Water Research 124 (2017) 654–662

Contents lists available at ScienceDirect

Water Research

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

Bioaccumulation of psychoactive pharmaceuticals in fish in an effluent dominated stream

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ARTICLE INFO

Article history:
Received 11 April 2017
Received in revised form
4 August 2017
Accepted 7 August 2017
Available online 8 August 2017

Keywords:
Brown trout
Contamination
Sewage water
Antidepressant
Real exposure

ABSTRACT

The treated effluent from sewage treatment plants (STP) is a major source of active pharmaceutical ingredients (APIs) that enter the aquatic environment. Bioaccumulation of 11 selected psychoactive pharmaceuticals (citalopram, clomipramine, haloperidol, hydroxyzine, levomepromazine, mianserin, mirtazapine, paroxetine, sertraline, tramadol and venlafaxine) was examined in Zivny Stream (tributary of the Blanice River, the Czech Republic), which is a small stream highly affected by effluent from the Prachatice STP. Six of the 11 pharmaceuticals were detected in grab water samples and in passive samplers. All pharmaceuticals were found in fish exposed to the stream for a defined time. The organs with highest presence of the selected pharmaceuticals were the liver and kidney; whereas only one pharmaceutical (sertraline) was detected in the brain of exposed fish. Fish plasma and muscle samples were not adequate in revealing exposure because the number of hits was much lower than that in the liver or kidney. Using the criterion of a bioaccumulation factor (BAF) $\geq$ 500, citalopram, mianserin, mirtazapine and sertraline could be classified as potential bioaccumulative compounds. In combination, data from integrative passive samplers and fish liver or kidney tissue samples were complimentary in detection of target compounds and simultaneously helped to distinguish between bioconcentration and bioaccumulation.

1. Introduction

Treated effluents from municipal sewage treatment plants (STPs) are important sources of aquatic environmental pollution (Gabet-Giraud et al., 2014; Golovko et al., 2014; Grabicova et al., 2015; Kosma et al., 2014; Roberts et al., 2016; Schultz et al., 2010; Verlicchi et al., 2012). Therefore, aquatic organisms living in streams affected by the effluent of STPs are exposed throughout their lives to mixtures of various compounds such as pharmaceuticals and personal care products (PPCPs) and other chemicals. PPCPs can affect the physiological functions of exposed aquatic organisms (Grabicova et al., 2013; Li et al., 2011; Mehinto et al., 2010; Sanchez et al., 2011; Steinbach et al., 2016). Recently, the ecological effects of pharmaceuticals have received increased attention, i.e., psychoactive drugs that can alter behaviours of aquatic organisms (Brandao et al., 2013; Brodin et al., 2013; De Lange et al., 2006; Huerta et al., 2016; Martin et al., 2017). The uptake of PPCPs from polluted water or via food can lead to bioconcentration/bioaccumulation of these compounds in aquatic organisms (Brooks et al., 2005; Gelsleichter and Szabo, 2013; Lajeunesse et al., 2011; Verlicchi et al., 2012; Zenker et al., 2014). The distribution of psychoactive pharmaceuticals varies among fish organs, with the highest bioconcentration/bioaccumulation factor found for organs with high lipid content: for the liver (as a primary metabolic organ) and the brain (as a target organ for some modes of action of psychoactive compounds; Grabicova et al., 2014). However, the exposure pathway through food webs could be important for some pharmaceuticals because these compounds could also bioaccumulate in lower levels of food chains (Grabicova et al., 2015).

Passive sampling is a relatively new method that can mimic bioconcentration uptake without the use of live organisms (Alvarez et al., 2004; Kot et al., 2000). The Polar Organic Chemical Integrative

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http://dx.doi.org/10.1016/j.watres.2017.08.018
0043-1354/

Sampler (POCIS) was developed for passive sampling of wide range of polar compounds including pharmaceuticals. By applying the POCIS approach, time average concentration of water-soluble pharmaceuticals can be achieved with its better representativeness and detection limits than that for grab water samples. The comparison of concentrations obtained from passive samplers and those in biota could be a useful tool in estimating the importance of bioconcentration and bioaccumulation of studied compounds.

The aim of this study was to investigate the bioaccumulation of selected psychoactive pharmaceuticals (citalopram, clomipramine, haloperidol, hydroxyzine, levomepromazine, mianserin, mirtazapine, paroxetine, sertraline, tramadol and venlafaxine) in fish exposed in the natural conditions of a stream affected by the effluent of a STP for defined time periods: 1, 3 and 6 months. Only active pharmaceuticals ingredients were included in this study since these almost always are more potent as well as more lipophilic than the metabolites. This combination of higher potency and higher lipophilicity makes the active pharmaceuticals more relevant starting points in an ecotoxicological context than their metabolites. As a component of this study, the concentrations of these pharmaceuticals in different fish tissues and in passive samplers were compared.

2. Materials and methods

2.1. Experimental design and sampling

Zivny Stream (a tributary of the Blanice River; 13 km long, 30 cm average depth, 3 m average width, flow 0.150–0.600 $m^3\ s^{-1}$) is a small watercourse in the southern part of the Czech Republic in which effluent from the STP at Prachatice contributes about 25% of the total stream flow. A detailed description of the stream and the treatment at the Prachatice STP are given in Grabicova et al. (2015). Two stretches of this stream were chosen for the experiment, which was conducted from October 2012 to April 2013. The first stretch (control, unpolluted site, site C) was 3–5 km upstream from the STP Prachatice, where only three small villages with max. 100 inhabitants and few summer cottages are located; and the second stretch (polluted site, site D) was 0.1–3 km downstream of the location at which the STP effluent enters the stream. The experimental stretches are separated by weirs, which prevent upstream fish migration. The same fish migration barriers are situated in the lower part of stretch D, separating Zivny Stream and the Blanice River, to prevent the back migration of escaped experimental fish monitored in stretch D. The sampling stretches are shown on a map (Fig. 1).

Brown trout (*Salmo trutta* m. *fario*; average total length 220 ± 20 mm, average weight 99 ± 33 g), which dominate to fish community in the stream, were caught by electrofishing (backpack electrofishing equipment EFKO FEG 1500, EFKO GmbH, Leutkirch im Allgäu, Germany) at the unpolluted site C, tagged by Visible Implant Elastomer tags (VIE; Northwest Marine Technology, Inc.), and immediately restocked into the site D downstream of the Prachatice STP. Trout were sampled at 1, 3 and 6 months after the restocking at both sites (C, D). Weight and length of sampled fish are given in SM1. Additionally, fish were sampled at locality D 18 months after the start of the experiment. Fish from each site were caught by electrofishing and transported to the laboratory for collection of blood samples. Then, the fish were sacrificed, and muscle (dorsal part of the fillet), liver, kidney and brain were collected and frozen at −20 °C until the chemical analyses. Blood was separated via centrifugation (Eppendorf centrifuge, 837 × g, 10 min, 4 °C) to obtain plasma samples, which were frozen and stored at −20 °C.

This study was performed in accordance with the principals of the EU-harmonized Animal Welfare Act of the Czech Republic. The unit is licensed (No. 53100/2013-MZE-17214) according to the Czech National Directive (the Law against Animal Cruelty, No. 246/1992).

Grab water samples were collected seven times within two weeks of the POCIS deployment period at the start and the end of experimental reach. Samples were filtered through regenerated cellulose filters (0.45 μm; purchased from Labicom, Olomouc, Czech Republic) and frozen at −20 °C until the analyses. Collected water samples were analysed within 60 days period from the sampling.

Passive samplers, POCIS type Pest (purchased from Nya Exposmeter AB, Tavelsjo, Sweden), were deployed at sites C and D in November 2012, January 2013 and April 2013. The linear integrative period for this sampling device is approximately three weeks (Fedorova et al., 2014b). After three weeks of exposure, the passive samplers were retrieved, cleaned and frozen at −20 °C until the analyses. Time table of the sampling is presented in SM2.

The temperature of the stream differs according to sampling periods. The average temperature was 6.9 ± 2.2 °C for locality C and 9.2 ± 2.1 °C for locality D in October and November 2012 (at the beginning of the experiment and after one months). In January 2013 (three months of exposure), the temperatures decreased to 1.3 ± 0.8 °C for control locality C and 4.9 ± 1.0 °C for locality D. The last sampling of fish was done in April 2013 (six months of exposure), when the temperature increased to 9.8 ± 1.3 and 10.8 ± 1.4 °C for locality C and D, respectively.

2.2. Chemicals

Chemicals and stocks used for chemical analyses are the same as described elsewhere (Grabicova et al., 2015). Briefly, methanol and acetonitrile were purchased from Merck (Germany) and formic acid for acidification from Sigma-Aldrich (Germany); both were LC/MS grade purity. Mass-labelled compounds of amitriptyline (D_6; CDN Isotopes), atenolol (D_6; Alsa Chim), carbamazepine (D_{10}; CDN Isotopes), clarithromycin (D_3; Toronto Research Chemicals), sulfamethoxazole ($^{13}C_6$; Cambridge Isotope Laboratories), and trimethoprim ($^{13}C_3$; Cambridge Isotope Laboratories) were used as internal standards. Individual stock solutions (1 mg mL^{-1}) were prepared from clomipramine hydrochloride, haloperidol, hydroxyzine dihydrochloride, mianserin hydrochloride, mirtazapine, paroxetine hydrochloride hemihydrate and tramadol hydrochloride (all from Sigma-Aldrich), citalopram, sertraline hydrochloride and venlafaxine hydrochloride (all from AK Scientific) and levomepromazine hydrochloride (EPL).

2.3. Water and passive sampler analyses

Water samples were thawed at room temperature. Ten nanograms of internal standards was added to 5 mL of filtered samples. The analyses were performed using a triple stage quadrupole MS/MS TSQ Quantum Ultra Mass Spectrometer coupled with an Accela 1250 and Accela 600 L C pumps (Thermo Fisher Scientific) according to methods described elsewhere (Fedorova et al., 2014a; Lindberg et al., 2014). The limits of quantification (LOQs) for water samples are given in Table 1.

POCIS extraction was performed according to standardized procedure (Alvarez et al., 2005), with a detail description of extraction and analysis in (Fedorova et al., 2014b). Briefly, the sorbent from POCIS was extracted by mixture of dichlormethane:-methanol:toluene (8:1:1), concentrated to approximately 0.5 mL, diluted with ultrapure water 1:1 and analysed using a Q-Exactive mass spectrometer (Thermo Fisher Scientific) coupled with an Accela 1250 L C pump (Thermo Fisher Scientific) and HTS XT-CTC autosampler (CTC Analytics AG). The LOQs for pharmaceuticals in