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A toxicity and hazard assessment of fourteen pharmaceuticals to *Xenopus laevis* larvae

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Abstract The toxicity of fourteen widely used human pharmaceuticals was determined using the Frog Embryo Teratogenesis Assay–*Xenopus* (FETAX). Stage 9 *Xenopus* blastulae were exposed for 96 h to single concentrations of commonly prescribed selective serotonin reuptake inhibitors (SSRIs), statin blood lipid regulators, non-steroidal anti-inflammatories, antibiotics, a stimulant, and an anti-epileptic. Toxicity, teratogenicity, minimum concentration to inhibit growth, and types and severity of associated malformations were determined. EC_{10}s ranged from 3.0 mg/l to >100 mg/l and LC_{10}s ranged from 3.6 mg/l to >100 mg/l. Toxicity varied between and within compound class of pharmaceutical. The fluoroquinolones, stimulants, anti-epileptics, and antibiotics tested were determined to be nontoxic and non-teratogenic at singular, water-soluble concentrations. The hazard quotients (HQ) for the pharmaceuticals ranged from 6.10×10^{-7} to 1.6×10^{-4}, all of which are orders of magnitude below EPA's levels for concern for harm to aquatic animals. Thus, based on the data from the present study, concentrations of individual pharmaceuticals currently detected in surface water are far below concentrations of effective and lethal concentrations.

Keywords Amphibian risk assessment · Pharmaceuticals · FETAX · Aquatic concentrations

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Introduction

Several widely used pharmaceuticals have been found in sewage treatment plant influent, effluent, and ultimately in surface water. Selective serotonin reuptake inhibitors (SSRIs), fluoroquinolones, statins, non-steroidal anti-inflammatories, antibiotics, stimulants, and anti-epileptics are among the pharmaceuticals detected in North American and European aquatic systems (Metcalfe and Miao, 2003a; Metcalfe et al. 2003b; Boyd et al. 2003; Kolpin et al. 2002; Jones-Lepp et al. 2001).

The potential for consumer introduction of pharmaceuticals via municipal sewage and/or agricultural runoff to surface water is significant (Kolpin et al. 2002). For example, up to 90% of some pharmaceuticals are excreted as the parent compound from the human body (Hirsch et al. 1999; NDC Health, 2005). Removal of pharmaceuticals by sewage treatment facilities may not fair much better; from 4% to 93% of the pharmaceutical total load can exit without change, depending on the facility and the pharmaceutical (Daughton and Ternes, 1999; Stumpf et al. 1999; Heberer, 2002). Even if the compounds are metabolized, some metabolites may remain physiologically active or even transformed back to the active parent form (Hirsch et al. 1999). The receptors and effects of these pharmaceuticals and their metabolites are well known in terrestrial mammals but not in aquatic organisms. Indeed, in aquatic organisms these compounds could act on different receptors than those of mammals thus producing different effects. These factors combined with the fact that pharmaceutical use is widespread and increasing (Farre et al. 2001), warrant

an assessment of potential pharmaceutical impact on aquatic organisms.

To examine possible effects on the selected pharmaceuticals, we chose the African clawed frog (*Xenopus laevis*) as the model organism to assess effects in anuran species. All toxicity testing in the present study was based on the Frog Embryo Teratogenic Assay-*Xenopus* (FETAX) method (ASTM, 2002).

Description of pharmaceutical classes and mode of action

The pharmaceuticals in the present study were chosen based on mechanism of action, prevalence of prescription, and surface water detection (Table 1). Although the typical SSRI mode of action in mammals is the inhibition of the reuptake of serotonin from the postsynaptic cleft (Schloss and Williams, 1998), serotonin plays a variety of roles, depending on the species, thus the potential effects of altering serotonin level will vary depending on species. Studies have shown that SSRIs can mimic the physiological actions of serotonin in aquatic organisms. For example, SSRIs induce spawning in zebra mussel (*Dreissena polymorpha*), parturition in fingernail clam (*Sphaerium corneum*) (Fong et al. 2003) and behavior regulation of snails and squids (Huber et al. 1997). Each SSRI has the same primary mode of action, though they are molecularly dissimilar and have both pharmacokinetic and side effect profiles that vary (Goodnick and Goldstein, 1998).

Atorvastatin and lovastatin belong to the statin family of blood lipid regulators. In mammals, the primary mode of action is the inhibition of the HMG CoA reductase enzyme, which effectively blocks the synthesis of cholesterol in the liver (von Keutz and Schluter, 1998). In mammals, the primary functions of cholesterol include the regulation of cell membrane fluidity, absorption of dietary lipids and synthesis of steroid hormones, bile acids and vitamin D (Zubay, 1999). Although not specifically tested, we hypothesize that these statins have similar modes of action in Xenopus. The potency of statins to *Xenopus* is addressed in the present study.

Acetaminophen and ibuprofen are two of the most widely used analgesic drugs available. These analgesics act by blocking the enzyme cyclooxygenase, which is responsible for converting arachidonic acid into prostaglandins (Morrow and Roberts, 2001). Prostaglandins are involved in a variety of physiological functions in amphibians, including roles in neurotransmitter release and water and ion transport across cellular membranes (Els and Helman, 1997; Arkhipova et al. 2005).

Ciprofloxacin and levofloxacin are fluoroquinolone antibiotic compounds. Quinolones inhibit the A

Table 1 Pharmaceuticals tested in the present study, their rank of prescription in the US (NDC Health, 2005), and the corresponding maximum concentrations detected in surface waters

Compound	Use	Rank of use	Maximum concentration detected (μg/l)
Acetaminophen	Analgesic	42	10.0[a,b]
Atorvastatin	Lipid Regulator	2	0.02[c]
Caffeine	Stimulant	No data	6.0[a,b]
Carbamazepine	Antiepileptic	252	1.3[d]
Chlortetracycline	Antibiotic	No data	0.69[a]
Ciprofloxacin	Antibiotic	82	0.03[a]
Fluoxetine	SSRI	28	0.01[a]
Ibuprofen	Analgesic	17	5.04[e]
Levofloxacin	Antibiotic	60	No data
Lovastatin	Lipid Regulator	106	No data
Paroxetine	SSRI	52	0.137[g]
Sertraline	SSRI	14	0.57[f]
Sulfamethoxazole	Antibiotic	119[h]	5.2[b]
Trimethoprim	Antibiotic	119[h]	0.71[a]

[a] Kolpin et al. (2002)

[b] Cahill et al. (2004)

[c] Metcalfe et al. (2003b)

[d] Zuehlke et al. (2004)

[e] Ashton et al. (2004)

[f] Thomas and Hilton (2004)

[g] Predicted environmental concentration Johnson et al. (2005)

[h] Often prescribed in tandem

subunit of DNA gyrase in bacteria (Chen and Lo, 2003). This gyrase, a type II topoisomerase, nicks and seals DNA during replication. Without the gyrase, DNA cannot be replicated. The quinolone action also inhibits relaxation of supercoiled (packed) DNA necessary for DNA replication and increases double-stranded DNA breakage (Chen and Lo, 2003).

Caffeine is the most widely consumed psychoactive drug in the world, and as such, it is one of the most commonly found drugs in surface water (Kolpin et al. 2002; Cahill et al. 2004). In mammals, caffeine works as an antagonist at adenosine receptors; thus, caffeine counteracts the normal sedating effects of adenosine in the central nervous system (O'Brien, 2001). In addition, exposure to caffeine results in increased norepinephrine and subsequent increases in neural activity in many parts of the brain.

Carbamazepine is an anticonvulsant that acts by inhibiting the repetitive firing of action potentials in the central nervous system (McNamara, 2001). It should be noted that carbamazepine's primary metabolite (10,11-epoxycarbamazepine) has the same mechanism of action (McLean and Macdonald, 1986), but to our knowledge, this metabolite has not been analyzed for in surface water. Thus, the concentrations of biologically-active carbamazepine may effectively be much greater than currently thought.

The non-quinolone antibiotics tested were chlortetracycline, sulfamethoxazole, and trimethoprim. At high concentrations, tetracyclines may impair protein synthesis of higher organisms such as mammals and possibly anurans (Chambers, 2001). Sulfamethoxazole and trimethoprim are prescribed most commonly in combination with each other. These antibiotics act on the synthesis of tetrahydrofolic acid (Petri, 2001) and ultimately the growth of microbes.

The present study was designed to characterize the toxicity of widely prescribed pharmaceuticals from a variety of classes ranging from 1.0 to 100.0 mg/l. The suite of compounds tested included three selective serotonin receptor inhibitors (SSRIs), two statin blood lipid regulators, two non-steroidal anti-inflammatories, four antibiotics (two fluoroquinolones and two non-specific), a stimulant, and an anti-epileptic.

Methods and materials

Xenopus blastulae, Stage 9, (Nieuwkoop and Faber, 1994) were exposed for 96 h to commonly prescribed SSRIs, fluoroquinolones, statins, non-steroidal anti-inflammatories, antibiotics, stimulants, and anti-epileptics, in single-compound, static-renewal tests. Toxicity, teratogenicity, minimum concentration to inhibit growth, and types and severity of associated malformations were determined.

Atorvastatin (98.0% purity) was purchased from Rugao Foreign Trade Corp. (Shanghai, China); acetaminophen (99.1% purity), caffeine (100.0% purity), chlortetracycline (80.0% purity), lovastatin (98.0% purity), paroxetine (98.0% purity), and sulfamethoxazole (99.0% purity) were purchased from Sigma-Aldrich (St. Louis, MO, USA); and carbamazepine (99.7% purity) was supplied by China Jiangsu Textiles (Nanjing, China). Ciprofloxacin (98.0% purity) and fluoxetine (99.9% purity) were obtained from Interchem (Paramus, NJ, USA). Levofloxacin (90.8% purity) was supplied by Zhejiang Wonderful Pharma & Chem Co. (Zhejiang, China); sertraline (100.0% purity) was supplied by Ranbaxy Laboratories Ltd. (New Delhi, India); and sulfamethoxazole and trimethoprim (100.0% purity) were supplied by BUFA B.V. Pharmaceutical Products (Uitgeest, Holland). All other reagents used to conduct the FETAX were obtained from Sigma-Aldrich Chemical Company (Oakville, ON, Canada).

Frog care, breeding, embryo acquisition and analysis were conducted according to the methods described in the American Society of Testing and Materials (ASTM) Standard Guide for Conducting FETAX E1439-98 (ASTM, 2002). Briefly, adults are reared in a fiberglass tank (2.2 m × 1.2 m × 0.9 m) filled with 175 l of dechlorinated water at 21–25°C on a 12:12 light–dark schedule. The water was replaced entirely every three days and monitored weekly to ensure that the pH and hardness ($CaCO_3$) were at acceptable levels (6.5–9 and 16–400 mg/l, respectively). Every three days the adults were fed ground beef liver that was supplemented with infant vitamin supplement (Polyvisol, Mead & Johnson, New Brunswick, NJ).

To induce breeding, males and females were injected in the dorsal lymph sac with 400 and 500 IU of human chorionic gonadotropin (HCG, Sigma-Aldrich, St. Louis, MO, USA), respectively. The pair was then placed in a breeding chamber fitted with a plastic grate on the bottom which the deposited eggs fell through, thus preventing damage from the *Xenopus* pair. Amplexus usually occurred between 2 and 7 h following injection, and egg deposition occurred from 9 to 16 h after injection.

Eggs were collected and treated with 5% cysteine solution (w/v, pH 8.0) to remove the associated jelly coat. Healthy eggs were then selected, sorted, and randomly placed in 60 mm petri dishes (25 eggs/dish) containing FETAX solution (625 mg NaCl, 96 mg $CaHCO_3$, 30 mg KCl, 15 mg $CaCl_2$, 60 mg $CaSO_4$,

$2H_2O$, and 75 mg $MgSO_4$ per liter of de-ionized water). Stock solutions of the pharmaceuticals were dissolved in either acetone or dimethyl sulfoxide (DMSO), depending on the nature of the compound before adding to the petri dishes. In all experiments, the concentration of the solvent did not exceed 1% (v/v), per ASTM (2002) guidelines. These concentrations have been found not to cause any adverse effects in FETAX (Fort et al. 1989). Solvent controls did not have significant effects on normal *Xenopus* development (i.e., at least 90% of controls survived without deformities throughout the duration of the test). Eggs, Stage 9, (Nieuwkoop and Faber, 1994) were exposed in duplicate groups of 25 for 96 h. At 24, 48, and 72 h test solutions were renewed 90% and status of tadpoles was recorded. Acetaminophen, fluoxetine, paroxetine, sertraline, lovastatin, and atorvastatin were tested with eggs produced by three different pairs of *Xenopus*. Thus three tests were conducted with each concentration of compound using 50 eggs (25 per dish) from three different couples for a total of 150 eggs. Chlortetracycline, ciprofloxacin, sulfamethoxazole, trimethoprim, acetaminophen, caffeine, and carbamazepine were tested using only two different pairs. These studies were truncated because no significant defects occurred in the tadpoles at concentrations up to 100 mg/l—concentrations orders of magnitude above environmental relevance. Embryos were cultured at 23 ± 3°C and on a 12:12 light–dark schedule throughout the test. Concluding the 96 h of exposure, surviving embryos were fixed in 3% formalin. The malformations types were then qualitatively characterized using a dissecting microscope at a magnification of 40*g*. Head-to-tail body length measurements (±0.5 mm) were conducted on fixed embryos. All concentrations reported herein are nominal.

Data analysis

For each sample with visually distinguishable malformations, probit analysis (USEPA, 1988) was used to calculate lethal and effective concentrations (e.g., LC_{10} and EC_{10}) with 95% confidence intervals. The teratogenic potential was determined using the Teratogenic Index ($TI = LC_{50}/ EC_{50}$), which is a measure of abnormal morphogenesis hazard. According to ASTM (2002), TI values higher than 1.5 indicated a greater potential for malformation. An ANOVA (SPSS, 2004) was conducted on each data set to distinguish if a correlation between the pharmaceutical concentration and decreasing body length was present ($\alpha = 0.05$). Pair-wise comparisons were then conducted using Scheffé's post-hoc analysis to determine significant differences between concentrations ($\alpha = 0.05$). Concentrations displaying significant changes in body length development are represented by the minimal concentration to inhibit growth (MCIG).

To evaluate the likelihood that exposure to a stressor can induce adverse ecological effects, a hazard assessment was performed based on the first tier of the EPA's Ecological Framework for Risk Assessment (USEPA, 1998). In order to determine the relative risk of each pharmaceutical, we calculated the hazard quotient (HQ) for each compound. The HQ can be viewed as an indicator of the likelihood of adverse effects due to the worst-case scenario aquatic concentration. The HQ was calculated as the ratio of the known maximum environmental concentration (MEC) to either the EC_{10} or the LC_{10} in an aquatic system (EC_{10} HQ and LC_{10} HQ, respectively). The HQ was then compared to the USEPA level of concern (LOC), which is an action level for further risk assessment (USEPA, 1998). The standard LOC for a HQ is 1. If the HQ is below 1, the USEPA does not consider harm to be likely.

Results

In general, EC_{10}s and LC_{10}s of the pharmaceuticals ranged from 3.0 mg/l and 3.6 mg/l, respectively, to >100 mg/l (Table 2). Although final length of the organisms in some trials was decreased (Fig. 1.), effect concentrations (EC_x calculations) in this study were calculated based on general malformations, irrespective of body length. Toxicity varied between and within class of compound. The fluoroquinolones, caffeine, acetaminophen, carbamazepine, and antibiotics were not toxic or teratogenic at >100 mg/l 96 h exposure.

Selective serotonin reuptake inhibitors (SSRIs)

Fluoxetine exposure resulted in an EC_{10} at 3.0 mg/l and EC_{50} at 4.9 mg/l. The two predominant malformations observed were tail flexures and mild to severe facial malformations (i.e., disruption of optic lobe development, head edema, Table 3). The fluoxetine MCIG was calculated to be 4.0 mg/l based on the body length of the control group (Scheffé's *F*-test, $P < 0.001$). The LC_{10} value was calculated to be 7.1 mg/l, while the LC_{50} was 7.5 mg/l. All embryos died at exposures of 9.0 mg/l or greater. The resultant TI value was 1.5. The EC_{10} HQ and LC_{10} HQ of fluoxetine were calculated to be 3.3×10^{-6} and 1.4×10^{-6}, respectively.

Table 2 Toxicity (mg/l) of 14 pharmaceuticals to *Xenopus laevis* following 96 h exposure

Compound	NOEC[a]	MCIG[b]	EC_{10}	LC_{10}	EC_{10} HQ[c]	LC_{10} HQ[d]	TI[e]
Acetaminophen	>100	>100	>100	>100	N/A	N/A	N/A
Atorvastatin	10.0	30.0	17.8	32.8	1.12×10^{-6}	6.10×10^{-7}	1.6
Caffeine	>100	>100	>100	>100	N/A	N/A	N/A
Carbamazepine	>100	>100	>100	>100	N/A	N/A	N/A
Chlortetracycline	>100	>100	>100	>100	N/A	N/A	N/A
Ciprofloxacin	>100	>100	>100	>100	N/A	N/A	N/A
Fluoxetine	2.0	4.0	3.0	7.1	3.3×10^{-6}	1.4×10^{-6}	1.5
Ibuprofen	20.0	30.0	30.7	50.8	1.6×10^{-4}	9.9×10^{-5}	1.4
Levofloxacin	>100	>100	>100	>100	N/A	N/A	N/A
Lovastatin	10.0	20.0	14.2	37.0	N/A	N/A	2.5
Paroxetine	2.0	3.0	3.6	4.4	3.8×10^{-5}	3.1×10^{-5}	1.1
Sertraline	1.0	2.0	3.0	3.6	N/A	N/A	1.1
Sulfamethoxazole	>100	>100	>100	>100	N/A	N/A	N/A
Trimethoprim	>100	>100	>100	>100	N/A	N/A	N/A

[a] No observable effects concentration

[b] Minimum concentration to inhibit growth

[c] Hazard quotient based on the EC_{10} (deformity)

[d] Hazard quotient based on the LC_{10}

[e] Teratogenicity index based on the LC_{50}/EC_{50}

The initial malformation in embryos exposed to paroxetine was tail flexures beginning at 4.0 mg/l. Predominant effects taken into consideration for paroxetine EC_x calculation included tail flexures and gut miscoiling, and thoracic edemas. The EC_{10} was calculated to be 3.6 mg/l and the EC_{50} was 4.1 mg/l. Paroxetine's MCIG was calculated to be 3.0 mg/l (Scheffé's F-test, $P < 0.001$). The LC_{10} and LC_{50} values were determined to be 4.4 and 5.12 mg/l, respectively. One-hundred percent embryo lethality occurred at 7.0 mg/l. The TI value of paroxetine was calculated to be 1.2.

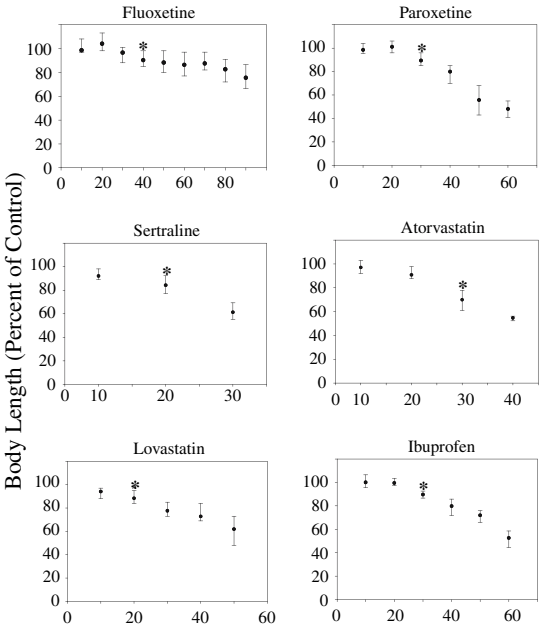

Fig. 1 Mean (±1SE) head-to-tail length of *Xenopus* larvae following 96-hour exposure to various concentrations of pharmaceuticals (all values are mg/l). Pair-wise comparisons ($\alpha = 0.05$) were conducted to establish statistical differences between the mean of each exposure group. The Minimal Concentration to Inhibit Growth (MCIG, indicated by an asterisk) is the lowest concentration of pharmaceutical that significant decreased head-to-tail body length ($P < 0.05$)

Sertraline exposure resulted in tail flexures and to a lesser extent, thoracic edemas, with an EC_{10} value of 3.0 mg/l and an EC_{50} value of 3.3 mg/l. The MCIG of sertraline was 2.0 mg/l (Scheffé's F-test, $P < 0.003$). The LC_{10} was 3.6 mg/l and LC_{50} was 3.9 mg/l. Concentrations of 5.0 mg/l and above resulted in total embryo lethality. Sertraline's TI was 1.2, similar to paroxetine.

Statins

Atorvastatin and lovastatin displayed teratogenic effects at similar concentrations. The most common malformation observed with both chemicals was abnormal gut coiling. The EC_{10} of atorvastatin was 17.8 mg/l (EC_{50} of 23.1 mg/l), while lovastatin had an EC_{10} of 14.2 mg/l (EC_{50} of 20.5 mg/l). Atorvastatin's MCIG was 30.0 mg/l (Scheffé's F-test, $P < 0.001$; Fig. 1), while the MCIG from lovastatin exposure was 20.0 mg/l (Scheffé's F-test, $P < 0.01$; Fig. 1). The LC_{10} and LC_{50} of atorvastatin were determined to be 32.8 mg/l and 38.6 mg/l, respectively. For lovastatin, the LC_{10} was 37.0 mg/l and the LC_{50} was 52.2 mg/l. One-hundred percent lethality occurred in atorvastatin-treated tadpoles at concentrations of 50.0 mg/l and greater, while lovastatin-treated tadpoles did not show

Table 3 Predominant *Xenopus* malformations elicited by 14 pharmaceuticals

Compound	Malformation	EC_{50}	EC_{16}	EC_{84}
Acetaminophen	N/A	>100	>100	>100
Atorvastatin	Abnormal Gut Coiling	23.1	18.1	29.5
Caffeine	N/A	>100	>100	>100
Carbamazepine	N/A	>100	>100	>100
Chlortetracycline	N/A	>100	>100	>100
Ciprofloxacin	N/A	>100	>100	>100
Fluoxetine	Tail Flexure	6.4	3.8	6.8
	Facial Malformations	6.6	5.2	6.7
Ibuprofen	Thoracic Edema	39.9	34.3	49.4
Levofloxacin	N/A	>100	>100	>100
Lovastatin	Abnormal Gut Coiling	20.5	16.2	27.6
Paroxetine	Tail Flexure	4.6	3.9	4.6
Sertraline	Tail Flexure	4.6	3.1	5.6
Sulfamethoxazole	N/A	>100	>100	>100
Trimethoprim	N/A	>100	>100	>100

The EC_{84} and EC_{16} indicate ±1 standard deviation from the EC_{50}, respectively (Newman, 1995). All values are mg/l

complete lethality until 60.0 mg/l. The TI of atorvastatin was calculated to be 1.6. Lovastatin had a higher index of 2.5. For atorvastatin, the EC_{10} HQ was calculated to be 1.12×10^{-6} and LC_{10} HQ was 6.10×10^{-7}.

Analgesics

The EC_{10} value for ibuprofen was 30.7 mg/l and the EC_{50} value was 39.9 mg/l. The main effect was thoracic edema. The MCIG for ibuprofen exposure compound was 30.0 mg/l (Scheffé's *F*-test, $P < 0.001$; Fig. 1). The LC_{10} was calculated to be 50.8 mg/l and the LC_{50} was 56.7 mg/l. There were no surviving embryos at concentrations of 70.0 mg/l and higher. The Teratogenic Index of ibuprofen was 1.4. Ibuprofen showed the largest calculated HQs in the present study due to the relatively high concentration measured in surface waters. The ibuprofen EC_{10}HQ was found to be 1.6×10^{-4} and the LC_{10}EQ was 9.9×10^{-5}.

At concentrations up to 100 mg/l of acetaminophen, there were no significant differences in lethality as compared to controls. However, 100% of tadpoles exposed to the highest concentration of acetaminophen, 100 mg/l, showed general malformations (i.e. tail flexures, edemas, gut miscoiling). No significant reductions in body length were noticed at any acetaminophen exposures. Since acetaminophen-associated effects only occurred at 100 mg/l, neither the HQ nor EQ was calculated.

Stimulants, anti-epileptics, antibiotics and fluoroquinolones

All embryos exposed to 100 mg/l caffeine appeared lighter in color and were significantly more active than the controls. This assessment is based on visual inspection alone and was not quantified. However, no other morphological abnormalities, including body length, seemed to be affected by the concentrations tested. Thus, caffeine was judged to be non-toxic to *Xenopus* larvae. Accordingly, concentrations of levofloxacin, ciprofloxacin, carbamazepine, sulfamethoxazole and trimethoprim ranging from 1.0 mg/l to 100 mg/l showed no signs of noticeable malformations or mortality.

Discussion

In an attempt to determine potential environmental threats of commonly used pharmaceuticals, we conducted a tier-one ecological risk assessment. The most sensitive measures of toxicity in our study involved macroscopic malformations of the organism. In all toxic compounds that displayed toxicity below 100 mg/l, except fluoxetine and lovastatin, the MCIG was the first concentration to display effects of pharmaceutical exposure.

Many of the compounds tested in the present study have not been examined in amphibians (except where noted below) or other ecological models. As human pharmaceuticals, these compounds have been thoroughly tested in mammalian models. Thus, the majority of the information available for mechanism of action and potency comparison comes from tests with mice and rats or human clinical trials.

In the present study, SSRIs were the most toxic and potentially teratogenic of the 14 pharmaceuticals tested, with effects and lethality beginning at 2.0 mg/l. However, the SSRIs did not show complete consistency between compounds in the types of induced malformations. For example, all three of the SSRI exposed groups showed signs of skeletal and muscular

kinking along the tail. Only fluoxetine, however, was associated with craniofacial defects such as flattened heads. Such facial defects have been attributed to serotonin regulation; Shuey et al. (1992) found that serotonin was linked to regulate craniofacial morphogenesis in mice embryos, and that the use of SSRIs by pregnant mothers was associated with craniofacial defects in humans. The mechanism of the SSRI-associated skeletal and muscular kinking in *Xenopus* is unknown; however, such spinal flexures in other species have been attributed to a neurotoxic mode of action (Lien et al. 1997; Teraoka et al. 2006).

The malformations (edemas) associated with ibuprofen exposure (1.0–100 mg/l) were localized to the thoracic region. While fluid retention and edema has been reported by humans administered ibuprofen and other antipyretics, the occurrence is relatively low (Schooley et al.; 1977).

The role of both atorvastatin and lovastatin is to ultimately decrease the amount of plasma lipids. Cholesterol and other products of cholesterol biosynthesis are essential to normal fetal development, especially for the synthesis of steroids and cell membranes (Moore and Persaud, 2003). The primary malformation associated with both drugs was abnormal gut formation. While abnormal gut coiling is not an uncommon effect seen in the FETAX, it is worthwhile to note that this type of deformity was predominantly associated with statin exposures in the present study. Studies conducted with pregnant rats and a HMG-CoA reductase inhibitor resulted in teratogenic effects in the offspring, including an increase in the incidence of abdominal defects (Minsker et. al., 1983).

The teratogenic potential of a compound can be estimated qualitatively by examining embryonic growth of the exposed organisms, including type and severity of the induced malformations, and quantifiably by creating the Teratogenic Index ($TI = LC_{50}/EC_{50}$). Generally, TI values greater than 1.5 indicate a teratogenic potential (Fabro et al. 1982). Fluoxetine, sertraline, and lovastatin all had TI values that exceeded 1.5, which indicates a potential for developmental toxicity. Paroxetine, atorvastatin, and ibuprofen all had TI values less than 1.5. In the present study, only distinct, macro malformations were accounted for, such as abnormal gut coiling or optic cup malformation. It did not account for relatively small deviations (less than 0.5 mm) in head–tail length, where even a slight shortening could alter the survivability and reproductive success of an organism (Richards and Kendall, 2003).

To put the risk of each compound in perspective, the MECs (Table 1) were divided by the EC_{10}s and LC_{10}s to determine the HQ of each pharmaceutical. These benchmark concentrations were used instead of the NOEC to provide a more meaningful, yet conservative estimate of effect (van der Hoeven, 1997a; van der Hoeven et al. 1997b). In the present study, the HQs ranged from 1.4×10^{-4} to 6.1×10^{-7} (Table 2), all of which fall far below the LOC, even with an uncertainty or safety factor of 1,000 applied. For special circumstances, such as a potentially exposed endangered species, the USEPA will use a lower LOC. However, even when the USEPA Acute LOC of 0.05 for endangered aquatic species (EAS LOC) is used, most compounds in the present study are still 1,000× lower than the EAS LOC, except for ibuprofen (HQ = 0.00016, 320 × lower than EAS LOC).

As mentioned, most of the compounds in the present study have not been tested elsewhere via the FETAX methodology or even with anuran models. To our knowledge, acetaminophen and caffeine are the only two compounds from the present study that have been tested elsewhere. We found no acetaminophen or caffeine associated mortality at concentrations up to 100 mg/l. Fraker and Smith (2004) results corroborate our findings. They exposed *Rana pipiens* for 28 days to 1.0 mg/l and 0.6 mg/l acetaminophen and caffeine, respectively. No effect on survivorship occurred in either case. Next, they exposed *X. laevis* for 11 days to maximum acetaminophen concentrations of 1.0 mg/l and found no significant effect on mass or survivorship (Fraker and Smith, 2005). In separate experiments with *Bufo americanus*, Smith and Burgett (2005) noted increased mortality associated with 1 mg/l acetaminophen exposure. However, their study was considerably different than ours. In addition to using a different anuran model, Smith and Burgett (2005) exposed the organisms for much longer (14 days) and used post-hatch tadpoles which are more sensitive than pre-hatch tadpoles (Richards and Kendall 2002, 2003). Despite these differences, Smith and Burgett (2005) did not see acetaminophen associated effects on body mass. Tadpoles in the present study did not have an increased mortality or growth reduction associated with 100 mg/l caffeine exposure. Sakamoto et al. (1993), however, report increased mortality and abnormalities at concentrations of 100–2,000 mg/l caffeine in 48-h *Xenopus* exposure. Fort et al. (1998) also report a greater *Xenopus* sensitivity to caffeine. Their FETAX yielded a caffeine MCIG of 50 mg/l. This could be due to a greater ability to detect smaller differences. Their head-to-tail measurements were conducted with the aid of digitizing software while our measurements were conducted by hand.

Based on our measurements and HQs, there is most likely no acute lethal or teratogenic threat to developing amphibian larvae posed by the singular exposure to pharmaceuticals tested herein. For example, if we consider *Xenopus* a suitable surrogate for North American anurans, in order for 10% of those organisms to be affected, the surface water concentrations of fluoxetine, paroxetine, and ibuprofen would have to be present in the environment at concentrations 301,000 times greater, 26,131 times greater, and 6083 times greater, respectively, than the maximum concentrations currently known.

While we maintain that singular acute exposures to these pharmaceuticals do not present a hazard to anurans, it would be remiss to not acknowledge the limitations of this study. The present study is a Tier 1 hazard assessment and is not suitable to use for hazard assessment of pharmaceuticals occurring in combination. The singular occurrence of pharmaceuticals in surface water is uncommon—most pharmaceutical occurrences will likely be in combination. The combined concentration of drugs that share the same mechanism of action in surface waters would most likely have an additive effect (ECETOC, 2001; Wang, 1987) and would therefore increase the effective concentration of pharmaceuticals.

Pharmaceuticals in combination should be considered for future research. Combining pharmaceuticals for human consumption is often contraindicated due to the potential that deleterious cross-reactive or additive effects may result. This is a distinct possibility in aquatic ecosystems where hundreds, perhaps thousands, of combinations may be possible. Indeed, some initial research shows that even with dissimilar modes of actions, combinations of pharmaceuticals have an increased physiological threat, whereas little toxicity was noted when singular administration was conducted (Pickrell, 2002).

Aside from the issue of additivity and contraindicaiton, chronic exposures should be assessed. Many of the compounds we tested herein are rapidly degraded in the presence of sunlight (Lam and Mabury, 2005). However, even pharmaceuticals with short environmental half-lives can take on the qualities of highly persistent contaminants when they are continually replenished by sewage treatment plants back into aquatic ecosystems.

Singular, 96-h exposure to caffeine, carbamazepine, fluoroquinolones or the other antibiotics did not result in any significant differences relative to the control in the parameters measured in the present study. However, our scope of parameters was narrow. To be completely confident that the compounds in the present study caused no effects, more assays should be conducted (e.g., behavioral, life-cycle, etc.). Indeed, concentrations of pharmaceuticals that do not affect survival or growth, do affect tadpole activity (Fraker and Smith 2004). In addition, further study should be conducted to determine the toxicity of pharmaceuticals with metabolic activation system (MAS) incorporation. MAS incorporates a class of detoxifying enzymes (cytochrome P450) that are present only in small amounts at the early developmental stages of *Xenopus* and are usually readily present in higher level organisms (Fort et al. 1998). The MAS was not incorporated into the present study. Many pharmaceuticals have even been proven to have an increased toxicity when these enzymes are present (Lin and Lu, 1998, Fort, 1998).

Conclusions

We have provided some insights to the sensitivity (or lack thereof) of developing *Xenopus* embryos to selected pharmaceuticals. The concentrations shown to be toxic in the present study are orders of magnitude above that which are currently detected surface waters. As the human population increases, ages, and as pharmaceutical use is expanded to include younger people, pharmaceutical use is continually expanding. Thus, the amount of gross pharmaceuticals entering surface waters is likely to increase. At this time, though, based on our Tier I HQ assessment and the assumptions described above, the pharmaceuticals tested herein are not likely to pose a threat to the aquatic anurans in single compound exposure.

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