence has been reported in natural waters, wastewater, sediments, and sludge. New studies reveal their occurrence in samples investigated worldwide [9–13].

The accurate quantification of pharmaceuticals, especially in environmental samples can be an analytical challenge, because of the complexity of the matrix and their low levels of occurrence. Several years ago, appropriate analytical techniques did not exist. Nowadays, gas and liquid chromatography (GC and LC) in combination with modern extraction, derivatization, and clean-up methods provide the opportunity to quantify many pharmaceutical compounds and metabolites down to ng L^{-1} levels. Capillary electrophoresis (CE) has also been used for analysis of pharmaceuticals. It is less complex and less expensive than GC and LC, but less sensitive than GC and LC, with detection limits in the μg L^{-1} range. Therefore CE methods are more appropriate for analysis of wastewater samples rather than surface water samples. In a continuous effort to optimize analytical techniques, several advances have recently been made in equipment and in sample preparation, derivatization, and clean-up procedures [14–17]. To confront such analytical problems in both GC and LC analytical procedures, a clean-up step is considered necessary and added before analysis of the final extract.

GC versus LC

Both GC and LC are applicable to the analysis of pharmaceuticals in environmental samples. GC is preferable for the analysis of non-polar and volatile compounds, but it can be applied for the analysis of low concentrations of pharmaceuticals by addition of a derivatization step. This step is very important and many optimization efforts have been made, because it can affect the accuracy of the method, because of the losses of analytes that can occur. The advantages of GC include very high selectivity and resolution, good accuracy and precision, wide dynamic range, and high sensitivity [18, 19]. Recently, GC×GC has been introduced in environmental analysis, providing even better separation and identification of the analytes in complex environmental samples [20]. LC is the preferred technique for separation of polar organic pollutants, and has the advantage of shorter analysis time, necessary for monitoring studies. The main drawback of HPLC analysis of pharmaceuticals in environmental samples is matrix effects (the ion-suppression phenomenon) which can reduce the sensitivity, linearity, accuracy, and precision of the method. For the detection of the analytes, tandem MS–MS is increasingly being used, replacing other detectors, in combination with LC (fluorescence, UV, PAD) and GC (FID, ECD) [21, 22].

Reduction of matrix effects

Matrix effects can be reduced by selective extraction, effective sample clean up after extraction, or improvement of the chromatographic separation. However attention should be paid to the possibility of analyte losses and there is the disadvantage of longer analysis times [22]. Other methods to reduce matrix effects include external calibration using matrix-matched samples, standard addition or internal standard calibration using structurally similar unlabeled pharmaceuticals or isotopically labeled standards, dilution of sample extracts, and isotope dilution (use of an isotopically labeled standard for each target compound) [23, 24].

Sample preparation

The sample-preparation procedure is one of the most important parts of the analysis of organic compounds in environmental matrices. The first step in sample preparation is filtration of an appropriate volume of wastewater (usually 500 mL) through <1-μm glass-fiber filters in order to avoid extraction inefficiencies because of the presence of suspended solids.

Extraction of pharmaceuticals from the sample into a small volume of solvent is the next step. This can be performed by several techniques, the most common being solid-phase extraction (SPE) and solid-phase microextraction (SPME). Other extraction techniques that have been applied include liquid-phase microextraction (LPME) and lyophilization [17].

In multi-residue methods, simultaneous extraction of all target analytes in one SPE step from water samples is the approach most widely used [25]. Another option is to combine two SPE materials in series to classify target compounds into two or more groups, according to their physicochemical properties [25]. Hydrophilic–lipophilic balanced polymers and silica-based bonded phase with strong hydrophobicity are the materials most widely used for pre-concentration and extraction of target compounds. Extraction with hydrophilic–lipophilic balanced polymers gives better results with neutral sample pH, whereas silica-based bonded phases with strong hydrophobicity needs sample pH adjustment before extraction, depending on the kind of analytes to be determined. There has been much research recently assessing SPE stationary phases. Other stationary phases used for pre-concentration and clean up of pharmaceuticals in aqueous samples are strong cation-exchange mixed-mode polymeric sorbent, or polystyrene–divinylbenzene resin modified with carboxyl groups [17]. The most commonly used elution solvents are methanol, acetone, and ethyl acetate. An interesting aspect of SPE procedures is their automation, which can improve the accuracy and speed of analysis of pharmaceuticals. Automated SPE can enable direct injection of untreated samples, automatically conducting conditioning, washing, and elution steps, requiring less time and lower amounts of solvent, improving reproducibility, reducing LODs (limit of detection), and reducing health risks during analysis [22].

SPME has recently started attracting particular interest for the analysis of many organic compounds in aqueous environmental samples, including pharmaceuticals [26]. The principle of SPME is extraction of the target compounds from a sample on to an absorptive layer of sorbent coated on a fiber. The quantity of the compound extracted by the fiber is proportional to its concentration in the sample, as long as equilibrium is reached or, for short-term pre-equilibrium, with the help of convection or agitation. After extraction, the SPME fiber is transferred to the injection port of the GC, where the target compounds are desorbed. SPME eliminates the need for solvents and combines sampling, isolation and enrichment in one step [26]. Determination of polar compounds by SPME can be performed by SPME derivatization, using in-coating, direct, or on-fiber derivatization. The difference between these techniques is that whereas in direct derivatization the derivatizing agent is first added to the sample vial and the derivatives are then extracted by the SPME fiber coating, for on-fiber derivatization, the derivatizing agent is loaded on the fiber, which is subsequently exposed to the sample and extracted [26].

After extraction of the pharmaceuticals from the aqueous sample, derivatization is necessary before GC–MS analysis of polar pharmaceuticals. The effectiveness of derivatization depends on the types of compound studied and on the type of derivatizing agent. The most common derivatizing agents used are acid anhydrides, benzyl halides, alkylchloroformates, and diazomethane, although use of diazomethane has been limited in recently developed methods because of its toxic and carcinogenic properties. In some cases, derivatization can be incomplete, affecting the results of the analysis or completely inhibiting the analysis of some compounds (e.g., β-blockers atenolol and sotalol, which cannot be analyzed by GC–MS for this reason). Moreover, some compounds are thermolabile and decompose during GC analysis (e.g., carbamazepine forms iminostilben as a degradation product) [27]. *N*-Methyl-*N*-(trimethylsilyl)-trifluoroacetamide) (MSTFA) has been reported to perform well as a derivatizing agent for the determination of metoprolol, nadolol, and propranolol (recoveries 85–94%) [28]. Butyldimethylsilylation was reported to perform better than trimethylsilylation, as it forms more stable derivatives after SPE [29]. During derivatization with TMAH (tetramethylammonium hydroxide), aniline can be formed as a by-product, which can interfere with compounds with similar retention times. An injection-port derivatization technique has also been reported [30], having the advantage of avoiding the handling of hazardous derivatizing agents.

Because of the complexity of environmental samples and the procedures of extraction and derivatization of samples containing low concentrations of analytes, a substantial number of interfering substances present in the matrix are frequently found in the extracts. Therefore, a final clean-up step after extraction is required to remove these compounds and enhance the accuracy and the reproducibility of the results. The clean-up step is usually performed using SPE cartridges, as described above. Sample extracts are therefore diluted with an appropriate volume of MilliQ water, until the organic solvent content is below 10%, in order to avoid losses of target compounds, that are finally retained on the SPE cartridge [10].

Recently developed multi-residue analytical methods

Multi-residue methods have the advantage of providing wider knowledge about the occurrence and fate of pharmaceuticals in the environment. However, for simultaneous analysis of compounds from diverse groups with different physicochemical properties, a compromise in the selection of experimental conditions is required, to accurately determine all analytes. This is the major challenge analysts are facing currently. Table 1 presents selected multi-residue analytical methods that have recently been developed.

As seen in the table, there is an increase in the number of compounds that can be analyzed simultaneously. SPE–LC–MS–MS techniques are predominant; in some cases

Table 1 Selected multi-residue analytical methods for pharmaceuticals

Number of pharmaceuticals studied	Sample preparation (phase type)	Separation method	Detection method	MS ionization mode	Mass analyzer type	Detection limit range (μg L^{-1})	Ref.
70	SPE (hydrophilic–lipophilic balanced polymer)	LC	MS–MS	ESI	Triple-quadrupole	0.02–20	[31]
16	Dual SPME (CW–TPR fibers)	LC	MS–MS	ESI	Ion-trap	0.005–0.05[a]	[32]
76	SPE (hydrophilic–lipophilic balanced polymer)	LC	MS–MS	ESI	Triple quadrupole	0.3–10	[33]
43	PLE+SPE (hydrophilic–lipophilic balanced polymer)	LC	MS–MS	ESI	Hybrid triple quadrupole-linear ion-trap	0.01–3.2	[34]
18	SPE (strong cation-exchange mixed-mode polymeric sorbent)	GC	MS	ESI	Single-quadrupole	0.0001–0.028	[35]
28	SPE (strong cation-exchange mixed-mode polymeric sorbent)	UPLC	MS–MS	ESI	Triple-quadrupole	0.0003–0.0005	[36]
29	SPE (hydrophilic–lipophilic balanced polymer)	UPLC	Q-TOF-MS	ESI	Wide-pass-quadrupole	0.01–0.5	[37]
30	SPE (strong cation-exchange mixed-mode polymeric sorbent)	Reversed phase HPLC	MS–MS	ESI	Triple-quadrupole	0.0001–0.005[a]	[38]

[a] LOQ

different extraction techniques were applied, e.g. pressurized liquid extraction (PLE) or dual SPME.

Identification and quantification of transformation products

Routes of introduction of pharmaceuticals into the environment include among others, transformation pathways, which are very important for understanding the fate and behavior of these compounds. Only a little information is currently available with regard to transformation products formed in the environment or wastewater-treatment plants. Besides the difficult selection of relevant transformation products for monitoring purposes, there are several challenges in analyzing transformation products in environmental samples. The generally low but nevertheless potentially toxicologically relevant concentrations in the ng L^{-1} range require enrichment, separation from the matrix, and sensitive detection. Another challenge is the identification of transformation products for which no reference standards exist. An additional challenge is the identification of previously unidentified transformation products, which have never been described in the literature. According to Hollender et al. [39] to unequivocally identify the molecular structure of a transformation product without a reference standard, nuclear magnetic resonance (NMR) analysis coupled with LC would be the method of choice. Although LC–NMR was successfully applied to environmental samples in a few cases it requires costly equipment and is not yet sufficiently sensitive for the low concentrations typically found in environmental samples. Although GC–MS–MS and LC–MS–MS enable quantification at concentrations down to a few ng L^{-1}, without reference standards, interpretation of complicated fragmentation pattern in MS–MS is necessary and may enable identification of unknown transformation products. A new approach to overcome the limitations discussed for GC–MS and LC–MS is to employ high-resolution mass spectrometric detection. Hybrid tandem mass spectrometers (which combine two mass spectrometric techniques, including one high-resolution technique, for example quadrupole time-of-flight tandem mass spectrometry (QTOF) or linear ion-trap–orbitrap tandem mass spectrometry (LTQ-Orbitrap) have been shown to enable fast, sensitive, and reliable detection and identification of low-molecular-weight substances because of their high mass accuracy and mass resolution [40, 41]. Full-scan chromatograms acquired with high mass accuracy and resolution enable selective searching for the molecular ions of transformation products based on their exact mass whereas MS–MS provides structural information from compound fragmentation [39].

Occurrence of pharmaceuticals in aqueous matrices and important issues related to this

Several thousand active pharmaceutical compounds are used for drugs in a large number of medicinal products, and the numbers are continuously increasing. After application, many drugs are excreted without any metabolism by the patients and consequently enter wastewater through the sewage systems either in their parent or metabolized form. Hence, these compounds after wastewater treatment may end up in the environment, because of their incomplete removal or partial mineralization at the treatment plants and the wastewater discharges or wastewater reuse practices. The existence of drugs in environmental waters was first

reported in the 1970s by Tabak and Brunch, [42], Norpoth et al. [43], and Garrison et al. [44], and the first studies reporting the existence of drugs in wastewater go back in the 1980s [45, 46]. From that time onward, numerous studies have confirmed the existence of pharmaceutical compounds in aquatic matrices, sediments, soils, and sludge.

From Fig. 1 it is clear that investigation of drugs in surface waters has been intense in recent years. The lower number of studies related to wastewater can be attributed to the complexity of the matrix and to the fact that only a small number of laboratories had until recently the capability to perform such analyses using sophisticated chemical analysis equipment and methods.

The most frequently detected classes of pharmaceuticals are anti-inflammatory drugs, analgesics, antibiotics, lipid regulators, steroids and related hormones, beta-blockers, and cancer therapeutics [47]. Carbamazepine, diclofenac, ibuprofen, gemfibrozil, atenolol, propranolol, erythromycin, ciprofloxacin, ofloxacin, sulfamethoxazole, and amoxicillin are some of the most popular compounds for which studies report widespread occurrence in the aquatic environmental including wastewater. The occurrence of drugs in the environment leads to various still unanswered questions with regard to their biological potency towards flora, fauna, and humans, for example endocrine-disruption activity and also other type of adverse effects. Some of the most commonly assays used are: algae (*M. aeruginosa, S. leopoliensis, C. vulgaris, S. capricornutum, S. acutus, D. tertiolecta, D. subspicatus, S. obliquus, C. Pyrenoidosa, S. acutus, S. quadricauda*), cnidarian (*H. attenuata*), mollusks (*P. carinatus, C. tentans, C. riparius*), grass shrimps (*P. pugio*), copepods (*N. spinipes*), amphipods (*H. azteca*), mosquito fishes (*G. affinis*), bacteria (*V. fischeri, P. putida, sewage sludge bacteria, A. salmonicida*), crustaceans (*D. magna, M. macrocopa, G. pulex, A. salina, T. pyriformis, C. dubia*), fish (*O. latipes, O. mykiss, Salmo trutta f. fario, P. promelas, D. rerio*), rotifiers (*B. calyciflorus*), diatoms (*C. meneghiniana*), weeds (*L. minor, A. retroflexus L., P. major L., R. acetosella L.*), aquatic macrophytes (*L. gibba, M. sibiricum, M. spicatum*), crop plants (*C. sativus, L. sativa, P. vulgaris, R. sativus, H. distichum, Z. mays L.*), earthworms and enchytreids.

The US Food and Drug Administration requires environmental risk assessments to be performed for human and veterinary medicines on the effects on aquatic and terrestrial organisms before a product can be marketed. In addition, the release of pharmaceutical compounds through wastewater discharges is, to some extent, dealt with by EMEA (European Medicines Agency) guidelines, because these are concerned with the release of medicinal products for human use into the environment [2]. For pharmaceuticals risk assessment, standard ecotoxicity tests are often used with short time scales focusing predominantly on mortality as the endpoint. Moreover, aquatic tests tend to focus on the water compartment and do not take into account pharmaceuticals residing in sediments or soil. In general, the effects observed in these studies occur at much higher concentrations than the relevant environmental ones. Currently, no single assessment factor seems to apply to all aquatic species across a wide diversity of pharmaceuticals. However, although the risk of acute toxic effects in the environment with the current use of pharmaceuticals is unlikely, chronic environmental toxic effects cannot be excluded because of lack of chronic ecotoxicity data [1].

Isidori et al. [48] performed a study that draws attention to endocrine interference caused by drugs. The YES-test (yeast estrogen system assay) and the E-screen assay have been performed to detect the capability of these substances to bind the human estrogenic receptor α (hERα) in comparison with 17β-estradiol. Of fourteen pharmaceuticals tested, nine were positive to YES-assay and eleven were positive to E-screen assay. In particular, furosemide and the fibrates (bezafibrate, fenofibrate, and gemfibrozil) gave the maximum estrogenic response. Tamoxifen showed its dual activity as agonist and antagonist of hERα. Even though tests were performed using drug concentrations higher than those in the environment, this result is alarming if one considers that drugs with the same activity can co-exist in natural environments and thus their overall concentration might be higher than those currently determined.

Plant uptake of pharmaceuticals, which occurs when treated wastewater is reused for irrigation, may also affect plant development. It is, in part, unclear whether the negative effects on plants originate from direct damage to the plant by the pharmaceuticals themselves or whether the antimicrobial action of pharmaceuticals on soil microorganisms is responsible for the damage by affecting the plant–microorganism symbiosis [49]. The latter is attributed to the fact that antibiotics in the soil may affect plant development indirectly by disrupting soil communities: the decrease in the number of soil bacteria leads to a lack of food for soil fauna (protozoa, nematodes, micro arthropods) and finally affects soil function: plant residues are decomposed more slowly, denitrification is slower, and therefore nutrients are recycled more slowly [50]. Risk assessment for uptake of pharmaceuticals in the edible portions of crops suggests that, because of the allergenic potential and long-term effects of antibiotics, the risk cannot be neglected [51]. More examples are given by Fatta-Kassinos et al. [2] in relation to mycotoxicity, plant development retardation potential, etc.

Another important issue is the widespread occurrence of antibiotics in the environment; this most often is reported in the literature in relation to the development of antibiotic-resistant bacteria, which is an evolutionarily conserved natural process. According to Kümmerer [52], the most

prominent medical examples are vancomycin-resistant enterococci, methicillin-resistant *Staphylococcus aureus*, and multi-resistant pseudomonads. The transfer of resistant bacteria to humans could occur via water or food if plants, for instance, are irrigated with water, wastewater, or sludge laden with antibiotic-resistant bacteria or genes which have escaped treatment. Wastewater constitutes one of the routes through which not only antibiotics but also resistance genes are introduced into natural bacterial ecosystems. In such systems, non-pathogenic bacteria could serve as a reservoir of resistance genes. Antibiotic resistance evolves in bacteria because of the effect of industrially produced antimicrobial agents on bacterial populations and communities [53]. Genetic reactors are places in which the occasion occurs for genetic evolution, particularly because of high biological connectivity, generation of variation, and the presence of specific selection. Beyond mutational events, significant genetic variation occurs as a consequence of recombinatorial events, frequently resulting from genetic exchanges among organisms inside populations and communities. According to Baquero et al. [53], there are four main genetic reactors in which antibiotic resistance evolves. The first is the human and animal microbiota on which therapeutic or preventive antibiotics exert their actions. The second is the hospitals, long-term care facilities, farms, or any other similar place in which susceptible individuals are crowded and can be exposed to bacterial exchange. The third reactor corresponds to the wastewater and to any other biological reactor type that can exist in places described above (e.g. hospitals, farms), including for instance lagoons, sewage treatment plants (STPs), or compost toilets, in which bacterial organisms from many different individuals have the opportunity to mix and genetically react. The fourth reactor is the soil, surface, or groundwater environments in which bacterial organisms originating in the other reactors mix and counteract with environmental organisms. Water is involved as a crucial agent in all four genetic reactors, but particularly in the last ones. The possibility of reducing the evolution of antibiotic resistance depends on the ability of humans to control the flow of active anti-microbial agents, bacterial clones, and genetically based biological information along these genetic reactors. This fact is of utmost importance in any wastewater discharge or reuse practices [53].

Furthermore, antibiotics might act, at very low concentrations, as signaling agents in microbial environments. Common receptors have been identified in plants for a number of antibiotics and disinfectants affecting chloroplast replication (fluoroquinolones), transcription–translation (tetracyclines, macrolides, lincosamides, aminoglycosides, pleuromutilins), folate biosynthesis (sulfonamides, and probably trimetoprim), fatty acid synthesis (triclosan), and sterol biosynthesis (azoles, statins) [53–56]. During recent years the environmental consequences of the release of triclosan in freshwater environments has been considered [57]. Ciprofloxacin affects stream microbial communities, including those colonizing senesced leaf materials [58].

According to Baquero et al. [53], a matter of major future concern is the effect of antibiotics and disinfectants released into the environment on *Cyanobacteria*, largely susceptible to antimicrobial agents, because these organisms account for more than 70% of total phytoplankton mass, and are responsible for more than a third of total free O_2 production or CO_2 fixation. What seems certain is that such alterations in microbial ecosystems either produced by release of antimicrobials or by the unexpected effective dispersal in water environments of resistant pathogenic organisms [59] might be relevant for public health.

Tables 2, 3 and 4 provide an overview of the various studies performed for determination of antibiotics in wastewater, surface, and groundwater, with the concentrations of the various pharmaceuticals. Most often few or even single monitoring campaigns are carried out. This, combined with the variety of sampling methods used, made it difficult to compare levels, which fluctuate substantially. For example in wastewater effluents, ciprofloxacin was found between 8 and 720 ng L^{-1}, cephalexin between 10 and 5070 ng L^{-1}, erythromycin between 38 and 4330 ng L^{-1}, and sulfamethoxazole between 4 and 9460 ng L^{-1} (Table 2). In surface waters, ciprofloxacin was found to range between 14.4 and 9660 ng L^{-1}, nalidixic acid between <10 and 750 ng L^{-1} and clarithromycin between 3 and 2330 ng L^{-1} (Table 3).

Hospitals are another major source of pharmaceuticals in the environment. This occurs through the hospital sewage system for admitted patients. Most of the work up on hospital effluents has focused on antibiotics but it has been emphasized that hospitals may be an important point source of some other classes of pharmaceuticals to the environment [102]. Hospital wastewater may contain a variety of organic xenobiotic compounds, for example pharmaceutical residues, radionuclides, solvents, and disinfectants used for medical purposes in a wide range of concentrations, because of laboratory and research activities or medicine excretion. Pharmaceutical residues may include prescription drugs, for example analgesics, antibiotics, blood-pressure regulating drugs, and hormones, but they can also contain residues from some other over-the-counter medicinal products. Additionally, this specific wastewater stream may contain other substances typically or almost exclusively administered in hospitals, for example X-ray contrast media, special diagnostic agents, cytostatic compounds, and some antibiotics used almost exclusively in hospitals, the objective being to limit the risk of development of resistant bacteria. Pharmaceuticals specifically administered in hospitals also include some strong highly effective analgesics classified

Table 2 Occurrence of antibiotics in urban wastewater effluents

Antibiotic	Concentrations (ng L^{-1})	Ref.
Amoxicillin (β-lactams)	50 / 30 / 64–1670	[60–62]
Ampicillin (β-lactams)	7 / 126	[63, 64]
Azithromycin (macrolides)	4–23 / 75 / 15	[63, 65, 82]
Cefaclor (β-lactams)	1800 / 60	[60, 61]
Cefotaxime (cephalosporins)	7 / 34	[63, 74]
Cephalexin (cephalosporins)	250 / 10–994 / 170–5070 / 283 / 240–1800 / 376	[60, 62–64, 74, 75]
Ciprofloxacin (quinolones)	720 / 240 / 132 / 627 / 140 / 400 / 8–73 / 220–450 / 62–106 / 108 / 251 / 42–392	[61, 63–73]
Chlortetracycline (tetracyclines)	250 / 50–280	[60, 83]
Clarithromycin (macrolides)	536 / 172 / 240 / 70–611 / 57–328 / 100 / 12–232 / 18	[63, 65, 67, 72, 75, 76, 81, 84]
Clindamycin (lincosamides)	70 / 15–33 / 51	[60, 63, 81]
Doxycycline (tetracyclines)	60–340 / 150 / 46 / 40	[60, 61, 76, 83]
Enrofloxacin (quinolones)	50 / 10	[60, 61]
Erythromycin-H_2O (macrolides)	361–811 / 300 / 838 / 246–4330 / 695 / 510–850 / 38–96 / 110–199	[62–64, 68, 74–76, 84]
Lincomycin (lincosamides)	300 / 60 / 30.5	[60, 61, 72]
Nalidixic acid (quinolones)	450 / 55 / 178	[60, 61, 63]
Norfloxacin (fluoroquinolones)	250 / 210 / 5.5–3700 / 85–320 / 112 / 120 / 36–73 /64	[60, 62, 66, 70, 71, 74, 76, 77]
Ofloxacin (fluoroquinolones)	183 / 53–991 / 506 / 110 / 96–7870 / 123 / 2–556 / 50–210 / 600 / 32–548 / 740–5700	[62–64, 67, 72, 73, 75, 76, 78–80]
Oxytetracycline (tetracyclines)	70 / 20 / 100–340 / 5–842 / 5	[60–62, 83, 85]
Roxithromycin (macrolides)	500 / 18 / 85–547 / 3 / 3–14 / 11–22 / 18	[60–64, 76, 84]
Sulfadiazine (sulfonamides)	6 / 16 / 34.3 / 19 / 4180	[63, 64, 76, 80, 89]
Sulfadimethoxine (sulfonamides)	2 / 310 / 2 / 9 / 12	[63, 81, 83, 85, 89]
Sulfamethazine (sulfonamides)	130–640 / 363 / 2 / 11 / 400	[63, 76, 83, 89]
Sulfamethoxazole (sulfonamides)	200 / 320 / 79–472 / 130–500 / 47–964 / 370 / 871 / 310 / 5–278 / 226 / 4–39 / 15–47 / 242 / 220–680 / 2000 / 289 / 127 / 132 / 9460	[60–65, 68, 69, 72, 75, 76, 78–81, 83, 85, 87, 89]
Sulfathiazole (sulfonamides)	600 / 5 / 2 / 54 / 4270	[60, 61, 63, 89, 90]
Tetracycline (tetracyclines)	20 / 30 / 31–34 / 190–360 / 16–38 / 850 / 977 / 3.5–1420 / 21 / 150–620 / 24 / 89 / 61–290	[60–64, 68, 69, 74–76, 81, 83, 85]
Trimethoprim (dihydrofolate reductase inhibitors)	250 / 70 / 2–37000 / 203–415 / 550 / 180 / 59–465 / 321 / 120–230 / 11–66 / 210–2400 / 1070 / 1288 / 105 / 140	[60–65, 68, 69, 74, 78, 79, 86–88]
Tylosin (macrolides)	3400 / 65 / 7	[60, 61, 63]

as opioid analgesics (derivatives of the opium poppy alkaloid morphine) and non-opioid analgesics [103]. Table 5 provides an overview of various studies performed during the last several years on the occurrence of pharmaceutical residues in hospital effluents along with the concentrations of the drugs. Some of the compounds were detected at high concentrations, for example ciprofloxacin at levels ranging from 751 to 101,000 ng L^{-1}; the same is true for compounds like ofloxacin (up to 35,500 ng L^{-1}), trimethoprim (up to 7,600 ng L^{-1}), and acetaminophen (up to 186,500 ng L^{-1}).

By their nature, it is expected that hospital effluents will contribute to some extent to the pharmaceutical load in the influent entering wastewater-treatment plants but the question is how significant this contribution is. Several studies have demonstrated that for some drugs the contribution might be more substantial than for others. The study performed by Langford and Thomas, [102] shows that point source discharges from hospitals typically make a small contribution to the overall pharmaceutical load when compared with municipal areas. However, this varies from substance to substance and is not true when a drug's use is primarily hospital-based. According to the same study, there is some uncertainty when looking at the hospital contributions of pharmaceutical compounds for which deconjugation seems to occur; for these drugs effluent concentrations were higher than in the influent. When measuring only the parent compound it is assumed that no deconjugation occurs in the sewage system before reaching the treatment plant. In reality it is possible that deconjugation occurs throughout the wastewater system so measuring the compounds in their conjugated form would be neces-

Table 3 Occurrence of antibiotics in surface waters

Antibiotic	Concentration (ng L^{-1})	Location	Ref.
β-Lactams			
Amoxicillin	200	River water system, Australia	[60]
Cefaclor	200	River water system, Australia	[60]
Penicillin G	250	River water system, Australia	[60]
Penicillin V	10	River water system, Australia	[60]
Cephalosporin			
Cephalexin	100	River water system, Australia	[60]
Quinolones			
Ciprofloxacin	1300	River water system, Australia	[60]
	17.4–588.5	Olona, Lambro, Po rivers, Italy	[67]
	<10	Seine River, France	[91]
	370–9660	Arc River, France	[92]
	14.4–26.2	Po and Lambro rivers, Italy	[72]
Danofloxacin	19	Seine River, France	[91]
Enoxacin	11	Seine River, France	[91]
Enrofloxacin	300	River water system, Australia	[60]
Flumequine	32	Seine River, France	[91]
Nalidixic acid	750	River water system, Australia	[60]
	<10	Seine River, France	[91]
Oxolonic acid	19	Seine River, France	[91]
Fluoroquinolones			
Norfloxacin	1150	River water system, Australia	[60]
	163	Seine River, France	[91]
	251	Pearl river, Guangzhou, China	[93]
	24–48	Lake and river water, India	[94]
Ofloxacin	19.3–306.1	Olona, Lambro, Po rivers, Italy	[67]
	8.1–634	Victoria Harbour, Hong Kong	[62]
	55	Seine River, France	[92]
	108	Pearl river, Guangzhou, China	[93]
	33.1–306.1	Po and Lambro rivers, Italy	[72]
Lincosamides			
Clindamycin	10	River water system, Australia	[60]
Lincomycin	50	River water system, Australia	[60]
	1.9–17.3	Olona, Lambro, Po rivers, Italy	[67]
	24.4–248.9	Po and Lambro rivers, Italy	[72]
Macrolides			
Clarithromycin	3.0–114.8	Olona, Lambro, Po rivers, Italy	[67]
	600–2330	Arc River, France	[92]
	190	River water, Germany	[95]
	1.6–20.3	Po and Lambro rivers, Italy	[72]
Erythromycin-H_2O	4.7–1900	Victoria Harbour, Hong Kong	[62]
	636	Pearl river, Guangzhou, China	[93]
Oleandomycin	20	River water system, Australia	[60]
Roxithromycin	350	River water, Australia	[60]
	169	Pearl river, Guangzhou, China	[93]
	190	Lutter river, Germany	[95]
	<30–40	Elbe river and tributaries, Germany	[96]
Spiramycin	3.3–459.5	Olona, Lambro, Po rivers, Italy	[67]

Table 3 (continued)

Antibiotic	Concentration (ng L^{-1})	Location	Ref.
	9.8–74.2	Po and Lambro rivers, Italy	[72]
Tylosin	60	River water system, Australia	[60]
Tetracyclines			
Chlortetracycline	600	River water system, Australia	[60]
	160	Cache La Poudre, USA	[83]
	1–180	Choptank watershed, USA	[97]
Democlocycline	120–440	Cache La Poudre, USA	[83]
Doxycycline	50–80	Cache La Poudre, USA	[83]
	13–146	Choptank watershed, USA	[97]
	400	River water system, Australia	[60]
Oxytetracycline	100	River water system, Australia	[60]
	7.7–105.1	Olona, Lambro, Po rivers, Italy	[67]
	80–130	Cache La Poudre, USA	[83]
	1–388	Choptank watershed, USA	[97]
	110–680	Arc River, France	[92]
	2–7	Alzette and Mess rivers, Luxembourg	[85]
	68000	River water, Japan	[98]
Tetracycline	80	River water system, Australia	[60]
	60–140	River water, USA	[83]
	1–5	Choptank watershed, USA	[97]
	7–8	Alzette and Mess rivers, Luxembourg	[85]
Sulfonamides			
N_4-Acetylsulfamethazine	0.7–316.8	Segre, Llobregat, Anoia rivers, Spain	[89]
Sulfadiazine	1.9–2312	Segre, Llobregat, Anoia rivers, Spain	[89]
	336	Pearl river, Guangzhou, China	[93]
Sulfadimethoxine	50–90	Cache La Poudre, USA	[83]
	1–9	Choptank watershed, USA	[97]
	3	Alzette and Mess rivers, Luxembourg	[85]
	1.5–182.4	Segre, Llobregat, Anoia rivers, Spain	[89]
Sulfadimidine	323	Pearl river, Guangzhou, China	[93]
Sulfamethazine	220	Cache La Poudre, USA	[83]
	<10	Seine River, France	[91]
	1.7–6192	Segre, Llobregat, Anoia rivers, Spain	[89]
Sulfamethoxazole	2000	River water system, Australia	[60]
	50–120	Cache La Poudre, USA	[83]
	300	Rio Grande, New Mexico	[78]
	1–7	Choptank watershed, USA	[97]
	480	Lutter river, Germany	[95]
	193	Pearl river, Guangzhou, China	[93]
	<30–70	Elbe river and tributaries, Germany	[96]
	544	Seine River, France	[71]
	1–22	Alzette and Mess rivers, Luxembourg	[85]
	6.4–1488	Segre, Llobregat, Anoia rivers, Spain	[89]
	47–96	Lake and river water, India	[94]
Sulfamethoxypyridazine	4.4–3704	Segre, Llobregat, Anoia rivers, Spain	[89]
Sulfapyridine	1.2–12000	Segre, Llobregat, Anoia rivers, Spain	[89]
Sulfasalazine	30	River water system, Australia	[60]
Sulfasoxazole	0.5–2.8	Segre, Llobregat, Anoia rivers, Spain	[89]

Table 3 (continued)

Antibiotic	Concentration (ng L^{-1})	Location	Ref.
Sulfathiazole	40	River water system, Australia	[60]
	1.5–332	Segre, Llobregat, Anoia rivers, Spain	[89]
	2	Mess River, Luxembourg	[85]
Chloramphenicol			
Chloramphenicol	266	Pearl river, Guangzhou, China	[93]
Dihydrofolate reductase inhibitors			
Trimethoprim	150	River water system, Australia	[60]
	<30–40	Elbe river and tributaries, Germany	[96]
	31	Seine River, France	[91]
	87	River Nakkavagu, India	[94]
	120	Lutter river, Germany	[95]

sary in order to confirm the load coming from hospitals compared with that from the public. Toxicity studies have highlighted the potential toxic effects of hospital effluent entering the aquatic environment [107, 108] and drug-resistant bacteria have also been observed where hospital effluents are present [109, 110].

Further to the above, recent studies have produced important new knowledge concerning the existence of

Table 4 Occurrence of antibiotics in groundwater

Antibiotic	Concentration (ng L^{-1})	Location	Ref.
Lincosamides			
Lincomycin	320	18 States, USA	[99]
Sulfonamides			
N_4-Acetylsulfamethazine	2.7	Barcelona, Spain	[89]
	0.02–56.95	Plana de Vic and La Selva, Catalonia, Spain	[100]
Sulfabenzamide	0.09–10.32	Plana de Vic and La Selva, Catalonia, Spain	[100]
Sulfacetamide	1.77–3461	Plana de Vic and La Selva, Catalonia, Spain	[100]
Sulfadimethoxine	0.2	Barcelona, Spain	[89]
Sulfadoxine	0.02–53.63	Plana de Vic and La Selva, Catalonia, Spain	[100]
Sulfaguanidine	3.3–91.78	Plana de Vic and La Selva, Catalonia, Spain	[100]
Sulfamerazine	0.11–744.7	Plana de Vic and La Selva, Catalonia, Spain	[100]
Sulfamethazine	360	18 States, USA	[99]
	76–215	Private wells, Idaho, USA	[101]
	0.03–106.8	Plana de Vic and La Selva, Catalonia, Spain	[100]
Sulfamethizole	0.22–9.29	Plana de Vic and La Selva, Catalonia, Spain	[100]
Sulfamethoxazole	9.9	Barcelona, Spain	[89]
	1110	18 States, USA	[99]
	0.08–312.2	Plana de Vic and La Selva, Catalonia, Spain	[100]
Sulfamethoxypyridazine	0.02–68.70	Plana de Vic and La Selva, Catalonia, Spain	[100]
Sulfanitran	0.04–568. 8	Plana de Vic and La Selva, Catalonia, Spain	[100]
Sulfapyridine	0.07–72.45	Plana de Vic and La Selva, Catalonia, Spain	[100]
Sulfaquinoxaline	0.01–112.1	Plana de Vic and La Selva, Catalonia, Spain	[100]
Sulfathiazole	0.01–16.78	Plana de Vic and La Selva, Catalonia, Spain.	[100]
Sulfisomidin	0.01–64.40	Plana de Vic and La Selva, Catalonia, Spain	[100]
Sulfisoxazole	0.21–4.43	Plana de Vic and La Selva, Catalonia, Spain	[100]

Table 5 Occurrence of pharmaceuticals residues in hospital effluents

Compound	Concentration (ng L^{-1})	Ref.	Compound	Concentration (ng L^{-1})	Ref.
Antibiotics					
Amoxicillin	900	[60]	Ampicillin	53	[64]
				5080	[63]
Azithromycin	227	[63]	Cefazolin	6221	[63]
Cefotaxine	413	[63]	Cephalexin	2457	[63]
Chloramphenicol	1	[63]	Chlortetracycline	11	[64]
Ciprofloxacin	1100–44000	[104]	Clarithromycin	721	[63]
	751	[63]			
	15000	[60]			
	3600–101000	[105]			
	2000	[78]			
Clindamycin	341	[63]	Colchicine	9	[63]
	90	[60]			
Dimetridazole	19	[63]	Doxycycline	200	[60]
				600–6700	[105]
Enrofloxacin	40	[63]	Erythromycin	676	[63]
	100	[60]		6110	[64]
				10–30	[106]
Flumequine	3	[63]	Lincomycin	24	[63]
				1700	[60]
				2000	[78]
Marbofloxacin	3	[63]	Metronidazole	1800–9400	[106]
				1591	[63]
				100–90200	[105]
Nalidixic acid	186	[63]	Norfloxacin	131	[63]
	40	[60]		900–17000	[104]
				200	[60]
Ofloxacin	35500	[78]	Oleandomycin	40	[60]
	1088	[63]			
	200–7600	[105]			
Oxolin acid	5	[63]	Oxytetracycline	14	[75]
Pefloxacin	62	[63]	Penicillin	10	[60]
				5200	[78]
Sulbutamol	22	[63]	Sulfadiazine	50	[63]
Sulfamerazine	1	[63]	Sulfamethoxazole	7350	[75]
				647	[63]
				400–12800	[105]
				300	[60]
				210	[78]
Sulfanilamide	19	[63]	Terbutaline	38	[63]
Tetracycline	455	[64]	Thiampenicol	4	[63]
	40	[60]			
Trimethoprim	600–7600	[105]			
	10–30	[106]			
	1040	[63]			
	300	[60]			
	5000	[78]			
β-Blockers					
Acebutolol	185	[63]	Atenolol	100–122000	[106]

Table 5 (continued)

Compound	Concentration (ng L^{-1})	Ref.	Compound	Concentration (ng L^{-1})	Ref.
				1607	[63]
Metopronol	145	[63]	Propranolol	225	[75]
				200–6500	[106]
				42	[63]
				15500–37500	[102]
NSAIDs					
Acetaminophen	186500	[64]	Diclofenac	286	[63]
	500–29000	[106]		70000	[75]
	36950	[63]		60–1900	[106]
Famotidine	94	[63]	Ibuprofen	300	[75]
				1500–151000	[106]
				282	[63]
Lipid regulators					
Bezafibrate	1	[63]	Clofibric acid	9	[63]
Gemfibrozil	134	[63]			
	1110	[64]			
Psychiatric drugs					
Carbamazepine	30–70	[106]			
	163	[63]			

illicit drugs in the urban water cycle. Residues of illicit drugs can reach STPs in substantial amounts, escaping degradation; these are then released into surface waters. The four most used classes of illicit drugs worldwide are cannabis, cocaine, opiates and amphetamine-like stimulants. Information about the occurrence of selected illicit drugs is given in Table 6.

Environmental concentrations are low, but risks to human health and the environment cannot be excluded. Morphine, cocaine, methamphetamine, and ecstasy all have potent pharmacological activity, and their presence, as complex mixtures in the aquatic or terrestrial environment may be toxic to aquatic organisms. Levels of residues in untreated wastewater have been used to estimate illicit drug consumption in the population. Given that current epidemiological methods are indirect and possibly biased, this evidence-based approach offers a new tool for estimating drug abuse in real time. "Sewage epidemiology" can be feasible because, according to van Nuijs et al. [123], their study achieved general good agreement between analytical results derived for illicit drugs and the percentage of population that uses illicit drugs at a given time based on data from Spain, Belgium, UK, Italy, Switzerland, and USA, and data from international organizations, for example the European Monitoring Centre for Drug and Drug Addiction (EMCDDA) and the United Nations Office on Drugs and Crime (UNODC).

The capacity of different technologies to remove pharmaceutical residues

The quality of the treated flow from an STP is measured by use of techniques which assess the removal of nitrogen and phosphate, pathogens, suspended solids, metals, and organic load. It is now well accepted that conventional treatment plants are not designed to quantitatively remove micropollutants such as pharmaceuticals and this results in their widespread environmental presence. During the last decade, intense efforts have been made to improve the performance of STPs in respect of micropollutants' removal, often by introducing new steps designed to remove such contaminants more efficiently [69, 124–126]. Nevertheless, many STPs in Europe and other parts of the world include only two treatment steps (physical and biological)—only a small number of plants use a tertiary or advanced treatment step (e.g. ultrafiltration, flocculation, ozonation, advanced oxidation, or osmosis) [127]. These last treatments are seldom used because of their high cost. However, they are under extensive investigation because of the improvements they yield in the removal of organic micropollutants. Enhanced tertiary chemical treatment processes, for example TiO_2 photocatalysis [128, 129] and several advanced oxidation processes [130] can be good engineering solutions to eliminate the residual micro-constituents derived from biological systems.

The large differences among STPs make the knowledge about treatment efficiency for pharmaceuticals somewhat

Table 6 Occurrence of selected illicit drugs in aquatic media (adapted from Ref. Huerta-Fontela et al. [163])

Drug	Source[a]	Concentration (ng L^{-1})	Samples[b]	Country	Ref.
Cocaine	ww	11	2 WWTPs	Italy	[111]
	ww	17	16 WWTP	Spain	[112]
	ww	47–138	1 WWTPs	Ireland	[113]
	ww	6.2–105	4 WWTPs	Spain	[114]
	ww	0.1–100	42 WWTP	Spain	[115]
	sw	1.2	River	Italy	[72]
	sw	6	River	Spain	[112]
	sw	25–33	River	Ireland	[113]
	sw	7–26	3 Streams	Belgium	[116]
	sw	4–183	4 Rivers	Italy	[117]
	sw	4–6	River	UK	[117]
	sw	9–60	River	Spain	[118]
	sw	1–115	28 Rivers	Belgium	[119]
	sw	1–7	2 Rivers	UK	[120]
Benzoylecgonine	ww	100–547	2 WWTPs	Italy	[111]
	ww	49	1 WWTP	Germany	[121]
	ww	0.1–1500	16 WWTPs	Spain	[112]
	ww	22–31	1 WWTP	Ireland	[113]
	ww	30–318	4 WWTPs	Spain	[114]
	ww	0.1–1500	42 WWTPs	Spain	[115]
	sw	25	River	Italy	[75]
	sw	3	River	Germany	[121]
	sw	77	River	Spain	[112]
	sw	44–191	3 Rivers	Belgium	[116]
	sw	0.5–44	4 Rivers	Italy	[117]
	sw	4–16	River	UK	[117]
	sw	15–150	River	Spain	[118]
	sw	1–520	28 rivers	Belgium	[119]
	sw	1–123	2 Rivers	UK	[120]
	dw	3–130	DWTP	Spain	[118]
Amphetamine	ww	4–210	16 WWTPs	Spain	[112]
	ww	0.5–3.3	4 WWTPs	Spain	[114]
	ww	4–210	42 WWTPs	Spain	[115]
	sw	6–9	River	UK	[120]
	sw	5–90	River	Spain	[118]
	sw	9–50	2 Rivers	UK	[120]
Morphine	ww	111	12 WWTP	Germany	[121]
	ww	55	2 WWTPs	Italy	[111]
	ww	12–30	4 WWTPs	Spain	[114]
	ww	21–81	5 WWTPs	Spain	[122]
	sw	3–38	4 Rivers	Italy	[117]
	sw	5–42	River	UK	[117]
Methadone	ww	9.1–36	2 WWTPs	Italy	[111]
	ww	4–25	5 WWTPs	Spain	[122]
	sw	3.4–8.6	4 Rivers	Italy	[117]
LSD	ww	10.2–1.6	4 WWTPs	Spain	[114]

[a] ww, wastewater; sw, surface water; dw, drinking water

[b] WWTPs, wastewater treatment plants; DWTP, drinking water treatment plant

vague. The efficiency of removal of pharmaceuticals in STPs can, indeed, vary substantially. Treatment efficiency in STPs is significantly affected by several factors, for example the physicochemical properties of pharmaceuticals, the treatment processes employed, the age of the activated sludge [131], the hydraulic retention time (HRT), and environmental conditions such as temperature and light intensity [132]. An important factor is also the sampling technique. Grab samples can only serve to obtain preliminary results in mostly screening studies. For calculating the loads or mass-fluxes in treatment plants, 24-hour composite sample collection should be carried out.

Knowing only the removal efficiency is not sufficient to understand whether the pharmaceuticals are adsorbed by sludge (often used for soil treatment), or whether they are biodegraded or abiotically degraded. Additionally, toxic degradation products occurring in the treated wastewater may not be identified if they are not explicitly addressed. Finally several pharmaceuticals are excreted as conjugates and can make a significant, but poorly understood, contribution after release of the active moiety by cleavage during treatment in STPs.

Concern is growing over incomplete removal during wastewater treatment, where microorganisms drive the key processes. The effect of pharmaceuticals on bacterial community structure in activated sludge was assessed by Kraigher et al. [133] in small-scale wastewater treatment bioreactors containing different concentrations (5, 50, 200, and 500 mg L^{-1}) of several commonly used pharmaceuticals (ibuprofen, naproxen, ketoprofen, diclofenac and clofibric acid). T-RFLP (terminal restriction fragment length polymorphism) analyses of the bacterial 16S rRNA genes indicated a minor but consistent shift in bacterial community structure in a bioreactor supplied with pharmaceuticals at a concentration of 50 mg L^{-1} (R50) compared with the control reactor operated without addition of pharmaceuticals (R0). In reactors operated with higher concentrations of pharmaceuticals, a greater structural divergence was observed. Bacterial community composition was further investigated by preparation of two clone libraries of bacterial 16S rRNA genes from reactors R0 and R50. Most clones in both libraries belonged to the betaproteo-bacteria, among which *Thauera*, *Sphaerotilus*, *Ideonella*, and *Acidovorax-related spp*. dominated. Nitrite-oxidizing bacteria of the genus Nitrospira sp., which are key organisms for the second stage of nitrification in wastewater-treatment plants, were found only in the clone library of the reactor without pharmaceuticals. In addition, diversity indices calculated for the two clone libraries were indicative of reduced diversity of activated sludge bacterial community in the reactor R50.

Removal rates are mostly reported as the difference in gram per day per inhabitant between influent and effluent [111, 134, 135]. Other information can also be found, though, for example the contribution of STP effluent to the presence of pharmaceuticals in natural waters [134], the ratio of removal by sorption to biological transformation, the concentration of pharmaceuticals after a treatment involving activated sludge, sand filtration, and ozonation [126], seasonal variations, and/or pharmaceutical concentration comparisons between the inlets and outlets of different STPs [116, 124, 135].

Table 7 provides selected examples for a variety of technology used mainly on an industrial scale and their removal efficiencies for several pharmaceutical compounds. The occurrence and removal of a variety of pharmaceuticals were studied by Zorita et al. [127] in the inlet and outlet of a tertiary STP in Sweden and between different treatment steps in the STP which includes conventional activated sludge step (Table 7). The HRT of each treatment step was considered for sampling and for calculation of removal rates. These rates were above 90%, except for clofibric acid and ofloxacin. Diclofenac was not eliminated during the treatment and in fact even higher concentrations were found in the effluent than in the inlet of the STP. The chemical treatment improved the removal of several pharmaceuticals, especially the antibiotics, for which step removal rates were between 55 and 70%. Despite the very low concentrations that escape the treatment process one cannot rule out the potential for chronic effects that may result from the degradation products, or ignore the ecological implications that may be caused by mixtures of compounds in nature. There are also cases, for example the study by Zorita et al., in which the method detection limit for a compound (in this case ethinylestradiol) was somewhat higher than the NOEC (no observable effect concentration), and therefore possible acute effects cannot be excluded.

Several pharmaceutically active compounds were monitored during one-year period in influent and effluent wastewater from STPs in Spain to evaluate their temporal evolution and removal from wastewater and to discover which variables affect their removal rates [137]. The compounds monitored were diclofenac, ibuprofen, ketoprofen, naproxen, and carbamazepine. All of the pharmaceutically active compounds monitored, except diclofenac, were detected in influent and effluent wastewater. Mean concentrations measured in influent wastewater were 0.48, 93.6, 1.83, and 5.41 μg L^{-1} for carbamazepine, ibuprofen, ketoprofen and naproxen, respectively, and those measured in effluent wastewater were 0.56, 8.20, 0.84 and 2.10 μg L^{-1}. Mean removal of the pharmaceuticals varied from ca. 8% (carbamazepine) to 88% (ibuprofen). The existence of relationships between the concentrations of the pharmaceutical compounds, their removal, the properties of influent wastewaters and WWTP design have been studied statistically (correlation and principal-component analysis).

Table 7 Efficiency and capacity of different treatments in respect of removal of pharmaceuticals

Ref.	Target drug	Information on the treatment process	Scale/type of wastewater	Results/findings
[127]	Fluoxetine	Mechanical: screens, grit-aerated chamber (sand trap), sedimentation basin	Industrial scale	Fluoxetine [1]3.5 – [2]<LOD – [3]>90
	Norfluoxetine	Biological: anoxic step followed by larger aerobic decomposition. In order to achieve a more efficient biological process in the aeration basin, an activated sludge process is used involving recycling of ca. 90% of the sludge giving an average solid retention time of 8±2 days	Sewage	Norfluoxetine [1]3.6 – [2]<LOD – [3]>99
	Ofloxacin			Ofloxacin [1]11.8 – [2]5.2 – [3]56
	Norfloxacin			Norfloxacin [1]9.3 – [2]<LOD – [3]>70
	Ciprofloxacin			Ciprofloxacin [1]168 – [2]16 – [3]90
	Ibuprofen	Removal of phosphorus using $FeCl_3$		Ibuprofen [1]3600 – [2]24 – [3]99
	Naproxen	Final sedimentation basin equipped with lamellar counter-current settler plates.		Naproxen [1]2560 – [2]146 – [3]94
	Clofibric acid	Final polishing step: sand filtration (HRT of the sand filter: ca. 0.5 h).		Clofibric acid [1]28 – [2]11 – [3]61
				[1] mg/day/1000inh (influent)
				[2] mg/day/1000inh (effluent)
				[3]Removal (%)
[136]	Salicylic acid	Screen, grit removal, activated sludge process, UV disinfection, microfiltration (MF), reverse osmosis (RO) and chlorination. It should be noted that the RO is only used for storm water treatment, which is supplied via a brickpit. Sewage is treated by MF only, because no salt removal is intended.	Industrial scale	The removal efficiencies were higher than 90% for all compounds except for ketoprofen (80%). The initial concentrations were:
	Acetaminophen		Sewage	salicylic acid: acetaminophen: 23.3 μg L^{-1}
	Ibuprofen			ibuprofen: 2.8 μg L^{-1}
	Naproxen			naproxen: 3.1 μg L^{-1}
[137]	Diclofenac	Four conventional WWTPs in Seville, Spain	Industrial scale	Removals:
	Ibuprofen		sewage	Ibuprofen, 80–88%
	Ketoprofen			Ketoprofen, 52–72%
	Naproxen			Naproxen, 43–71%
	Carbamazepine			Carbamazepine, 8–15%
				The initial concentrations were:
				Ibuprofen, 69.7–115
				Ketoprofen, 1.58–2.07
				Naproxen, 4.28–8.07
				Carbamazepine, 0.41–0.53
[138]	Atenolol, metoprolol, propranolol, bezafibrate, erythromycin, sulfamethoxazole, trimethoprim, diclofenac, indomethacin, ketoprofen, mefenamic acid, carbamazepine, omeprazole	1) The biological treatment worked under a traditional nitrification–denitrification stage and enhanced phosphorus removal by phosphorus-accumulating microorganisms. The treatment takes place in three zones: anaerobic, anoxic, and oxic. The nitrate produced in the oxic zone is recycled, with mixed liquor, to the anoxic zone where denitrification takes place. The return sludge from the settler is recycled to the anaerobic zone where the influent and sludge are mixed under anaerobic conditions.	Industrial scale	1) For all compounds removal efficiencies were below 20% in the STP treatment.

Table 7 (continued)

Ref.	Target drug	Information on the treatment process	Scale/type of wastewater	Results/findings
		2) Ozonation with doses lower than 90 μmol L^{-1}	Sewage and industrial wastewater	2) With ozonation, all compounds except ketoprofen and bezafibrate were totally removed.
[139]	Clofibric acid, diclofenac, ketoprofen, mefenamic acid, carbamazepine, primidone	1) Pilot-scale MBR + nanofiltration (NF)/RO	Pilot-scale MBR	The presence of organic macromolecules increased removal by an NF membrane, whereas an RO membrane resulted in very high removal irrespective of the presence of organic macromolecules.
		2) CAS + media filtration + NF/RO (industrial scale)	Conventional activated sludge (CAS) + media filtration (industrial scale)	This was attributed to modification of the membrane surface because of membrane fouling and association between the macromolecules and the pharmaceuticals.
		The NF/RO part of was on the bench scale	sewage	The results obtained in this study revealed that characteristics of organic macromolecules differed depending on the type of wastewater treatment used for pretreatment for subsequent NF/RO processes (e.g., MBRs or conventional tertiary treatments).
[140]	Sulfadiazine, sulfamethoxazole, norfloxacin, ofloxacin, ciprofloxacin, tetracycline, enalapril, salbutamol, famotidine, ranitidine, cimetidine, glibenclamide, nadolol, atenolol, bezafibrate, gemfibrozil, atorvastatin, propyphenazone, ketoprofen, naproxen, ibuprofen, diclofenac, acetaminophen, salicylic acid, furosemide	7 WWTPs Most of the plants have primary and secondary treatment operating with conventional activated sludge, except one in which biological treatment is with biological filters, but the main differences between them lie in their hydraulic retention times.	Industrial scale Sewage	Removal efficiency (% ± RSD): Sulfadiazine (69±32) Sulfamethoxazole (74±22) Norfloxacin (57±54) Ofloxacin (40±64) Ciprofloxacin (66±35) Tetracycline (71±33) Enalapril (96±11) Salbutamol (60±44) Famotidine (50±59) Ranitidine (66±39) Cimetidine (50±64) Glibenclamide (46±39) Nadolol (60±51) Atenolol (59±50) Bezafibrate (69±39) Gemfibrozil (67±48) Atorvastatin (58±44) Propyphenazone (44±68) Ketoprofen (69±40) Naproxen (86±13) Ibuprofen (91±13) Diclofenac (58±53) Acetaminophen (99±1) Salicylic acid (96±8) Furosemide (50±59)

With both statistical analyses, high correlations were obtained between the concentration of the pharmaceutical compounds and the properties of influent wastewaters, and between removal of the pharmaceutical compounds, removal the pollution load of influent wastewaters, and WWTP hydraulic retention times. Principal-component analysis showed the existence of two main components accounting for 76% of the total variability. The first component reflects a close correlation between the total Kjeldahl nitrogen (TKN) content of influent wastewater, removal of BOD, COD, TKN, and oil, removal of most of the pharmaceutical compounds monitored, and the operating condition HRT. The second component reflects a close correlation between TSS, BOD, TP, and oil and the concentration of all of the pharmaceutical compounds monitored.

Rosal et al. [138] have reported a systematic survey of over seventy individual pollutants in a STP receiving urban wastewater. The compounds include mainly pharmaceuticals and personal care products, and some metabolites. For the group of compounds seen in Table 7 removal efficiency was below 20% in STP treatment. Ozonation with doses lower than 90 mmol L^{-1} enabled the removal of many individual pollutants including some of those more refractory to biological treatment. The results showed that the hydroxyl radical reaction was the major pathway for oxidative transformation of these compounds.

High-pressure-driven membranes for example nanofiltration (NF) membrane and reverse osmosis (RO) membranes, are believed to be effective for control of pharmaceuticals in wastewater treatment. According to Kimura et al. [139], in practical applications of NF/RO membranes to municipal wastewater treatment, feed water for the membranes always contains organic macromolecules at concentrations of up to 10 mg TOC L^{-1}, which are mainly composed of soluble microbial products (SMPs) produced during biological wastewater treatment, for example an activated sludge process. In this study, the effect of these organic macromolecules on the removal of six pharmaceuticals by NF/RO membranes was investigated. Two types of biological treatment (a conventional activated sludge process followed by media filtration (i.e., tertiary treatment) and treatment with a membrane bioreactor (MBR)) were examined as pretreatments for NF/RO membranes in this study. In the filtration tests with wastewater effluents, removal of the pharmaceuticals was higher than that seen with deionized pure water spiked with the pharmaceuticals. The increase was significant for the NF membrane. Both alteration of membrane surface properties, because of membrane fouling, and association of the pharmaceuticals with organic macromolecules contributed to the increase in removal of pharmaceuticals by the membranes. Characteristics of the organic macromolecules contained in the wastewater effluents differed depending on the type of treatment, implying that removal of pharmaceuticals by NF/RO membranes is affected by the type of pretreatment used.

Concluding, as mentioned also by Gros et al. [140], reported overall removal rates vary strongly between individual pharmaceuticals and among studies. It is therefore difficult to establish a general trend for each of the therapeutic groups; in most cases, however, results indicate that elimination of most of the substances is incomplete. For serotonin reuptake inhibitors, benzodiazepines, carbamazepine, and macrolide antibiotics, negative removal is often observed during conventional treatment. This is usually attributed to biotransformation of conjugates. What is also important to note is that currently it is not fully possible to elucidate which factors explain the behavior of the various pharmaceutical compounds, because in most studies insufficient operating data are reported for the STPs [140]. Besides compound physicochemical properties, other influencing factors can be the temperature of operation (higher removal efficiencies have been observed in summer periods in comparison with colder seasons), different kinetic behavior (degradation rates) of compounds, redox conditions, sludge retention time, and hydraulic retention time.

Transformation products

Recently, there have been studies on the metabolites and oxidation products of pharmaceuticals [16, 141], because it is important to investigate their presence and, especially, their possible effects on the environment and human health, which are still largely unknown for these compounds.

Carbamazepine 10,11-epoxide has been detected in STP influents at levels far lower than the parent compound [141]. Acetylsalicylic acid (ASA) and its metabolites have been detected in STP influents, whereas usually only salicylic acid has been found in effluents, depending mostly on the influent concentrations. ASA is easily degraded by deacetylation into salicylic acid and two other metabolites, *ortho*-hydroxyhippuric acid and the hydroxylated metabolite gentisic acid. The metabolites of ibuprofen usually detected are the corresponding hydroxy and carboxy compounds [141]. Caffeine is detected in many environmental matrices and is used as an anthropogenic marker for wastewater contamination of natural waters. Its main metabolite, 1,7-dimethylaxanthine, has been detected in STP effluents [141]. Clofibric acid, the major metabolite of lipid regulators (e.g., clofibrate, etofibrate, etofyllinclofibrate), and fenofibric acid, the major metabolite of fenofibrate, have been detected [142].

Metabolites of illicit drugs have also been detected in environmental samples at trace levels; examples include cocaine's metabolites benzoylecgonine (BE) and coca-

ethylene (CE) [37]. CE is a transesterification product formed when cocaine is consumed with ethanol, and transforms rapidly into the metabolites norcocaethylene and ecgonine ethyl ester. Heroin is subject to rapid hydrolysis to morphine and 6-acetylmorphine. Lysergic acid diethylamide (LSD) and its metabolites nor-LSD, nor-iso-LSD, and 2-oxo-3-hydroxy-LSD (O-H-LSD), have been detected at very low concentrations [37]?. Phenylethylamine ephedrine, 3,4-methylenedioxymetamphetamine hydrochloride (MDMA or "ecstasy"), methylenedioxyethylamphetamine (MDE, MDEA, or "Eve"), and 3,4-methylenedioxyamphetamine (MDA or "Love pills", and metabolites of both MDE and MDMA), have been detected frequently at ng L^{-1} levels. 11-nor-9-Carboxy THC (nor-THC) and 11-hydroxy-THC (OH-THC), both metabolites of Δ9-tetrahydrocannabinol (THC), the most physiologically active constituent of cannabis, have also been detected [143, 144].

Once released into the environment via the discharge of treated or untreated wastewater, pharmaceuticals can undergo the same potential transport and degradation processes as all other organic contaminants. The main elimination processes can be biotic or abiotic. Following the current state of knowledge for the microbial degradation of pharmaceuticals and taking into account the fact that in general they have a designed resistance to biodegradation, it is considered that microbial degradation for most of the drugs may not be an important loss process in the aquatic environment. Many are expected to be eliminated from the environment by abiotic degradation processes (e.g. hydrolysis, photolysis, redox reactions, etc) [145]. Among these, direct photolysis and indirect photodegradation processes including reaction with photo-excited dissolved organic matter (DOM), and transient reactive species, for example singlet oxygen ($^1O_2$), the hydroxyl radical ($HO^\bullet$), peroxy radicals ($^\bullet OOR$), and solvated electrons (e_{aq}^-), generated by irradiation of various aquatic components (DOM, NO_3^-, Fe^{3+}), may be an important removal process for these compounds, because usually their structure contains aromatic rings, heteroatoms, and other functional groups that can either directly absorb solar radiation or react with the above-mentioned photogenerated transient species in natural waters [145]. The effect of light in the destruction of organic chemicals has also proved to be useful in water treatment technology [130]. Only a few studies have dealt with biotic natural attenuation processes and proved that such processes can, indeed, contribute to the elimination of drugs. Recent work by Lin et al. [146] during a two-week simulation study found that for acetaminophen, biodegradation was an important attenuation process whereas sorption was the dominant mechanism of removal for propranolol and acebutolol. For caffeine, both sorption and biodegradation were primary attenuation processes. The term "biodegradation" refers for the purposes of the particular study to the elimination of the parent compound without any knowledge whether the compound is mineralized or transformed to a metabolite.

Table 8 summarizes some of the chemical oxidation products that have been detected during the application of different oxidation processes to wastewater containing pharmaceuticals.

Another crucial issue related to photo-transformation products is that only few specialized reports are available with regard to potential toxic effects of these compounds. Bioassays have been performed on bacteria, algae, rotifers, and microcrustaceans to assess acute and chronic toxicity, and the SOS chromotest and the Ames fluctuation test have been used to detect the genotoxic potential of the investigated photoderivatives. Results obtained so far from assessment of the ecotoxicity of the photoproducts of diclofenac, naproxen, and the fibrates, for instance, constitute well-established evidence that acute and chronic toxicity can be greater for the photoproducts than for the parent compounds, and genotoxic and mutagenic effects cannot be excluded [145].

Policies and socioeconomic measures for pharmaceuticals' pollution mitigation control

The problem related to the "*pharmaceuticals in the environment*" field, may find solutions using various socioeconomic measures. Mitigation measures, i.e. approaches used for management of pollutant flows, may be required because end-of-pipe solutions (e.g. wastewater treatment) are not always adequate for solving these problems. Bodies such as authorities and industrial chambers may undertake mitigation measures leading to prevention of release of pharmaceuticals into the environment. Moreover, the quality of the sludge at the STPs will improve and the microbial communities and processes at STPs will be free from such biologically active compounds.

Methods for prevention of release of pharmaceuticals into the environment, including "control at the source" by segregation of sources, improvement of disposal systems for expired medicines, and application of pharmaceutical-return programs, and the development of "green" pharmaceuticals, have been proposed [154–157]. Source separation can be regarded as an effective means of preventing release of pharmaceuticals into the aquatic environment. For example, segregating sources of pharmaceuticals, e.g. hospital wastewater and urine source separation at the household level, could prevent the release of pharmaceuticals and metabolites into wastewater. Unused and expired pharmaceuticals are frequently disposed of via the sink/toilet or in household waste ending up in landfill sites,

Table 8 Oxidation products of pharmaceuticals

Pharmaceutical	Oxidation process	Oxidation product	Ref.
Sulfadiazine	$TiO_2/h\nu$	4-Methyl-2-aminopyrimidine	[147]
Sulfamethoxazole	Ozonation	Hydroxylamine	[148]
Sulfamethoxine	$TiO_2/h\nu$	2,6-Dimethoxy-4-aminopyrimidine 2-Aminothiazole	[147]
Sulfathiazole	$TiO_2/h\nu$	2,6-Dimethoxy-4-aminopyrimidine 2-Aminothiazole	[147]
Sulfamerazine	$TiO_2/h\nu$	4-Methyl-2-aminopyrimidine	[147]
Busperidone	$TiO_2/h\nu$	Hydroxybusperidone Dihydroxybusperidone Dipyrimidinylbusperidone 1-Pyrimidinylpiperazine	[149]
Carbamazepine	Ozonation	1-(2-Benzaldehyde)-4-hydro(1*H*,3*H*)quinazoline-2-one	[150]
	H_2O_2/UV	1-(2-Benzaldehyde)-(1*H*,3*H*)quinazoline-2,4-dione 1-(2-Benzoic acid)-(1*H*,3*H*)quinazoline-2,4-dione Acridine, salicylic acid, catechol, anthranilic acid	[151]
Paracetamol	Ozonation	2-[(2,6-Dichlorophenyl)amino]-5-hydroxyphenylacetic acid	[152]
	H_2O_2/UV	2,5-Dihydroxyphenylacetic acid *N*-4-Hydroxyphenylacetamide	[153]

which, via leaching, reach groundwater. To confront this problem, the US Federal Prescription Drug-Disposal Guidelines (2007) [158] allows flushing when it is safe to do so, and return of unused, unneeded, or expired medicines to pharmaceutical take-back locations for safe disposal. Through pharmaceutical-return programs, residual medications can be collected from the public at take-back locations and disposed of in an environmentally sound manner. The US EPA has suggested the desirability of national regulation for disposal of unwanted and expired pharmaceuticals and personal care products, and of implementation of an "extended producer responsibility" for manufacturers and distributors, and has listed these issues as outstanding research needs [159]. Pharmaceutical return programs to collect unused and expired medicines have been established by the pharmaceutical industry in Spain and in British Columbia, Canada, where provincial waste-management regulations require all brand owners of pharmaceutical products to fund and organize pharmaceutical-return programs involving efficient collection and safe disposal of leftover medicines returned by the public [160].

Replacement of persistent pharmaceuticals with more "environmentally friendly" or "green" pharmaceuticals would be an effective way of facilitating their rapid removal upon release to the environment, [161] but this of course requires much effort and time. Although achieving such a replacement still seems a challenging task for the distant future, research relevant to this field has started in Sweden's Stockholm County Council for assessment and classification of pharmaceuticals according to their environmental impact: persistence, bioaccumulation, and toxicity (PBT). Each of these properties is assigned a value on a scale of 0 to 3 and the sum of these values constitutes the PBT index for the pharmaceutical. A PBT value of zero in each category means that a drug is biodegradable, does not bioaccumulate, and has low toxicity, whereas a total of 9 indicates the highest level for these three unfavorable properties for the particular pharmaceutical (least environmentally friendly). In this way, an environment label is being introduced in Sweden with the assistance of the chemical industry, which would enable the physician and the patient, where medications of similar action and efficiency are available, to select the treatment that is more environment-friendly [1, 162]. PBT testing for environmental pollutants however, is a topic for debate as pharmaceuticals are constantly being re-infused into the environment. Therefore, even if degraded rather rapidly, chronic toxic exposure may still result. This has to be taken into consideration during development and application of all relevant methods, including PBT testing.

Current knowledge and future prospects

Pharmaceuticals are inherently biologically active compounds and often very potent. They are also designed to be resistant to biodegradation because metabolic stability usually improves their desired pharmacological action. This however, contributes to their environmental persistence. Pharmaceuticals have such physicochemical characteristics

that make them quite unique contaminants. For this reason, their behavior cannot be simulated or compared with other chemicals, for example pesticides.

Concerning pharmaceutical analysis, in recent years, advanced analytical methods have been developed and optimized, with the objective of improving precision and sensitivity, to enable accurate quantification of trace concentrations of pharmaceuticals present in the aquatic environment. The analytical instrumentation used includes GC–MS, GC–MS–MS, LC–MS and LC–MS–MS.

Various methods and materials are used for sample preparation. SPE is the most popular and well-established sample-preparation technique, with which the best sensitivity is obtained. SPME has also been applied recently because it has several advantages over SPE in terms of sample handling and minimizing solvents used.

However, despite the techniques available and the optimizations already performed, rapid, accurate analysis of trace concentrations of pharmaceuticals in complex environmental matrices continues to be a fascinating challenge for many researchers working in the field. Moreover, identification of unknown compounds including transformation by-products is still an open question. Further research is needed to improve method accuracy and sensitivity. There is also a need to expand on-going scientific research to assess the impact of pharmaceuticals, and their metabolites and transformation products on the aquatic environment. The effluent organic matter of treated wastewater needs to be characterized to a greater extent especially when wastewater is reused for irrigation.

Much more research effort should thus be directed toward elucidation of the structure, fate, and behavior of pharmaceutical metabolites and transformation products. The role of indirect photochemistry, and, especially, the involvement of photoexcited dissolved organic matter, reactive radical species, and other naturally occurring compounds in the photodegradation in the aquatic environment should be investigated.

More data and further refinement of risk assessment are required to estimate the acute and chronic potential effects of these compounds and by-products in the environment. In addition, further investigations on the ecotoxic potential of their mixtures are required.

The current consensus on treatment in the research community is that no single technology can completely remove pharmaceuticals. For example, the fact that the membrane bioreactor has higher sludge retention times than conventional activated sludge treatment does not solve the problem, because concentrations of such compounds can still escape. Nanofiltration and reverse osmosis can be quite successful in removing some pharmaceuticals but the rejected water and brine can still contain recalcitrant organic load. Advanced chemical oxidation can be quite efficient in removing such compounds. These processes however, have the drawback of producing oxidation by-products that can, occasionally, be more biologically active than the parent compounds. Hence, integration of removal technology may prove essential to handling of today's mixtures of compounds in wastewater.

Little has been done to prevent pharmaceuticals from entering the environment in the first place. Various approaches have been discussed and applied, including the control of pharmaceuticals at source, segregation of sources, and improvement of disposal systems for expired medicines. Because such approaches have been implemented only rarely, pharmaceuticals end up in wastewater-treatment plants and hence into the environment as a result of incomplete removal.

In conclusion, although there is much uncertainty about possible detrimental effects of pharmaceuticals on aquatic and terrestrial ecosystems, the precautionary principle should be considered and applied.

Currently, the OSPAR convention is the only regulatory body to consider pharmaceuticals as a threat and this refers to the marine environment. The pharmaceutical agents clotrimazole, (a common antifungal agent) and diosgenin (steroid) have been listed for priority action, and other drugs have been recognized as being of possible concern. According to the European policy framework, the precautionary principle may be invoked when urgent measures are needed because of the possibility of danger to human, animal, or plant health, or to protect the environment where scientific data do not enable complete evaluation of the risk. Therefore, despite the fact that science has not yet established direct cause and effect relationships, the principle may give rise to stricter standards for wastewater treatment, for instance, in the near future.

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