Science of the Total Environment 621 (2018) 782–790

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Concentrating mixtures of neuroactive pharmaceuticals and altered neurotransmitter levels in the brain of fish exposed to a wastewater effluent

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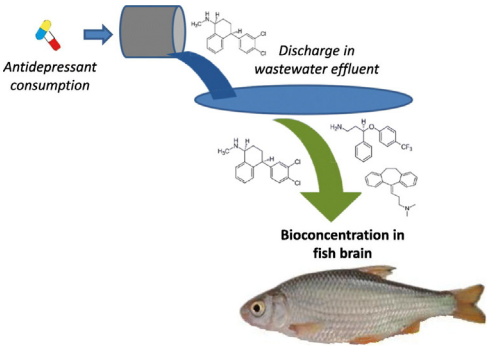
HIGHLIGHTS

- A complex mixture of neuroactive pharmaceuticals accumulated in the brain and plasma of effluent-exposed roach.
- Bioconcentration factors of the pharmaceuticals were 3-40 fold higher in brain compared with blood plasma
- Fish plasma concentrations of pharmaceuticals were 33-5714 fold below human therapeutic plasma concentrations
- Disruption in neurotransmitter concentrations were observed in brain regions of effluent-exposed fish.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 September 2017
Received in revised form 22 November 2017
Accepted 23 November 2017
Available online xxxx

Editor: D. Barcelo

Keywords:
Fish
Antidepressants
Antipsychotics
SSRIs
Neurotransmitters
Wastewater effluents

ABSTRACT

Fish can be exposed to a variety of neuroactive pharmaceuticals via the effluent discharges from wastewater treatment plants and concerns have arisen regarding their potential impacts on fish behaviour and ecology. In this study, we investigated the uptake of 14 neuroactive pharmaceuticals from a treated wastewater effluent into blood plasma and brain regions of roach (*Rutilus rutilus*) after exposure for 15 days. We show that a complex mixture of pharmaceuticals including, 6 selective serotonin reuptake inhibitors, a serotonin-noradrenaline reuptake inhibitor, 3 atypical antipsychotics, 2 tricyclic antidepressants and a benzodiazepine, concentrate in different regions of the brain including the telencephalon, hypothalamus, optic tectum and hindbrain of effluent-exposed fish. Pharmaceuticals, with the exception of nordiazepam, were between 3–40 fold higher in brain compared with blood plasma, showing these neuroactive drugs are readily uptaken, into brain tissues in fish. To assess for the potential for any adverse ecotoxicological effects, the effect ratio was calculated from human therapeutic plasma concentrations (HtPCs) and the measured or predicted fish plasma concentrations of pharmaceuticals. After accounting for a safety factor of 1000, the effect ratios indicated that fluoxetine, norfluoxetine, sertraline, and amitriptyline warrant prioritisation for risk assessment studies. Furthermore, although plasma concentrations of all the pharmaceuticals were between 33 and 5714-fold below HtPCs, alterations in serotonin, glutamate, acetylcholine and tryptophan concentrations were observed in different brain regions of effluent-exposed fish. This study highlights the importance of determining the potential health effects arising from the concentration of complex environmental mixtures in risk assessment studies.

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https://doi.org/10.1016/j.scitotenv.2017.11.265
0048-9697/

1. Introduction

After human ingestion, many pharmaceuticals are excreted in their native form or as metabolites and they enter aquatic systems mainly via effluents from wastewater treatment works (WwTWs) (Fent et al., 2006; Gardner et al., 2012; Kostich et al., 2014; Petrie et al., 2015; Tanoue et al., 2015). Recently, concerns have arisen regarding the presence of neuroactive pharmaceuticals, such as selective serotonin reuptake inhibitors (SSRIs) and benzodiazepines, in WwTW effluents because these pharmaceuticals have been shown to alter behaviour of different fish species under experimental laboratory conditions (Brodin et al., 2013; Huerta et al., 2016; Margiotta-Casaluci et al., 2014; Valenti et al., 2012). In addition to SSRIs, other types of antidepressants including serotonin-noradrenaline reuptake inhibitors (SNRIs) and tricyclic antidepressants are also regularly detected in effluents from WwTWs (Grabicova et al., 2014; Lajeunesse et al., 2011; Petrie et al., 2015). In comparison to SSRIs, the effects of tricyclic antidepressants on fish behaviour have been less studied, but a member of this class, amitriptyline, is extensively prescribed in England to treat depression and psychiatric disorders (>10,000 kg in England in 2012) (Petrie et al., 2015; Ziarrusta et al., 2017).

Recent studies investigating the effects of neuroactive pharmaceuticals on fish have focused on behavioural endpoints as these are likely to be the primary effects of exposure to neuroactive pharmaceuticals according to the read-across hypothesis (Margiotta-Casaluci et al., 2014; Valenti et al., 2012). This hypothesis has been suggested to be a useful tool in risk assessment studies and is based on the theory that blood plasma concentrations of pharmaceuticals in non-target vertebrates similar to human therapeutic plasma concentrations are likely to cause pharmacological effects in organisms such as fish (Huggett et al., 2003; Rand-Weaver et al., 2013). However, there is uncertainty as to whether environmental water concentrations of neuroactive drugs such as SSRIs bioconcentrate to high enough concentrations in the plasma or target tissues to cause behavioural effects in fish (Sumpter et al., 2014). Mean measured concentrations of neuroactive pharmaceuticals in WwTW effluents have been reported at between 1 and 330 ng/L for SSRIs (e.g., fluoxetine, sertraline and citalopram), SNRIs (venlafaxine), tricyclic antidepressants (amitriptyline) and benzodiazepines (diazepam) (Grabicova et al., 2014; Petrie et al., 2015), and these are far below water concentrations for SSRIs fluoxetine, sertraline and citalopram (in the range of 1–116 μg/L) and for venlafaxine (between 250 and 500 μg/L) reported to affect the behaviour of different fish species in laboratory experiments (Bisesi et al., 2014; Kellner et al., 2016; Margiotta-Casaluci et al., 2014; Valenti et al., 2012; Xie et al., 2015). In contrast with these reports however, there have been some studies indicating that fluoxetine at concentrations below 1 μg/L (0.3 μg/L and 0.54 μg/L), and therefore similar to environmental concentrations, could affect fish behaviour (Barry, 2013; Dzieweczynski and Hebert, 2012). Furthermore, non-monotonic shifts in behavioural responses have been reported for neuroactive compounds such as oxazepam (Huerta et al., 2016). More information is needed on the uptake and tissue partitioning of mixtures of neuroactive pharmaceuticals in fish to support the environmental risk assessments of these drugs in aquatic environments.

In a recent study, we used a holistic untargeted chemical profiling approach to identify pharmaceuticals accumulating in roach (*Rutilus rutilus*) exposed to a treated wastewater effluent. We identified 14 different neuroactive compounds in extracts of either gonad, liver, gills, kidney or plasma (David et al., 2017). The aim of the study presented here was to use targeted analyses to quantify the concentrations of this pharmaceutical mixture in plasma and brain and investigate for biological responses in the brain via measurement of neurotransmitter concentrations as a consequence of effluent exposure. We provide novel information on the accumulation pattern of neuroactive pharmaceuticals in four different regions of the brain, i.e. the telencephalon, hypothalamus, optic tectum and hindbrain. We show that even though plasma concentrations of all neuroactive drugs were well below the human therapeutic plasma concentrations, concentrations of neurotransmitters were altered in the hypothalamus of effluent-exposed fish, highlighting the need to understand the effects of exposure to complex chemical mixtures on fish behaviour and physiology.

2. Materials and methods

2.1. Chemicals and reagents

Certified standards of glutamate, acetylcholine, serotonin, tryptophan, fluoxetine (Fluo), sertraline (Ser), paroxetine (Par), clozapine (Clo), venlafaxine (Venlaf), and also formic acid, ammonium hydroxide, phosphoric acid were obtained from Sigma Aldrich, Dorset, UK. Norfluoxetine (norFluo), norsertraline (norSer), citalopram (Cit), norclozapine (norClo), quetiapine (Quet), amitriptyline (Ami), noramitriptyline (norAmi), diazepam (Diaz), nordiazepam (norDaz), serotonin-d4, norfluoxetine-d6 were purchased from LGC standards UK. Venlafaxine-d6, fluoxetine-d5 were purchased from QMX Laboratories Limited UK. All standards were >99% compound purity and deuterated standards >97% isotopic purity. HPLC grade acetonitrile, methanol and water were obtained from Rathburns UK. Oasis HLB cartridges (1 g) were obtained from Waters, Manchester, UK and Strata-X-C Polymeric Reversed Phase 96-Well Plates (33 μm sized particles, 30 mg/well) from Phenomenex, Macclesfield UK.

2.2. Fish exposure and sample collections

A population of male and female sexually mature roach (*Rutilus rutilus*, age 2+, mean ± SEM length of 14.5 ± 1.3 cm and weight 45.4 ± 12.1 g) was exposed for 15 days to either a treated effluent from a WwTW or to clean water for the control population (60 fish per treatment and 120 fish in total). The WwTW received 95% influent from domestic sources (population equivalent of 117,574) and 5% input from industrial wastewaters. The influent was treated by fine screens, chemically assisted settlement, biological aerated flooded filter processes and ultraviolet disinfection. The pH of the effluent was 7.3, concentrations of suspended solids 21 mg/L, biochemical oxygen demand 11 mg/L, chemical oxygen demand 67 mg/L and total ammonia 29 mg/L during the exposure period (South West Water data). Roach were exposed in triplicate tanks (20 fish per 200 L tank) to either dechlorinated water or 100% final effluent for fifteen days. The flow rate was 10 L/min and the effluent and water was continually aerated. The fish were maintained at an average temperature of 12 ± 1 °C for both treatments under a constant photo-period (15 h light: 9 h dark).

After 15 days, fish were terminated using an overdose of phenoxyethanol and according to UK Home Office regulations and local ethical guidelines. Blood was collected from the caudal vein using heparinised needles (BD Microlance 3 25G 0.5 × 25 mm), centrifuged (5 min, 10,000 g) and the plasma supernatant stored at −70 °C. Four different parts of the brain, i.e. the telencephalon, the hypothalamus, the optic tectum and the hindbrain were carefully dissected and immediately snap frozen in liquid nitrogen. All samples were stored at −70 °C until analysis.

Wastewater effluent samples (2 × 2.5 L) were collected in solvent washed, acid rinsed amber glass bottles at the beginning of the fish exposure (day 0), after 7 days, and at the end of the exposure period (day 15) in order to monitor the concentrations of neuroactive pharmaceuticals in the wastewater effluent throughout the exposure period. Samples were stored at 4 °C with the addition of acetic acid (1%) and methanol (5%) and were extracted within 12 h after collection.

2.3. Sample preparation

2.3.1. Brain tissues and plasma samples

Telencephalon (mean ± standard deviation; 18 ± 4 mg), hypothalamus (15 ± 3 mg), hindbrain (56 ± 8 mg) and optic tectum (80 ±