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Review

Ecotoxicology of human pharmaceuticals

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Abstract

Low levels of human medicines (pharmaceuticals) have been detected in many countries in sewage treatment plant (STP) effluents, surface waters, seawaters, groundwater and some drinking waters. For some pharmaceuticals effects on aquatic organisms have been investigated in acute toxicity assays. The chronic toxicity and potential subtle effects are only marginally known, however. Here, we critically review the current knowledge about human pharmaceuticals in the environment and address several key questions. What kind of pharmaceuticals and what concentrations occur in the aquatic environment? What is the fate in surface water and in STP? What are the modes of action of these compounds in humans and are there similar targets in lower animals? What acute and chronic ecotoxicological effects may be elicited by pharmaceuticals and by mixtures? What are the effect concentrations and how do they relate to environmental levels? Our review shows that only very little is known about long-term effects of pharmaceuticals to aquatic organisms, in particular with respect to biological targets. For most human medicines analyzed, acute effects to aquatic organisms are unlikely, except for spills. For investigated pharmaceuticals chronic lowest observed effect concentrations (LOEC) in standard laboratory organisms are about two orders of magnitude higher than maximal concentrations in STP effluents. For diclofenac, the LOEC for fish toxicity was in the range of wastewater concentrations, whereas the LOEC of propranolol and fluoxetine for zooplankton and benthic organisms were near to maximal measured STP effluent concentrations. In surface water, concentrations are lower and so are the environmental risks. However, targeted ecotoxicological studies are lacking almost entirely and such investigations are needed focusing on subtle environmental effects. This will allow better and comprehensive risk assessments of pharmaceuticals in the future.

Keywords: Pharmaceuticals; Ecotoxicological effects; Environmental toxicity; Chronic effects; Environmental risk assessment

Contents

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1. Introduction

It came as a surprise when an unusually high death rate among three species of vulture in India and Pakistan was reported in 2004 to be caused by diclofenac, a widely used analgesic and antiinflammatory drug (Oaks et al., 2004). The oriental white-backed vulture (*Gyps bengalensis*) is one of the most common raptors in the Indian subcontinent and a population decline of >95% makes this species as being critically endangered. Whereas a population decline has started in the 1990s, recent catastrophic declines also involve *Gyps indicus* and *Gyps tenuirostris* across the Indian subcontinent (Prakash et al., 2003; Risebrough, 2004). High adult and subadult mortality and resulting population loss is associated with renal failure and visceral gout, the accumulation of uric acid throughout the body cavity following kidney malfunction. A direct correlation between residues of diclofenac and renal failure was reported both by experimental oral exposure and through feeding vultures diclofenac-treated livestock. Hence, the residues of diclofenac were made responsible for the population decline (Oaks et al., 2004). This drug has recently come into widespread use in

these countries as a veterinary medicine, but is also widely used as in human medicine since the 1970s. Vultures are natural scavengers feeding on carrion of wildlife and domestic livestock and cattle. The three vulture species continue to decline in Pakistan, India, Bangladesh and southern Nepal. Apart from this severe case, never having been anticipated, potential ecotoxicological effects of drug residues in the environment on wildlife are largely unknown.

Pharmaceuticals are a class of emerging environmental contaminants that are extensively and increasingly being used in human and veterinary medicine. These chemicals are designed to have a specific mode of action, and many of them for some persistence in the body. These features among others make pharmaceuticals to be evaluated for potential effects on aquatic flora and fauna. The current investigations are mainly driven by advances in environmental residue analysis, particularly after the establishment of chemical analysis methods able to determine more polar compounds such as liquid chromatography–tandem mass spectrometry, which allows the identification of trace quantities of polar organic pollutants without derivatization (Ternes et al., 1998, 2001; Kolpin et al., 2002; Kümmerer, 2004). Accordingly, many environmental analyses have been performed in various countries, which are summarized by various reports (e.g. Halling-Sorensen et al., 1998; Daughton and Ternes, 1999; Kümmerer, 2004). These monitoring studies demonstrate that drug residues in treated wastewater and surface water are very widespread.

In contrast, only little is known about ecotoxicological effects of pharmaceuticals on aquatic and terrestrial organisms and wildlife, and a comprehensive review on ecotoxicological effects is lacking. Aquatic organisms are particularly important targets, as they are exposed via wastewater residues over their whole life. Standard acute ecotoxicity data have been reported for a number of pharmaceuticals, however, such data alone may not be suitable for specifically addressing the question of environmental effects, and subsequently in the hazard and risk assessment (Fent, 2003). The current lack of knowledge holds in particular for chronic effects that have only very rarely been investigated. In spite of the sizeable amounts of human drugs released to the environment, concise regulations for ecological risk assessment are largely missing. Only in the last few years, regulatory agencies have issued detailed guidelines on how pharmaceuticals should be assessed for possible unwanted effects on the environment. The first requirement for ecotoxicity testing as a prerequisite for registration of pharmaceuticals was established in 1995 according to the European Union (EU) Directive 92/18 EEC and the corresponding “Note for Guidance” (EMEA, 1998) for veterinary pharmaceuticals. The European Commission released a draft guideline (Directive 2001/83/EC) specifying that an authorization for a medicinal product for human use must be accompanied by an environmental risk assessment (EMEA, 2005). The U.S. Food and Drug Administration (FDA) published a guidance for the assessments of human drugs; according to this, applicants in the U.S.A. are required to provide an environmental assessment report when the expected introduction concentration of the active ingredient of the pharmaceutical in the aquatic environment is $\geq 1\,\mu g/L$ (FDA-CDER, 1998), which corresponds to about 40 t as a trigger level. In contrast, environmental assessments of veterinary pharmaceuticals is required by the U.S. FDA since 1980 (Boxall et al., 2003).

The objective of our paper is to compile and critically review the present knowledge about the environmental occurrence and fate of human pharmaceuticals in the aquatic environment, to discuss potential mechanisms of action based on knowledge from mammalian studies, and to describe the acute and chronic ecotoxicological effects on aquatic organisms. We also identify major gaps in the current knowledge and future research needs. We concentrate on pharmaceuticals used in human medicine, some of which are also applied in veterinary medicine, thereby focusing on environmentally important compounds belonging to different drug categories, namely nonsteroidal antiinflammatory drugs, beta-blockers, blood lipid lowering agents, cancer therapeutics and neuroactive compounds. These classes differ for their modes of actions and were chosen because of their consumption volumes, toxicity and persistence in the environment. We will not address the environmental effects of antibiotics and biocides (Halling-Sorensen et al., 1998; Daughton and Ternes, 1999; Hirsch et al., 1999), hormones (used in contraceptives and in therapy) (Damstra et al., 2002) and special veterinary pharmaceuticals (Montforts et al., 1999; Boxall et al., 2003) as the cited reports provide detailed information.

The current knowledge indicates that residues of pharmaceuticals at trace quantities are widespread in aquatic systems. Pharmaceuticals in the environment are suggested to pose only a low risk for acute toxicity. For chronic effects, the situation may be different, but there is a considerable lack of information. Investigation of multigenerational life-cycle effects or at various life stages is lacking, although many aquatic organisms are exposed for their entire life. There is a need to focus on long-term exposure assessment regarding specific modes of action of pharmaceuticals to better judge the implications of pharmaceutical residues in aquatic systems. Only after filling these gaps, more reliable environmental risk assessments with much lower uncertainty can be performed.

2. Sources

The consumption of pharmaceuticals is substantial. In the European Union (EU) about 3000 different substances are used in human medicine such as analgesics and antiinflammatory drugs, contraceptives, antibiotics, beta-blockers, lipid regulators, neuroactive compounds and many others. Also a large number of pharmaceuticals are used in veterinary medicine, among them antibiotics and antiinflammatory. Sales figures are relatively high as reported for several countries (Table 1). In England, Germany and Australia, the amounts for the most frequently used drugs are in the hundreds of tons per year (Jones et al., 2002; Huschek et al., 2004; Khan and Ongerth, 2004). The pattern of consumed pharmaceuticals for the different countries is not identical and some drugs may be forbidden or replaced by related drugs. However, as listed in Table 1, some drugs are regularly documented within the most frequently applied range: the class of non-steroidal antiinflammatory drugs (NSAID) including acetylsalicylic acid (e.g. 836 t in Germany in 2001), paracetamol (e.g. 622 t in Germany in 2001), ibuprofen (e.g. 345 t in Germany in 2001), naproxen (e.g. 35 t in England in 2000) and diclofenac (86 t in Germany in 2001), the oral antidiabetic metformin (e.g. 517 t in Germany 2001) and the antiepileptic carbamazepine (e.g. 88 t in Germany 2001). Data representing the annual sales or consumptions include mainly prescribed drugs, some include also sales over-the-counter, some a mixture of both, and internet sales are not included. Therefore, the real amounts of applied drugs is uncertain, but probably significantly higher for some of the pharmaceuticals reported than the figures in Table 1. Figuring out the annual consumption of a certain drug is difficult and often based on estimates. For example, based on sales, estimates of the U.S. production of the antiepileptic carbamacepine (which is also used for other treatments) ranged from 43 t in 2000 to 35 t in 2003 (Thaker, 2005).

Pharmaceuticals are excreted after application in their native form or as metabolites and enter aquatic systems via different ways. The main pathway from humans is ingestion following excretion and disposal via wastewater. Municipal wastewater is therefore the main route that brings human pharmaceuticals after normal use and disposal of unused medicines into the environment. Hospital wastewater, wastewater from manufacturers and landfill leachates (Holm et al., 1995) may contain significant concentrations of pharmaceuticals. Pharmaceuticals not readily degraded in the sewage treatment plant (STP) are being discharged in treated effluents resulting in the contamination of rivers, lakes, estuaries and rarely, groundwater and drinking water. Where sewage sludge is applied to agricultural fields, contamination of soil, runoff into surface water but also drainage may occur. In addition, veterinary pharmaceuticals may enter aquatic systems via manure application to fields and subsequent runoff, but also via direct application in aquaculture (fish farming). Of environmental concern is not necessarily a high production volume of a certain pharmaceutical per se, but the environmental persistence and critical biological activity (e.g. high toxicity, high potency for effects on biological key functions such as reproduction). As exemplified by the synthetic steroid hormones in contraceptive pills, such as 17α-ethinylestradiol (EE2), the annual production lies in a couple of hundreds kilograms per year in the EU, yet it is extremely potent, quite persistent in the environment and shows estrogenic activity in fish at 1–4 ng/L, or lower. Hence, pharmaceuticals having environmental relevance share the following properties: often, but not always, high production volume combined with environmental persistence and biological activity, mainly after long-term exposure.

Table 1
Annual consumption of different classes of prescribed drugs for different countries

Compounds	Germany 1999[a]	Germany 2000[a]	Germany 2001[a]	Austria 1997[b]	Denmark 1997[c]	Australia 1998[d]	England 2000[e]	Italy 2001[f]	Switzerland 2004[g]
Analgesics, antipyretics and anti-inflammatory									
Acetylsalicylic acid	902.27 (1)	862.60 (1)	836.26 (1)	78.45 (1)	0.21 (7)	20.4 (9)			43.80 (3)
Salicylic acid	89.70 (12)	76.98 (17)	71.67 (17)	9.57 (11)					5.30 (6)
Paracetamol	654.42 (2)	641.86 (2)	621.65 (2)	35.08 (2)	0.24 (6)	295.9 (1)	390.9 (1)		95.20 (1)
Naproxen				4.63 (16)		22.8 (7)	35.07 (12)		1.70 (12)
Ibuprofen	259.85 (5)	300.09 (5)	344.89 (5)	6.7 (13)	0.03 (19)	14.2 (13)	162.2 (3)	1.9 (15)	25.00 (4)
Diclofenac	81.79 (16)	82.20 (14)	85.80 (14)	6.14 (15)			26.12 (16)		4.50 (7)
β-Blocker									
Atenolol							28.98 (13)	22.07 (4)	3.20 (9)
Metoprolol	67.66 (18)	79.15 (16)	92.97 (11)	2.44 (20)					3.20 (10)
Antilipidemic									
Gemfibrazol						20 (10)			0.399 (18)
Bezafibrate				4.47 (17)				7.60 (8)	0.757 (15)
Neuroactive									
Carbamazepine	86.92 (13)	87.71 (13)	87.60 (12)	6.33 (14)		9.97 (18)	40.35 (8)		4.40 (8)
Diazepam					0.21 (8)				0.051 (21)
Antiacidic									
Ranitidine	85.41 (15)	89.29 (12)	85.81 (13)			33.7 (5)	36.32 (10)	26.67 (3)	1.60 (13)
Cimetidine							35.65 (11)		0.063 (20)
Diuretics									
Furosemide					3.74 (1)			6.40 (19)	1.00 (14)
Sympatomimetika									
Terbutalin					0.46 (3)				0.0099 (23)
Salbutamol					0.17 (9)				0.035 (22)
Various									
Metformin	368.01 (4)	433.46 (4)	516.91 (3)	26.38 (3)		90.9 (2)	205.8 (2)		51.40 (2)
Estradiol					0.12 (13)				
Iopromide	64.93 (19)	63.26 (19)	64.06 (19)						6.90 (5)

For every country a top 20 sold-list is taken into account. Data in bracket represent the position in the ranking list within a country. Data are in t/year.

[a] Huschek et al. (2004).
[b] Sattelberger (1999).
[c] Stuer-Lauridsen et al. (2000).
[d] Khan and Ongerth (2004).
[e] Jones et al. (2002).
[f] Calamari et al. (2003).
[g]

3. Fate in the environment

The behavior and fate of pharmaceuticals and their metabolites in the aquatic environment is not well known. The low volatility of pharmaceuticals indicates that distribution in the environment will occur primarily through aqueous transport, but also via food chain dispersal. In *wastewater treatment*, two elimination processes are generally important: adsorption to suspended solids (sewage sludge) and biodegradation. Adsorption is dependent on both hydrophobic and electrostatic interactions of the pharmaceutical with particulates and microorganisms. Acidic pharmaceutical such as the NSAID acetylsalicylic acid, ibuprofen, fenoprofen, ketoprofen, naproxen, diclofenac and indomethacin having pK_a values ranging from 4.9 to 4.1, as well as clofibric acid, bezafibrate (pK_a 3.6) and gemfibrozil occur as ion at neutral pH, and have little tendency of adsorption to the sludge. But adsorption increases with lower pH. At neutral pH, these negatively charged pharmaceuticals therefore occur mainly in the dissolved phase in the wastewater. For these compounds and the antitumor agent ifosfamide sorption by non-specific interactions seems not to be relevant (Kümmerer et al., 1997; Buser et al., 1998b). In general, sorption of acidic pharmaceuticals to sludge is suggested to be not very important for the elimination of pharmaceuticals from wastewater and surface water. Therefore, levels of pharmaceuticals in digested sludge and sediments are suggested to be relatively low, as was demonstrated in several monitoring studies (Ternes et al., 2004; Urase and Kikuta, 2005). However, basic pharmaceuticals and zwitterions can adsorb to sludge to a significant extent, as has been shown for fluoroquinolone antibiotics (Golet et al., 2002). For the hydrophobic EE2 (log K_{ow} 4.0) sorption to sludge is likely to play a role in the removal from wastewater. Degradation in sludge seems not significant. As a consequence, EE2 occurs in digested sludge, where concentrations of 17 ng/g were reported (Temes et al., 2002).

In case a pharmaceutical is occurring mainly in the dissolved phase, biodegradation is suggested to be the most important elimination process in wastewater treatment. It can occur either in aerobic (and anaerobic) zones in activated sludge treatment, or anaerobically in sewage sludge digestion. In general, biological decomposition of micro-pollutants including pharmaceuticals increases with increase in hydraulic retention time and with age of the sludge in the activated sludge treatment. For example, diclofenac was shown to be significantly biodegraded only when the sludge retention time was at least 8 days (Kreuzinger et al., 2004). In contrast, data from Metcalfe et al. (2003a,b) indicate that the neutral drug carbamazepine, which is hardly biodegradable, is only poorly eliminated (normally less than 10%), independent from hydraulic retention times. Pharmaceuticals are often excreted mainly as non-conjugated and conjugated polar metabolites. Conjugates can, however, be cleaved in sewage treatment plants (STP), resulting in the release of active parent compound as shown for estradiol (Panter et al., 1999; Ternes et al., 1999), and the steroid hormone in the contraceptive pill, 17α-ethinylestradiol (D'Ascenzo et al., 2003).

Studies on the elimination rates during the STP process are mainly based on measurements of influent and effluent concentrations in STPs, and they vary according to the construction and treatment technology, hydraulic retention time, season and performance of the STP. Some studies (Ternes, 1998; Stumpf et al., 1999; Carballa et al., 2004) indicate elimination efficiencies of pharmaceuticals to span a large range (0–99%). The average elimination for specific pharmaceuticals varied from only 7 to 8% for carbamazepine (Ternes, 1998; Heberer, 2002; Clara et al., 2004) up to 81% for acetylsalicylic acid, 96% for propranolol, and 99% for salicylic acid (Ternes, 1998; Ternes et al., 1999; Heberer, 2002). Lowest average removal rates were found for diclofenac (26%), the removal of bezafibrate was 51%, but varied significantly between STPs, and high removal rates were found for naproxen (81%) (Lindqvist et al., 2005). Table 2 shows that removal rates are variable, even for the same pharmaceutical between different treatment plants. Very high total elimination of 94–100% of ibuprofen, naproxen, ketoprofen and diclofenac was found in three STPs in the U.S.A. (Thomas and Foster, 2004). Efficient removal took place mainly in the secondary treatment step (51–99% removal), whereas in the primary treatment only 0–44% were removed. X-ray contrast media (diatrizoate, iopamidol, iopromide, iomeprol), to the contrary, were not significantly eliminated (Ternes and Hirsch, 2000). Also, the anticancer drug tamoxifen (antiestrogen) was not eliminated (Roberts and Thomas, 2005). This variation in elimination rates is

Table 2
Influent and effluent concentrations and removal efficiency in sewage treatment plants (different equipment, different countries, sampling in different seasons)

Compound	Influent concentration (μg/L)	Effluent concentration (μg/L)	Maximal removal (%)	Reference
Analgesics and antiinflammatory drugs				
Acetylsalicylic acid	3.2	0.6	81	Ternes et al. (1999)
Salicylic acid	57	0.05	99	Metcalfe et al. (2003a)[a]
	330	3.6		Carballa et al. (2004)
Dextropropoxyphene	0.03	0.06	0	Roberts and Thomas (2005)[a]
Diclofenac	3.0	2.5	17	Heberer (2002)
	n.r.	n.r.	69	Ternes (1998)[b]
	0.33–0.49	n.r.	75 (10–75)	Andreozzi et al. (2003a)[c]
	[5]	[1.5]	53–74	Strenn et al. (2004)[a]
	1.3	n.r.		Metcalfe et al. (2003a)[a]
	0.47–1.9	0.31–0.93		Buser et al. (1998b)
	2.8	1.9	23 ± 30	Quintana et al. (2005)[b]
	0.4–1.9	0.4–1.9	0	Tauxe-Wuersch et al. (2005)[c]
	0.35 ± 0.1	0.17–0.35	9–60	Lindqvist et al. (2005)[c]
	1.0	0.29	71	Roberts and Thomas (2005)[a]
Ibuprofen	3		96	Buser et al. (1999)
	38.7	4	>90	Metcalfe et al. (2003a)[a]
	9.5–14.7	0.01–0.02	99	Thomas and Foster (2004)
	[0.54]	[0.08–0.28]	22–75 99 (52–99)	Andreozzi et al. (2003a)[c]
	[1.5]	[0.01]	12–86	Strenn et al. (2004)[a]
	2.6–5.7	0.9–2.1	60–70	Carballa et al. (2004)[a]
	5.7	0.18	97 ± 4	Quintana et al. (2005)[b]
	28.0	3.0	98	Roberts and Thomas (2005)[a]
	2–3	0.6–0.8	53–79	Tauxe-Wuersch et al. (2005)[c]
	13.1 ± 4	0–3.8	78–100	Lindqvist et al. (2005)[c]
Ketoprofen	0.41–0.52	0.008–0.023	98	Thomas and Foster (2004)
	[0.55]	[0.18–0.3]	48–69	Stumpf et al. (1999)[b]
	5.7	n.r.		Metcalfe et al. (2003a)[a]
	0.47	0.18	62 ± 21	Quintana et al. (2005)[b]
	0.25–0.43	0.15–0.24	8–53	Tauxe-Wuersch et al. (2005)[c]
	2.0 ± 0.6	0–1.25	51–100	Lindqvist et al. (2005)[c]
Mefenamic acid	1.6–3.2	0.8–2.3	2–50	Tauxe-Wuersch et al. (2005)[c]
	0.20	0.34	0	Roberts and Thomas (2005)[a]
Naproxen			66	Ternes (1998)[b]
	40.7	12.5	40–100	Metcalfe et al. (2003a)
	10.3–12.8	n.d.-0.023	100	Thomas and Foster (2004)
	[0.6]	[0.1–0.54]	15–78	Stumpf et al. (1999)[b]
			93 (42–93)	Andreozzi et al. (2003a)[c]
	1.8–4.6	0.8–2.6	40–55	Carballa et al. (2004)[a]
	0.95	0.27	71 ± 18	Quintana et al. (2005)[b]
	4.9 ± 1.7	0.15–1.9	55–98	Lindqvist et al. (2005)[c]
Paracetamol	6.9	0	100	Roberts and Thomas (2005)[a]
β-Blocker				
Metoprolol	n.r.	n.r.	83	Ternes (1998)[b]
	n.r.	n.r.	10 (0–10)	Andreozzi et al. (2003a)[c]

Table 2 (*Continued*)

Compound	Influent concentration (μg/L)	Effluent concentration (μg/L)	Maximal removal (%)	Reference
Propranolol	n.r.	n.r.	96	Ternes (1998)[b]
	70	304	0	Roberts and Thomas (2005)[a]
Atenolol	n.r.	n.r.	<10 (0–10)	Andreozzi et al. (2003a)[c]
Blood lipid lowering agents				
Bezafibrate	[1.18]	[0.6–0.84]	27–50	Stumpf et al. (1999)[b]
	n.r.	n.r.	83	Ternes (1998)[b]
	[5]	[0.01]	10–97	Strenn et al. (2004)[a]
	0.6	0.2		Metcalfe et al. (2003a)[a]
	2.6	0.24	91 ± 4	Quintana et al. (2005)[b]
	0.42 ± 0.3	0–0.85	15–100	Lindqvist et al. (2005)[c]
Gemfibrozil	n.r.	n.r.	69	Ternes (1998)[b]
	[0.3]	[0.18–0.28]	16–46	Stumpf et al. (1999)[b]
	n.r.	n.r.	75 (10–75)	Andreozzi et al. (2003a)[c]
	0.7	1.3	n.r.	Metcalfe et al. (2003a)[a]
Fenofibric acid	[0.44]	[0.22–0.4]	6–45	Stumpf et al. (1999)[b]
	n.r.	n.r.	64	Ternes (1998)[b]
Clofibric acid	n.r.	n.r.	6–50	Stumpf et al. (1996)
	[1]	[0.68–0.88]	15–34	Stumpf et al. (1999)[b]
	n.r.	n.r.	51	Ternes (1998)[b]
	0.15–0.25	0.15–0.25	0	Tauxe-Wuersch et al. (2005)[c]
	0.34	0	91	Roberts and Thomas (2005)[a]
Neuroactive compounds				
Carbamazepine	n.r.	n.r.	7–8	Ternes (1998)[b]
	0.7	0.7	<50	Metcalfe et al. (2003a)[a]
	n.r.	n.r.	8	Heberer (2002)
	[1.5]	n.r.	4	Clara et al. (2004)[a]
	n.r.	[1.5]	53 (0–53)	Andreozzi et al. (2003a)[c]
Diazepam	0.59–1.18	0.1–0.66	93	Van Der Hoeven (2004)
Various				
Ethinylestradiol	0.003	0.0004	85	Baronti et al. (2000)
Clotrimazole	0.031	0.14	55	Roberts and Thomas (2005)[a]
Ifosfamide	0.007–0.029	0.010–0.043	0	Kümmerer et al. (1997)[a]
Tamoxifen	0.15	0.20	0	Roberts and Thomas (2005)[a]
X-ray contrast media	0.18–7.5	0.14–8.1	0	Ternes and Hirsch (2000)[b]

Data estimated from graphical data are in square brackets. n.r.: not reported.
[a] Median concentrations or percent.
[b] Average concentrations or percent.
[c] Maximal concentrations or percent.

not surprising, since pharmaceuticals form a heterogeneous group consisting of compounds with diverse chemical properties. Independent from the chemical characteristics of the compounds, the efficiencies of various STPs also vary for the same compound due to their equipment and treatment steps but also to other factors such as temperature and weather. For instance, diclofenac showed largely different elimination rates between 17% (Heberer, 2002) and 69% (Ternes, 1998), and 100% (Thomas and Foster, 2004).

Once in *surface waters*, biotransformation through biodegradation occurs, but abiotic transformation

reactions are probably more important. Whereas hydrolysis is generally negligible for environmentally relevant human drugs, photodegradation sometimes plays an important role at the water surface. Photolysis has been shown to be the main removal process for diclofenac in surface water (Buser et al., 1998b). For additional pharmaceuticals (sulfamethoxazole, ofloxacin and propranolol) laboratory experiments indicate direct and indirect photolysis as an important removal process (Andreozzi et al., 2003b). Carbamazepine and clofibric acid, both compounds that are marginally processed in STP, have been shown to undergo slow photodegradation in salt- and organic-free water with estimated half-lives in the range of 100 days at latitudes of 50°N in winter (Andreozzi et al., 2003b). The efficiency of photodegradation depends, besides substance properties, on the strength of the solar irradiation, and therefore on latitude and season, and on constituents present in the water that may act as photosensitizers generating hydroxyl radicals and singlet oxygen (i.e. nitrates, humic acids). Some adsorption to particles may occur. Laboratory batch studies to characterize the sorption behavior of carbamazepine, diclofenac and ibuprofen in sandy sediments show that sorption coefficients were generally quite low (Scheytt et al., 2005). Diclofenac and ibuprofen are carboxylic acids with pK_a values of 4.16 and 4.52 and these weak acids are negatively charged at pH of ambient water and sediment.

There is no information about the *bioaccumulation* potential of pharmaceuticals in biota or food webs with the exception of diclofenac, accumulating in the prey of vultures (Oaks et al., 2004), fluoxetine, sertraline and the SSRI metabolites norfluoxetine and desmethylsertraline detected in fish (Brooks et al., 2005). Diclofenac bioconcentration factors were 10–2700 in the liver of fish and 5–1000 in the kidney, depending on exposure concentrations (Schwaiger et al., 2004).

A few cases were reported, where pharmaceuticals were detected in *drinking water* (Heberer and Stan, 1996) and groundwater (Holm et al., 1995; Ternes et al., 2001). Ozonation, granulated activated carbon, and advanced oxidation have been shown as efficient removal processes. In drinking water, this has been shown for diclofenac, while clofibric acid and ibuprofen were oxidized in laboratory experiments mainly by ozone/H_2O_2 (Zwiener and Frimmel, 2000). The elimination of selected compounds (bezafibrate, clofibric acid, carbamazepine, diclofenac) during drinking water treatment was investigated in laboratory experiments and waterworks (Ternes et al., 2002). No significant removal was observed in batch experiments with sand, indicating low sorption properties and persistence. Flocculation using iron(III) chloride was ineffective, but ozonation was in some cases very effective in eliminating these polar pharmaceuticals. However, clofibric acid was stable and not eliminated, even with filtration using granular activated carbon, which was effective for the other compounds. The removal of pharmaceuticals and other polar micro-pollutants can therefore only be assured using more advanced techniques such as ozonation, activated carbon or membrane filtration (Ternes et al., 2002). However, the economic consequences have to be evaluated carefully before investing into these advanced treatment technologies on a larger scale.

4. Environmental concentrations

The occurrence of pharmaceuticals was first reported in the U.S.A. in treated wastewater, where clofibric acid in the range of 0.8–2 μg/L was found (Garrison et al., 1976). Subsequently, pharmaceuticals were detected in the U.K. in 1981 in rivers up to 1 μg/L (Richardson and Bowron, 1985), and ibuprofen and naproxen were identified in wastewaters in Canada (Rogers et al., 1986). In the last few years, knowledge about the environmental occurrence of pharmaceuticals has increased to a large extent due to new analytical techniques able to determine polar compounds at trace quantities. This also holds for the steroid hormones contained in contraceptive pills such as 17α-ethinylestradiol (EE2), which is linked to biological effects in fish (Stumpf et al., 1996; Desbrow et al., 1998). Data on environmental concentrations up to 2004 have been compiled and reviewed (e.g. Halling-Sorensen et al., 1998; Daughton and Ternes, 1999; Kümmerer, 2001; Heberer, 2002; Kümmerer, 2004). Here, we give a summary on environmental concentrations focusing on most recent analytical data with the ultimate aim to relate them to ecotoxicological data. First, we give a general overview on the occurrence of pharmaceuticals in general and in different environmental media, and subsequently present data on the different pharmaceutical classes.

Recent studies reported concentrations of a wide range of about 80–100 pharmaceuticals from many classes of drugs (antiinflammatory, beta-blockers, sympathomimetics, antiepileptics, lipid regulators, antibiotics, etc.) and some of their metabolites in many countries in treated sewage, rivers and creeks, seawater, groundwater and even drinking water. Ternes (1998) reported on the occurrence of 32 pharmaceuticals belonging to different medicinal classes in German municipal STP effluents, river and stream waters. Twenty different drugs and four corresponding metabolites including antiinflammatory drugs (salicylic acid, diclofenac, ibuprofen, indometacine, naproxen, phenazone), lipid regulators (bezafibrate, gemfibrozil, clofibric acid, fenofibric acid), beta-blockers (metoprolol, propranolol) and carbamazepine were found to be ubiquitously present in streams and river water in the ng/L range. In an extended monitoring study concentrations of 95 micro-pollutants in water samples of 139 streams downstream of urban areas and livestock production across the U.S.A. were detected (Kolpin et al., 2002). In some sites as many as 38 of the targeted 95 compounds were detected in a single water sample (average number of compounds in a sample was seven). Among the most frequently detected compounds were steroids (although some data had to be withdrawn subsequently), an insect repellant (*N*,*N*-diethyltoluamide), caffeine, triclosan (an antimicrobial compound), antibiotics, a fire retardant, 4-nonylphenol and some pharmaceuticals. Analysis of the distribution of different drugs in the river Elbe and its tributaries between the source and the city of Hamburg, Germany, showed the presence of many pharmaceuticals. The main substances found were diclofenac, ibuprofen, carbamazepine, various antibiotics and lipid regulators (Wiegel et al., 2004). A similar contamination pattern was found in Italy in the river Po and Lambro (Calamari et al., 2003) where at all sampling sites atenolol, bezafibrate, furosemide, and antibiotics were found and ranitidine, clofibric acid, diazepam were often detected. Kolpin et al. (2004) collected water samples upstream and downstream of selected towns and cities in Iowa, U.S.A., during high-, normal- and low-flow conditions to determine the contribution of urban centres to concentrations of pharmaceuticals and other organic wastewater contaminants in streams under varying flow conditions. Prescription drugs were only frequently detected during low-flow conditions.

Environmental concentrations of pharmaceuticals were mainly reported in STP effluents and in surface water in many countries, often at locations near STPs (Halling-Sorensen et al., 1998; Kolpin et al., 2002; Ashton et al., 2004; Gross et al., 2004). The occurrence of selected pharmaceuticals was also reported in the Tyne estuary in the U.K. with concentrations ranging from 4 to 2370 ng/L (Roberts and Thomas, 2005). Fig. 1 gives a summary on the concentrations of most frequently assessed pharmaceuticals in wastewater and surface water reported so far. In STP effluents a number of different pharmaceuticals occur at concentrations generally in the ng/L to μg/L range. In rivers, lakes and seawaters, they are in the ng/L range (Buser et al., 1998b; Kolpin et al., 2002; Weigel et al., 2002; Ashton et al., 2004; Thomas and Hilton, 2004). The rather persistent antiepileptic carbamazepine, and clofibric acid, a metabolite of the lipid lowering agents clofibrate, etofibrate and etofyllin clofibrate, have been detected with few exceptions in STP effluents, freshwater (rivers and lakes) and even in seawater (Buser et al., 1998b; Weigel et al., 2002). In surface water, carbamazepine is found with maximal concentrations of 1.2 μg/L (Wiegel et al., 2004) and clofibric acid at 0.55 μg/L (Ternes, 1998). Carbamazepine contamination is widespread. In 44 rivers across the U.S.A. average levels were 60 ng/L in water and 4.2 ng/mg in the sediment (Thaker, 2005). Frequently, the analgesic ibuprofen and its metabolites were detected in STP effluents (Ternes, 1998; Buser et al., 1999; Boyd et al., 2003; Weigel et al., 2004), in surface water of up to 1 μg/L (Kolpin et al., 2002), and in seawater (Thomas and Hilton, 2004; Weigel et al., 2004). In a monitoring study in the U.K. propranolol (median level 76 ng/L) was always found in STP effluents, whereas diclofenac (median 424 ng/L) was found in 86%, ibuprofen (median 3086 ng/L) in 84%, mefenamic acid (median 133 ng/L) in 81%, dextropropoxyphene (median 195 ng/L) in 74%, and trimethoprim (median 70 ng/L) in 65% of the samples (Ashton et al., 2004). In the corresponding receiving streams, fewer compounds and lower levels were found.

Some drinking waters (Heberer and Stan, 1996; Stumpf et al., 1999; Putschew et al., 2000; Zuccato et al., 2000; Stackelberg et al., 2004), groundwaters (Holm et al., 1995; Ternes et al., 2001), and landfill leachates (Holm et al., 1995) contain pharmaceuticals in the ng/L range, in some cases up to μg/L. Phenazone,

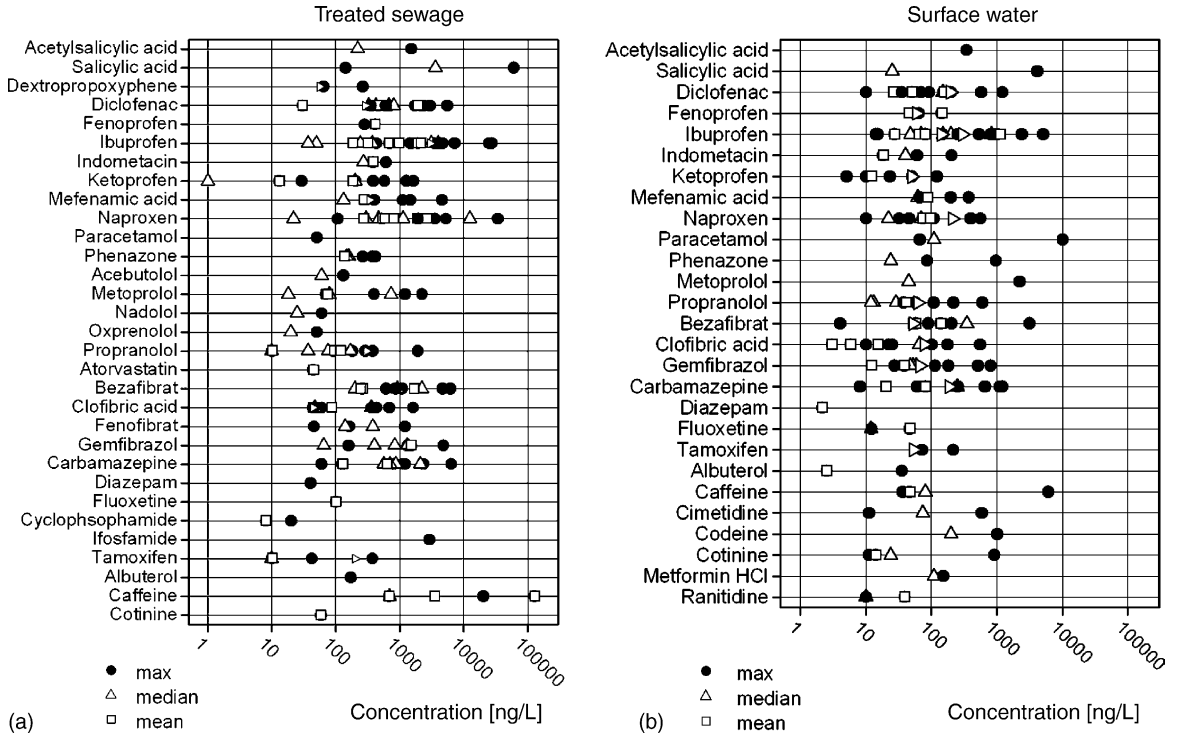

Fig. 1. Concentration of pharmaceuticals in treated wastewater (a) and surface water (b). *References*: Halling-Sorensen et al. (1998), Ternes (1998), Stuer-Lauridsen et al. (2000), Jones et al. (2002), Kolpin et al. (2002), Andreozzi et al. (2003b), Calamari et al. (2003), Metcalfe et al. (2003a,b), Gross et al. (2004), Khan and Ongerth (2004), Kümmerer (2004), Stackelberg et al. (2004), Thomas and Hilton (2004), Weigel et al. (2004), Lindqvist et al. (2005), Quintana et al. (2005), Roberts and Thomas (2005) and Tauxe-Wuersch et al. (2005).

propiphenazone and clofibric acid were found in samples of potable water collected in the vicinity of Berlin, Germany (Heberer and Stan, 1997; Reddersen et al., 2002). Several polar pharmaceuticals such as clofibric acid, carbamazepine, and X-ray contrast media can occur in groundwater. In the following, current knowledge about major pharmaceuticals of different therapeutic classes is summarized.

4.1. Analgesics and antiinflammatory drugs

The widely used non-steroidal antiinflammatory drugs (NSAID) ibuprofen, naproxen, diclofenac and some of their metabolites (e.g. hydroxyl-ibuprofen and carboxy-ibuprofen) are very often detected in sewage and surface water. Ternes (1998) reported levels in sewage exceeding 1 μg/L, and in effluents of conventional STP (mechanical clarification and biological treatment) concentrations often approach or exceed 0.1 μg/L in the U.S.A. (Gross et al., 2004). The deacylated, more active form of acetylsalicylic acid, salicylic acid, has been found in many municipal wastewaters at levels up to 4.1 μg/L (Ternes, 1998), 13 μg/L (Farré et al., 2001; Heberer, 2002) or even 59.6 μg/L with median levels of 3.6 μg/L (Metcalfe et al., 2003a). However, salicylic acid may also derive from other sources. Similar to acetylsalicylic acid, acetaminophen (paracetamol) is well removed from STP. However, up to 10 μg/L (median 0.11 μg/L) acetaminophen has been found in 24% of samples from U.S. streams (Kolpin et al., 2002). The analgesic codeine was detected in 7% of samples at median concentrations of 0.01 μg/L.

In many countries diclofenac was frequently detected in wastewater in the μg/L range, and in surface water at lower levels (Heberer and Stan, 1997; Buser et al., 1998b; Ternes, 1998; Stumpf et al., 1999; Farré et al., 2001; Sedlak and Pinkston, 2001; Heberer,

2002). This also holds for ibuprofen (Heberer and Stan, 1997; Ternes, 1998; Buser et al., 1999; Stumpf et al., 1999). Sometimes, high levels of up to 85 μg/L (Farré et al., 2001), or 24.6 μg/L (median 4.0 μg/L) were detected in STP effluents (Metcalfe et al., 2003a). In Norway, ibuprofen and its metabolites occurred in all sewage samples, and in seawater at concentrations of 0.1–20 μg/L (sum of ibuprofen and metabolites) (Weigel et al., 2004). In U.K. estuaries maximal concentration of 0.93 μg/L (median 0.05 μg/L) occurred (Thomas and Hilton, 2004). Ibuprofen is significantly removed during sewage treatment, and metabolites such as hydroxy-ibuprofen occur in STP effluents. Kolpin et al. (2002) found ibuprofen in 10% of stream water samples with maximal concentrations of 1 μg/L (median 0.2 μg/L). In two stormwater canals levels of ibuprofen were up to 674 ng/L and of naproxen up to 145 ng/L (Boyd et al., 2004). Naproxen was also found at much higher level in Canadian STP effluents with median levels of 12.5 μg/L and maximal levels of up to 33.9 μg/L (Metcalfe et al., 2003a). Moreover, several other analgesics have been detected in sewage and surface water, but also in ground water and drinking water samples.

4.2. Beta-blockers

Several beta-blockers were identified in wastewater (Ternes, 1998; Sedlak and Pinkston, 2001). Propranolol, bisoprolol and metoprolol were found at highest levels (0.59, 2.9 and 2.2 μg/L, respectively, in surface water), with lower levels of nadolol (in surface water) and betaxolol (0.028 μg/L in surface water) (Ternes, 1998). Propranolol, metoprolol and bisoprolol have also been found in surface water, and sotalol in groundwater (Sacher et al., 2001).

4.3. Blood lipid lowering agents

Clofibric acid, the active metabolite from a series of widely used blood lipid regulators (clofibrate, etofyllin clofibrate, etofibrate) belongs to the most frequently found and reported pharmaceutical in monitoring studies. It has been found in numerous wastewaters, surface waters, in seawater (Stumpf et al., 1996; Buser et al., 1998a; Ternes, 1998), and at rather high concentrations in groundwater (4 μg/L) (Heberer and Stan, 1997) and drinking water (0.07–0.27 μg/L) (Stumpf et al., 1996; Heberer and Stan, 1997). Bezafibrate occurred in maximal concentrations of up to 4.6 and 3.1 μg/L (median 2.2 and 0.35 μg/L, respectively) in wastewater and surface water, respectively (Stumpf et al., 1996; Ternes, 1998). In addition, gemfibrozil, clofibric acid and fenofibric acid (metabolite of fenofibrate) have also been detected in sewage up to the μg/L level and in surface water (Ternes, 1998; Stumpf et al., 1999; Farré et al., 2001; Heberer, 2002). Gemfibrozil was detected in 4% of streams at maximal levels of 0.79 μg/L (Kolpin et al., 2002).

4.4. Neuroactive compounds (antiepileptics, antidepressants)

Of this category, the antiepileptic carbamazepine was detected most frequently and in highest concentration in wastewater (up to 6.3 μg/L) (Ternes, 1998), and at lower levels in other media (Heberer et al., 2002; Andreozzi et al., 2003b; Metcalfe et al., 2003b; Wiegel et al., 2004). Carbamazepine was found in every Canadian STP effluent sample at concentration up to 2.3 μg/L (Metcalfe et al., 2003b). This compound was found to occur ubiquitously in the river Elbe and its tributaries, Germany (Wiegel et al., 2004), exceeding 1 μg/L in other German surface waters (Ternes, 1998; Heberer, 2002) and occurred in groundwater (Seiler et al., 1999; Sacher et al., 2001; Ternes et al., 2001). In U.S. rivers average levels were 60 ng/L in water and 4.2 ng/mg in the sediment (Thaker, 2005). Carbamazepine was also found at average levels of 20.9 ng/mg solids of STP. Diazepam was present in 8 of 20 STPs in Germany at relatively low concentrations of up to 0.04 μg/L (Ternes, 1998) whereas in Belgium it was found at concentration up to 0.66 μg/L (van der Ven et al., 2004). The antidepressant fluoxetine was also detected in STP effluents samples in Canada (Metcalfe et al., 2003a), and in U.S. streams, median concentrations of 0.012 μg/L were estimated (Kolpin et al., 2002). Primidone, an antiepileptic drug, has also been detected in sewage up to 0.6 μg/L (Heberer, 2002).

4.5. Antineoplastics and antitumor agents

Pharmaceuticals used in cancer chemotherapy occur primarily in hospital effluents and only at lower concentrations in municipal wastewater. Ifosfamide and

cyclophosphamide occur in concentrations of up to 4.5 μg/L in hospital wastewaters (Steger-Hartmann et al., 1997), and at ng/L in municipal wastewater (Kümmerer et al., 1997; Steger-Hartmann et al., 1997). The occurrence of the antiestrogen tamoxifen used in breast cancer therapy was reported in U.K. wastewater, where concentrations in STP effluents ranged between 146 and 369 ng/L (Roberts and Thomas, 2005). Tamoxifen was not reduced in the STP, and even found in estuarine waters (Tye estuary) at concentrations ranging from 27 to 212 ng/L with a median level of 53 ng/L (Thomas and Hilton, 2004; Roberts and Thomas, 2005).

4.6. Various other compounds

Many additional pharmaceuticals have been detected in sewage and surface water (Daughton and Ternes, 1999; Heberer, 2002; Kolpin et al., 2002). Here only a few of them will be mentioned. The stimulant caffeine and the nicotine metabolite cotinine were generally present in STP effluents and surface waters contaminated by drugs (Metcalfe et al., 2003b). Caffeine was generally found in U.S. streams at maximal levels of 6.0 μg/L (median 0.1 μg/L) (Kolpin et al., 2002) and this compound can even serve as an anthropogenic marker in aquatic systems due to its ubiquity in surface water, seawater (Weigel et al., 2004), and also in groundwater (Fig. 1). The antiacid cimetidine and ranitidine were estimated to occur in U.S. streams at concentrations of 0.58 and 0.01 μg/L, respectively, and they were detected at a frequency of 10 and 1%, respectively (Kolpin et al., 2002). X-ray contrast media are very persistent. Iopamidol has been found in municipal wastewater as high as 15 μg/L, in surface water (0.49 μg/L) and groundwater (Putschew et al., 2000; Ternes and Hirsch, 2000). Iopromide was detected at 2–4 μg/L in surface water, and up to 21 μg/L in STP (Putschew et al., 2000), but showed degradation in the laboratory (Steger-Hartmann et al., 2002). Hospital wastewater was also a source of gadolinium (Kümmerer and Helmers, 2000). The antidiabetic compound metformin was observed in 5% of stream water samples with estimated levels of 0.11 μg/L (Kolpin et al., 2002). Bronchodilators (β_2-sympathomimetics terbutalin and salbutamol) were also detected in sewage in a few cases not exceeding 0.2 μg/L (Ternes, 1998).

4.7. Steroidal hormones

Steroidal hormones have been reported on in many reports, and in our review we only summarize knowledge about the synthetic estrogen EE2 and mestranol contained in contraceptive pills. These steroids have been found in numerous studies in many countries in Europe, Canada, the U.S.A., Japan, Brazil, etc. both in wastewater and surface water. A survey in the U.S.A. showed that maximal and median EE2 concentrations were as high as 831 and 73 ng/L, respectively, and levels of mestranol were 407 and 74 ng/L, respectively (Kolpin et al., 2002). They were detectable in 16 and 10% of the streams sampled. Generally, median concentrations are much lower being in the range of non-detectable up to 9 ng/L in treated wastewater in several countries (Baronti et al., 2000). Typical wastewater effluent concentrations are 0.5 ng/L and they are even lower in surface water. However, these concentrations must put into the perspective of their high biological activity accounting for potential estrogenic effects in fish.

Exposure and fate models are increasingly being used to estimate environmental concentrations without analytical chemical measurements. Some exposure models have been developed for drugs (e.g. PhATE), others have been extended from general chemicals to pharmaceuticals (e.g. EPIWIN, GREAT-ER). These tools have been developed both for estimation of predicted environmental concentrations (PEC) and the behavior of pharmaceuticals in the environment. A pharmaceutical assessment and transport evaluation model (PhATE) was developed to estimate concentrations of active pharmaceutical ingredients in U.S. surface waters (Anderson et al., 2004). The PhATE model uses some for most hydrologic regions of the U.S. representative watersheds. For European surface waters an exposure simulation was developed for pharmaceuticals with the GREAT-ER (Geo-referenced Regional Exposure Assessment Tool for European Rivers) model, a tool developed for use within ecological risk assessment (ERA) schemes and river basin management (Schowanek and Webb, 2002). The GREAT-ER software calculates the distribution of PEC's of consumer chemicals in surface waters, for individual stretches, as well as representative average PEC's for entire catchments. The system uses an ARC/INFO-ArcView (ESRI®) based Geographical

Information System (GIS) for data storage and visualization, combined with simple mathematical models for prediction of the fate of chemicals.

For some estimates, measured environmental concentrations (MEC) are in agreement with the estimated PEC's, however, often, they are not as large differences occur between the models and the real world situation. The main reason is that different assumptions are made, which not always correspond to the real conditions in the environment. Consumption figures, metabolism in the organism, removal during sewage treatment plants and fate in the environment contain all uncertainties that may result in inappropriate estimates of PEC's. Moreover, detailed situations at a given site is not reflected by models integrating large geographical areas. Poor prediction performance of current models for many pharmaceuticals is one of the outstanding scientific issues with regard to the question of pharmaceuticals in the environment. It is hoped that the models are improving by further refining the mentioned uncertainties and may be developing to a useful and readily applicable regulatory tool (Sanderson et al., 2004b).

5. Modes of actions in humans and mammals and occurrence of target biomolecules in lower vertebrates and invertebrates

Here, we briefly summarize the modes of actions of pharmaceutical classes and ask, whether or not similar target receptors and biomolecules exist in lower vertebrates and invertebrates. Knowledge about similar targets exists primarily for fish. In general, very little is known about possible counterparts of human target biomolecules of pharmaceuticals in invertebrates. In addition, some of the side effects in humans are discussed, giving hints to possible adverse effects in lower animals.

5.1. Analgesics and non-steroidal antiinflammatory drugs (NSAID)

Non-steroidal antiinflammatory drugs act by inhibiting either reversibly or irreversibly one or both of the two isoforms of the cyclooxygenase enzyme (COX-1 and COX-2), which catalyze the synthesis of different prostaglandins from arachidonic acid (Vane and Botting, 1998). Classical NSAID inhibit both COX-1 and COX-2 at different degrees, whereas new NSAID act more selectively on COX-2, the inducible form responsible for the inflammatory reactions. Differences in binding site size are responsible for the selectivity of these drugs (Kurumbail et al., 1997; Penning et al., 1997; Gierse et al., 1999). NSAIDS are commonly used to treat inflammation and pain and to relieve fever, and sometimes they are also used for long-term treatment of rheumatic diseases.

Prostaglandins play a variety of physiological roles according to their cells source and target molecules. They are known to be involved in process such as inflammation and pain, regulation of blood flow in kidney, coagulation processes and synthesis of protective gastric mucosa (Smith, 1971; Vane, 1971; Mutschler, 1996). Since NSAID inhibit non-specifically prostaglandin synthesis, most side effects, at least after long-term treatment, are related to the physiological function of prostaglandins. In the kidney, prostaglandins are involved in maintenance of the equilibrium between vasoconstriction and vasodilatation of the blood vessel that supply glomerular filtration. Renal damages and renal failure after chronic NSAID treatment seems to be triggered by the lack of prostaglandins in vasodilatation-induction. Gastric damages are thought to be caused by inhibition of both COX isoforms (Wallace, 1997; Wallace et al., 2000). In contrast, liver damages are apparently due to building of reactive metabolites (e.g. acyl glucuronides) rather than inhibition of prostaglandins synthesis (Bjorkman, 1998).

The mode of action of paracetamol is not yet fully elucidated. It seems that this drugs acts mainly by inhibiting the cyclooxygenase of the central nervous system and it does not have antiinflammatory effects, because of the lack of inhibition of peripheral cyclooxygenase involved in inflammatory processes. Adverse effects of paracetamol are mainly due to formation of hepatotoxic metabolites, primarily *N*-acetyl-*p*-benzoquinone imine, synthesized when the availability of glutathione is diminished in liver cells. Acetaminophen widely used in many analgesic/antipyretic medications induces proliferation of cultured breast cancer cells via estrogen receptors without binding to them, but has no estrogenic activity in rodents (Harnagea-Theophilus et al., 1999). The consequences of these observations are not clear.

In fish an inducible COX-2 homologue has been found to be expressed in macrophages in rainbow trout (*Oncorhynchus mykiss*) and the translation product of the COX gene was found to have a high homology of 83–84 and 77% to its human counterpart COX-2 and COX-1, respectively (Zou et al., 1999). Also in goldfish, macrophages express a COX enzyme, which is an equivalent to mammalian COX-2 (Zou et al., 1999). A COX-1 and COX-2 homologue was cloned from brook trout ovary (Roberts et al., 2000), and recently, a shark COX was cloned in dogfish *Squalus acanthias* having 68 and 64% homology to mammalian COX-1 and COX-2, respectively (Yang and Carlson, 2004). Prostaglandins are formed in a diverse range of vertebrates and invertebrates. However, in lower invertebrates such as corals, their synthesis is independent of COX, involving other enzymes (Song and Brash, 1991). In arthropods and molluscs, COX-like activity is apparently responsible for the formation of prostaglandins, but these enzymes have not been purified and characterized (Pedibhotla et al., 1995). In birds, prostaglandins play a role in the biosynthesis of egg shells and treatment with the COX-inhibitor indometacine resulted in egg shell thinning (Lundholm, 1997).

5.2. Beta-blockers

Beta-blocker act by competitive inhibiting beta-adrenergic receptors and they are used in the treatment of high blood pressure (hypertension), and to treat patients after heart attack to prevent further attacks. The adrenergic system is involved in many physiological functions such as regulation of the heart oxygen need and beating, vasodilatation mechanisms of blood vessels, and bronchodilation. Furthermore, the adrenergic system is also known to interact with carbohydrate and lipid metabolisms, mainly in response to stress needs such as starvation (Jacob et al., 1998).

β-Adrenoceptors are 7-transmembrane receptor proteins coupled with different G-proteins that ultimately enhance the synthesis of the second messenger signaling molecules cAMP (Rang et al., 2003). According to medical needs beta-blockers may selectively inhibit one or more β-receptors types; for example β_2-blockers are used to treat hypertension avoiding cardiac effects, since this receptor subtype is not found in the heart. Selectivity is based on difference in chemical groups added to compounds that are able to enhance the interactions with amino acids of the transmembrane domains. Some of the beta-blockers (e.g. propranolol, a beta1-adrencoceptor antagonist) have the ability to cause cell membrane stabilization, whereas other (e.g. metoprolol) have no membrane stabilizing activity (Doggrell, 1990). Side effects of this therapeutic class are mainly bronchoconstriction and disturbed peripheral circulations (Hoffman and Lefkowitz, 1998; Scholze, 1999). Due to their lipophilicity they are supposed to pass the blood brain barrier and to act in the central nervous system (Soyka, 1984, 1985).

β-Adrenoceptors were found in fish (*O. mykiss*) liver, red and white muscle with a high degree of sequence conservation with other vertebrate homologues. They are also supposed to play similar role as in humans (Nickerson et al., 2001). The presence of a β_2-adrenoceptor subtype was also suggested by binding studies to occur in liver membranes of other fish and amphibians. β_2-Adrenoceptors of rainbow trout (Nickerson et al., 2001) show a high degree of amino-acid sequence conservation with other vertebrate β_2-adrenoceptors. Frog- (Devic et al., 1997) and turkey β_1-adrenoceptors (Yardeny et al., 1986) are similar to mammalian β_1-adrenoceptors. In rainbow trout, the β_2-adrenoceptor gene is highly expressed in the liver, red and white muscle, with lower expression in gills, heart, kidney and spleen (Nickerson et al., 2001). Clenbuterol or ractopamine that function in mammals as β-agonist were found in rainbow trout to show a somewhat different reaction. Clenbuterol displayed only partial agonist activities and the small effects of ractopamine may be related to low affinity for the trout β_2-adrenoceptor. Agonist regulation of the trout hepatic β_2-adrenoceptors may involve down-regulation of the receptors without affecting responsiveness (Dugan et al., 2003). Differences in the structure and function of the receptors may be responsible for differences in the affinity with β-blockers and mechanisms triggered by these drugs.

Whereas mammals have three α_2-adrenoceptors, five distinct α_2-adrenoceptor genes have been found expressed in zebrafish (Ruuskanen et al., 2005). Localization of the α-adrenoceptors in zebrafish shows marked conservation when compared with mammals. The α_2-adrenergic system is functional in zebrafish as demonstrated by marked locomotor inhibition and lightening of skin color induced by the specific

α_2-adrenoceptor agonist dexmedetomidine, similar to mammals. Both effects were antagonized by the specific α_2-adrenoceptor antagonist atipamezole. The α-adrenoceptor agonists medetomidine and clonidine are being investigated as potential antifouling agents preventing the settlement of barnacles on ship halls (Dahlstrom et al., 2004). Settlement of larvae is inhibited at low concentrations of 0.25–2.5 μg/L. Additional pharmacological and biochemical investigations on α- and β-adrenoceptors of fish and other lower organisms are needed.

5.3. Blood lipid lowering agents

There are basically two types of antilipidemic drugs, namely statins and fibrates, the latter have been targeted analytically more often in the aquatic environment than the former. Both types are used to decrease the concentration of cholesterol (statins and fibrates) and triglycerides (fibrates) in the blood plasma. Statins as inhibitors of cholesterol synthesis act by inhibiting the 3-hydroxymethylglutaril coenzyme A reductase (HMG-CoA), responsible for the limiting step in the cholesterol synthesis, namely the conversion of HMG-CoA to mevalonate (Laufs and Liao, 1998). As a consequence of the intracellular cholesterol depletion, the expression of LDL receptors in hepatocyte membranes is increased and therefore, the resorption of LDL-cholesterol from blood plasma. Due to interactions of statins with mevalonate metabolism, multiple additional effects occur (antiinflammatory, antioxidative). There is also evidence that statins affect juvenile hormone synthesis in insects (Debernard et al., 1994), as fluvastatin completely suppressed its biosynthesis in vitro, and in the mandibular organo of lobsters (Li et al., 2003).

In contrast, effects of fibrates are mediated, at least in part, through alterations in transcription of genes encoding for proteins controlling lipoprotein metabolism. Fibrates act probably by activating the lipoprotein lipase enzyme, which is mainly responsible for the conversion of very low density lipoprotein (VLDL) to high density lipoproteins (HDL), decreasing therefore plasma triglycerides concentration (Staels et al., 1998). Binding of fibrates to peroxisome proliferator-activated receptors (PPARs), nuclear receptors known to be activated during different cellular pathways, stimulates the expression of several lipid regulatory proteins such as, for example, the lipoprotein lipase (Staels et al., 1998). To date, three subtypes of PPAR have been described; PPARα is involved in peroxisome proliferation and plays a pivotal role in controlling hepatic lipid metabolism (Schoonjans et al., 1996), whereas PPARβ has diverse roles in basic lipid metabolism, and PPARγ plays a key role in the differentiation of adipocytes (Kersten et al., 2000). Heterodimerization of PPARs with the retinoid X receptor and their binding to response elements in the promoter regions of genes leads to their activation.

Fibrates stimulate cellular fatty acid uptake, conversion to acetyl-CoA derivatives, and catabolism by the beta-oxidation pathways, which, combined with a reduction in fatty acid and triglyceride synthesis, results in a decrease in VLDL production (Staels et al., 1998). Hepatic damages may occur after chronic exposure to fibrates in rat (Qu et al., 2001) and this is thought to be related to inhibition of mitochondrial oxidative phosphorylation (Keller et al., 1992). Furthermore, fibrates caused in rodents a massive proliferation of peroxisomes (Hess et al., 1965). Strong correlation between fibrates exposure and hepatocarcinogenicity in rodents were found, while this was not observed in humans (Cajaraville et al., 2003). These findings increase the interest for ecotoxicological impact of this therapeutic class of drugs.

PPAR genes have been found in fish such as plaice (Leaver et al., 1998) and Atlantic salmon (Ruyter et al., 1997) and zebrafish (Ibabe et al., 2002). Fish PPARs display an amino acid sequence identity of 43–48% to the human and amphibian PPARγ (Andersen et al., 2000). All PPAR forms have been found in zebrafish, and PPARα was mainly expressed in hepatocyte and tissues that catabolize high amounts of fatty acids (Ibabe et al., 2002). Furthermore, PPARγ was shown to be induced in response to clofibrate and bezafibrate in salmon hepatocytes (Ruyter et al., 1997), although their PPARγ seem to be less responsive than PPARγ of rodents (Andersen et al., 2000). All three PPAR receptors were found to already been expressed in the larval stage, with a similar tissue distribution pattern to that found in adult zebrafish (Ibabe et al., 2005a). Activators of PPARα include a variety of endogenously present fatty acids, leukotrienes and hydroxyeicosatetraenoic acids and drugs, such as fibrates (Cajaraville et al., 2003). PPARβ activators include fatty acids, prostaglandin A_2 and prostacyclin. PPARγ

is the most selective receptor and prostaglandin J2 has been described to be a specific ligand (Ibabe et al., 2005b). In isolated zebrafish hepatocytes, mRNA of both PPARα and PPARγ was induced by clofibrate at 0.5–2 mM, although to a low extent (Ibabe et al., 2005b). The physiological and toxicological roles of PPARs have yet to be investigated, and their involvement in potential effects of lipid lowering drugs is not yet known. With regard to invertebrates, no information is currently available on the existence of PPARs, although extensive searches for nuclear receptors in cnidarians and platyhelminthes have been performed (Escriva et al., 1997).

5.4. Neuroactive compounds (antiepileptics, antidepressants)

Among the many drugs interacting with the central nerve system (CNS), only a few will be considered as with respect to its occurrence in the aquatic environment. Antiepileptic drugs act on the CNS by decreasing the overall neuronal activity. This can be achieved either by blocking voltage-dependent sodium channels of excitatory neurons (e.g. carbamazepine), or by enhancing of inhibitory effects of the GABA neurotransmitter by binding on a specific site in the gamma subunit of the corresponding receptor (e.g. diazepam, member of benzodiazepine family) (Study and Barker, 1981; MacDonald and Olsen, 1994; Rogers et al., 1994). Evidence of the occurrence of the GABA system in fish (*O. mykiss*, Cole et al., 1984; Meissl and Ekstrom, 1991) was found, whereas no studies have been found indicating the occurrence of sodium voltage dependent channels in fish or lower invertebrate.

Fluoxetine is a widely used antidepressant, which acts by inhibiting the re-uptake of serotonin. This neurotransmitter is involved in many mechanisms, namely hormonal and neuronal, and it is also important in functions such as food intake and sexual behavior. A pump directs serotonin from the synapse space back to the presynapse, and selective serotonin re-uptake inhibitors (SSRI) inhibit this pump, thus increasing the serotonin level in the synapse space. Serotonin as a neurotransmitter occurs in lower vertebrates and invertebrates (Fong, 1998), however, the effects associated with this transmitter are different, and so are possibly the effects of SSRI. Serotonin mediates, among others, endocrine functions in aquatic organisms such as fingernail claims (*Sphaerium striatinum*, Fong et al., 1998) and Japanese medaka (*Oryzias latipes*, Fong et al., 1998; Foran et al., 2004). Fluoxetine and sertraline and the SSRI metabolites norfluoxetine and desmethylsertraline have been detected in fish sampled from wild in the U.S., and therefore reflect a bioaccumulation potential (Brooks et al., 2005). Whether the accumulated levels of 1.6 ng/g fluoxetine and 4.3 ng/g sertraline found in brain have effects on the nervous system of fish has yet to be investigated.

5.5. Cytostatics compounds and cancer therapeutics

Another potential interesting class of compound is represented by cytostatic pharmaceuticals interacting with cell proliferation. There are different modes of actions of the different compounds. For example methotrexate acts as a potent inhibitor of the folate dehydroreductase enzyme, which is responsible for the purine and pyrimidine synthesis (Schalhorn, 1995; Rang et al., 2003). Doxorubicin is an intercalating substance inducing DNA-strand brakes (in humans, heart arrhythmia may be a side effect). Tamoxifen as an antiestrogenic drug is used for breast cancer treatment and acts by competitive inhibiting the estrogenic receptor at least in mammary gland (Rang et al., 2003).

5.6. Various compounds

Cimetidine and ranitidine are compounds, which act by inhibiting the histamine receptors type 2 in the gastric system, thus inhibiting the acid secretion (antacid). These drugs are used to treat gastric ulceration. Since H_2-histamine receptors are found also in the brain, both drugs may elicit central nervous system reactions and side effects (Cannon et al., 2004). Peitsaro et al. (2000) demonstrated the presence of H_3-histamin receptors in central nervous system of zebrafish (*Danio rerio*), but the lack of histamine in the periphery of this fish was also reported. However, interspecies differences may occur; cod and carp seem to have histamine and H_2-receptors in the periphery (Peitsaro et al., 2000).

Metformin is an antidiabetic agent, which mechanisms of actions are not well understood. It seems that this drugs acts by increasing the cellular use of glucose and inhibiting the gluconeogenesis. Metformin seems to act on insulin receptor by direct stimulation of the

insulin receptor or indirectly through inhibition of tyrosine phosphatase (Holland et al., 2004).

6. Ecotoxicological effects

Pharmaceuticals are designed to target specific metabolic and molecular pathways in humans and animals, but they often have important side effects too. When introduced into the environment they may affect the same pathways in animals having identical or similar target organs, tissues, cells or biomolecules. As shown above, certain receptors in lower animals resemble those in humans, others however, are different or lacking, which means that dissimilar modes of actions may occur in lower animals. It is important in this respect to recognize that for many drugs, their specific modes of actions are not well known and often not only one, but many different modes of actions occur. Among other reasons, this makes specific toxicity analysis in lower animals difficult to perform. Despite this, toxicity experiments should be targeted and designed for specific targets of the pharmaceutical even in lower vertebrates and invertebrates, based on the hypothesis of similarity of modes of actions. However, current toxicity testing is not designed in this way, rather general and established test systems and traditional organisms according to guidelines are being used and traditional end points such as mortality are assessed.

Thus far, ecotoxicity testing merely provided indications of acute effects in vivo in organisms of different trophic levels after short-term exposure, and only rarely after long-term (chronic) exposures. These data are ultimately used for ecological risk assessments. Because of animal welfare and screening purposes, in vitro analyses are becoming more important, but they are not sufficient for assessing the toxicological profiles of a compound, particularly as a basis for risk analysis (Fent, 2001). Beyond laboratory investigations, some mathematical models were developed to estimate or predict ecotoxicological effects. The most often applied quantitative structure–activity relationship (QSAR) program is ECOSAR (online http://www.epa.gov/oppt/newchems/sarman.pdf) (Sanderson et al., 2004b). Despite serious drawbacks such as an inadequate structure coverage for pharmaceuticals, the program has been repeatedly applied to estimate pharmaceutical baseline toxicities (Jones et al., 2002; Sanderson et al., 2004b; Cleuvers, 2005). Both methods are helpful in estimating potential toxicity or the behavior of a compound in the environment, but they cannot replace in vivo or in vitro assays.

The current literature about ecotoxicological effects of human pharmaceutical deals mainly with the acute toxicity in standardized tests and it is generally focused on aquatic organisms. The influence of environmental parameters such as pH on toxicity has only rarely, or not yet been investigated. Such studies would be of importance for instance for acidic pharmaceuticals that may induce different toxicities depending on speciation at different ambient pH. Moreover, effects of drug metabolites have rarely been investigated. Phototransformation products of naproxen, for instance, showed higher toxicities than the parent compound, while genotoxicity was not found (Isidori et al., 2005). At contaminated sites, aquatic life is exposed over the entire life cycle to these compounds. Chronic effects are less investigated and often even related to relative short-term exposures. However, long-term exposures are needed for an accurate environmental risk assessment. Here we summarize the current ecotoxicological data, focusing on specific modes of action of different therapeutic classes of pharmaceuticals, and covering many differences in methods, species and time of exposure. These data are then related to environmental levels in order to assess the potential hazard for the different classes of pharmaceuticals and identify current research and knowledge gaps.

6.1. Acute effects

Pharmaceuticals are assessed for their acute toxicity by traditional standard tests according to established guidelines (e.g. OECD, U.S. EPA, ISO) using established laboratory organisms such as algae, zooplankton and other invertebrates and fish. Acute toxicity data of pharmaceuticals were compiled by Halling-Sorensen et al. (1998) and Webb (2001), whereby in the latter, a list of about 100 human pharmaceuticals from different sources is given. By comparing different trophic levels, Webb (2001) suggested that algae were more sensitive to the listed pharmaceuticals than *Daphnia magna*, and fish were even less sensitive. However, such generalizations do not focus enough on the different modes of actions of a given pharmaceutical, and hence, differences in toxicity in different phyla. In the attempt

to compare the different classes of pharmaceuticals in terms of acute toxicity, Webb (2001) noted that the most toxic classes were antidepressants, antibacterials and antipsychotics, but the range of responses within each of these categories was large, typically several orders of magnitude. In our present review, we provide and summarize additional and new data and discuss its ecotoxicological relevance covering different classes of human pharmaceuticals. The data originate from different sources, and studies were performed under different quality criteria (i.e. nominal versus measured exposure concentrations), making comparisons difficult.

6.1.1. Analgesics and non-steroidal antiinflammatory drugs (NSAID)

In general, toxicity data vary for each pharmaceutical, however, diclofenac seems to be the compound having highest acute toxicity within the class of NSAID, since for all the tests performed the effect concentrations were below 100 mg/L (Fig. 2). Short-term acute toxicity was analyzed in algae and invertebrates (Webb, 2001; Cleuvers, 2003), phytoplankton was found to react more sensitive [lowest EC_{50} (96 h) = 14.5 mg/L (Ferrari et al., 2004)] than zooplankton [lowest EC_{50} (96 h) = 22.43 mg/L (Ferrari et al., 2004)]. There is no correlation between the acute toxicity in *Daphnia* and the lipophilicity as represented by log K_{ow} (Fig. 3). In general, not much is known about the acute toxicity to fish.

6.1.2. Beta-blockers

As shown in Fig. 2, the acute toxicity of beta-blockers is not extensively studied, with the exception of propranolol. This compound shows the highest acute toxicity and highest log K_{ow} as compared to other beta-blockers (Fig. 3). This and the fact that it is a strong membrane stabilizer, whereas other investigated beta-blockers are not, may in part explain its higher toxicity (Doggrell, 1990; Huggett et al., 2002).

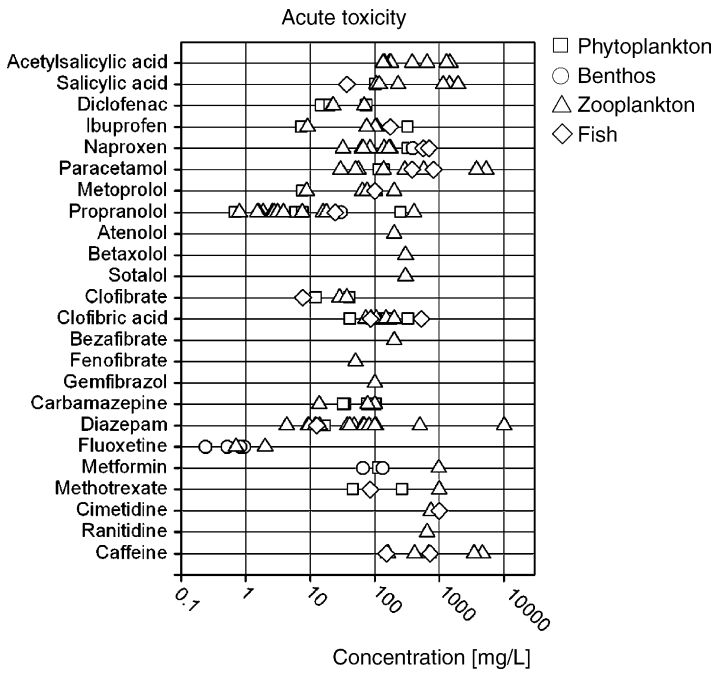

Fig. 2. Acute toxicity of 24 different pharmaceuticals, belonging to different therapeutic classes to aquatic organisms. EC_{50} and LC_{50} for different organisms and different endpoint and exposure time are summarized. See text for details. *References*: Calleja et al. (1993, 1994), Lilius et al. (1994), Henschel et al. (1997), Fong (1998), Halling-Sorensen et al. (1998), Webb (2001), Huggett et al. (2002), Brooks et al. (2003), Cleuvers (2003, 2004), Villegas-Navarro et al. (2003), Ferrari et al. (2004), Henry et al. (2004), Hernando et al. (2004), Kümmerer (2004), Marques et al. (2004a,b), Nunes et al. (2004) and Isidori et al. (2005).

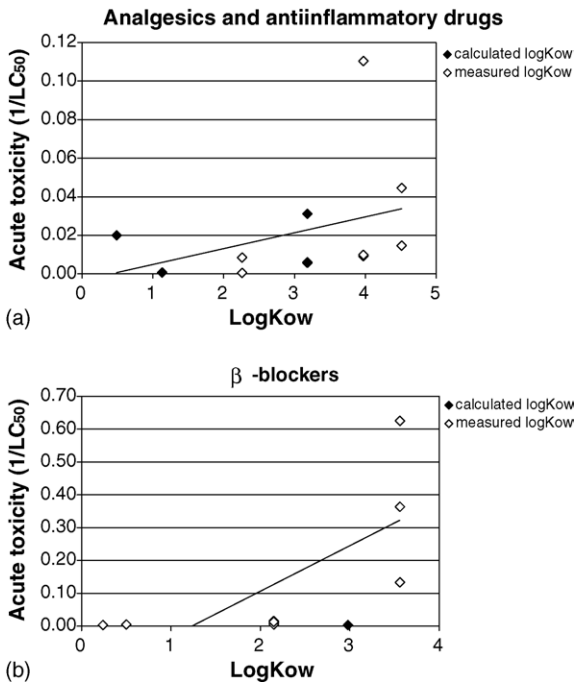

Fig. 3. Relation between acute toxicity (LC_{50}) of analgesics and anti-inflammatory drugs ($y = 0.0082x - 0.0034$; $R^2 = 0.1202$; ANOVA not significant) (a) and β-blockers ($y = 0.1386x - 0.1709$; $R^2 = 0.4301$; ANOVA significant; $p < 0.02$) (b) and octanol–water partition coefficients of the compounds ($\log K_{ow}$); calculated and measured values are given in different symbols. Acute toxicity of *Daphnia magna* refers to immobilization after 48 h (LC_{50} value). *References*—acute toxicity: Calleja et al. (1993), Lilius et al. (1994), Henschel et al. (1997), Halling-Sorensen et al. (1998), Huggett et al. (2002), Brooks et al. (2003), Cleuvers (2003), Villegas-Navarro et al. (2003), Cleuvers (2004), Ferrari et al. (2004), Hernando et al. (2004), Marques et al. (2004a,b). $\log K_{ow}$, in between parentheses: acetylsalicylic acid (1.13) (Sanderson et al., 2003); salicylic acid (2.26) (Hansch et al., 1995); diclofenac (4.51), ibuprofen (3.97) (Avdeef et al., 1998); naproxen (3.18) (Cleuvers, 2004); paracetamol (0.49) (Henschel et al., 1997); atenolol (0.5) (Griffin et al., 1999); betaxolol (2.98) (Sanderson et al., 2003); metoprolol (2.15), propranolol (3.56) (Hardman et al., 1996); sotalol (0.24) (Hansch et al., 1995).

Comparison of toxicity is difficult in this case, since other beta-blockers, except metoprolol, were only analyzed in *D. magna* (Hernando et al., 2004). Metoprolol and verapamil caused the acceleration of the heart beat rate at low concentration, but lowered it at high concentrations in *D. magna* (Villegas-Navarro et al., 2003). For propranolol it seems that phyto- and zooplankton are more sensitive than fish. *Ceriodaphnia dubia* [EC_{50} (48 h) = 0.8 mg/L; Ferrari et al., 2004] displayed higher sensitivity than *D. magna* [EC_{50} (48 h) = 1.6 mg/L; Huggett et al., 2002] or other zooplankton organisms. Within phytoplankton, the microorganism *Synechococcus leopolensis* reacted most sensitive [EC_{50} (96 h) = 0.668 mg/L; Ferrari et al., 2004].

6.1.3. Blood lipid lowering agents

Similar to beta-blockers, acute toxicity of lipid lowering agents is not extensively reported. Clofibrate showed LC_{50} values in the range of 7.7–39.7 mg/L and can be classified as harmful to aquatic organisms. The fish *Gambusia holbrooki* [LC_{50} (96 h) = 7.7 mg/L; Nunes et al., 2004] seems the most sensitive organism to acute clofibrate concentrations studied so far. The known rodent peroxisome proliferator gemfibrozil injected to rainbow trout led to significant increases in fatty acyl-CoA oxidase (FOA) activity at doses of 46–152 mg/kg/day (Scarano et al., 1994). Significant dose-related increases in peroxisomal FOA were observed after exposure of rainbow trout primary hepatocytes to clofibric acid, and ciprofibrate, but not with gemfibrozil (Donohue et al., 1993). The in vitro activity in these fishes is weak.

6.1.4. Neuroactive compounds (antiepileptics, antidepressants)

The serotonin re-uptake inhibitor fluoxetine is apparently the most acute toxic human pharmaceutical reported so far with acute toxicity ranging from EC_{50} (48 h, alga) = 0.024 mg/L (Brooks et al., 2003) to LC_{50} (48 h) = 2 mg/L (Kümmerer, 2004). For benthic organisms, acute toxicity is in the range of 15–43 mg/kg sediment [*Chironomus tentans* LC_{50} (10 days) = 15.2 mg/kg, *Hyalella azteca* LC_{50} (10 days) = 43 mg/kg; Brooks et al., 2003]. Fluoxetine seems to stronger affect phytoplankton than other aquatic organisms.

Diazepam and carbamazepine, both antiepileptics, can be classified as potentially harmful to aquatic organisms, because most of the acute toxicity data are below 100 mg/L. For both compounds it seems that *D. magna* is affected more than other species, but the reasons for the higher susceptibility is not known. Acute toxicity of carbamazepine was found at 17.2 mg/L in *Daphnia* and at 34.4 mg/L in midges, but growth was inhibited at 12.7 mg/L in *Daphnia* and at 9.2 mg/L in midges (Thaker, 2005).

6.1.5. Cytostatic compounds and cancer therapeutics

Acute toxicity of methotrexate on highly proliferative species, namely the ciliate *Tetrahymena pyriformis*, indicated acute effects [EC_{50} (48 h) = 45 mg/L; Henschel et al., 1997]. Teratogenicity in fish embryos was observed at even higher concentrations [EC_{50} (48 h) = 85 mg/L; Henschel et al., 1997].

The acute toxicity data summarized in Fig. 2 shows that 17% of the pharmaceuticals displayed an acute toxicity below 100 mg/L, and for fluoxetine, all toxicity values were below 10 mg/L. On the other hand, 38% of the pharmaceuticals such as acetylsalicylic acid, betaxolol, sotalol, bezafibrate, gemfibrozil, bezafibrate, cimetidine and ranitidine displayed LC_{50} values higher than 100 mg/L, which, according to EU-Directive 93/67/EEC (Commission of the European Communities, 1996), are classified as not being harmful for aquatic organisms. The other pharmaceuticals (45%) displayed a considerable variability of acute toxicity values, spreading over a wide range, thus making a classification difficult.

Variability of data both within the same and between different species is obvious. Different actual exposure concentrations (only nominal concentrations were used in the determination of the endpoints), different sensitivities of used clones, different laboratory performances are among the reasons for variability within the same species (for example, clofibric acid toxicity in *D. magna* varies between 72 and 200 mg/L; the LC_{50} (48 h) of acetylsalicylic acid varies between168 mg/L (Calleja et al., 1994) and 1468 mg/L (Lilius et al., 1994); the LC_{50} (24 h) of diazepam varies between 9.6 mg/L (Calleja et al., 1993) and 10000 mg/L (Calleja et al., 1994)). Depending on the quantity and quality of data, ranges of acute toxicity values span one to two orders of magnitude, in some cases such as propranolol or diazepam, the species differences are quite large, spanning three to four orders of magnitude. When different categories are compared, a tendency of lower LC_{50} (EC_{50}) values is found for beta-blockers and neuroactive drugs as compared to antiinflammatory drugs or various other compounds.

Often, acute toxicity is related to non-specific mode of actions, and not to mechanisms involving specific target molecules. The compounds are thought to interact with cellular membranes leading to unspecific membrane toxicity. This general mechanism may be only one, additional ones (e.g. oxidative stress) come into play with particular pharmaceuticals. We evaluated whether the acute toxicity data of the different classes of pharmaceuticals correlate with the $\log K_{ow}$ of the compound, as the lipophilicity determined by $\log K_{ow}$ is an important parameter for membrane toxicity. However, no correlation was found between the $\log K_{ow}$ of pharmaceuticals of a certain category or of all pharmaceuticals, and the acute toxicity either of a certain species, a group of organisms, or all of them. The best relation between measured and estimated $\log K_{ow}$ of one class of pharmaceuticals and acute toxicity in one species, *D. magna*, is depicted in Fig. 3. Reasons for the variability of the data are probably based on laboratory differences, nominal concentration differences, clone susceptibility differences, but also on the fact that $\log K_{ow}$ may not be the best model for lipophilicity. This holds in particular for ionizable compounds, where the pH-dependent speciation is of significant influence (Fent and Looser, 1995; Looser et al., 1998).

In conclusion, acute toxicity to aquatic organisms is unlikely to occur at measured environmental concentrations, as acute effects concentrations are 100–1000 times higher than residues found in the aquatic environment. For example, the lowest acute effect concentration of fluoxetine was 20 μg/L, whereas the highest estimated environmental concentration was 0.01 μg/L; the lowest acute effect of salicylic acid was 37 mg/L, whereas the highest environmental concentration was ∼60 μg/L. Therefore, acute toxicity is only relevant in case of spills.

6.2. Chronic effects

Many aquatic species are continuously exposed over long periods of time or even over their entire life cycle. Evaluation of the chronic potential of micropollutants, e.g. pharmaceuticals, is therefore important. However, there is a lack of chronic data, and where available, chronic toxicity is marginally known. The available chronic data do often not investigate the important key targets, nor do they address the question in different organisms. Toxicity experiments are usually performed according to established guidelines. More specific investigations including analysis of possible targets of the pharmaceutical, or over different life stages, are lacking, or have only rarely

been performed. Moreover, life-cycle analyses are not reported, except for EE2 (Länge et al., 2001; Parrott and Blunt, 2005), and toxicity to benthic and soil organisms have very rarely been evaluated. In this chapter, we review the current literature according to the different pharmaceutical classes and summarize the data in Fig. 4.

The best knowledge exists for the *synthetic steroid* EE2 contained in contraceptive pills, showing estrogenic effects at extremely low and environmentally relevant concentrations. This steroid has been shown

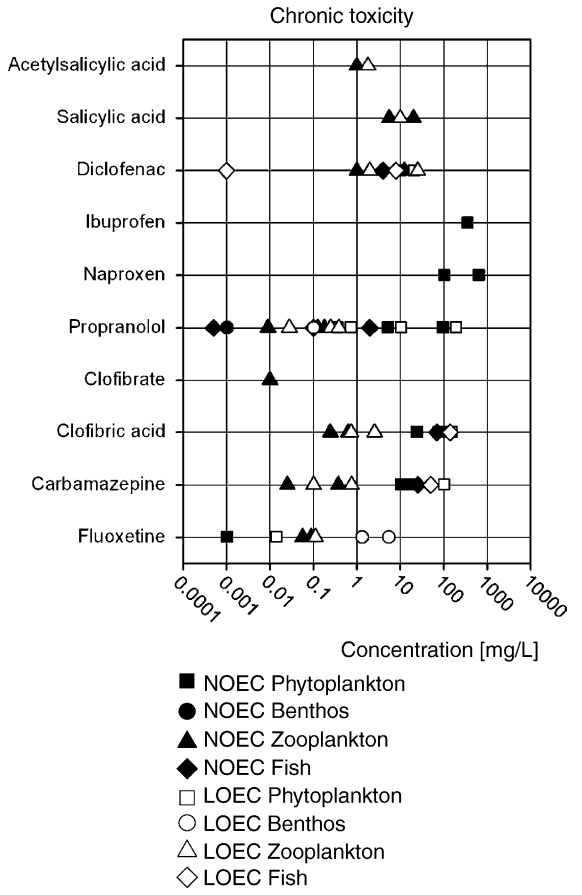

Fig. 4. Chronic toxicity of 10 different pharmaceuticals, belonging to different therapeutic classes. Given are lowest observed effect concentrations (LOEC) and no observed effect concentrations (NOEC) for different aquatic organism, different endpoints and exposure times. See text for details. *References*: Webb (2001), Huggett et al. (2002), Brooks et al. (2003), Ferrari et al. (2003, 2004), Cleuvers (2004), Henry et al. (2004), Kümmerer (2004), Marques et al. (2004a,b), Schwaiger et al. (2004) and Triebskorn et al. (2004).

in many fish to induce estrogenic effects at extremely low concentrations. Exposure of fathead minnows over their life cycle indicates reproductive effects at low concentrations of EE2 (Länge et al., 2001). The NOEC values of the F_0 generation F_1 embryo hatching success and larval survival were $\geq$1 ng/L. Male fish exposed to EE2 at 4 ng/L failed to develop normal secondary sexual characteristics and the sex ratio was altered. No testicular tissue was observed in any fish exposed to EE2 at 4 ng/L. A recent study shows vitellogenin (VTG) induction in fathead minnows with an EC_{50} value as low as 1 ng/L; EE2 was 25–30 times more potent than estradiol (Brian et al., 2005), confirming previous reports on VTG induction at concentrations between 0.1 and 1 ng/L (Pawlowski et al., 2004). Decreased egg fertilization and sex ratio (skewed toward females), both of which were significantly affected at extremely low concentrations of 0.32 ng/L EE2 (Parrott and Blunt, 2005). The next most sensitive parameter was demasculinization (decreased male secondary sex characteristic index) of males exposed to an EE2 concentration of 0.96 ng/L. Full life-cycle exposure of zebrafish to 3 ng/L EE2 lead to elevation of VTG and caused gonadal feminization in all exposed fish and thus inhibited reproduction (Fenske et al., 2005). Life-long exposure of zebrafish to 5 ng/L in the F_1 generation caused a 56% reduction in fecundity and complete population failure with no fertilization. Infertility in the F_1 generation was due to disturbed sexual differentiation with males having no functional testes and intersex gonads (Nash et al., 2004).

In hazard and risk assessment, the ratio between acute to chronic toxicity is often taken for evaluation of chemicals. For pharmaceuticals, this is difficult, because only very rarely, a systematic analysis of a given drug in both acute and chronic toxicity in a single species is performed. Apart from EE2, there are only a few NSAID, from which acute to chronic ratios can be deduced. Table 3 shows that even for similar drugs, these ratios in *D. magna* vary by two orders of magnitude. For all other drugs, only partial information is available on a given species. Ratios derived on the basis of a number of different species are not accurate, giving questionable information. The examples in Table 3 confirm again that chronic toxicity cannot be derived from acute toxicity by simple calculations. This is often neglected in risk assessment.

Table 3
Ratio between acute and chronic toxicity in *Daphnia magna* and *Ceriodaphnia dubia* (48 h/21days)

Drug	Acute (mg/L)	Chronic (mg/L)	Ratio
Acetylsalicylic acid	1293.1	1.4	924
Salicylic acid	1031.7	13.3	77
Clofibrate	28.2	0.01	2820
Naproxen	66.4	0.33	201
Naproxen Na	43.6	0.68	64

Data after Marques et al. (2004a,b) (acetylsalicylic acid and salicylic acid, *D. magna*), Webb (2001) (clofibrate, *D. magna*) and Isidori et al. (2005) (naproxen and naproxen Na, *Ceriodaphnia dubia*).

6.2.1. Analgesics and non-steroidal antiinflammatory drugs

NSAID inhibit the synthesis and release of prostaglandins via COX inhibition and these compounds are the most consumed category of drugs. About NSAID commonly found in the aquatic environment, most chronic data are reported. Acetylsalicylic acid affected reproduction in *D. magna* and *D. longispina* at concentrations of 1.8 mg/L (Marques et al., 2004a). Diclofenac is commonly found in wastewater at median concentration of 0.81 μg/L (Ternes, 1998) whereas the maximal concentration in wastewater and surface water is up to 2 μg/L (Stumpf et al., 1996; Ternes, 1998; Schwaiger et al., 2004). Traditional chronic toxicity studies with diclofenac were reported in invertebrates (Ferrari et al., 2003, 2004). A recent study demonstrated chronic histopathological effects in rainbow trout after 28 days of exposure. At the LOEC of 5 μg/L renal lesions (degeneration of tubular epithelia, interstitial nephritis) and alterations of the gills occurred in rainbow trout (Schwaiger et al., 2004), and subtle subcellular effects even at 1 μg/L (Triebskorn et al., 2004). Impairment of renal and gill function is likely to occur after long-term exposure. The kidney was also found to be a target of diclofenac in vultures, acute renal failure was probably the reason for the visceral gout (Oaks et al., 2004) and the occurrence of extensive deposits of uric acid on and within internal organs (Gilbert et al., 2002). In zebrafish embryos, no effect of diclofenac on embryonic development was observed, except delayed hatching at 1 and 2 mg/L (Hallare et al., 2004). Additional side effects of diclofenac have been observed in humans in the liver with degenerative and inflammatory alterations (Banks et al., 1995), in lower gastrointestinal tract and in the esophagus (Bjorkman, 1998), but not in fish.

6.2.2. Beta-blockers

As fish contain β_2-receptors in heart and liver (Gamprel et al., 1994) and probably in reproductive tissues (Haider and Baqri, 2000), unspecific antagonists such as propranolol may be active in fish. In fact, propranolol indicated chronic toxicity not only on the cardiovascular system, but also on reproduction. The no-observed-effect-concentration (NOEC) and lowest-observed-effect-concentration (LOEC) of propranolol affecting reproduction in *C. dubia* were 125 and 250 μg/L, and reproduction was affected after 27 days of exposure in *H. azteca* at 100 μg/L (Huggett et al., 2002). In fish *O. latipes*, significant changes in plasma steroid levels occurred after 14 days of exposure. The number of eggs released by fish was reduced at 0.5 μg/L after a 4-week exposure to 0.5 and 1 μg/L, but not at 50 and 100 μg/L (Huggett et al., 2002). No alteration in vitellogenin levels was observed. It was suggested that alteration in sex steroids let to decreased oxytocin excretion, which could decrease the number of eggs released. Propranolol was also analyzed in invertebrates. LOEC and NOEC for different organisms span several orders of magnitude (Fig. 4), partly due to differences between laboratories, but also species differences. These data should be compared to the environmental concentrations; propranolol, metoprolol and nadolol were identified in U.S. wastewater samples up to 1.9, 1.2 and 0.36 μg/L, respectively (Huggett et al., 2002).

6.2.3. Blood lipid lowering agents

Data on this class of compounds are rare. Fibrates have been evaluated by traditional toxicity tests. The following NOEC were found for clofibric acid in *C. dubia* [NOEC (7 days) = 640 μg/L], the rotifer *B. calyciflorus* [NOEC (2 days) = 246 μg/L], and in early life stages of zebrafish [NOEC (10 days) = 70 mg/L] (Ferrari et al., 2003). Gemfibrozil occurred in blood plasma of goldfish after exposure over 14 days at 113-times higher levels than in water (bioconcentration factor of 113). Plasma testosterone was reduced by over 50% after exposure to 1.5 and 10 mg/L, as well as levels of steroid acute regulatory protein transcript in goldfish testes (Mimeault et al., 2005).

6.2.4. Neuroactive compounds

Most data were reported for the antiepileptic carbamazepine and selective serotonin re-uptake inhibitors

(SSRI), other neuroactive compounds were very rarely or not evaluated (Fig. 4). Traditional toxicity tests showed chronic toxicity of carbamazepine in *C. dubia* [NOEC (7 days) = 25 μg/L], in the rotifer *B. calyciflorus* [NOEC (2 days) = 377 μg/L], and in early life stages of zebrafish [NOEC (10 days) = 25 mg/L] (Ferrari et al., 2003). Carbamazepine is considered carcinogenic in rats but is not mutagenic in mammalian cells. Sublethal effects occurred in *Daphnia* at 92 μg/L and the lethal concentration in zebra fish was 43 μg/L (Thaker, 2005). In a study with the cnidarian *Hydra vulgaris*, diazepam was shown to inhibit polyp regeneration at 10 μg/L (Pascoe et al., 2003).

Most chronic studies focused on SSRI. Serotonin is a neurotransmitter found in lower vertebrates and invertebrates, and SSRI may adversely influence the function of the nervous and associated hormonal systems of these organisms as well. Besides having important functions as a neurotransmitter, serotonin may directly act on the immune system, alters appetite, influences behavior and modulates sexual function. The role of serotonin in reproduction varies between different phyla and effects of SSRI as well. Fong (1998) found that SSRI (fluvoxamine, paroxetine) led to induction (at 10 nM to 100 μM) and fluoxetine to potentiation (at 5 μM, and if co-applied with 7–100 μM serotonin, but not at other concentrations) of parturition in fingernail clams. Fong (1998) found an induction of spawning in zebra mussels by fluvoxamine concentrations as low as 0.032 μg/L. Induction of mussel spawning point to an interference with serotonin action, as in invertebrates, serotonin may stimulate ecdysteroids, ectysone and juvenile hormone, responsible for controlling oogenesis and vitellogenesis (Nation, 2002). A reproductive stimulation was also found in *D. magna* exposed to 36 μg/L fluoxetine for 30 days, and in *C. dubia* fecundity was increased at 56 μg/L (Flaherty et al., 2001), but reduced in another study (Brooks et al., 2003). An evaluation of five SSRI (fluoxetine, fluvoxamine, paroxetine, citalopram, sertraline) showed negative effects on *C. dubia* reproduction by reduction of the number of neonates or brood per female after 7–8 days of exposure. For the most active compound, sertraline, the LOEC was 45 μg/L and the NOEC 9 μg/L (Henry et al., 2004). Fluoxetine has been detected in sewage and stream water at concentrations of 12 ng/L (Kolpin et al., 2002) and 99 ng/L (Metcalfe et al., 2003b), respectively.

In medaka (*O. latipes*), serotonin induced oocyte maturation (Iwamatsu et al., 1993), but a contrary action was reported in mummichog (*F. heteroclitus*) (Cerda et al., 1998). Serotonin was indicated to potentiate effects of gonadotropin-releasing hormone on gonadotropin release from the pituitary (Khan and Thomas, 1994). When medaka were exposed for 4 weeks to fluoxetine concentrations of 0.1–5 μg/L, vitellogenin plasma content, plasma steroids, fecundity, egg fertilization or hatching rate were not affected (Foran et al., 2004). This indicates no reproduction impairment in this fish up to 5 μg/L fluoxetine. Taken together the chronic effects of SSRI on reproduction of fish and invertebrates are not yet clear, interference with reproduction occurred at much higher concentrations than measured in surface waters.

Chronic data on various other compounds are lacking, although they have been shown to occur in considerable amounts in surface waters (Fig. 2). This holds in particular for fish. For the anticancer compound, tamoxifen, chronic data are found for *Acartia tonsa* [EC50 = 49 μg/L; Andersen et al., 2001]. Various morphological and developmental effects (early embryonic mortality) were induced in sea urchin embryos after exposure to 10^{-8} to 10^{-5} M tamoxifen, which corresponded to oxidative stress. ROS production was increased and lead to oxidative damage and it is thought to represent a pro-oxidant mode of action explaining carcinogenicity in humans and rodents (Pagano et al., 2001).

The antiandrogenic compound flutamide and aromatase inhibitor fadrozole were also analyzed for effects in fish, mainly as a positive control for the evaluation of effects suspected for other environmental chemicals. Short-term reproduction assays in fathead minnows show that flutamide at 0.9 mg/L significantly reduced male sex characteristics in male fish. Fadrozole significantly inhibited ovarian growth and induced testis growth at 0.05 and 0.96 mg/L after 21 days, and inhibited VTG in females and induced VTG synthesis in males (Panter et al., 2004). Flutamide at 0.5 mg/L also reduced fecundity of the fish after 21 days. Embryo hatch was reduced and alterations in gonadal histology were observed (Jensen et al., 2004). Ovaries from females indicated a decrease in mature oocytes and males exhibited spermatocyte degeneration and necrosis. Concentration-dependent VTG and testosterone increase were observed in females. Flutamide had an

antiandrogenic effect and reduced fecundity, yet at rather high concentrations. Moreover, in adult male guppy, reduction in ejaculated sperms, reduced sex coloration and smaller testes occurred. The male courtship behavior was also disrupted at 1 and 10 mg/kg in feed (Baatrup and Junge, 2001). The aromatase inhibitor fadrozole reduced fecundity after 21 days at water concentrations of 10 and 50 μg/L and inhibited brain aromatase activity (Ankley et al., 2002). In females a concentration dependent reduction in plasma estradiol and VTG was observed. In males, androgens in plasma were significantly increased and resulted in a marked accumulation of sperm in the testes.

6.3. In vitro studies

Several pharmaceuticals have been investigated in in vitro systems. They were mainly analyzed for acute cytotoxicity in fish cell lines and in primary fish cell cultures. Cytotoxicity of clofibrate, fenofibrate, carbamazepine, fluoxetine, diclofenac, and propranolol to the fish cell line PLHC-1 (hepatoma cells derived from topminnow) and primary cultures of trout hepatocytes was reported (Laville et al., 2004). Fibrates are known to enhance β-oxidation of lipids, which increases the amount of reactive oxidative species (ROS) in cells. Fenofibrate [EC_{50} (24 h) = 3.25 mg/L] and clofibrate [EC_{50} (24 h) = 0.46 mg/L] were the most active compounds (Laville et al., 2004). Cytotoxicity was higher in PLHC-1 than in primary hepatocytes. Oxidative stress is thought to be responsible for the cytotoxicity, at least for these fibrates (Laville et al., 2004). Cytotoxicity of fluoxetine [EC_{50} (24 h, PLHC-1) = 1.73 mg/L] was also mediated in part by oxidative stress. Besides cytotoxicity and ROS production, the pharmaceuticals were analyzed for their potential to induce cytochrome P4501A monooxygenase activity (CYP1A), which can be regarded as important for chronic toxicity. Among the tested drugs, the β-adrenergic receptor antagonist propranolol was the only CYP1A inducer in primary hepatocytes, the other six pharmaceuticals lead to inhibition of basal activity (Laville et al., 2004).

Furthermore, Henschel et al. (1997) evaluated the cytotoxicity of salicylic acid, paracetamol, clofibrinic acid and methotrexate to BF-2 fish cell line (fibroblasts derived from bluegill sunfish). For three out of four compounds the concentrations inducing in vitro cytotoxicity were lower as compared to in vivo studies with highly proliferating ciliates. The particular mode of action of methotrexate [EC_{50} (48 h) = 3 mg/L; Henschel et al., 1997] may negatively interact with cell proliferation and therefore survival. Sensitivity of cells to toxicants may vary within species, as demonstrated by a direct comparison between cytotoxicity on fish and rat cell lines (Rau et al., 2004), or depending on their origin, e.g. PLHC-1 cell lines are more sensitive than trout primary hepatocytes (Laville et al., 2004). Some of the differences may be based on the difference in the ability of the cells to metabolize toxicants. These in vitro studies indicate their usefulness for the acute toxicity evaluation, but also for investigations of the modes of action of pharmaceuticals including chronic toxicity parameters. Among the advantages of in vitro systems based on fish cells or reporter gene systems are their potential for screening and first evaluation of potential toxicity (Fent, 2001). They are important alternatives to animal testing able to identify general toxicity and specific cellular targets and processes, and they are economic.

6.4. Toxicity of pharmaceutical mixtures and community effects

There are only a few studies dealing with the effects of mixtures of pharmaceuticals. Cleuvers (2003, 2004) has evaluated the ecological potential of antiinflammatory drugs and of diverse acting pharmaceuticals in different sets of biotests using different aquatic organisms. A mixture of NSAID (diclofenac, ibuprofen, naproxen, acetylsalicylic acid) has been evaluated using acute *Daphnia* and algal tests. Toxicity of the mixture was found at concentrations at which the single compound showed no or only little effects. The mixture toxicity followed the concept of concentration addition, which means that the concentrations of each compound behaved in an additive fashion.

Acute toxicity tests using *D. magna*, alga (*Desmodesmus subspicatus*) and macrophyte (*Lemna minor*) were performed to analyze for acute toxicity of nine drugs having different modes of action (clofibric acid, carbamazepine, ibuprofen, propranolol, metoprolol, diclofenac, naproxen, captopril, metformin). The combined effects of two substances, clofibric acid and carbamazepine, followed the concept of concentration addition in the *Daphnia* test, whereas in the algal tests, the concept of independent action was adequate. When

a combination of NSAID, ibuprofen and diclofenac, was analyzed, the effect on algae followed the concentration addition concept, whereas for *Daphnia*, the combination effect was stronger. These data indicate that for the acute toxicity of these pharmaceuticals, concentration addition can be assumed, which means that the concentration of each individual pharmaceutical has to be added for the combination effects. This implies that compounds occurring at concentrations below their individual NOEC can nevertheless contribute to the total effect of the mixture.

Only few pharmaceuticals have been analyzed in ecologically more realistic *model ecosystems*, namely, microcosms and mesocosms. In two recent studies, outdoor aquatic microcosms of a total volume of 12,000 L containing water and sediment were used to analyze the effects of combination of pharmaceuticals. (Brain et al., 2004a) evaluated the effects of combinations of eight pharmaceuticals at three concentration levels on macrophytes *Lemnea gibba* and *Myriophyllum sibiricum* over a 35 days period. Atorvastatin, a blood lipid regulator, was among antibiotics the pharmaceutical eliciting phytotoxicity. Using similar microcosms effects on phyto- and zooplankton were assessed after exposure for 35 days at three concentrations to two pharmaceuticals (ibuprofen, fluoxetine) and an antibiotic (ciprofloxacin) (Richards et al., 2004). The microcosms contained periphyton, phytoplankton, zooplankton, algae and benthic communities, and in addition, juvenile sunfish were exposed in mesh cages. Species abundance and number of phytoplankton and zooplankton were affected at the medium (60–100 μg/L each compound), and high treatment level (600–1000 μg/L each), whereas at the low treatment (6–10 μg/L each), only trends were visible, but no significant effects occurred. Unexpected high lethality occurred in fish at the high and medium treatments, lethality was observed in plants in addition to decreased growth. Decreased diversity of both phytoplankton and zooplankton communities and increased abundance of both communities may have important ecological implications. However, the cause of the decline in diversity and the other effects was unclear (whether caused directly or indirectly and by what pharmaceutical having different modes of action). Maximal concentrations of ibuprofen, fluoxetine and ciprofloxacin detected in the U.S. were 1.0, 0.012 and 0.03 μg/L, respectively (Kolpin et al., 2002). Richards et al. (2004) concluded that a low probability exists that these three pharmaceuticals are currently present in surface waters at concentrations negatively affecting aquatic communities. By comparing calculated whole-body therapeutic doses – and not human and fish plasma levels – the authors note that all responses occurred at levels well below the equivalent pharmacologically active concentrations in mammals. Concentrations of pharmaceuticals in fish can reach significantly higher concentrations in plasma than in the ambient water (Mimeault et al., 2005).

7. Comparison of environmental concentrations and ecotoxicological effects concentrations

The potential risk of a substance to the environment is often characterized by comparing the Predicted Environmental Concentration (PEC) with the Predicted No Effect Concentration (PNEC). PEC of pharmaceuticals are often estimated using calculations, which include usage or sales figures, population density, wastewater production and dilution in watersheds to generate likely concentrations in surface waters (Halling-Sorensen et al., 1998; Jones et al., 2002; Straub, 2002; Sanderson et al., 2003; Bound and Voulvoulis, 2004). Due to the lack of experimental data (in particular chronic) in the public domain on the ecotoxicity of pharmaceuticals, estimation of PNEC, and therefore hazard and risk assessment, is difficult or even impossible to perform. In the open literature or databases, for less than 1% of pharmaceuticals data are available, and only a small number of new pharmaceuticals have been undergone risk assessment using ecotoxicological tests (Halling-Sorensen et al., 1998; Jones et al., 2002; Sanderson et al., 2003) In the absence of experimental data, information is often derived from quantitative structure–activity relationships (QSAR) predictions, for example by applying the EPA's ECOSAR program (Jones et al., 2002; Sanderson et al., 2004a). While being a pragmatic approach for identifying hazards or prioritizing critical substances, this concept is not sufficiently precise for accurate hazard and risk assessments of pharmaceuticals.

Here, we summarize and compare the currently available empirical data in the open literature on maximal STP effluent concentrations with chronic LOEC

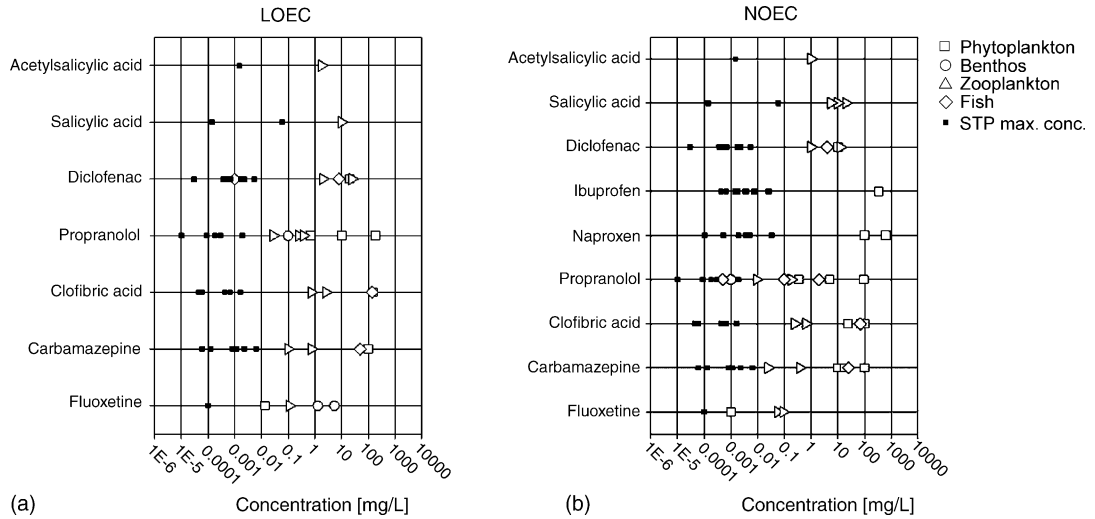

Fig. 5. Comparison between maximal concentrations of pharmaceuticals in treated wastewater and their chronic toxicity in aquatic organisms. (a) Lowest observed effect concentrations (LOEC); (b) no observed effect concentrations (NOEC) for different aquatic organism, different endpoints and exposure times. References see Fig. 1 (wastewater concentrations) and Fig. 4 (chronic toxicity).

and NOEC concentrations of individual pharmaceuticals (Fig. 5). This approach is based on experimental data allowing to prioritize pharmaceuticals according to their ecotoxicological potential and to gain knowledge about the worst case situation. As can be deduced from Fig. 5, LOEC and NOEC values of the pharmaceuticals for different aquatic organisms are about one to two orders and two orders of magnitude, respectively, higher than maximal concentrations in STP effluents. For diclofenac, the LOEC for fish toxicity was in the range of wastewater concentrations, whereas the LOEC of propranolol and fluoxetine for zooplankton and benthic organisms were near to maximal measured STP effluent concentrations. This shows that for diclofenac, propranolol and fluoxetine the margin of safety is narrow, and chronic effects at highly contaminated sites cannot be completely ruled out, in particular, when the combined effects of pharmaceutical mixtures are taken into account. However, median sewage effluent concentrations are lower and dilution in receiving waters result in lower levels in surface waters reducing the environmental risk. It should be noted, however, that more experimental data on chronic toxicity and on the bioaccumulation potential is needed to fully judge the environmental risk posed by individual pharmaceuticals.

8. Discussion

Pharmaceuticals have been tested in traditional ways. A set of mainly acute toxicity tests using traditional species such as an algae (mainly *Scenedesmus quadricauda*), zooplankton (*D. magna*) and fish (species according to OECD guidelines) has been performed. In general, only very few pharmaceuticals have been assessed for acute and chronic toxicity in fish. Moreover, only a few pharmaceuticals have been analyzed for chronic toxicity, again in the traditional way according to guidelines (OECD, U.S. EPA). Based on these studies, no one would probably have been able to anticipate the current population decline of three species of vultures due to diclofenac exposure. Furthermore, these tests alone are not sufficient for deriving an accurate profile of the possible hazards and risks of the pharmaceutical in question. Current tests cover only a small set of laboratory organisms, which are often not sensitive enough and often not able to unravel adverse effects of pharmaceuticals. As a consequence, more specific tests are needed. Only chronic toxicity investigations using more specific toxicity parameters will lead to a more meaningful ecological risk assessment. The following working hypotheses should be addressed in future ecotoxicological investigations:

1. Pharmaceuticals as biologically active compounds may have similar (chronic) effects in non-mammalian animals (and even plants) as in mammals as target receptors and/or biomolecules are similar and conserved during evolution. Therefore, similar adverse (chronic) effects as in humans and mammals may occur in lower vertebrates and invertebrates.
2. Some pharmaceuticals may have unexpected (chronic) effects in lower organisms due to biological differences in pharmacodynamics, pharmacokinetics and physiology.
3. In vitro studies of pharmaceuticals are important for screening, elucidating the modes of action in non-target organisms, and designing specific in vivo studies.

One approach to address these hypotheses is to include histopathological investigations in chronic fish toxicity studies. By focusing on specific tissues and organs, more detailed answers about possible adverse effects may be obtained. This is exemplified by a study on chronic effects of diclofenac in fish (Schwaiger et al., 2004; Triebskorn et al., 2004). Another approach is to use the existing knowledge about possible side effects of the compound of interest in mammals and humans for the design of specific analysis in aquatic organisms. Furthermore, known drug–drug interactions in humans may be relevant for compound mixtures in the environment. Both are based on the hypothesis that targets of the pharmaceutical may be identical or similar in lower organisms as receptors, biochemical pathways and enzymes are conserved in evolutionary terms. This holds true for nuclear steroid receptors that are very similar in organisms of different evolutionary levels (Wilson et al., 2004), nuclear peroxisome proliferator-activated receptors (PPAR's) (Escriva et al., 1997), adrenoceptors such as β_1- and β_2-receptors (Nickerson et al., 2001), but also for insulin receptor, insulin-like growth factor and glucagon receptors being present in lower vertebrates and invertebrates (Navarro et al., 1999). Also, basic mechanisms like signal transduction, cell division, and key metabolizing enzymes such as cytochrome P450s are conserved in a large variety of organisms (Nelson et al., 1996). As a consequence, analysis of pharmaceuticals should specifically be directed to

- specific and identical targets (biomolecules, tissues, organs): target specificity
- known adverse side effects in humans and mammals: side effect specificity
- general chronic effects for accounting physiological differences: species specificity

Our proposed strategy for future research on the ecotoxicology of pharmaceuticals is exemplified by a few examples. When the ecotoxicity of NSAID is studied, effects on inhibition of prostaglandin synthesis and COX inhibition should be addressed in lower organisms, and at the same time, on side effects already known in mammals. Diclofenac has been known for causing side effects on the kidney (and other organs such as liver) in mammals, subsequently being found in vultures (Oaks et al., 2004), and fish (Schwaiger et al., 2004). Cardiovascular pharmaceuticals should be analyzed for their possible effects on the cardiovascular system in lower vertebrates. Lipid lowering agents such as fibrates are also known to act by enhancing or reducing PPAR (Kliewer et al., 1997). These nuclear receptors play key roles in the catabolism and storage of fatty acids and are important for blood lipid regulation. Indeed, PPAR's are affected in amphibians (Kliewer et al., 1997) and fish by clofibrate, benzafibrate and fenofibrate (Ruyter et al., 1997).

Beta-blockers bind to the beta-adrenergic receptors and block its activation by physiological agonists. These receptors are located in mammals in many tissues including heart, and its blockade causes a decrease in heart rate and contraction. Beta-blockers differ in specificity to the different receptor subtypes, some are non-specifically acting on β_1- and β_2-receptors (e.g. propranolol), while others are specific for the β_1-receptor subtype (e.g. atenolol). In *D. magna*, heart beat rate, fecundity and biomass were reduced after chronic exposure to 0.11 mg/L (Dzialowski et al., 2003), although it is not know whether β_2-receptors occur. Long-term exposure to propranolol reduced reproduction in *C. dubia* at 250 μg/L and in *H. azteca* at 100 μg/L (Huggett et al., 2002).

A class of antihyperlipidemic drugs inhibit their target enzyme hydroxymethylglutaryl-CoA reductase (HMG-CoA reductase) in mammals (Seiler, 2002). Whether these enzymes are also inhibited in lower animals should be addressed in future investigations. Surprisingly, atorvastatin was even found in a plant

(duckweed, *Lemna gibba*) to have effects, but the mode of action is unclear (Brain et al., 2004b).

Many antineoplastic drugs used in cancer therapy have a high mutagenic and cancerogenic potential. Parent compounds are often bioactivated leading to formation of mutagenic metabolites (e.g. cyclophosphamide, ifosfamide). In case organisms in the environment are able to metabolize these pharmaceuticals, enhanced mutation frequencies and cancer risk will result. In addition, these drugs often have significant side effects on humans such as nausea, cytotoxicity, reduction in proliferation of cells in various tissues etc. One would expect mutagenicity and cancerogenicity to occur in exposed aquatic organisms as well. However, such studies are lacking besides the analysis of hospital wastewater, in which the genotoxic potential was based on antibiotics such as ciprofloxacin (Hartmann et al., 1998).

It should be noted, however, that besides known targets additional or other target tissues and organs may be affected alternatively. This would result in *unexpected effects* not targeted by the investigations. Examples are effects on sex hormones in blood plasma of fish and reduced reproduction in *C. dubia* and *H. azteca* induced by the beta-blocker propranolol after long-term exposure (Huggett et al., 2002), and the effects of serotonin-re-uptake inhibitors on reproduction of mollusks (Fong, 1998; Fong et al., 1998). As the effects of the antiestrogen tamoxifen indicate, pharmaceuticals may have not only one, but multiple modes of action, such as oxidative damage in addition in case of tamoxifen (Pagano et al., 2001). This fact complicates the strategy to analyze for chronic effects. However, many of these unexpected chronic responses will be elucidated in the context of careful chronic toxicity analyses including histopathology and reproduction. However, such analyses are more expensive and probably only justified for important pharmaceuticals occurring in significant concentrations in the environment. But in the light of the limitations of traditional (acute) toxicity testing for use in environmental risk assessment, more specific toxicity analyses should be performed in forthcoming studies, taking full advantage of the available knowledge that is generated during the pharmaceutical drug development process (e.g. mechanisms of action, pharmacokinetic behavior and metabolism, target organs and side effects in mammals).

In vitro studies are important for screening and evaluation of possible cellular targets in ecotoxicology (Fent, 2001). They are also important in the reduction of animal experiments, in conjunction with other proposed new strategies (Hutchinson et al., 2003). Effects of pharmaceuticals have been evaluated in fish primary cells and fish cell lines indicating this potential (Laville et al., 2004). We assume that investigating pharmaceuticals in in vitro systems will not only allow a reduction of animal experiments, but also a better and more accurate characterization of possible targets of pharmaceuticals. These test systems not only allow the analysis of specific receptor interactions and target enzymes in animal and plant cells, but also a rapid screening of a large number of compounds.

Pharmaceuticals are analyzed for possible ecotoxicological effects as single compounds and only rarely as *mixtures* (Cleuvers, 2003). However, as other environmental pollutants pharmaceuticals are present in the environment in mixtures. Effects of mixtures most probably follow the concept of concentration addition, hence, the overall toxicity is the result of the sum of the individual concentration of each compound. Therefore effects may occur even at the NOEC of individual compounds. It should also be recognized that even subtle changes of normal homeostasis including behavioral alterations may have direct and indirect effects, even if only minor ones, that eventually result in significant deteriorating effects on a species or population in the ecological context. The extreme case of the dramatic poisoning and population declines of Indian vultures is a case in point. The dimension of this population decline has no parallel in birds since the disappearance of peregrine falcons and other predatory birds in the 1960s due to the pesticide DDT.

9. Conclusions and future directions

One important aspect to solve the load of pharmaceutical residues in wastewater and surface water is to optimize STP processes. There is a need to increase the knowledge about the fate of pharmaceuticals during sewage treatment for implementation of better removal techniques. Future work on STP treatment optimization will show to what extend pharmaceuticals can be removed from wastewater and to what extent the implementation of an improved technology is feasible,

taking into account other macro- and micro-pollutants as well as the broad variety of complex wastewater matrices.

Our present knowledge about residues of pharmaceuticals in aquatic systems indicate that they are unlikely to pose a risk for acute toxicity. Environmental concentrations are in the range of 10^3 to 10^7 times lower than known LC_{50} or EC_{50} values (ratio of 10^3 between lowest acute effect of fluoxetine and highest environmental concentration; difference of 10^7 between highest LC_{50} of diazepam and highest environmental concentration). However, as the collapse of vulture populations in the Indian subcontinent indicates, important adverse effects can occur under certain circumstances.

There is a general lack of chronic toxicity data on pharmaceuticals, in particular in fish. Many pharmaceuticals need more investigation about potential long-term ecotoxicological effects, particularly with respect to potential disturbances in hormonal homeostasis (endocrine disruption), immunological status, or gene activation and silencing during long-term exposure. For better understanding of possible effects, a mechanism-based approach focused on target molecules, tissues and organs should yield more meaningful results and insights than traditional acute toxicity testing. Current data on acute and chronic toxicity of pharmaceuticals support to the conclusion that more target- or biomolecule-oriented, or mode-of-action-based investigations, will allow more relevant insights into effects on survival, growth and reproduction than traditional standard ecotoxicity testing. Often, similar target biomolecules are present in non-mammalian organisms and so are the adverse effects. In vitro systems are very important tools for both elucidating modes of action in lower vertebrates, and for screening of the ecotoxicological potential of pharmaceuticals prior to fish toxicity testing. Unless more is known about possible chronic effects of individual pharmaceuticals and mixtures thereof, conclusions concerning hazards or risks of pharmaceuticals to the aquatic ecosystem are premature.

Drugs may also induce unexpected effects in non-mammalian organisms, however. This is based on the difference in pharmacokinetics and pharmacodynamics, important parameters for occurring species differences. Disturbances of the reproductive system and hormone system, immune depression, neurobehavioral changes, to name some key targets, may have far reaching effects on the population level. This has become evident for endocrine disrupters such as steroid hormones used in contraceptives resulting in important adverse effects at environmentally relevant concentrations (Jobling et al., 1998; Länge et al., 2001; Thorpe et al., 2003; Parrott and Blunt, 2005).

Comparison of available chronic toxicity data with environmental concentrations indicate that for most investigated pharmaceuticals concentrations are too low in aquatic systems to induce chronic effects on traditional laboratory organisms such as inhibition of algal growth and reproduction in *Daphnia*. For diclofenac, the LOEC for fish toxicity on an organ level was in the range of wastewater concentrations, however (Schwaiger et al., 2004), whereas the LOEC of propranolol and fluoxetine for zooplankton and benthic organisms were near to maximal measured STP effluent concentrations. Whether or not the margin of safety is narrow for additional human pharmaceuticals should be investigated in future studies. The future requirement of chronic testing with algae, daphnids and fish instead of only traditional acute toxicity studies is an important step forward (EMEA, 2005). Moreover, the potential of combined effects of pharmaceutical mixtures should be addressed. In the ecological context, subtle changes and disturbances may have negative consequences for the organism's fitness. As a consequence much more should be known about the potential for chronic effects of pharmaceuticals in the aquatic system.

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