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Toxicokinetics, disposition and metabolism of fluoxetine in crabs

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HIGHLIGHTS

- Fluoxetine in crabs shows a high volume of distribution into peripheral tissues, notably nervous tissue.
- Fluoxetine is extensively transformed into its active metabolite nor-fluoxetine.
- Fluoxetine and its metabolite nor-fluoxetine remain present in the crabs bodies for more than 10 days.
- Oral bioavailability of fluoxetine was less than 20%, but prolongs half-life of the parent compound fluoxetine.

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ABSTRACT

The disposition and metabolism of fluoxetine in the European shore crab and the Dungeness crab were assessed. Crabs received intracardiac doses of either 0.13 μg/kg or 0.5 mg/kg fluoxetine, respectively. In addition, fluoxetine was administered to *Metacarcinus cancer* by oral gavage at 7.8 mg/kg. The distribution of fluoxetine was quantified in haemolymph and digestive gland for both crabs, as well as brain, muscle, and testis of *Carcinus maenas*, over 12 days. The metabolite norfluoxetine, was also measured in *C. maenas*. Fluoxetine was mainly found in lipid rich tissues. Distribution coefficients increased for digestive gland until three days after fluoxetine administration and then decreased until the end of the observations. The highest distribution coefficients were obtained for brain. Norfluoxetine displayed continuously high levels in digestive gland and brain. The strong decrease in fluoxetine and the concomitant increase in norfluoxetine demonstrates that decapod crustaceans metabolise fluoxetine into the more biologically active norfluoxetine. Fluoxetine levels in the haemolymph of *M. cancer* declined within 20 h, but showed a second peak 25 h later, suggesting remobilisation from tissues sequestering the compound. The steady state volume distribution and the total body clearance of fluoxetine were high, consistent with high diffusion of fluoxetine into the peripheral tissues and biotransformation as an important elimination pathway. Oral administration of fluoxetine prolonged its half-life in *M. cancer*, but bioavailability was low. These results confirm the high distribution into nervous tissue, extensive biotransformation into the highly active norfluoxetine and a half-life similar to that observed in vertebrates.

Abbreviations: AUC, area-under-the-curve; B, brain; Cl_b, body clearance; DC, distribution coefficient; DG, digestive gland; ESI, electrospray ionization; FLX, fluoxetine; GC, gas chromatography; H, haemolymph; IC, intra-cardiac; LC, liquid chromatography; LOQ, limit of quantification; 5-HT, 5-Hydroxytryptamine (serotonin); MS, mass spectrometry; Mu, muscle; nor-FLX, norfluoxetine; QuEChERS, Quick Easy Cheap Effective Rugged Safe; SER, sertraline; SSRIs, selective serotonin reuptake inhibitors; T, testis; $t_{½}$, half-life; V_{ss}, steady-state volume of distribution.

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1. Introduction

In most OECD countries the use of antidepressants has increased considerably over the last decade (Silva et al., 2012; OECD, 2013). Amongst them, selective serotonin reuptake inhibitors (SSRIs) have been widely marketed since the mid-1980s. Fluoxetine (FLX) was the first SSRI to be approved by the US Food and Drug administration in 1987. In the following years, many other SSRIs have been commercialised, including Citalopram, Paroxetine and Sertraline (SER) among others (Nielsen and Gøtzsche, 2011). A second class of psychotropic medication, the serotonin-norepinephrine reuptake inhibitors, also acts on brain chemistry by altering serotonin (5-HT) levels. Together these medications are widely used in the treatment of depression, compulsive disorders, panic disorder, anxiety and social phobia (Schultz and Furlong, 2008). Already in 2001, FLX was prescribed to 34 million people worldwide and since then the use of SSRIs has increased dramatically (Schultz and Furlong, 2008; Kosjek and Heath, 2010; Nielsen and Gøtzsche, 2011). Global sales of antidepressants amounted to $20 billion in 2010, and SSRIs like SER and FLX figured amongst the top five psychoactive drugs (Hayman, 2012). The high prescription volumes of SSRIs and the relative environmental stability of these drugs (Kwon and Armbrust, 2006) as well as their potential for accumulation in biota have rendered them emerging micropollutants in the 2000s. After oral administration, FLX is metabolized into a principal active metabolite, the chiral and demethylated metabolite norfluoxetine (nor-FLX) (Silva et al., 2012), the half-life of which is even longer than that of FLX (Kim et al., 2004). Conventional wastewater treatment plants (WWTP) do not efficiently remove many SSRIs and their effluents are the primary source of FLX and nor-FLX release into aquatic environments (Auriol et al., 2006; Heberer, 2002).

Fluoxetine and other SSRIs influence the level of one of the major neuromodulators, the monoamine 5-HT, widely distributed in the central nervous system (CNS) of both vertebrates and invertebrates. FLX blocks the reuptake of the neurotransmitter into the presynaptic cells by binding to serotonin transporters, thus increasing the levels of 5-HT in the synaptic cleft (Wong et al., 1995; Roman et al., 2003). 5-HT signalling is phylogenetically highly conserved and modulates a wide range of physiological processes in aquatic invertebrates (Turlejski, 1996; Hay-Schmidt, 2000; Kreke and Dietrich, 2008). FLX is therefore potentially capable of interfering with the regulation of a wide range of neurological functions including behavioural endpoints in non-target species (Mesquita et al., 2011; Franzellitti et al., 2013; Weinberger and Klaper, 2014; Bossus et al., 2014; Bidel et al., 2016; Robert et al., 2016). Consequently, robust analytical tools for determining the environmental occurrence, the fate and the transformation SSRIs residues and their metabolite in water and other biological samples have been developed, either based on gas chromatography coupled mass spectrometry (GC-MS) (Pablo Lamas et al., 2004; Brooks et al., 2005), or high performance liquid chromatography-electrospray ionization tandem mass spectrometry (HPLC/ESI/MS/MS) detection (Schultz and Furlong, 2008; Vasskog et al., 2008).

FLX is the most investigated of the SSRIs and its metabolite nor-FLX also inhibits 5-HT reuptake (Torres et al., 2003; Qu et al., 2009; Silva et al., 2012, 2015; Davis et al., 2016). Both compounds have been frequently monitored in surface waters, sediment and WWTPs. Concentrations in WWTP effluents can vary widely for both FLX and nor-FLX with reported values ranging from 0.4 to 841 ng/L for FLX and from 0.7 to 9.3 ng/L for nor-FLX (Schultz et al., 2010; Lajeunesse et al., 2008; Santos et al., 2010; Thomas and Klaper, 2012). Surface water concentrations of FLX and nor-FLX measured downstream from WWTPs have been reported to range from 1 to 12 ng/L for FLX and 1.3–4 ng/L for nor-FLX (Kolpin et al., 2002; Fent et al., 2006; Kim et al., 2007; Lajeunesse et al., 2008). Caged fish placed downstream of a WWTP effluent discharge site accumulated several SSRIs up to 3.2 μg/kg (Metcalfe et al., 2010). This level of exposure is comparatively low relative to human use where daily doses range from 10 to 40 mg/kg, *i.e.*, 32.3–129.3 μmol/kg (http://pi.lilly.com/us/prozac.pdf; www.fda.gov/ohrms/dockets/ac/04/briefing/2004-4065b1-35-PROZAC-CLASS-LABELING) resulting in serum concentrations between 200 and 1000 ng/mL (Sawyer and Howell, 2011). If, however, FLX is equally well distributed and highly accumulated in crustacean tissues as reported for mammals (*e.g.*, Hiemke and Härtter, 2000), one may assume that chronic low-level exposures could result in minimal effective concentrations in crustacean species. This particularly applies to FLX, as the half-live (t½) of its active metabolite nor-FLX may be up to two weeks (Hiemke and Härtter, 2000; Brooks, 2014). Numerous ecotoxicological studies also demonstrated that even a low dose of SSRIs can impact aquatic wildlife, especially invertebrates (Franzellitti et al., 2014; Fong and Ford, 2016). FLX and nor-FLX at concentrations below the μg/L range have been demonstrated to affect reproduction, growth and behaviour in vertebrates and invertebrates (Brooks et al., 2003a, 2003b; Henry et al., 2004; Flaherty and Dodson, 2005; Péry et al., 2008; Santos et al., 2010; Bossus et al., 2014; Di Poi et al., 2014). The main question that has to be asked is what are the concentrations of FLX and nor-FLX in tissues where SSRIs exert their action, *i.e.*, brain and neuronal tissues of aquatic animals? Brooks et al. (2005) measured 1.58 ng/g and 8.86 ng/g in fish brain for FLX and nor-FLX, respectively. Schultz et al. (2010) confirmed FLX and nor-FLX bioaccumulation in brain tissue of fish collected in different river systems: although concentrations of 0.02–0.18 ng/L for FLX and 0.07–0.9 ng/L for nor-FLX were lower than those reported by Brooks et al. (2005), significant bioaccumulation was observed for FLX when compared to the concentrations in the surface water. It is assumed the majority of the nor-FLX present in the brains of these fish came from metabolism of the parent compound, as nor-FLX concentration in surface waters was extremely low.

The European shore crab, *Carcinus maenas*, is considered as a suitable model for ecotoxicological research (Rodrigues and Pardal, 2014). Where *C. maenas* is not present or endemic, other crab species, such as the Dungeness crab (*Metacarcinus cancer*) can serve for the same purpose. Dungeness crabs are an economically important species abundant throughout the West coast of North America. Thus, in a more general context, crabs, due to their availability and bioaccumulation capacity can be considered suitable indicator species for marine biomonitoring. Studies on antidepressants in *C. maenas* and other crabs have suggested potential effects of SSRIs on biochemical, physiological and behavioural responses (Santos et al., 2001; Mesquita et al., 2011; Rodrigues et al., 2014, 2015; Hamilton et al., 2016; Robert et al., 2016). However, little is known about the relation between exogenous FLX exposure and internal tissue concentrations in crabs and other crustaceans. Particularly, in the case of FLX, the persistent and apparently more potent nor-FLX should be taken into account. Robert et al. (2016) observed delayed responses following single dose applications of FLX to *C. maenas* with effects on glucose regulation and steroid hormone metabolism occurring several hours after injection. Hence, compared to the immediate changes triggered by 5-HT, FLX may need to be metabolized before it fully effects its action concomitantly to a continuous increase of nor-FLX. Such transition of FLX into nor-FLX is suggested for fish by the studies of Schultz et al. (2010, 2011), where nor-FLX was the only metabolite to be consistently detected in the brain tissue. Knowledge about similar dynamics in invertebrate species, however, is lacking. Efficient absorption, tissue accumulation and t½ of FLX and nor-FLX of up to 4 and 15 d, respectively (Hiemke and Härtter, 2000) may establish a