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Review

Ecotoxicological aspects related to the presence of pharmaceuticals in the aquatic environment

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ABSTRACT

Pharmaceuticals are biologically active and persistent substances which have been recognized as a continuing threat to environmental stability. Chronic ecotoxicity data as well as information on the current distribution levels in different environmental compartments continue to be sparse and are focused on those therapeutic classes that are more frequently prescribed and consumed. Nevertheless, they indicate the negative impact that these chemical contaminants may have on living organisms, ecosystems and ultimately, public health. This article reviews the different contamination sources as well as fate and both acute and chronic effects on non-target organisms. An extensive review of existing data in the form of tables, encompassing many therapeutic classes is presented.

Contents

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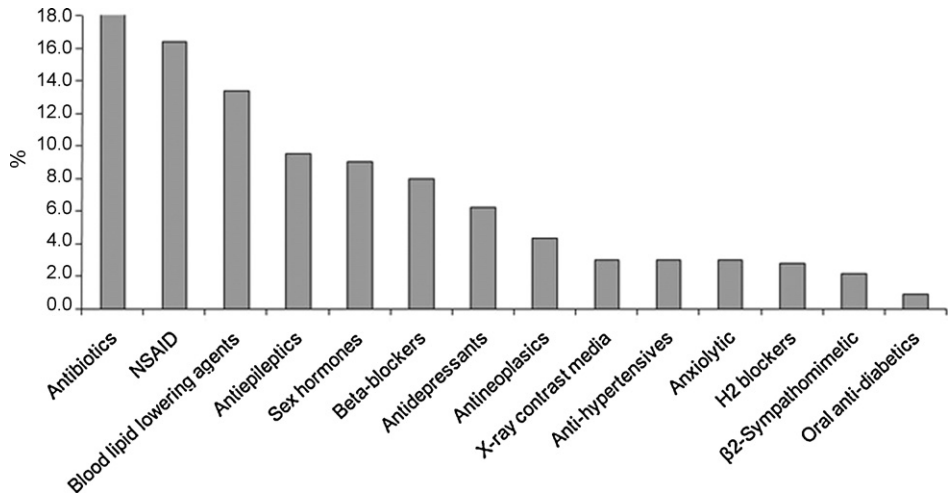

Fig. 1. Percentage of published studies on different therapeutic classes, expressed in relative percentage, described on 183 articles published between 1996 and 2009.

1. Introduction

The presence of medicines in the environment has become a recent research topic. Initially, the problem was highlighted in the US back in the 1970s [1,2] and almost a decade later in England (UK) [3–5]. Yet, it was only in the mid 90s with advances in analytical techniques that important knowledge on environmental contamination by those compounds grew. Powerful hyphenated chromatographic-detection techniques enabling detection limits within the ng L^{-1} to μg L^{-1} range allowed researchers to quantify a large number of medicines components (i.e. drugs and excipients) in the environment, thus compelling the scientific community to consider this contamination type as a potential issue meriting concern [6–8]. In fact, tons of them are produced annually worldwide to be consumed by humans or animals [9,10]. They are conceived primarily to have particular physiological modes of action and frequently to resist to inactivation before exerting their intended therapeutic effect. However, these same properties are paradoxically responsible either for bioaccumulation and toxic effects in aquatic and terrestrial ecosystems [10,11]. In a different way from some conventional pollutants (such as pesticides, detergents, fuels, among others), medicines are continuously delivered at low levels which might give rise to toxicity even without high persistence rates [11–13]. Wide dissemination at low concentrations mainly in the aquatic environment is evident today. Such concentrations have been detected in aquatic compartments such as influents [14–16] and effluents [17–19] from sewage treatment plants (STPs), surface waters (rivers, lakes, streams, estuaries, among others) [20–24], seawater [25], groundwater [26–28] and drinking water [29–32]. The scientific community is in broad agreement with the possibility that adverse effects may arise from the presence of pharmaceuticals not only for human health but also for aquatic organisms. Several, almost negligible effects have been shown to occur from continuous exposure during the life cycle of aquatic vertebrates and invertebrates to sub-therapeutic drug concentrations [33,34]. These effects slowly accumulate to manifest themselves into a final irreversible condition which is frequently only noticed several generations' later, affecting sustainability of aquatic organisms' populations [35].

This review presents an updated survey of the acquired knowledge regarding the sources, spreading conditions, occurrence and induced toxic effects on non-target organisms by drugs in the environment. Fig. 1 illustrates the clear predominance of studies on non-steroidal anti-inflammatory drugs (NSAIDs), antibiotics and blood lipid lowering agents from the literature, drawn from human prescription and consumption. Most of the reported data concerns the occurrence of drugs of each therapeutic class in the aquatic environment and is included in the form of tables to facilitate easy comparison between regional sample sources and ecotoxicological data. Current EU and US legislation compels new medicines to undergo an environmental impact assessment and consequently, new evaluation methods for acute as well as chronic effects are being implemented. However, a significant lack of knowledge persists particularly concerning toxicological data from synergistic pharmaceuticals interactions.

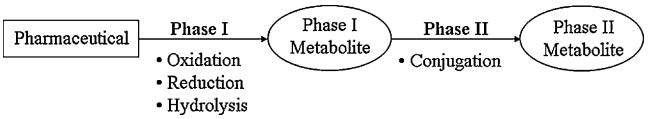

Fig. 2. Schematic representation of pharmaceutical biotransformation to increase their polarity (adapted from Reference [35]).

2. Sources of environmental contamination

The most obvious pathway for environmental contamination of medicines is via the unaltered excretion in urine and faeces although other anthropogenic mechanisms should be assumed, namely:

a) Metabolism post-consumption; since many drugs are metabolised as the organism attempts to convert hydrophobic compounds into more easily excreted polar residues. Their bioconversion into one or more metabolites can occur throughout Phase I[1] and Phase II[2] reactions as shown in Fig. 2 [36].
b) Diagnostic compounds; such as X-ray contrast media are directly discharged in their native forms.
c) Household Disposal; either topic formulations or unused medicines (out-of-date or unwanted) are discarded through the sink/toilet or via waste collection [9,37,38], before being taken to

[1] Phase I reactions include oxidation, reduction and hydrolysis to modify the original molecule structure by introducing functional groups more receptive to phase II reactions.
[2] Phase II reactions (or conjugation reactions) consist of the addition of endogenous groups (like glucuronic acid, sulphate, glutathione, etc.) to receptive functional groups present in the original molecule or in its metabolite derived from phase I.

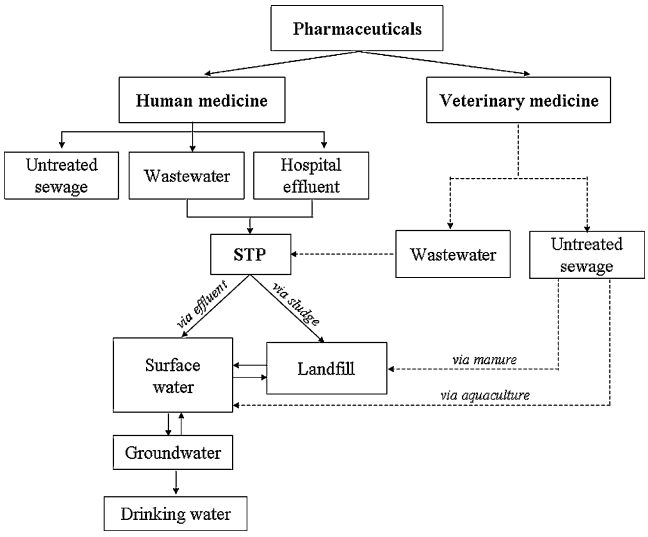

Fig. 3. Representative sources and fate of pharmaceuticals in the environment (adapted from Reference [6]).

landfill sites where they appear as terrestrial ecosystem contaminants. Alternatively, they may possibly leak into surrounding water compartments [39,40].

d) Impacts due to anthropogenic activities; as, for instance, Sewage Treatment Plant (STP) sludge, which can carry non-suspected drugs and is frequently used as a fertilizer on agricultural land [41,42]; veterinary medicines, which are also excreted in urine and faeces by animals before being spread onto land via manure application as fertilisers. Apart from the potential for direct soil contamination, there is also the risk of run-off with heavy rain, thus potentially contaminating both the surrounding surface and groundwater [42–44]. Other example of an anthropogenic activity is aquaculture, whose pharmaceuticals employed, as well as their metabolites and degradation products, are directly discharged into surface waters [45,46]. Another important source of environmental contamination by pharmaceuticals is the effluents of pharmaceutical production facilities [47–49].

At a higher level, existing geographical information on environmental contamination sources is sparse and limited. Countries and regions worldwide differ concerning the prevalence of diseases, waste treatment processes, cultural habits or economic constraints related to the pharmaceutical market [8]. Nevertheless, it seems that urban regions are major sources of contamination due to the proximity of hospitals and STP facilities. Additionally, the contribution of rural regions where agriculture, animal husbandry and aquaculture represent important ways of life should be considered as important.

3. Environmental fate

The fate and behaviour of medicines in the environment still requires further elucidation. As previously stated, drugs (used in human and/or in veterinary medicine) and their metabolites are spread into the environment in different ways, namely through STP effluents, heavy rain on agricultural land provokes (surface) water run-off, and occasionally, through untreated sewage (domestic wastes and flooding, among others) (Fig. 3). Some of them do reach surface waters (rivers, lakes and estuaries, among others) and eventually groundwaters [11,35,39] after resisting the intended biological degradation. However, in surface waters they may be degraded through different processes such as photolysis whose efficiency depends on factors such as intensity of solar irradiation, latitude, season of the year and presence of photosensitizes (e.g. nitrates, humic acids) [50,51].

In the case of drugs that have low volatility and high polarity distribution is mainly made by aqueous transport or even via food chain dispersion [35,52]. Usually, wastewaters are conducted to STPs, which play a key role in the entrance of pharmaceuticals in the environment. However, in some regions or even countries these kinds of facilities may not exist and the environmental problem is still worse. The evaluation of removal efficiency in STPs (by comparing influent and effluent contents) has been studied in detail, showing removal rates that can differ by up to 99% [22,53–55]. Depending both on the particular technology resorted to and the active substance properties they may undergo: (i) degradation (mineralization) to low molecular weight compounds (e.g. CO_2 and water); (ii) entrapment by suspended solids; (iii) discharge of the parent compound through chemical cleavage of the respective conjugate forms and (iv) conversion to a more hydrophilic, persistent form which will short-circuit the treatment process [39,41,56,57]. Thus, in hospitals use of specific antibiotics, antineoplasic or diagnostic agents subsequently requires a sewage treatment process more embracing and directed to these kind of drugs, which are only used in hospitals [35,58], and that must be different to the more specific procedure adopted at STPs receiving industrial discharges from drug manufactures [47–49,59]. In both, the form and extension of the final contamination risk will also depend on geographical location of the STP facility. Low adsorption coefficients that make active substances remain in the aqueous phase, favour their mobility through the STP and into nearby surface waters [53]. Adsorption to suspended solids depending on both hydrophobic and electrostatic interactions established between each will follow the same destiny [11,41]. On the other hand, hydrophobic metabolites will be held on STP sludge, provoking terrestrial contamination, thus affecting microorganisms and invertebrates. Aerobic/anaerobic bio-conversion occurring either during sewage sludge digestion or during activated sludge treatment seems to be the most efficient process to eliminate chemical contaminants from the aquatic environment. Usually, the best biodegradation results are obtained when activated sludge treatment is conducted through an increase in hydraulic retention time and the use of mature sludge [10]. However, one should be aware of the fact that if a particular pharmaceutical is not detected in a STP effluent, this does not imply that it has been fully removed. On some occasions, it may have been degraded and give rise to unsuspecting metabolites that will subsequently contaminate surface waters [35,39,60]. Notwithstanding that some drugs and their metabolites show a stable nature, nowadays is still difficult to establish a complete contamination pattern in final receiving surface waters, due to the water dilution, the treatment and discharging processes [54].

4. Ecotoxicology

Continuous consumption of drugs even at sub-therapeutic concentrations represents a potential threat to public health although one should bear in mind that it is still impossible to evaluate the effects of exposure on human health [35,60,61]. In turn, many non-target organisms (which possess human- and animal-alike metabolic pathways, similar receptors or biomolecules) are therefore inadvertently exposed to active substances released into the environment [10,35]. A comprehensive manner to evaluate the toxicity effects on non-target organisms must include the development of specific tests embracing either acute effects (where mortality rates are often registered) or chronic effects (by means of exposure to different concentrations of a chemical compound over a prolonged period of time). In the latter, effects are measured

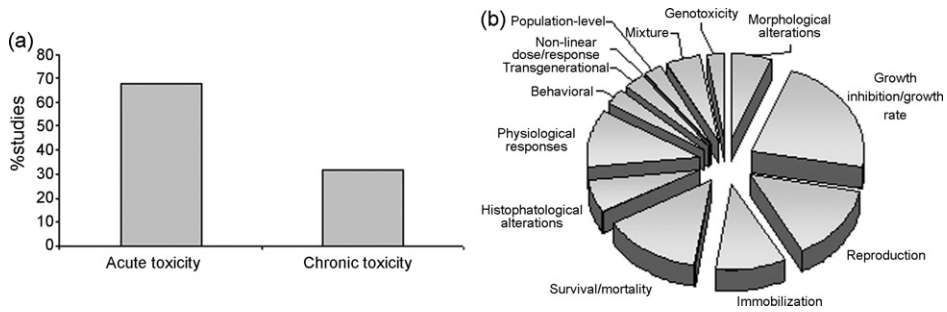

Fig. 4. (a) Acute vs. chronic ecotoxicological studies. (b) Principal endpoints used in ecotoxicological studies, expressed in relative percentage (data collected from 94 articles published between 1996 and 2009).

through specific parameters such as growth index or reproduction rates [52]. Unfortunately, studies on acute effects in organisms belonging to different trophic levels (i.e. algae, zooplankton and other invertebrates and fish) predominate relatively to chronic ones (Fig. 4). Acute toxicity data is only valuable when accidental discharge of the drugs occurs, since the environmental concentrations usually reported for these compounds are low, typically in a factor of one thousand. Bioaccumulation and chronic toxicity tests are scarce [10,35] probably due to the complex experimental work involved. However, recent development of sensitive methods for identification and quantification of drugs enabled to devise their distribution patterns in several environmental samples, thus highlighting the more relevant therapeutic classes in terms of environmental contamination (Fig. 5). These data is useful to set out the most appropriate active substances to be used in ecotoxicity tests. According to data present in literature, scientific community has mainly concerned their attention on therapeutic classes such as, non-steroidal anti-inflammatory drugs, blood lipid lowering agents, antibiotics and sex hormones. By those reasons, this review will focus in the drugs belonging to those therapeutic classes.

Within this context, some of the acute and chronic toxicity effects caused by drugs belonging to different therapeutic classes and mixtures of them in non-targets organisms deserve further analysis and are discussed in the following section. For a critical analysis of the ecotoxicological data present in the literature relatively to different drugs, we decide to group them according to their main pharmacological activity. Therefore, toxicity data will be related to the environmental concentrations found by several authors, to establish the severity of the situation.

4.1. Non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs are weak acids acting by reversible or irreversible inhibition of one or both isoforms of the cyclooxygenase enzymes, COX-1 and COX-2, involved in the synthesis of different prostaglandins from arachidonic acid [62]. A cyclooxygenase enzyme similar to human COX-2 has been found in fish thereby making them a potential target for aquatic contamination [63]. Prostaglandins also play an important role in the synthesis of bird eggshells and from inhibiting its synthesis, shell thinning has been observed [64]. Among the NSAID, diclofenac showed the most acute toxic nature with effects being observed at concentrations below 100 mg L^{-1} [65]. Chronic toxicity trials performed on rainbow trout (*Oncorhynchus mykiss*) evidenced cytological changes in the liver, kidneys and gills after 28 days of exposure to just 1 μg L^{-1} of diclofenac. For a concentration of 5 μg L^{-1} renal lesions were evident as well as drug bioaccumulation in the liver, kidneys, gills and muscle [66,67]. Brown trout (*Salmo trutta* f. *fario*) showed similar cytological damage and a reduction of haematocrit values after 21 days of exposure to 0.5 μg L^{-1} of this active substance [68]. Schmitt-Jansen et al. [69] evaluated both diclofenac phytotoxicity and its photochemical products on the unicellular chlorophyte *Scenedesmus vacuolatus*. Inhibition of algal reproduction by the parent compound only occurred at a concentration of 23 mg L^{-1}, hence indicating no specific toxicity. However, the threat significantly increased when metabolites were produced from 53 h of exposure to daylight. Diclofenac also inhibited the growth of marine phytoplankton *Dunaliella tertiolecta* for concentrations of 25 mg L^{-1} and above [70]. For this organism, 96 h EC_{50} of

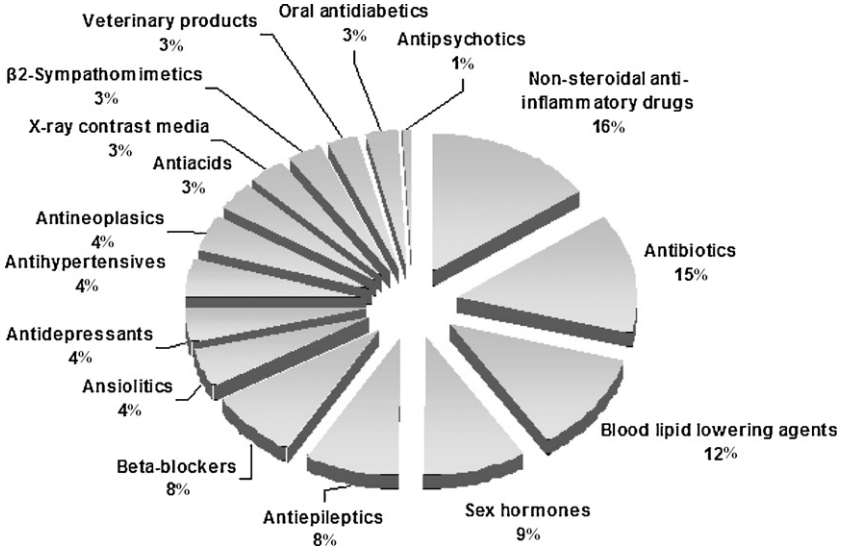

Fig. 5. Therapeutic classes detected in the environment, expressed in relative percentage. Data collected from 134 articles published between 1997 and 2009.

Table 1
Examples of concentrations (ng L^{-1}) of non-steroidal anti-inflammatory drugs measured in different aquatic environments.

Compound	CAS number	Sample	Country	Analytical procedure	LOD (ng L^{-1})	Concentration reported (ng L^{-1})	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
Acetylsalicylic acid	50-78-2	Somes river water	Romania	SPE-GC–MS	30 (LOQ)	<30–37.2 (±4.6)	[20]	Algae	*D. subspicatus*	EC_{50} (growth inhibition)	106.7 mg L^{-1}	[95]
Acetylsalicylic acid		STP influent	Japan	SPE-GC–MS	10 (LOQ)	470–19,400	[86]	Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	88.1 mg L^{-1}	[95]
		STP effluent				38.0–111						
Salicylic acid	69-72-7	STP effluent	Canada	SPE-GC–MS/MS	0.1	554.3–2178.2	[17]	Bacteria	*V. fischeri*	EC_{50} (30 min)	90 mg L^{-1}	[83]
		River water				130.4–371.5						
		Lake water				286.7						
Salicylic acid		STP influent	Canada	SPE-GC–MS	10	2820–12,700	[18]	Algae	*Scenedesmus subspicatus*	EC_{50} (72 h)	>100 mg L^{-1}	[83]
		STP effluent				10–320						
								Crustacean	*D. magna*	EC_{50} (24 h) (immobilization)	118 mg L^{-1}	[83]
								Ciliates	*Tetrahymena pyriformis*	EC_{50} (48 h) (growth inhibition)	>100 mg L^{-1}	[83]
								Fish	*B. rerio (zebra fish)*	LC_{50} (48 h)	37 mg L^{-1}	[83]
Diclofenac	15307-79-6	STP influent	Spain	SPE-GC–MS	100	200–3600	[14]	Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	68 mg L^{-1}	[65]
		STP effluent				140–2200						
Diclofenac		STP influent	Switzerland	SPE-GC–MS	6	1300–2900	[15]	Algae	*D. subspicatus*	EC_{50} (growth inhibition)	72 mg L^{-1}	[65]
		STP effluent				1300–2400						
Diclofenac		STP effluent	Canada	SPE-GC–MS/MS	1.0	32–448	[17]	Duckweed	*L. minor*	EC_{50} (7 d) (growth inhibition)	7.5 mg L^{-1}	[65]
Diclofenac		STP influent	Canada	SPE-GC–MS	10	50–2450	[18]	Fish	*Oncorhynchus mykiss*	LOEC (28 days) (histopathological alterations)	5 μg L^{-1}	[66]
		STP effluent				70–250						
Diclofenac		STP influent	Greece	SPE-GC–MS	1	12–560	[19]	Fish	*Oncorhynchus mykiss*	LOEC (28 days) (cytological alterations)	1 μg L^{-1}	[67]
		STP effluent				10–365						
Diclofenac		STP influent	Sweden	SPE-GC–MS	—†	160	[21]	Fish	*Salmo trout* f. *fario*	NOEC (21 days) (histopathological alterations)	0.5 μg L^{-1}	[68]
		STP effluent				120						
		Höje river water				10–120						
Diclofenac		Paraíba do Sul river water	Brazil	SPE-GC–MS	10	20–60	[22]	Algae	*Dunaliella tertiolecta*	EC_{50} (96 h) (growth inhibition)	185,690 μg L^{-1}	[87]
		Drinking water										
						<10–50						
Diclofenac		Groundwater	Germany	SPE-GC–MS	29	590	[26]	Algae	*D. subspicatus*	EC_{50} (growth inhibition)	71.9 mg L^{-1}	[95]
								Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	68.0 mg L^{-1}	[95]
								Bacteria	*V. fischeri*	EC_{50} (30 min)	11,454 μg L^{-1}	[96]
Diclofenac		Drinking water	USA	SPE-LC–MS/MS	0.25	<0.25	[32]	Algae	*P. subcapitata*	NOEC (96 h) (growth inhibition)	10,000 μg L^{-1}	[96]
Diclofenac		Hospital effluent	Taiwan	SPE-HPLC–MS/MS	10	328	[47]			LOEC (96 h) (growth inhibition)	20,000 μg L^{-1}	[96]

Table 1 (*Continued*)

Compound	CAS number	Sample	Country	Analytical procedure	LOD (ng L^{-1})	Concentration reported (ng L^{-1})	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
		Pharmaceutical production facility effluent				53						
Diclofenac		STP influent	United Kingdom	SPE-HPLC–MS/MS	20	901–1036	[53]	Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	22,430 μg L^{-1}	[96]
		STP effluent				261–598						
Diclofenac		STP influent	Spain	SPE-HPLC–MS/MS	7	21–148	[71]		*C. dubia*	EC_{50} (48 h) (immobilization)	22,704 μg L^{-1}	[96]
		STP effluent	Belgium			32–1420						
		River water	Germany			26–72						
		Drinking water	Slovenia			<7						
Diclofenac		Elber river water Alster lake water	Germany	SPE-GC–MS	0.08 (LOQ)	42–67	[72]			NOEC (7 d) (reproduction)	1000 μg L^{-1}	[96]
						26						
Diclofenac		Hospital effluent	Spain	SPE-HPLC–MS/MS	30	60–1900	[73]			LOEC (7 d) (reproduction)	2000 μg L^{-1}	[96]
Diclofenac		STP influent	Taiwan	SPE-HPLC–MS/MS	—†	3–347	[87]	Fish	*D. rerio*	NOEC (10 d) (survival)	4000 μg L^{-1}	[96]
		STP effluent				4–101						
Diclofenac		Pearl Rivers water	China	GC-NCI-MS	1.1	ND–147 (± 5)	[88]			LOEC (10 d) (survival)	8000 μg L^{-1}	[96]
Diclofenac		STP influent	Luxembourg	SPE-LC–MS/MS	0.3	2–43	[89]	Fish	*O. mykiss*	LOEC (21 d) (liver cytopathology)	1 μg L^{-1}	[97]
		STP effluent				0.3–78						
		Alzette river water				0.3–55						
		Mess river water				0.3–19						
Diclofenac		STP effluent	South Korea	SPE-LC–MS/MS	1.0	8.8–127	[90]			LOEC (21 d) (kidney cytopathology)	1 μg L^{-1}	[97]
		Surface water				1.1–6.8						
Diclofenac		STP effluent	Spain	SPE-LC-QqLIT-MS	4 (LOQ)	890–1440	[91]			LOEC (21 d) (gills cytopathology)	1 μg L^{-1}	[97]
Diclofenac		STP effluent	United Kingdom	SPE-HPLC–MS/MS	20	350–460	[94]					
		Surface water				<20–91						
Fenoprofen	53746-45-5	STP influent	Japan	SPE-GC–MS	1 (LOQ)	9.68–80.6	[86]					
		STP effluent				1.59–9.22						
Ibuprofen	15687-27-1	STP influent	Spain	SPE-GC–MS	23	34,000–168,000	[14]	Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	108 mg L^{-1}	[65]
		STP effluent				240–28,000						
Ibuprofen		STP influent	Switzerland	SPE-GC–MS	8	1750–4500	[15]	Algae	*D. subspicatus*	EC_{50} (growth inhibition)	315 mg L^{-1}	[65]
		STP effluent				100–1200						
Ibuprofen		STP effluent	Canada	SPE-GC–MS	0.8	2235.2–6718.3	[17]	Duckweed	*L. minor*	EC_{50} (7 d) (growth inhibition)	22 mg L^{-1}	[65]
Ibuprofen		STP influent	Canada	SPE-GC–MS	10	4100–10,210	[18]	Crustacean	*Daphnia magna*	EC_{50} (48 h) (immobilisation)	10–100 mg L^{-1}	[75]
		STP effluent				110–2170						
Ibuprofen		Somes river water	Romania	SPE-GC–MS	30 (LOQ)	<30–115.2	[20]			EC_{50} (14 d) (reproduction)	13.4 mg L^{-1}	[75]

					(±20.7)						
Ibuprofen	STP influent	Sweden	SPE-GC–MS	–†	3590	[21]			NOEC (14 d) (survival)	20 mg L^{-1}	[75]
	STP effluent				150				LOEC (14 d) (survival)		
	Höje river water				10–220					80 mg L^{-1}	
Ibuprofen	Paraíba do Sul river water	Brazil	SPE-GC–MS	10	<10	[22]			LOEC (14 d) (population growth)	20 mg L^{-1}	[75]
	Drinking water				<10						
Ibuprofen	Po river water	Italy	SPE-HPLC–MS/MS	4.2 (LOQ)	ND–9.76	[24]	Crustacean	*Gammarus pulex*	LOEC (behaviour)	10 ng L^{-1}	[76]
	Lambro river water				78.50						
Ibuprofen	Groundwater	USA	SPE-LC–MS	18	3110	[28]	Duckweed	*Lemna minor*	EC_{50} (7 d) (growth inhibition)	4.01 mg L^{-1}	[77]
Ibuprofen	Hospital effluent	Taiwan	SPE-HPLC–MS/MS	25	119	[47]	Crustacean	*T. platyurus*	LC_{50} (24 h) (mortality)	19.59 mg L^{-1}	[78]
	Pharmaceutical production facility effluent				45,875						
Ibuprofen	STP influent	United Kingdom	SPE-HPLC–MS/MS	20	7741–33,764	[53]	Fish	*O. latipes*	LC_{50} (96 h) (mortality)	>100 mg L^{-1}	[79]
	STP effluent				1979–4239						
	Tyne river water				144–2370						
Ibuprofen	STP influent	Spain	SPE-HPLC–MS/MS	12	37–860	[71]	Mollusc	*P. carinatus*	LC_{50} (72 h) (survival)	17.1 mg L^{-1}	[79]
	STP effluent	Belgium			18–1860						
	River water	Germany			60–152						
	Drinking water	Slovenia			<12						
Ibuprofen	Elber river water	Germany	SPE-GC–MS	0.05 (LOQ)	8.7–32	[72]			NOEC (21 d) (survival)	5.36 mg L^{-1}	[79]
	Alster lake water				4.9						
Ibuprofen	Hospital effluent	Spain	SPE-HPLC–MS/MS	31	1500–151,000	[73]			NOEC (21 d) (growth)	1.02 mg L^{-1}	[79]
Ibuprofen	STP effluent	USA	SPE-GC–MS	10	18 (±14%)	[81]			LOEC (21 d) (growth)	2.43 mg L^{-1}	[79]
Ibuprofen	STP influent	Japan	SPE-GC–MS	1 (LOQ)	407–1130	[86]			NOEC (21 d) (reproduction)	2.43 mg L^{-1}	[79]
	STP effluent				1.41–177						
Ibuprofen	STP influent	Taiwan	SPE-HPLC–MS/MS	–†	711–17,933	[87]	Algae	*D. subspicatus*	EC_{50} (growth inhibition)	342.2 mg L^{-1}	[95]
	STP effluent				313–3777						
Ibuprofen	STP influent	Luxembourg	SPE-LC–MS/MS	0.3	82–3080	[89]	Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	101.2 mg L^{-1}	[95]
	STP effluent				3–359						
	Alzette river water				10–295						
	Mess river water				9–2383						
Ibuprofen	STP effluent	South Korea	SPE-LC–MS/MS	1.0	10–137	[90]	Cnidarian	*Hydra attenuata*	LC_{50} (96 h) (morphology)	22.36 mg L^{-1}	[98]
	Surface water				11–38						

Table 1 (*Continued*)

Compound	CAS number	Sample	Country	Analytical procedure	LOD (ng L^{-1})	Concentration reported (ng L^{-1})	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
		Drinking water				<1.0						
Ibuprofen		STP effluent	Spain	SPE-LC-QqLIT-MS	13 (LOQ)	100–340	[91]			EC_{50} (96 h) (morphology)	1.65 mg L^{-1}	[98]
Ibuprofen		Mankyung river water	South Korea	SPE-LC–MS/MS	5	<5–414 (±13)	[92]			LOEC (96 h) (morphology)	1 mg L^{-1}	[98]
Ibuprofen		STP effluent	United Kingdom	SPE-HPLC–MS/MS	20	1700–3800	[94]			NOEC (96 h) (morphology)	0.1 mg L^{-1}	[98]
		Surface water				<20						
										EC_{50} (96 h) (feeding)	3.85 mg L^{-1}	[98]
Carboxy-ibuprofen*	—†	STP influent	Sweden	SPE-GC–MS	—†	10,750	[21]					
		STP effluent				430						
		Höje river water				230–680						
Carboxy-ibuprofen*		Elber river water	Germany	SPE-GC–MS	0.21	11–32	[72]					
		Alster lake water				9.5						
Hydroxy-ibuprofen*	—†	STP influent	Sweden	SPE-GC–MS	—†	990	[21]					
		STP effluent				50						
		Höje river water				20–60						
Hydroxy-ibuprofen*		Elber river water	Germany	SPE-GC–MS	0.38	32–101	[72]					
		Alster lake water				18						
Indomethacin	53-86-1	STP influent	Canada	SPE-GC–MS	10	30–430	[18]					
		STP effluent				40–490						
Indomethacin		STP effluent	Spain	SPE-LC-QqLIT-MS	8 (LOQ)	160–390	[91]	Crustacean	*T. platyurus*	LC_{50} (24 h) (mortality)	16.14 mg L^{-1}	[78]
Indomethacin		Mankyung river water	South Korea	SPE-LC–MS/MS	1	<1–33.5 (±8)	[92]	Fish	*O. latipes*	LC_{50} (96 h) (mortality)	81.92 mg L^{-1}	[78]
Ketoprofen	22071-15-4	STP effluent	Canada	SPE-GC–MS/MS	1.0	8–351	[17]					
Ketoprofen		STP influent	Canada	SPE-GC–MS	10	60–150	[18]					
		STP effluent				40–90						
Ketoprofen		STP influent	Sweden	SPE-GC–MS	—†	940	[21]					
		STP effluent				330						
		Höje river water				10–70						
Ketoprofen		Hospital effluent	Taiwan	SPE-HPLC–MS/MS	10	9.6	[47]					
		Pharmaceutical production facility effluent				ND						
Ketoprofen		STP influent	Spain	SPE-HPLC–MS/MS	26	131	[71]					
		STP effluent	Belgium			<26						
		River water	Germany			<26						
		Drinking water	Slovenia			<26						
Ketoprofen		STP effluent	USA	SPE-GC–MS	9	23 (±6.8%)	[81]					
Ketoprofen		STP influent	Japan	SPE-GC–MS	0.3 (LOQ)	108–369	[86]					
		STP effluent				68.1–219						

Ketorolac	74103-06-3	Hospital effluent	Spain	SPE-HPLC–MS/MS	26	200–59,500	[73]					
Mefenamic acid	61-68-7	STP influent	United Kingdom	SPE-HPLC–MS/MS	50	136–363	[53]	Crustacean	*T. platyurus*	LC_{50} (24 h) (mortality)	3.95 mg L^{-1}	[78]
		STP effluent				290–396						
Mefenamic acid		STP effluent	Japan	SPE-GC–MS	1 (LOQ)	4.45–396	[86]	Fish	*O. latipes*	LC_{50} (96 h) (mortality)	8.04 mg L^{-1}	[78]
Mefenamic acid		Pearl Rivers water	China	GC-NCI-MS	2.2	ND–22.4 (±3.1)	[88]					
Mefenamic acid		STP effluent	Spain	SPE-LC-QqLIT-MS	3 (LOQ)	40–60	[91]					
Mefenamic acid		Mankyung river water	South Korea	SPE-LC–MS/MS	10	<10–326 (±21)	[92]					
Mefenamic acid		STP effluent	United Kingdom	SPE-HPLC–MS/MS	50	720–1100	[94]					
		Surface water				<50–65						
Naproxen	22204-53-1	STP effluent	Canada	SPE-GC–MS/MS	0.5	271.4–7962.3	[17]	Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	174 mg L^{-1}	[65]
Naproxen		STP influent	Canada	SPE-GC–MS	10	1730–6030	[18]	Algae	*D. subspicatus*	EC_{50} (growth inhibition)	>320 mg L^{-1}	[65]
		STP effluent				360–2540						
Naproxen		STP influent	Sweden	SPE-GC–MS	—†	3650	[21]	Duckweed	*L. minor*	EC_{50} (7 d) (growth inhibition)	24.2 mg L^{-1}	[65]
		STP effluent				250						
		Höje river water				90–250						
Naproxen		Paraíba do Sul river water	Brazil	SPE-GC–MS	10	<10–50	[22]	Rotifers	*B. calyciflorus*	LC_{50} (24 h)	62.48 mg L^{-1}	[80]
		Drinking water				<10–30						
Naproxen		Drinking water	USA	SPE-LC–MS/MS	0.5	<0.5	[32]	Rotifers	*T. platyurus*	LC_{50} (24 h)	84.09 mg L^{-1}	[80]
Naproxen		Hospital effluent	Taiwan	SPE-HPLC–MS/MS	10	698	[47]	Crustaceans	*C. dubia*	EC_{50} (24 h) (immobilization)	66.37 mg L^{-1}	[80]
		Pharmaceutical production facility effluent				ND						
Naproxen		STP influent	Spain	SPE-HPLC–MS/MS	26	109–455	[71]	Algae	*P. subcapitata*	EC_{50} (72 h) (growth inhibition)	31.82 mg L^{-1}	[80]
		STP effluent	Belgium			625						
		River water	Germany			70						
		Drinking water	Slovenia			<26						
Naproxen		STP effluent	USA	SPE-GC–MS	9	31 (±5.5%)	[81]	Rotifers	*B. calyciflorus*	EC_{50} (48 h) (growth inhibition)	0.56 mg L^{-1}	[80]
Naproxen		STP influent	Japan	SPE-GC–MS	0.3 (LOQ)	38.0–230	[86]	Crustaceans	*C. dubia*	EC_{50} (7 d) (population growth inhibition)	0.33 mg L^{-1}	[80]
		STP effluent				12.0–139						
Naproxen		Pearl Rivers water	China	GC-NCI-MS	1.3	ND–118 (±10.1)	[88]	Algae	*D. subspicatus*	EC_{50} (growth inhibition)	625.5 mg L^{-1}	[95]
Naproxen		STP effluent	South Korea	SPE-LC–MS/MS	1.0	20–483	[90]	Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	166.3 mg L^{-1}	[95]
		Surface water				1.8–18						
								Cnidarian	*Hydra attenuata*	LC_{50} (96 h) (morphology)	22.36 mg L^{-1}	[98]
										EC_{50} (96 h) (morphology)	2.62 mg L^{-1}	[98]

Table 1 (*Continued*)

Compound	CAS number	Sample	Country	Analytical procedure	LOD (ng L^{-1})	Concentration reported (ng L^{-1})	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
										LOEC (96 h) (morphology)	5 mg L^{-1}	[98]
										NOEC (96 h) (morphology)	1 mg L^{-1}	[98]
										EC_{50} (96 h) (feeding)	2.68 mg L^{-1}	[98]
Paracetamol	103-90-2	STP influent	Spain	SPE-GC–MS	32	29,000–246,000	[14]	Bacteria	*V. fischeri*	EC_{50} (15 min)	567.5 mg L^{-1}	[82]
		STP effluent				<32–4300						
Paracetamol		Groundwater	USA	SPE-LC–MS	9	380	[28]	Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	30.1 mg L^{-1}	[82]
Paracetamol		Hospital effluent	Taiwan	SPE-HPLC–MS/MS	2	62,250	[47]		*D. magna*	EC_{50} (96 h) (immobilization)	26.6 mg L^{-1}	[82]
		Pharmaceutical production facility effluent				124						
Paracetamol		STP influent	United Kingdom	SPE-HPLC–MS/MS	20	5529–69,570	[53]	Fish	*O. latipes*	LC_{50} (48 h)	>160 mg L^{-1}	[82]
		STP effluent				<20						
Paracetamol		Hospital effluent	Spain	SPE-HPLC–MS/MS	47	500–29,000	[73]		*O. latipes*	LC_{50} (96 h)	>160 mg L^{-1}	[82]
Paracetamol		Danube river water	Serbia	SPE-LC–MS/MS	0.50	78,170	[84]	Bacteria	*V. fischeri*	EC_{50} (30 min)	650 mg L^{-1}	[83]
		Sava river water				610						
		Tamiš river water				310						
Paracetamol		STP effluent	South Korea	SPE-LC–MS/MS	1.0	1.8–19	[90]	Algae	*Scenedesmus subspicatus*	EC_{50} (72 h)	134 mg L^{-1}	[83]
		Surface water				4.1–73						
Paracetamol		STP influent	Korea	SPE-LC–MS	5	13,046–56,944	[93]	Crustacean	*D. magna*	EC_{50} (immobilization)	50 mg L^{-1}	[83]
		STP effluent				<5–9						
		Han river water				<5–127						
Paracetamol		STP effluent	United Kingdom	SPE-HPLC–MS/MS	50	<50	[94]	Ciliates	*Tetrahymena pyriformis*	EC_{50} (48 h) (growth inhibition)	112 mg L^{-1}	[83]
		Surface water				<50						
								Fish	*B. rerio (zebra fish)*	LC_{50} (48 h)	378 mg L^{-1}	[83]

*—Metabolite; †—Data not available; ND—Not detected; SPE—Solid Phase Extraction; GC–MS—Gas Chromatography with Mass Spectrometry Detection; GC–MS/MS—Gas Chromatography with Tandem Mass Spectrometry Detection; GC-NCI-MS—Gas Chromatography-Negative Chemical Ionization-Mass Spectrometry; HPLC–MS/MS—High Performance Liquid Chromatography with Tandem Mass Spectrometry Detection; LC-QqLIT-MS—Liquid chromatography-quadrupole-linear ion trap-mass spectrometry detection.

185.69 mg L^{-1} was found [70]. Diclofenac was detected in STP effluents at maximum concentrations of 2.4 [15] and 1.42 μg L^{-1} [71] in Switzerland and Belgium respectively (Table 1) which highlighted that the effects cited are of sufficient magnitude to suspect chronic toxicity in aquatic organisms. Diclofenac has also been found in rivers [21,22,72], groundwater [26], hospital effluents [47,73] and drinking water [22,32,71] but at concentrations in the order of ng L^{-1}.

Ibuprofen is another NSAID with documented chronic toxicity. Female Japanese medaka (the Japanese killifish, *Oryzias latipes*) exposed to different concentrations of the drug over six weeks, showed a sharp rise in liver weight together with enhanced egg production, yet with a reduction in the number of weekly spawning events [74]. Authors associated these phenomena with changes in the spawning process and vitellogenin production, a glycoprotein precursor in yolk formation. With the water flea *Daphnia magna* population growth rate was significantly reduced for concentrations ranging from 0 to 80 mg L^{-1} [75]. Reproduction was affected at all concentrations and completely inhibited at the highest pharmaceutical levels. An activity decrease of the freshwater amphipod *Gammarus pulex* was noticed when in contact with ibuprofen concentrations of 1 and 10 ng L^{-1}, the latter value corresponding to the LOEC[3] obtained for behaviour change [76]. Regarding aquatic photosynthetic organisms, specific effects have been noticed. A 5-day exposure to concentrations in the 1–1000 μg L^{-1} range stimulated the growth of the cyanobacterium *Synechocystis* sp. while inhibiting that of the duckweed plant *Lemna minor* after 7 days [77]. Ibuprofen has been detected in STP effluents at concentrations that can reach 28 μg L^{-1} [14] (Spain) (Table 1). Two metabolites of ibuprofen (carboxyl-ibuprofen and hydroxyl-ibuprofen) were also found in surface waters and in a Swedish STP (influent and effluent) [21,72]. Due to demonstrable chronic toxicity, this may represent a real threat to non-target organisms, even at those lower concentrations. Ibuprofen was also found in rivers [20–22,24,72] and drinking water [22] which may broaden the scope of the problem to public health. However, effects in humans caused by chronic exposure to this active substance still remain unknown.

The ecotoxicity of naproxen and its photoderivative products have also been envisaged. Acute toxicity tests performed on the rotifer *Brachionus calyciflorus*, the water flea *Ceriodaphnia dubia* and the fairy shrimp *Thamnocephalus platyurus*, showed that naproxen had LC_{50}[4] and EC_{50}[5] values within the 1–100 mg L^{-1} range, with the photolysis products being significantly more toxic [80]. Highly chronic toxic properties were equally noticed with algae being the less sensitive organisms. Yet again, degradation products were shown to be more toxic with EC_{50} values of 26 and 62 μg L^{-1} for *C. dubia*, relative to growth inhibition. Naproxen had been found in STP effluents in a concentration range between 31 ng L^{-1} [81] and 7.96 μg L^{-1} [17] and in surface waters [21,22,71], at concentration levels that can reach 250 ng L^{-1} [21]. This active substance was also detected in drinking water [22,32,71].

The highly prescribed paracetamol (or acetaminophen) is a weak inhibitor of the cyclooxygenase enzyme, whose side effects are mainly associated with the formation of hepatotoxic metabolites, such as *N*-acetyl-*p*-benzoquinone imine (NAPQI) when the levels of liver glutathione are low [36]. Tests were carried out on algae, water fleas, fish embryos, luminescent bacteria and ciliates. The most sensitive species was shown to be *D. magna* for which EC_{50} values of 30.1 [82] or 50 mg L^{-1} [83] were reported. Some authors reported the presence of paracetamol in STP effluents at concentrations below to 20 ng L^{-1} [53] to 4.3 μg L^{-1} [14], and in surface waters, values can reach 78.17 μg L^{-1} [84] (Table 1), which are values higher than the predicted no-effect concentration (PNEC) of 9.2 μg L^{-1} [85]. Hence, paracetamol might represent a threat for non-target organisms.

4.2. Blood lipid lowering agents

Modulating drugs for lipid metabolism are frequently prescribed in the developed world and aim to decrease the concentration of blood circulating cholesterol and triglycerides. Pharmaceuticals belonging to this therapeutic class can be divided into two main groups: statins and the group most frequently detected in the environment, fibrates [99]. Statins act by inhibiting the 3-hydroxymethylglutaryl coenzyme A reductase (HMG-CoA), an enzyme involved in feedback control of cholesterol synthesis. In response, the number of LDL lipoprotein receptors at hepatocyte surfaces increases, thus lowering the circulating LDL cholesterol [100]. Toxicity data of statins on different organisms is very limited and restricted to the active substances simvastatin and atorvastatin. After an exposure of 96 h to simvastatin, larval and adult grass shrimp (*Palaemonetes pugio*) showed a LC_{50} of 1.18 mg L^{-1} and upper 10 mg L^{-1}, respectively [101], while the harpacticoid copepod *Nitocra spinipes* had a 96-h LC_{50} of 0.81 mg L^{-1} [102]. Dahl et al. (2006) [102] also reported a significantly increase in development time and body length of the copepod for a range of concentrations between 0.16 and 1.6 μg L^{-1}. On the other hand, simvastatin exhibited an EC_{50} of 22.8 mg L^{-1}, after 96 h, for the marine phytoplankton *D. tertiolecta* [70]. Relatively to atorvastatin, this active substance can affect the development of the duckweed *Lemna gibba*, showing a LOEC of 300 μg L^{-1} for parameters such as wet mass, frond number, chlorophyll-*a* and carotenoids content, for a time of exposure of 7 days [103]. Apart from statins had also the ability to suppress synthesis of the juvenile hormone in insects [104]. Statins were found in untreated sewage samples (Table 2) at concentrations between 4 and 117 ng L^{-1} and in treated sewage samples at 1–59 ng L^{-1} [105,106]. Additionally, they were also detected in surface water [105] and drinking water [32] at concentrations that can reach 1 ng L^{-1}. In turn, fibrates act by activating specific transcription factors belonging to the nuclear hormone receptor super family, known as peroxisome proliferator-activated receptors (PPARs) [107]. There are three types of PPARs related to different cellular events. PPAR-α and PPAR-β play key roles in catabolism and storage of fatty acids while PPAR-γ plays an important role in cellular differentiation [108]. Some authors have reported a proliferation of peroxisomes in rodent livers caused by fibrates [10]. Embryonic development of non-target organisms that share these receptors can be stopped by simply inhibiting cellular differentiation. Fibrates present in the micromolar concentration range are sufficient to cause it in zebrafish (*Danio rerio*) [109,110] and amphibians [111]. Raldúa et al. [110] demonstrated that, when exposed to 0.5–1 mg L^{-1} of clofibrate, zebrafish larvae had a significantly shorter body length and their morphologic characteristics were also altered. Clofibrate-exposed zebrafish larvae had also lethargic behaviour. It was evidenced that gemfibrozil and bezafibrate significantly affect feeding, attachment and hydrant growth of the cnidarian *Hydra attenuata* [98]. According to Quinn et al. [98], gemfibrozil could be classified as toxic (EC_{50} between 1 and 10 mg L^{-1}) and bezafibrate as harmful for non-target organisms (EC_{50} between 10 and 100 mg L^{-1}). Toxic properties of gemfibrozil were also respectively investigated on the inhibition of the bacterium *Vibrio fischeri* luminescence, growth inhibition of the alga *Chlorella vulgaris* and on the immobilization of the *D. magna*. In this study both the bacteria and the water flea were shown to be sensitive to gemfibrozil with the latter being the most sensitive, having an EC_{50} of 30 mg L^{-1} after 72 h [112]. Proliferative inhibition

[3] LOEC—Lowest Observed Effect Concentration.
[4] LC_{50}—Half Maximal Lethal Concentration.
[5] EC_{50}—Half Maximal Effective Concentration.

Table 2
Examples of concentrations (ng L^{-1}) of blood lipid lowering agents measured in different aquatic environments.

Compound	CAS number	Sample	Country	Analytical procedure	LOD (ng L^{-1})	Concentration reported (ng L^{-1})	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
Fibrates												
Bezafibrate	41859-67-0	Paraíba do Sul river water	Brazil	SPE-GC–MS	25	<25	[22]	Cnidarian	*Hydra attenuata*	LC_{50} (96 h) (morphology)	70.71 mg L^{-1}	[98]
		Drinking water				<25						
Bezafibrate		Po river water	Italy	SPE-HPLC–MS/MS	0.3	0.79–2.75	[24]			EC_{50} (96 h) (morphology)	25.85 mg L^{-1}	[98]
		Lambro river water				57.15						
Bezafibrate		STP effluent	Spain	SPE-LC-QqLIT-MS	3 (LOQ)	40–130	[91]			LOEC (96 h) (morphology)	1 mg L^{-1}	[98]
Bezafibrate		STP effluent	Italy	SPE-HPLC–MS/MS	0.1 (LOQ)	0.3–117	[118]			NOEC (96 h) (morphology)	0.1 mg L^{-1}	[98]
										EC_{50} (96 h) (feeding)	8.59 mg L^{-1}	[98]
								Rotifer	*B. calyciflorus*	LC_{50} (24 h) (mortality)	60.91 mg L^{-1}	[113]
										EC_{50} (48 h) (population growth inhibition)	0.44 mg L^{-1}	[113]
										NOEC (48 h)	0.156 mg L^{-1}	[113]
										LOEC (48 h)	0.3125 mg L^{-1}	[113]
								Crustacean	*T. platyurus*	LC_{50} (24 h) (mortality)	39.69 mg L^{-1}	[113]
									D. magna	EC_{50} (24 h) (immobilization)	100.08 mg L^{-1}	[113]
									C. dubia	EC_{50} (48 h) (immobilization)	75.79 mg L^{-1}	[113]
										EC_{50} (7 d) (population growth inhibition)	0.13 mg L^{-1}	[113]
										NOEC (7 d)	0.023 mg L^{-1}	[113]
										LOEC (7 d)	0.047 mg L^{-1}	[113]
Clofibrate	82115-62-6							Fish	*D. rerio*	LC_{50} (96 h) (mortality)	0.89 mg L^{-1}	[110]
Fenofibrate	49562-28-9							Rotifer	*B. calyciflorus*	LC_{50} (24 h) (mortality)	64.97 mg L^{-1}	[113]
										EC_{50} (48 h) (population growth inhibition)	1.44 mg L^{-1}	[113]
										NOEC (48 h)	0.156 mg L^{-1}	[113]
										LOEC (48 h)	0.3125 mg L^{-1}	[113]
								Crustacean	*D. magna*	EC_{50} (24 h) (immobilization)	50.12 mg L^{-1}	[113]
									C. dubia	EC_{50} (7 d) (population growth inhibition)	0.76 mg L^{-1}	[113]
										NOEC (7 d)	0.039 mg L^{-1}	[113]
										LOEC (7 d)	0.078 mg L^{-1}	[113]
								Algae	*P. subcapitata*	EC_{50} (72 h) (growth inhibition)	19.84 mg L^{-1}	[113]
										NOEC (72 h)	3.12 mg L^{-1}	[113]
										LOEC (72 h)	6.25 mg L^{-1}	[113]
Clofibric acid*	882-09-7	STP influent	Greece	SPE-GC–MS	1.8	ND	[19]	Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	72 mg L^{-1}	[65]

		STP effluent				5						
Clofibric acid*		Paraíba do Sul river water	Brazil	SPE-GC–MS	10	<10–30	[22]	Algae	*D. subspicatus*	EC_{50} (growth inhibition)	115 mg L^{-1}	[65]
		Drinking water				<10–20						
Clofibric acid*		Po river water	Italy	SPE-HPLC–MS/MS	0.3	0.41–5.77	[24]	Duckweed	*L. minor*	EC_{50} (7 d) (growth inhibition)	12.5 mg L^{-1}	[65]
		Lambro river water				ND						
Clofibric acid*		North Sea water	—†	SPE-GC–MS	0.008	ND–18.6	[25]	Bacteria	*V. fischeri*	EC_{50} (30 min)	100 mg L^{-1}	[83]
Clofibric acid*		STP influent	United Kingdom	SPE-HPLC–MS/MS	20	<20–651	[53]	Algae	*Scenedesmus subspicatus*	EC_{50} (72 h)	89 mg L^{-1}	[83]
		STP effluent				<20–44						
Clofibric acid*		STP influent	Spain	SPE-HPLC–MS/MS	17	25–58	[71]	Crustacean	*D. magna*	EC_{50} (immobilization)	106 mg L^{-1}	[83]
		STP effluent	Belgium			22–107						
		River water	Germany			24–35						
		Drinking water	Slovenia			<17						
Clofibric acid*		Elbe river water	Germany	SPE-GC–MS	0.26 (LOQ)	3.2–7.6	[72]	Ciliates	*Tetrahymena pyriformis*	EC_{50} (48 h) (growth inhibition)	175 mg L^{-1}	[83]
		Alster lake water				2.4						
Clofibric acid*		STP influent	Taiwan	SPE-HPLC–MS/MS	—†	36–2593	[87]	Fish	*D. rerio*	LC_{50} (48 h)	86 mg L^{-1}	[83]
		STP effluent				47–487						
Clofibric acid*		STP effluent	Spain	SPE-LC-QqLIT-MS	4 (LOQ)	36–51	[91]	Algae	*Dunaliella tertiolecta*	EC_{50} (96 h) (growth inhibition)	224,180 μg L^{-1}	[87]
Clofibric acid*		STP effluent	United Kingdom	SPE-HPLC–MS/MS	50	<50	[94]	Bacteria	*V. fischeri*	EC_{50} (30 min)	91,827 μg L^{-1}	[96]
		Surface water				<50						
Clofibric acid*		STP effluent	Italy	SPE-HPLC–MS/MS	0.36 (LOQ)	ND–82	[118]	Algae	*P. subcapitata*	NOEC (96 h) (growth inhibition)	75,000 μg L^{-1}	[96]
Clofibric acid*		Groundwater	Germany	SPE-GC–MS	2 (LOQ)	2–40	[119]			LOEC (96 h) (growth inhibition)	150,000 μg L^{-1}	[96]
								Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	>200,000 μg L^{-1}	[96]
									C. dubia	EC_{50} (48 h) (immobilization)	>200,000 μg L^{-1}	[96]
										NOEC (7 d) (reproduction)	640 μg L^{-1}	[96]
										LOEC (7 d) (reproduction)	2560 μg L^{-1}	[96]
								Fish	*D. rerio*	NOEC (10 d) (survival)	70,000 μg L^{-1}	[96]
										LOEC (10 d) (survival)	140,000 μg L^{-1}	[96]
								Fish	*O. mykiss*	LOEC (21 d) (liver cytopathology)	>100 μg L^{-1}	[97]
										LOEC (21 d) (kidney cytopathology)	>100 μg L^{-1}	[97]
										LOEC (21 d) (gills cytopathology)	5 μg L^{-1}	[97]
Gemfibrozil	25812-30-0	STP effluent	Canada	SPE-GC–MS/MS	0.3	80.1–478.2	[17]	Cnidarian	*Hydra attenuata*	LC_{50} (96 h) (morphology)	22.36 mg L^{-1}	[98]
		River water				ND–18.4						
		Lake water				ND						

Table 2 (*Continued*)

Compound	CAS number	Sample	Country	Analytical procedure	LOD (ng L^{-1})	Concentration reported (ng L^{-1})	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
Gemfibrozil		STP influent	Canada	SPE-GC–MS	10	120–36,530	[18]			EC_{50} (96 h) (morphology)	1.18 mg L^{-1}	[98]
		STP effluent				80–2090						
Gemfibrozil		STP influent	Sweden	SPE-GC–MS	—†	710	[21]			LOEC (96 h) (morphology)	1 mg L^{-1}	[98]
		STP effluent				180						
		Höje river water				1–170						
Gemfibrozil		Drinking water	USA	SPE-LC–MS/MS	0.25	0.43	[32]			NOEC (96 h) (morphology)	0.1 mg L^{-1}	[98]
Gemfibrozil		Hospital effluent	Taiwan	SPE-HPLC–MS/MS	1.0	760	[47]			EC_{50} (96 h) (feeding)	1.76 mg L^{-1}	[98]
		Pharmaceutical production facility effluent				1795						
Gemfibrozil		Pearl rivers water	China	SPE-GC-NCI-MS	1.8	ND–22.4 (±3.1)	[88]	Bacteria	*V. fischeri*	EC_{50} (24 h) (bioluminescence)	64.6 mg L^{-1}	[112]
Gemfibrozil		STP effluent	South Korea	SPE-LC–MS/MS	1.0	3.9–17	[90]			EC_{50} (48 h) (bioluminescence)	45.1 mg L^{-1}	[112]
		Surface water				1.8–9.1						
Gemfibrozil		STP effluent	Spain	SPE-LC-QqLIT-MS	4 (LOQ)	470–3550	[91]	Algae	*Chlorella vulgaris*	EC_{50} (24 h) (growth)	195 mg L^{-1}	[112]
										EC_{50} (48 h) (growth)	161 mg L^{-1}	[112]
										EC_{50} (72 h) (growth)	150 mg L^{-1}	[112]
								Crustacean	*D. magna*	EC_{50} (24 h) (immobilization)	57.1 mg L^{-1}	[112]
										EC_{50} (48 h) (immobilization)	42.6 mg L^{-1}	[112]
										EC_{50} (72 h) (immobilization)	30.0 mg L^{-1}	[112]
								Bacteria	*V. fischeri*	EC_{50} (30 min) (bioluminescence)	85.74 mg L^{-1}	[113]
								Rotifer	*B. calyciflorus*	LC_{50} (24 h) (mortality)	77.30 mg L^{-1}	[113]
										EC_{50} (48 h) (population growth inhibition)	0.44 mg L^{-1}	[113]
										NOEC (48 h)	0.156 mg L^{-1}	[113]
										LOEC (48 h)	0.312 mg L^{-1}	[113]
								Crustacean	*T. platyurus*	LC_{50} (24 h) (mortality)	161.05 mg L^{-1}	[113]
									D. magna	EC_{50} (24 h) (immobilization)	74.30 mg L^{-1}	[113]
									C. dubia	EC_{50} (7 d) (population growth inhibition)	0.53 mg L^{-1}	[113]
										NOEC (7 d)	0.078 mg L^{-1}	[113]
										LOEC (7 d)	0.156 mg L^{-1}	[113]
								Algae	*P. subcapitata*	EC_{50} (72 h) (growth inhibition)	15.19 mg L^{-1}	[113]
										NOEC (72 h)	3.125 mg L^{-1}	[113]
										LOEC (72 h)	6.25 mg L^{-1}	[113]
Statins												
Atorvastatin	134523-03-8	Drinking water	USA	SPE-LC–MS/MS	0.25	<0.25	[32]	Duckweed	*L. gibba*	LOEC (7 d) (growth parameters)	300 μg L^{-1}	[103]

Atorvastatin		STP influent STP effluent Otonabee river water	Canada	SPE-LC–MS/MS	0.1	76 (±3) 37 (±2) 1 (±0)	[105]					
Atorvastatin		STP effluent	Canada	SPE-LC–MS/MS	0.001	22.4 (±1.4)	[106]					
o-hydroxy atorvastatin*	214217-86-6	Drinking water	USA	SPE-LC–MS/MS	0.50	<0.50	[32]					
p-hydroxy atorvastatin*	214217-88-6	Drinking water	USA	SPE-LC–MS/MS	0.50	<0.50	[32]					
Lovastatin	81739-26-6	STP influent STP effluent Otonabee river water	Canada	SPE-LC–MS/MS	0.1	49 (±2) 14 (±1) ND	[105]					
Pravastatin	81131-70-6	STP influent STP effluent Otonabee river water	Canada	SPE-LC–MS/MS	1.0	117 (±6) 59 (±2) ND	[105]					
Simvastatin	79902-63-9	STP influent	Canada	SPE-LC–MS/MS	0.1	4 (±0)	[105]	Algae	*Dunaliella tertiolecta*	EC_{50} (96 h) (growth inhibition)	22,800 μg L^{-1}	[70]
		STP effluent Otonabee river water				1 (±0) ND		Grass shrimp	*Palaemonetes pugio*	LC_{50} (96 h) (larvae survival)	1.18 mg L^{-1}	[101]
										NOEC (larvae survival)	0.625 mg L^{-1}	[101]
										LOEC (larvae survival)	1.25 mg L^{-1}	[101]
										LC_{50} (96 h) (adult survival)	>10 mg L^{-1}	[101]
										NOEC (adult survival)	5.00 mg L^{-1}	[101]
										LOEC (adult survival)	10.0 mg L^{-1}	[101]
								Copepod	*Nitocra spinipes*	LC_{50} (96 h) (growth rate)	810 μg L^{-1}	[102]
										LOEC (growth rate)	0.16 μg L^{-1}	[102]

*—Metabolite; †—Data not available; ND—Not detected; SPE—Solid Phase Extraction; GC–MS—Gas Chromatography with Mass Spectrometry Detection; GC–MS/MS—Gas Chromatography with Tandem Mass Spectrometry Detection; GC-NCI-MS—Gas Chromatography-Negative Chemical Ionization-Mass Spectrometry; HPLC–MS/MS—High Performance Liquid Chromatography with Tandem Mass Spectrometry Detection; LC–MS/MS—Liquid Chromatography with Tandem Mass Spectrometry Detection.

of *C. vulgaris* was only observed for concentrations up to 150 mg L^{-1} [112]. Isidori et al. [113] studied the acute and chronic toxicities caused by bezafibrate, fenofibrate and gemfibrozil and their photoproducts on non-target organisms, considering that they did not significantly affect the exposed organisms (LC_{50} values ranged from 39.69 to 161.05 mg L^{-1}). When goldfish (*Carassius auratus*) were exposed to 1.5 μg L^{-1} of gemfibrozil for 14 days, a decrease of more than 50% in plasma testosterone levels was noticed [114], thereby proving that this pharmaceutical may also act as an endocrine disruptor. As the main active metabolite of several fibrate compounds, clofibric acid is frequently used to assess toxicity due to its high degree of persistence in the environment. In acute toxicity tests on bacteria, ciliates, daphnids and fish embryos, Ferrari et al. [96] noticed low toxicity when at concentrations up to 14 mg L^{-1}. These results are in agreement with the tests performed on three estuarine species: algae *D. tertiolecta*, crustacean *P. pugio* and fish *Fundulus heteroclitus* [115]. For concentrations ≤1000 μg L^{-1}, clofibric acid did not significantly affect cell density and growth rate of the first, neither did it affect the survival of the remainder. This is in agreement with the 96-h EC_{50} of 224.18 mg L^{-1} found for *D. tertiolecta* [87]. On the contrary, exposure to concentrations above 10 μg L^{-1} and up to 100 μg L^{-1} increased the proportion of male offspring produced by *D. magna* [116]. Rotifers have also shown to be sensitive and a NOEC[6] value of 250 μg L^{-1} was deduced [96]. Fathead minnow fish (*Pimephales promelas*) showed alterations in reproductive function expressed by a reduction in sperm motility and plasma androgen concentration [117] while cytological changes in gills were noticed in rainbow trout exposed to 5 μg L^{-1} of this metabolite [97]. Fibrates (as bezafibrate and gemfibrozil) have been detected in several environmental samples (Table 2). Bezafibrate was found in STP effluents [91,118] and in the Paraíba do Sul river (Brazil) [22] as was gemfibrozil [17,18,21] and further identified in surface waters [17,21,88]. Due to its greater persistence, clofibric acid has been found in STP influents [19,71] and effluents [19,53,71], surface water [22,24,71], drinking water [71,119] and North Sea water [25]. All of these pharmaceuticals were shown to be present at concentration levels in the order of ng L^{-1} or low μg L^{-1}, which indicates that their exposure may represent a threat for non-target organisms.

4.3. Antibiotics

Antibiotics come within a therapeutic class where human health preservation and environmental disturbance are closely related. The major concern is associated with the development of resistance mechanisms by bacteria which can subsequently compromise public health by means of treatment effectiveness [52,108]. According to Jones et al. [120], antibiotics could be classified as extremely toxic to microorganisms (EC_{50} below 0.1 mg L^{-1}) and very toxic to algae (EC_{50} between 0.1 and 1 mg L^{-1}). Chronic toxicity tests performed on algae have shown high sensitivity to antibacterial agents as deduced from growth inhibition measurements [121,122]. Vertebrates (such as fish) put directly in contact with low levels of antimicrobials apparently did not yield observable effects [123,124]. Accordingly, a LC_{50} value above 100 mg L^{-1} for Japanese medaka concerning sulfonamides was reported [81]. However, one must bear in mind that algae constitute the basis of the food chain and a decrease in their population will directly affect the entire aquatic ecosystem equilibrium [123,125]. Exposure of *D. magna* to erythromycin, lincomycin, sulfamethoxazole or trimethoprim in a concentration ranging from 1 to 100 μg L^{-1} did not affect the degree of survival, nor morphology in adults or neonates, nor fecundity or sex ratio [116]. Similar results were obtained after chronic exposure to 10 μg L^{-1} of sulfamethoxazole [116]. Amoxicillin concentrations ranging from 50 ng L^{-1} to 50 mg L^{-1} were tested on four different algae without observable effects, unless for the blue-green algae *Synechococcus leopolensis* for which a NOEC of 0.78 μg L^{-1} was achieved [126]. Isidori et al. [124] tested erythromycin, oxytetracyclin, sulfamethoxazole, ofloxacin, lincomycin and clarithromycin on aquatic organisms belonging to different trophic levels (bacteria, algae, rotifers, crustaceans and fish). Results showed that acute toxicity level was in the order of mg L^{-1} while chronic toxicity appeared at concentrations in the order of μg L^{-1}, mainly for algae. The antibiotics tested were shown to be less active against rotifers, crustaceans and fish where no effect was noticed even for concentrations up to 1000 mg L^{-1}. After a 48 h exposure period of the microalga *Scenedesmus obliquus* to a concentration range of norfloxacin between 0 and 60 mg L^{-1} was noticed a growth inhibition (EC_{50} = 38.49 mg L^{-1}) and a reduction in chlorophyll-*a* concentration [127].

Most antibiotics used in veterinary medicine are aimed at preventing and treating diseases in livestock production or aquaculture. Even considering their use at sub-therapeutically concentrations, many studies suggest the development of bacterial resistance and further potential appearance of cross-resistance between different classes of antibiotics shared with humans [43,58,120,128]. Antibiotics used in livestock production are excreted in the urine and faeces of animals and often appear in manure. From here they can cause some problems in terrestrial ecosystems such as adverse effects on nitrifying bacteria [11] or growth inhibition of crop plants and weeds by bioaccumulation [129]. The presence of antibiotics in STP influents may also impair treatment processes that use bacteria and cause toxic effects in the downstream aquatic and/or terrestrial ecosystems at different trophic levels [11]. Bacterial cultures from sewage bioreactors receiving waters from a STP were tested for resistance against six antibiotics, showing that all were resistant to at least two of the antibiotics, whilst bacteria isolated from receiving waters were only resistant to erythromycin and ampicillin [130]. Aquatic photosynthetic organisms can also be affected. A study performed both on the cyanobacterium *Synechocystis* sp. and the duckweed *L. minor* showed growth inhibition in the presence of 1–1000 μg L^{-1} erythromycin while another antibiotic, tetracycline, inhibited growth of the former when at concentrations between 10 and 100 μg L^{-1} while stimulating the latter [77]. Eguchi et al. [131] studied the influence of several antimicrobial agents used as veterinary drugs in Japan on the growth of the green algae *Selenastrum capricornutum* and *C. vulgaris* by considering the growth inhibitory activity. Erithromycin showed the strongest activity against *S. capricornutum* with an EC_{50} value of 37 μg L^{-1} followed by dihydrostreptomycin (EC_{50} = 110 μg L^{-1}), oxytetracycline (EC_{50} = 340 μg L^{-1}) and tylosin (EC_{50} = 410 μg L^{-1}). Sulfonamides exhibited lower inhibitory activity with EC_{50} values between 1.53 and 2.30 mg L^{-1}. In contrast, ampicillin and cefalozin did not show any effect even at concentrations as high as 1000 mg L^{-1}. The authors also showed the arousal of a synergistic inhibitory growth activity from the very common combination of sulfamethoxazole with trimethoprim in medicines, when compared to the respective individual activities. Yamashita et al. [132] evaluated the growth inhibition of the algae *P. subcapitata* by two antibiotics, levofloxacin and clarithromycin, showing that the last one had a more pronounced toxic effect with an EC_{50} of 11 μg L^{-1} and a LOEC and a NOEC of 6.3 and 3.1 μg L^{-1}, respectively. Toxic effects of sulfachlorpyridazine and oxytetracycline were also tested on the aquatic plant *L. minor*, showing EC_{50} values of 2.33 and 4.92 mg L^{-1}, respectively [133]. Assays on *D. magna* showed that following 48 h of exposure, oxolinic acid and tiamulin were the most toxic compounds, with EC_{50} values of

[6] NOEC—Non-Observed Effect Concentration.

Table 3
Examples of concentrations (ng L^{-1}) of antibiotics measured in different aquatic environments.

Compound	CAS number	Sample	Country	Analytical procedure	LOD (ng L^{-1})	Concentration reported (ng L^{-1})	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
(Fluor)quinolones												
Ciprofloxacin	85721-33-1	Surface water	USA	SPE-LC–MS	20	20	[23]					
Ciprofloxacin		Po river water	Italy	SPE-HPLC–MS/MS	0.3	ND–26.15	[24]					
		Lambro river water				14.36						
Ciprofloxacin		STP influent	USA	SPE-HPLC–MS/MS	20	ND–1000	[138]					
		STP effluent				ND						
		Hospital effluent				ND–2000						
		Rio Grande river water				ND						
Ciprofloxacin		STP influent	Portugal	SPE-LC-FD	25 (LOQ)	418.8–667.1	[139]					
		STP effluent				100.8–309.2						
		Hospital effluent				127.0–10,962.5						
Ciprofloxacin		STP influent	USA	SPE-LC–MS	50	150	[140]					
		STP effluent				60						
Ciprofloxacin		STP influent	Sweden	SPE-LC–MS	6 (LOQ)	90–300	[141]					
		STP effluent				<6–60						
Ciprofloxacin		Mondego river water	Portugal	SPE-LC-FD	25	79.6–119.2	[142]					
Enrofloxacin	93106-60-6	STP influent	Portugal	SPE-LC-FD	50 (LOQ)	121.8–447.1	[139]	Bacteria	*V. fischeri*	EC_{50} (15 min) (luminescence)	326.8 mg L^{-1}	[13]6
		STP effluent				53.7–211.5						
		Hospital effluent				<50						
Enrofloxacin		STP influent	USA	SPE-LC–MS	50	250	[140]	Crustacean	*D. magna*	EC_{50} (24 h) (immobilization)	131.7 mg L^{-1}	[136]
		STP effluent				270						
Enrofloxacin		Mondego river water	Portugal	SPE-LC-FD	25	67.0–102.5	[142]			EC_{50} (48 h) (immobilization)	56.7 mg L^{-1}	[136]
										EC_{50} (21 d) (adult survival)	11.47 mg L^{-1}	[136]
										LOEC (21 d) (reproduction)	15 mg L^{-1}	[136]
										NOEC (21 d) (reproduction)	5 mg L^{-1}	[136]
									M. macrocopa	EC_{50} (24 h) (immobilization)	285.7 mg L^{-1}	[136]
Levofloxacin	100986-34-5	Mankyung river water	South Korea	SPE-LC–MS/MS	5	ND–87.4 (±13)	[92]	Crustacean	*T. platyurus*	LC_{50} (24 h) (mortality)	>100 mg L^{-1}	[78]
								Fish	*O. latipes*	LC_{50} (96 h) (mortality)	>100 mg L^{-1}	[78]
								Algae	*P. subcapitata*	EC_{50} (96 h) (growth inhibition)	1200 μg L^{-1}	[132]
										LOEC (96 h) (growth inhibition)	630 μg L^{-1}	[132]
										NOEC (96 h) (growth inhibition)	310 μg L^{-1}	[132]
								Crustacean	*D. magna*	EC_{50} (21 d) (reproduction)	340 μg L^{-1}	[132]
										LOEC (21 d) (reproduction)	63 μg L^{-1}	[132]

Table 3 (*Continued*)

Compound	CAS number	Sample	Country	Analytical procedure	LOD (ng L^{-1})	Concentration reported (ng L^{-1})	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
										NOEC (21 d) (reproduction)	31 μg L^{-1}	[132]
Nalidixic acid	389-08-2	STP influent	Taiwan	SPE-HPLC–MS/MS	—†	26–372	[87]					
		STP effluent				40–200						
Norfloxacin	70458-96-7	Surface water	USA	SPE-LC–MS	20	120	[23]	Algae	*S. obliquus*	IC_{50} (48 h) (growth inhibition)	38.49 mg L^{-1}	[127]
Norfloxacin		STP influent	Portugal	SPE-LC-FD	25 (LOQ)	191.2–455.0	[139]	Algae	*S. capricornutum*	EC_{50} (growth inhibition)	16.6 mg L^{-1}	[131]
		STP effluent				29.6–35.0						
		Hospital effluent				<25–334.0						
Norfloxacin		STP influent	Sweden	SPE-LC–MS	7 (LOQ)	72–174	[141]			NOEC (growth inhibition)	4.01 mg L^{-1}	[131]
		STP effluent				<6–37						
Norfloxacin		Mondego river water	Portugal	SPE-LC-FD	25	ND	[142]		*C. vulgaris*	EC_{50} (growth inhibition)	10.4 mg L^{-1}	[131]
Norfloxacin		Surface seawater	China (Hong Kong)	SPE-HPLC–MS/MS	13	<13–8.00	[144]			NOEC (growth inhibition)	4.02 mg L^{-1}	[131]
Norfloxacin		Victoria Harbour seawater	China	SPE-HPLC–MS	3.2 (LOQ seawater)	9.4–12.3	[145]	Rotifer	*B. calyciflorus*	LC_{50} (24 h) (mortality)	29.88 mg L^{-1}	[124]
		Pearl River water			10 (LOQ river water)							
						12–150						
Ofloxacin	82419-36-1	STP influent	Taiwan	SPE-HPLC–MS/MS	—†	115–1274	[87]			EC_{50} (48 h) (population growth inhibition)	0.53 mg L^{-1}	[124]
		STP effluent				53–991						
Ofloxacin		STP influent	USA	SPE-HPLC–MS/MS	10	ND–1000	[138]	Crustacean	*T. platyurus*	LC_{50} (24 h) (mortality)	33.98 mg L^{-1}	[124]
		STP effluent				110						
		Hospital effluent				ND–35,500						
		Rio Grande river water				ND						
Ofloxacin		STP influent	Portugal	SPE-LC-FD	250	ND	[139]		*D. magna*	EC_{50} (24 h) (immobilization)	31.75 mg L^{-1}	[124]
		STP effluent				ND						
		Hospital effluent				ND–10,675.5						
Ofloxacin		STP influent	Sweden	SPE-LC–MS	6 (LOQ)	<6–287	[141]		*C. dubia*	EC_{50} (24 h) (immobilization)	17.41 mg L^{-1}	[124]
		STP effluent				<6–52						
Ofloxacin		Victoria Harbour seawater	China	SPE-HPLC–MS	2.6 (LOQ seawater)	5.2–10	[145]			EC_{50} (7 d) (population growth inhibition)	3.13 mg L^{-1}	[124]
		Pearl River water			10 (LOQ river water)							
						11–77						
								Algae	*P. subcapitata*	EC_{50} (72 h) (growth inhibition)	1.44 mg L^{-1}	[124]
Oxolinic acid	14698-29-4							Algae	*M. aeruginosa*	EC_{50} (72 h) (growth inhibition)	0.180 mg L^{-1}	[122]

									R. salina	EC_{50} (72 h) (growth inhibition)	10 mg L^{-1} [122]
									S. capricornutum	EC_{50} (72 h) (growth inhibition)	16 mg L^{-1} [122]
								Crustacean	*D. magna*	EC_{50} (24 h) (immobilization)	5.9 mg L^{-1} [134]
										EC_{50} (48 h) (immobilization)	4.6 mg L^{-1} [134]
										NOEC (21 d) (reproduction)	0.38 mg L^{-1} [134]
Sarafloxacin	98105-99-8							Algae	*R. salina*	EC_{50} (72 h) (growth inhibition)	24 mg L^{-1} [122]
									S. capricornutum	EC_{50} (72 h) (growth inhibition)	16 mg L^{-1} [122]
β-Lactams											
Amoxicillin	81030-75-3							Algae	*M. aeruginosa*	EC_{50} (72 h) (growth inhibition)	0.0037 mg L^{-1} [122]
									S. capricornutum	NOEC (72 h) (growth inhibition)	250 mg L^{-1} [122]
								Algae	*S. leopoliensis*	EC_{50} (growth inhibition)	2.22 μg L^{-1} [126]
										NOEC (growth inhibition)	0.78 μg L^{-1} [126]
										LOEC (growth inhibition)	1.56 μg L^{-1} [126]
								Bacteria	*V. fischeri*	EC_{50} (15 min) (luminescence)	3597 mg L^{-1} [136]
Ampicillin	69-53-4	Hospital effluent	Taiwan	SPE-HPLC–MS/MS	10	21	[47]	Bacteria	*V. fischeri*	EC_{50} (15 min) (luminescence)	2627 mg L^{-1} [136]
		Pharmaceutical production facility effluent				ND					
Penicillin G (Benzylpenicillin)	69-57-8	STP influent	China	SPE-LC–MS	930 (LOQ)	153,000 ± 4000	[48]	Algae	*M. aeruginosa*	EC_{50} (growth rate)	0.006 mg L^{-1} [121]
		STP effluent				1680 ± 480					
									S. capricornutum	NOEC (growth rate)	100 mg L^{-1} [121]
Cephalosporins											
Cephalexin	66905-57-5	STP influent	Taiwan	SPE-HPLC–MS/MS	—†	1563–4367	[87]				
		STP effluent				10–994					
Cephalexin		Surface seawater	China (Hong Kong)	SPE-HPLC–MS/MS	13	<13–182	[144]				
Lincosamide											
Lincomycin	154-21-2	Surface water	USA	SPE-LC–MS	50	60	[23]	Rotifer	*B. calyciflorus*	LC_{50} (24 h) (mortality)	24.94 mg L^{-1} [124]
Lincomycin		Po river water	Italy	SPE-HPLC–MS/MS	0.3	3.13–248.90	[24]			EC_{50} (48 h) (population growth inhibition)	0.68 mg L^{-1} [124]
		Lambro river water				24.40					
Lincomycin		Groundwater	USA	SPE-LC–MS	50	320	[28]	Crustacean	*T. platyurus*	LC_{50} (24 h) (mortality)	30.00 mg L^{-1} [124]
Lincomycin		Hospital effluent	USA	SPE-HPLC–MS/MS	10	ND–2000	[138]		*D. magna*	EC_{50} (24 h) (immobilization)	23.18 mg L^{-1} [124]
		Livestock effluent				ND–6600					
									C. dubia	EC_{50} (24 h) (immobilization)	13.98 mg L^{-1} [124]

Table 3 (*Continued*)

Compound	CAS number	Sample	Country	Analytical procedure	LOD (ng L^{-1})	Concentration reported (ng L^{-1})	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
										EC_{50} (7 d) (population growth inhibition)	7.20 mg L^{-1}	[124]
								Algae	*P. subcapitata*	EC_{50} (72 h) (growth inhibition)	0.07 mg L^{-1}	[124]
Macrolides												
Clarithromycin	81103-11-9	Po river water	Italy	SPE-HPLC–MS/MS	0.3	0.49–20.30	[24]	Rotifer	*B. calyciflorus*	LC_{50} (24 h) (mortality)	35.46 mg L^{-1}	[124]
		Lambro river water				8.31						
Clarithromycin		STP influent	Taiwan	SPE-HPLC–MS/MS	—†	59–1433	[87]			EC_{50} (48 h) (population growth inhibition)	12.21 mg L^{-1}	[124]
		STP effluent				12–232						
Clarithromycin		Mankyung river water	South Korea	SPE-LC–MS/MS	1	ND–443 (±14)	[92]	Crustacean	*T. platyurus*	LC_{50} (24 h) (mortality)	94.23 mg L^{-1}	[78]
								Fish	*O. latipes*	LC_{50} (96 h) (mortality)	>100 mg L^{-1}	[78]
								Crustacean	*T. platyurus*	LC_{50} (24 h) (mortality)	33.64 mg L^{-1}	[124]
									D. magna	EC_{50} (24 h) (immobilization)	25.72 mg L^{-1}	[124]
									C. dubia	EC_{50} (24 h) (immobilization)	18.66 mg L^{-1}	[124]
										EC_{50} (7 d) (population growth inhibition)	8.16 mg L^{-1}	[124]
								Algae	*P. subcapitata*	EC_{50} (72 h) (growth inhibition)	0.0020 mg L^{-1}	[124]
								Algae	*P. subcapitata*	EC_{50} (96 h) (growth inhibition)	11 μg L^{-1}	[132]
										LOEC (96 h) (growth inhibition)	6.3 μg L^{-1}	[132]
										NOEC (96 h) (growth inhibition)	3.1 μg L^{-1}	[132]
								Crustacean	*D. magna*	EC_{50} (21 d) (reproduction)	40 μg L^{-1}	[132]
										LOEC (21 d) (reproduction)	6.3 μg L^{-1}	[132]
										NOEC (21 d) (reproduction)	3.1 μg L^{-1}	[132]
Erithromycin	114-07-8	Po river water	Italy	SPE-HPLC–MS/MS	0.3	1.40–15.90	[24]	Duckweed	*Lemna minor*	EC_{50} (7 d) (growth inhibition)	5.62 mg L^{-1}	[77]
		Lambro river water				4.50						
Erithromycin		STP effluent	South Korea	SPE-LC–MS/MS	1.0	8.9–294	[90]	Crustacean	*T. platyurus*	LC_{50} (24 h) (mortality)	>100 mg L^{-1}	[78]
		Surface water				1.8–4.8						
Erithromycin		Mankyung river water	South Korea	SPE-LC–MS/MS	1	ND–137 (±15)	[92]	Fish	*O. latipes*	LC_{50} (96 h) (mortality)	>100 mg L^{-1}	[78]
								Rotifer	*B. calyciflorus*	LC_{50} (24 h) (mortality)	27.53 mg L^{-1}	[124]
										EC_{50} (48 h) (population growth inhibition)	0.94 mg L^{-1}	[124]

								Crustacean	*T. platyurus*	LC_{50} (24 h) (mortality)	17.68 mg L^{-1}	[124]
									D. magna	EC_{50} (24 h) (immobilization)	22.45 mg L^{-1}	[124]
									C. dubia	EC_{50} (24 h) (immobilization)	10.23 mg L^{-1}	[124]
										EC_{50} (7 d) (population growth inhibition)	0.22 mg L^{-1}	[124]
								Algae	*P. subcapitata*	EC_{50} (72 h) (growth inhibition)	0.020 mg L^{-1}	[124]
								Algae	*S. capricornutum*	EC_{50} (growth inhibition)	0.0366 mg L^{-1}	[131]
										NOEC (growth inhibition)	0.0103 mg L^{-1}	[131]
									C. vulgaris	EC_{50} (growth inhibition)	33.8 mg L^{-1}	[131]
										NOEC (growth inhibition)	12.5 mg L^{-1}	[131]
Erithromycin-H_2O*	114-07-8	Hospital effluent	Taiwan	SPE-HPLC–MS/MS	1.0	938	[47]					
		Pharmaceutical production facility effluent				110						
Erithromycin-H_2O*		STP influent	Taiwan	SPE-HPLC–MS/MS	—†	226–1537	[87]					
		STP effluent				361–811						
Erithromycin-H_2O*		Surface seawater	China (Hong Kong)	SPE-HPLC–MS/MS	13	9.50–486	[144]					
Erithromycin-H_2O*		Victoria Harbour seawater	China	SPE-HPLC–MS	2.0 (LOQ seawater)	3.3–3.4	[145]					
		Pearl River water			5 (LOQ river water)							
						30–460						
Roxithromycin	80214-83-1	Surface water	USA	SPE-LC–MS	30	50	[23]					
Roxithromycin		Victoria Harbour seawater	China	SPE-HPLC–MS	2.0 (LOQ seawater)	5.1–6.1	[145]					
		Pearl River water			5 (LOQ river water)							
						16–66						
Spiramycin	67262-35-5	Po river water	Italy	SPE-HPLC–MS/MS	0.3	ND–43.80	[24]	Algae	*M. aeruginosa*	EC_{50} (growth rate)	0.005 mg L^{-1}	[121]
		Lambro river water				74.20						
									S. capricornutum	EC_{50} (growth rate)	2.3 mg L^{-1}	[121]
Tylosin	1401-69-0	Surface water	USA	SPE-LC–MS	50	40	[23]	Algae	*M. aeruginosa*	EC_{50} (growth rate)	0.034 mg L^{-1}	[121]
Tylosin		Po river water	Italy	SPE-HPLC–MS/MS	0.3	ND–0.30	[24]		*S. capricornutum*	EC_{50} (growth rate)	1.38 mg L^{-1}	[121]
		Lambro river water				2.77						
								Algae	*S. capricornutum*	EC_{50} (growth inhibition)	0.411 mg L^{-1}	[131]
										NOEC (growth inhibition)	0.206 mg L^{-1}	[131]
								Crustacean	*D. magna*	LOEC (24 h) (immobilization)	700 mg L^{-1}	[134]

Table 3 (*Continued*)

Compound	CAS number	Sample	Country	Analytical procedure	LOD (ng L^{-1})	Concentration reported (ng L^{-1})	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
										EC_{50} (48 h) (immobilization)	680 mg L^{-1}	[134]
										NOEC (21 d) (reproduction)	45 mg L^{-1}	[134]
Sulfonamides												
Sulfachloropyridazine	80-32-0	STP influent	Korea	SPE-LC–MS	30	<30–476	[93]	Bacteria	*V. fischeri*	EC_{50} (15 min)	26.4 mg L^{-1}	[82]
		STP effluent				<30–149						
								Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	375.3 mg L^{-1}	[82]
									D. magna	EC_{50} (96 h) (immobilization)	233.5 mg L^{-1}	[82]
								Fish	*O. latipes*	LC_{50} (48 h)	589.3 mg L^{-1}	[82]
									O. latipes	LC_{50} (96 h)	535.7 mg L^{-1}	[82]
								Aquatic plant	*Lemna minor*	EC_{50} (48 h) (n° of green fronds)	2.33 mg L^{-1}	[133]
Sulfadiazine	68-35-9	Tevere river water	Italy	SPE-LC–MS	21 (LOQ)	236	[143]	Algae	*M. aeruginosa*	EC_{50} (72 h) (growth inhibition)	0.135 mg L^{-1}	[122]
Sulfadiazine		Victoria Harbour seawater	China	SPE-HPLC–MS	0.5 (LOQ seawater)	ND	[145]		*S. capricornutum*	EC_{50} (72 h) (growth inhibition)	7.8 mg L^{-1}	[122]
		Pearl River water			1 (LOQ river water)	38–209						
								Algae	*S. capricornutum*	EC_{50} (growth inhibition)	2.19 mg L^{-1}	[131]
										NOEC (growth inhibition)	<1.00 mg L^{-1}	[131]
								Crustacean	*D. magna*	LOEC (24 h) (immobilization)	150 mg L^{-1}	[132]
										EC_{50} (48 h) (immobilization)	221 mg L^{-1}	[132]
										EC_{50} (21 d) (reproduction)	13.7 mg L^{-1}	[132]
								Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	212 mg L^{-1}	[135]
Sulfadimethoxine	122-11-2	Surface water	USA	SPE-LC–MS	50	60	[23]	Bacteria	*V. fischeri*	EC_{50} (15 min)	>500 mg L^{-1}	[82]
Sulfadimethoxine		Groundwater	USA	SPE-LC–MS/MS	30	46–68	[27]	Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	248.0 mg L^{-1}	[82]
Sulfadimethoxine		Hospital effluent	Taiwan	SPE-HPLC–MS/MS	1.0	ND	[47]		*D. magna*	EC_{50} (96 h) (immobilization)	204.5 mg L^{-1}	[82]
		Pharmaceutical production facility effluent				0.8						
Sulfadimethoxine		STP influent	Luxembourg	SPE-LC–MS/MS	0.3	0.3–26	[89]	Fish	*O. latipes*	LC_{50} (48 h)	>100 mg L^{-1}	[82]
		STP effluent				0.3–9						
		Alzette river water				0.3–3						
		Mess river water				<0.3						
Sulfadimethoxine		STP influent	Korea	SPE-LC–MS	10	<10–213	[93]		*O. latipes*	LC_{50} (96 h)	>100 mg L^{-1}	[82]
		STP effluent				<10–70						
		Han river water				<10–13						
Sulfadimethoxine		Tevere river water	Italy	SPE-LC–MS	8	28	[143]	Algae	*S. capricornutum*	EC_{50} (growth inhibition)	2.30 mg L^{-1}	[131]

		Trigno river water				74						
		Drinking water				11						
										NOEC (growth inhibition)	0.529 mg L^{-1}	[131]
									C. vulgaris	EC_{50} (growth inhibition)	11.2 mg L^{-1}	[131]
										NOEC (growth inhibition)	<20.3 mg L^{-1}	[131]
								Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	270 mg L^{-1}	[135]
								Crustacean	*D. magna*	EC_{50} (24 h) (immobilization)	639.8 mg L^{-1}	[136]
									M. macrocopa	EC_{50} (24 h) (immobilization)	296.6 mg L^{-1}	[136]
										EC_{50} (48 h) (immobilization)	183.9 mg L^{-1}	[136]
Sulfamethazine	57-68-1	Groundwater	USA	SPE-LC–MS/MS	20	76–215	[27]	Bacteria	*V. fischeri*	EC_{50} (15 min)	344.7 mg L^{-1}	[82]
Sulfamethazine		Groundwater	USA	SPE-LC–MS	50	360	[28]	Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	174.4 mg L^{-1}	[82]
Sulfamethazine		Hospital effluent	Taiwan	SPE-HPLC–MS/MS	0.5	ND	[47]		*D. magna*	EC_{50} (96 h) (immobilization)	158.8 mg L^{-1}	[82]
		Pharmaceutical production facility effluent				178						
Sulfamethazine		STP influent	Luxembourg	SPE-LC–MS/MS	0.3	0.3–2	[89]	Fish	*O. latipes*	LC_{50} (48 h)	>100 mg L^{-1}	[82]
		STP effluent				<0.3						
		Alzette river water				<0.3						
		Mess river water				<0.3						
Sulfamethazine		STP influent	USA	SPE-LC–MS	50	160	[140]		*O. latipes*	LC_{50} (96 h)	>100 mg L^{-1}	[82]
		STP effluent				ND						
								Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	202 mg L^{-1}	[135]
										EC_{50} (21 d) (reproduction)	4.25 mg L^{-1}	[135]
										LOEC (21 d) (reproduction)	3.125 mg L^{-1}	[135]
										NOEC (21 d) (reproduction)	1.563 mg L^{-1}	[135]
								Crustacean	*D. magna*	EC_{50} (24 h) (immobilization)	506.3 mg L^{-1}	[136]
										EC_{50} (48 h) (immobilization)	215.9 mg L^{-1}	[136]
									M. macrocopa	EC_{50} (24 h) (immobilization)	310.9 mg L^{-1}	[136]
										EC_{50} (48 h) (immobilization)	110.7 mg L^{-1}	[136]
Sulfamethoxazole	723-46-6	Surface water	USA	SPE-LC–MS	50	150	[23]	Bacteria	*V. fischeri*	EC_{50} (15 min)	78.1 mg L^{-1}	[82]
Sulfamethoxazole		Groundwater	USA	SPE-LC–MS	23	1110	[28]	Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	189.2 mg L^{-1}	[82]
Sulfamethoxazole		Drinking water	USA	SPE-LC–MS/MS	0.25	0.32	[32]		*D. magna*	EC_{50} (96 h) (immobilization)	177.3 mg L^{-1}	[82]
Sulfamethoxazole		Hospital effluent	Taiwan	SPE-HPLC–MS/MS	1.0	1335	[47]	Fish	*O. latipes*	LC_{50} (48 h)	>750 mg L^{-1}	[82]

Table 3 (*Continued*)

Compound	CAS number	Sample	Country	Analytical procedure	LOD (ng L^{-1})	Concentration reported (ng L^{-1})	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
		Pharmaceutical production facility effluent				34						
Sulfamethoxazole		STP influent	Taiwan	SPE-HPLC–MS/MS	—†	179–1760	[87]		*O. latipes*	LC_{50} (96 h)	562.5 mg L^{-1}	[82]
		STP effluent				47–964						
Sulfamethoxazole		STP influent	Luxembourg	SPE-LC–MS/MS	0.3	13–155	[89]	Cnidarian	*Hydra attenuata*	LC_{50} (96 h) (morphology)	>100 mg L^{-1}	[98]
		STP effluent				4–39						
		Alzette river water				1–22						
		Mess river water				0.3–5						
Sulfamethoxazole		STP effluent	South Korea	SPE-LC–MS/MS	1.0	3.8–407	[90]			LOEC (96 h) (morphology)	10 mg L^{-1}	[98]
		Surface water				1.7–36						
Sulfamethoxazole		STP influent	Korea	SPE-LC–MS	5	156–984	[93]			NOEC (96 h) (morphology)	5 mg L^{-1}	[98]
		STP effluent				25–492						
		Han river water				<5–82						
Sulfamethoxazole		STP influent	USA	SPE-HPLC–MS/MS	12	ND–1000	[138]	Bacteria	*V. fischeri*	EC_{50} (30 min) (luminescnce)	23.3 mg L^{-1}	[124]
		STP effluent				310						
		Hospital effluent				ND–2100						
		Rio Grande river water				ND–300						
Sulfamethoxazole		STP influent	USA	SPE-LC–MS	50	300	[140]	Rotifer	*B. calyciflorus*	LC_{50} (24 h) (mortality)	26.27 mg L^{-1}	[124]
		STP effluent				200						
Sulfamethoxazole		STP influent	Sweden	SPE-LC–MS	80 (LOQ)	<80–674	[141]			EC_{50} (48 h) (population growth inhibition)	9.63 mg L^{-1}	[124]
		STP effluent				<80–304						
Sulfamethoxazole		Tevere river water	Italy	SPE-LC–MS	9	402	[143]	Crustacean	*T. platyurus*	LC_{50} (24 h) (mortality)	35.36 mg L^{-1}	[124]
		Drinking water				13–80						
Sulfamethoxazole		Victoria Harbour seawater	China	SPE-HPLC–MS	0.8 (LOQ seawater)	ND	[145]		*D. magna*	EC_{50} (24 h) (immobilization)	25.20 mg L^{-1}	[124]
		Pearl River water			1 (LOQ river water)							
						37–134						
									C. dubia	EC_{50} (24 h) (immobilization)	15.51 mg L^{-1}	[124]
										EC_{50} (7 d) (population growth inhibition)	0.21 mg L^{-1}	[124]
								Algae	*P. subcapitata*	EC_{50} (72 h) (growth inhibition)	0.52 mg L^{-1}	[124]
								Algae	*S. capricornutum*	EC_{50} (growth inhibition)	1.53 mg L^{-1}	[131]

											NOEC (growth inhibition)	0.614 mg L^{-1} [131]
								Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	123.1 mg L^{-1} [136]
									M. macrocopa	EC_{50} (24 h) (immobilization)	84.9 mg L^{-1} [136]
										EC_{50} (48 h) (immobilization)	70.4 mg L^{-1} [136]
Sulfapyridine	7238-91-7	Tevere river water	Italy	SPE-LC–MS	12	<12–121	[143]	Cnidarian	*Hydra attenuata*	LC_{50} (96 h) (morphology)	>100 mg L^{-1}	[98]
		Trigno river water				66						
										EC_{50} (96 h) (morphology)	21.61 mg L^{-1}	[98]
										LOEC (96 h) (morphology)	5 mg L^{-1}	[98]
										NOEC (96 h) (morphology)	1 mg L^{-1}	[98]
Sulfathiazole	72-14-0	STP influent	Luxembourg	SPE-LC–MS/MS	0.3	0.3–2	[89]	Bacteria	*V. fischeri*	EC_{50} (15 min)	>1000 mg L^{-1}	[82]
		STP effluent				<0.3						
		Alzette river water				<0.3						
		Mess river water				0.3–2						
Sulfathiazole		STP influent	Korea	SPE-LC–MS	30	<30–531	[93]	Crustacean	*D. magna*	LOEC (21 d) (reproduction)	35 mg L^{-1}	[136]
		STP effluent				<30						
										NOEC (21 d) (reproduction)	11 mg L^{-1}	[136]
								Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	149.3 mg L^{-1}	[82]
									D. magna	EC_{50} (96 h) (immobilization)	85.4 mg L^{-1}	[82]
								Fish	*O. latipes*	LC_{50} (48 h)	>500 mg L^{-1}	[82]
									O. latipes	LC_{50} (96 h)	>500 mg L^{-1}	[82]
								Crustacean	*D. magna*	EC_{50} (24 h) (immobilization)	616.7 mg L^{-1}	[136]
									M. macrocopa	EC_{50} (24 h) (immobilization)	430.1 mg L^{-1}	[136]
										EC_{50} (48 h) (immobilization)	391.1 mg L^{-1}	[136]
Tetracyclines												
Chlortetracycline	57-62-5	Surface water	USA	SPE-LC–MS	100	420	[23]	Algae	*M. aeruginosa*	EC_{50} (growth rate)	0.05 mg L^{-1}	[121]
Chlortetracycline		Hospital effluent	Taiwan	SPE-HPLC–MS/MS	5.0	ND	[47]		*S. capricornutum*	EC_{50} (growth rate)	3.1 mg L^{-1}	[121]
		Pharmaceutical production facility effluent				5.7						
								Bacteria	*V. fischeri*	EC_{50} (15 min) (luminescence)	13.0 mg L^{-1}	[136]
								Crustacean	*D. magna*	EC_{50} (24 h) (immobilization)	380.1 mg L^{-1}	[136]
										EC_{50} (48 h) (immobilization)	225 mg L^{-1}	[136]
									M. macrocopa	EC_{50} (24 h) (immobilization)	515 mg L^{-1}	[136]
										EC_{50} (48 h) (immobilization)	272 mg L^{-1}	[136]

Table 3 (*Continued*)

Compound	CAS number	Sample	Country	Analytical procedure	LOD (ng L^{-1})	Concentration reported (ng L^{-1})	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
								Fish	*O. latipes*	LC_{50} (24 h) (mortality)	88.4 mg L^{-1}	[136]
										LC_{50} (48 h) (mortality)	78.9 mg L^{-1}	[136]
Oxytetracycline	79-57-2	Surface water	USA	SPE-LC–MS	100	340	[23]	Cnidarian	*Hydra attenuata*	LC_{50} (96 h) (morphology)	>100 mg L^{-1}	[98]
Oxytetracycline		Po river water	Italy	SPE-HPLC–MS/MS	0.3	ND–19.2	[24]			EC_{50} (96 h) (morphology)	40.13 mg L^{-1}	[98]
		Lambro river water				14.35						
Oxytetracycline		Hospital effluent	Taiwan	SPE-HPLC–MS/MS	2.0	2.9	[47]			LOEC (96 h) (morphology)	100 mg L^{-1}	[98]
		Pharmaceutical production facility effluent				23						
Oxytetracycline		STP influent	Luxembourg	SPE-LC–MS/MS	0.3	0.3–7	[89]			NOEC (96 h) (morphology)	50 mg L^{-1}	[98]
		STP effluent				0.3–5						
		Alzette river water				0.3–2						
		Mess river water				0.3–7						
								Algae	*M. aeruginosa*	EC_{50} (72 h) (growth inhibition)	0.207 mg L^{-1}	[122]
									R. salina	EC_{50} (72 h) (growth inhibition)	1.6 mg L^{-1}	[122]
									S. capricornutum	EC_{50} (72 h) (growth inhibition)	4.5 mg L^{-1}	[122]
								Bacteria	*V. fischeri*	EC_{50} (30 min) (luminescnce)	64.50 mg L^{-1}	[124]
								Rotifer	*B. calyciflorus*	LC_{50} (24 h) (mortality)	34.21 mg L^{-1}	[124]
										EC_{50} (48 h) (population growth inhibition)	1.87 mg L^{-1}	[124]
								Crustacean	*T. platyurus*	LC_{50} (24 h) (mortality)	25.00 mg L^{-1}	[124]
									D. magna	EC_{50} (24 h) (immobilization)	22.64 mg L^{-1}	[124]
									C. dubia	EC_{50} (24 h) (immobilization)	18.65 mg L^{-1}	[124]
										EC_{50} (7 d) (population growth inhibition)	0.18 mg L^{-1}	[124]
								Algae	*P. subcapitata*	EC_{50} (72 h) (growth inhibition)	0.17 mg L^{-1}	[124]
								Algae	*S. capricornutum*	EC_{50} (growth inhibition)	0.342 mg L^{-1}	[131]
										NOEC (growth inhibition)	0.183 mg L^{-1}	[131]
									C. vulgaris	EC_{50} (growth inhibition)	7.05 mg L^{-1}	[131]
										NOEC (growth inhibition)	<3.58 mg L^{-1}	[131]

								Aquatic plant	*Lemna minor*	EC_{50} (48 h) (n° of green fronds)	4.92 mg L^{-1}	[133]
								Crustacean	*D. magna*	LOEC (48 h) (immobilization)	100 mg L^{-1}	[134]
										EC_{50} (21 d) (reproduction)	46.2 mg L^{-1}	[134]
								Bacteria	*V. fischeri*	EC_{50} (15 min) (luminescence)	87.0 mg L^{-1}	[136]
								Crustacean	*D. magna*	EC_{50} (24 h) (immobilization)	831.6 mg L^{-1}	[136]
										EC_{50} (48 h) (immobilization)	621.2 mg L^{-1}	[136]
									M. macrocopa	EC_{50} (24 h) (immobilization)	137.1 mg L^{-1}	[136]
										EC_{50} (48 h) (immobilization)	126.7 mg L^{-1}	[136]
								Fish	*O. latipes*	LC_{50} (24 h) (mortality)	215.4 mg L^{-1}	[136]
										LC_{50} (48 h) (mortality)	110.1 mg L^{-1}	[136]
Tetracycline	60-54-8	Surface water	USA	SPE-LC–MS	100	110	[23]	Duckweed	*Lemna minor*	EC_{50} (7 d) (growth inhibition)	1.06 mg L^{-1}	[77]
Tetracycline		Hospital effluent	Taiwan	SPE-HPLC–MS/MS	2.0	89	[47]	Algae	*M. aeruginosa*	EC_{50} (growth rate)	0.09 mg L^{-1}	[121]
		Pharmaceutical production facility effluent				25						
Tetracycline		STP influent	Taiwan	SPE-HPLC–MS/MS	—†	46–234	[87]		*S. capricornutum*	EC_{50} (growth rate)	2.2 mg L^{-1}	[121]
		STP effluent				16–38						
Tetracycline		STP influent	Luxembourg	SPE-LC–MS/MS	0.3	0.3–85	[89]	Crustacean	*D. magna*	NOEC (48 h) (immobilization)	340 mg L^{-1}	[134]
		STP effluent				0.3–24						
		Alzette river water				0.3–8						
		Mess river water				0.3–7						
Tetracycline		STP influent	USA	SPE-LC–MS	50	520	[140]			EC_{50} (21 d) (reproduction)	44.8 mg L^{-1}	[134]
		STP effluent				170						
Tetracycline		Surface seawater	China (Hong Kong)	SPE-HPLC–MS/MS	13	<13–122	[144]					
Others												
Chloramphenicol	85666-84-8	Victoria Harbour seawater	China	SPE-HPLC–MS	4.1 (LOQ seawater)	ND	[145]					
		Pearl River water			5 (LOQ river water)							
						41–127						
Metronidazole	99616-64-5	STP influent	Taiwan	SPE-HPLC–MS/MS	—†	1–294	[87]	Crustacean	D. magna	LOEC(48 h) (immobilization)	1000 mg L^{-1}	[134]
		STP effluent				10–126						
										NOEC (21 d) (reproduction)	250 mg L^{-1}	[134]
Trimethoprim	738-70-5	Surface water	USA	SPE-LC–MS	30	150	[23]	Bacteria	*V. fischeri*	EC_{50} (15 min)	176.7 mg L^{-1}	[82]
Trimethoprim		Drinking water	USA	SPE-LC–MS/MS	0.25	<0.25	[32]	Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	167.4 mg L^{-1}	[82]

Table 3 (*Continued*)

Compound	CAS number	Sample	Country	Analytical procedure	LOD ($ng\,L^{-1}$)	Concentration reported ($ng\,L^{-1}$)	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
Trimethoprim		Danube river water	Serbia	SPE-LC–MS/MS	0.34	25	[84]		*D. magna*	EC_{50} (96 h) (immobilization)	120.7 $mg\,L^{-1}$	[82]
		Tamiš river water				24						
		Lake Očaga water				174						
		Groundwater				100						
Trimethoprim		STP influent	Taiwan	SPE-HPLC–MS/MS	—†	259–949	[87]	Fish	*O. latipes*	LC_{50} (48 h)	>100 $mg\,L^{-1}$	[82]
		STP effluent				203–415						
Trimethoprim		STP effluent	South Korea	SPE-LC–MS/MS	1.0	10–188	[90]		*O. latipes*	LC_{50} (96 h)	>100 $mg\,L^{-1}$	[82]
		Surface water				3.2–5.3						
Trimethoprim		STP influent	Korea	SPE-LC–MS	10	<10–496	[93]	Cnidarian	*Hydra attenuata*	LC_{50} (96 h) (morphology)	>100 $mg\,L^{-1}$	[98]
		STP effluent				<10–174						
		Han river water				<10–26						
Trimethoprim		STP influent	USA	SPE-HPLC–MS/MS	10	ND–1400	[138]			LOEC (96 h) (morphology)	>100 $mg\,L^{-1}$	[98]
		STP effluent				180						
		Hospital effluent				ND–5000						
		Rio Grande river water				ND						
Trimethoprim		STP influent	USA	SPE-LC–MS	50	330	[140]			NOEC (96 h) (morphology)	>100 $mg\,L^{-1}$	[98]
		STP effluent				170						
Trimethoprim		Surface seawater	China (Hong Kong)	SPE-HPLC–MS/MS	13	<13–21.8	[144]	Algae	*M. aeruginosa*	EC_{50} (72 h) (growth inhibition)	112 $mg\,L^{-1}$	[122]
									R. salina	EC_{50} (72 h) (growth inhibition)	16 $mg\,L^{-1}$	[122]
									S. capricornutum	EC_{50} (72 h) (growth inhibition)	130 $mg\,L^{-1}$	[122]
								Algae	*S. capricornutum*	EC_{50} (growth inhibition)	80.3 $mg\,L^{-1}$	[131]
										NOEC (growth inhibition)	25.5 $mg\,L^{-1}$	[131]
								Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	149 $mg\,L^{-1}$	[135]
								Crustacean	*D. magna*	EC_{50} (24 h) (immobilization)	155.6 $mg\,L^{-1}$	[135]
										EC_{50} (48 h) (immobilization)	92.0 $mg\,L^{-1}$	[135]
									M. macrocopa	EC_{50} (24 h) (immobilization)	144.8 $mg\,L^{-1}$	[135]
										EC_{50} (48 h) (immobilization)	54.8 $mg\,L^{-1}$	[135]
								Crustacean	*D. magna*	LOEC (21 d) (reproduction)	20 $mg\,L^{-1}$	[136]
										NOEC (21 d) (reproduction)	6 $mg\,L^{-1}$	[136]

ND—Not detected; †—Data not available; SPE—Solid Phase Extraction; GC–MS—Gas Chromatography with Mass Spectrometry Detection; HPLC–MS/MS—High Performance Liquid Chromatography with Tandem Mass Spectrometry Detection; LC-FD—Liquid Chromatography with Fluorescence Detection; LC–MS—Liquid Chromatography with Mass Spectrometry Detection; LC–MS/MS—Liquid Chromatography with Tandem Mass Spectrometry Detection.

4.6 and 40 mg L^{-1} respectively [134], while sulfamethazine had an EC_{50} of 202 mg L^{-1} [135]. Reproduction was also impaired by oxytetracycline, sulfadiazine, tetracycline and tiamulin at concentrations between 5 and 50 mg L^{-1}. Oxolinic acid, streptomycin and tylosin were revealed to be lethal after long-term exposure [134]. Chronic toxicity effects were also observed on the reproduction of the crustacean *D. magna*, when were exposed to levofloxacin and clarithromycin, with EC_{50} values of 340 and 40 μg L^{-1}, respectively [132]. Eleven commonly used antibiotics were evaluated in organisms belonging to different trophic levels (*V. fischeri*, *D. magna*, *Moina macrocopa*, and *O. latipes*). Neomycin showed significant effects on *D. magna* (EC_{50} = 42.1 mg L^{-1}), *M. macrocopa* (EC_{50} = 34.1 mg L^{-1}) and *O. latipes* (LC_{50} = 80.8 mg L^{-1}) while beta-lactam antibiotics (ampicillin and amoxycillin) were the less toxic to all tested organisms [136]. Neomycin showed chronic toxicity by affecting the reproduction and adult survival of *D. magna* and *M. macrocopa* even at low mg L^{-1} levels of exposure (EC_{50}s of 0.09 and 0.74 mg L^{-1}, respectively). Other pharmaceuticals such as sulfathiazole, trimethoprim and enrofloxacin also showed similar effects on those two cladocerans in a dose-dependent manner. Luminescence inhibition on *V. fischeri* occurred after irradiation of tetracycline, proving that photolytic products become more toxic than the parent compound [137]. Antibiotics belonging to different classes have been found in different aquatic environments (Table 3). Lincomycin was detected in hospital and livestock effluents at concentrations of 2 and 6.6 μg L^{-1}, respectively [138]. Fluorquinolone antibiotics as ciprofloxacin were found in hospital effluents [138,139] at values between 2 and 11 μg L^{-1}, in STP influents (90–1000 ng L^{-1}) and effluents (<6–310 ng L^{-1}) [138–141] as well as in surface waters, i.e. the Lambro river (Italy) (14.36 ng L^{-1}) [24] and Mondego river (Portugal) (79.6–119.2 ng L^{-1}) [142]. Enrofloxacin, a fluorquinolone used by the veterinary medicine, was detected in STP influents (121.8–447.1 ng L^{-1}) and effluents (53.7–270 ng L^{-1}) in Portugal [139] and the US [140] as well as in surface waters from the Mondego river (Portugal) (67.0–102.5 ng L^{-1}) [142]. Sulfonamides have been found in several aquatic systems as STP influents and effluents [138,140,141], surface waters [23,143], groundwaters [27,28] and drinking waters [143] in concentrations ranging from ng L^{-1} to a few μg L^{-1}. Regarding the tetracyclines, oxytetracycline was detected in the Po and Lambro rivers (Italy) at concentrations up to 248.90 and 24.40 ng L^{-1} respectively [24], in combination with tetracycline [140] in American STP influent (47 μg L^{-1}) and effluent (4.2 μg L^{-1}) [140] and in surface waters (340 ng L^{-1}) [23]. In addition to aquatic systems, antibiotics belonging to the fluorquinolones class have also been found in sediments at concentrations that can reach 4.8 mg kg^{-1} [141]. This finding may represent a potential risk warning of persistence in the environment.

4.4. Sex hormones

Sex hormones are extremely active biological compounds producing intense therapeutic effects even at very low doses. Today, they are commonly prescribed as oral contraceptives thus indirectly contributing to the increase in environmental concentrations [52,108]. Estrogens are the sex hormones most commonly found in the environment. These can exist as either natural or synthetic substances, mimicking the effects of endogenous estrogens as endocrine-disrupting compounds (EDCs) [146] through binding to specific receptors common to non-target organisms (invertebrates, fish, reptiles, birds and mammals) [108]. In fish, estrogens are involved in several physiological functions including: (i) vitellogenin synthesis; (ii) vitelline envelope (eggshell) protein production; (iii) gonadal differentiation; (iv) development of secondary sexual characteristics; (v) gonadotropin secretion; (vi) synthesis of estrogen receptors; (vii) pheromonal communication; (viii) bone formation; and (ix) calcium homeostasis [146]. The enhanced production of the vitellogenin found in the blood of male and juvenile fish provides a useful biomarker of aquatic contamination by compounds with estrogenic activity [52,146]. Wild fish (roach; *Rutilus rutilus*) exposed to such compounds in UK rivers receiving STP effluents suffered adverse reproductive effects. Male fish were shown to be intersex, i.e. they had simultaneous male and female gonadal characteristics besides a high plasma vitellogenin concentration [147]. Ethinylestradiol (EE_2) is a synthetic estrogen found in oral contraceptive pills with marked estrogenic effects in fish. The life-cycle exposure of fathead minnows to EE_2 concentrations below 1 ng L^{-1} caused a significant reduction in fertilization success, an increased egg production and decreased expression of secondary male sex characteristics [148]. Similar findings were obtained by Pawlowski et al. [149] in trials extended over a reduced period of three weeks. Concentrations below 1 ng L^{-1} gave rise to an increased female population and for EE_2 concentrations above 3.5 ng L^{-1}, fish became completely feminized [148]. Concentrations above 1 ng L^{-1} of EE_2 also induced higher vitellogenin plasma levels in both males and females [149,150]. Nash et al. [151] registered similar findings for zebrafish males by simply performing the assay with 0.5 ng L^{-1} of EE_2. Life-long exposure of zebrafish to 5 ng L^{-1} of EE_2 has led to reproductive failure due to the absence of secondary male sex characteristics and normal testes [151]. Exposure of juveniles to estrogen has caused skewed sex ratios in favour of females for concentrations of 1 ng L^{-1} [150]. Sex reversal was complete at levels of 2 ng L^{-1} [150]. Xu et al. [152] also exposed zebrafish to EE_2 during their period of sex differentiation, showing that, after 90 days post-hatch, there was already an increase in mortality rate and sex ratio for fish exposed to concentrations of 2 ng L^{-1}. When the concentration was increased to 10 ng L^{-1} was observed a significantly decrease in the weight and length body. On the other hand, 180 days post-hatch were found abnormal testicular morphologies in male fish, namely malformations of the sperm duct, an altered proportion of germ cell types, and a reduced number of spermatozoa, for those levels of EE_2 [152]. Exposure of male roach to EE_2 concentrations up to 4 ng L^{-1} in early life disrupted normal sexual development causing a feminized response, characterized by the presence of an ovarian cavity and induced plasma vitellogenin production [153]. Kidd et al. [34] conducted a 7-year, whole-lake experiment, proving that chronic exposure of fathead minnow to concentrations of EE_2 in the order of 5–6 ng L^{-1}, led to feminization of males fish, through production of vitellogenin and disruption in gonadal development, causing intersex, and altered oogenesis in females. Those reproductive alterations led to a collapse of the fathead minnow population due to the loss of the young generations, expressed in a loss of smaller sizes classes of fish, what contribute, in a last case, to leave this species from the lake near of extinction [34]. The natural estrogen 17β-estradiol (E_2) can also negatively affect fish at low concentrations. Japanese medaka exposed to 33.5 ng L^{-1} of this estrogen in early life enhanced their body length and body weight. Additionally, the males also exhibited testis-ova after 14 days of exposure [154]. When the E_2 concentration was increased to 140.6 ng L^{-1}, testis-ova were observed in males (after 12 days exposure) and complete gonadal transformation to an ovary occurred after 20 days [154]. The exposure of adult fish to concentrations from 29.3 to 463 ng L^{-1} over 21 days gave rise to testis-ova development and induced vitellogenin production in males to all tested concentrations [155]. At the higher level, a decrease in the number of eggs produced and fertility [155] was also observed. Amphibians and reptiles exposed to environmental estrogens showed sex reversal as well as significant changes in secondary sex characteristics [156,157]. Concerning invertebrates such as the amphipod *Hyalella azteca* it was observed that at sub-lethal concentrations of EE_2 (0.1–10 μg L^{-1}) sexual development of males was affected

Table 4
Examples of concentrations (ng L^{-1}) of sex hormones measured in different aquatic environments.

Compound	CAS number	Sample	Country	Analytical procedure	LOD (ng L^{-1})	Concentration reported (ng L^{-1})	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
Diethylstilbestrol	8053-00-7	River water	China	SPME-GC–MS	2	20 (±0)	[162]					
17α-Estradiol	57-91-0	Surface water	USA	LLE-GC–MS	5	30	[23]					
17α-Estradiol		Groundwater	France	SPE-LC–MS/MS	0.03	0.8–3.5	[164]					
17β-Estradiol	50-28-2	Surface water	USA	LLE-GC–MS	5	9	[23]	Fish	*O. latipes*	NOEC (21 d) (testis-ova induction)	<29.3 ng L^{-1}	[15]5
17β-Estradiol		Drinking water	USA	SPE-LC–MS/MS	0.50	<0.50	[32]			LOEC (21 d) (testis-ova induction)	<26.3 ng L^{-1}	[155]
17β-Estradiol		Hospital effluent	Taiwan	SPE-HPLC–MS/MS	25	25	[47]			NOEC (21 d) (VTG induction)	29.3 ng L^{-1}	[155]
		Pharmaceutical production facility effluent				ND						
17β-EStradiol		STP influent	Japan	SPE-GC–MS	0.1 (LOQ)	13.3–25.8	[86]					
		STP effluent				0.49–12.4						
17β-Estradiol		Pearl Rivers water	China	SPE-GC–MS	0.3	ND–7.5 (±0.4)	[88]					
17β-Estradiol		STP influent	Luxembourg	SPE-LC–MS/MS	1.0	1.0–102	[89]					
		STP effluent				1.0–85						
		Alzette river water				1.0–35						
		Mess river water				1.0–6						
17β-Estradiol		STP effluent	South Korea	SPE-LC–MS/MS	1.0	<1.0	[90]					
		Surface water				ND						
17β-Estradiol		STP effluent	Japan	SPE-LC–MS/MS	0.3	0.3–2.5	[160]					
		Tamagawa river water				0.6–1.0						
		Kasumigaura lake water				<0.3						
17β-Estradiol		STP influent	Germany	SPE-LC–MS/MS	2.0 (LOQ STP influent)	11.8 (±5.1)	[161]					
		STP effluent			0.4 (LOQ STP effluent)	0.8 (±0.3)						
		Berlin surface water			0.2 (LOQ surface water)	<0.2						
17β-Estradiol		River water	China	SPME-GC–MS	9	100 (±20)	[162]					
17β-Estradiol		STP influent	Italy	SPE-LC–MS/MS	1.9 (STP influent)	10–31	[163]					
		STP effluent			0.8 (STP effluent)	3–8						
		Tibre river water			0.2 (Tibre river water)	2–6						
17β-Estradiol		Groundwater	France	SPE-LC–MS/MS	0.01	0.3–1.3	[164]					
Estriol	50-27-1	Surface water	USA	LLE-GC–MS	5	19	[23]					
Estriol		STP influent	Japan	SPE-GC–MS	0.2 (LOQ)	83.0–255	[86]					
		STP effluent				0.31–0.84						
Estriol		STP effluent	South Korea	SPE-LC–MS/MS	5.0	8.9–25	[90]					
		Surface water				ND						
Estriol		STP influent	Italy	SPE-LC–MS/MS	7.0 (STP influent)	23–48	[163]					
		STP effluent			0.5 (STP effluent)	ND–1						
		Tibre river water			0.3 (Tibre river water)	2–5						
Estrone	53-16-7	Surface water	USA	LLE-GC–MS	5	27	[23]					
Estrone		Drinking water	USA	SPE-LC–MS/MS	0.20	<0.20	[32]					

Estrone		Hospital effluent	Taiwan	SPE-HPLC–MS/MS	25	126	[47]				
		Pharmaceutical production facility effluent				ND					
Estrone		STP influent	Japan	SPE-GC–MS	0.6 (LOQ)	28.7–197	[86]				
		STP effluent				2.80–110					
Estrone		Pearl Rivers water	China	SPE-GC–MS	0.2	ND–75.0 (±5.3)	[88]				
Estrone		STP influent	Luxembourg	SPE-LC–MS/MS	0.3	0.3–9	[89]				
		STP effluent				0.3–14					
		Alzette river water				0.3–6					
		Mess river water				0.3–27					
Estrone		STP effluent	South Korea	SPE-LC–MS/MS	1.0	2.2–36	[90]				
		Surface water				1.7–5.0					
Estrone		STP effluent	Japan	SPE-LC–MS/MS	0.1	2.5–34	[160]				
		Tamagawa river water				3.4–6.6					
		Kasumigaura lake water				0.2–0.8					
Estrone		STP influent	Germany	SPE-LC–MS/MS	1.0 (LOQ STP influent)	188 (±92)	[161]				
		STP effluent			0.2 (LOQ STP effluent)	12.6 (±7.0)					
		Berlin surface water			0.1 (LOQ surface water)	0.16 (± 0.05)					
Estrone		River water	China	SPME-GC–MS	18	180 (±20)	[162]				
Estrone		STP influent	Italy	SPE-LC–MS/MS	1.2 (STP influent)	15–60	[163]				
		STP effluent			0.8 (STP effluent)	5–30					
		Tibre river water			0.1 (Tibre river water)	5–12					
Estrone		Surface water	France	SPE-LC–MS/MS	0.02	0.3	[164]				
		Groundwater				0.8–3.5					
17α-Ethinylestradiol	57-63-6	Surface water	USA	LLE-GC–MS	5	73	[23]	Fish	*P. promelas*	LOEC (21 d) (plasma VTG induction)	1 ng L^{-1}	[149]
17α-Ethinylestradiol		Drinking water	USA	SPE-LC–MS/MS	1.0	<1.0	[32]			LOEC (21 d) (ultrastructure testes)	1 ng L^{-1}	[149]
17α-Ethinylestradiol		Hospital effluent	Taiwan	SPE-HPLC–MS/MS	25	32	[47]			LOEC (21 d) (ultrastructure liver)	1 ng L^{-1}	[149]
		Pharmaceutical production facility effluent				ND						
17α-Ethinylestradiol		STP influent	Luxembourg	SPE-LC–MS/MS	2.0	2.0–24	[89]			LOEC (21 d) (fertilization rate)	10 ng L^{-1}	[149]
		STP effluent				<2.0						
		Alzette river water				<2.0						
		Mess river water				<2.0						
17α-Ethinylestradiol		STP effluent	South Korea	SPE-LC–MS/MS	1.0	1.3	[90]	Fish	*D. rerio*	LOEC (38 dph) (plasma VTG induction)	2 ng L^{-1}	[150]

Table 4 (*Continued*)

Compound	CAS number	Sample	Country	Analytical procedure	LOD ($ng\,L^{-1}$)	Concentration reported ($ng\,L^{-1}$)	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
		Surface water				ND						
17α-Ethinylestradiol		STP influent	Germany	SPE-LC–MS/MS	2.0 (LOQ STP influent)	8.8 (±8.0)	[161]					
		STP effluent			0.4 (LOQ STP effluent)	1.7 (±1.3)						
		Berlin surface water			0.2 (LOQ surface water)	<0.2						
17α-Ethinylestradiol		STP influent	Italy	SPE-LC–MS/MS	1.6 (STP influent)	ND	[163]					
		STP effluent			1.1 (STP effluent)	ND						
		Tibre river water			0.4 (Tibre river water)	ND–1						
17α-Ethinylestradiol		Groundwater	France	SPE-LC–MS/MS	0.20	0.5–3.0	[164]					
Mestranol	72-33-3	Surface water	USA	LLE-GC–MS	5	74	[23]					

ND—Not detected; SPE—Solid Phase Extraction; SPME—Solid Phase Microextraction; LLE—Liquid–Liquid Extraction; GC–MS—Gas Chromatography with Mass Spectrometry Detection; LC–MS/MS—Liquid Chromatography with Tandem Mass Spectrometry Detection; dph—days post-hatch.

[158]. On the other hand, the estrogens E_2 and EE_2 did not show significant effects on reproduction or survival of *C. dubia* even at concentrations of 1 and 0.5 $mg\,L^{-1}$, respectively [159]. According to many authors, the concentrations of estrogens detected in the environment may not post a threat to humans. However regarding these compounds, there is the possibility of bioaccumulation within aquatic organisms, thereby reaching humans through the food chain or directly through drinking water [146]. Estrogens have been found in water samples (Table 4) at low $ng\,L^{-1}$ concentrations but they represent a greater risk for non-target organisms as already proved. For example, 17β-estradiol was detected in rivers [23,160–163] at levels ranging from 0.6 to 100 $ng\,L^{-1}$ and in STP effluents at concentrations between 0.3 [160] and 85 $ng\,L^{-1}$ [89]. Ethinylestradiol was also found in surface waters in the US (73 $ng\,L^{-1}$) [23] and Italy (the Tibre river) at 1 $ng\,L^{-1}$ [163].

4.5. Antiepileptics

Antiepileptic drugs act in the central nervous system (CNS) by reducing the overall neuronal activity. This can be achieved either by blocking voltage-dependent sodium channels (e.g. carbamazepine) or by enhancement of the inhibitory effects of the γ-aminobutyric acid (GABA) neurotransmitter (e.g. benzodiazepines) [99]. Carbamazepine is carcinogenic to rats but does not have mutagenic properties in mammals [165]. Moreover, this drug is lethal to zebrafish at the 43 $\mu g\,L^{-1}$ level and produces sub-lethal changes in *Daphnia* sp. at 92 $\mu g\,L^{-1}$ [165]. Regarding aquatic organisms, it can be deduced that carbamazepine does have harmful proclivity since most of the acute toxicity data were harvested from trial concentrations between 10 and 100 $mg\,L^{-1}$ [98]. In fact, *D. magna* growth was shown to be sensitive to this compound, being inhibited for concentrations of carbamazepine above 12.7 $mg\,L^{-1}$ and with acute toxicity being evident at 17.2 $mg\,L^{-1}$ [165]. The EC_{50} value (considering the motility as indicator) was approximately 13.8 $mg\,L^{-1}$ after 48 h of exposure [96]. Female *D. pulex* exposed to 1 $\mu g\,L^{-1}$ of carbamazepine showed a tendency to mature and reproduce earlier (with more offspring), suggesting that this pharmaceutical may slightly induce stimulatory effects [166]. For *C. dubia*, chronic toxicity studies revealed a NOEC of 25 $\mu g\,L^{-1}$ [96] while the activity of *G. pulex* was slightly reduced by exposure to a concentration range from 1 to 10 $ng\,L^{-1}$ [76]. Continuous exposure of *H. attenuata* to carbamazepine caused a significant reduction in feeding, with an EC_{50} of 3.76 $mg\,L^{-1}$ [98]. Japanese medaka showed a LC_{50} of 35.4 $mg\,L^{-1}$ [82] and ultrastructural changes in the liver, kidney and gill tissues of carps were induced by this pharmaceutical [97]. The changes observed in the kidney were shown to occur as a cellular response to impaired kidney function. In gills, the effects were more pronounced for concentrations above 20 $\mu g\,L^{-1}$. Another important issue concerning carbamazepine is that it can adsorb to sediments, in this way threatening aquatic organisms which feed on organic matter. Oetken et al. [167] showed that exposure of the invertebrate *Chironomus riparius* to this pharmaceutical through sediments caused a blockade of pupation and decreased emergence with EC_{50} values of 160 and 280 $\mu g\,kg^{-1}$ of dry weight, respectively. Carbamazepine is ubiquitously present in the environment, having an extremely low removal rate in STPs (7%) [54] and consequently being detected in rivers [16,20,21,54,92] at concentrations up to 595 $ng\,L^{-1}$ [92] (Table 5). In addition to surface waters, carbamazepine has also been found in groundwater [26,119] at concentrations that can reach 900 $ng\,L^{-1}$. A monitoring programme performed on the river Rhine (Germany) over a decade, showed the regular detection of carbamazepine, with an annual average concentration of 100 $ng\,L^{-1}$ [168]. These results support the idea that the presence of carbamazepine in the environment may represent a real threat.

Table 5
Examples of concentrations (ng L^{-1}) of antiepileptic drugs measured in different aquatic environments.

Compound	CAS number	Sample	Country	Analytical procedure	LOD (ng L^{-1})	Concentration reported (ng L^{-1})	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
Carbamazepine	298-46-4	STP influent	Spain	SPE-GC–MS	30	120–310	[14]	Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	>100 mg L^{-1}	[65]
		STP effluent				110–230						
Carbamazepine		STP influent	Finland	SPE-HPLC–MS/MS	1.4	290–400	[16]	Algae	*D. subspicatus*	EC_{50} (growth inhibition)	74 mg L^{-1}	[65]
		STP effluent				380–470						
		Vantaa river water				<1.4–66						
		Luhtajoki river water				23						
Carbamazepine		Somes river water	Romania	SPE-GC–MS	30	<30–75.1 (±6.1)	[20]	Duckweed	*L. minor*	EC_{50} (7 d) (growth inhibition)	25.5 mg L^{-1}	[65]
Carbamazepine		STP influent	Sweden	SPE-LC–MS/MS	—†	1680	[21]	Crustacean	*Gammarus pulex*	LOEC (behaviour)	10 ng L^{-1}	[76]
		STP effluent				1180						
		Höje river water				<1–500						
Carbamazepine		Groundwater	Germany	SPE-GC–MS	32	900	[26]	Crustacean	*T. platyurus*	LC_{50} (24 h) (mortality)	>100 mg L^{-1}	[78]
Carbamazepine		Drinking water	USA	SPE-LC–MS/MS	0.5	6.8	[32]	Fish	*O. latipes*	LC_{50} (96 h) (mortality)	45.87 mg L^{-1}	[78]
Carbamazepine		STP effluent	Germany	SPE-LC–MS/MS	50 (STP effluent)	2100	[54]	Bacteria	*V. fischeri*	EC_{50} (15 min)	52.2 mg L^{-1}	[82]
		Surface water			30 (surface water)	250						
Carbamazepine		Hospital effluent	Spain	SPE-HPLC–MS/MS	7	30–70	[73]	Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	>100 mg L^{-1}	[82]
Carbamazepine		Danube river water	Serbia	SPE-LC–MS/MS	0.27	8–130	[84]		*D. magna*	EC_{50} (96 h) (immobilization)	76.3 mg L^{-1}	[82]
		Sava river water				29–50						
		Tamiš river water				30						
		Lake Očaga water				30						
		Groundwater				6–23						
Carbamazepine		STP influent	Japan	SPE-GC–MS	6	14.9–270	[86]	Fish	*O. latipes*	LC_{50} (48 h)	35.4 mg L^{-1}	[82]
		STP effluent				10.8–163						
Carbamazepine		STP influent	Taiwan	SPE-HPLC–MS/MS	—†	82–357	[87]		*O. latipes*	LC_{50} (96 h)	35.4 mg L^{-1}	[82]
		STP effluent				93–214						
Carbamazepine		STP effluent	South Korea	SPE-LC–MS/MS	1.0	73–729	[90]	Bacteria	*V. fischeri*	EC_{50} (30 min)	>81,000 μg L^{-1}	[96]
		Surface water				4.5–61						
		Drinking water				<1.0						
Carbamazepine		Mankyung river water	South Korea	SPE-LC–MS/MS	1	ND–595 (±14)	[92]	Algae	*P. subcapitata*	NOEC (96 h) (growth inhibition)	>100,000 μg L^{-1}	[96]
Carbamazepine		STP influent	Korea	SPE-LC–MS	5	<5–451	[93]			LOEC (96 h) (growth inhibition)	>100,000 μg L^{-1}	[96]
		STP effluent				<5–195						
		Han river water				<5–36						
Carbamazepine		STP effluent	Italy	SPE-HPLC–MS/MS	1.3	ND–1318	[118]	Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	>13,800 μg L^{-1}	[96]
Carbamazepine		Groundwater	Germany	SPE-GC–MS	2 (LOQ)	45	[119]		*C. dubia*	EC_{50} (48 h) (immobilization)	77,700 μg L^{-1}	[96]
Carbamazepine		STP influent	France	SPE-LC–MS	2.4	193–420	[169]			NOEC (7 d) (reproduction)	25 μg L^{-1}	[96]
		STP effluent				86–258						

Table 5 (*Continued*)

Compound	CAS number	Sample	Country	Analytical procedure	LOD ($ng\,L^{-1}$)	Concentration reported ($ng\,L^{-1}$)	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
										LOEC (7 d) (reproduction)	100 $\mu g\,L^{-1}$	[96]
								Fish	*D. rerio*	NOEC (10 d) (survival)	25,000 $\mu g\,L^{-1}$	[96]
										LOEC (10 d) (survival)	50,000 $\mu g\,L^{-1}$	[96]
								Fish	*O. mykiss*	LOEC (21 d) (liver cytopathology)	>100 $\mu g\,L^{-1}$	[97]
										LOEC (21 d) (kidney cytopathology)	1 $\mu g\,L^{-1}$	[97]
								Cnidarian	*Hydra attenuata*	LC_{50} (96 h) (morphology)	29.4 $mg\,L^{-1}$	[98]
										EC_{50} (96 h) (morphology)	15.52 $mg\,L^{-1}$	[98]
										LOEC (96 h) (morphology)	5 $mg\,L^{-1}$	[98]
										NOEC (96 h) (morphology)	1 $mg\,L^{-1}$	[98]
										EC_{50} (96 h) (feeding)	3.76 $mg\,L^{-1}$	[98]
Carbamazepine-10,11-epoxide*	–†	STP influent	Spain	SPE-GC–MS	70	300–500	[14]					
		STP effluent				<70–300						
Carbamazepine-10,11-epoxide*		STP influent	France	SPE-LC–MS	5.2	ND–27	[169]					
		STP effluent				<5.2–29						

*—Metabolite; †—Data not available; ND—Not detected; SPE—Solid Phase Extraction; GC–MS—Gas Chromatography with Mass Spectrometry Detection; HPLC–MS/MS—High Performance Liquid Chromatography with Tandem Mass Spectrometry Detection; LC–MS—Liquid Chromatography with Mass Spectrometry Detection; LC–MS/MS—Liquid Chromatography with Tandem Mass Spectrometry Detection.

4.6. Beta-blockers

Beta-blockers act by competitive inhibition of beta-adrenergic receptors, a class of receptors critical for normal functioning in the sympathetic branch of the vertebrate autonomic nervous system in vertebrates. Within the most commonly used β-blockers propranolol is a non-specific antagonist, blocking both β_1 and β_2-receptors while metoprolol and atenolol present β_1-receptors specificity [99]. Fish, like other vertebrates, possess β-receptors in the heart, liver and reproductive system [170,171] so that prolonged exposure to drugs belonging to this therapeutic class may cause deleterious effects. From a two weeks study, it was observed that exposure to 500 $\mu g\,L^{-1}$ of propranolol reduced growth rates of Japanese medaka [172]. Plasma steroid levels were altered in both male and female fish even at concentrations as low as 1 $\mu g\,L^{-1}$ propranolol. Exposure to concentrations of 0.5 and 1 $\mu g\,L^{-1}$ resulted in a decreased egg production. On the other hand, acute exposure of rainbow trout to 70.9 $\mu g\,L^{-1}$ of propranolol showed no significant reduction in its heart rate [173]. However, for concentrations of metoprolol of 1 $\mu g\,L^{-1}$, ultrastructural changes in the liver and kidney were observed as well in gills if the concentration rose above 20 $\mu g\,L^{-1}$ [97]. Fathead minnows exposed to atenolol during embryo-larval development showed NOEC and LOEC values for growth rate of 3.2 and 10 $mg\,L^{-1}$, respectively [174]. Furthermore, a reproduction study performed in adults over a 21-day exposure period demonstrated that the male fish condition index was the most sensitive endpoint with NOEC and LOEC values of 1.0 and 3.2 $mg\,L^{-1}$, respectively [174]. These data suggest that atenolol has a low chronic toxicity to fish when compared to propranolol.

As invertebrates do not possess β-receptors a different potential impact on these organisms would be expected. Accordingly, the acute toxicity of propranolol, metoprolol and nadolol was assessed on the invertebrates *H. azteca*, *D. magna*, *D. lumholtzi* and *C. dubia*. Following a 48-h exposure to propranolol, LC_{50} values of 29.8, 1.6 and 0.8 $mg\,L^{-1}$ were obtained for *H. azteca*, *D. magna* and *C. dubia* respectively [172] while acute exposure to nadolol did not affect the survival of the invertebrates [172]. Regarding metoprolol, *D. magna* and *C. dubia* exhibited LC_{50} values of 63.9 and 8.8 $mg\,L^{-1}$, respectively [172]. However, Cleuvers [175] obtained a higher EC_{50} value (438 $mg\,L^{-1}$) in an acute toxicity test performed on *D. magna*. Reproduction in invertebrates decreased following propranolol exposure with NOEC values of 1 and 125 $\mu g\,L^{-1}$ for *H. azteca* and *C. dubia* respectively [172]. Propranolol inhibited the growth of the green algae *Desmodesmus subspicatus*, showing an EC_{50} of 7.7 $mg\,L^{-1}$ [175] while atenolol almost failed to register a toxic effect (EC_{50} of 620 $mg\,L^{-1}$). Chronic exposure of *D. magna* to propranolol (9 days) resulted in a significant reduction in heart rate, fecundity and biomass with LOECs values of 55, 110 and 440 $\mu g\,L^{-1}$ respectively [176] while chronic exposure to metoprolol showed LOECs of 12.5 $mg\,L^{-1}$ (body mass) and 6.15 $mg\,L^{-1}$ (reproduction). At the highest concentrations (25 and 50 $mg\,L^{-1}$) reproduction ceased and at the highest levels, all organisms died before the end of the test. A reduced heart rate for *D. magna* was evident for a 3.2 $mg\,L^{-1}$ level of metoprolol. Chronic toxicity tests performed in algae also evidenced their sensitivity to β-blockers with NOEC values below 1 $mg\,L^{-1}$ [52].

Collectively, this data might indicate a possible environmental risk since propranolol has been detected in STP effluents [21,53,94] at concentrations from 30 to 373 $ng\,L^{-1}$ and in surface waters [21,53,92,94] at levels of $ng\,L^{-1}$ (Table 6). This pharmaceutical has also been found in hospital effluent (Spain) at concentrations that can reach 6.5 $\mu g\,L^{-1}$ [73]. Other β-blockers such as atenolol, metoprolol and solatol have also been detected in environmental samples [16,21,24,73,118] including groundwater [26] at concentrations up to 122 $\mu g\,L^{-1}$.

Table 6
Examples of concentrations (ng L^{-1}) of β-blockers agents measured in different aquatic environments.

Compound	CAS number	Sample	Country	Analytical procedure	LOD (ng L^{-1})	Concentration reported (ng L^{-1})	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
Acebutolol	37517-30-9	STP influent	Finland	SPE-HPLC–MS/MS	0.8	390–510	[16]					
		STP effluent				80–230						
		Vantaa river water				<0.8–8						
		Luhtajoki river water				8						
Atenolol	29122-68-7	STP influent	Finland	SPE-HPLC–MS/MS	11.8	510–800	[16]	Crustacean	*T. platyurus*	LC_{50} (24 h) (mortality)	>100 mg L^{-1}	[78]
		STP effluent				40–440						
		Vantaa river water				<11.8–25						
		Luhtajoki river water				<11.8						
Atenolol		STP influent	Sweden	SPE-LC–MS/MS	—†	30	[21]	Fish	*O. latipes*	LC_{50} (96 h) (mortality)	>100 mg L^{-1}	[78]
		STP effluent				160						
		Höje river water				10–60						
Atenolol		Po river water	Italy	SPE-HPLC–MS/MS	0.3 (LOQ)	3.44–39.43	[24]	Algae	*D. subspicatus*	EC_{50} (growth inhibition)	620 mg L^{-1}	[17]5
		Lambro river water				241						
Atenolol		Drinking water	USA	SPE-LC–MS/MS	0.25	0.47	[32]	Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	313 mg L^{-1}	[175]
Atenolol		Hospital effluent	Spain	SPE-HPLC–MS/MS	28	100–122,000	[73]	Fish	*P. promelas*	NOEC (28 d) (growth)	3.2 mg L^{-1}	[174]
Atenolol		Mankyung river water	South Korea	SPE-LC–MS/MS	30	ND–690 (±26)	[92]			LOEC (28 d) (growth)	10 mg L^{-1}	[174]
Atenolol		STP influent	Taiwan	SPE-HPLC–MS/MS	—†	738–2883	[87]			NOEC (21 d) (condition index)	1.0 mg L^{-1}	[174]
		STP effluent				210–681						
Atenolol		STP effluent	Italy	SPE-HPLC–MS/MS	1.07 (LOQ)	27–1168	[118]			LOEC (21 d) (condition index)	3.2 mg L^{-1}	[174]
										NOEC (21 d) (reproduction)	10 mg L^{-1}	[174]
										LOEC (21 d) (reproduction)	>10 mg L^{-1}	[174]
Metoprolol	83-43-2	STP influent	Finland	SPE-HPLC–MS/MS	3.8	980–1350	[16]	Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	>100 mg L^{-1}	[65]
		STP effluent				910–1070						
		Vantaa river water				<3.8–116						
		Luhtajoki river water				38						
Metoprolol		STP influent	Sweden	SPE-LC–MS/MS	—†	160	[21]	Algae	*D. subspicatus*	EC_{50} (growth inhibition)	7.3 mg L^{-1}	[65]
		STP effluent				190						
		Höje river water				30–70						
Metoprolol		STP influent	Taiwan	SPE-HPLC–MS/MS	—†	14–597	[87]	Duckweed	*L. minor*	EC_{50} (7 d) (growth inhibition)	>320 mg L^{-1}	[65]

Table 6 (*Continued*)

Compound	CAS number	Sample	Country	Analytical procedure	LOD (ng L^{-1})	Concentration reported (ng L^{-1})	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
		STP effluent				12–199						
								Fish	*O. mykiss*	LOEC (21 d) (liver cytopathology)	1 μg L^{-1}	[97]
										LOEC (21 d) (gills cytopathology)	20 μg L^{-1}	[97]
								Crustacean	*H. azteca*	LC_{50} (48 h) (mortality)	>100 mg L^{-1}	[172]
									C. dudia	LC_{50} (48 h) (mortality)	8.8 mg L^{-1}	[172]
									D. magna	LC_{50} (48 h) (mortality)	63.9 mg L^{-1}	[172]
								Fish	*O. latipes*	LC_{50} (48 h) (mortality)	>100 mg L^{-1}	[172]
								Algae	*D. subspicatus*	EC_{50} (48 h) (growth inhibition)	7.9 mg L^{-1}	[177]
								Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	438 mg L^{-1}	[175]
								Crustacean	*D. magna*	NOEC (9 d) (body mass)	6.15 mg L^{-1}	[176]
										LOEC (9 d) (body mass)	12.5 mg L^{-1}	[176]
										LOEC (9 d) (reproduction)	6.15 mg L^{-1}	[176]
										LOEC (9 d) (heart rate)	3.2 mg L^{-1}	[176]
Propranolol	525-66-6	STP influent	Sweden	SPE-LC–MS/MS	—†	50	[21]	Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	7.5 mg L^{-1}	[65]
		STP effluent				30						
		Höje river water				<1–10						
Propranolol		Hospital effluent	Taiwan	SPE-HPLC–MS/MS	0.5	54	[47]	Algae	*D. subspicatus*	EC_{50} (growth inhibition)	5.8 mg L^{-1}	[65]
		Pharmaceutical production facility effluent				ND						
Propranolol		STP influent	United Kingdom	SPE-HPLC–MS/MS	10	60–119	[53]	Duckweed	*L. minor*	EC_{50} (7 d) (growth inhibition)	114 mg L^{-1}	[65]
		STP effluent				195–373						
		Tyne river water				35–107						
Propranolol		Hospital effluent	Spain	SPE-HPLC–MS/MS	8	200–6500	[73]	Crustacean	*T. platyurus*	LC_{50} (24 h) (mortality)	10.31 mg L^{-1}	[78]
Propranolol		Mankyung river water	South Korea	SPE-LC–MS/MS	10	ND–40.1 (±3)	[92]	Fish	*O. latipes*	LC_{50} (96 h) (mortality)	11.40 mg L^{-1}	[78]
Propranolol		STP effluent	United Kingdom	SPE-HPLC–MS/MS		130–180	[94]	Crustacean	*H. azteca*	LC_{50} (48 h) (mortality)	29.8 mg L^{-1}	[172]
		Surface water				<10–37						
										NOEC (27 d) (reproduction)	0.001 mg L^{-1}	[172]

											LOEC (27 d) (reproduction)	0.1 mg L^{-1} [172]
									C. dudia	LC_{50} (48 h) (mortality)	0.8 mg L^{-1} [172]
										NOEC (7 d) (reproduction)	0.125 mg L^{-1} [172]
										LOEC (7 d) (reproduction)	0.25 mg L^{-1} [172]
									D. magna	LC_{50} (48 h) (mortality)	1.6 mg L^{-1} [172]
								Fish	*O. latipes*	LC_{50} (48 h) (mortality)	24.3 mg L^{-1} [172]
								Algae	*D. subspicatus*	EC_{50} (48 h) (growth inhibition)	0.7 mg L^{-1} [175]
								Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	7.7 mg L^{-1} [175]
								Duckweed	*L. minor*	EC_{50} (growth rate)	113 mg L^{-1} [175]
								Crustacean	*D. magna*	NOEC (9 d) (body mass)	0.22 mg L^{-1} [176]
										LOEC (9 d) (body mass)	0.44 mg L^{-1} [176]
										NOEC (9 d) (reproduction)	0.055 mg L^{-1} [176]
										LOEC (9 d) (reproduction)	0.11 mg L^{-1} [176]
										LOEC (9 d) (heart rate)	0.055 mg L^{-1} [176]
Sotalol	959-24-0	STP influent	Finland	SPE-HPLC–MS/MS	3.9	640–830	[16]					
		STP effluent				160–300						
		Vantaa river water				<3.9–52						
		Luhtajoki river water				37						
Sotalol		Groundwater	Germany	SPE-HPLC–MS/MS	8.0	560	[26]					

†—Data not available; ND—Not Detected; SPE—Solid Phase Extraction; HPLC–MS/MS—High Performance Liquid Chromatography with Tandem Mass Spectrometry Detection; LC–MS/MS—Liquid Chromatography with Tandem Mass Spectrometry Detection.

4.7. Antidepressants

Serotonin (or 5-hydroxytryptamine) is an important neurotransmitter in hormonal and neuronal mechanisms. It participates in different regulatory and endocrine functions so that altered levels may cause changes in appetite, immune system, reproduction and other behavioural functions [10,35]. It is also important to lower vertebrates and invertebrates though being associated with different physiological mechanisms from those observed for mammals. In therapeutics, the selective serotonin reuptake inhibitors (SSRIs) fluoxetine, fluvoxamine, paroxetine and sertraline are the most widely used synthetic antidepressants. They act by inhibiting the reuptake of serotonin from the pre-synaptic nerve cleft. It is thus obvious that from the presence of SSRIs in the environment (even at low concentrations (ng or $\mu g\,L^{-1}$)), adverse effects on aquatic organisms could arise [177]. In fact, fluvoxamine at a concentration of $0.32\,\mu g\,L^{-1}$ or fluoxetine at higher concentrations were capable of inducing spawning and oocyte maturation of zebra mussels (*Dreissena polymorpha*) [178]. On the contrary, a NOEC value of $0.47\,\mu g\,L^{-1}$ was deduced for the ability of fluoxetine to reduce reproduction of the freshwater mudsnail *Potamopyrgus antipodarum* [179]. Japanese medaka were exposed to a range of fluoxetine from 0.1 to $5\,\mu g\,L^{-1}$ over four weeks, revealing that fecundity, egg fertilization and hatching success were unaffected. However, an increase in developmental abnormalities in fish embryos was observed and plasma estradiol concentrations were significantly raised in females [180]. Following an one-week exposure of western mosquitofish (*Gambusia affinis*) neonates to fluoxetine, a LC_{50} value of $546\,\mu g\,L^{-1}$ was obtained [181]. Although chronic exposure to concentrations from 0.05 to $5\,\mu g\,L^{-1}$ increased lethargy, it did not affect survival, growth or sex ratio [181]. In turn, *G. affinis* exposed to $71\,\mu g\,L^{-1}$ of fluoxetine from juvenile through adult life stages showed a delay in the development of mature sexual morphology in both male and female fish [181].

Another SSRI, sertraline, exhibits highly toxic properties. Following a 96-h exposure of rainbow trout to sertraline, a LC_{50} of $0.38\,mg\,L^{-1}$ was deduced [182]. The same authors also found that those surviving fish exposed to $0.32\,mg\,L^{-1}$ of sertraline for 72 h, died following irreparable physiological damage after being removed to control water. Fish exposed to higher concentrations of this pharmaceutical showed a decreased respiration and a loss of movement coordination.

SSRIs were also tested on algae by evaluating the growth inhibition induced. Chronic toxicity tests proved that the organisms were sensitive with NOEC values below $1\,mg\,L^{-1}$ [52]. *C. vulgaris* was shown to be the least sensitive species for all SSRIs tested [183]. On the contrary, *Pseudokirchneriella subcapitata* was the most sensitive species mainly regarding fluoxetine with a reported EC_{50} of $24\,\mu g\,L^{-1}$ after 48 h [177,184] or $45\,\mu g\,L^{-1}$ when the exposure time was increased to 96 h [183]. Cell deformities in these green algae were noticed with just $13.6\,\mu g\,L^{-1}$ of fluoxetine. Similar EC_{50} values were determined for acute toxic effects caused by sertraline on *P. subcapitata* and *Scenedesmus acutus* (12.1 and $99\,\mu g\,L^{-1}$ respectively) [183]. By reducing the exposure time from 96 to 72 h, *P. subcapitata* showed an EC_{50} of $0.14\,mg\,L^{-1}$ [182]. Fluvoxamine gave rise to the highest EC_{50} values for all algae species tested (3563–10,208 $\mu g\,L^{-1}$) [183]. An exposure of 96 h of the marine phytoplankton *D. tertiolecta* to fluoxetine showed an EC_{50} of $169.81\,\mu g\,L^{-1}$ [70], which is higher than growth rate EC_{50}s reported previously to algae species.

Tests performed on the invertebrates *C. dubia*, *D. magna* and on fathead minnow fish showed LC_{50} values of 234, 820 and $705\,\mu g\,L^{-1}$ respectively, after 48 h of exposure to fluoxetine [184]. On the other hand, for paroxetine, *D. magna* showed an EC_{50} of $2.5\,mg\,L^{-1}$ [185]. Regarding the invertebrates, fluoxetine may cause a stimulation of reproduction as is the case of *C. dubia* when exposed to $56\,\mu g\,L^{-1}$ of this pharmaceutical [184]. This same effect was also found for *D. magna* after 30 days of exposure to a concentration of $36\,\mu g\,L^{-1}$ [116] which resulted in an increase in total number of offspring produced. Higher concentrations of fluoxetine were tested (e.g. $223\,\mu g\,L^{-1}$) and proven to exert the opposite effect [184] in a similar way to that observed for sertraline, exhibiting an EC_{50} of $0.066\,mg\,L^{-1}$ and a LOEC of $0.1\,mg\,L^{-1}$ [182]. A multigenerational study was performed by exposing *D. magna* and their newborns to fluoxetine [33]. The highest effects were found on the development of the embryos. The newborns length was affected (NOEC = $8.9\,\mu g\,L^{-1}$ and LOEC = $31\,\mu g\,L^{-1}$), what had consequences in their future reproduction, that was significantly reduced for a concentration of $31\,\mu g\,L^{-1}$ [33]. The exposure of the invertebrate *P. antipodarum* to fluoxetine caused a decrease in reproduction, resulting in a NOEC of $13\,\mu g\,L^{-1}$ and a LOEC of $69\,\mu g\,L^{-1}$ [33]. In contrast, *H. azteca* reproduction was not affected by this SSRI, but a significant effect on growth was noticed, showing a NOEC and a LOEC of 33 and $100\,\mu g\,L^{-1}$, respectively [33].

The behaviour of aquatic invertebrates was also shown to be affected by SSRIs as illustrated by the amphipod *G. pulex* in the presence of 10 and $100\,ng\,L^{-1}$ of fluoxetine [76]. Fairy shrimps *T. platyurus* are more sensitive to sertraline compared to *D. magna*. For the former an EC_{50} of $0.6\,mg\,L^{-1}$ after 24 h was obtained and with *D. magna* corresponding EC_{50} values were 3.1 and $1.3\,mg\,L^{-1}$ after 24 and 48 h, respectively [182]. Nematoceran flies *Chironomus tentans* and hydras *H. azteca* were exposed to fluoxetine by sediments, showing growth inhibition with LOECs of 1.3 and $5.6\,mg\,kg^{-1}$ respectively [184]. However, hydras reproduction was stimulated for all concentrations tested ($1.4–22.4\,mg\,kg^{-1}$) as well as blackworms *Lumbriculus variegatus* when exposed to 0.94 and $2.34\,mg\,kg^{-1}$ of fluoxetine [179]. In *C. tentans*, this kind of exposure caused a reduction in emergence with a LOEC of $1.12\,mg\,kg^{-1}$. On the other hand, Péry et al. [33] did not observed toxic effects on *C. riparius* growth, emergence and reproduction, even when exposed to $59.5\,mg\,kg^{-1}$ of fluoxetine.

SSRIs contaminate different aquatic environments at concentrations in the order of $ng\,L^{-1}$ (Table 7). Fluoxetine is a typical example, being detected in STP influents at concentrations of 0.4–18.7 $ng\,L^{-1}$ and in effluents in the lower range of 0.12–8.4 $ng\,L^{-1}$ [186–188]. This pharmaceutical was also detected in surface waters [23,188], groundwaters [28] and drinking water [32]. Other SSRIs, such as fluvoxamine, sertraline and paroxetine have also been detected in STP influents and effluents [186–188] as well as seawater (Norway) [187]. Antidepressants were detected at low concentrations ($ng\,L^{-1}$) which may not represent isolated threats to non-target organisms when considering the respective contribution. However, since they exert similar effects and are present in the environment as a mixture, it is possible that chronic exposure of aquatic organisms may induce toxicity.

4.8. Antineoplasics

Antineoplasic drugs are designed to kill cells that are proliferating excessively such as those found in pathological cancer conditions. Therefore, a similar effect on any other growing eukaryotic organisms is expected [189]. Pharmaceuticals belonging to this therapeutic class possess genotoxic, mutagenic, carcinogenic, teratogenic and fetotoxic properties and can constitute (in their native form) from 14 to 53% of the administered drug excreted in urine [108]. Cyclophosphamide and ifosfamide ecotoxicity predicted by ECOSAR have yielded EC_{50} values of 8.2 and $70\,mg\,L^{-1}$ for algae and fish respectively, whereas the freshwater flea *D. magna* registered a LC_{50} of $1795\,mg\,L^{-1}$ [108]. Toxicity tests performed on the algae *P. subcapitata* and the invertebrate *D. magna* showed that cyclophosphamide slightly increased the growth of the former (NOEC above $100\,mg\,L^{-1}$) and reduced offspring number in the lat-

Table 7
Examples of concentrations ($ng\,L^{-1}$) of antidepressants measured in different aquatic environments.

Compound	CAS number	Sample	Country	Analytical procedure	LOD ($ng\,L^{-1}$)	Concentration reported ($ng\,L^{-1}$)	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
Amitriptyline	—†	STP influent	Canada	SPE-LC–MS/MS	0.077	17.6 (±0.8)–20.8 (±1.2)	[188]					
		STP effluent				15.6 (±0.8)–21.0 (±1.5)						
		St. Lawrence River water				0.87 (±0.07)–3.7 (±0.2)						
Nortriptyline*	—†	STP influent	Canada	SPE-LC–MS/MS	0.057	3.1 (±0.1)–4.5 (±0.4)	[188]					
		STP effluent				1.5 (±0.1)–3.8 (±0.4)						
		St. Lawrence River water				0.41 (±0.02)–0.73 (±0.06)						
Citalopram	59729-33-8	STP influent	Norway	SPE-HPLC–MS	0.16	13.0–612	[186]					
		STP effluent				9.2–382						
Citalopram		STP influent	Norway	HF-LPME-HPLC–MS	0.017	62.9 (±30.7)–303.6 (±4.3)	[187]					
		STP effluent				21.9 (±13.5)–238.4 (±23.6)						
Citalopram		STP influent	Canada	SPE-LC–MS/MS	0.077	52.2 (±3.7)–52.7 (±4.9)	[188]					
		STP effluent				46.8 (±1.2)–57.8 (±0.3)						
		St. Lawrence River water				3.4 (±0.2)–11.5 (±0.8)						
Fluoxetine	54910-89-3	Surface water	USA	SPE-LC–MS	18	12	[23]	Amphipod	*H. azteca*	LOEC (28 d) (growth)	$100\,\mu g\,L^{-1}$	[33]
Fluoxetine		Groundwater	USA	SPE-HPLC–MS	18	56	[28]			NOEC (28 d) (growth)	$33\,\mu g\,L^{-1}$	[33]
Fluoxetine		Drinking water	USA	SPE-LC–MS/MS	0.50	0.64	[32]	Crustacean	*D. magna*	NOEC (21 d) (newbornes lenght)	$8.9\,\mu g\,L^{-1}$	[33]
Fluoxetine		STP effluent	South Korea	SPE-LC–MS/MS	1.0	1.7	[90]			LOEC (21 d) (newbornes lenght)	$31\,\mu g\,L^{-1}$	[33]
		Surface water				ND						
Fluoxetine		STP influent	Norway	SPE-HPLC–MS	0.12	0.4–2.4	[186]	Freshwater snail	*P. antipodarum*	NOEC (reproduction)	$13\,\mu g\,L^{-1}$	[33]
		STP effluent				<0.12–1.3						
Fluoxetine		STP influent	Norway	HF-LPME-HPLC–MS	0.15	1.1 (±22.9)–18.7 (±0.9)	[187]			LOEC (reproduction)	$69\,\mu g\,L^{-1}$	[33]
		STP effluent				0.6 (±20.0)–8.4 (±22.9)						
Fluoxetine		STP influent	Canada	SPE-LC–MS/MS	0.05	3.1 (±0.3)–3.5 (±0.3)	[188]	Crustacean	*Gammarus pulex*	LOEC (behaviour)	$100\,ng\,L^{-1}$	[76]
		STP effluent				2.0 (±0.1)–3.7 (±0.1)						
		St. Lawrence River water				0.42 (±0.01)–1.3 (±0.1)						
								Algae	*Dunaliella tertiolecta*	EC_{50} (96 h) (growth inhibition)	$169.81\,\mu g\,L^{-1}$	[70]
									P. subcapitata	EC_{50} (120 h) (growth)	$24\,\mu g\,L^{-1}$	[17]7
										LOEC (growth)	$13.6\,\mu g\,L^{-1}$	[177]
								Crustacean	*C. dubia*	LC_{50} (48 h)	$234\,\mu g\,L^{-1}$	[177]
										NOEC	$56\,\mu g\,L^{-1}$	[177]
										LOEC	$112\,\mu g\,L^{-1}$	[177]

Table 7 (*Continued*)

Compound	CAS number	Sample	Country	Analytical procedure	LOD (ng L^{-1})	Concentration reported (ng L^{-1})	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
									D. magna	LC_{50} (48 h)	820 μg L^{-1}	[177]
								Fish	*P. pimelas*	LC_{50} (48 h)	705 μg L^{-1}	[177]
								Midge	*C. tentans*	LC_{50} (10 d)	15.2 mg kg^{-1}	[177]
										LOEC (10 d) (growth)	1.3 mg kg^{-1}	[177]
								Amphipod	*H. azteca*	LOEC (growth)	5.4 mg kg^{-1}	[177]
								Freshwater snail	*P. antipodarum*	EC_{10} (56 d) (n° embryos whitout shell)	0.81 μg L^{-1}	[179]
										NOEC (56 d) (n° embryos whitout shell)	0.47 μg L^{-1}	[179]
								Midge	*C. riparius*	LOEC (28 d) (emergence)	1.12 mg kg^{-1}	[179]
								Mosquitofish	*Gambusia affinis*	LC_{50} (7 d) (lethality)	546 μg L^{-1}	[181]
								Algae	*P. subscapitata*	IC_{50} (96 h) (growth inhibition)	44.99 μg L^{-1}	[183]
									S. acutus	IC_{50} (96 h) (growth inhibition)	91.23 μg L^{-1}	[183]
									S. quadricauda	IC_{50} (96 h) (growth inhibition)	212.98 μg L^{-1}	[183]
									C. vulgaris	IC_{50} (96 h) (growth inhibition)	4339.25 μg L^{-1}	[183]
								Algae	*P. subscapitata*	EC_{50} (120 h) (growth)	39 μg L^{-1}	[184]
								Crustacean	*C. dubia*	LC_{50} (48 h) (survival)	234 μg L^{-1}	[184]
									D. magna	LC_{50} (48 h) (survival)	820 μg L^{-1}	[184]
								Fish	*P. promelas*	LC_{50} (48 h) (survival)	705 μg L^{-1}	[184]
								Midge	*C. tentans*	LC_{50} (10 d) (survival)	15.2 mg kg^{-1}	[184]
										LOEC (10 d) (growth)	1.3 mg kg^{-1}	[184]
								Amphipod	*H. azteca*	LOEC (10 d) (growth)	5.6 mg kg^{-1}	[184]
Norfluoxetine*	83891-03-6	Drinking water	USA	SPE-LC–MS/MS	0.50	0.77	[32]					
Norfluoxetine*		STP influent	Norway	HF-LPME-HPLC–MS	0.16	0.7 (±13.1)–9.3 (±4.6)	[187]					
		STP effluent			0.54 (LOQ)	<0.54–2.4 (±14.5)						
Norfluoxetine*		STP influent	Canada	SPE-LC–MS/MS	0.087	1.8 (±0.3)–4.2 (±0.5)	[188]					
		STP effluent				1.7 (±0.1)–1.8 (±0.3)						
		St. Lawrence River water				1.2 (±0.1)–1.3 (±0.1)						
Fluvoxamine	54739-18-3	STP influent	Norway	SPE-HPLC–MS	0.15	0.4–3.9	[186]	Algae	*P. subscapitata*	IC_{50} (96 h) (growth inhibition)	4002.88 μg L^{-1}	[183]
		STP effluent				<0.15–0.8						
Fluvoxamine		STP influent	Norway	HF-LPME-HPLC–MS	0.129	0.8 (±38.2)–1.7 (±18.6)	[187]		*S. acutus*	IC_{50} (96 h) (growth inhibition)	3620.24 μg L^{-1}	[183]
		STP effluent			0.379 (LOQ)	<0.379–0.8 (±38.2)						
		Seawater				0.5 (±0.5)–0.8 (±0.3)						
									S. quadricauda	IC_{50} (96 h) (growth inhibition)	3563.34 μg L^{-1}	[183]
									C. vulgaris	IC_{50} (96 h) (growth inhibition)	10,208.47 μg L^{-1}	[183]

Paroxetine	61869-08-7	STP influent	Norway	SPE-HPLC–MS	0.12	0.6–12.3	[186]	Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	2.5 mg L^{-1}	[185]
		STP effluent				0.5–1.6						
Paroxetine		STP influent	Norway	HF-LPME-HPLC–MS	0.053	2.9 (±19.0)–12.9 (±29.4)	[187]					
		STP effluent				1.0 (±15.7)–11.7 (±36.8)						
		Seawater				0.6 (±0.4)–1.4 (±0.4)						
Paroxetine		STP influent	Canada	SPE-LC–MS/MS	0.096	4.6 (±0.3)–5.3 (±0.2)	[188]					
		STP effluent				4.3 (±0.2)–5.2 (±0.5)						
		St. Lawrence River water				1.3 (±0.1)–3.0 (±0.1)						
Sertraline	79617-96-2	STP influent	Norway	SPE-HPLC–MS	0.29	1.8–2.5	[186]	Bacteria	*V. fischeri*	EC_{50} (30 min) (inhibition)	10.72 mg L^{-1}	[182]
		STP effluent				0.9–2.0						
Sertraline		STP influent	Norway	HF-LPME-HPLC–MS	0.16	8.4 (±4.5)–19.8 (±10.8)	[187]			NOEC (30 min) (inhibition)	2.25 mg L^{-1}	[182]
		STP effluent			0.52 (LOQ)	3.7 (±16.3)–14.6 (±4.2)						
		Seawater				<0.52						
Sertraline		STP influent	Canada	SPE-LC–MS/MS	0.048	6.0 (±0.4)–6.1 (±0.3)	[188]			LOEC (30 min) (inhibition)	4.5 mg L^{-1}	[182]
		STP effluent				5.1 (±0.3)–5.8 (±0.8)						
		St. Lawrence River water				0.84 (±0.09)–2.4 (±0.1)						
								Algae	*P. subcapitata*	EC_{50} (72 h) (inhibition)	0.14 mg L^{-1}	[182]
										NOEC (72 h) (inhibition)	0.05 mg L^{-1}	[182]
										LOEC (72 h) (inhibition)	0.075 mg L^{-1}	[182]
								Shrimp	*T. platyurus*	LC_{50} (24 h) (lethality)	0.6 mg L^{-1}	[182]
										NOEC (24 h) (lethality)	0.4 mg L^{-1}	[182]
										LOEC (24 h) (lethality)	0.6 mg L^{-1}	[182]
								Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	1.3 mg L^{-1}	[182]
										NOEC (48 h) (immobilization)	0.10 mg L^{-1}	[182]
										LOEC (48 h) (immobilization)	0.18 mg L^{-1}	[182]
										EC_{50} (21 d) (reproduction)	0.066 mg L^{-1}	[182]
										NOEC (21 d) (reproduction)	0.032 mg L^{-1}	[182]
										LOEC (21 d) (reproduction)	0.1 mg L^{-1}	[182]
										LC_{50} (21 d) (lethality)	0.12 mg L^{-1}	[182]
										NOEC (21 d) (lethality)	0.032 mg L^{-1}	[182]
										LOEC (21 d) (lethality)	0.1 mg L^{-1}	[182]
								Fish	*O. mykiss*	LC_{50} (96 h) (lethality)	0.38 mg L^{-1}	[182]
										NOEC (96 h) (lethality)	0.1 mg L^{-1}	[182]

Table 7 (*Continued*)

Compound	CAS number	Sample	Country	Analytical procedure	LOD (ng L^{-1})	Concentration reported (ng L^{-1})	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
										NOEC (96 h) (lethality)	0.32 mg L^{-1}	[182]
								Algae	*P. subcapitata*	IC_{50} (96 h) (growth inhibition)	12.10 μg L^{-1}	[183]
									S. acutus	IC_{50} (96 h) (growth inhibition)	98.92 μg L^{-1}	[183]
									S. quadricauda	IC_{50} (96 h) (growth inhibition)	317.02 μg L^{-1}	[183]
									C. vulgaris	IC_{50} (96 h) (growth inhibition)	763.66 μg L^{-1}	[183]
Desmethylsertraline*	87857-41-8	STP influent	Canada	SPE-LC–MS/MS	0.072	4.2 (±0.6)–5.0 (±0.8)	[188]					
		STP effluent				3.6 (±0.3)–4.7 (±0.5)						
		St. Lawrence River water				2.3 (±0.1)–4.5 (±0.4)						
Venlafaxine	99300-78-4	STP influent	Canada	SPE-LC–MS/MS	0.10	195.7 (±25.3)–213.0 (±8.2)	[188]					
		STP effluent				175.9 (±12.7)–214.6 (±3.6)						
		St. Lawrence River water				12.9 (±0.1)–45.9 (±2.0)						
Desmethylvenlafaxine*	—†	STP influent	Canada	SPE-LC–MS/MS	0.097	274.3 (±26.5)–345.9 (±19.8)	[188]					
		STP effluent				222.5 (±16.8)–330.0 (±9.8)						
		St. Lawrence River water				21.0 (±0.5)–68.7 (±3.1)						

*—Metabolite; ND—Not Detected; †—Data not available; SPE—Solid Phase Extraction; HF-LPME—Hollow Fibre Supported Liquid Phase Microextraction; HPLC–MS—High Performance Liquid Chromatography with Mass Spectrometry Detection; LC–MS—Liquid Chromatography with Mass Spectrometry Detection; LC–MS/MS—Liquid Chromatography with Tandem Mass Spectrometry Detection.

Table 8
Examples of concentrations ($ng\,L^{-1}$) of antineoplasic drugs measured in different aquatic environments.

Compound	CAS number	Sample	Country	Analytical procedure	LOD ($ng\,L^{-1}$)	Concentration reported ($ng\,L^{-1}$)	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
Cyclophosphamide	50-18-0	Somes river water	Romania	SPE-GC–MS	30 (LOQ)	<30–64.8 (±8.0)	[20]	Algae	*P. subcapitata*	EC_{50} (72 h) (growth inhibition)	>100 $mg\,L^{-1}$	[190]
Cyclophosphamide		STP effluent	Italy	SPE-HPLC–MS/MS	1.9 (LOQ)	<1.9–9.0	[118]			NOEC (72 h) (growth inhibition)	>100 $mg\,L^{-1}$	[190]
Cyclophosphamide		STP influent	—†	SPE-GC–MS	6	<6–143	[192]	Crustacean	*D. magna*	EC_{50} (21 d) (reproduction)	>100 $mg\,L^{-1}$	[190]
		STP effluent				6–15						
		Hospital effluent				19–4486						
Cyclophosphamide		STP influent	Switzerland	SPE-LC–MS/MS	0.3	2.0–6	[193]			LOEC (21 d) (reproduction)	100 $mg\,L^{-1}$	[190]
		STP effluent				2.1–4						
										NOEC (21 d) (reproduction)	56 $mg\,L^{-1}$	[190]
Ifosfamide	84711-20-6	STP influent	Switzerland	SPE-LC–MS/MS	0.3	<0.3–5	[193]					
		STP effluent				1.7–6						
Methotrexate	59-05-2	STP effluent	Italy	SPE-HPLC–MS/MS	0.83 (LOQ)	<0.83–12.6	[118]	Bacteria	*V. fischeri*	EC_{50} (30 min)	1220 $mg\,L^{-1}$	[83]
								Algae	*Scenedesmus subspicatus*	EC_{50} (72 h)	260 $mg\,L^{-1}$	[83]
								Crustacean	*D. magna*	EC_{50} (immobilization)	>1000 $mg\,L^{-1}$	[83]
								Ciliates	*Tetrahymena pyriformis*	EC_{50} (48 h) (growth inhibition)	45 $mg\,L^{-1}$	[83]
								Fish	*D. rerio*	LC_{50} (48 h)	85 $mg\,L^{-1}$	[83]
Tamoxifen	74899-71-1	STP influent	United Kingdom	SPE-HPLC–MS/MS	10	143–215	[53]	Rotifer	*B. calyciflorus*	LC_{50} (24 h) (mortality)	0.97 $mg\,L^{-1}$	[191]
		STP effluent				146–369						
		Tyne river water				27–212						
Tamoxifen		STP effluent	United Kingdom	SPE-HPLC–MS/MS	10	<10	[94]			EC_{50} (48 h) (population growth inhibition)	0.25 $mg\,L^{-1}$	[191]
		Surface water				<10						
Tamoxifen		STP influent	United Kingdom	SPE-LC–MS/MS	0.003	0.2–15	[194]	Crustacean	*T. platyurus*	LC_{50} (24 h) (mortality)	0.40 $mg\,L^{-1}$	[191]
		STP effluent				0.2–0.7						
									D. magna	EC_{50} (24 h) (immobilization)	1.53 $mg\,L^{-1}$	[191]
									C. dubia	EC_{50} (7 d) (population growth inhibition)	8.1×10^{-4} $mg\,L^{-1}$	[191]

†—Data not available; SPE—Solid Phase Extraction; GC–MS—Gas Chromatography with Mass Spectrometry Detection; HPLC–MS/MS—High Performance Liquid Chromatography with Tandem Mass Spectrometry Detection; LC–MS/MS—Liquid Chromatography with Tandem Mass Spectrometry Detection.

ter at all tested concentrations of the drug (10–100 mg L^{-1}), with a NOEC of 56 mg L^{-1} [190]. Methotrexate revealed teratogenicity for fish embryos with an EC_{50} of 85 mg L^{-1} after 48 h of exposure [83] and acute effects in the ciliate *Tetrahymena pyriformis* with an EC_{50} for 48 h of 45 mg L^{-1} [83]. Acute and chronic toxicity of tamoxifen and its photoproducts was studied by DellaGreca et al. [191], showing that both the active pharmaceutical and its photoproducts affected the rotifer *B. calyciflorus* and crustacean *T. platyurus* with LC_{50} values ranging from 0.95 to 1.31 mg L^{-1} and 0.40 to 1.59 mg L^{-1} respectively. In chronic toxicity tests, *C. dubia* proved the most sensitive organism. An EC_{50} value of 0.81 μg L^{-1} for tamoxifen and EC_{50} values ranging from 0.41 to 2.8 μg L^{-1} for its photoproducts, relative to population growth inhibition, were found after a 7-day trial [191].

The antineoplasic drug cyclophosphamide has been detected in hospital effluents at concentrations ranging from 19 ng L^{-1} to 4.5 μg L^{-1} [192], in STP influents [192,193] and effluents [118,192,193] and in surface waters [20] (Table 8). Other antineoplasic pharmaceuticals detected to date have been in the order of ng L^{-1}. However, as chronic toxicity data is very sparse, further studies are required to elucidate the potential effect of life-cycle exposure to these compounds in aquatic organisms.

4.9. X-ray contrast media

Contrast media are used as diagnostic tools for capturing detailed X-ray images of soft tissues. Iodinated X-ray contrast media are highly hydrophilic substances that are widely used and eliminated almost non-metabolised. STP removal processes are usually ineffective and for this reason they persist for a long time in the environment. As X-ray contrast media do not show biological activity, their presence might not represent a threat to public health [35,195,196]. Toxicity tests have shown that iopromide or its main metabolite do not have a toxic effect in luminescent bacteria, algae (*Scenedesmus subspicatus*), daphnids or fish (*D. rerio*, *Leuciscus idus*) even at concentrations as high as 1 g L^{-1} [196,197]. Contamination by X-ray contrast media has been reported in different aquatic environments (Table 9). Media have been detected in STP influents and effluents [198–201], surface waters [199,201–203], groundwaters [26,199,200] and even drinking water [200,202,203] at concentrations that can reach few μg L^{-1}. Although accepting that X-ray contrast media do not exhibit toxic effects at high concentration levels, additional studies should be undertaken with a view to evaluating chronic effects, due to continuous exposure of aquatic organisms to these pharmaceuticals.

4.10. Mixture effects

Presently environmental risk assessment of pharmaceuticals is based on single compounds ecotoxicity studies. However, pharmaceuticals do not occur alone in the environment, but as a mixture of different active substances, their metabolites and transformation products [23,205,206]. Ecotoxicological data showed that mixtures might have different effects than single compounds [65,70,207], but in general knowledge about the toxicity of the mixture of active substances is still sparse. There are some examples of toxicity studies in literature showing that mixture of pharmaceuticals at environmentally relevant concentrations may exhibit additive effects [70]. In some cases, lower levels than expected may lead to toxic effects when in the presence of a mixture of active substances [70]. For instance, Cleuvers [65] showed that a mixture of diclofenac and ibuprofen had a stronger toxicity than predicted in *D. magna*, and when the author added more two NSAIDs (naproxen and acetylsalicylic acid) to the last two, a considerable toxicity on *Daphnia* was also reported, even at concentrations at which the single NSAIDs do not exhibit effects [95]. The exposure of the cnidarian *H. attenuata* to a mixture of eleven pharmaceuticals, belonging to different therapeutic classes, showed also sub-lethal effects for environmentally relevant concentrations (μg–ng L^{-1}) [207]. Acute exposure of *D. magna* to a mixture of 36 μg L^{-1} of fluoxetine and 100 μg L^{-1} of clofibric acid caused a significant mortality and malformation, while there are no apparent effects for the same concentrations of individual pharmaceuticals [116]. The mixture of trimethoprim with sulfamethoxazole and sulfadiazine increased significantly the growth inhibition of the algae *S. capricornutum* [131]. On the other hand, the exposure of *H. azteca* to a mixture of seven pharmaceuticals did not reveal significant effects on their survival, mating, body size or reproduction, but there was a slight increase in the number of males [208]. Identical results were observed for fish. Apparently, a life-cycle exposure of fathead minnows to a mixture of six pharmaceuticals, in the order of ng L^{-1}, did not affect their survival, growth or egg production, however it increased the number of deformities in their offspring [209]. The examples here highlighted showed that the simultaneous presence of several pharmaceuticals in the environment might result in a greater toxicity to non-target organisms than the predicted one for individual active substances. However, more ecotoxicological studies should be done to evaluate the impact of different mixtures of pharmaceuticals in non-target organisms, once that most of the published studies are focused on mixture of NSAIDs, antibiotics and blood lipid lowering agents.

5. Pharmaceuticals and legislation: what does legislation say?

Every day an increasing number of pharmaceuticals reach the environment all over the world. However, there is a gap in legislation regarding environmental contamination by pharmaceuticals. This probably arises because available data is insufficient to quantify a precise contamination profile. Abundant conclusive studies concerning chronic toxicity are also lacking so that it becomes impossible to infer the risks of long-term exposure of pharmaceuticals and their metabolites on fauna and flora. In this section, EU and US laws concerning the necessity of environmental risk assessment studies to obtain a marketing authorisation for pharmaceuticals is approached.

The European Union Directive 92/18/EEC [210] introduced for the first time, the requirement for an environmental risk assessment, as a prerequisite to obtain marketing authorization for veterinary pharmaceuticals. For this purpose, the European Agency for the Evaluation of Medicinal Products (EMEA) published a "Note for Guidance" [211] where guidelines to assess the environmental risk of veterinary medicinal products are established. The European Commission extended its concerns to pharmaceuticals for human use by publishing Directive 2001/83/EC which was subsequently amended by Directive 2004/27/EC [212]. These directives established that marketing authorization for new medical products for human use should be accompanied by an environmental risk assessment, whose guidelines were set out by EMEA [213]. Nevertheless, the environmental impact does not provide sufficient grounds for a refusal. Environmental risk assessment of both veterinary and human pharmaceuticals is assessed in a step-wise approach, divided into two phases. In Phase I, environmental exposure to the pharmaceutical or its metabolites is estimated. Phase II comprises its fate and effects in the environment. For this reason, Phase II is sub-divided into two parts: Tier A, in which possible fate and effects of the pharmaceutical and/or its major metabolites are evaluated; and Tier B, which focuses on the effects on fauna and flora within environmental compartments that are likely to be affected [211,213]. However, medicinal products for human use

Table 9
Examples of concentrations (ng L^{-1}) of X-ray contrast media measured in different aquatic environments.

Compound	CAS number	Sample	Country	Analytical procedure	LOD (ng L^{-1})	Concentration reported (ng L^{-1})	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
Diatrizoate	131-49-7	STP effluent	Germany	SPE-LC–MS/MS	50 (LOQ STP effluent)	250	[199]					
		Surface water			10 (LOQ surface water; groundwater)	<10–8700						
		Groundwater				30						
Diatrizoate		Surface water	Germany	SPE-HPLC–MS	—†	2000	[203]					
		Drinking water				1200						
Diatrizoate		STP effluent	Germany	SPE-LC–MS/MS	50 (LOQ STP effluenT)	1140	[204]					
		Rhine river water			10 (LOQ surface and drinking water)	110–140						
		Drinking water				60						
Iohexol	66108-95-0	STP influent	Australia	DI-LC–MS/MS	800	2800–4760	[201]					
		STP effluent				<800						
Iohexol		Danube river water	Germany	SPE-HPLC–MS/MS	40	40–86	[202]					
Iomeprol	78649-41-9	STP effluent	Germany	SPE-LC–MS/MS	50 (LOQ STP effluent)	370	[199]					
		Surface water			10 (LOQ surface water)	10–890						
Iomeprol		STP influent	Australia	DI-LC–MS/MS	730	<730	[201]					
		STP effluent				<730						
Iomeprol		Danube river water	Germany	SPE-HPLC–MS/MS	40	100–160	[202]					
Iopamidol	60166-93-0	Groundwater	Germany	SPE-HPLC–MS/MS	14	300	[26]					
Iopamidol		STP effluent	Germany	SPE-LC–MS/MS	50 (LOQ STP effluent)	660	[199]					
		Surface water			10 (LOQ surface water; groundwater)	170–2800						
		Groundwater				160						
Iopamidol		STP influent	Australia	DI-LC–MS/MS	220	400–620	[201]					
		STP effluent				<220						
Iopamidol		Danube river water	Germany	SPE-HPLC–MS/MS	40	210	[202]					
Iopamidol		STP effluent	Germany	SPE-LC–MS/MS	50 (LOQ STP effluent)	590	[204]					
		Rhine river water			10 (LOQ surface and drinking water)	180–300						
		Drinking water				70						
Iopromide	73334-07-3	STP effluent	South Korea	SPE-LC–MS/MS	1.0	1170–4030	[90]	Crustacean	*D. magna*	EC_{50} (48 h) (immobilization)	>1 g L^{-1}	[15]7
		Surface water				20–361						
		Drinking water				<1.0						
Iopromide		STP influent	Spain	SPE-LC–MS/MS	6.7	6600	[198]	Fish	*D. rerio*	NOEC (28 d) (hatchability, post-hatch survival, body length, weight)	>100 mg L^{-1}	[157]
		STP effluent				9300						
Iopromide		STP effluent	Germany	SPE-LC–MS/MS	50 (LOQ STP effluent)	4400	[199]	Bacteria	*V. fischeri*	EC_{50} (30 min) (luminescence)	>10.0 g L^{-1}	[158]
		Surface water			10 (LOQ surface water; groundwater)	11–910						
		Groundwater				<10						
Iopromide		STP influent	USA	SPE-LC–MS/MS	0.577	ND–17	[200]		*P. putida*	EC_{10} (16 h) (growth inhibition)	>10.0 g L^{-1}	[158]
		STP effluent				4.6						
		Ohio river water				2.2						
		Drinking water				4.6						

Table 9 (*Continued*)

Compound	CAS number	Sample	Country	Analytical procedure	LOD ($ng\,L^{-1}$)	Concentration reported ($ng\,L^{-1}$)	Ref.	Taxon	Species	Toxicological endpoint	Ecotoxicity data	Ref.
Iopromide		Groundwater	Australia	SPE-LC–MS/MS	0.577	168	[200]	Algae	*S. subspicatus*	EC_{50} (72 h) (growth inhibition)	>10.0 $g\,L^{-1}$	[158]
Iopromide		STP effluent	South Korea	SPE-LC–MS/MS	0.577	152–2670	[200]	Crustacean	*D. magna*	EC_{50} (24 h) (immobilization)	>10.0 $g\,L^{-1}$	[158]
Iopromide		STP influent	Australia	DI-LC–MS/MS	200	430–1350	[201]					
		STP effluent				<200						
Iopromide		Danube river water	Germany	SPE-HPLC–MS/MS	40	76–100	[202]					
Iopromide		Surface water	Germany	SPE-HPLC–MS	50	1600	[203]					
		Drinking water				<50						
Iopromide		STP effluent	Germany	SPE-LC–MS/MS	50 (LOQ STP effluenT)	3070	[204]			EC_{50} (22 d) (reproduction)	>1.0 $g\,L^{-1}$	[158]
		Rhine river water			10 (LOQ surface and drinking water)	150						
		Drinking water				40						
								Fish	*D. rerio*	LC_{50} (96 h) (mortality)	>10.0 $g\,L^{-1}$	[158]
									L. idus	LC_{50} (48 h) (mortality)	>10.0 $g\,L^{-1}$	[158]

†—Data not available; ND—Not detected; DI-LC–MS/MS—Direct Injection Liquid Chromatography-Tandem Mass Spectrometry; SPE—Solid Phase Extraction; HPLC–MS—High Performance Liquid Chromatography with Mass Spectrometry Detection; HPLC–MS/MS—High Performance Liquid Chromatography with Tandem Mass Spectrometry Detection; LC–MS/MS—Liquid Chromatography with Tandem Mass Spectrometry Detection.

only require Phase II studies if the predicted environmental concentration in surface water is equal to or above 0.01 $\mu g\,L^{-1}$ [213].

In the US, issues concerning pharmaceuticals in the environment are regulated by the U.S. Food and Drug Administration (FDA). This institution requires environmental assessments to obtain marketing authorisations which are specified in the "Guidance for Industry—Environmental Assessment of Human Drug and Biologic Applications" [214]. However, an environmental assessment is required only if the estimated environmental concentration of the pharmaceutical at the point of the entry is above 1 $\mu g\,L^{-1}$ [214]. As EMEA, the FDA also requires environmental assessments for veterinary medicinal products, using a tiered approach. With a view to harmonising the guidelines that govern these environmental impact assessments, the EU, US and Japan elaborated two guidelines: "Environmental Impact Assessment (EIAs) for Veterinary Medicinal Products (VMPs)—Phase I" [215] and "Environmental Impact Assessment for Veterinary Medicinal Products—Phase II Guidance" [216] so that environmental fate and toxicity data obtained could be used to obtain marketing authorisation in all these regions.

6. Conclusions

Today, the presence of pharmaceuticals in the environment is being reported worldwide. Furthermore, new data on the sources, fate and effects of pharmaceuticals in the environment, seems to indicate the possibility of a negative impact on different ecosystems and imply a threat to public health. For this assumption, data from acute and chronic ecotoxicity tests on species belonging to different trophic levels such as bacteria, algae, crustaceans and fish among others, is relevant to illustrate the several adverse effects that environmental exposure to measured concentrations of these contaminants can have. On literature, the principal toxicological endpoints/studies that are described are growth, survival, reproduction and immobilization of species, comparatively to transgenerational and population level studies that are still sparse. This demonstrates the lack of data relatively to long-term exposure of non-target organisms and principally how a continuous exposure, during several generations, may affect a whole population. To our knowledge, just one work followed the impact of a pharmaceutical in a fish population throughout seven years, showing how ethinylestradiol negatively affect the fish population, leaving them near of the extinction. In the near future, the evaluation of chronic toxicity effects should be set out as a priority for the scientific community since simultaneous exposure to pharmaceuticals, metabolites and transformation products of several therapeutic classes are unknown and whose probable effects on subsequent generations should be assumed. Another example of missing data is what occurs with statins. Nowadays, they are the blood lipid lowering agents most used all over the world, although toxicity data relatively to them is almost non-existent and limited to the active substances simvastatin and atorvastatin. It is also important to assess the presence of pharmaceuticals and/or their metabolites and transformation products in several environmental compartments in different countries with a view to gaining reliable knowledge of the contamination levels. Only with further available information will be easier to improve existing legislation in order to protect humans, animals and ecosystems from the threat posed by the presence of pharmaceuticals in the environment.

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