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Uptake and depuration of the anti-depressant fluoxetine by the Japanese medaka (*Oryzias latipes*)

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ABSTRACT

The selective serotonin reuptake inhibitor (SSRI) class of anti-depressants is among the most widely prescribed groups of pharmaceuticals. Consequently, aquatic ecosystems impacted by municipal wastewater discharges are predicted to receive substantial annual loadings of these compounds. Although SSRIs have been detected in fish tissues, little is known of their uptake and depuration in freshwater fish species. In this study, Japanese medaka (*Oryzias latipes*) were exposed to fluoxetine at a nominal concentration of 0.64 μg L^{-1} for 7 d and subsequently allowed to depurate in clean water over a 21 d period. Fluoxetine uptake by medaka was observed within the first 5 h of exposure and the biologically active metabolite, norfluoxetine, was also detected in medaka tissues during this timeframe. A maximum fluoxetine concentration was measured in medaka by the third day of the uptake phase, yielding an uptake rate constant (k_1) of 5.9 ± 0.5 (d^{-1}). During the depuration phase of the experiment, a half life of 9.4 ± 1.1 d was determined for fluoxetine. Using these data, bioconcentration factor (BCF) values of 74 and 80 were estimated for fluoxetine and a pseudo-BCF (the ratio of the concentration of norfluoxetine in medaka and the aqueous fluoxetine concentration) of 117 was calculated for norfluoxetine. These results indicate longer persistence and greater potential for the bioaccumulation of fluoxetine and norfluoxetine in fish tissues than would be predicted from prior half life estimates derived using mammalian species.

1. Introduction

Anti-depressant pharmaceuticals and their metabolites from the class of selective serotonin reuptake inhibitors (SSRIs) have been detected in water and municipal wastewater in North America and in northern Europe (Weston et al., 2001; Kolpin et al., 2002; Metcalfe et al., 2003; Zorita et al., 2007; Schultz and Furlong, 2008). Fluoxetine, which is the active ingredient in Prozac™, was one of the first SSRIs developed for clinical use as an anti-depressant, and it remains one of the most widely prescribed pharmaceuticals in North America (Preskorn, 1996; NDC Health, 2008). Fluoxetine persists in water for a relatively long time relative to other pharmaceuticals (Johnson et al., 2005; Kwon and Armbrust, 2006). Octanol water partition coefficients ($\log K_{ow}$) for fluoxetine have been variously reported as 1.57 at pH 7 by Brooks et al. (2003), 1.8 by Huggett et al. (2004), 4.05 by Christensen et al. (2007), and 4.51 by Lienert et al. (2007), indicating at least moderate potential for bioaccumulation. Attempts to extend the concept of K_{ow} from hydrophobic organic compounds to more polar or charged molecules have included determination of octanol water distribution ratio (D_{ow}) at a given pH and with a given ionic strength (Scherrer, 1984). Given these constraints it should not be surprising that there is considerable variation in the reporting $\log K_{ow}$ values for pharmaceuticals. A recent study demonstrated that the liposome/water distribution ratio ($D_{lip-wat}$) may be a more useful descriptor than D_{ow} for predicting pharmaceutical bioaccumulation and toxicity as there is very little ionic strength dependence for the partitioning of compounds across liposome membranes (Nakamura et al., 2008).

In mammals, fluoxetine is biotransformed to norfluoxetine (Fig. 1) through the activity of cytochrome P450 enzymes (Hiemke and Härtter, 2000). Pharmacokinetics data for SSRIs in mammalian models and in humans indicate that fluoxetine has a relatively long half life of 1–4 d, in comparison to values of ⩽1 d for other SSRIs (Hiemke and Härtter, 2000). However, little is currently known regarding the pharmacokinetics of fluoxetine and other SSRIs in fish. SSRIs are known to induce biological effects in fish, including delays in reproductive and physiological development (Foran et al., 2004), decreased aggressiveness (Perrault et al., 2003), and inhibition of feeding responses (Stanley et al., 2007).

Annual rates of discharge of pharmaceuticals from municipal wastewater treatment plants (WWTPs) into receiving waters may reach kilogram levels (Daughton and Ternes, 1999; Lindberg et al., 2005). Under conditions of chronic exposure, there is potential for pharmaceuticals to bioaccumulate in fish and other aquatic organisms. Mimeault et al. (2005) observed bioaccumulation of the cholesterol reducing drug, gemfibrozil, in the plasma of goldfish to

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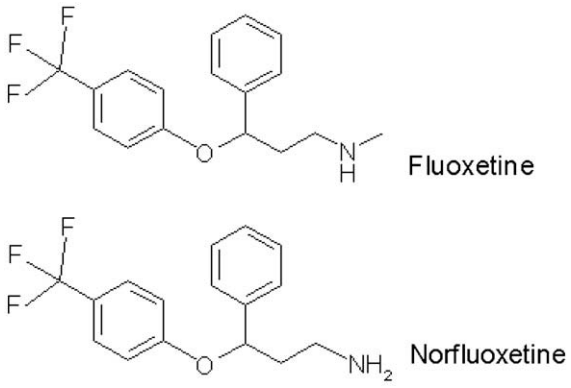

Fig. 1. Structure of fluoxetine and its active metabolite, norfluoxetine.

levels equivalent to a bioconcentration factor (BCF) of 89. Combined concentrations of fluoxetine and its metabolite, norfluoxetine, were >10 μg kg^{-1} in the tissues of fish collected downstream of a municipal wastewater treatment plant (Brooks et al., 2005). Fluoxetine and norfluoxetine were detected at concentrations of 1.02 and 1.08 μg kg^{-1}, respectively, in a gizzard shad (*Dorosoma cepedianum*) collected from an urbanized embayment of western Lake Ontario, Canada (Chu and Metcalfe, 2007). Aquatic systems with substantial inputs of municipal wastewater effluent or whose flow is dominated represent the worse case exposure scenarios for emerging pollutants such as pharmaceuticals and personal care products (Brooks et al., 2006). Our ability to predict the extent of bioaccumulation and thresholds for toxicity in aquatic organisms exposed to pharmaceuticals in such habitats is dependent on knowledge of the rates of uptake and elimination of these compounds. In this study, the aquarium fish species, the Japanese medaka (*Oryzias latipes*) was exposed to aqueous concentrations of fluoxetine to determine the kinetics of uptake and depuration for both the parent compound and the metabolite, norfluoxetine. This research is among the first to study the kinetics of both the uptake and elimination of a pharmaceutical in an aquatic species.

2. Materials and methods

2.1. Exposure

Japanese medaka were obtained from a broodstock that has been continuously maintained at Trent University over the past 15 years. A total of 46 adult Japanese medaka averaging 0.36 ± 0.02 g in mass were used in the uptake and depuration experiments. Throughout the exposures, fish were held in dechlorinated tap water in 2 × 10 L glass aquaria (23 fish per aquarium) at 25 ± 2 °C under a 16:8 h light dark cycle. Water quality parameters, including pH, alkalinity, and hardness, were monitored throughout the study (Table 1). Over the duration of the experiment, fish were fed to satiation with live artemia two times daily. Animals were maintained and handled throughout the experiment according to the Canadian Council for Animal Care Guidelines.

A stock solution was prepared by dissolving fluoxetine hydrochloride (USP, Rockville, MD, USA) in acetone to a concentration of 640 μg of fluoxetine per mL of acetone. Volumes of stock solution (10 μL) were added to dechlorinated tap water in the exposure aquaria to produce a nominal fluoxetine concentration of 0.64 μg L^{-1} and a solvent concentration of 0.014 μmol L^{-1}. This nominal concentration is approximately an order of magnitude higher than the ng L^{-1} concentrations of fluoxetine that have been reported in surface waters in North America (Kolpin et al., 2002; Metcalfe et al., 2003).

Table 1
Ranges and average values for water quality parameters measured over 28 d experimental duration

Parameter	Units	Range	Average
pH	N/A	7.2–7.5	7.4
Alkalinity	mg L^{-1}	60–75	68
Hardness	mg L^{-1}	75–90	83
Fluoxetine concentration[a]	μg L^{-1}	0.67–0.18	0.55

Fluoxetine concentration is the average measured over the 24 h renewal period.
N/A, unitless.
[a] Fluoxetine concentration measured in water during the 24 h renewal period.

Exposures were conducted under static conditions for 7 d, with complete daily renewal of the exposure solution. After the 7 d uptake phase, the medaka were transferred to aquaria with clean water and allowed to depurate for 21 d, with daily renewal of the water. A minimum of 4 and a maximum of 7 fish were collected on each of days 0.2 (i.e. 5 h), 3 and 7 during the uptake phase, and on days 7, 14, and 21 during the depuration phase. To minimize potential bias due to fish size, individuals observed to be much smaller or larger than the average fish at the time of collection were returned to their respective tanks. Control fish were maintained in a separate aquarium and sampled on days 0 and 7 of the uptake phase and day 21 of the depuration phase ($n = 5$ per sampling period). An equivalent volume (10 μL) of the acetone carrier solvent was added to the control tank on a daily basis during the 7 d uptake phase.

In separate, preliminary experiments to determine the concentrations of fluoxetine in aquaria over the 24 h renewal period, a 10 μL aliquot of the stock solution was added to a 10 L aquarium containing 23 medaka. Samples of water (50 mL) were collected in triplicate for analysis at 0, 6, and 24 h post-addition (Table 1). Exposure conditions, stock solutions, and reagents throughout the experimental procedure were identical to those of the preliminary procedures.

2.2. Extraction and analysis

Water samples were extracted for the analysis of fluoxetine and norfluoxetine as described by Metcalfe et al. (2003). Briefly, samples were extracted by solid phase extraction (SPE) with Oasis HLB cartridges (Waters Inc., Milford, MA, USA), and the analytes were then eluted from the cartridges with methanol. Medaka were extracted for the analysis of fluoxetine and norfluoxetine using the methods described previously by Chu and Metcalfe (2007). Briefly, whole medaka were homogenized and mixed with Hydromatrix® medium (Varian Inc., Palo Alto, CA, USA) and then extracted into methanol by pressurized liquid extraction (PLE) using an ASE 300 accelerated solvent extraction system (Dionex Corp., Sunnyvale, CA, USA). The PLE extracts were cleaned up using Oasis MCX SPE cartridges (Waters Inc.), as described by Chu and Metcalfe (2007).

The extracts from water and fish were analyzed by liquid chromatography tandem mass spectrometry (LC–MS/MS) using methods described by Chu and Metcalfe (2007). Liquid chromatography was performed on an Agilent Series 1100 HPLC system (Agilent Technologies Canada, Mississauga, ON, Canada) and the analytes were separated chromatographically on a Genesis C18 column (150 × 2.1 mm i.d., 4 μm particle size) purchased from Chromatographic Specialties Inc. (Brockville, ON, Canada). The HPLC system was coupled to a Q-Trap tandem mass spectrometer (Applied Biosystems/MDS Sciex, Concord, ON, Canada) with an atmospheric pressure chemical ionization (APCI) source operated in positive ion mode. Detection was by multiple reaction monitoring (MRM) of two transition ions. Quantification was performed by an isotope dilution method using fluoxetine-D5 (Sigma–Aldrich, St. Louis,