

Chapter 12

In Silico Models for Ecotoxicity of Pharmaceuticals

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Abstract

Pharmaceuticals and their active metabolites are one of the significantly emerging environmental toxicants. The major routes of entry of pharmaceuticals into the environment are industries, hospitals, or direct disposal of unwanted or expired drugs made by the patient. The most important and distinct features of pharmaceuticals are that they are deliberately designed to have an explicit mode of action and designed to exert an effect on humans and other living systems. This distinctive feature makes pharmaceuticals and their metabolites different from other chemicals, and this necessitates the evaluation of the direct effects of pharmaceuticals in various environmental compartments as well as to living systems. In this background, the alarming situation of ecotoxicity of diverse pharmaceuticals have forced government and nongovernment regulatory authorities to recommend the application of in silico methods to provide quick information about the risk assessment and fate properties of pharmaceuticals as well as their ecological and indirect human health effects. This chapter aims to offer information regarding occurrence of pharmaceuticals in the environment, their persistence, environmental fate, and toxicity as well as application of in silico methods to provide information about the basic risk management and fate prediction of pharmaceuticals in the environment. Brief ideas about toxicity endpoints, available ecotoxicity databases, and expert systems employed for rapid toxicity predictions of ecotoxicity of pharmaceuticals are also discussed.

Key words Database, Ecotoxicity, Endpoints, Expert system, In silico, Pharmaceuticals, QSAR

1 Introduction

A significant amount of pharmaceuticals and their metabolites have been found in the various environmental compartments causing damage to the environment and hazard to the living systems. Due to an increase in application of human and veterinary medicines manifold, pharmaceuticals and their metabolite residues have been found in rivers, sewage effluents, streams and in surface, ground, and potable water, creating a big concern for the ecologists [1]. The primary routes of entrance of pharmaceuticals into the environment are domestic, hospital, and industrial wastes [2]. Pharmaceuticals are excreted in urine or feces as a mixture of unchanged chemicals and metabolites and enter into the environment through septic tank and sewage systems [1]. On the other

hand, ecotoxicity data of pharmaceuticals are available in the literature for less than 1 % of the drugs, and only a small number of pharmaceuticals and their residues have been subjected to risk assessment employing ecotoxicological tests.

Pharmaceuticals are intentionally designed to have a specific mode of action and exert an effect on specific organs, tissues, cells, or biomolecules in humans, mammals, or other vertebrates, and many of them are persistent in the body [3]. As a consequence, when pharmaceuticals and their unaltered metabolites enter into the environment by different means, they can affect humans as well as other living species. There are many drugs whose specific effects or modes of action are not well known, and they often produce effects through several modes of action. These distinguished features make pharmaceuticals dissimilar from others and this is the sole reason to assess the potential acute and chronic effects of pharmaceuticals in diverse environmental compartments. It is quite apparent that the toxic effects of pharmaceuticals on diverse organisms in aquatic as well as nonaquatic environment are due to their long persistent and bio-accumulative nature [4]. In view of the serious issue of pharmaceutical toxicity to the environment, it is vital to categorize the proper source, occurrence, effects, and fate of each individual pharmaceutical product as well as to perform the risk assessment and risk management of ecotoxicological effects of the pharmaceutical chemicals and their metabolites [1, 2].

Antibiotics are one of the majorly used pharmaceuticals in human and veterinary medicines. The world consumption of antibiotics has risen radically in the last decade, also increasing the elimination of their metabolites in their original form. Most antibiotics are poorly metabolized after ingestion, probably resulting in a fraction of antibiotics from 25 to 75 % leaving the bodies in an unaltered form after consumption [5]. Additionally, a high percentage of the antibiotics added to the animal feed are excreted in urine or manure. In some cases, as much as 90 % of the antibiotic administered orally may pass through the animal unchanged and excreted in urine and manure. Thereafter, these antibiotics can enter surface and groundwater and be strongly adsorbed in soils and are not readily degradable [6]. Vidaver [7] estimates that 53,000 ha of fruit and vegetable plants are sprayed annually with antibiotics. For example, streptomycin and oxytetracycline are registered by the US Environment Protection Agency (USEPA) for use in plant agriculture. Utilization of transgenic plants to produce inexpensive antibiotics may also be a cause of environmental hazards due to the existence of crop residues, roots, and root exudates in the soil which can act as a continuous source of residual antibiotics to soil fauna and flora [8].

While pharmaceuticals and their metabolite residues are detected in rivers, streams, sewage influents and effluents, surface,

ground, and potable waters [9], it may be noted that the drinking water treatment methods reduce residues, but they are incapable of removing the contaminant pharmaceuticals absolutely. According to a nationwide study of “emerging pollutants” in waters, the US Geological Survey (USGS) tested for pharmaceuticals in 139 rivers in 30 states of the USA, detecting diverse therapeutic classes of biologically active compounds [10]. The cardiovascular drug propranolol has been reported downstream from the sewage treatment plant [11]. The antiepileptic drugs carbamazepine and clofibrate are two most persistent pharmaceuticals which have been detected in the environment [2]. Major detected drugs in rivers were beta blockers (e.g., metoprolol up to 1.54 $\mu\text{g}/\text{l}$) and beta-sympathomimetics, estrogens (e.g., 17 β -estradiol up to 0.013 $\mu\text{g}/\text{l}$) [12], analgesic and anti-inflammatory drugs (e.g., Diclofenac up to 1.2 $\mu\text{g}/\text{l}$) [13], and also antibiotics (e.g., erythromycin up to 1.7 $\mu\text{g}/\text{l}$) [12], as well as lipid-lowering agents (e.g., clofibrinic acid up to 0.2 $\mu\text{g}/\text{l}$) [14] and antiepileptic drugs (e.g., carbamazepine up to 2.1 $\mu\text{g}/\text{l}$) [13]. Presence of clofibrinic acid, propylphenazone, and diclofenac has been reported in the drinking water of Berlin in the concentration range of several hundreds of nanograms per liter [15]. Paracetamol, diclofenac, and carbamazepine were monitored in drinking water in Southern France [16], and clofibrinic acid and diazepam were detected in treated drinking water in Milan, Italy [17]. Psychoactive and illicit drugs amphetamine, cocaine and its metabolite benzoylecgonine, morphine, 6-acetylmorphine, 11-nor-9-carboxy-delta-9-tetrahydrocannabinol, methadone and its main metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine have been detected in surface and waste waters [18]. Schultz and Furlong found highest concentrations of antidepressant drugs venlafaxine, citalopram, and bupropion 1000 \pm 400 ng/l, 90 \pm 20 ng/l, and 60 \pm 40 ng/l, respectively, in samples collected downstream from a water reclamation plant [19]. The maximum determined concentration of fluoxetine was 0.099 ng/l in wastewater treatment plant (WWTP) effluents in Canada [20].

Nonprescription drugs like caffeine, cotinine, and acetaminophenone are found in samples of potable water collected near Atlanta, Georgia [21]. Tauber detected carbamazepine and gemfibrozil in drinking waters in ten cities in Canada that were examined for a 44-drug subset consisting pharmaceuticals including sulfonamides, quinolones, tetracyclines, and macrolide antibiotics [22]. Oraine and Pettigrove identified and quantified ibuprofen (0.93 $\mu\text{g}/\text{l}$) and ibuprofen methyl ester (4.95 $\mu\text{g}/\text{l}$) in finished water in alarming quantity [23]. Median concentrations of 0.02 $\mu\text{g}/\text{l}$ and 0.12 $\mu\text{g}/\text{l}$ were reported for ciprofloxacin and norfloxacin, respectively, for samples from 139 surface streams across the USA. Ciprofloxacin in the range 0.7–124.5 $\mu\text{g}/\text{l}$ was found in wastewater of a Swiss hospital [24]. Hellweger et al. [25] claimed

that environmental concentrations of tetracycline in surface waters are usually less than 0.11 mg/l, although higher values of up to 6.8 mg/l have been observed. Estrogens, a sex hormone, have been detected in plasticizers and preservatives, while 17 α -ethinylestradiol (EE2) used as a component of contraceptive pills has been identified in ground and tap water samples [26].

The presence of human and veterinary pharmaceuticals and their residues into the environment has impelled the introduction of different risk assessment guidelines in the European Union by the European Medicines Evaluation Agency (EMA) and in the USA by the Food and Drug Administration (FDA). According to the European Commission guideline [27], a medicinal product for human use must be accompanied by environmental risk assessment data. The EMA has released a guideline for the assessment of potential environmental risks in 2006 [28]. According to the US FDA guidelines for the risk assessments of human drugs, applicants have to provide an environmental assessment report when the expected concentration of the active pharmaceuticals in the aquatic environment is ≥ 1 $\mu\text{g/l}$ [29]. Additionally, the FDA Center for Drug Evaluation and Research (CDER) issued a guidance document “*Guidance for Industry for the Submission of an Environmental Assessment in Human Drug Application and Supplements*” in 1995 [30]. In case of veterinary medicines, environmental risk assessments have been required in the USA since about 1980 and Europe since 1997 [31].

The need for a practical approach in gathering data on the environmental toxic effects of pharmaceuticals has been identified by the European Union Commission’s scientific committee on toxicity, ecotoxicity, and environment (CSTEE). The four classes of special environmental feature-specific concerns, which are stereotypically not evaluated in traditional ecotoxicity testing under EU directive 1488/94 [28] are antibiotics [resistance issue], anti-neoplastics [mutagenicity], sex hormones [endocrine disruption], and cardiovascular high potential hazard. Therefore, it is acknowledged that a prioritization technique needs to be developed for environmental risk assessment of pharmaceuticals, and this should follow the general scheme for chemicals according to the REACH guidelines [27], where the implication of in silico methods specifically the quantitative structure–activity relationship (QSAR) method is stressed.

In this perspective, to make the information regarding ecotoxicity of diverse pharmaceuticals available, different government and nongovernment regulatory authorities are recommending the application of fast and economical in silico methods for prediction of the elementary physicochemical and fate properties of pharmaceuticals as well as their ecological and direct human health effects before they reach into market for usage. Computer-aided toxicity models allow for the effects of pharmaceuticals (physicochemical

properties, toxicological activity, distribution, fate, etc.) to be easily predicted. These predictions may be obtained from the knowledge of chemical structure alone, provided that the structure can be described in two or three dimensions. Employing these methods, ecotoxicity information on pharmaceuticals may be obtained without toxicity testing, and/or even before synthesis of the compound. Therefore, use of QSAR as one of the non-experimental methods is significant in order to lessen time, animal usage and cost involvement in design, development, and discovery process of drugs and/or pharmaceuticals.

There is a significant lack of knowledge about the environmental fate of a huge number of pharmaceuticals and their metabolites. On the contrary, only a limited number of in silico models have been developed so far to predict the risk of pharmaceuticals to the environment. This chapter aims to provide information regarding occurrence of pharmaceuticals and their residues in the environment, their persistence, environmental fate, and toxicity as well as application of in silico methods to predict risk and fate properties of pharmaceuticals to the environment. Concise ideas about ecotoxicity endpoints, available ecotoxicity databases and expert systems employed for rapid ecotoxicity predictions of pharmaceuticals are discussed in this chapter.

2 Ecotoxicity of Pharmaceuticals: A General Overview

2.1 Source and Entry Routes

Identification of proper sources and routes of entry of pharmaceuticals into diverse environmental compartments is the first step to get a proper view of the ecotoxicity problem due to pharmaceuticals. The most obvious and common pathways for environmental contamination of pharmaceuticals are discussed below.

- (a) *Urine and feces*: Major and most common entry routes for pharmaceuticals into the environment are via urine and feces of the patients. Not only active ingredients, but also the metabolites are excreted through the urine and feces as many drugs are metabolized into hydrophilic compounds for excretion. The risk of these metabolites is completely different from the parent drugs in majority of cases which make the risk assessment study more critical one.
- (b) *Direct exposure of diagnostic compounds*: Contrast media like diatrizoate, iohexol, iomeprol, and iopromide are used as diagnostic tools for capturing detailed X-ray images of soft tissues. Iodinated X-ray contrast media are highly hydrophilic substances which are extensively applied and eliminated without proper treatment; as a result they persist for a long time in the environment [32].

- (c) *Household disposal*: Either out-of-date or unwanted medicines are discarded through the sink/toilet or via waste collection, before being taken to landfill sites where they appear as terrestrial ecosystem contaminants. Less than 20 % users had ever been given instructions about medication dumping by a health care provider. In a study, causes for possessing unused medication were found to be due to an alteration of medication by the doctor (48.9 %), or self-discontinuation (25.8 %) [33]. The most common method of disposal was to throw unused medicines in the trash (76.5 %) or flush them down the drain (11.2 %) [33].
- (d) *Manufacturers*: According to the regulation of the Good Manufacturing Practices (GMP), the active pharmaceutical emissions during manufacturing have been thought to be insignificant. But recently it has been found that in Asian countries concentrations up to several milligrams per liter can be found in effluents for single compounds [34].
- (e) *Hospital influent and effluent*: Point sources such as hospital effluents are likely to be another significant source. There are up to 16 pharmaceuticals including antiepileptics and anti-inflammatories which were found in the hospital waste water according to a study [35]. Several studies suggested the existence of the pharmaceuticals in the effluent and influent of the sewage treatment plants and it was proved that the elimination of the pharmaceuticals is partial [35].
- (f) *Animal husbandry and veterinary medicine*: Veterinary medicines and their metabolites are also excreted through urine and feces. 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. Other sources include direct application in aqua farming, manure run-off, run-off from the application of sewage sludge and manure on farmland as fertilizers, or, finally, via landfill leaching [36].
- (g) *Aquaculture*: Sewage Treatment Plant (STP) sludge is habitually employed as fertilizer on agricultural land which is a rich source of non-suspected drugs [37]. According to the Food and Agriculture Organization (FAO), antibiotics have been utilized in aquaculture primarily for therapeutic purposes and as prophylactic agents. Antibiotics authorized for use in aquaculture are florfenicol, oxytetracycline, sarafloxacin, premix, erythromycin sulfonamides potentiated with ormethoprim, or trimethoprim [38].
- (h) *Plant agriculture*: Antibiotics are comprehensively employed to control bacterial diseases of plants. Streptomycin with oxytetracycline to a minor extent is very commonly used antibiotic in

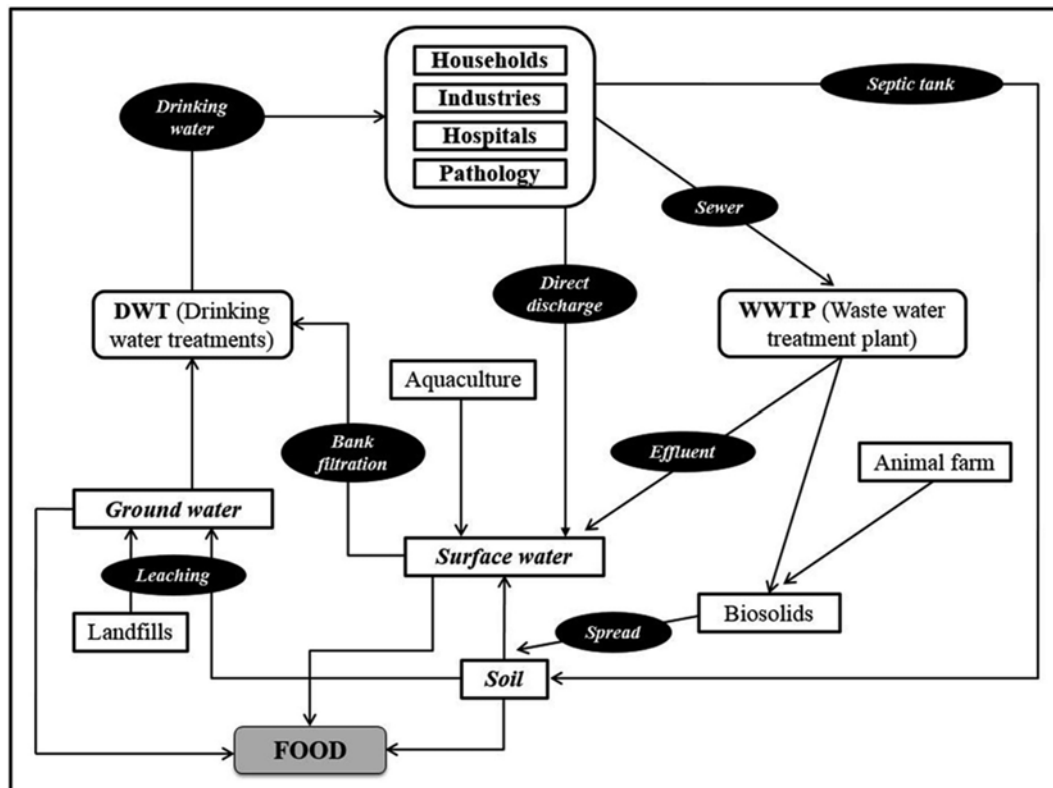


Fig. 1 Common sources, routes and fate of pharmaceuticals

plant agriculture in controlling bacterial diseases of tree fruits. Primary uses are on apple, pear, and related fruit trees for the control of fire blight caused by *Erwinia amylovora*. According to a report, antibiotics applied to plants account for less than 0.5 % of total antibiotic use in the USA [39]. In Fig. 1, we have represented different sources, routes, fate of pharmaceuticals.

2.2 Occurrence

Pharmaceuticals are among the most common personal care products in day to day life. Medicines are regularly used in human and veterinary health care, farming, and aquaculture in the modern era. Country specific consumption for groups of drugs in defined daily doses (DDDs) can be found for Europe on the European Surveillance of Antimicrobial Consumption (ESAC) homepage [40]. In the last decade, a large number of studies covering occurrence of pharmaceuticals in water bodies, sewage treatment plants, manure, soil, and air dust have been published. The most concerning issue is that under the environmental conditions, these molecules can be neutral, cationic, anionic, or zwitterionic which make the risk assessment study of pharmaceuticals more difficult. In Table 1 we have presented the reported concentrations of

Table 1

Various therapeutic classes of pharmaceuticals and their reported concentration in different samples of different countries as well as toxicological endpoints and probable ecotoxicity data

Class of drugs	Name of drugs	Country	Sample	Concentrations reported (ng/l)	Toxicological endpoint	Ecotoxicity data (mg/l)	Ref.
NSAIDs	Acetylsalicylic acid	Romania	River water	<30–37.2 (±4.6)	<i>D. subspicatus</i> EC ₅₀ (growth inhibition)	106.7	[41–45]
		Japan	STP influent	470–19,400	<i>D. magna</i> EC ₅₀ (48 h) (immobilization)	88.1	[45]
	Salicylic acid	Canada	STP influent River Water	554.3–2178.2, 130.4–371.5	<i>V. fischeri</i> EC ₅₀ (30 min)	90	[46]
		Spain	STP influent	200–3600	<i>D. magna</i> EC ₅₀ (48 h) (immobilization)	68	[41]
		Switzerland	STP influent	1300–2900	<i>D. subspicatus</i> EC ₅₀ (growth inhibition)	72	[41]
		Canada	STP influent	32–448	<i>L. minor</i> , EC ₅₀ (7 days) (growth inhibition)	7.5	[46]
		Greece	STP influent	12–560	<i>O. mykiss</i> LOEC (28 days) (cytological alterations)	0.001	[47]
		Germany	Groundwater	590	<i>D. subspicatus</i> EC ₅₀ (growth inhibition)	71.9	[45]
		USA	Drinking water	<0.25	<i>P. subcapitata</i> NOEC (96 h) (growth inhibition)	10	[44]
		UK	STP influent	901–1036	<i>D. magna</i> EC ₅₀ (48 h) (immobilization)	22.43	[44]
	Fenoprofen	Japan	STP influent	9.68–80.6	–	–	[45]
	Ibuprofen	Spain	STP influent	34,000–168,000	<i>D. magna</i> EC ₅₀ (48 h) (immobilization)	108	[41]
		Switzerland	STP influent	1750–4500	<i>D. subspicatus</i> EC ₅₀ (growth inhibition)	315	[41]

	Canada	STP influent	2235.2–6718.3	<i>L. minor</i> EC ₅₀ (7 days) (growth inhibition)	22	[41]
	Sweden	STP influent	3590	NOEC (14 days) (survival)	20	[47]
	Italy	River water	78.50	<i>Gammarus pulex</i> LOEC (behavior)	0.01	[48]
	USA	Groundwater	3110	<i>L. minor</i> EC ₅₀ (7 days) (growth inhibition)	4.01	[49]
	UK	STP influent	7741–33,764	<i>O. latipes</i> LC ₅₀ (96 h) (mortality)	>100	[50]
	South Korea	STP influent	10–137	<i>Hydra attenuata</i> LC ₅₀ (96 h) (morphology)	22.36	[41]
	Germany	River water	8.7–32	NOEC (21 days) (survival)	5.36	[50]
	Spain	STP effluent	160–390	<i>T. platyurus</i> LC ₅₀ (24 h) (mortality)	16.14	[3]
	South Korea	River water	<1–33.5	<i>O. latipes</i> LC ₅₀ (96 h) (mortality)	81.92	[3]
	Canada	STP effluent	8–351	–	–	[46]
	Sweden	STP effluent	940	–	–	[47]
	Spain	STP effluent	131	–	–	[51]
	Germany	River water	<26	–	–	[51]
	USA	STP effluent	23 (±6.8 %)	–	–	[51]
	UK	STP influent	136–363	<i>T. platyurus</i> LC ₅₀ (24 h) (mortality)	3.95	[52]
	Japan	STP influent	4.45–396	<i>O. latipes</i> LC ₅₀ (96 h) (mortality)	8.04	[52]
	China	River water	ND to 22.4 (±3.1)	–	–	[52]
	Spain	STP effluent	40–60	–	–	[52]
	Canada	STP effluent	271.4–7962.3	<i>D. magna</i> EC ₅₀ (48 h) (immobilization)	174	[46]
	Sweden	STP influent	3650	<i>L. minor</i> EC ₅₀ (7 days) (growth inhibition)	24.2	[47]
	USA	Drinking water	<0.5	<i>T. platyurus</i> LC ₅₀ (24 h)	84.09	[53]

(continued)

Table 1
(continued)

Class of drugs	Name of drugs	Country	Sample	Concentration reported (ng/l)	Toxicological endpoint	Ecotoxicity data (mg/l)	Ref.	
		Spain	STP influent	109–455	<i>P. subcapitata</i>	31.82	[53]	
		USA	River water	31 ($\pm 5.5\%$)	EC ₅₀ (72 h) (growth inhibition)	0.56	[53]	
		South Korea	STP effluent	20–483	<i>B. calyciflorus</i>	166.3	[45]	
		Spain	STP influent	29,000–246,000	EC ₅₀ (48 h) (growth inhibition)	567.5	[54]	
	Paracetamol	USA	Groundwater	380	<i>D. magna</i>	30.1	[54]	
		UK	Surface water	<50	EC ₅₀ (48 h) (immobilization)	378	[3]	
		UK	STP influent	5529–69,570	<i>D. rerio (zebrafish)</i>	>160	[54]	
		Taiwan	Hospital effluent	62,250	LC ₅₀ (48 h)	26.6	[54]	
		South Korea	STP effluent	1.8–19	<i>O. latipes</i>	134	[54]	
					LC ₅₀ (48 h)			
	Blood lipid lowering agents	Bezafibrate	Italy	River water	0.79–2.75	EC ₅₀ (96 h) (morphology)	25.85	[55]
			Brazil	River water	<25	<i>Hydra attenuata</i>	70.71	[55]
	Clofibrate	Clofibrate	Spain	STP effluent	40–130	LC ₅₀ (96 h) (morphology)	1	[55]
			Brazil	Drinking water	<10–30	LOEC (96 h) (morphology)	115	[41]
		Italy	River water	0.41–5.77	<i>D. subspicatus</i>	12.5	[48]	
					EC ₅₀ (growth inhibition)			
					<i>L. minor</i>			
					EC ₅₀ (7 days) (growth inhibition)			

	UK	STP influent	<20–651	<i>S. subspicatus</i> EC ₅₀ (72 h)	89	[48]
	Spain	STP influent	25–58	<i>D. magna</i> EC ₅₀ (immobilization)	106	[51]
	Greece	STP influent	ND	<i>D. magna</i> EC ₅₀ (48 h) (immobilization)	72	[51]
	Germany	Groundwater	2–40	<i>D. magna</i> EC ₅₀ (48 h) (immobilization)	>200	[3]
Gemfibrozil	Canada	STP effluent	80.1–478.2	<i>Hydra attenuata</i> LC ₅₀ (96 h) (morphology)	22.36	[55]
	Sweden	STP influent	710	LOEC (96 h) (morphology)	1	[55]
	USA	Drinking water	0.43	NOEC (96 h) (morphology)	0.1	[55]
	China	River water	ND to 22.4 (±3.1)	<i>V. fischeri</i> EC ₅₀ (24 h) (bioluminescence)	64.6	[56]
	South Korea	STP effluent	3.9–17	<i>V. fischeri</i> EC ₅₀ (48 h) (bioluminescence)	45.1	[56]
	Spain	STP effluent	470–3550	<i>D. magna</i> EC ₅₀ (48 h) (immobilization)	42.6	[56]
Atorvastatin	USA	Drinking water	<0.25	<i>L. gibba</i> LOEC (7 days) (growth parameters)	0.3	[57]
	Canada	STP influent	76 (±3)	–	–	[57]
Lovastatin	Canada	STP influent	49 (±2)	–	–	[57]
Pravastatin	Canada	STP influent	117 (±6)	–	–	[57]
Simvastatin	Canada	STP influent	4 (±0)	–	22.8	[57]
Antibiotics	USA	Surface water	20	–	–	[42]
Ciprofloxacin	USA	WWTP effluent	100–160	–	–	[68]
	USA	STP influent	ND to 1000	–	–	[58]
	Germany	Surface water	60	–	–	[68]
	Germany	WWTP effluent	600	–	–	[68]

(continued)

Table 1
(continued)

Class of drugs	Name of drugs	Country	Sample	Concentration reported (ng/l)	Toxicological endpoint	Ecotoxicity data (mg/l)	Ref.
		Switzerland	Surface water	5–18	–	–	[68]
		Switzerland	WWTP effluent	55–405	–	–	[68]
		France	WWTP effluent	60	–	–	[68]
		Italy	River water	ND to 26.15	–	–	[48]
		Sweden	WWTP effluent	30	–	–	[68]
		Sweden	STP influent	90–300	–	–	[58]
	Enrofloxacin	Portugal	STP influent	121.8–447.1	<i>V. fischeri</i> EC ₅₀ (15 min) (luminescence)	326.89	[58]
		Japan	WWTP influent	7–85	–	–	[68]
		USA	STP influent	250	<i>D. magna</i> EC ₅₀ (48 h) (immobilization)	131.7	[58]
	Levofloxacin	South Korea	River water	ND to 87.4 (±13)	<i>D. magna</i> EC ₅₀ (21 days) (reproduction)	0.34	[52]
		Japan	WWTP influent	255–587	–	–	[68]
	Norfloxacin	USA	Surface water	120	<i>S. obliquus</i> IC ₅₀ (48 h) (growth inhibition)	38.49	[42]
		Portugal	STP influent	191.2–455.0	<i>S. capricornutum</i> EC ₅₀ (growth inhibition)	16.6	[59]
		Sweden	STP influent	72–174	NOEC (growth inhibition)	4.01	[59]
		China	Surface seawater	<13	NOEC (growth inhibition)	4.02	[59]
		China	WWTP influent	460	–	–	[68]
		China	WWTP effluent	85–320	–	–	[68]

	Japan	WWTP influent	155–486	–	–	[68]
Ofloxacin	USA	STP influent	ND to 1000	<i>T. platyurus</i> LC ₅₀ (24 h) (mortality)	33.98	[60]
	USA	WWTP effluent	110–1000	–	–	[68]
	Portugal	STP influent	ND	<i>D. magna</i> EC ₅₀ (24 h) (immobilization)	31.75	[60]
	Sweden	STP influent	<6–287	<i>C. dubia</i> EC ₅₀ (24 h) (immobilization)	17.41	[60]
	China	Harbor seawater	5.2–10	<i>C. dubia</i> EC ₅₀ (7 days) (growth inhibition)	3.13	[60]
Naldixic acid	Japan	WWTP influent	7–40	–	–	[68]
	Taiwan	STP influent	26–372	–	–	[3]
Ampicillin	Taiwan	Hospital effluent	21	<i>V. fischeri</i> EC ₅₀ (15 min) (luminescence)	2627	[58]
Penicillin G	China	STP influent	153,000 ± 4000	<i>M. aeruginosa</i> EC ₅₀ (growth rate)	0.006	[43]
Cephalexin	China	Surface seawater	<13–182	–	–	[60]
Lincomycin	USA	Surface water	60	<i>B. calyciflorus</i> LC ₅₀ (24 h) (mortality)	24.94	[60]
	USA	Groundwater	320	<i>T. platyurus</i> LC ₅₀ (24 h) (mortality)	30.00	[60]
	Italy	River water	3.13–248.90	<i>B. calyciflorus</i> EC ₅₀ (48 h) (growth inhibition)	0.68	[60]
Clarithromycin	Italy	River water	0.49–20.30	<i>B. calyciflorus</i> LC ₅₀ (24 h) (mortality)	35.46	[60]
	Taiwan	STP influent	59–1433	<i>B. calyciflorus</i> EC ₅₀ (48 h) (growth inhibition)	12.21	[60]
	South Korea	River water	ND to 443 (±14)	<i>D. magna</i> EC ₅₀ (24 h) (immobilization)	25.72	[52]

(continued)

Table 1
(continued)

Class of drugs	Name of drugs	Country	Sample	Concentration reported (ng/l)	Toxicological endpoint	Ecotoxicity data (mg/l)	Ref.
Erythromycin		Italy	Po River water	1.4–15.9	<i>L. minor</i> EC ₅₀ (7 days) (growth inhibition)	5.62	[3]
		South Korea	STP effluent	8.9–294	<i>T. platyurus</i> LC ₅₀ (24 h) (mortality)	>100	[3]
Sulfachloropyridazine		Korea	STP influent	<30–476	<i>V. fischeri</i> EC ₅₀ (15 min)	26.4	[3]
		Italy	River water	236	<i>M. aeruginosa</i> EC ₅₀ (72 h) (growth inhibition)	0.135	[3]
Sulfadiazine		USA	Surface water	60	<i>V. fischeri</i> EC ₅₀ (15 min)	>500	[54]
Sulfamethoxine		USA	Groundwater	46–68	<i>D. magna</i> EC ₅₀ (48 h) (immobilization)	248	[54]
		Taiwan	Hospital effluent	ND	<i>D. magna</i> EC ₅₀ (96 h) (immobilization)	204.5	[54]
		Luxembourg	STP influent	0.3–6	<i>O. latipes</i> LC ₅₀ (48 h)	>100	[54]
		Italy	River water	28	<i>S. capricornutum</i> EC ₅₀ (growth inhibition)	2.30	[59]
Sulfamethazine		USA	Groundwater	76–215	<i>V. fischeri</i> , EC ₅₀ (15 min)	344.7	[54]
		USA	STP influent	160	<i>O. latipes</i> , LC ₅₀ (96 h)	>100	[54]
		Luxembourg	STP influent	0.3–2	<i>O. latipes</i> LC ₅₀ (48 h)	>100	[54]
Sulfamethoxazole		USA	Surface water	150	<i>V. fischeri</i> EC ₅₀ (15 min)	78.1	[54]
		USA	Groundwater	1110	<i>D. magna</i> EC ₅₀ (48 h) (immobilization)	189.2	[54]
		USA	Drinking water	0.32	<i>D. magna</i> EC ₅₀ (96 h) (immobilization)	177.3	[54]
		Taiwan	STP influent	179–1760	<i>O. latipes</i> LC ₅₀ (96 h)	562.5	[54]

Sulfathiazole	South Korea	STP effluent	3.8–407	LOEC (96 h) (morphology)	10	[55]
	Sweden	STP influent	<80–674	EC ₅₀ (48 h) (growth inhibition)	9.63	[60]
	Italy	Drinking water	13–80	<i>T. platyurus</i> LC ₅₀ (24 h) (mortality)	35.36	[60]
Sulfathiazole	Luxembourg	STP influent	0.3–2	<i>V. fischeri</i> EC ₅₀ (15 min)	>1000	[3]
	South Korea	STP influent	<30–531	<i>D. magna</i> LOEC (21 days) (reproduction)	35	[3]
Chlortetracycline	USA	Surface water	420	<i>M. aeruginosa</i> EC ₅₀ (growth rate)	0.05	[43]
	Taiwan	Hospital effluent	ND	<i>S. capricornutum</i> EC ₅₀ (growth rate)	3.1	[43]
Oxytetracycline	USA	Surface water	340	<i>Hydra attenuata</i> LC ₅₀ (96 h) (morphology)	>100	[55]
	Italy	River water	ND to 19.2	<i>Hydra attenuata</i> EC ₅₀ (96 h) (morphology)	40.13	[55]
	USA	Surface water	110	<i>L. minor</i> EC ₅₀ (7 days) (growth inhibition)	1.06	[60]
Tetracycline	Taiwan	STP influent	46–234	<i>S. capricornutum</i> EC ₅₀ (growth rate)	2.2	[43]
	Luxembourg	STP influent	0.3–85	<i>D. magna</i> NOEC (48 h) (immobilization)	340	[58]
Metronidazole	China	Surface seawater	<13–122	–	–	[58]
	Taiwan	STP influent	1–294	<i>D. magna</i> LOEC (48 h) (immobilization)	1000	[3]
Trimethoprim	USA	Drinking water	<0.25	<i>D. magna</i> EC ₅₀ (48 h) (immobilization)	167.4	[54]
	Serbia	River water	25	<i>D. magna</i> EC ₅₀ (96 h) (immobilization)	120.7	[54]
	South Korea	STP effluent	10–188	<i>O. latipes</i> LC ₅₀ (96 h)	>100	[54]
Trimethoprim	China	Surface seawater	<13–21.8	<i>D. magna</i> EC ₅₀ (48 h) (immobilization)	149	[58]

(continued)

Table 1
(continued)

Class of drugs	Name of drugs	Country	Sample	Concentration reported (ng/l)	Toxicological endpoint	Ecotoxicity data (mg/l)	Ref.
Sex hormones	17 α -Estradiol	USA	Surface water	30	-	-	[42]
	17 β -Estradiol	France USA	Groundwater Surface water	0.8–3.5 9	- <i>O. latipes</i> NOEC (21 days)	- <0.0293	[56] [42]
Estradiol	Estradiol	Japan	STP influent	13.3–25.8	-	-	[62]
		China	Rivers water	ND to 7.5 (\pm 0.4)	-	-	[62]
		South Korea	STP effluent	<1.0	-	-	[62]
		Germany	STP influent	11.8 (\pm 5.1)	-	-	[62]
		France	Groundwater	0.3–1.3	-	-	[62]
		USA	Surface water	19	-	-	[3]
		Italy	STP influent	23–48	-	-	[3]
		South Korea	STP effluent	8.9–25	-	-	[3]
		USA	Surface water	27	-	-	[3]
		USA	Drinking water	<0.20	-	-	[3]
Estrone	Estrone	Japan	STP influent	28.7–197	-	-	[3]
		USA	Surface water	73	-	0.000001	[63]
17 α -Ethinylestradiol	17 α -Ethinylestradiol	USA	Drinking water	<1.0	<i>P. promelas</i> LOEC (21 days) (plasma VTG induction)	0.000001	[63]
					<i>P. promelas</i> LOEC (21 days) (ultrastructure testes)		
					-		
Ethinylestradiol	Ethinylestradiol	Germany Italy France	STP influent STP influent Groundwater	8.8 (\pm 8.0) ND 0.5–3.0	-	-	[63] [63] [61]

Antiepileptic	Carbamazepine	Spain	STP influent	120–310	<i>D. magna</i> EC ₅₀ (48 h) (immobilization)	>100	[41]		
		Finland	STP influent	290–400	<i>D. subspicatus</i> EC ₅₀ (growth inhibition)	74	[41]		
		Romania	River water	<30–75.1 (±6.1)	<i>L. minor</i> EC ₅₀ (7 days) (growth inhibition)	25.5	[41]		
		Sweden	STP influent	1680	<i>Gammarus pulex</i> LOEC (behavior)	0.00001	[47]		
		Germany	Groundwater	900	<i>T. platyurus</i> LC ₅₀ (24 h) (mortality)	>100	[52]		
		USA	Drinking water	6.8	<i>O. latipes</i> LC ₅₀ (96 h)	45.87	[52]		
		Japan	STP influent	14.9–270	<i>O. latipes</i> LC ₅₀ (48 h)	35.4	[54]		
		South Korea	Drinking water	<1.0	<i>V. fischeri</i> EC ₅₀ (30 min)	>81	[44]		
		Spain	STP influent	300–500	-	-	[3, 41]		
		France	STP influent	ND-27	-	-	[3, 41]		
		β-Blockers	Atenolol	Finland	STP influent	510–800	<i>T. platyurus</i> LC ₅₀ (24 h) (mortality)	>100	[52]
				Sweden	STP influent	30	<i>O. latipes</i> LC ₅₀ (96 h)	>100	[52]
				Italy	River water	3.44–39.43	<i>D. subspicatus</i> EC ₅₀ (growth inhibition)	620	[48]
				USA	Drinking water	0.47	<i>D. magna</i> EC ₅₀ (48 h) (immobilization)	313	[64]
Spain	Hospital effluent			100–122,000	<i>P. promelas</i> NOEC (28 days) (growth)	3.2	[64]		
South Korea	River water			ND to 690 (±26)	<i>P. promelas</i> LOEC (28 days) (growth)	10	[64]		

(continued)

Table 1
(continued)

Class of drugs	Name of drugs	Country	Sample	Concentration reported (ng/l)	Toxicological endpoint	Ecotoxicity data (mg/l)	Ref.
	Metoprolol	Finland	STP influent	980–1350	<i>D. magna</i> EC ₅₀ (48 h) (immobilization)	>100	[41]
		Sweden	STP influent	160	<i>D. subspicatus</i> EC ₅₀ (growth inhibition)	7.3	[47]
		Taiwan	STP influent	14–597	<i>L. minor</i> EC ₅₀ (7 days) (growth inhibition)	>320	[41]
	Sotalol	Finland	STP influent	640–830	–	–	[3]
		Germany	Ground water	560	–	–	[3]
	Propranolol	Sweden	STP influent	50	<i>D. magna</i> EC ₅₀ (48 h) (immobilization)	7.5	[47]
		Taiwan	Hospital effluent	54	<i>D. subspicatus</i> EC ₅₀ (growth inhibition)	5.8	[41]
		UK	STP influent	60–119	<i>L. minor</i> , EC ₅₀ (7 d) (growth inhibition)	114	[41]
		Spain	Hospital effluent	200–6500	<i>T. platyurus</i> LC ₅₀ (24 h) (mortality)	10.31	[52]
		South Korea	River water	ND to 40.1 (±3)	<i>O. latipes</i> LC ₅₀ (96 h)	11.40	[52]
Antidepressants	Fluoxetine	USA	Surface water	12	<i>H. azteca</i> LOEC (28 days) (growth)	0.1	[65]
		USA	Groundwater	56	<i>H. azteca</i> NOEC (28 days) (growth)	0.033	[65]
		USA	Drinking water	0.64	<i>D. magna</i> NOEC (21 days) (newborns' length)	0.0089	[65]
		South Korea	STP effluent	1.7	<i>D. magna</i> LOEC (21 days) (newborns' length)	0.031	[65]
		Norway	STP influent	0.4–2.4	<i>P. antipodarum</i> NOEC (reproduction)	0.013	[65]

	Canada	STP influent	3.1 (± 0.3)–3.5	<i>Gammarus pulex</i> LOEC (behavior)	0.0001	[65]
Norfluoxetine	USA	Drinking water	0.77	–	–	[66]
	Norway	STP influent	0.7 (± 13.1)–9.3	–	–	[66]
	Canada	STP influent	1.8 (± 0.3)–4.2	–	–	[66]
Fluvoxamine	Norway	STP influent	0.4–3.9	<i>P. subcapitata</i> IC ₅₀ (96 h) (growth inhibition)	4.003	[3]
	Norway	STP influent	0.6–12.3	<i>D. magna</i> EC ₅₀ (48 h) (immobilization)	2.5	[3]
Sertraline	Norway	STP influent	1.8–2.5	<i>V. fischeri</i> EC ₅₀ (30 min) (inhibition)	10.72	[66]
	Canada	STP influent	6.0 (± 0.4)	<i>V. fischeri</i> LOEC (30 min) (inhibition)	4.5	[66]
Antineoplastic	Romania	River water	<30–64.8 (± 8.0)	<i>P. subcapitata</i> EC ₅₀ (72 h) (growth inhibition)	>100	[67]
Cyclophosphamide	Italy	STP influent	<1.9–9.0	<i>P. subcapitata</i> NOEC (72 h) (growth inhibition)	>100	[67]
	Switzerland	STP influent	2.0–6	<i>D. magna</i> LOEC (21 days) (reproduction)	100	[67]
Methotrexate	Italy	STP influent	<0.83–12.6	<i>V. fischeri</i> EC ₅₀ (30 min)	1220	[3]
Tamoxifen	UK	STP influent	143–215	<i>B. calyciflorus</i> LC ₅₀ (24 h) (mortality)	0.97	[67]

(continued)

Table 1
(continued)

Class of drugs	Name of drugs	Country	Sample	Concentration reported (ng/l)	Toxicological endpoint	Ecotoxicity data (mg/l)	Ref.
X-ray contrast media	Diatrizoate	Germany	STP effluent	250	-	-	[3]
	Iohexol	Australia	STP influent	2800–4760	-	-	[3]
	Iopamidol	Germany	Ground water	300	-	-	[3]
		Australia	STP influent	400–620	-	-	[3]
	Iopromide	South Korea	STP effluent	1170–4030	<i>D. magna</i> EC ₅₀ (48 h) (immobilization)	>1000	[3]
		Germany	STP effluent	4400	<i>V. fischeri</i> EC ₅₀ (30 min)	>10,000	[3]
		Australia	Ground water	168	<i>S. subspicatus</i> EC ₅₀ (72 h) (growth inhibition)	>10,000	[3]
		USA	STP influent	ND to 17	<i>P. putida</i> EC ₁₀ (16 h) (growth inhibition)	>10,000	[3]
		Spain	STP influent	6600	<i>D. rerio</i> NOEC (28 days)	>100	[3]
		Australia	STP influent	<730	-	-	[3]

LOEC lowest-observed-effect concentration, *NOEC* no-observed-effect-concentration, *STP* sewage treatment plant, *WWTP* waste water treatment plants

diverse pharmaceuticals from various therapeutic classes in different samples of different countries and probable ecotoxicity data to particular toxicological endpoints [3, 41–68].

2.2.1 Waterbodies

The presence of pharmaceuticals in the various waterbodies in the environment has been quite extensively studied by different research groups. Quinolones (predominantly ciprofloxacin) and other pharmaceuticals have been detected in the effluent of hospitals up to a low $\mu\text{g}/\text{l}$ range. Another study reveals that β -lactams (including penicillins, cephalosporins, carbapenems, monobactams, β -lactamase inhibitors) were detected in the lower $\mu\text{g}/\text{l}$ range in hospital effluent and in the influent of a municipal STP [69]. NSAIDs have the higher concentrations recorded in surface water, ranging between 0.4 ng/l and 15 $\mu\text{g}/\text{l}$, diclofenac, paracetamol, and ibuprofen being the most quantitatively found [70]. Drugs like caffeine with a maximum concentration of 6 $\mu\text{g}/\text{l}$ and sulfamethoxazole with 1.9 $\mu\text{g}/\text{l}$ in the USA, carbamazepine up to 1.3 $\mu\text{g}/\text{l}$ in Germany and in Canada, gemfibrozil up to 790 ng/l , ranitidine up to 580 ng/l , atenolol with 241 ng/l in Italy, and metformin up to 150 ng/l are detected in surface water [71]. In the effluent of WWTP and STP, the concentrations of estrogenic compounds usually are below 50 ng/l , but there are unexpected high concentrations of estriol and 17α -estradiol (about 590 ng/l and 180 ng/l respectively) found in the USA [72].

2.2.2 Manure and Soil

Antibiotics have been detected in soil in concentrations in the mg/kg range [73]. Generally, the concentrations of pharmaceuticals detected in the soils are quite low when compared with that of pharmaceuticals in water resource. According to the literature, the six most common pharmaceuticals found in soil are the antibacterials (trimethoprim, sulfadiazine, and triclosan), analgesics (ibuprofen and diclofenac) and antiepileptic (Carbamazepine). Extensive studies have detected tetracyclines and sulfonamides in liquid manure at concentrations of up to 20 and 40 mg/l , respectively. Antibiotics like virginiamycin, sarafloxacin, tetracycline, oxytetracycline, chlortetracycline, and cyclosporine A have quite slow biodegradability in soil. Tylosin disappeared soon after the application of manure. Hamscher et al. [74] detected tetracycline and chlortetracycline in 10 out of 12 soil samples. The highest average concentration of 86.2 $\mu\text{g}/\text{kg}$ (0–10 cm), 198.7 $\mu\text{g}/\text{kg}$ (10–20 cm), 171.7 $\mu\text{g}/\text{kg}$ (20–30 cm) tetracycline, and 4.6–7.3 $\mu\text{g}/\text{kg}$ (in all three sublayers) chlortetracycline were found. Carbamazepine is the most frequent compound detected in soil among five studies [75].

2.2.3 Air Dust

Several comprehensive reports have been published on environmental concentrations of antibiotics in dust originating from a pig-fattening house [76]. In a large-scale pig production, veterinary antibiotics are hugely used. This production system is represented as a considerable source of dust.

2.3 Effects

2.3.1 Antibiotics

Pharmaceuticals may have potential adverse effects on aquatic and terrestrial organisms by directly reaching into the environment. Organisms like bacteria, fungi, and microalgae are primarily affected as antibiotics are designed to inhibit the microorganisms. Antibiotics have the potential to affect the microorganisms in sewage systems and waste water treatment plant too. The inhibition of wastewater bacteria may seriously affect organic matter degradation and nitrification process which is a vital step in wastewater purification and elimination of toxic ammonia [77]. Lincomycin showed significant inhibition of the nitrification activity [78]. Ciprofloxacin was found to be active against *Vibrio fischeri* at a concentration of 5 mg/l [79]. Thomulka and McGee [80] have performed two bioassays to evaluate the toxicity of antibiotics like novobiocin, chloramphenicol, tetracycline, ampicillin, and streptomycin to *Vibrio harveyi*, and approximately no toxic effects were identified after short incubation times where the employed endpoint was luminescence. Common receptors have been identified in plants for a number of antibiotics affecting transcription and translation (tetracyclines, macrolides, lincosamides, aminoglycosides, and pleuromutilins), metabolic pathways such as folate biosynthesis (sulfonamides), chloroplast replication (fluoroquinolones), and fatty acid biosynthesis (triclosan) [81].

Antimicrobials can affect the degradation of organic matter in large extent as well as have effects upon sediment's microbial community [82]. Strong inhibitory effects on several bacteria and diminution in the length of the hyphae of lively molds in forest soil have been observed when antibiotics are added in concentrations of 10 mg/kg soil. A transitory effect on sulfate reduction was detected when antibiotics were mixed to sediment [83]. Allergic risks may arise from the high exposure of antibiotics dust particle in the air. Tylosin and sulfamethazine, which occurred in 80 % and 65 % of the samples respectively, are drugs with known allergic potential. Therefore, the high incidence of the asthma disease occurred among children living on farms. A survey on dust in pig fattening buildings in Europe exposed an average concentration of inhalable airborne dust of 2.2 mg/m³ [84]. Chloramphenicol is extensively employed in farming resulting in severe hazardous effects including myelosuppression to farmers; that is why it was totally banned for food-producing animals within the EU and the USA in 1994 [85].

Another important aspect is the emergence of resistance due to enormous application of antibiotics in human medicine, veterinary medicine, and animal husbandry. Resistance is one of the most concerning issue in medical field due to its accumulating and accelerating nature. On the contrary, the techniques combating resistance are diminishing in power and number. Antibiotics in sub-inhibitory concentrations can have an influence on cell

functions and modify the genetic expression of virulence factors or the transfer of antibiotic resistance. The most prominent medical examples are vancomycin-resistant enterococci (VRE), methicillin-resistant *Staphylococcus aureus* (MRSA), and multiresistant pseudomonads [86].

2.3.2 Analgesics and Nonsteroidal Anti-inflammatory Drugs (NSAIDs)

Cleuvers [45] evaluated that acute toxicities of NSAIDs were relatively low, with half-maximal effective concentration (EC_{50}) values obtained using *Daphnia* in the range from 68 to 166 mg/l and from 72 to 626 mg/l in the algal test. With EC_{50} values of 23.6 mg/l (ibuprofen), 23.8 mg/l (diclofenac), and 38.2 mg/l (naproxen), chronic ecotoxicity was somewhat higher, but still the values are far above the concentrations detected in surface water. A prominent confirmation of diclofenac residues in dead cattle has been observed in Pakistan [87]. Only in Germany, in 2002, 93.5 million prescriptions for NSAIDs were made with a transaction volume of about 1562 million Euros [88]. Due to higher usage and pharmacokinetic and pharmacodynamic properties, analgesics and anti-inflammatory drugs can reach considerable (up to $>1 \mu\text{g/l}$) concentrations in the environment. Few NSAIDs are detected in very low doses even in drinking water. Reports suggested the presence a concerning amount of diclofenac and ibuprofen in Swiss lakes and rivers, as well as in water bodies from the UK, Spain, Brazil, Greece, and the USA [15].

Diclofenac seems to be the compound having the highest acute toxicity with the effective concentrations below 100 mg/l within the class of NSAIDs. Short-term acute toxicity was analyzed in algae and invertebrates, phytoplankton was found to react more sensitively [lowest EC_{50} (96 h) = 14.5 mg/l] than zooplankton [lowest EC_{50} (96 h) = 22.43 mg/l] [89]. Diclofenac is commonly found in wastewater at median concentration of 0.81 $\mu\text{g/l}$ whereas the maximal concentration in wastewater and surface water is up to 2 $\mu\text{g/l}$ [90]. Acetylsalicylic acid affected reproduction in *D. magna* and *D. longispina* at concentrations of 1.8 mg/l [90]. Water flea *Daphnia magna* population growth rate was considerably reduced for concentrations ranging from 0 to 80 mg/l due to chronic toxicity of ibuprofen. Acute toxicity tests showed that naproxen had LC_{50} and EC_{50} values within the 1–100 mg/l range for the water flea *Ceriodaphnia dubia*, the rotifer *Brachionus calyciflorus*, and the fairy shrimp *Thamnocephalus platyurus*. But the most sensitive reported species was *D. magna* for which EC_{50} values were 30.1 or 50 mg/l. Another most commonly prescribed NSAID is paracetamol which is present in concentration below to 20 ng/l to 4.3 $\mu\text{g/l}$ in STP effluents; in surface waters, the values can reach 78.17 $\mu\text{g/l}$, which are values higher than the predicted no-effect concentration (PNEC) of 9.2 $\mu\text{g/l}$ [3]. Hence, paracetamol might represent a threat for nontarget organisms.

2.3.3 Blood Lipid-Lowering Agents

Statins have the capability to subdue synthesis of the juvenile hormone in insects and may also produce detrimental effect to protozoan parasites, inhibiting growth and development. Reports suggested that a proliferation of peroxisomes in rodent livers is caused by fibrates. Embryonic development of nontarget 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*) and amphibians [3]. Quinn et al. [91] classified gemfibrozil as toxic (EC_{50} between 1 and 10 mg/l) and bezafibrate as harmful for nontarget organisms (EC_{50} between 10 and 100 mg/l).

Clofibrate is classified as harmful to aquatic organisms as it showed LC_{50} values in the range of 7.7–39.7 mg/l. The fish *Gambusia holbrooki* [LC_{50} (96 h)=7.7 mg/l] seems to be the most sensitive organism to acute clofibrate concentrations [92]. Clofibrate has an immunosuppressive action in mammalian hosts, suppressing the production of IgM but not IgE antibodies, allowing an amplified number of encysted larvae of the nematodes *T. spiralis* and *Trichinella nelsoni* to occur and a decrease in the rate of exclusion of adult worms from the intestines, although the effects differed between parasite species and host strain [93]. Fibrates have been assessed by conventional toxicity tests and the following no-observed-effect-concentration (NOEC) were found for clofibric acid in *C. dubia* [NOEC (7 days)=640 μ g/l], the rotifer *B. calyciflorus* [NOEC (2 days)=246 μ g/l], and in early life stages of zebrafish [NOEC (10 days)=70 mg/l] [94]. Clofibrate was observed to produce no effect on in vitro growth of *T. bruceii* but did reduce the incidence of *P. berghei* and the invasiveness and development of *Acanthamoeba culbertsoni* in exposed mammalian hosts [95]. Lovastatin hinders the egg production of the trematode *S. mansoni* and subsequently there is a decline in pathogenic granulomas typically associated with the eggs in the mammalian liver [96].

2.3.4 Beta-blockers

Beta-blockers act by competitive inhibition of beta-adrenergic receptors which is critical for normal functioning in the sympathetic branch of the vertebrate autonomic nervous system. Among beta-blockers, propranolol shows the highest acute toxicity and highest $\log K_{ow}$ which proves the fact that it is a strong membrane stabilizer than other examined beta-blockers [97]. Undefined antagonists such as propranolol may be active in fish as they contain β_2 -receptors in heart and liver as well as in reproductive tissues [98]. There is a prominent evidence that propranolol not only has chronic cardiovascular toxicity, but also has toxic effect on reproduction system. The NOEC and lowest-observed-effect-concentration (LOEC) of propranolol affecting reproduction in *C. dubia* were 125 and 250 μ g/l, and reproduction was affected after 27 days of exposure in *H. azteca* at 100 μ g/l [97].

Beta-blockers may also affect parasite functional biology. Aqueous exposure of propranolol may negatively affect swimming behavior, survival, and phototaxis of free living aquatic stages of trematodes. Propranolol may also considerably decrease the number of *Dirofilaria immitis* nematode larvae capable of finishing third-stage molt, and in vitro prevent the growth of the malaria parasite *Plasmodium falciparum* [99]. Fathead minnows exposed to atenolol throughout embryo-larval growth showed NOEC and LOEC values for growth rate of 3.2 mg/l and 10 mg/l, respectively [3]. At 48-h exposure to propranolol, LC₅₀ values of 29.8, 1.6, and 0.8 mg/l were obtained for *H. azteca*, *D. magna*, and *C. dubia*, respectively, while acute exposure to nadolol did not affect the survival of the invertebrates [3]. Encystment of the protozoan *Entamoeba invadens* was inhibited in the presence of metoprolol [100].

2.3.5 Antineoplastic Drugs

Antineoplastic drugs are designed to kill the proliferating cells in cancer. As a consequence, a parallel effect can be expected on normally growing eukaryotic organisms. It is expected that antineoplastic drugs possess mutagenic, genotoxic, teratogenic, carcinogenic, and fetotoxic properties, and 14–53 % of the administered drugs can be excreted in unchanged form through urine [101]. Methotrexate revealed teratogenicity for fish embryos with an EC₅₀ of 85 mg/l after 48 h of exposure and acute effects in the ciliate *Tetrahymena pyriformis* with an EC₅₀ for 48 h of 45 mg/l [102]. Due to immunosuppressant property, methotrexate and cyclophosphamide are reported to cause a proliferation in disease incidence and intensity in host–parasite systems [103]. Acute toxicity of methotrexate is reported on highly proliferative species like the ciliate *Tetrahymena pyriformis* [EC₅₀ (48 h)=45 mg/l] [104]. On the contrary, cyclophosphamide appears to have a little effect on them. Methotrexate has been shown to have no or little effect on certain protozoans including *Toxoplasma gondii*, *Babesia bovis*, and *Leishmania tropica*, perhaps as they have different mechanisms of drug metabolism [105]. Development and growth of helminths in both mammalian and bird hosts were detrimentally effected by methotrexate and cyclophosphamide. Abnormal teratogenicity was noticed in fish embryos at higher concentrations [EC₅₀ (48 h)=85 mg/l]. *Biomphalaria glabrata*, a freshwater snail is largely affected with the long-term exposure to methotrexate [106]. Doxorubicin, tamoxifen, and methotrexate have all been reported as effective parasiticide agents against many protozoan species [107].

2.3.6 Neuroactive Compounds (Antiepileptics, Antidepressants)

A very limited number of studies on the effects of neurological agents on host–parasite dynamics have been studied, despite phenothiazine has been used as a parasiticide for long time [108]. The serotonin re-uptake inhibitor (SSRI) fluoxetine is deceptively the

most acute toxic human pharmaceutical with toxicity ranging from EC_{50} (48 h, alga) = 0.024 mg/l to LC_{50} (48 h) = 2 mg/l so far [2]. Sertraline exhibits highly toxic properties to rainbow trout (LC_{50} of 0.38 mg/l) at a 96-h exposure [109]. 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 [110]. *C. vulgaris* was shown to be the least sensitive species for all SSRIs tested. Fluvoxamine provided escalation to the highest EC_{50} values for all algae species tested (3563–10,208 μ g/l).

Under the category of benzodiazepines, diazepam and nitrazepam were identified to increase the number of microfilariae of *Setavia cervi* liberated from the lungs into the peripheral blood circulation in rats [111]. Caffeine was found to stimulate the growth of *Plasmodium gallinaceum* and *P. falciparum*, while the antipsychotic haloperidol and the mood stabilizer valproic acid effectively inhibited the in vitro growth of *T. gondii* [112]. Diazepam and carbamazepine (antiepileptics) are classified as potentially detrimental to aquatic organisms as most of the acute toxicity data are below 100 mg/l. Conventional toxicity studies showed chronic toxicity of carbamazepine in *C. dubia* [NOEC (7 days) = 25 μ g/l], in the rotifer *B. calyciflorus* [NOEC (2 days) = 377 μ g/l], and in early life stages of zebrafish [NOEC (10 days) = 25 mg/l] [94]. Carbamazepine is carcinogenic to rats but does not have mutagenic properties in mammals [113]. It is also lethal to zebrafish at the 43 μ g/l level and produces sublethal changes in *Daphnia* sp. at 92 μ g/l [113]. Growth of *D. magna* was inhibited for concentrations of carbamazepine above 12.7 mg/l, showing acute toxicity at 17.2 mg/l [113].

2.3.7 Sex Hormones

Sex hormones are one of the extremely important biologically active compounds emerged as most serious aquatic environmental toxicants due to extensive use of human contraceptives. Exposure of mammalian hosts infected with the blood trematode *S. mansoni* to contraceptive pills resulted in a noteworthy modification in a range of liver cell's ultrastructure and function. Ethinylestradiol (EE2) is a synthetic estrogen found in oral contraceptive pills with noticeable estrogenic effects in fish. The life-cycle exposure of fathead minnows to EE2 concentrations below 1 ng/l produced a noteworthy decline in fertilization success, an increased egg production and decreased expression of secondary male sex characteristics. Life-long exposure of zebrafish to 5 ng/l to EE2 has led to reproductive failure due to the nonexistence of secondary male sex characteristics [63]. Exposure to 17β -estradiol caused an increased susceptibility to the protozoan *T. gondii* in mice, while increased pathology occurred in mammals infected with *Leishmania mexicana amazonensis* and exposed to either estradiol or testosterone [114]. Estradiol increased the susceptibility of cyprinids to

hemoflagellates by the suppression of lymphocyte proliferation [115]. At relatively high concentrations, hydrocortisone can cause an increase in the intensity of ectoparasitic infections in fish.

2.3.8 Antiparasitic Compounds

A study was performed on farms in the UK and the report suggested that concentrations of antiparasitic compounds of 0.112 mg/kg (doramectin) to 1.85 (ivermectin) mg/kg in dung were found. On the contrary, in same place, concentrations of these drugs in soil were considerably lower up to 0.046 mg/kg [116]. In a study performed in Slovenia, it was found that high concentrations of abamectin and doramectin were found in feces (0.2–0.8 mg/kg and 0.4–1.2 mg/kg, respectively) during the first 20 days after treatment, reaching concentrations of about 0.2 mg/kg after 70 and 50 days, respectively [117]. Grønvold et al. [118] found that ivermectin and fenbendazole affect the survival of the nematode *Pristionchus maupasi* at concentrations higher than 3 mg dung/kg (w/w) and 10–20 mg dung/kg, respectively. Svendsen et al. [119] showed that ivermectin and the fenbendazole did not affect earthworms. However, the disappearance of dung was affected by the avermectin but not by the fenbendazole. Avermectin B_{1A} with LC₅₀ value of 17.1 mg/kg in soil was found with the compost worm *Eisenia fetida* [120]. Eprinomectin did not affect survival or biomass of the earthworm species *Lumbricus terrestris* in laboratory tests at concentrations up to 0.43 mg/kg dung (w/w) or 3.3 mg/kg dung [121].

2.3.9 Antivirals

Tamiflu [oseltamivir ethylester-phosphate (OP)] and Relenzas (zanamivir) belong to a novel class of antiviral drugs under the neuraminidase inhibitors category. National storing of neuraminidase inhibitors in the USA began with the emergence of the 2009 influenza pandemic (H1N1) [122]. Tamiflu tablet largely dominated Relenza (disk inhaler) due to its relative ease of administration. Tamiflu is a prodrug, which is converted to the active drug oseltamivir carboxylate (OC) in the liver. About 80 % of an oral dose of Tamiflu is excreted as OC in the urine and the remaining portions are excreted as OP in the feces. Therefore, both the parent chemical and its bioactive metabolite eventually are projected to reach a mean of 2–12 mg/l in WWTPs during a moderate and severe pandemic [122]. Current evidences suggested that rivers receiving WWTP effluent would also be exposed to OC throughout a pandemic. The OC concentrations between 293 and 480 ng/l have been recorded in rivers receiving WWTP effluent during the 2009 pandemic [123].

2.3.10 Pharmaceuticals Mixtures

Pharmaceuticals are identified as multicomponent mixtures rather than isolated pure substance in diverse environmental compartments. Majority of pharmaceuticals will either be transformed by physical and chemical means and/or subsequently biotransformed

by some organisms. Multicomponent mixtures are the foremost concerning issue for the ecotoxicity. The following characteristics also make their joint toxic effects a major issue for hazard and risk assessment:

1. The toxicity of a mixture has always a synergistic effect than the effects produced by a single component.
2. A mixture can have a substantial ecotoxicity, even if all components exist only in low concentrations that do not aggravate noteworthy toxic effects if acting separately on the exposed systems.

A combination of fluoxetine and clofibric acid is lethal for more than 50 % of a water-flea (*Daphnia*) population after an exposure of 6 days, although the individual drugs did not show any significant effect when present separately at same concentrations [124]. A substantial swing in sex ratio was perceived after an exposure to a three-component mixture of erythromycin, triclosan, and trimethoprim. Again, individual components did not elicit significant individual effects. These studies are very important to show that mixture effects have to be taken into consideration to identify the effects of pharmaceuticals.

2.4 The Environmental Risk Assessment

Exposure assessment is the procedure of determining or assessing the intensity, frequency, and extent of environment and human exposure to an existing pharmaceutical product, or of estimating theoretical exposure that might rise from the discharge of new pharmaceuticals into the environment. The concept of “exposomics,” which integrates a top-down and bottom-up approach to identification of relevant exposure biomarkers, will be an important component of future exposure science [125]. The major aims of environmental risk assessment (ERA) should be risk mitigation and risk management. In order to alleviate or accept risks, a risk assessment has to be performed both for products and for activities followed by generation of report based on the characteristics of the product, its possible environmental exposure, fate and effects, and risk extenuation strategies. The inference of the report should be based on sound scientific reasoning supported by adequate studies. If other applicable data are accessible, they should also be submitted.

The outline of the registration process and the ERA consist of European Commission and Council directives and regulations on registration, European policy, case law, and global (trade) agreements. The decision-making process and the risk models should elevate the expenses to society in terms of ecotoxicity and financial loss. Also the assessment method itself should obstruct neither product development nor timely action to eradicate hazards.

2.4.1 Risk Assessment Approaches

The most commonly employed approaches for risk assessment are hazard identification, dose-response assessment, exposure assessment, and risk characterization of pharmaceuticals and its metabolites in various environment compartment [126].

Hazard Identification

The first step for risk assessment is hazard identification which supports the intensity of risk for a particular product. Although in vitro test studies provide useful data on the toxicity of environmental hazards, the majority of scientists rely heavily on the outcome of animal toxicity tests for hazard identification. As a consequence, a greater stress should be provided on the implication of in vitro assays in human cells and QSAR analysis, as well as the use of computational techniques in systems biology [127].

Dose-Response Assessment

Identification of the threshold dose of the toxic effect of any product is very much essential for scientific risk assessment. Dose-response information over a wide range of test concentrations should be assessed employing Quantitative high throughput screening (q-HTS). There should be availability of sensitive assays capable of detecting toxicity at very low doses or below environmental levels experienced by human populations. Statistical approaches can be used to estimate yardstick concentrations for adaptive and adversarial responses and to assess critical concentrations [128]. As discussed in subheading “Hazard Identification”, the extrapolation techniques will be required to interpret in vitro test results to in vivo utilizing an appropriate internal tissue dose metric [129].

Dose and Species Extrapolation

The major problems of risk assessment are low-dose and interspecies extrapolation. In silico models and expert systems have supported such extrapolations, including linear and threshold models for low-dose extrapolation and body weight or surface area alterations for interspecies extrapolation. New extrapolation complications are dose extrapolation of molecular and cellular pathway responses, and extrapolation from the short-term in vitro to longer term in vivo exposure. In vitro to in vivo extrapolation and physiologically based pharmacokinetic (PBPK) models are amenable to sensitivity, variability, and uncertainty analysis employing conventional tools [130]. Computational biology systems will back the application of tools for determining variability and uncertainty from the pharmacologically based pharmacokinetics (PBPK) information as the pathway components imitate more targeted molecular elements and their interactions [131].

Exposure Assessment

In present scenario, human exposure assessment is made principally on the measured levels of environmental agents in the human environment [132]. In few cases, internal dose measures may also be calculated using biomonitoring [133] or pharmacokinetic modeling [134]. For superior exposure assessment, the focus should be

more on direct measures of critical toxicity pathway agitations in humans by employing innovative biomonitoring techniques coupled with advanced new high throughput approaches [135].

2.4.2 Environmental Risk Assessment Modeling of Pharmaceuticals

The risk assessment model consists of the risk assessment process, including their harmonization and communication with the risk management process. The risk model interprets the safety issues in quantities like probabilities, concentrations, dosages, and risk quotients of each pharmaceutical product. The simplest approaches to estimating concentrations of a pharmaceutical in diverse compartments are provided in the guidance for environmental assessments for regulatory drug approvals by the US FDA [30] or the EU EMA [28]. In Fig. 2, the risk assessment is harmonized with risk management process.

Before designing or modeling a toxicological study, it is very beneficial to assess exposure of any pharmaceutical by the following way [136]:

- The exposure is measured in form of the environmental concentration (occurrence) to which the biological system is exposed, the duration and frequency being not on the concentrations to which each individual is actually exposed. The actual exposure is subjected to many other factors such as, the fate, sorption effects, metabolism and transformation processes.
- The life stage and behavioral patterns should also be taken into account for any organism or living system.

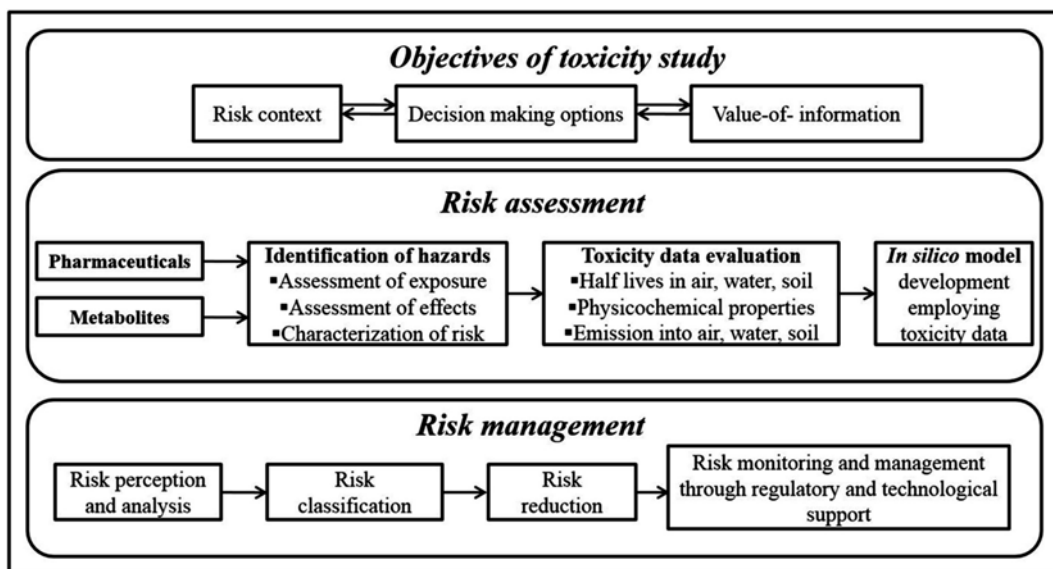


Fig. 2 Possible steps for risk assessment and risk management

- The bioavailability and toxicokinetics of the drug are studied.
- The pathways and target sites in the biological system are explored.
- The mode of action which depicts steps and processes to molecular and functional effects is determined.
- As pharmacokinetics and pharmacodynamics can influence the dose of pharmaceuticals, one has to consider these aspects to assess the dose which ultimately reaches the environment taking into account possible absorption, distribution, and elimination mechanisms.
- The hazard due to the inherent toxicity of the pharmaceutical according to its chemical properties is also studied.

2.5 Risk Management

Risk management is “the process of identifying, evaluating, selecting, and implementing actions to reduce risk to human health and to ecosystems. The goal of risk management is scientifically sound, cost-effective, integrated actions that reduce or prevent risks while taking into account social, cultural, ethical, political, and legal considerations” [137]. For eco-friendly risk management, one may select a combination of apposite tactics to balance risks, costs and benefits, taking into account social values and economic considerations.

2.5.1 Implementation of Precautionary Measures

The application of pharmaceuticals and their after use toxic effects cannot be stopped but the probable risk of pharmaceutical products related to environmental can be controlled by implementing proper precaution and safety measures. The EMEA 2006 guideline demonstrates following steps as safety measures for risk management:

1. Calculation of product risks initially
2. Proper product labeling and summary product characteristics (SPC)
3. Package leaflet (PL) for each pharmaceutical for patient use to inform the probable toxic effects
4. Appropriate and safe storage of pharmaceutical product
5. Safe and proper scientific disposal of pharmaceuticals

2.5.2 Lessening the Input of Pharmaceuticals into the Environment

To diminish the occurrence of pharmaceuticals into the different compartments of the environment, one has to follow the principle of sustainability where the entire life cycle of a pharmaceutical has to be taken into consideration to categorize the opportunities for risk management. For diminishing the input of pharmaceuticals into the environment, following steps can be employed effectively [138].

Training and Awareness

The most important step to reduce the occurrence of pharmaceuticals in the environment is proper training and awareness of users who are the major source points. A proper usage and disposal of pharmaceutical is the responsibility of the shareholders and people using the compounds, including patients, doctors and nurses, and pharmacists. Industrial sectors should have the major role to treat the failed active pharmaceutical under quality control category properly before it reach to the environment. Additionally, each pharmaceutical product should consist of materials safety data sheet (MSDS) intended to provide workers and emergency personnel with procedures for handling or working with that substance in a safe manner, information such as physical data, toxicity, health effects, first aid, reactivity, storage, disposal, protective equipment, and spill-handling procedures. Appropriate and effective risk management strategies need basic knowledge of entry routes of pharmaceuticals. Therefore, one has to identify the bulk of drug flows connected with the diverse sources of pharmaceuticals such as households, industries, hospitals and pharmacy.

Improved Sewage Treatment

The most technical and extensively considered approach for risk management is improvement of sewage treatment. Analyzing Table 1, one can easily identify the presence of threatening amount of pharmaceutical wastes after sewage and waste water treatment also. The purpose of advanced and improved sewage and waste water treatment is to further reduce the ecotoxicity, hormonal effects and pathogenic effects of the effluent. In recent years, advanced effluent treatment has been studied extensively. The advanced treatment of sewage influents and effluents as well as waste water treatment can be done employing photochemical oxidation processes, filtration, and application of powdered charcoal and constructed wetlands [139].

Green and Sustainable Pharmacy

The third approach is evolving from the knowledge of green and sustainable pharmacy which states that substitution of the compound with a more environmentally benign compound [138]. Though this approach is less practiced, in terms of sustainability, it appears to be the most encouraging one in the long run. The prime principle of green chemistry is easy and fast degradability of pharmaceuticals after their application. Understanding of full life cycle of drugs will lead to a different understanding of the functionality necessary for a pharmaceutical.

Additionally, other crucial issues like (a) development of improved drug delivery systems so that lower doses are required; (b) upgradation of packaging and package sizes to prolong shelf life and lessen the amount of the product that expires and rejection of unused products; and (c) changes in prescription and animal farming practices are substantial options for minimizing or

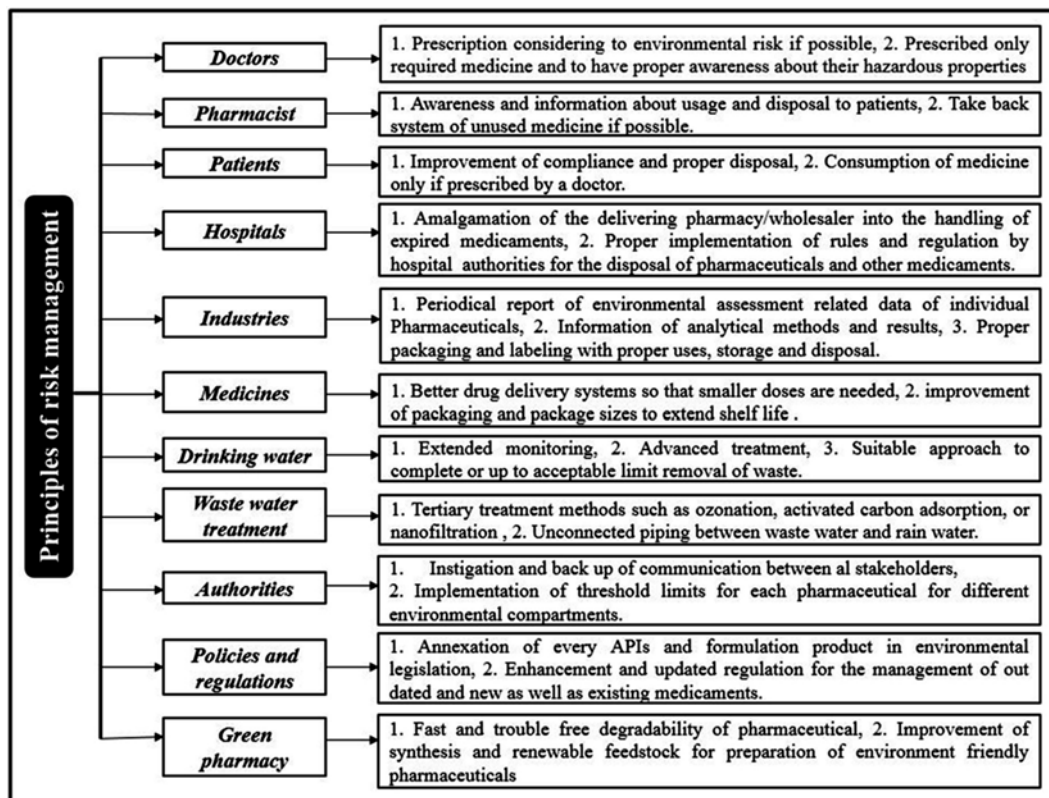


Fig. 3 Probable actions to be taken for reduction of the occurrence of pharmaceuticals in the environment by different stakeholders

eliminating emissions to the environment. Potential processes and measures to decrease the environmental toxicity by carious stakeholders are addressed in Fig. 3 for a better understanding.

3 Regulatory Agencies for the Risk Assessment and Management of Ecotoxicity Pharmaceuticals

Immense exposure of pharmaceuticals and their metabolites to the environment is a matter of concern and a burning global issue at recent times. The risk effects are not only related with the environment, it is also directly related to human health to a large extent. As a consequence, release of these pharmaceutical products, their risk assessment as well as risk management are controlled and regulated at local, national and international levels by different governments and regulatory agencies worldwide. As experimental data of environmental fate and toxicity of pharmaceuticals are absent or some time not sufficient, there is a strong urge to predict physical and chemical properties, environmental fate, ecological effects and health effects of pharmaceuticals and their metabolites. Several

government organizations have been applying the approaches of structure–activity relationship (SAR) and QSAR to develop the predictions for untested existing as well as newly introduced pharmaceuticals. To establish proper identification of environmental hazards, their risk assessment and fate modeling, SAR and QSAR approaches along with other predictive in silico tools are employed by Australian, Canadian, Danish, European, German, Japanese, Dutch, and US Government organizations [28, 30, 140–144].

QSAR models can be generated for prediction of the following ecotoxicity related properties or effects:

1. Physicochemical properties
2. Toxic potential and potency
3. Environmental distribution and fate in different compartments (air, water and soil) of environment
4. Biokinetic processes (absorption, distribution, metabolism, and excretion) of pharmaceuticals and their metabolites

Areas where QSARs can be applied by governmental regulatory agencies are as follows:

1. Prioritization of existing pharmaceuticals for toxicity testing to environment.
2. Classification and labeling of new pharmaceuticals according to their safe use.
3. Risk assessment of new and existing pharmaceuticals.
4. Guiding experimental design of regulatory tests or testing strategies.
5. Providing mechanistic information
6. Filling up the large data gaps.
7. Building a proper database of each pharmaceutical to different species regarding environmental toxicity.
8. Development of expert systems for each therapeutic classes for different compartments of the environment.
9. Construction of efficient interspecies models to extrapolate data from one species to another species when data of a particular species is absent.

Global regulatory authorities and agencies [28, 30, 140–144] for the risk identification, risk assessment and finally risk management of ecotoxicity pharmaceuticals are listed in Table 2.

The most common endpoints associated with various test methods proposed under Organization for Economic Co-operation and Development (OECD) are the following ones:

- *Physical-chemical properties:* Most commonly evaluated properties are melting point, boiling point, vapor pressure, octanol–water partition coefficient, organic carbon–water partition coefficient, and water solubility.

Table 2
Global regulatory bodies and agencies for the hazard and risk assessment of ecotoxicity pharmaceuticals

Regulatory agencies	Note
EMEA (European agency for the evaluation of medicinal products)	<p>The EMEA has prepared a draft guideline for the environmental risk assessment of human medicinal products. It demonstrates the scope and legal basis for risk assessment of pharmaceuticals and outlines the general considerations and the recommended step-wise procedure for their risk assessment. As the risks cannot be excluded completely, this guideline outlines precautionary and safety measures to be considered. The guideline is based on the risk assessment paradigms for industrial chemicals and biocides, but it also considers the specific features of pharmaceuticals, e.g., the use of available pharmacological information. Previously environmental risk assessments were mainly based on acute ecotoxicity data, but in recent times EMEA draft has proposed to include pharmacokinetic and pharmacodynamic data for environmental risk assessment. Such an approach is presently also taken within the European Union project ERA Pharm.</p> <p>The phased approach in the environmental risk assessment by EMEA is divided into three different Phases:</p> <ol style="list-style-type: none"> 1. Phase I: <i>Pre-screening</i> and <i>estimation of exposure</i> based only on the drug substance, irrespective of its route of administration, pharmaceutical form, metabolism and excretion. 2. Phase II Tier A: <i>Screening</i> and <i>initial prediction of risk</i>. In this phase, all relevant data should be taken into account, e.g., data on physical-chemical properties, primary and secondary pharmacodynamics, toxicology, metabolism, excretion, degradability, and persistence of the drug substance and/or relevant metabolites. 3. Phase II Tier B: <i>Extended and substance and compartment-specific risk assessment</i>. At the end of Tier B, information from the refined data set is available comprising information on route(s) of excretion; and qualitative and quantitative information on excreted compounds, and possibly additional long-term toxicity data
EU-CSTEE (European Union Commission's scientific committee on toxicity, ecotoxicity and environment)	<p>The CSTEE identified the need for a proactive approach in obtaining data on the environmental effects of pharmaceuticals. Thus, it is recognized that a prioritization procedure needs to be developed for environmental risk assessment of pharmaceuticals, and that this should follow the general scheme for chemicals described in the White Paper for the EU chemicals policy, i.e., REACH guideline, where the uses of QSTRs are stressed. QSTR is the first step in gaining more general knowledge on the risk assessment issue as an alternative to non-animal method. In contrast to the amount of analytical data, information about the ecotoxicological effects of drug residues is scrubby. To create a broader basis for the evaluation of the ecotoxicological relevance of pharmaceutical compounds, proper documentation of their effects and the reason should be identified.</p>

(continued)

Table 2
(continued)

Regulatory agencies	Note
<p><i>US-FDA</i> (US Food and Drug Administration) and <i>CDER</i> (Center for Drug Evaluation and Research)</p>	<p>An assessment of risk to the environment is required for manufacture, use and distribution of human drugs under the National Environment Policy Act of 1969 and an environmental assessment procedure was developed by the FDA as a part of the registration procedure for new human pharmaceutical drugs. Along with it, in 1995, the FDA-CDER issued a new guidance document for the Submission of an Environmental Assessment in Human drugs. In the same year, the US FDA initiated a retrospective review on ecotoxicity data submitted in environmental assessments over the preceding decade. In this respect, in 1997 the FDA implemented a Note for Guidance paper in which all drugs entering the aquatic compartment at levels below 1 µg/l, Predicted Environmental Concentration ($PEC_{EFFLUENT}$) were exempted from a detailed risk assessment.</p>
<p><i>MHLW</i> (The Ministry of Health, Labor and Welfare of Japan)</p>	<p>The MHLW constructed a research group to build up a concept on the regulation of pharmaceuticals for environmental safety in 2007. The regulation system is similar to that of general chemicals in Japan and the Guideline by EMEA. The main function of this group is to establish a risk-benefit analysis committee for the pharmaceuticals which have a high risk for environmental organisms and to human health. The risk assessment is judged by the PEC/PNEC (Predicted Environmental Concentration/Predicted No Effect Concentration) ratio or $\Sigma PEC_i/PNEC_i$. In addition, the Organization for Pharmaceutical Safety and Research (OPSR) conducted compliance reviews on application data. This was followed by the integration of the aforementioned Evaluation Center, OPSR, and part of the Medical Devices Center to form a new independent administrative organization, the Pharmaceutical and Medical Devices Agency (PMDA). The MHLW and PMDA handle a wide range of activities from clinical studies to approval reviews, reviews throughout post-marketing stage, and pharmaceutical safety measures.</p>
<p><i>NICNAS</i></p>	<p>The National Industrial Chemicals Notification and Assessment Scheme (NICNAS) is a statutory scheme administered by the Australian Government Department of Health. NICNAS was established in July 1990 under the <i>Industrial Chemicals (Notification and Assessment) Act 1989</i> (the Act). A range of state, territory and Commonwealth government agencies share regulatory responsibility for chemical safety in Australia, with each chemical being regulated according to its use, whether as a pharmaceuticals, veterinary medicine, pesticide, food additive or industrial chemical. The major responsibility of NICNAS are:</p> <ul style="list-style-type: none"> • Assessing new industrial chemicals for human health and/or environmental effects • Maintaining the Australian Inventory of Chemical Substances (AICS) • Circulation of information on the human health and environmental impacts of chemicals and recommending on their safe use • Registering new industrial chemicals

<p><i>UBA</i> (Federal Environment Agency)</p>	<p>The German Medicines Act provides that the Federal Environment Agency (UBA) is responsible for the environmental risk assessment. The UBA started assessing the environmental impact of veterinary and human pharmaceuticals in an authorization routine in 1998 and 2003, respectively. The UBA already assessed around 180 veterinary and around 240 human pharmaceutical formulations. Filtering concepts established between UBA and the authorization agency responsible for veterinary medicines focused the ERA on antibiotics, parasiticial substances and analgesics. Cytostatic medicines, hormones and contrast agents dominated the human medicine dossiers assessed by UBA.</p>
<p><i>SECS</i> (Swedish Environmental Classification and Information System for pharmaceuticals)</p>	<p>The SECS is an authorized regulatory body which was initiated in 2005 by the Swedish Association for the Pharmaceutical Industry. The rationale of the classification system is to offer the public and health care sectors with environmental information about all active pharmaceutical ingredients (API) on the Swedish market up to till date. In 2004, the Swedish Medical Product Agency (MPA) concluded in a report to the Swedish government that there is a lack of environmental toxicity data for the majority of the APIs available on the Swedish market. To improve risk management decision making, sufficient knowledge about environmental exposures and effects in nontarget species for all relevant pharmaceutical substances is needed. Within SECS, the pharmaceutical companies provide environmental data and classify their products according to predefined criteria and a guidance document. The guidance document is developed for the purposes of SECS, but it is based on the European Medicines Agency (EMA) guideline for environmental risk assessment of pharmaceuticals and the European Commission Technical Guidance Document (TGD).</p>
<p><i>AEA</i> (Australian Environment Agency)</p>	<p>The AEA applies the latest methodologies to environmental risk assessment. It advises clients on the environmental hazards and potential risks associated with the production, use, and disposal of chemicals. AEA has undertaken extensive reports for the Department of Sustainability, Environment, Water, Population and Communities (DSEWPaC), particularly with respect to their environmental assessments performed on new and existing agricultural and veterinary chemicals for the Australian Pesticides and Veterinary Medicine Authority (APVMA), and industrial chemicals for the National Industrial Chemicals Notification and Assessment Scheme (NICNAS).</p>
<p><i>VICH</i> (International Cooperation on Harmonization of Technical Requirements for Veterinary Medicinal Products)</p>	<p>VICH is a trilateral (EU–Japan–USA) program aimed at harmonizing technical requirements for veterinary product registration was officially launched in April 1996. The initiative to begin the harmonization process came about in 1983 when the first International Technical Consultation on Veterinary Drug Registration (ITCVDJR) was held. Veterinary medicinal products (VMPs) are regulated for environment safety as described in Environmental Impact Assessment for VMPs; Phase I in 2000 and Phase II in 2004.</p>

- *Ecological effects on endpoints*: Acute fish-toxicity, long-term toxicity, acute Daphnia toxicity, algal toxicity, terrestrial toxicity, marine organism toxicity, microorganism toxicity in sewage treatment plant.
- *Environmental fate*: Biodegradation, hydrolysis in water, atmospheric oxidation, and bioaccumulation;
- *Human health effects*: Acute oral, acute dermal, acute inhalation, eye irritation, skin irritation, skin sensitization, repeated dose toxicity, genotoxicity, reproductive toxicity, developmental toxicity, systemic toxicity, mutagenicity, carcinogenicity, etc.

OECD's database on risk assessment models:

In silico models that are employed by the OECD countries to predict health or environmental hazards, exposure potential, and probable effects were organized into a searchable database. This database is intended as an information resource only. The models are listed by countries and by the property or effect included. The models can be useful as a screening tool, when there is a lacking of chemical-specific data, for establishing priorities for chemical assessment and for identifying issues of potential concern [140]. Areas of assessment and category of information for predicting human health and environment according to OECD's guidelines are represented in Figs. 4 and 5, respectively.

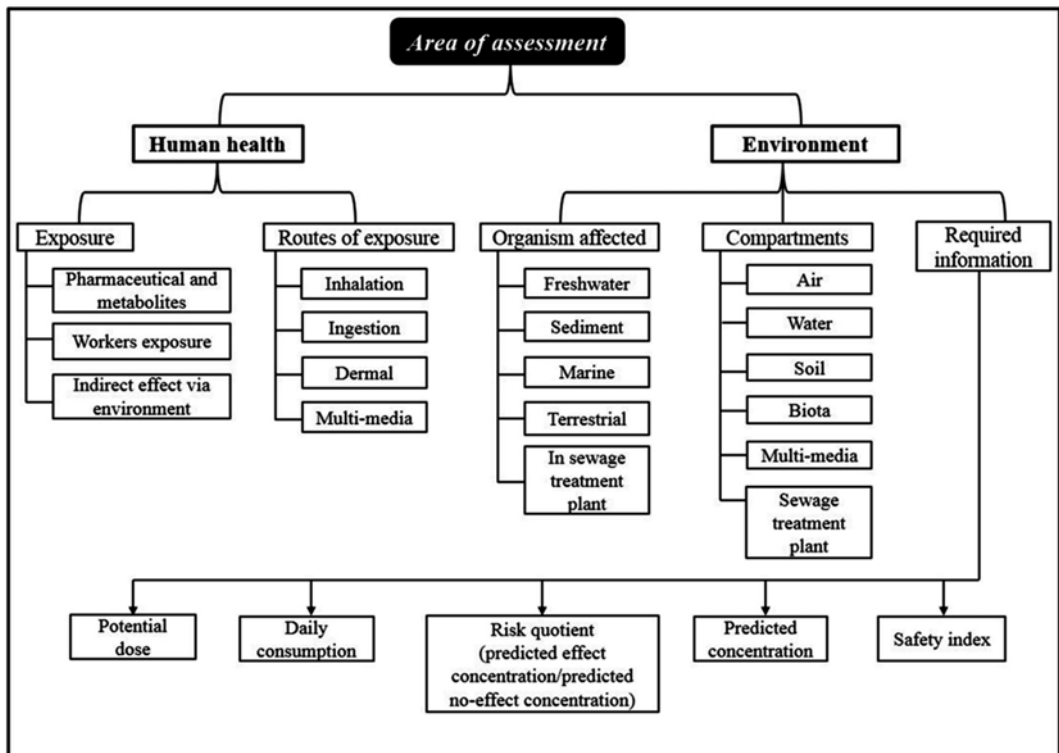


Fig. 4 Areas of assessment and risk models for predicting human health and environment according to the OECD database

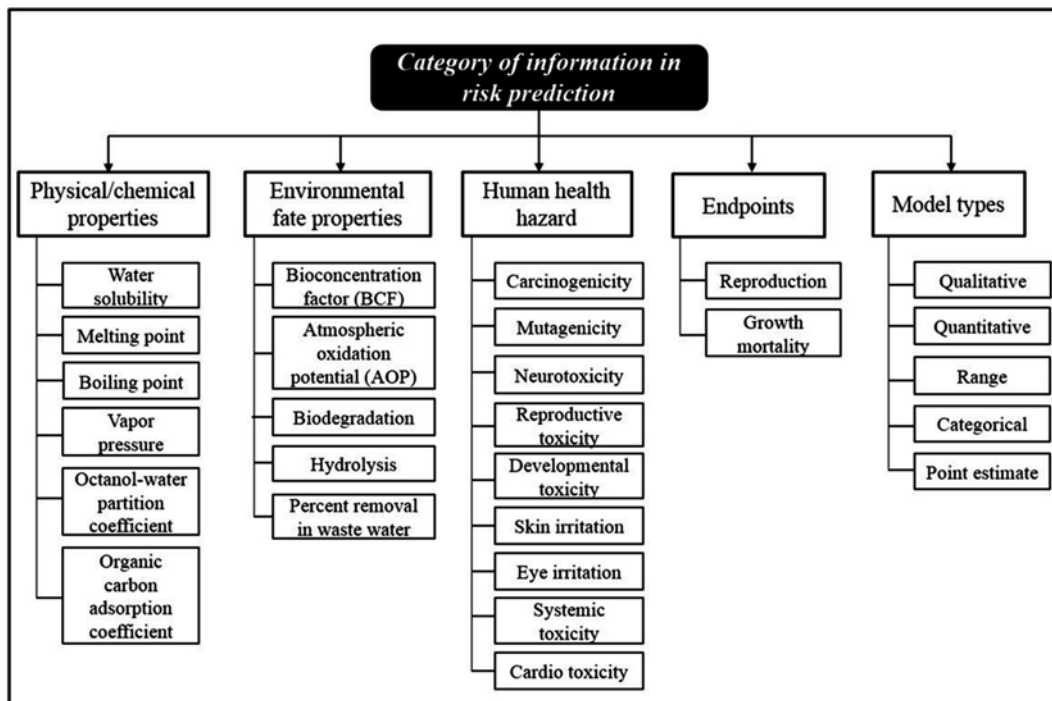


Fig. 5 Category of information included in predicting health and environmental effects according to the OECD guidelines

4 In Silico Modeling of Ecotoxicity Using SAR and QSAR Approaches

The toxic potential of large quantities of industrial chemicals including pharmaceuticals, cosmetics, pesticides and other synthetic or semisynthetic chemicals is often required to be assessed by using standard animal models, comprising the basic test protocol for risk assessments for their approval as a registered product to launch into the market. With increasing concern about the environmental pollution and human health, the manufacture, storage, distribution, and release of these hazardous substances after their application to the environment are controlled and regulated at various levels by different governments and regulatory agencies worldwide. Applications of analogues, SAR and QSAR of different pharmaceuticals are also providing useful information in a regulatory decision making context in the absence of experimental data [140]. Most commonly employed predictive in silico tools are depicted in Fig. 6.

Among the available in silico predictive models for ecotoxicity, majority of the models are constructed employing QSAR techniques. Therefore, in this book chapter, a special importance is given to the discussion of QSAR models. The QSAR approach

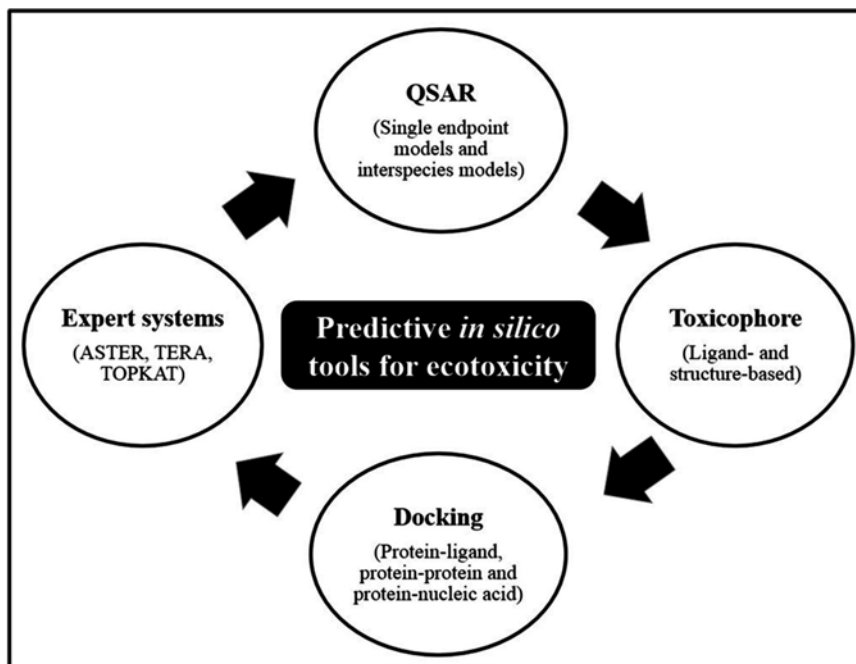


Fig. 6 Predictive in silico tools for the prediction of ecotoxicity of pharmaceuticals

attempts to correlate structural/molecular properties with biological activities/toxicities, for a set of compounds by means of statistical methods. As a result, a simple mathematical relationship is established:

$$\text{Biological activity or toxicity} = f(\text{chemical structure or property}).$$

Applications of QSAR can be extended to any molecular design purpose, prediction of different kinds of biological activities and toxicities, lead compound optimization, classification, diagnosis, and elucidation of mechanisms of drug action, toxicity prediction of environmental toxicants (pollutant pharmaceuticals, chemicals, gas, etc.), and prediction of drug-induced toxicity [145]. The major objective of structure–activity/toxicity relationship modeling is to investigate and identify the decisive factors for the measured activity/toxicity for a particular system, in order to have an insight of the mechanism and behavior of the studied system. For such a purpose, the employed strategy is to generate a mathematical model that connects experimental measures with a set of chemical descriptors determined from the molecular structure for a set of compounds. The derived model should have as good predictive capabilities as possible to predict the studied biological/toxicological or physicochemical behavior for new compounds. The factors governing the events in a biological system are represented by a

multitude of physicochemical descriptors, which can include parameters to account for hydrophobicity, electronic properties, steric effects, and topology, among others [145].

With the constant progress of QSAR techniques, many methods, algorithms, and techniques have been discovered and applied in QSAR studies. The development of a QSAR model follows five major steps:

1. Selection of a dataset with series of known response data
2. Calculation of descriptors
3. Splitting of the dataset into training and test sets for model development and its subsequent validation
4. Construction of models using different chemometric tools, and
5. Validation of the developed model based on internal and external validation statistics

Additionally, the development of 3D-QSAR models includes two more steps for their successful execution: conformation analysis of the molecules and their alignment status with respect to the most active compound. The most important feature for an acceptable and reliable QSAR model is predictive capability for new set of compounds. The predictive quality of the developed model is determined based on different validation statistics. Thus, validation of QSAR models plays the most crucial role in defining the applicability of the QSAR model for the prediction of untested compounds. Initially, verification of the correlation between chemical features of the molecules and the biological activity/toxicity was of prime interest during the development of a QSAR model. Later, the focus gradually shifted toward the predictive power of the model than simply unveiling the quantitative relationships [146].

To validate a QSAR model, one has to follow OECD principles for acceptable predictions in order to make the model as a reliable screening tool for future toxicity prediction of untested pharmaceuticals. A meeting of QSAR experts held in Setúbal, Portugal in March 2002 reported guidelines for the validation of QSAR models for regulatory purposes. The OECD principles were agreed by OECD member countries, QSAR and regulatory communities at the 37th Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology in November 2004. These principles are listed here: Principle 1: a defined endpoint; Principle 2: an unambiguous algorithm; Principle 3: a defined domain of applicability; Principle 4: appropriate measures of goodness-of fit, robustness, and predictivity; Principle 5: a mechanistic interpretation, if possible [147]. Different quality metrics for QSAR models can be categorized into two classes: one determining the fitting ability of the model while the other analyzing the predictive potential of the developed model [146].

4.1 Why In Silico Models Should Be Developed for Ecotoxicity Predictions of Pharmaceuticals?

4.1.1 The 3R Concept

The 3R concept represents three words “Reduction,” “Replacement,” and “Refinement”. The concept brought about an imperative notification about animal experimentation in the scientific communities. The word ‘Reduction’ refers to the diminution in number of animals used to get results of a defined precision. Next, ‘Replacement’ corresponds to the use of nonliving resources to replace conscious living higher animals, and ‘Refinement’ means decline in the severity or cruelty of inhuman methodologies applied to the experimental animals [148]. As a consequence, to establish the 3R concept, in silico techniques are one of the front runners.

4.1.2 Ban of Animal Experimentation

There are different social as well as governmental organizations that consider reduction or complete ban of animal experimentations [149]. Here, we have listed a few of them:

1. The European Centre for the Validation of Alternative Methods (ECVAM) was established in the year 1991 that agrees the principle of 3Rs.
2. The European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Procedures.
3. Council Directive 86/609/EEC on the Approximation of Laws, Regulations and Administrative Provisions of the Member States Regarding the Protection of Animals Used for Experimental and Other Scientific Purposes.
4. Johns Hopkins Center for Alternatives to Animal Testing (CAAT), a US based organization focussing on the reduction of animal experimentations.
5. The testing ban on the finished cosmetic products applies since 11 September 2004; the testing ban on ingredients or combination of ingredients applies since 11 March 2009. The marketing ban applies since 11 March 2009 for all human health effects with the exception of repeated-dose toxicity, reproductive toxicity, and toxicokinetics. For these specific health effects, the marketing ban applies since 11 March 2013, irrespective of the availability of alternative non-animal tests [150].
6. India and Israel have also banned animal testing for cosmetic products, while the USA has no such ban in place [151].
7. China is the only major market where testing all cosmetics on animals is required by law, and foreign companies distributing their products to China must also have them tested on animals. [152] China has announced that its animal testing requirement will be waived for shampoo, perfume, and other so-called “non-special use cosmetics” manufactured by Chinese companies after June 2014. “Special use cosmetics,” including hair regrowth, hair removal, dye and permanent wave products, antiperspirant, and sunscreen, will continue to warrant mandatory animal testing.

4.1.3 Regulatory Decision Making

SARs and QSARs are employed to predict aquatic toxicity, physical or chemical properties, and environmental fate parameters as well as to predict specific health effects of organic chemicals by Australian, Canadian, Danish, European, German, Japanese, Dutch, and US Government organizations.

4.1.4 Filling Data Gaps

Acceptable toxicity data of pharmaceuticals to environment and human health is considerably less than 5 % [153]. Computer-aided prediction has the competency to assist in the prioritization of pharmaceuticals for testing, and for predicting specific toxicities to allow for classification. As the number of reliable models for toxicity predictions is increasing, they can be employed as one of the major sources for filling the missing data of pharmaceutical toxicity to ecosystem.

4.1.5 Development of Understanding of Biology and Chemistry

In the modeling of acute toxicological endpoints, much has been gained regarding mechanisms of action. For many modeling approaches, it may be assumed that compounds fitting the same QSAR are acting by the same mechanism of action. This has allowed workers to define the chemical domain of certain mechanisms. There are countless examples where knowledge of biology and chemistry has been advanced by modeling in the field of toxicological and fate effects [154].

4.1.6 Cost and Time Reduction

Toxicity study is very costly in terms of the animals employed for testing and time taken. Even a simple ecotoxicological assay may cost several thousand dollars, and a 2-year carcinogenicity assay may cost several million dollars. Cost is a clear issue to fill the data gaps for the many new compounds that have not been tested. On the other hand, prediction of various toxicity endpoints for pharmaceuticals at an early stage of design can save a large amount of expenses for such compounds which may be found toxic at a later stage of drug development program [155].

4.1.7 Identification of New Toxicological Problems

The development of computational techniques not only allows for the prediction of the potential risk of pharmaceuticals but also allows for rational direction to be given to the testing programs.

5 Review of Literature on In Silico Ecotoxicity Modeling of Pharmaceuticals

Kar and Roy [156] have constructed robust quantitative interspecies toxicity correlation models for *Daphnia magna* and fish evaluating the ecotoxicity of structurally diverse 77 pharmaceuticals. They have demonstrated that the keto group and the $\text{Y}(\text{C})$ (aasC) fragment are principally responsible for higher toxicity of pharmaceuticals to *D. magna*. On the other hand, for fish toxicity, along with the keto group, structural fragments like $\text{X}=\text{C}=\text{X}$, $\text{R}-\text{C}(=\text{X})-\text{X}$, and

$R-C\equiv X$ are largely responsible for the toxicity. The interspecies models were also used to predict fish toxicities of 59 pharmaceuticals (for which *Daphnia* toxicities were present) and *Daphnia* toxicities of 30 pharmaceuticals (for which fish toxicities were present). They established that the interspecies correlation study would permit an improved and inclusive risk assessment of pharmaceuticals for which toxicity data was missing for a particular endpoint.

Das et al. [157] attempted to develop interspecies correlation models taking rodent toxicity as dependent variable so that any drug without reported rodent toxicity can be predicted using fish, *daphnia*, or algae toxicity data which can be further extrapolated to human toxicity. Interspecies extrapolation QSAR models were developed employing multiple validation strategies. Analyzing the models, the authors concluded that heteroatom atom count and charge distribution were significant determinants of the rodent toxicity, and that the atom level $\log P$ contributions of various structural fragments and various extended topochemical atom (ETA) indices reflecting electronic information and branching pattern of molecules were important determinants for the rodent toxicity. In addition, from interspecies aquatic toxicity modeling, it was established that apart from the algae toxicity, atom level $\log P$ contributions of different fragments, charge distribution, shape, and ETA parameters were important in describing the *daphnia* and fish toxicities in the interspecies correlation models with algae toxicity. The toxicity of chemicals to rodents bears minimum interspecies correlation with other mentioned nonvertebrate and vertebrate toxicity endpoints.

The acute toxicity was predicted (>92 %) using a generic quantitative structure–toxicity relationship (QSTR) model developed by Sanderson and Thomsen [158] suggesting a narcotic mechanism of action (MOA) of 275 pharmaceuticals. An analysis of model prediction error suggests that 68 % of the pharmaceuticals have a nonspecific MOA. Authors have compared the measured effect data to the predicted effect concentrations using ECOSAR regarding the predictability of ecotoxicity of pharmaceuticals and accurate hazard categorization relative to Global Harmonized System (GHS). Molecules were predicted using the model resulting in 71 % algae, 74 % *daphnia*, 83 % fish datasets that could be compared.

Escher et al. [159] constructed QSAR models with the total toxic potential of mixtures of the β -blockers and related human metabolites for the phytotoxicity endpoint. They have assumed two scenarios for this study. In the first scenario, the metabolites lose their explicit activity and act as baseline toxicants. In the second scenario, the metabolites reveal the identical specific mode of action like their parent drug. β -Blockers are secondary amines and are, therefore, fully protonated at environmental pH. The authors accounted for their positive charge in the QSAR analysis and have

experimentally determined the liposome–water partition ratios at pH 7 to make QSAR analysis more robust.

Berninger and Brooks [160] considered the mammalian Acute to Therapeutic Ratio (ATR) to predict pharmaceuticals which may result in comparatively high Acute to Chronic Ratio (ACR) in fish models. The authors identified a statistically significant relationship between mammalian ATRs and fish ACRs ($p < 0.001$, $r^2 = 0.846$). In this model, they only included chronic responses of fish to pharmaceuticals which appear to have been elicited through a therapeutic MOA for calculating ACRs and for statistical analysis of the relationship with mammalian ATRs. Utilizing this approach, mammalian ATR values can be used for predicting pharmaceuticals with higher fish ACRs if the chronic response used in ACR calculation is reasonably linked to the therapeutic MOA of a pharmaceutical.

Sanderson et al. [161] employed the US EPA generic aquatic (Q)SAR model ECOSAR to screen more than 2800 pharmaceuticals and provided a baseline to fill the screening data regarding parent pharmaceuticals environmental toxicity. The model can be used to predict both acute and chronic aquatic toxicity.

Sanderson and Thomsen [162] overestimated the toxicity for 70 % of the 59 pharmaceuticals by ECOSAR v3.20 which contains both measured and modeled data. For the remaining 30 % pharmaceuticals, more than 94 % of the predictions underestimated toxicity by less than a factor of 10. This is an indication that a narcosis based model is conservative relative to experimental values around 70 % of the time, thus implying that for at least 70 % of the Active Pharmaceutical Ingredients (APIs), the acute mode of action (MOA) can be elucidated by baseline toxicity. The authors have observed determination coefficients (r^2) ranging from 0.73 to 0.76 between all the modeled Log EC₅₀ and Log K_{ow}. The slopes of the Log EC₅₀–Log K_{ow} regressions based on measured data from the USA National Oceanic and Atmospheric Administration (NOAA) database for both fish and daphnia equal -0.86 which suggest a narcotic MOA.

Lienert et al. [163] assessed the ecotoxicological risk potential of 42 pharmaceuticals from 22 therapeutic classes, including metabolites formed in humans. They considered each parent drug and its metabolites as a mixture of equally acting compounds, and in case when effect data were missing, they estimated these with QSAR models. They have collected data on the identity and excretion pathways of human metabolites and, where available, experimental ecotoxicity data (EC/LC₅₀) from pharmaceutical compilations and from diverse literature sources. They have compiled physicochemical data like structure, molecular weight, octanol–water partition coefficient K_{ow}, acidity constant pK_a mainly from the Physical Properties Database (<http://www.syrres.com/esc/physprop.htm>). Moreover, they have generated a risk quotient

($RQ_{mixture}$) using simple predictions of drug concentrations in wastewater which can be useful for risk assessment of pharmaceuticals.

Christen et al. [164] developed VirtualTox Lab [165] to predict the effects of pharmaceuticals in the aquatic system. The study leads to the inference that the mode of action perception is most appropriate for the identification of highly active compounds (HC). As suggested by the authors, modification can be done by balancing this concept by the QSAR model (VirtualTox Lab), whereas the fish plasma model seemed to be less apposite due to the requirement of environmental concentration above 10 ng/l for the identification of a risk. The practice of the VirtualTox Lab will support the mode of action concept and may be beneficial to recognize surplus targets of the pharmaceutical to assess the ecotoxicity.

Escher et al. [166] predicted baseline toxicity of the 100 molecules using established QSARs for algae, daphnia, and fish. The QSARs were selected from the Technical Guidance Document of the EU. The logarithm of D_{lipw} (liposome water distribution coefficient) was employed in the model development for baseline toxicity to calculate the toxicity of the compound towards the stated species.

The environmental risk assessment of 26 pharmaceuticals and personal care products have been performed by De García et al. [167] based on the ecotoxicity values generated by bioluminescence and respirometry assays. Then the compounds were classified following the Globally Harmonized System of Classification and Labelling of Chemicals by predictions using the US EPA ecological structure–activity relationship (ECOSAR™). The real risk of impact of these pharmaceuticals in wastewater treatment plants (WWTPs) and in the aquatic environment was predicted according to the criteria of the European Medicines Agency. According to their studies, in at least two ecotoxicity tests, 65.4 % of the PPCPs showed prominent toxicity to aquatic organisms. Their study showed some type of risk for the aquatic environments and/or for the activated sludge of WWTPs for pharmaceuticals like acetaminophen, ciprofloxacin, clarithromycin, clofibrate, ibuprofen, omeprazole, triclosan, parabens, and 1,4-benzoquinone.

Here we have discussed available *in silico* models on ecotoxicity of pharmaceuticals. Due to the limited availability of the *in silico* models on ecotoxicity of pharmaceuticals, there is a need to develop more *in silico* models in order to reduce time and cost involvement as well as reduction of animal usage in getting relevant data and for better and fast risk assessment of pharmaceuticals. It is not possible to experimentally study toxic effects of each pharmaceutical in different species. Most active pharmaceutical ingredients have available rodent toxicity information. As a result, if this data could be extrapolated or modeled to different other species,

this would be a noteworthy resource for prioritization of pharmaceuticals with regards to diverse environment hazards. However, very limited papers have been published on interspecies models to predict environmental toxicity for pharmaceuticals, and there are relatively few statistical models available to bridge the chronic toxicity data information gap [168, 169].

6 Endpoints

Toxicity of a molecule should be assayed on specific toxicity endpoints for the generation of data which are employed commonly to develop in silico models. This why a clear concept is required about the endpoints or test batteries as they are employed for the experimental toxicity studies and for understanding the mode of toxicity with respect to that particular endpoint [110]. We list the most commonly employed endpoints for this purpose in Table 3.

7 Databases

A good quality of ecotoxicological data of pharmaceuticals and their metabolites is required for the development of accurate and reproducible in silico models. A significant number of chemical/drug/agrochemical/pesticide toxicity databases towards environment are publicly accessible, and such numbers are growing. But one cannot deny that the existing databases are very few compared to drug discovery compound libraries. Recent initiatives requiring superior use of in silico technologies have called for transparency and expansion of toxicity database information that is available to the public at no cost. Table 4 represents publicly available toxicity databases describing environmental as well as human health effects of pharmaceuticals useful in risk assessment, risk management, safety evaluation, and hazard characterization.

8 Expert Systems

Expert systems allow for the direct entry of a structure into software followed by the calculation or prediction without the requirement to compute descriptors and re-perform the modeling process. This makes expert system a more convenient option for toxicity prediction over traditional QSARs. Expert systems have been frequently employed by regulatory agencies, academia and industries worldwide for more efficient and fast prediction. The foremost criterion of toxicity prediction is to differentiate between toxicologically active and inactive molecules. Multiple mechanisms can lead to the identical toxic effect and this intricacy requires the

Table 3
Most commonly employed test batteries (endpoints) for the modeling of ecotoxicity

Endpoints	Species	Portrayal
Algae	<i>Chlorella vulgaris</i>	Chlorella is a unicellular green alga comprising a major component of the phytoplankton.
	<i>Chlorella pyrenoidosa</i>	One of the prime producers of the aquatic ecosystem and ideal test organisms for toxicological studies. Ecotoxicity is measured by growth rate inhibition of green alga <i>P. subcapitata</i> .
	<i>Pseudokirchneriella subcapitata</i> (<i>Selenastrum capricornutum</i>)	
	<i>Scenedesmus obliquus</i>	<i>Scenedesmus obliquus</i> (Chlorophyta, Chlorococcales) is a common cosmopolitan green alga, often occurring as almost a pure culture in fresh water plankton. It can grow in industrial wastewaters of different origins showing good adaptation ability and it is a very versatile microalga as a test battery.
	<i>Scenedesmus vacuolatus</i>	A green alga of the Chlorophyceae. It is colonial and non-motile. The species has been used in the prediction of photoinduced toxicity of polycyclic aromatic hydrocarbons by in silico modelers. Also, Predictive modeling studies has been performed for the ecotoxicity of ionic liquids (ILs) towards the <i>Scenedesmus vacuolatus</i> .
Bacterium	<i>Escherichia coli</i>	A gram-negative, facultatively anaerobic, rod-shaped bacterium of the genus <i>Escherichia</i> . <i>E. coli</i> is frequently used as a model organism in microbiology and modeling studies.
	<i>Vibrio fischeri</i>	A gram-negative rod-shaped bacterium found globally in marine environments which has bioluminescent properties and is found predominantly in symbiosis with various marine animals. Predominantly employed in the research of microbial bioluminescence, quorum sensing, and bacterial–animal symbiosis.
	<i>Bacillus flexus</i> , <i>Pseudomonas fluorescens</i> and <i>Vibrio natriegens</i>	They are good model systems for studying marine biofouling.

Crustaceans	<i>Daphnia magna</i> <i>Daphnia pulex</i> <i>Daphnia ambigua</i> <i>Daphnia melanica</i> <i>Thamnocephalus platyurus</i>	<i>Daphnia</i> are members of the order Cladocera , and are one of the small aquatic crustaceans commonly called water fleas . These invertebrate species in aquatic food webs have been used as a representative test species for ecotoxicological evaluation of industrial chemicals. Generally, immobilization test is done by <i>Daphnia</i> . Small crustaceans employed as a biological species for ecological toxicity testing of chemicals.
Duckweed	<i>Lemna minor</i> <i>Lemna gibba</i>	<i>L. minor</i> , one form of aquatic vascular plant, is most commonly used among duckweeds for in silico models. Growth inhibition tests of duckweeds are used to identify the chemical toxicity. <i>Lemna gibba</i> is used in testing the phytotoxicity of pesticides and other environmental chemicals to higher plants.
Enzyme	Acetylcholinesterase	It plays the most important role in autonomic nervous system function. It catalyzes the hydrolysis of acetylcholinesters with a relative specificity for acetylcholine. Commonly, (a) enzyme inhibition data of the acetylcholinesterase from electric eel (<i>Electrophorus electricus</i>), (b) the AMP deaminase and (c) the antioxidant enzyme system of mouse liver are important for toxicity prediction and in silico model development.
Fish	Channel Catfish Ovary (CCO) Zebrafish (<i>Danio rerio</i>) Fathead minnow (<i>Pimephales promelas</i>)	CCO is the cell line of choice for the propagation and diagnosis of Channel Catfish Virus (CCV). It is the standard for diagnosing Channel Catfish Virus Disease (CCVD) in farm reared Channel Catfish. Prediction of ILs has been performed by using this endpoint in recent time. Zebrafish plays an important role in ecotoxicology as a prominent model vertebrate. It is standardized under the OECD and is employed to test chemicals, pharmaceuticals as well as industrial effluents. <i>Pimephales promelas</i> is the EPA recommended vertebrate species for freshwater chronic toxicity tests. In these tests, larvae are exposed for 7 days to different concentrations of effluent or to receiving water. Test results are based on the survival and weight of the larvae. As the fathead minnow is fairly tolerant of harsh conditions, it can be found in bodies of water that may be uninhabitable to other fish, such as waste drainage sites. It has also been studied to investigate the effects of these waste materials on the aquatic life. Effects induced by progestins are largely studied employing Fathead minnow.

(continued)

Table 3
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Endpoints	Species	Portrayal
Mammalian cells	Human keratinocyte cell line (HaCaT)	HaCaT cells are a spontaneously immortalized, human keratinocyte line that has been widely used for studies of skin biology and differentiation. In recent times, this cell line is modeled for the cytotoxicity prediction of metal oxide by different group of researchers.
	CaCo-2	A continuous cell of heterogeneous human epithelial colorectal adenocarcinoma cells. Permeability coefficients across the cellular membranes of CaCo-2 cells are generally employed for modeling.
	HeLa	A prototypical cells of the human epithelium used in scientific research. It is the oldest and most commonly used human cell line which was derived from cervical cancer cells. Largely employed for anticancer activity.
	Prostate cancer cell line (PC3)	A human prostate cancer cell line used in modeling of prostate cancer inhibitors.
	Human malignant melanoma (Fem-X)	Not so popular, but recently used by group of authors to derive QSAR models.
	HT-29	HT-29 is a human colorectal adenocarcinoma cell line with epithelial morphology. These cells are sensitive to the chemotherapeutic drugs used in standard treatment for colorectal cancer.
	Rat cell line—IPC-81	Promyelocytic leukemia rat cell line IPC-81 is frequently used in cytotoxicity assays of ILs.
Protozoa	<i>Tetrahymena thermophila</i> <i>Tetrahymena pyriformis</i>	Free-living unicellular ciliated protozoa and one of the largely employed endpoint for the assessment of the environmental toxicity.
Tadpoles	<i>Bufo vulgaris formosus</i> <i>Rana japonica</i> (Japanese brown frog)	Tadpoles, a common and sensitive species, the larva of the frogs. They are typical amphibious animals bridging the gap between aquatic and terrestrial animals. They are recurrently used for toxicity testing purposes and risk assessments and have been recommended by the EU-TGD.
Yeast	<i>Saccharomyces cerevisiae</i>	One form of budding yeast and one of the most intensively studied eukaryotic model organisms in molecular and cell biology. Small in size, accessible, reproduction time quick, and potentially economic. Considered as important species for ecotoxicity prediction.

Table 4
List of available databases comprising information of the ecotoxicity due to pharmaceuticals^a

Database name	Web accessibility	Information
ACToR	http://actor.epa.gov/actor/faces/ACToRHome.jsp	Publicly available data on industrial chemicals, pesticides and drinking water contaminants by US EPA National Center for Computational Toxicology. The database consists of chemical structure, physicochemical values, and provides in vitro and in vivo toxicology data
BDSM	http://systemsanalysis.louisville.edu/	Developmental toxicity database and open-source software for discovery of developmental toxicants by University of Louisville
Cal/EPA	http://www.oehha.ca.gov/risk/ChemicalDB/index.asp	State of California EPA Toxicity Criteria Database of chronic reference exposure levels and cancer potency information
CCRIS	http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS	Chemical carcinogenesis research information system (CCRIS) created by US National Library of Medicine (NLM) contains carcinogenicity and mutagenicity test results for over 8000 chemicals
CPDB	http://potency.berkeley.edu/	Carcinogenic potency database (CPDB) is a widely used resource of the results of 6540 chronic, long-term animal cancer tests on 1547 chemicals developed by University of California, Berkeley. The CPDB provides easy access to the bioassay literature and analyses of experiments that have been published over the past 50 years in the general literature through 2001 and by the National Cancer Institute/National Toxicology Program through 2004
Danish (Q)SAR Database	http://ecbQSTR.jrc.ec.europa.eu/	Repository of estimates from over 70 QSTR models and health effects for 166,072 chemicals created by Danish EPA
DEMETERA	http://www.demetra-tox.net/	DEMETERA is a EC-funded project in which a huge number of QSTR models have been developed for the prediction of different ecotoxicological endpoints namely rainbow trout LC ₅₀ (96 h), water flea LC ₅₀ (48 h), honey bee LD ₅₀ (48 h)
DevTox	http://www.devtox.org	Developmental toxicity study data and control database for various strains of common laboratory animals

(continued)

Table 4
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Database name	Web accessibility	Information
DSSTox	http://www.epa.gov/ncct/dsttox/index.html	Distributed Structure-Searchable Toxicity database (DSSTox) developed by National Center for Computational Toxicology, US EPA. It is a network of downloadable, structure-searchable, standardized chemical structure files associated with toxicity data
ECOTOX	http://cfpub.epa.gov/ecotox/	Another USEPA database of single chemical toxicity information for aquatic and terrestrial life
ESIS	http://ecb.jrc.ec.europa.eu/esis/	European Chemical Substances Information system (ESIS) provides information on chemicals related to risk and safety
EXTOXNET	http://extoxnet.orst.edu/ghindex.html	EXTension TOXicology NETwork is a cooperative effort of University of California-Davis, Oregon State University, Michigan State University, Cornell University, and the University of Idaho. Databases related to pesticide toxicology, Pesticide Information Profiles (PIPs) and Toxicology Information Briefs (TIBs) can be obtained
GAC	http://www.niehs.nih.gov/research/resources/databases/gac/index.cfm	Genetic Alterations in Cancer (GAC) is a database that quantifies specific mutations found in cancers induced by environmental chemicals. Its created by US NIH/NIEHS
GAP	http://www.ils-inc.com/services/information-sciences	US EPA and IARC Genetic Activity Profile (GAP) database; it provides quantitative genotoxicity results of ~500 chemicals to support hazard classification of human carcinogens
Gene-Tox	http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?GENETOX	Created by US NLM, consists of genetic toxicology test data for over 3000 chemicals
HERA	http://www.heraproject.com/RiskAssessment.cfm	Human and Environmental Risk Assessment (HERA) is a voluntary industry program in Brussels, Belgium. The database consists of toxicity assessment on ingredients and household cleaning products; toxicity and risk data on ingredients supplied and formulated by European manufacturers
Household Products Database	http://householdproducts.nlm.nih.gov/	Database with MSDSs of household products with health effects ratings and produce chemical information

IARC Monograph	http://monographs.iarc.fr/	International Agency for Research on Cancer Monograph (IARC) is developed by World Health Organization (WHO) which identifies environmental factors that can increase the risk of human cancer. These include chemicals, complex mixtures, occupational exposures, physical agents, biological agents, and lifestyle factors
IRIS	http://cfpub.epa.gov/ncea/iris/index.cfm	Integrated Risk Information System (IRIS) is under National Center for Environmental Assessment (NCEA), US EPA. The database is a compilation of electronic reports on 540 environmental chemical substances and their potential to cause human health effects
ISSCAN	http://www.iss.it/ampp/dati/cont.php?id=233&lang=1&tipo=7	ISSCAN is a database on chemical carcinogens (long-term carcinogenicity bioassay on rodents) created by Istituto Superiore di Sanità, Italy
ITER	http://www.tera.org/iter/	International Toxicity Estimates for Risk (ITER) is developed by TERA (Toxicity Excellence for Risk Assessment). The database consists of human health risk values and cancer classifications for over 600 environmental chemicals
IUCLID	http://iuclid.echa.europa.eu/	International Uniform Chemical Information Database (IUCLID) is a software application to capture, store, maintain and exchange data on intrinsic and hazard properties of chemical substances
JECDB	http://dra4.nihs.go.jp/mhlw_data/jsp/SearchPageENG.jsp	JECDB is a Chemical Toxicity Database by Japanese Ministry of Health, Labor and Welfare which contains toxicity test reports of environmental chemicals
JRC QSTR Database	http://ecb.jrc.ec.europa.eu/QSTR/background/	European Commission, Joint Research Centre's database of REACH relevant QSTRs
KATE	http://kate.nies.go.jp	KAshinhou Tool for Ecotoxicity (KATE) is created by Japanese National Institute for Environmental Studies (NIES), Ministry of the Environment (MoE), Government of Japan. It uses a structural domain named C-judgement and performs categorization of chemicals as potential hazards
LAZAR	http://lazar.in-silico.de/	Structure-Activity Relationships database provides QSTR predictions for liver toxicity, mutagenicity, and carcinogenicity

(continued)

Table 4
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Database name	Web accessibility	Information
MRL	http://www.atsdr.cdc.gov/mrls/index.html	Minimal Risk Levels for hazardous substances (MRL) is developed by US DHHS/ATSDR. The MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse non-cancer health effects over a specified duration of exposure
N-class database, Kemi	http://apps.kemi.se/nclass/	Based on substance environmental hazard data (available), the N-Class database calculates aquatic classifications of preparations both according to Directive 1999/45/EEC and to the Globally Harmonized System (GHS)
NTP	http://ntp.niehs.nih.gov/	National Toxicology Program initiated by US NIH/NIEHS
OECD HPV database	http://cs3-hq.oecd.org/scripts/hpv/	It stores at least a minimum set of data (including acute aquatic toxicity) necessary to determine a potential hazard. Compounds are searchable by chemical name, CAS, sponsoring country and stage
RAIS	http://rais.ornl.gov/	Risk Assessment Information System (RAIS); it deals with chemical-specific toxicity values Sponsored by the US Department of Energy (DOE), Office of Environmental Management, Oak Ridge Operations (ORO) Office through a contract between Bechtel Jacobs Company LLC and the University of Tennessee
Riskline, Kemi	http://apps.kemi.se/riskline/	It contains information on both environment and health. The database is produced by the Swedish Chemicals Inspectorate, Sweden
RITA	http://www.item.fraunhofer.de/remi/public/rita/index.php	Registry of Industrial Toxicology Animal-data (RITA) is generated by Fraunhofer Institute of Toxicology and Experimental Medicine (ITEM) Hannover for comparing and interpreting rodent carcinogenicity studies and tumor data. As per May 2011, RITA includes total toxicity case-studies of 60,896 on rat and 25,335 on mice
SCOGS	http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASSubstancesSCOGSDatabase/default.htm	Database resource on toxicology and safety reports made by the selected Committee on GRAS Substances by US FDA/CFRAN

STITCH	http://stitch.embl.de/	Search Tool for Interactions of Chemicals (STITCH) is developed at the Novo Nordisk Foundation Center for Protein Research; University of Copenhagen, European Molecular Biology Laboratory, the Swiss Institute of Bioinformatics, the Biotechnology Center (BIOTEC) of the Technische Universität Dresden and the University of Zurich. It is a knowledge database to explore known and predicted interactions between proteins and small-molecule chemicals for understanding of molecular and cellular functions. Contains interactions for over 74,000 small molecules and over 2.5 million proteins in 630 organisms
TEXTRATOX	http://www.vet.utk.edu/TEXTRATOX/index.php	TEXTRATOX is a product of the University of Tennessee Institute of Agriculture. It is a collection of aquatic toxic potency data for more than 2400 industrial organic compound
TOXNET	http://toxnet.nlm.nih.gov/	Databases on toxicology, hazardous chemicals, environmental health, and toxic releases by US NLM
ToxRefDB	http://www.epa.gov/ncct/toxrefdb/	Database of standard toxicity test results for pesticides and other environmental chemicals including acute, subchronic, chronic, reproductive, and developmental toxicity in support of the ToxCast program by US EPA
Toxtree	http://ecb.jrc.ec.europa.eu/QSTR/QSTR-tools/index.php?c=TOXTREE	Open-source application that places chemicals into categories and predicts various kinds of toxic effects by applying decision tree approaches created by European Commission, Joint Research Centre
TSCATS	http://www.ntis.gov/products/ots.aspx	Toxic Substances Control Act Test Submissions (TSCATS) is an online database of chemical testing results and adverse effects of chemicals on health and ecological systems constructed by US Department of Commerce National Technical Information Service Alexandria, Virginia. The collection currently exceeds 25,000 titles of studies that are submitted to the US Environmental Protection Agency by US Industry under several section of the Toxic Substance Control Act
USGS	http://137.227.231.90/data/acute/acute.html	US Geological Survey (USGS), Columbia Environmental Research Center developed the database for the aquatic acute toxicity tests
WikiPharma	www.wikipharma.org	WikiPharma provides an easily accessible, comprehensive and up-to-date overview of effects caused by pharmaceuticals on nontarget organisms developed within the Swedish research program MistraPharma (www.mistrapharma.se). The database currently contains basic information for 831 active pharmaceutical ingredients (APIs) representing 35 different drug classes. Effect data have been identified and included for 116 of these substances and ecotoxicity test data have been extracted from 156 different sources

^aNote that many database contains pharmaceuticals, organic pollutants, agrochemicals and pesticides under the categories of chemicals

accessibility of predictive tools that are able to discriminate manifold regions in the activity space. This necessitates the development of so-called expert systems, which try to cover broader structural and activity regions in comparison to the local models. Table 5 summarizes different freely available and commercial expert systems to predict endpoints related toxicity predictions.

9 Green and Ecological Pharmacy

The role of green chemistry and principles are very important for risk management of pharmaceuticals. The principles of green chemistry state that the functionality of a chemical should not only comprise the properties of a chemical essential for its application, but also quick and trouble free degradability after its usage. Improvement of synthesis and renewable feedstock are very important issues for preparation of environment friendly pharmaceuticals. Employing these principles and the awareness of green chemistry to pharmaceuticals are necessary [138]. In this perspective, a system called “benign by design” can be considered which means easy degradability after application is considered even before a pharmaceutical’s synthesis. This approach is not completely new. For instance, it is a general practice during the development of pharmaceuticals that adverse side effects are to be taken into consideration. This can also result in economic rewards in the long run and will fit into green pharmacy [170]. But one has to note that a pharmaceutical may also lose its specific therapeutic action due to the structural modification while introducing green chemistry. However, this approach can be employed at least for the optimized and new synthesis routes [170]. Again, it is true that finding good lead compounds is a major task even without considering the environment toxicity issue. However, there is no requirement to find a new lead compound at first. The modification of known lead structures can be the best option to do. Responding to the green and justifiable pharmacy challenge may also result in new marketing opportunities with help of appropriate and scientific research within industry and academia.

10 Overview and Conclusion

A huge number of reports and publications have been published in the last decade about the ecotoxicity due to human and veterinary pharmaceuticals, but it is still too meager to permit us to execute a systematic and precise risk assessment and appropriate risk management. There is still a huge need of filling the missing data gaps in our knowledge. Due to biologically active and persistence nature of pharmaceuticals, they are one of the most serious threats to

Table 5
A complete list of expert systems to predict various endpoints of ecotoxicity

Expert system	Website	Explanatory note
<i>ASTER</i> (Assessment Tools for the Evaluation of Risk)	http://www.epa.gov/med/Prods_Pubs/aster.htm	ASTER is an integration of ECOTOX database and a structure–activity based expert system. ASTER is freely available to provide high quality data for discrete chemicals, when available in the associated databases (i.e., ECOTOX and EcoChem), and QSTR models estimate when data are lacking.
<i>CAESAR</i> (Computer Assisted Evaluation of industrial chemical Substances According to Regulations)	http://www.caesar-project.eu/	CAESAR is an EC funded project which is specifically dedicated to develop QSTR models for the REACH legislation. Five endpoints considered under CAESAR are bioconcentration factor, skin sensitization, carcinogenicity, Mutagenicity, developmental toxicity.
<i>DEREK</i> (Deductive estimation of risk from existing knowledge)	http://www.lhasalimited.org/index.php?cat=2&sub_cat=64	DEREK, a Knowledge-based expert system, developed in collaboration with industrial partners, which makes its predictions based on structural alerts, reasoning rules and examples contained within its knowledge base. Currently 21 structural alerts for teratogenicity, or teratogenic endpoints are considered under this expert system.
<i>DfW</i>	–	Knowledge-based expert system created with knowledge of structure–toxicity relationships. It consists of 361 alerts covering a wide range of toxicological endpoints. The skin sensitization knowledge base in DfW was initially developed in collaboration with Unilever in 1993 using its historical database of GPMT data for 294 chemicals. The DfW version 9.0.0 contains 64 alerts for skin sensitization.
<i>ECOSAR</i> (Ecological Structure–Activity Relationships)	http://www.epa.gov/oppt/exposure/docs/episuitecdl.htm	ECOSAR is freely available from the US EPA which utilizes a number of class-specific $\log K_{ow}$ -based QSTRs to predict the toxicity (both short-term and long-term) of chemicals. Hazard assessment of environmentally occurring pharmaceuticals to fish, daphnids, and green algae can be performed employing ECOSAR.

(continued)

Table 5
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Expert system	Website	Explanatory note
<i>HazardExpert Pro</i>	http://www.computdrug.com/	Teratogenicity, reproductive toxicity predicted based on structural fragments.
<i>MCASE/MC4PC</i>	http://www.multicase.com/products/products.htm	MCASE is a knowledge-based commercial system which develops QSTR models and evaluates the structural features for non-congeneric molecules and identifies the substructures that may be responsible for the response. MCASE contains models for several fish species (blue gill, FHM, rainbow trout, red killifish). There are more than 180 modules covering various areas of toxicology and pharmacology endpoints including skin sensitization currently marketed by MultiCASE Inc. Available databases are as follows: Retinoids (76 compounds); developmental toxicity (66 antifungal triazole alcohols; composite dataset of 275 compounds); developmental toxicants (mouse 101, rat 134, rabbit 66, humans 119, hamster 192 compounds); FDA/TERIS developmental toxicity (humans 323 compounds); developmental toxicants in FDA teratogenicity (rabbit 812, rat 1286, mouse 794, miscellaneous mammal 1409 compounds)
<i>OASIS & TIMES</i>	http://www.oasis-lmc.org/software.php	OASIS is commercial software which uses the response-surface approach for modeling acute toxicity for two types of toxico-chemical domains: reversible acting chemicals (noncovalent ones) and irreversible (covalent ones) bioreactive chemicals. Interspecies correlations for acute toxicity to 17 aquatic species, such as fish, snail, tadpole, hydrozoan, crustacean, insect larvae, and bacteria have been developed. The TIssue MEtabolism Simulator (TIMES) platform is used to predict the individual and interspecies models for acute aquatic toxicity.
<i>OECD (Q)SAR Application Toolbox</i>	http://www.oecd.org/document/23/0,3343,en_2649_34379_33957015_1_1_1_1,00.html	It allows the user to develop categories and perform read-across, QSTR and trend analyses. A platform that will allow chemical information management, similarity searches and toxicological profiling.
<i>ONCOLOGIC</i>	http://www.epa.gov/oppt/sf/pubs/oncologic.htm	OncoLogic is a desktop computer program that evaluates the probability that a chemical may induce cancer. OncoLogic predicts cancer-causing potential by applying the rules of structure activity relationship (SAR) analysis, mimicking the decision logic of human experts, and incorporating knowledge of how chemicals cause cancer.

<p><i>OSIRIS</i> property explorer</p>	<p>http://www.organic-chemistry.org/prog/peo/tox.html</p>	<p><i>OSIRIS</i> is an on-line system which predicts reproductive effects on the basis of structural fragments which developed from the analysis of 3570 compounds with reproductive effects listed in RTECS.</p>
<p><i>PASS</i></p>	<p>http://ibmc.p450.ru/PASS//</p>	<p><i>PASS</i> assesses similarity of molecules to those with known activity. It predicts over 30 endpoints relevant to reproductive toxicity. The employed endpoints are abortion inducer, alkylator, carcinogenic, DNA intercalator, DNA repair enzyme inhibitor, DNA synthesis inhibitor, DNA topoisomerase ATP hydrolyzing inhibitor, DNA topoisomerase inhibitor, DOPA decarboxylase inhibitor, embryotoxic, estradiol 17β-dehydrogenase stimulant, estrogen agonist, estrogen antagonist, ER modulator, estrone sulfatase inhibitor, estrone sulfotransferase stimulant, fertility enhancer, gynecological disorders treatment, menopausal disorders treatment, mutagenic, retinoic acid α-receptor agonist, retinoic acid β-receptor agonist, retinoic acid receptor agonist, retinoic acid receptor antagonist, retinoid X receptor agonist, retinoid acid receptor agonist, spermicide, teratogen, testosterone agonist, toxic, uterine relaxant, uterine stimulant.</p>
<p><i>SARET</i> (Structure-Activity Relationships for Environmental Toxicology)</p>	<p>http://www.ibmh.msk.ru</p>	<p><i>SARET</i>base and <i>SARET</i>model are used as computer programs for computation of descriptors. <i>SARET</i>base includes the information on more than 190 characteristics for 8500 substances: chemical structure, physicochemical properties (density, boiling and melting points, partition coefficients of octanol-water, etc.), adverse effect doses and concentrations for acute and chronic exposure. The <i>SARET</i>model is prepared for statistical analysis of data and calculation of unknown parameters of substances on the basis of (Q)SARs. The application of <i>SARET</i> provides the information essential to assess the hazard of chemicals and to approximate their unknown characteristics.</p>
<p><i>TERA</i> (Tools for environmental risk assessment)</p>	<p>http://www.ogm-dss.isprambiente.it/index.xhtml</p>	<p><i>TÉRA</i> is based on a fuzzy inference engine using the personal experience and knowledge of ERA experts. <i>TERA</i> includes the information on approximately 200 characteristics for more than 13,000 chemical substances. All information collected in <i>SARET</i> and <i>TERA</i> is verified and specified on the basis of both Russian and foreign literature data including official documents, open publications. In addition, <i>TERA</i> contains information for 194 mixtures, 182 polymers, 346 dyes, 1080 non-organic compounds, 1407 remedies, 1260 agrochemicals. More than 1000 compounds contained in <i>TERA</i> are not presented in the Registry of Toxic Effects of Chemical Substances (RTECS) <i>TERA</i> contains information useful for human, environmental and ecological risk assessment and management. <i>TERA</i> is a tool for assessment of multi-domain risk, assessment of carcinogenic potency risk, Prediction of lead concentrations in blood of fetus, children, adults (system LRISK), health risk connected with lead exposure, prediction of emission of chemical substances and there distribution in different media, parameters used for setting priority of chemical substances in risk assessment and risk assessment using epidemiological data</p>

(continued)

Table 5
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Expert system	Website	Explanatory note
<i>TerraQSTR-FHM</i>	http://www.terrabase-inc.com	It is a commercial software and a stand-alone neural network-based program to compute the acute toxicity of organic chemicals to the FHM using a proprietary neural network algorithm
<i>TIMES-SS</i> (Times MEtabolism Simulator platform)	Marketed by LMC University "As Zlatarov," Bourgas, Bulgaria	<i>TIMES-SS</i> is a hybrid expert system which can encode structure toxicity and structure metabolism relationships through a number of transformations simulating skin metabolism and interaction of the generated reactive metabolites with skin proteins. The skin metabolism simulator mimics metabolism using 2D structural information. The covalent reactions with proteins are described by 47 alerting groups.
<i>TOPKAT</i> (TOxicity Prediction by C(K)omputer Assisted Technology)	http://accelrys.com/products/discovery-studio/admet.html	<i>TOPKAT</i> is a statistical commercial expert system. Under <i>TOPKAT</i> , <i>QSTR</i> models developed from a huge number of heterogeneous databases of toxicological information using substructural fragments and (electro)-topological indices. Developmental toxicity potential are taken from FDA/TERIS. The program uses a range (Q)SAR models for assessing acute toxicity to FHM and Daphnia. The <i>TOPKAT LD50</i> (acute oral toxicity) modeling approach has been used by the Danish EPA in their project to develop <i>QSTR</i> models for evaluation of dangerous properties of around 47,000 organic substances on the European Inventory of Existing Commercial chemical Substances (EINECS) list.
<i>Toxmatch</i>	http://ccb.jrc.ec.europa.eu/QSTR/QSTR-tools/index.php?c=TOXMATCH	An open-source computer program of Joint Research Centre (EC) that encodes several chemical similarity indices in order to facilitate the grouping of chemicals, thereby supporting the development of chemicals categories and the application of read-across between analogues.

human health and environment stability. Additionally, their specific modes of action and specific effects on living systems make pharmaceuticals distinctly different from other chemicals. This sole feature is sufficient reason to assess the potential effects of pharmaceuticals in diverse environmental compartments. The problem is more horrifying as the occurrence level of pharmaceuticals in different environmental compartments is largely varied. The variations in drug occurrences from country to country and also within the different regions of a country make the assessment of pharmaceuticals a troublesome job for the environmental scientist. The interactions between pharmaceuticals and natural stressors of aquatic and terrestrial communities remain to be unexplained. Along with that, the proper risk assessment of mixtures of pharmaceutical products is another area where more introspection is required in present times.

In this book chapter, the hazardous effects of the most common therapeutic classes of pharmaceutical to the living ecosystems and environment are discussed. Furthermore, specific information on the sources, fate, and effects of pharmaceuticals in the environment and their possible negative impact on different ecosystems are explored. There is a lack of sufficient information and scientific data on effects of long-term exposure to nontarget organisms. It is also important to assess the presence of pharmaceuticals and their metabolites and transformation products in several environmental compartments. One can find only a few reports on the quantitative effects of pharmaceuticals, but the effects of metabolites are not sufficiently explored by the scientific community. One has to accept that the identification of risk assessment and management are not sufficient if they are not properly implemented in right way. In these perspectives, the major role should be played by government authorities and agencies by implementing various guidelines and rules for the reduction of toxicity of pharmaceuticals to the environment.

Scarcity of adequate ecotoxicity data related to the diverse classes of pharmaceuticals and their metabolites has stalled appropriate computational modeling and development of expert systems. As a consequence, there are only a very limited number of models developed so far for the risk assessment of pharmaceuticals and their metabolites as well as for the pharmaceutical mixtures. Hence, a sufficient number of models should be developed to address the risk assessment and risk management in an efficient way by minimizing the requirement of time, animal testing and cost. This will also help in gathering the ecotoxicity data as soon as a new pharmaceutical product comes to the market. In this perspective, expert systems are more reliable and results may be easily available in no time. There is a need of more expert systems for prediction of toxicity of pharmaceuticals from diverse classes of therapeutic actions and their metabolites against different endpoints. It is true

that *in silico* techniques cannot substitute “wet” experiments but both of them can be utilized together for a better risk management of pharmaceuticals in near future.

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