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Assessing the effects of the antidepressant venlafaxine to fathead minnows exposed to environmentally relevant concentrations over a full life cycle☆

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ABSTRACT

Venlafaxine is an antidepressant drug that has been detected in municipal wastewater effluents at low μg/L concentrations. To assess the potential of this compound to affect the survival, development and reproductive capacity of fish, we exposed fathead minnow (*Pimephales promelas*) over a full lifecycle in a flow-through system to nominal venlafaxine concentrations of 0.88, 8.8, and 88 μg/L. Mean measured venlafaxine concentrations in these treatments were 1.0, 9.3 and 75 μg/L. During the 167–168 d exposure, no significant changes were observed in survival, or the weights and lengths of fathead minnows. At maturity, there were no significant differences relative to controls in condition factor, liver-somatic index, or secondary sex characteristics in the venlafaxine exposed male or female fish. Fathead minnows from the highest venlafaxine treatment (i.e. 88 μg/L) produced 46% more eggs per female than control fish ($p = 0.031$). Egg quality, % fertilization, % hatching, and % deformities in F1 fry were unaffected by exposure of the parent fish to venlafaxine at any of the test concentrations. Venlafaxine exposure at environmentally relevant concentrations (i.e. 0.88 and 8.8 μg/L) caused no adverse effects in fathead minnows. This study is the first to assess the potential for effects in fish exposed to the antidepressant venlafaxine over a full lifecycle.

1. Introduction

Venlafaxine is a pharmaceutical widely prescribed to manage depression and anxiety (Vaswani et al., 2003). Venlafaxine acts at the synaptic junction to prevent the re-uptake of serotonin, and to a lesser extent noradrenalin, and it has a weak effect on dopamine re-uptake (Asnis et al., 2004; Caccia, 1998; Preskorn et al., 1994). In humans, over 60% of the compound is excreted via urine as metabolites (Caccia, 1998). Therefore, both the parent compound and metabolites have the potential to be transported in sewage to municipal wastewater treatment plants (WWTPs). There is evidence that venlafaxine and metabolites are not removed efficiently during wastewater treatment. In one Canadian study of antidepressants in a WWTP serving a city in the province of Ontario, venlafaxine was detected at mean concentrations of 1.1 μg/L and 0.8 μg/L in the influent and effluent, respectively (Metcalfe et al., 2010). In another Canadian study of the WWTP serving the population of Montreal in the province of Quebec, concentrations ranging from 0.18 to 0.21 μg/L in the influent and effluent, respectively, were observed over two seasons (Lajeunesse et al., 2008). Data from the USA show similar patterns, with venlafaxine concentrations of 0.93 μg/L in influent and 0.87 μg/L in effluent measured in the WWTP serving the city of Boulder, Colorado (Schultz and Furlong, 2008).

Mean concentrations of venlafaxine measured in surface waters range from 0.01 to 0.46 μg/L (Lajeunesse et al., 2008) in the St. Lawrence River downstream of the Montreal WWTP to 0.4–0.9 μg/L in the Grand River immediately downstream of a WWTP in Ontario (Metcalfe et al., 2010), and 0.3–1.0 μg/L in rivers close to WWTP discharges in Texas and Colorado (Schultz and Furlong, 2008). In addition to the parent compound, elevated concentrations of O-desmethyl venlafaxine and other metabolites have been detected in these river systems, so total concentrations of venlafaxine and metabolites in surface waters exceeded 2 μg/L

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(Lajeunesse et al., 2008; Metcalfe et al., 2010).

Fish can accumulate venlafaxine, but the concentrations in tissues are quite low compared to the rates of accumulation of other antidepressants that have higher log Kow values (Schultz et al., 2010). Fathead minnows (*Pimephales promelas*) caged for 2 weeks downstream of a WWTP that discharged effluents containing about 1 μg/L venlafaxine accumulated this compound in tissues at mean concentrations up to 1.2 ng/g wet weight (Metcalfe et al., 2010). In wild white suckers (*Catostomus commersoni*) captured downstream of WWTPs (in waters where measured concentrations of venlafaxine ranged from 0.12 to 0.69 μg/L), venlafaxine was not detected in the brain tissue of the majority of the animals, but in fish that had detectable levels, the concentrations ranged from 0.1 to 1 ng/g in brain tissue (Schultz et al., 2010). In controlled lab experiments, venlafaxine did not accumulate in the brain tissue of fathead minnows exposed to 0.3 and 1.1 μg/L venlafaxine for 21 d (Schultz et al., 2011).

Biological effects have been observed in fish exposed to venlafaxine in the laboratory in relatively short-term experiments. Reduced survival was observed in adult male fathead minnow exposed to 0.3 and 1.1 μg/L venlafaxine for 21 d, but this response was not concentration-dependent, with about 36% mortality at 0.3 μg/L and 18% mortality at 1.1 μg/L (Schultz et al., 2011). In tests with zebrafish (*Danio rerio*) over 7 weeks of exposure to venlafaxine, egg production was reduced in treatments with measured concentrations of 5–6 μg/L (Galus et al., 2013). Fish behaviour can also be affected by exposure to venlafaxine. Escape responses were slowed in larval fathead minnows exposed to 5 μg/L venlafaxine for 12 d (Painter et al., 2009). Time to capture prey was increased in hybrid striped bass (*Morone saxatilis* x *Morone chrysops*) exposed to 0.36–4.65 μg/L venlafaxine for 3–6 d (Bisesi et al., 2014). Disrupted circadian rhythm with decreased locomotion during the day was seen in adult mosquitofish (*Gambusia holbrooki*) exposed to 100 μg/L venlafaxine for 7 d (Melvin, 2017).

Venlafaxine can also affect invertebrate responses, behaviours, and reproduction. Newly hatched cuttlefish (*Sepia officinalis*) exposed for 20 d to 0.1 μg/L venlafaxine had reduced camouflage ability (Bidel et al., 2016). Venlafaxine increased locomotion in marine star snail (*Lithopoma americanum*), with a LOEC of 157 μg/L (Fong et al., 2015). In lifecycle exposures of *Daphnia magna*, cumulative reproduction was decreased by exposure to 100 μg/L venlafaxine in the first generation, but recovered back to control levels of reproduction in the following generation (Minguez et al., 2015).

There are currently no data reported in the literature on the effects of venlafaxine in fish exposed over an entire lifecycle. These types of experiments are important for assessing lethal and sublethal responses to contaminants of emerging concern, as they follow fish from the egg stage through all critical stages of development. Previously, we used the fathead minnow lifecycle test to study the biological effects from exposure to other contaminants of emerging concern such as propranolol (Parrott and Balakrishnan, 2017), ethinylestradiol (Parrott and Blunt, 2005), and a mixture of 6 pharmaceuticals and triclosan (Parrott and Bennie, 2009).

In the present study, we monitored fathead minnows exposed continuously to environmentally relevant concentrations of venlafaxine through the embryo stage to hatching (after day 5), growth of larvae (days 7–20) and juveniles (days 30–60), maturation of fish into adults (days 70–90), and mating and reproductive stages (days 90–168). The endpoints monitored in the lifecycle test included survival, % egg fertilization, % deformities in hatched fry, the overall success of hatch in the F1 generation, as well as the body condition, secondary sex characteristics and gonadal sex of the adults. The lowest venlafaxine concentrations tested were environmentally relevant, at nominal concentrations of 0.88 and 8.8 μg/L. The highest venlafaxine treatment (nominal concentration of 88 μg/L) was approximately 40 times above the highest venlafaxine concentrations detected in WWTPs in North America.

2. Materials and methods

2.1. Flow through exposures

Flow-through exposures of fathead minnows to venlafaxine were accomplished with a modified Mount & Brungs-type diluter, as described previously (Parrott and Bennie, 2009). Water flowed through the diluter and into the mixing cells that received venlafaxine stock solutions from 3 peristaltic pumps attached to 3 separate Marriott bottles containing venlafaxine solutions. Venlafaxine-HCl (CAS # 99300-78-4, 99.9% purity, lot # A-1237-145) was purchased from SynFine Research (Richmond Hill, ON, Canada). Nominal concentrations were 0 (i.e. control), 0.88, 8.8, 88 μg/L. A venlafaxine super-stock solution of 17.67 g/L (500 mg venlafaxine hydrochloride in 25 mL) was prepared with distilled water, so no solvent controls were required for the exposures. For the highest concentration of venlafaxine, 1 mL of super-stock was used to prepare 1 L of stock (20 mg/L). During each 3.5 min cycle, 2 mL of this stock flowed via peristaltic pump to a 400 mL mixing chamber in the diluter. This 400 mL volume then flowed to the randomly assigned tanks receiving the highest concentration of venlafaxine (i.e. 88 μg/L). For the two lower venlafaxine treatments, the stock solution was diluted 10-fold and 100-fold, respectively, and separate 1 L Marriott bottles and peristaltic pumps were used to deliver 2 mL per cycle to 400 mL water in the mixing chamber. The 400 mL aliquots supplied 4 replicate aquaria per venlafaxine concentration, so the flow rate was 100 mL/3.5 min into each 12 L volume aquaria for a solution replacement time of about 3.4 tank volumes per day. A schematic of the aquaria set-up showing stock solutions, pumps, diluter mixing cells, and delivery of solutions to aquaria is shown in Fig. S1.

2.2. Analysis

The solid phase extraction (SPE) methods used to extract water to measure the concentrations of venlafaxine and its primary metabolite, O-desmethyl venlafaxine in the various treatments were identical to those published previously (Metcalfe et al., 2010). Briefly, water samples were extracted using Oasis MCX solid phase extraction, followed by analysis by liquid chromatography with tandem mass spectrometry (LC-MS/MS). Stable isotope internal standards of venlafaxine-d_{10} hydrochloride, and O-desmethyl venlafaxine-d_{10} purchased from SynFine Research were added to all samples before extraction.

Venlafaxine, O-desmethyl venlafaxine and their respective stable isotope surrogates were analyzed by LC-MS/MS with an AB Sciex (Mississauga, ON, Canada) Q-Trap 5500 instrument with an electrospray ionization source, and this instrument was operated in negative ion mode. This system was equipped with an Agilent 1100 series HPLC system. The analytes were separated chromatographically using a Genesis C18 column that was 150 mm long, 2.1 mm ID and 4 μm particle size (Chromatography Specialities, Brockville, ON, Canada) with a guard column with the same stationary phase (Genesis C18, 10 × 2.1 mm and 4 μm). MS detection was performed using multiple reactions monitoring with the ion transitions previously described by Metcalfe et al. (2010). For quantification, an internal standard method was used with a nine-point calibration graph covering the range of anticipated analyte concentrations. Internal standards (i.e. stable isotope labelled compounds) were used to correct for analyte recovery and matrix effects. The Limits of Detection (LODs) and Limits of Quantification (LOQs) were