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Impact of Emergent Contaminants in the Environment: Environmental Risk Assessment

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Abstract Human pharmaceuticals enter the environment mainly through regular domestic use. Their presence in the aquatic environment has been recorded in the range ng L^{-1} to $\mu g\,L^{-1}$. Knowledge of the risk associated with the use of pharmaceuticals involves establishing the ratio between predicted environmental concentrations (PECs) and predicted no effect concentration (PNECs). The European Union (EMEA) and USA (FDA) have implemented two-tiered strategies for environmental risk assessment (ERA) of pharmaceuticals. Advances in analytical techniques have allowed us to measure pharmaceuticals in the environmental compartment and the refinement of ERA. On the other hand, for calculation of PNECs, acute and chronic toxicity tests are employed; a critical analysis of the available information was carried out, indicating that acute toxicity was only likely for spills, although an exception to this general behavior is shown by endocrine-active substances. Studies including mixtures of pharmaceuticals are not common in the study of pharmaceutical effects. Only for a limited number of drugs, are the ecotoxicity data available adequate for risk assessment. Selection of model compounds with a priori knowledge about the target biological compounds, and the selection of

species, life stages and endpoints would be helpful. New technologies such as proteomics and genomics could be valuable resources to be included in the framework of pharmaceutical environmental risk assessment.

Keywords Ecotoxicology · Environmental concentration · Pharmaceuticals · Risk assessment

Abbreviations

AF	Assessment factor
BAF	Bioaccumulation factor
CPMP	Committee for Proprietary Medicinal Products
EC_{50}	Effect concentration 50%
EE2	Ethynilestradiol
EEC	Expected environmental concentration
EIC	Expected introduction concentration
EMC	Endocrine modulating chemicals
EMEA	European Medicines Agency
ERMS	European Risk Management Strategy
FDA	Food and Drug Administration
GMOs	Genetically modified organisms
ICH	International Conference on Harmonization of Pharmaceuticals for Human Use
ISO	International Organization for Standardization
LC_{50}	Lethal concentration 50%
LC-MS	Liquid chromatography tandem mass spectrometry
LOQ	Limit of quantification
NOEC	No observed effect concentration
OA	Oxolonic acid
OECD	Organization for Economic and Cooperation Development
OTC	Oxytetracycline
PBDEs	Polybromated diphenylethers
PEC	Predicted environmental concentration
PNEC	Predicted no effect concentration
PPCPs	Pharmaceutical and personal care products
QSARs	Quantitative structure—activity relationships
SSRI	Selective serotonin re-uptake inhibitors
STP	Sewage treatment plant
TGD	Technical Guide Document in Support of Commission Directive 93/67/EEC

1 Introduction

Emergent contaminants are not easy to define because they represent a changing reality, dependent on perspective and timing [1]. The permanence in this status is dependent on its persistence in the environment, effects on humans and ecotoxicity. In this sense, knowledge of new properties of chemicals that are well known can re-introduce them as emergent contaminants. Recently,

an editorial of Environmental Toxicology and Chemistry [2] pointed out that the level of concern about the new emergent contaminants is unknown and it is necessary to evaluate their significance for human and ecological health.

Four broad categories have been established for emergent contaminants: (a) pharmaceuticals and personal-care products (PPCPs); (b) polybromated diphenylethers (PBDEs) and other persistent organic contaminants; (c) endocrine modulating chemicals (EMCs) and (d) nanotechnology products. These categories are not totally separated because a compound could be at the same time a PPCP and an EMC.

Herein we will focus on the environmental risk assessment of human pharmaceuticals because the ERA of the different types of emergent contaminants pointed out above is beyond the scope of this work.

Entry of human pharmaceuticals and PCCPs to the environment is mainly via regular domestic use [3]. After their use, pharmaceuticals are excreted, some of them are partially metabolized (slightly transformed or conjugated to polar molecules) and released into the aquatic environment via wastewater effluent. Unused drugs are stored until the expiration date and finally exposed of down drains reaching the aquatic environment. Consequently, they can potentially affect drinking water quality. The entry path scenarios for human pharmaceutical products have been summarized by the Committee for Proprietary Medicinal Products (CPMP) (Fig. 1) [4].

Variable quantities of pharmaceuticals are present in surface waters, ground waters, and sediment, ranging in concentrations between $ng\,L^{-1}$ to $\mu g\,L^{-1}$ [5, 6]. Knowledge of pharmaceuticals in environmental compartments has been supported by the great advance in analytical techniques, which has improved detection levels of these compounds in the environment. New chemical methods, such as liquid chromatography tandem mass

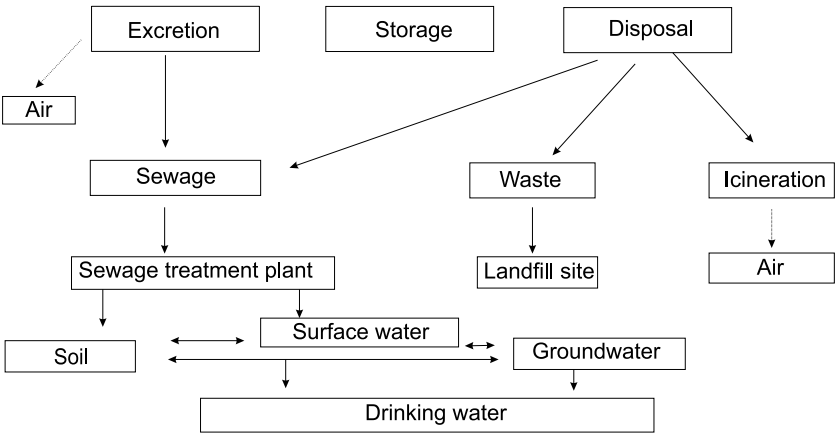

Fig. 1 Routes of entry to the environment for human pharmaceuticals [4]

spectrometry (LC-MS), are able to determine more organic polar compounds without derivatization [7–9]. As a consequence, several monitoring programs have been carried out in different countries that have demonstrated the presence of drug residues to be widely distributed.

On the other hand, knowledge concerning the ecotoxicological effects of pharmaceuticals on aquatic and terrestrial organisms and wildlife is scarce, especially the aspects related to chronic toxicity and more-subtle effects [10]. Most of the published aquatic toxicity data and risk assessments for human pharmaceuticals are based on short-term acute studies [5, 11, 12]. Nevertheless, information about the chronic effects of human pharmaceuticals on aquatic organisms has been recently reviewed by Crane et al. [13].

Although the amounts of human drugs released to the environment are quite high, only recently have detailed guidelines been developed about how pharmaceuticals should be assessed.

2
Environmental Risk Assessment Regulations

Environmental risk assessment is a process that evaluates the likelihood that adverse effects may occur as a result of exposure to one or more stressors [15]. The characterization of the risk involves knowing the ratio between predicted environmental concentration (PEC) and predicted no effect concentration (PNEC); if this value is less than 1 there is no risk to the ecosystem, but if the value is equal to or higher than 1 there is a risk and regulation activities will be needed.

Although the market for pharmaceuticals is highly globalized, and harmonization for testing guidelines have been supported by the International Conference on Harmonization of Pharmaceuticals for Human Use (ICH), for the ERA of human pharmaceuticals different strategies have been followed in different countries according to specific regulations.

2.1
Regulations in the EU

The European Commission has released a guideline about the environmental risk assessment of medicinal products for human use, in accordance with Article 8(3) of Directive 2001/83/EC, as amended, the evaluation of the potential environmental risks posed by medicinal products, their environmental impact should be assessed and, on a case-by-case basis, specific arrangements to limit the impact should be considered [14]. The ERA should accompany any application for a marketing authorization for a medicinal product for human use and the evaluation of the environmental impact should be made also if there is an increase in the environmental exposure. Nevertheless, this guide-

line does not apply to medicinal products consisting of genetically modified organisms (GMOs).

The evaluation of risk assessment to the environment is a step-wise process, consisting of two phases. The first phase (Phase I) includes checking the exposure of the environment to the drug substance against the action limit assessment. If the result is lower than the limit assessment the ERA is finished. Alternatively, second-phase information about the fate and effect of the drug substance should be carried out. This Phase II is divided into two parts (Tier A and B). In Table 1, the phase approach of environmental risk assessment according to the guidelines of EMEA is shown [14]. Phase I is considered a pre-screening and it is independent of route administration, pharmaceutical characteristics, metabolism, and excretion. The calculation of PEC is restricted to the aquatic environment and some restrictions are considered:

- A market penetration factor (Fpen) is defined, the value can be a default value or refined according to specific data (eg. Epidemiological data).
- The amount is distributed along the year and the considered geographic area.
- The sewage system is the main route of entry for the substances.
- No biodegradation of the substance is taken into account during the treatment in the sewage treatment plant (STP).
- Metabolism in the patient is not considered.

For calculation of the PEC the following equation is applied [14]:

$$\mathrm{PEC}_{\mathrm{surfacewater}} = \frac{\mathrm{Dose}_{\mathrm{ai}} \times \mathrm{F}_{\mathrm{pen}}}{\mathrm{Wastewater}_{\mathrm{inh}} \times \mathrm{Dilution}} \tag{1}$$

Table 1 The phase approach in environmental risk assessment according to the Committee for Medicinal Products for Human Use [14]

Stage in regulatory evaluation	Stage in risk assessment	Objective	Method	Test/data requirement
Phase I	Pre-screening	Estimation of exposure	Action limit	Consumption data, log K_{ow}
Phase II Tier A	Screening	Initial prediction of risk	Risk assessment	Base set aquatic toxicology and fate
Phase II Tier B	Extended	Substance and compartment – specific refinement and risk assessment	Risk assessment	Extended data set on emission, fate and effects

where $Dose_{ai}$ ($mg\,inh^{-1}\,d^{-1}$) is the maximum daily dose consumed per inhabitant; F_{pen} is the percentage of market penetration and represents the proportion of the population being treated daily with a specific substance; $Wastewater_{inh}$ ($L\,inh^{-1}\,d^{-1}$) corresponds to the amount of wastewater per inhabitant and per day and Dilution is the dilution factor.

When the $PEC_{surfacewater}$ value is below $0.01\ \mu g\,L^{-1}$ and there are no other environmental concerns it is assumed that the pharmaceutical is not a risk. In the case where the $PEC_{surfacewater}$ is above this value, a Phase II environmental fate and effect analysis should be carried out. In drugs that have a $PEC_{surfacewater}$ lower than $0.01\ \mu g\,L^{-1}$ but may affect reproduction a strategy including Phase II evaluation should be carried out.

In the Phase II assessment, the evaluation of the PEC/PNEC ratio is based on aquatic toxicology data and predicted environmental concentration (Tier A). For drugs where a potential impact can be weighted a refinement of the values should be realized in Tier B. The guidelines for experimental bioassays of the Organization for Economic Cooperation and Development (OECD) or the International Organization for Standardization (ISO) should be followed and all relevant data about physical-chemical properties, metabolism, excretion, biodegradability, persistence, and pharmacodynamic processes must be taken into account.

For the aquatic effect analysis standard long-term toxicity tests in fish, *daphnia*, and algae are proposed (OECD 201, 210, and 211) [16] and to determine the $PNEC_{water}$ an assessment factor (AF) is applied to the no-observed effect concentration (NOEC).The AF applied is a default value of 10 and it represents the uncertainty associated to intra-species variability and interspecies sensitivities and extrapolation from lab to field studies.

The refinement of the risk when it has been identified in Tier A involves refining PEC and PNEC values for the compounds using data on transformation of the substance in the environment. The equation that should be applied is:

$$PEC_{surfacewater} = \frac{Elocal_{water} \times F_{stpwater}}{Waste_{inh} \times Capacity_{stp} \times Factor \times Dilution} \tag{2}$$

$$Elocal_{water} = Dose_{ai} \times F_{excreta} \times F_{pen} \times Capacity_{stp} \tag{3}$$

$Waste_{inh}$ = amount of wastewater per inhabitant per day

$Capacity_{stp}$ = capacity of local sewage treatment plant

$F_{stpwater}$ = fraction of emission directed to surface water

Factor = factor to take into account the adsorption to suspended matter

Dilution = dilution factor

$Elocal_{water}$ = local emission to wastewater of the relevant residue.

If the pharmaceuticals can be adsorbed on soil or sediment, an effect analysis on sediment-dwelling organisms should be carried out and compared

Table 2 Terrestrial fate and effects studies recommended in Phase II Tier B, according to the Committee for Medicinal Products for Human Use [14]

Study type	Recommended protocol
Aerobic and anaerobic transformation in soil	OECD 307
Soil microorganisms: Nitrogen transformation test	OECD 216
Terrestrial plants, Growth test	OECD 208
Earthworm, Acute toxicity tests	OECD 207
Collembola, Reproduction test	ISO 11267

to $PEC_{sediment}$ (OCDE 308) [16]. For compounds with $K_{OC} > 10\,000\,L\,kg^{-1}$, unless they are readily biodegradable, methodologies such as TGD [17] are recommended for risk assessment including PEC_{soil} calculation. The bioassays recommended for Phase II Tier B in soils are shown in Table 2.

Recently, the European Risk Management Strategy (ERMS) work programme for 2008 and 2009 has been adopted, which will focus on improvement of the EU Pharmacovigilance system and the science and methodologies which give support to the safety monitoring of medicines for human use [17].

2.2 Regulations in USA

The National Environmental Policy Act of 1969 requires the Food and Drug Administration (FDA) to take into account the environmental impact of approving drug and biologic applications as an integral part of its regulatory process. A guidance was prepared by the direction of the Chemistry Manufacturing Controls Coordinating Committee, Center for Drug Evaluation and Research (CDER) and the Center for Biologics Evaluation and Research (CBER) and it represents the current thinking on environmental assessment. This guidance [18] involves several topics, among them: the content and format of environmental assessment (EAs), test methods and specific guidance for the environmental issues that are associated with human drugs.

According to this guidance, the EA is required when the estimated concentration of the compound is: (a) equal or higher than $1\,\mu g\,L^{-1}$; (b) when the substance occurs naturally but its application alters significantly its concentration or distribution or its metabolites and (c) when the expected exposure levels can potentially generate harm to the environment. A tiered approach is employed to assess the environmental fate and effects of pharmaceuticals (Fig. 2).

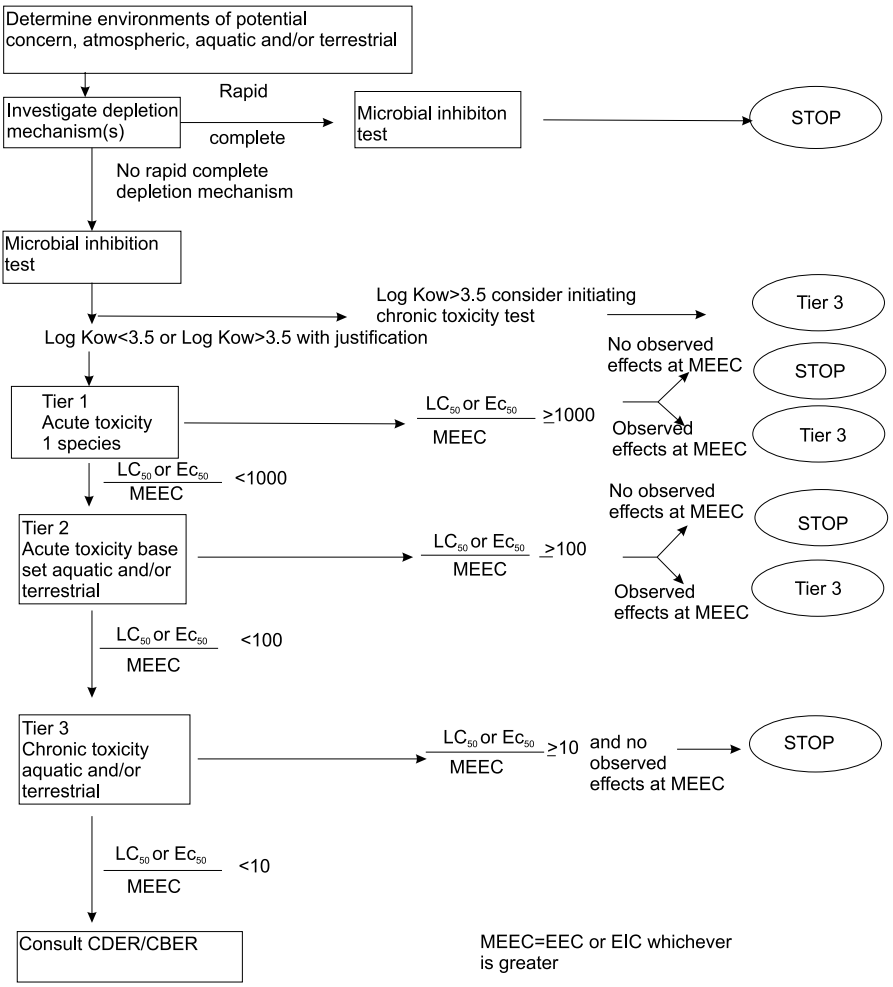

Fig. 2 Tiered approach of FDA for fate and testing [18]

The expected introduction concentration (EIC) should be estimated and the method for calculating this value in aquatic media is:

$$\text{EIC} - \text{aquatic(ppb)} = A \times B \times C \times D$$

$A = \text{kg y}^{-1}$ produced for direct use
$B = 1/\text{L}$ per day entering in STP
$C = \text{year}/365$ days
$D = 10^9\ \mu\text{g kg}^{-1}$

Some kinds of drug may enter the terrestrial environment when biosolids from waste water treatment plant facilities with adsorbed material are applied to soil. The calculation of this concentration is carried out considering the typical treatment, disposal, and application processes. A metabolizing process (biodegradation) occurs during the waste treatment process and it should be considered for calculating EIC.

The PEC is calculated using EIC and taken into account are the processes which affect the compound (spatial or temporal variations, dilution, degradation, sorption, etc.). Normally, EPA applies a dilution factor of 10 to the EIC-aquatic to estimate the PEC.

In summary, the fate of the substance should be provided for the environmental compartment and the transport between compartments should be taken into account if it is of interest to the environmental behavior of the compound.

The evaluation of the effect of pharmaceuticals is oriented to the aquatic compartment because their effect will be on aquatic organisms. Nevertheless, for compounds with high adsorption capacity or high degradation rate, its effects in the aquatic environment could not be considered. For the terrestrial environment, fate and effects testing should be considered when the substance has a $K_{OC} > 10^3$.

Testing of the environmental effects of the pharmaceuticals should be carried out according to the tiered approach as was indicated in Fig. 2. If the compound is not removed from the environment quickly, its persistence and the associated toxic effects should be taken into account. A tiered approach should be used (as was proposed in the guidance), thus the ratio between LC_{50} or EC_{50} and the EIC or EEC is employed as the assessment factor (10, 100, and 1000) to carry out toxicity tests at different levels. The toxicity tests should be performed according to the protocols defined by FDA, OECD, and other peer-reviewed literature if they are appropriate for environmental studies.

3
Pharmaceutical Environmental Concentrations

3.1
Predicted Environmental Concentration

The ERA requires one to know the occurrence and concentration of compounds in the environmental compartments. The exposure assessment should take into account the fate of the substance released to the environment and predict the environmental concentration [19]. The lack of information about measured levels of pharmaceuticals in environmental compartments mean that to carry out the ERA for pharmaceuticals the $PECs_{surfacewater}$

have been estimated, in many cases, according to the recommendations of EMEA or FDA [14, 18]. A review of 111 substances, corresponding to the highest-selling human drugs that have annual sales in Germany of more than 5000 kg, has been carried out. For all compounds the values were higher than 0.01 $\mu g\,L^{-1}$ [20]. According to the scheme developed by EMEA a Phase II process should be carried out for evaluating the exposure. The $PEC_{surfacewater}$ for pharmaceuticals according to data for its use in Germany, Sweden, France and UK [19–23] are presented in Fig. 3. The differences among $PEC_{surfacewater}$ should be related to drug prescription patterns in the countries. These data correspond to the worst case because degradability is not considered. Thus, for paracetamol the PEC is 367.3 $\mu g\,L^{-1}$ [19], although a high degree of elimination, around 98%, has been observed during activated-sludge wastewater treatment [7]. On the other hand, for other compounds such as oxytetracycline (OTC), human metabolism is limited [24], and the compound will be excreted without transformation. It has been observed that biodegradation

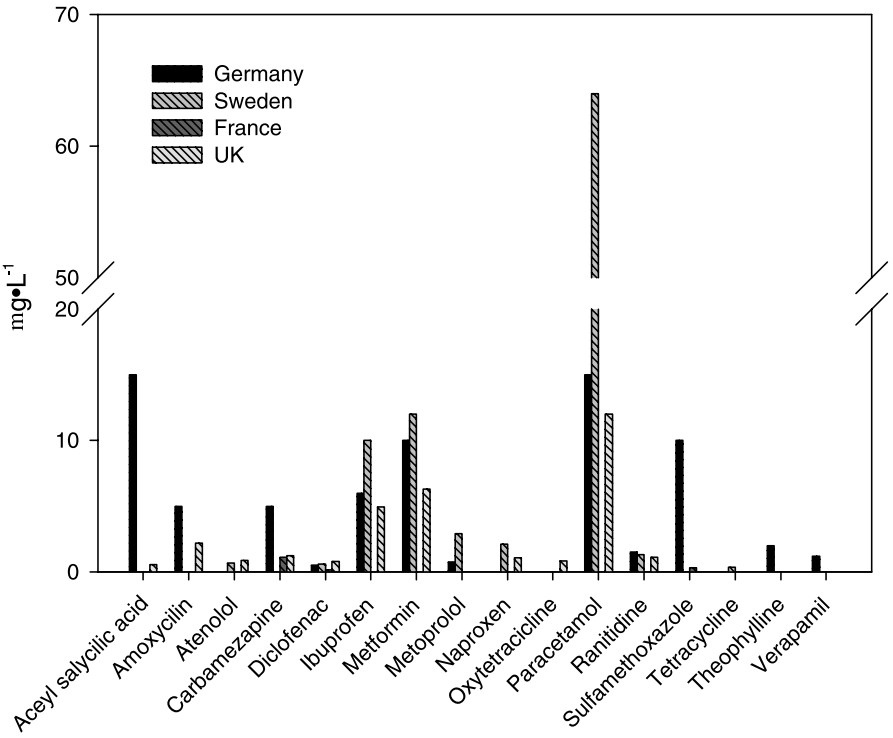

Fig. 3 Predicted environmental concentration (PEC) for pharmaceuticals in surface water of several countries (Germany, Sweden, France, and UK). Data were extracted from [20, 21, 23, 57]

for OTC is limited [25]; and its PEC will be equal to 0.62 $\mu g\,L^{-1}$ after applying a dilution factor of 10.

3.2 Measured Environmental Concentration

3.2.1 Effluent Sewage Treatment Plant

The first work on the presence of drug residues in STP effluents was carried out in the USA and it was focused on clofibric acid, the metabolite of three lipid regulators: clofibrate, etofyllin clofibrate and etofibrate at $\mu g\,L^{-1}$ concentration levels in treated sewage [26]. Later, significant advances in analytical techniques have allowed one to measure pharmaceuticals in environmental compartments [27]. The main drawback of the conventional analytical approach is target-compound monitoring which is insufficient to assess the environmental relevance of emerging contaminants, and the lack of knowledge about the transformation products. Other problems relating to conjugated metabolites (e.g. glucuronides and sulfate conjugates) which can be deconjugated by microbial actions in STP have been pointed out [28].

The pharmaceutical levels in the effluents of STP in many countries are high. Table 3 presents information on the levels for individual compounds in the effluents of STP in Germany, Greece, Spain, and Switzerland. The highest concentrations were recorded in the effluent of STP in Seville (Spain) for two anti-inflammatory drugs, ibuprofen and naproxen, with concentrations of 48.2 and 4.3 $\mu g\,L^{-1}$, respectively [29]. The differences between influent and effluent showed the degradability of these compounds. The values recorded for ibuprofen in the Seville STPs are very high, because the concentrations are below 1 $\mu g\,L^{-1}$, normally. Acetylsalicylic can be degraded into its metabolites, although they are eliminated in the STP process; thus only the metabolite salicylic acid has been detected in sewage effluents [30, 31]. The ubiquity of target compounds can be related to the metabolism, sales, and practices carried out in each country. Therefore, analgesics and antibiotics are detected frequently because they are excreted as the unchanged parent compound; in addition the high loads of analgesic and anti-inflammatories, in comparison with other therapeutic groups is attributed to the higher consumption. The removal efficiency is related to the treatment applied in each plan and the compound physicochemical characteristics and hydraulic retention time [32].

3.2.2 Environmental Levels

In developed countries, production and use of pharmaceuticals are increasing annually [33]. The measurement of these compounds in environmental

Table 3 Concentration range and mean concentration in $\mu g\,L^{-1}$ of pharmaceuticals and metabolites in effluents of municipal STPs of several countries

Drug	Germany	Greece	Spain	Switzerland	Canada
Acetyl salycilic acid	0.32–0.92	na	na	na	na
Diclofenac	0.21–1.11	0.20–0.34	blq–0.38	0.1–0.7	0.015–0.039
Ibuprofen	0.32–0.58	na	0.78–48.24	0.005–1.5	2.2–3.5
Naproxen	0.12–0.53	nd	0.22–4.28	0.1–3.5	1.0–1.7
Indometazine	0.07–0.11	na	na	na	0.048–0.075
Benzafibrate	0.72–1.2	nd–0.15	na	na	0.13–0.28
Gemfribozil	0.12–0.35	na	na	na	0.37–0.60
Fenofibric acid	0.32–0.44	nd	na	na	na
Clofibric acid	0.42–0.69	na	na	nd–0.06	na
Carmabezapine	1.31–2.2	na	blq–1.29	0.1–0.8	na
Phenazone	0.12–0.20	na	na	na	na
Porpanolol	0.34–0.48	na	na	na	na
Metoprolol	1.72–2.44	na	na	na	na
Bisoprolol	0.12–0.16	na	na	na	na
Betaxolol	0.14–0.20	na	na	na	na
Terbutalin	0.10–0.12	na	na	na	na
Carazolol	0.05–0.09	na	na	na	na
Dihydrocodeine	1.47	na	na	na	na
Hydrocodone	0.72	na	na	na	na
Ketoprofen	nd	0.27–0.82	blq–3.48	nd–0.20	0.015
Mefenamic acid	nd	0.08–0.22	na	na	na
Primidone	nd–0.88	nd	na	na	na
Propyphenazone	nd–0.74	nd	na	na	na
Salycilic acid	nd–0.65	0.64–2.0	0.57	na	0.054–0.46
Caffeine	na	na	0.15–3.20	na	na

* Data were extracted from [6, 29, 36]
na not analysed, *nd* not detected, *blq* below limit quantification

compartments can improve knowledge about the occurrence and persistence of the compounds in the environment. The advances in analytical techniques have allowed one to measure extremely low concentrations of pharmaceuticals in surface water, rivers, streams, etc. [34]. The occurrence of organic wastewater contaminants is high in the environment, 80% of 139 streams sampled in the USA [35] showed at least one organic wastewater contaminant, although the authors pointed out that the results were influenced by the design of the study and it can not be considered as representative of the global situation in USA streams. The concentrations were, in general, less than $1\,\mu g\,L^{-1}$ but their presence in many streams indicated that compounds survived biodegradation.

Pharmaceuticals in effluents of wastewater treatment plants are diluted when entering river waters being detected in the $ng\,L^{-1}$ range. However,

the same spectrum of compounds that are found in the STP are found in the Ebro river basin where analgesics (diclofenac, naproxen, ibuprofen), lipid regulators (gemfibrozil, bezaibrate), antibiotics (azithomycin, trimethoprim, and sulfamethoxazole), antipiletic (carbamezapine), antihistamic (ratidine), and β-blockers (atenolol and sotanol) are the recorded compounds, which are consumed at high levels in Spain [32]. Drugs in a large body of receiving water are in many cases below detection limits although in small receiving streams were around 15–30% effluent median concentration [36]. The availability of occurrence data for pharmaceuticals in estuarine or marine waters is less common than stream and river waters. In the North Sea, for clofibric acid concentrations of $1\,\mathrm{ng\,L^{-1}}$ have been reported, whilst in seawater samples ibuprofen has not been measured above $0.2\,\mathrm{ng\,L^{-1}}$ [37, 38]. Pharmaceutical residues are present as contaminants in UK estuaries [39], but the authors only detected above the detection limits the following targeted compounds/metabolites: clofibric acid, clotrimazolem dextropropoxyphene, dicofenac, ibuprofen, mefenamic acid propanolol, tamoxifen, and trimethoprim, with ibuprofen showing the highest detected concentration ($928\,\mathrm{ng\,L^{-1}}$). In the Victoria Harbor of Hong Kong, antibiotics (belonging to the class quinolones, macrolides, sulfonamides, β-lactam, and chloramphenicol) were mainly below the limit quantification (LOQ). However, they were found in the Pearl River during the high and low water seasons in the range $10–100\,\mathrm{ng\,L^{1}}$. The level of antibiotics in the high water season is controlled by daily sewage discharge patterns and in the low season may be controlled by water column dynamics [40].

There is less knowledge about pharmaceutical concentrations in soil and sediment than for the aquatic environment. This was due to the lack of suitable sensitive analytical methods for the detection of compounds [41]. The persistence of a drug in a sediment or soil mostly depends on its photostability, its binding and adsorption capability, its degradation rate, and leaching in water [42]. The main route of entry for antibiotics for human use is related to the use of sewage sludge for fertilizing the soil. The occurrence of fluoroquinolones, ciprofloxacin, and norfloxacin in sewage sludge has been detected at concentrations ranging between 1.4 to $2.4\,\mathrm{mg\,kg^{-1}}$ [43], which is in the same range as can be measured in digested sludge, indicating a high affinity to the solid phase. Most of the literature on pharmaceuticals in solid environmental samples is related to veterinary drugs, especially those employed in fish farming, which are principally antibiotics.

Pharmaceuticals, as other chemical compounds, can be accumulated by aquatic or benthic organisms. Oxytetracycline (OTC, tetracycline) and oxolonic acid (OA, quinolone) are accumulated by the blue mussel, preferentially being accumulated in the viscera for OTC and in the gills for OA. Bioaccumulation factors (BAF) were low (< 0.5) regardless of the analyzed bivalve part. The application of K_{ow} for antibiotic bioaccumulation can predict a weak accumulation in mussel for antibiotics with $K_{ow} < 2$, whereas

antibiotics such as macrolides with $K_{ow} > 2$ accumulate at a higher level [44]. Fluoxetine and sertraline are prescribed as antidepressants and their occurrence has been detected in surface water or effluent discharges [35, 45]. The analysis of these compounds in streams from a reference site and an effluent-dominated stream showed that these compounds were not detected in the reference site whereas they were detected in all tissues analyzed from fish from the effluent-dominated stream, including *P. nigromaculatus*, L. *macrochirus*, and *I. punctatus*, with a preferential accumulation in the brain, although they also accumulate in muscle at concentrations higher than the limits of quantitation, and subsequently an exposure route to humans in this way should be considered [46]. The influence of pH on the bioconcentration factor of fluoxetine in the fish *Oryzia latipes* has been analyzed [47], showing that BCF values were lower at pH 7 and higher at pH 9 because of an increase of hydrophobicity at pH values closer to pK_a.

4 Ecotoxicology of Human Pharmaceuticals

4.1 Acute Toxicity

Aquatic organisms are targets to analyze the effect of human pharmaceuticals because they are exposed via wastewater over their whole life. Drugs are designed to have a specific mode-of-action along the target pathway. Hypotheses about the mode-of-action in lower animals in many cases are not well supported, because many of the organisms lack the required receptors. Although a mode-of-action for a pharmaceutical should be taken into account when an experiment is designed, this approach may not be appropriate because the mode-of-action could be different or not well known [48].

The ecotoxicological effects of human pharmaceuticals are focused on acute and standard tests. More than three-hundred-and-six endpoints for pharmaceutical ecotoxicity data have been collected for macroinvertebrates, fish, and algae, and over one-hundred for human pharmaceuticals [12]. The selection of three trophic levels (algae, *Daphnia*, and fish) showed that sensitivity followed the order algae > *Daphnia magna* > fish. However, the range of acute toxicity endpoints varied from $> 15\,000\ mg\ L^{-1}$ (for atropine sulfate-anthicolorgenic/mydriatic) [49] to $< 0.003\ mg\ L^{-1}$ for fluvoxamine (antidepressant) [50]. The ecotoxicity effects for therapeutic classes showed the following order: antidepressants, antibacterials, and antipsychotics [12]. A recent review [48] summarized the ecotoxicity data, taking into account the ecological relevance and the different classes of human pharmaceuticals: analgesic and non-steroidal anti-inflammatory drugs, beta-blockers, blood lipid-lowering agents, neuroactive compounds, and cytostatic compounds

and cancer therapeutics. Seventeen percent showed acute toxicity below 100 mg L^{-1} and 38% above 100 mg L^{-1}, which is classified as not harmful for aquatic organisms according to EU Directive 93/67/EEC. The rest of the compounds (45%) showed high variability in acute toxicity tests. The difference between the acute toxicity data and the environmental levels for human pharmaceuticals demonstrate that only in the case of spills will the toxicity be relevant.

4.2 Chronic Toxicity

The standard acute toxicity tests have as endpoints the lethality and they do not seem appropriate for risk assessment of pharmaceuticals, because of the nature of these compounds. The use of chronic tests over the life-cycle of organisms for different trophic levels could be more appropriate [51]. Nevertheless, the database for this kind of bioassay is very limited.

Most chronic aquatic toxicity data for human pharmaceuticals are available for algae because they are the quickest to perform and therefore less expensive. The sensitivity to antimicrobial substances is higher in Cyanobacteria such as *Microcystis aureginosa* than standard algal toxicity tests (*Pseudokirchneriella subcapitata*) although there are no differences for non-antimicrobial substances [52].

Only in the case of the synthetic steroid EE2, which is present in contraceptive pills, has an effect been observed at environmentally relevant concentrations. In a recent study [53], vitellogenin induction in fathead minnows was reported at an EC_{50} value of 1 ng L^{-1}. The life-cycle exposure of zebrafish to 3 ng L^{-1} EE2 provoked an increase of vitellogenin and caused gonadal feminization [54]. The exposure of some invertebrate taxa (snails) to EE2 also caused effects at very low concentrations $\sim$ 1 ng L^{-1} [55]. Fish are also sensitive to other sex hormones such as methyltestosterone and beta-adrenergic receptor blockers [56].

Analgesic and non-steroidal anti-inflammatory drugs are the most-consumed drugs, and a chronic study with diclofenac has been reported in invertebrates [22, 57]. A chronic study with rainbow trout showed renal lesions at 5 μg L^{-1} [58]. Regarding beta-blockers, propanolol showed chronic toxicity not only on the cardiovascular system in fish but also in the reproductive system [48]. The number of eggs released by fish was reduced at 0.5 μg L^{-1} after four weeks of exposure but not at 50 and 100 μg L^{-1} [59]. The blood lipid-lowering agents have been evaluated by traditional toxicity tests and NOEC in the range of 246 μg L^{-1} to 70 mg L^{-1} have been recorded for *B. caliciflorus* (2 days) and early life stages of zebrafish (10 days), respectively [57].

Chronic toxicity tests have been carried out with carbamezapine (an antiepileptic) and *C. dubia* showed a NOEC (7 days) = 25 μg L^{-1} [57]. Lethal

concentration in zebrafish was reported at 43 $\mu g\,L^{-1}$ [60]. Chronic studies have been carried out on selective serotonin re-uptake inhibitors (SSRI). Serotonin is a neurotransmitter found in vertebrates and invertebrates. SSRI may affect the function of the nervous and associated hormonal systems. The role of serotonin varies between phyla and in consequence also the effects of SSRI; in medaka (*O. latipes*) serotonin induced oocyte maturation [61] but the opposite action was reported in mummichog (*F. heteroclitus*) [62]. The chronic effects of SSRI on reproduction in fish and invertebrates are not yet clear, interference in the reproduction occurred at concentrations not ecologically relevant [48].

To date, chronic toxicity data using marine or estuarine species have been very scarce. The results with different classes of compounds (carbamezapine, acetaminophen, and ibuprofen) and the endpoint inhibition growth at 72 h for the marine microalgae *Phaeodactylum tricornutum* did not show toxicity below 2.0 $mg\,L^{-1}$.

Studies concerning the effects of mixtures of pharmaceuticals are very limited in the scientific literature [63, 64]. The mixture of diclofenac, ibuprofen, naproxen, and acetylsalicylic acid has been evaluated using *Daphnia* and algae, the toxicity of the mixture followed the concept concentration addition. Nevertheless, the effects of mixtures of compounds with different modes-of-action depends on the species and they do not all act in the same way. Few studies concerning the toxicity of mixtures of pharmaceuticals in realistic ecological systems (microcosms and mesocosms) have been carried out. The effect of a combination of eight pharmaceuticals at three levels on *Lemna gibba* and *Myriophyllium sibiricum* has been tested [65]. In a similar microcosm (periphyton, phytoplankton, zooplankton, algae, and benthic communities), three pharmaceuticals with different modes-of-action were analyzed at three levels [66]. At low concentrations (6–10 $\mu g\,L^{-1}$) only trends were appreciable and no significant effects could be recorded. The comparison of assayed treatment with current concentrations in the environment did not allowed to establish a risk situation for this mixture. Nevertheless, many pharmaceuticals are present in the environment and the effect of this "cocktail" could affect to aquatic communities.

5 Environmental Risk Assessment

The objective of environmental risk assessment is to determine the nature and likelihood of the effects of human actions (in this case the use of pharmaceuticals) on animals, plants, and the environment [67]. According to this principle, operational monitoring in support of this concept should be adequate for characterizing exposure and effects [68]. The two-tiered approach (EU and USA) is employed normally for risk assessment of pharmaceuticals

(see Sects. 2.1 and 2). In both risk strategies trigger values are selected for further research via tiered assessment 0.01 $\mu g\,L^{-1}$ and 0.1 $\mu g\,L^{-1}$, respectively. The use of this value permits a reduction in the need to carry out many assessments which facilitates the release of new drugs to the market. However, for some compounds this trigger value is insufficient; this is the case for endocrine disruptors which at 1 $ng\,L^{-1}$ showed environmental effects, below the stricter trigger value.

The potential effect of pharmaceuticals is calculated according to the ratio between PEC and PNEC. The PEC is calculated in many cases using figures such as sales, density of population, etc., representing the worst case. In order to get a refinement of this value more precise environmental risk assessment should be carried out; data for biodegradation adsorption, and abiotic factors (pH, temperature) of the environment should be taken into account. The use of measured concentrations allows one to establish more realistic ERA. The other data which should be available is the PNEC, but the lack of chronic toxicity data has made it difficult to perform this assessment. The use of the assessment factor when only acute data are available involves the reduction of uncertainty associated with its use [22]. Though the use of a quantitative structure—activity relationship has been pointed out as a possibility for identifying hazard or prioritizing substances to be analyzed it is not sufficiently precise for risk assessment [48].

The risk of an acute toxic effect from pharmaceuticals in the environment is unlikely [21]. However, many drugs have been designed to affect specific biological systems in target organisms at relatively low dose and exposure concentrations. For this reason, the long-term sublethal effects of pharmaceuticals could be a greater potential concern than acute effects. With the exception of a limited number of drugs, available ecotoxicity data could be inadequate for risk assessment and an extensive suite of chronic sublethal tests may be necessary [69].

6 Concluding Remarks

Although human pharmaceuticals are found at low concentrations in the environment and acute toxicity is not frequent, a broad database with chronic and subtle toxicity tests is necessary to carry out the ERA of these compounds. A priori knowledge about the target biological pathway can identify compounds with higher priority for testing and the species, life stages, and endpoints suitable for testing. In this sense, the selection of estuarine and marine species should be considered.

On the other hand, biomarkers as responses to molecular or biochemical changes can be useful for ecological risk assessment. In vitro systems can be appropriate tools for screening the ecotoxicological effect of pharmaceuticals

before fish toxicity testing is carried out. The lack of toxicity tests for pharmaceutical mixtures should be taken into account in order to improve the risk assessment because of the additive, antagonistic, or synergetic effects that can be present. Finally, new technologies such as proteomics and genomics, which are powerful tools for human diagnosis, are under development and they may be helpful to validate effects in the environment and should be included in the framework of ERA, although its use is limited by the current knowledge of the impacted biota.

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