Arch Environ Contam Toxicol (2012) 62:455–464
DOI 10.1007/s00244-011-9723-6

Early Life-Stage Toxicity of Eight Pharmaceuticals to the Fathead Minnow, *Pimephales promelas*

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Received: 25 April 2011 / Accepted: 17 October 2011 / Published online: 4 November 2011

Abstract Human pharmaceuticals are routinely being detected in the environment, and there is growing concern about whether these drugs could elicit effects on aquatic organisms. Regulatory paradigms have shifted accordingly, with a greater emphasis on chronic toxicity data compared with acute data. The Organisation for Economic Co-operation and Development 210 Early Life Stage Test has been proposed as a good measure of the potential for pharmaceuticals to elicit chronic toxicity. To begin building a data set regarding the early life-stage toxicity of pharmaceuticals to fish, fathead minnows (FHM) were exposed to amiodarone, carbamazepine, clozapine, dexamethasone, fenofibrate, ibuprofen, norethindrone, or verapamil. Survival and growth were used to assess chronic toxicity in FHM at 28 days posthatch. Exposure of FHM to carbamazepine, fenofibrate, and ibuprofen resulted in no significant adverse effects at the concentrations tested. FHM survival was not impacted by verapamil exposure; however, growth was significantly decreased at 600 μg/L. Dexamethasone-exposed FHM showed a significant decrease in survival at a concentration of 577 μg/L; however, growth was not impacted at the concentration tested. Norethindrone exposure resulted in a significant decrease in survival and dry weight at 14.8 and 0.74 μg/L, respectively. Exposure to amiodarone and clozapine resulted in a significant decrease in survival and a significant increase in growth at concentrations of 1020 and 30.8 μg/L, respectively. Although the effect levels derived in this study are greater then concentrations observed in the environment, these data suggest that synthetic progestins may require additional research.

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During the last several decades, the use of human pharmaceuticals has increased dramatically (Crockett 2005). As such, pharmaceuticals are being routinely detected in wastewater effluents and surface waters across the United States (Kolpin et al. 2002). The major route of pharmaceuticals into the environment is considered to be post-consumer use, followed by discharge from wastewater treatment plants (WWTPs) (Petrovic et al. 2003). However, pharmaceuticals are not completely removed by WWTPs, and there is a growing concern that pharmaceuticals and their metabolites may have an impact on aquatic organisms (Ankley et al. 2007).

The strongest evidence for the potential environmental impact of pharmaceuticals involves endocrine disruption and the associated alteration of fish reproduction by oral contraceptive ingredients (Länge et al. 2001; Gross-Sorokin et al. 2006; Kidd et al. 2007). The mechanism by which this alteration of reproduction occurs is considered to be similar to its intended therapeutic use in humans (e.g., estrogen receptormediated response) (Caldwell et al. 2008). This has led to speculation that other pharmaceuticals whose therapeutic targets are conserved across species may pose a risk to the environment (Huggett et al. 2003; Ankley et al. 2007; Gunnarsson et al. 2008).

The concern about pharmaceuticals as biologically active environmental contaminants has led to updated regulatory guidelines from the European Medicines Agency (2006). One of these new guideline suggests that drugs whose predicted environmental concentration (PEC)

is >10 ng/L be evaluated using a fish early life-stage (ELS) test. One ancillary approach would be to evaluate reference pharmaceuticals from various therapeutic classes to prioritize which pharmaceuticals or classes should undergo a more detailed testing program (Ankley et al. 2005). This type of prioritization approach would also limit unnecessary testing and align well with the need to decrease, refine, and replace vertebrate animal testing (Mehlman et al. 1989).

The goal of this study was to begin building a database of ELS fish toxicity data related to various pharmaceutical classes. Specifically, fathead minnows (FHM) were exposed to amiodarone, carbamazepine, clozapine, dexamethasone, fenofibrate, ibuprofen, norethindrone, or verapamil from egg to 28 days posthatch, and survival and growth were evaluated. Each of these drugs represents a different mechanism of action, with their therapeutic target identified in fish (Huggett et al. 2003; Gunnarsson et al. 2008). Amiodarone is an antiarrhythmic used for the treatment of arrhythmias (Vassallo & Trohman 2007). Carbamazepine is an antiepileptic used in the treatment of epileptic seizures (McNamara 2001). Clozapine, an atypical antipsychotic, is indicated for use in schizophrenia (Baldessarini & Tarazi 2001). Dexamethasone is a corticosteroid with a wide range of therapeutic uses (Schimmer & Parker 2001). Fenofibrate is a lipid-lowering fibric-acid derivative used in the treatment of hypercholesterolemia (Mahley & Bersot 2001). Ibuprofen is a nonspecific cyclooxygenase (COX) inhibitor used for the treatment of pain and inflammation (Rang et al. 2007). Norethindrone is a synthetic progestin, commonly used in oral contraceptives and hormone-replacement therapy (Loose-Mitchell & Stancel 2001). Verapamil is a L-type calcium-channel blocker used for the treatment of angina, hypertension, and arrhythmias (Rang et al. 2007).

Materials and Methods

Clozapine, norethindrone, (*R,S*) -ibuprofen, verapamil, and associated deuterated internal chemical standards (d_6-norethindrone, d_3-ibuprofen, d_6-verapamil, d_8-clozapine, d_4-amiodarone, d_8-carbamazepine, and d_6-fenofibrate) were purchased from Toronto Research Chemicals (Ontario, Canada). Amiodarone, carbamazepine, dexamethasone, fenofibrate, prednisolone, dimethylformamide, ethyl 3-aminobenzoate methanesulfonate (MS-222), and analytical-grade methanol, acetonitrile, and formic acid were purchased from Sigma-Aldrich (St. Louis, MO). Analytical-grade hexane and ethyl acetate were purchased from Fisher Scientific (Pittsburgh, PA).

Dilution water used in this study was City of Denton, TX, activated carbon-treated, dechlorinated tap water amended to hard water characteristics (American Public Health Association 2005). None of the pharmaceuticals used in this study were detected in the dilution water during the study period. Dilution water was monitored for water quality before use and from the experimental test vessels. Temperature and pH were maintained between 22.0°C and 24.0°C and 8.3 and 8.5, respectively. Alkalinity and hardness were maintained between 100 and 130 and 110 and 150 mg/L $CaCO_3$, respectively. Conductivity ranged from 500 to 560 μmhos/cm, and dissolved oxygen ranged from 7.5 to 8.0 mg/L.

Test solutions for carbamazepine, clozapine, dexamethasone, fenofibrate, ibuprofen, and norethindrone were prepared by performing serial dilutions from DMF-concentrated stocks. The resultant DMF concentration in the test vessels was ≤0.001%. Test solutions for amiodarone were prepared in the same fashion, except that methanol was used due to the insolubility of amiodarone in DMF. The final methanol concentration in each test vessel was 0.005%. Due to the high water solubility of verapamil, dechlorinated tap water was used to prepare test solutions as opposed to DMF or methanol. Exposure concentrations were initially selected based on acute-toxicity test and a 7-day growth range-finding study. In addition, based on published environmental concentration data and corresponding relevance, the upper concentration limit was set at 1 mg/L. Amiodarone, carbamazepine, dexamethasone, fenofibrate, ibuprofen, and verapamil nominal concentrations were 1000, 500, 250, 125, and 62.5 μg/L, respectively. Initial studies with norethindrone resulted in increased mortality in most of the exposure concentrations relative to the controls. Thus, norethindrone nominal concentrations were 10, 1, 0.5, 0.25, and 0.125 μg/L. The nominal concentrations for clozapine were 100, 50, 25, 12.5 and 6.25 μg/L.

Fathead minnow eggs <48 h old were obtained from breeding cultures at the University of North Texas (UNT) Aquatic Toxicology Facility and commercially from Aquatic BioSystems, Inc. (Fort Collins, CO). Early life-stage tests followed the Organization of Economic Cooperation and Development (OECD) 210 guidelines (1992), and all protocols were approved by the Animal Care and Use Committee. For each exposure concentration, 25 eggs were placed in each of 4 replicate 600-mL glass test vessels. The test vessels contained a stainless-steel wire mesh screen in the bottom on which the eggs rested. Aeration was applied to the test vessels, which provided continuous agitation for the eggs as well as maintained the oxygen levels at saturation during the experimental period. Each study contained a water-only control as well as a solvent control (DMF or methanol) where applicable. All test vessels were placed in a walk-in environmental chamber maintained at 23°C ± 1°C. The light-to-dark cycle was set at 16:8,

respectively. Test solutions were renewed daily. After hatch, mesh screens were removed from the test vessels. Fish were fed *ad libitum* daily.

Survival of the larvae was monitored daily, and fish weight and length were determined at test termination. At 28 days posthatch, larvae were killed in MS-222. Fish were blotted dry, and individual and pooled mean dry weights were determined using a Mettler H51AR analytical balance after drying overnight at 60°C. Individual larval lengths (cm) were measured using a Zeiss Axiocam HRc digital camera attached to a Zeiss Stemi 2000-cstereoscope. Axiovision 4 image software (Zeiss; Munich, Germany) was used for data processing and analysis. Five larval fish were selected at random from each replicate for length measurement. Lengths of FHM were determined after exposure to ibuprofen, norethindrone, and verapamil, which were the first three compounds tested. Length was not more sensitive than dry weight when assessing impacts on growth. Therefore, dry weight was analyzed as an indicator of growth in the remaining studies.

At two periods during each experiment, mean measured pharmaceutical concentrations were determined during the 24-h period using gas or liquid chromatography-mass spectrometry (GC-MS or LC-MS) or liquid chromatography-tandem mass spectrometry (LC-MS/MS). Specifically, water samples were taken immediately and then again 24 h after redosing. Isotope dilution, using a labeled internal standard for each chemical, was used for quantification of each compound. A deuterated analog of dexamethasone was not available for this analysis. Thus, prednisolone (m/z 452 > 95) was used as an internal standard for dexamethasone because it is structurally similar. Norethindrone, ibuprofen, verapamil, and clozapine analytes were extracted from water samples using a liquid–liquid extraction method (1:1 mixture of hexane and ethyl acetate). Dexamethasone, and its associated internal standard, required a derivatization process using a mixture of hydrazinopyridine and trifluoroacetic acid for analysis (Hala et al. 2011). All other compounds did not require an extraction process and were analyzed directly from the collected water sample.

Analysis of norethindrone and ibuprofen was conducted using an Agilent 6890 gas chromatographer coupled to an Agilent 5973 mass spectrometer for selective ion monitoring of m/z 335 and 161, respectively (Gomez et al. 2010; Nallani et al. 2011). Verapamil and clozapine were analyzed using an Agilent 1100 LC coupled to an Agilent SL ion trap mass spectrometer for selective ion monitoring of m/z 303 and multiple reaction monitoring of m/z (327 > 270), respectively (Kollroser & Schober 2002, Sun et al. 2004). Fenofibrate, dexamethasone, amiodarone, and carbamazepine were all analyzed using a Waters 2695 LC coupled to a Micromass Quattro Ultima tandem mass spectrometer (Kuhn et al. 2010; Vazquez-Roig et al. 2010; Zhang et al. 2011). The following m/z transitions were analyzed using multiple reaction monitoring: fenofibrate (360 > 233), dexamethasone (484 > 95), and carbamazepine (237 > 194). Amiodarone was analyzed using selective ion monitoring of m/z 646.

Lowest observed-effect concentrations (LOECs) and no observed-effect concentrations (NOECs) for survival and dry weight were determined by testing for statistical significance of treatment levels relative to controls. Shapiro–Wilk's and Bartlett's test was used to test for normality and homogeneity, respectively. One-way analysis of variance (ANOVA) with Dunnett's multiple comparison *posthoc* test was used to determine significance relative to the controls. If the assumptions for the ANOVA were not met, then significance was determined with nonparametric Kruskal–Wallis test. Significance was reported at $\alpha = 0.05$. GraphPad Prism 5 software was used for statistical and graphical analysis of data.

Results

The pharmaceuticals used in this study were not detected in dilution water controls. Measured drug concentrations in the exposure vessels varied. On average, renewed solutions of the pharmaceuticals ranged from 32% to 172% of nominal concentrations. Solutions of the pharmaceuticals before renewal (i.e., 24 h old) ranged from 0% to 124% of nominal concentrations. Fenofibrate and verapamil were not detected in solutions before renewal. The mean measured concentrations (SE) during the 24-h period for amiodarone, carbamazepine, clozapine, dexamethasone, fenofibrate, ibuprofen, norethindrone, and verapamil were, on average, 91 (11.2), 93 (5.4), 35 (2.8), 105 (6.3), 16 (1.8), 68 (4.4), 148 (12.3), and 60 (4.6)% of nominal values, respectively. Based on the first part of the sentence it specifies for instance that 91% represents a mean measured concentration and that (11.2) is the standard error or SE for each drug in the list.

Hatching success in control and exposure replicates across experiments was ≥ 85%. Initial studies with norethindrone resulted in high mortality at exposure concentrations ≥ 100 μg/L (data not shown). The 28-day ELS study with amiodarone resulted in a significant difference in survival at 1020 μg/L (Fig. 1a). The LC_{50} was determined to be 526 μg/L, and the NOEC and LOEC were 623 and 1020 μg/L, respectively (Table 1). Clozapine significantly decreased survival at 30.8 μg/L (Fig. 1c). However, this decrease remained >50%; therefore, the LC_{50} could not be determined. The NOEC and LOEC of clozapine were 17.9 and 30.8 μg/L, respectively (Table 1). There was a significant decrease in survival for dexamethasone at 577 and 1160 μg/L (Fig. 1d). The LC_{50} of dexamethasone was calculated to be 254 μg/L, and the NOEC and LOEC of

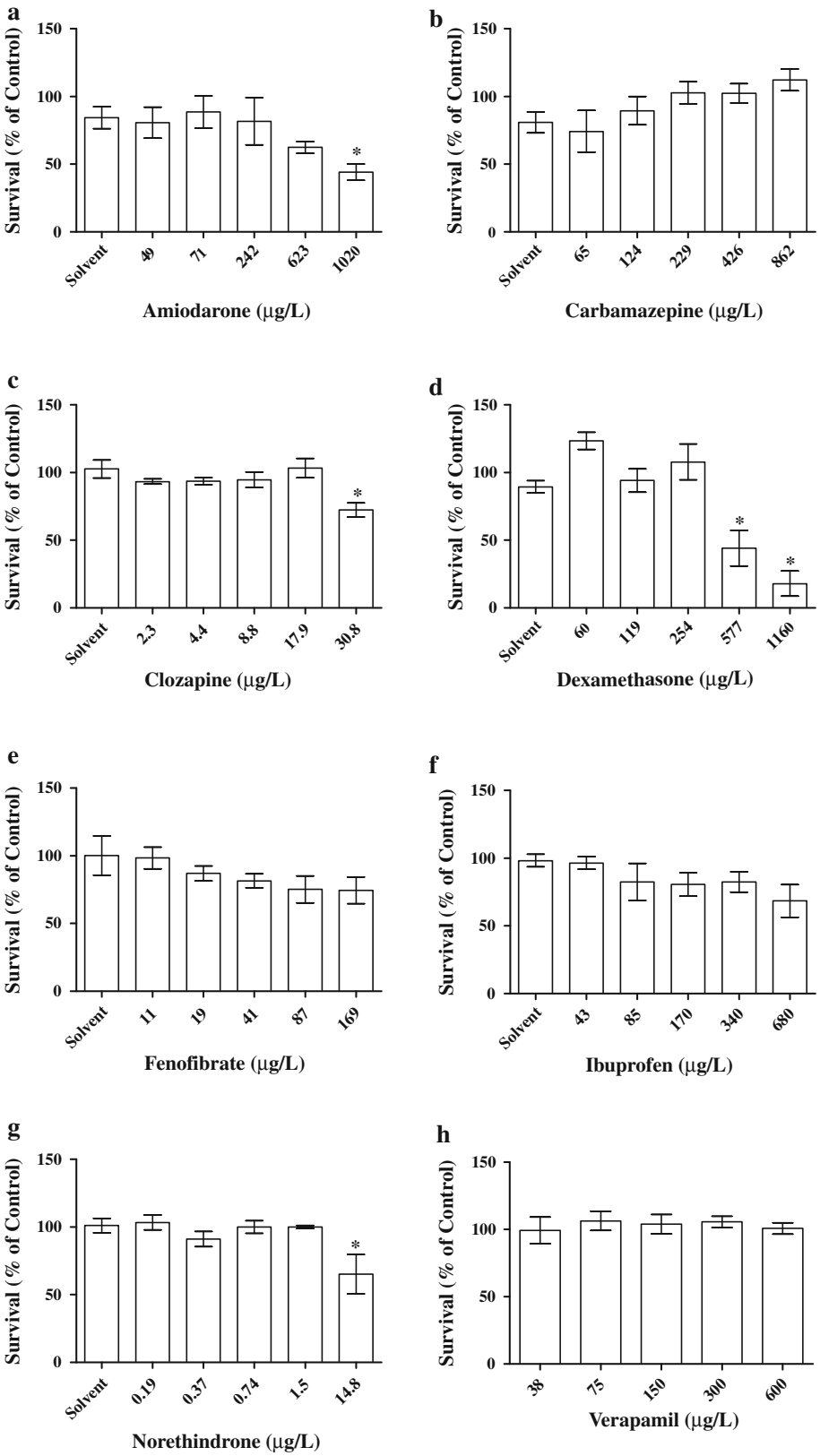


Fig. 1 Survival (% of control) of fathead minnow larvae in the early life stage tests exposed to (**a**) amiodarone, (**b**) carbamazepine, (**c**) clozapine, (**d**) dexamethasone, (**e**) fenofibrate, (**f**) ibuprofen, (**g**) norethindrone, and (**h**) verapamil. *Significant difference compared with controls ($p < 0.05$). Error bars = SEM

Table 1 Summary of LC_{50}, NOEC and LOEC values (μg/L) for end points associated with the fathead minnow early life-stage studies[a]

Pharmaceutical	$NOEC_{Survival}$	$LOEC_{Survival}$	LC_{50}	$NOEC_{Growth}$	$LOEC_{Growth}$
Amiodarone	623	1020	526	623	1020
Carbamazepine	862	>862	>862	862	>862
Clozapine	17.9	30.8	>30.8	17.9	30.8
Dexamethasone	254	577	254	1160	>1160
Fenofibrate	169	>169	>169	169	>169
Ibuprofen	680	>680	>680	680	>680
Norethindrone	1.5	14.8	>14.8	0.37	0.74
Verapamil	600	>600	>600	300	600

[a] Values are based on mean measured pharmaceutical concentrations

dexamethasone were determined to be 254 and 577 μg/L, respectively (Table 1). Exposure to norethindrone resulted in a statistical difference in survival at 14.8 μg/L (Fig. 1g). However, survival was >50%, so the LC_{50} could not be calculated. The NOEC and LOEC for norethindrone were 1.5 and 14.8 μg/L, respectively (Table 1). There were no significant changes in survival for carbamazepine, fenofibrate, ibuprofen, or verapamil ($p > 0.05$).

Dilution water and solvent control larval weights and lengths at 28 days were within the range of FHM values reported in the literature (Bogers et al. 2006; Lizotte et al. 1999). At 28 days in all experiments, FHM appeared normal (i.e., no visible deformities or changes in behavior). Norethindrone was the most potent of the chemicals tested in this study, with a significant decrease in pooled mean dry weight at 0.74 μg/L (Fig. 2g). The NOEC and LOEC for growth after norethindrone exposure were 0.37 and 0.74 μg/L, respectively (Table 1). Verapamil caused a significant decrease in dry weight at 600 μg/L (Fig. 2h). The NOEC and LOEC for FHM growth after verapamil exposure were 300 and 600 μg/L, respectively (Table 1). Amiodarone had a significant increase in growth at 1020 μg/L (Fig. 2a), resulting in a NOEC and LOEC for growth of 623 and 1020 μg/L, respectively (Table 1). There was also a significant increase in growth with clozapine at 30.8 μg/L (Fig. 2c). Therefore, the NOEC and LOEC for growth of clozapine were 17.9 and 30.8 μg/L, respectively (Table 1). Carbamazepine, dexamethasone, fenofibrate, and ibuprofen did not result in any significant changes in FHM growth at concentrations tested in this study.

Lengths of FHM were determined after exposure to ibuprofen, norethindrone, and verapamil (Fig. 3), which were the first three compounds tested. Length was significantly decreased in FHM exposed to norethindrone and verapamil at 0.74 and 600 μg/L, respectively. Ibuprofen had no impact on length of FHM at the concentrations tested. Length was not more sensitive than dry weight when assessing impacts on growth. Therefore, dry weight was analyzed as an indicator of growth in the remaining studies.

Discussion

Early development in fish may be a particularly sensitive time period for toxicant-induced effects (van Aerle et al. 2002; Oxendine et al. 2006). The OECD 210 ELS test incorporates this critical window of sensitivity and thus may provide a good estimation of chemical safety (Lizotte et al. 1999). However, there is a paucity of data regarding 28-day fish ELS studies with pharmaceuticals published in peer-reviewed literature. Winter et al. (2008) reported a 28-day growth NOEC and LOEC for the β_1-adrenergic receptor antagonist atenolol to be 3.2 and 10 mg/L, respectively. However, decreases in growth during this 28-day study may be transient and not representative of impacts during an entire fish full life cycle (Williams et al. 2007).

Amiodarone is an iodine-rich benzofuranic derivative used for the treatment of arrhythmias by prolonging myocardial repolarization by way of blocking the potassium channel. Other effects include the blocking of sodium and calcium channels and β-adrenergic receptors (Vassallo & Trohman 2007). Amiodarone decreases thyroxine levels at a nominal concentration of 1 μM (681.77 μg/L), which leads to developmental arrest of the gastrointestinal system, swim bladder, and lower jaw cartilage and, ultimately, lethality in zebrafish larvae (Liu and Chen 2002; Raldúa and Babin 2009). Besse and Garric (2008) suggested that amiodarone be classified as a priority pharmaceutical due to its PEC of 555 ng/L. However, in this study, only the highest concentration tested (1020 μg/L) altered survival and growth in FHM. Amiodarone has not yet been detected in the environment.

Carbamazepine is a derivative of iminostilbene, which is commonly used for the treatment of seizures. Its pharmacological action is achieved by binding to the inactivated state of the sodium channel, thus producing a decrease in action potential transduction (McNamara 2001; Brodie 2010). A zebrafish 72-h fish embryo toxicity test and 10-day early life-stage test reported NOECs of 30.6 and 25 mg/L, respectively (Ferrari et al. 2003; van den Brandhof and Montforts 2010). No signs of malformations

or mortality were reported in *Xenopus laevis* larvae when exposed to carbamazepine at concentrations ranging from 1.0 to 100 mg/L (Richards & Cole 2006). In this study, we report a NOEC of 862 μg/L in a 28-day early life-stage study in FHM. Carbamazepine concentrations are as great as 6.3 μg/L in wastewater and 1.16 ng/g in muscle tissue of fish, which is considerably lower than effect concentrations reported in this study (Ternes 1998; Ramirez et al. 2007).

Clozapine is an atypical antipsychotic that has dopaminergic and serotonergic activity and appears to be most effective for treatment-resistant schizophrenia. However, its use is limited due to the risk of agranulocytosis, myocarditis, sedation, and convulsions (Baldessarini & Tarazi 2001). Weight gain is also a common side effect associated with the administration of clozapine to schizophrenic patients, which mirrors observations observed with FHM (Bai et al. 2011). Akande et al. (2010) reported that zebrafish embryos exposed to clozapine failed to hatch and resulted in several abnormalities, such as coagulation, eye defects and periocardial edema at 10 mg/L. The PEC for hospital wastewater is 0.97 μg/L, although no reports of its presence in environmental samples have been made (Escher et al. 2011). This study demonstrated clozapine to have significant effects on survival and growth at 30.8 μg/L.

Dexamethasone is a corticosteroid with a wide range of uses, including treatment of inflammation, autoimmune diseases, adrenal insufficiencies, and chemotherapy (Schimmer & Parker 2001). In this study, only survival was impacted at 577 μg/L. DellaGreca et al. (2004) reported 24-h *D. magna* EC_{50} and 7-day *Ceriodaphnia dubia* chronic values to be 48.3 and 0.05 mg/L, respectively. However, surface water concentrations of dexamethasone range from 0.02 to 0.31 ng/L (Chang et al. 2007).

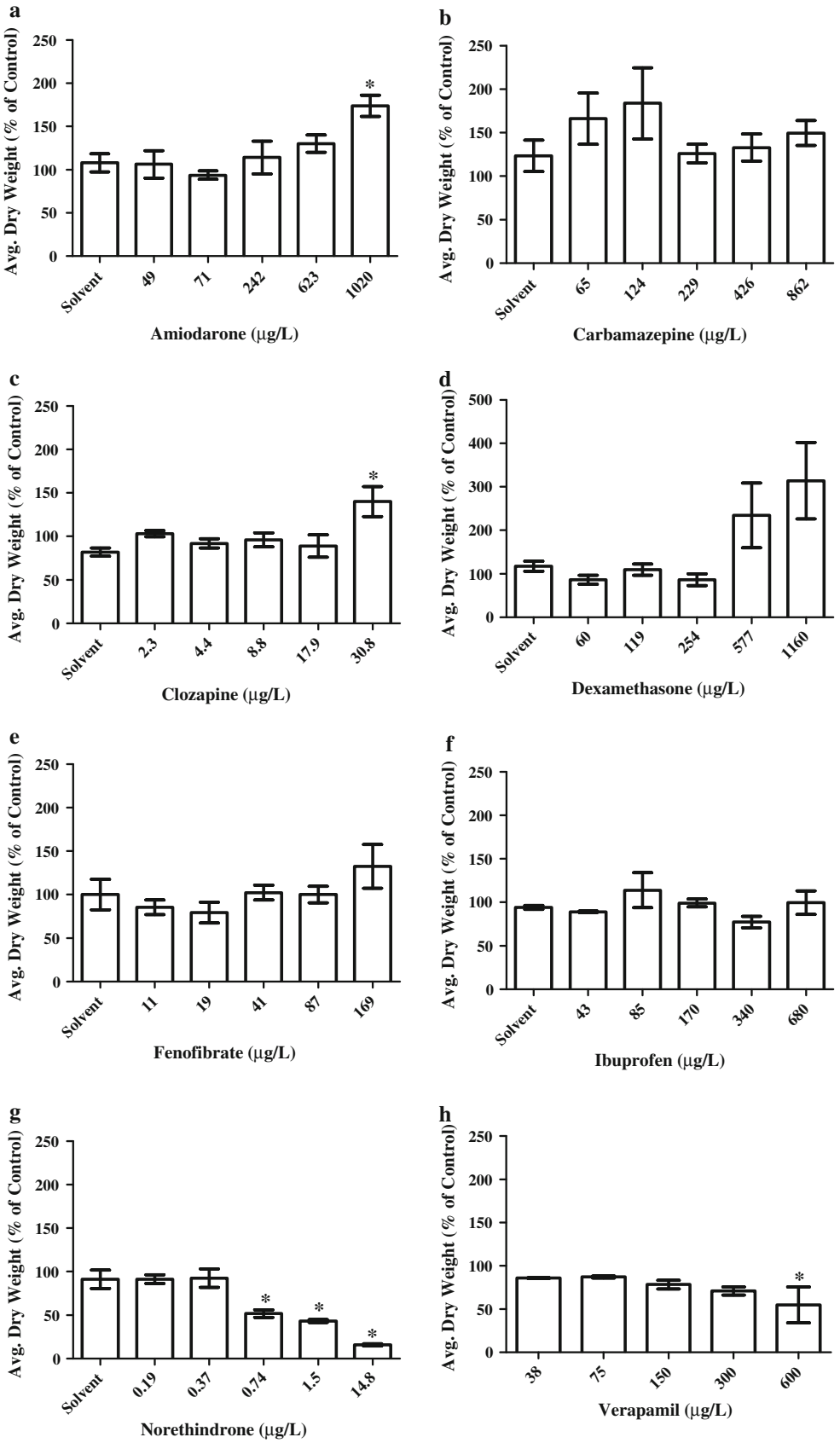
Fenofibrate is a fibric-acid lipid-lowering derivative used in the treatment of hypercholesterolemia (Mahley & Bersot 2001). Fenofibrate undergoes rapid hydroxylation to fenofibric acid in water, which makes detection in water difficult (Sacher et al. 2001; Stolker et al. 2004). Thus, the 16% recovery of nominal values for fenofibrate could be explained by this mechanism. Fenofibric acid has been detected in sewage treatment effluents and surface waters at a range of 0.28 to 0.34 ng/L (Ternes 1998; Stumpf et al. 1999). Although this study reports no significant adverse effects at concentrations tested, the EC_{50} for *Daphnia magna* is 4.90 mg/L for fenofibric acid (Rosal et al. 2010). Other fibric acid derivatives, such as bezafibrate, clofibric acid, and gemfibrozil, have EC_{50} values ranging from 10.4 to >200 mg/L in *D. magna* (Henschel et al. 1997; Cleuvers 2003; Ferrari et al. 2003; Hernando et al. 2004; Han et al. 2006; Zurita et al. 2007). A NOEC of 70 mg/L was reported for clofibric acid in a 10-day zebrafish ELS study (Ferrari et al. 2003).

Fig. 2 Growth (% of control) of fathead minnow larvae in the early life stage tests exposed to (**a**) amiodarone, (**b**) carbamazepine, (**c**) clozapine, (**d**) dexamethasone, (**e**) fenofibrate, (**f**) ibuprofen, (**g**) norethindrone, and (**h**) verapamil. *Significant difference compared with controls ($p < 0.05$). Error bars = SEM

Ibuprofen is a nonselective inhibitor of COX, which is associated with decreasing pain and inflammation (Rang et al. 2007). Nonsteroidal anti-inflammatories, such as ibuprofen, cause developmental toxicity in mammalian test organisms (Cappon et al. 2003). However, no change in survival, growth, or incidence in abnormalities were observed in FHM larvae at the concentrations tested in this study. Ibuprofen alters the pattern of reproduction in medaka and the heat-shock response in trout (Flippin et al. 2007; Gravel & Vijayan 2007). Studies with zebrafish and the nonspecific COX inhibitor diclofenac demonstrated that normal fish development was not impacted after exposure to 2000 μg/L (Hallare et al. 2004). However, diclofenac causes histopathological effects in brown trout after chronic exposure (Hoeger et al. 2005). Santos et al. (2009) detected ibuprofen in wastewater effluent at a maximum concentration of 8.2 μg/L, which is well below the NOEC for this study.

As suggested by Ankley et al. (2007), hormonally active compounds in the environment may present a risk to aquatic organisms. In this study, norethindrone was the most potent pharmaceutical tested, with a NOEC based on growth of 0.37 μg/L. Norethindrone is a synthetic progestin commonly used in combination oral contraceptives and hormone-replacement therapy. Zeilinger et al. (2009) reported that the synthetic progestin levonorgestrel altered reproduction and secondary sexual characteristics at concentrations ≥ 0.8 ng/L. Norethindrone causes feminization in turtles (Wibbels and Crews 1995). *D. magna* reproduction was not altered by norethindrone at concentrations ≤ 500 μg/L, but it did alter reproduction in combination with subeffect levels of 17α-ethinylestradiol (Goto & Hiromi 2003). These data suggest that fish may be more susceptible to progestin toxicity compared with invertebrates. Norethindrone has been measured in the environment, with wastewater effluent concentrations ranging from lower than analytical detection to 56 ng/L (Petrovic et al. 2002; Viglino et al. 2008). The NOEC derived in this study is ≥ 6.6 times greater these reported effluent concentrations.

Verapamil is a L-type calcium-channel blocker commonly used for the treatment of angina, arrhythmias, and hypertension (Rang et al. 2007). Burgess and Vere (1989) noted that calcium-channel blockers, such as verapamil, alter the development of *Xenopus*. Only at the highest concentration tested (i.e., 600 μg/L) was FHM growth altered in this study. Hummel et al. (2006) reported verapamil in surface water at concentrations ≤ 0.006 μg/L,


a
Avg. Dry Weight (% of Control)
250
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Solvent
49
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242
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Amiodarone (μg/L)
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Avg. Dry Weight (% of Control)
250
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Solvent
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862
Carbamazepine (μg/L)
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Clozapine (μg/L)
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600
Verapamil (μg/L)

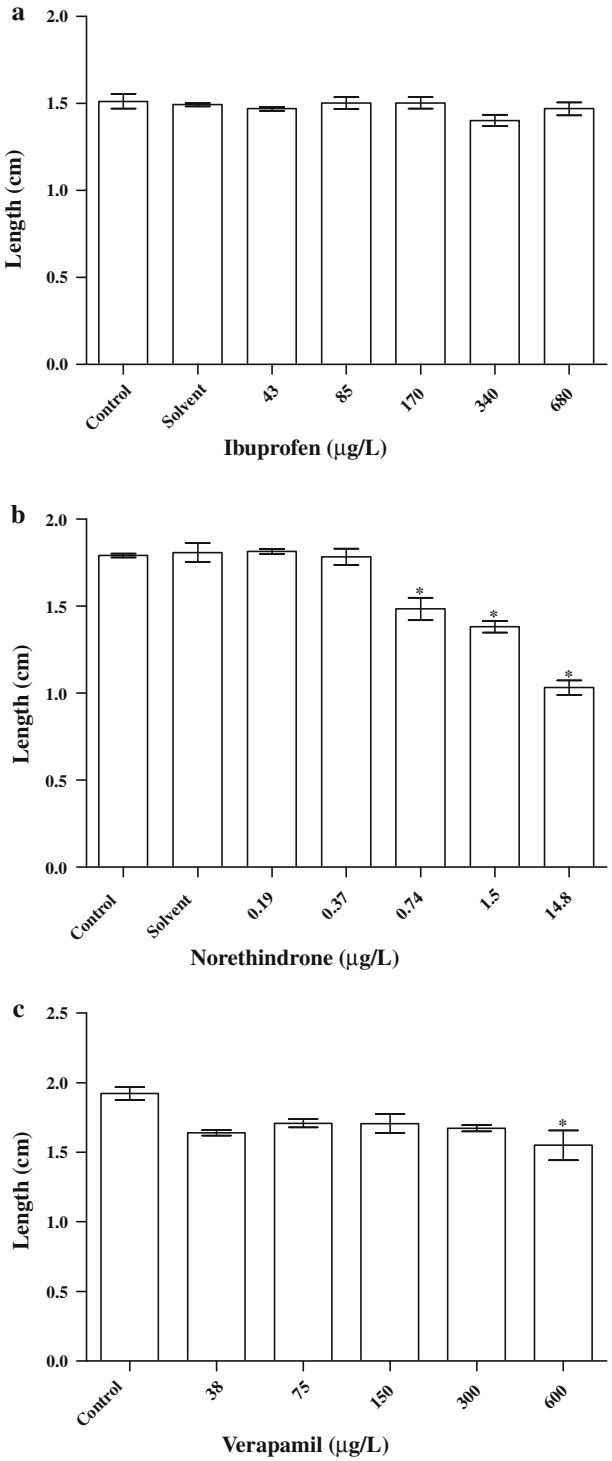

Fig. 3 Mean larval length (cm) of fathead minnow larvae in the early life stage tests exposed to (**a**) ibuprofen, (**b**) norethindrone, and (**c**) verapamil. *Significant difference compared with controls ($p < 0.05$). Error bars = SEM

which is well below than the effect concentrations observed in this study.

This study evaluated the FHM early life-stage toxicity of eight representative pharmaceuticals with different mechanisms of action. Testing representative drugs from various pharmaceutical classes with known mammalian mechanisms of action may help prioritize testing needs for new and existing pharmaceuticals as well as support the reduction of vertebrate animal testing. Norethindrone was the most potent drug tested: Developmental effects were observed at concentrations <1 μg/L. These data, in combination with limited environmental concentration data, suggest that synthetic progestins should undergo a more systematic ecotoxicological evaluation for their potential risk to the environment. Amiodarone and clozapine both resulted in significant decreases in survival and increases in growth. Thus, additional research is warranted on both antiarrhythmics and antipsychotics to assess their risk to aquatic organisms. Dexamethasone exposure significantly decreased survival; however, concentrations tested were higher than those detected in the environment. Verapamil elicited a significant decrease in growth only at the highest concentration tested, suggesting that calcium-channel blockers present a low developmental risk to fish. Carbamazepine, fenofibrate, and ibuprofen exposures did not adversely alter survival or growth of FHM. In combination with other peer-reviewed data, antiepileptics, fibric-acid derivatives, and nonsteroidal anti-inflammatory agents may not present a low developmental risk to fish.

Acknowledgments The authors thank Tom Waller at the University of North Texas for review and critique of an earlier version of this manuscript. Funding for this project was supplied by Pfizer Global Research and Development.

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