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Occurrence of antidepressant residues in the sewage-impacted Vistula and Utrata rivers and in tap water in Warsaw (Poland)

Joanna Giebułtowicz [a,*], Grzegorz Nałęcz-Jawecki [b]

[a] Department of Bioanalysis and Drugs Analysis, Faculty of Pharmacy, Medical University of Warsaw, 1 Banacha Street, Warsaw 02097, Poland
[b] Department of Environmental Health Sciences, Faculty of Pharmacy, Medical University of Warsaw, 1 Banacha Street, Warsaw 02097, Poland

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ABSTRACT

Antidepressants, even at low concentrations, can reveal some adverse effects on aquatic life due to disturbing homeostasis throughout the central and peripheral nervous system both in vertebrates and invertebrates. To date there have not been any reports regarding the presence of these pharmaceuticals in surface and tap waters in Eastern Europe. Therefore the aim of this study was to determine the presence of 21 antidepressant pharmaceuticals at specific points of the main Polish river – the Vistula, a smaller river of the Warsaw region – the Utrata, as well as in tap water samples of Warsaw. Samples were collected twice at one month intervals and analysed using solid-phase extraction (SPE) technique coupled with the liquid chromatography–electrospray ionisation–tandem mass spectrometer (LC–MS/MS) method operated under the multiple reaction monitoring mode (MRM).

This is the first study where active compounds such as moclobemid or trazodone in the environment have been investigated.

Environmental risk assessment of antidepressants in Poland was estimated on the basis of annuals sale data extracted from the NFZ (Narodowy Fundusz Zdrowia—National Health Service) base of reimbursed pharmaceuticals[1]. Predicted environmental concentration (PEC) of target pharmaceuticals were compared with their measured concentration (MEC).

Moreover, the application of the EMEA/CHMP guideline for environmental risk assessment of the antidepressants was discussed. The highest concentration of antidepressants was observed in the small river Utrata. In tap water only trace amounts of antidepressants including citalopram (up to 1.5 ng/l), mianserin (up to 0.9 ng/l), sertraline (<3.1 ng/l), moclobemid (up to 0.3 ng/l) and venlafaxine (up to 1.9 ng/l) were detected. However this highlights their inadequate elimination in the drinking waste treatment facility. The presence of antidepressants in drinking water and the aquatic environment could have long-term effects even at low exposure level, especially since synergy amongst pharmaceutical pollutants may occur.

1. Introduction

Antidepressants are frequently prescribed pharmaceuticals. The latest Eurobarometer on mental health (2010) reported that 6 percent of Polish and 7 percent of European citizens were treated for mental disorders with antidepressants in 2009. About 17 percent took antidepressants regularly for less than four weeks, 33 percent in excess of four weeks, and 50 percent from time to time, when they felt the need. Recently, increasing numbers of people suffer from mental disorders. The Global Burden of Disease Study identified depression as the second most serious illness and the leading cause of disability throughout the world in 2010 (Ferrari et al., 2013).

Selective serotonin reuptake inhibitors (SSRIs) are the first-line treatment for depression. Among SSRIs (Anatomical Therapeutic Chemical (ATC) classification system code: N06AB) available in the market in Poland are: fluoxetine, citalopram, paroxetine, sertraline, fluvoxamine and escitalopram. Other groups of antidepressants include non-selective monoamine reuptake inhibitors (N06AA)—amitriptyline, clomipramine, opipramol, doxepin and maprotiline, monoamine oxidase A inhibitors (N06AG)—moclobemide and non-classified other antidepressants (N06AX) like mianserin, trazodone, mirtazepine, bupropion, tianeptine, milnacipran, reboxetine, agomelatine and serotonin-norepinephrine reuptake inhibitors (SNRIs)—duloxetine and venlafaxine.

Significant antidepressant usage among both humans (due to depression, anxiety and chronic pain) and animals (in treatment of

* Corresponding author.
E-mail addresses: joanna.giebultowicz@wum.edu.pl (J. Giebułtowicz), grzegorz.nalecz@wum.edu.pl (G. Nałęcz-Jawecki).

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obsessive, compulsive and fearful behaviour or separation anxiety) results in the prevalence of pharmaceuticals in the environment. Since conventional activated sludge treatments reveal only moderate potential to remove antidepressants, the discharge of municipal wastewater effluent is a primary route of introducing them to the aquatic environment (Lajeunesse et al., 2012). Pharmaceuticals enter wastewater treatment plants (WWTPs) by wastewater from the disposal of unused or expired drugs in toilets as well as human excretion. Human excretion is considered to be the primary source of the pharmaceuticals in the environment. Even though antidepressants are excreted mainly as metabolites (biologically active or not), part of the administrated dose remains unchanged. The percentage of the parent compound in urine depends on the pharmaceutical, e.g. citalopram is excreted unchanged in approximately 12–20 percent, whereas fluoxetine in 10 percent, paroxetine 3 percent and sertraline 0.2 percent (Hiemke and Härtter, 2000; Silva et al., 2012). Besides phase I of metabolism which include the most common modification–hydroxylation and desmethylation, the conjugation of pharmaceuticals (modified or not) also occurs. Therefore another source of the parent drug in effluent is deconjugation of pharmaceutical conjugates in WWTPs (Ternes, 1998).

Antidepressants even at low concentrations at micrograms per litre or even nanograms per litre can reveal some adverse effect on aquatic life. The pharmaceuticals modify the neurotransmitter regulation, such as serotonin, norepinephrine and dopamine and disturb homeostasis throughout the central and peripheral nervous system both in vertebrates and invertebrates. Psychiatric pharmaceuticals are one of the most toxic drugs to aquatic organisms. A large number of studies are focused on the biological activity of selective serotonin reuptake inhibitors, especially fluoxetine. Serotonine is an important neurotransmitter in hormonal and neuronal mechanisms (Santos et al., 2010). Because of their serotonergic action, SSRIs can influence the reproductive behaviour of invertebrates and vertebrates. The compounds negatively affected *Ceriodaphnia dubia* reproduction by reducing the number of neonates per female, with NOEC of 9 μg/l, 89 μg/l and 220 μg/l for sertraline, fluoxetine and paroxetine, respectively (Henry et al., 2004). Similar toxicity of sertraline to *Daphnia magna* reported Minagh et al. (2009) with NOEC=32 μg/l. Nentwig (2007) noticed reduced reproduction of freshwater mudsnail *Potamopyrgus antipodarum* at concentrations of fluoxetine as low as 0.81 μg/l. Fluvoxamine at a concentration of 0.32 μg/l is capable of inducing spawning of zebra mussels (*Dreissena polymorpha*) (Calisto and Esteves, 2009). The acute toxicity of the pharmaceuticals is lower than the chronic toxicity, but still high. Survival test with *D. magna* and *Pimephales promelas* revealed the LOEC values of 170 μg/l and 101 μg/l, respectively (Calisto and Esteves, 2009). The effects of SSRIs on the growth inhibition of algae depended on the species. *Pseudokirchneriella subcapitata* was affected by fluoxetine (48 h-EC_{50}=24 μg/l) and sertraline (EC_{50}=12.1 μg/l) (Santos et al., 2010), while *Desmodesmus subspicatus* was 10 fold more sensitive for fluoxetine with EC_{50} value of 2.1 μg/l (Oakes et al., 2010). Venlafaxine is a relatively novel antidepressant and little is known about its toxicity to freshwater organisms. The antidepressant affected fathead minnow's predatory avoidance at a concentration of 0.5 μg/l (Painter et al., 2009) and is toxic to plants at chronic exposure of 0.1 μg/l (Feito et al., 2013).

The aim of this study was to determine the presence of 21 antidepressant compounds at specific points of the main river in Poland – the Vistula, a smaller river of the Warsaw region – Utrata as well as in tap water samples of Warsaw. All the aforementioned antidepressants from N06AA, N06AB and N06AB as well as about half from the N06AX group were studied. Additionally the presence of some non-selective monoamine reuptake inhibitors formerly often used were also determined: desipramine, imipramine, trimipramine, nortriptyline and protriptyline.

To the best of our knowledge, this work is the first one assessing contamination levels of antidepressants in Eastern Europe surface and tap water. Moreover, for the first time the presence of such antidepressants as e.g. moclobemid or trazodone in the environment was investigated.

2. Materials and methods

2.1. Chemicals

All the pharmaceutical standards for target compounds were of high purity grade (> 98 percent). Doxepin, fluoxetine, fluvoxamine, desipramine, imipramine, maprotyline, trimipramine, amitriptyline, nortriptyline, protriptyline and clomipramine were purchased from Sigma–Aldrich (Germany). Citalopram, mianserin, mirtazapine, moclobemid, opipramol, paroxetine, sertraline, tianeptine, trazodone, venlafaxine were a gift from the Drug Research Institute in Warsaw, Poland.

Fluoxetine-D5 (internal standard) was supplied by Sigma–Aldrich (Germany).

The solvents, HPLC gradient grade methanol (LiChrosolv), acetonitrile hypergrade for LC–MS (LiChrosolv) and formic acid 98 percent were provided by Merck (Darmstadt). Ultrapure water was obtained from a Millipore water purification system (Milli-Q water). The cartridges used for solid-phase extraction were Oasis HLB (400 mg, 3 ml) from Waters Corporation (Milford, MA, U.S.). The individual standard stock solutions as well as isotopically labelled internal standard solution were prepared on a weight basis in methanol at a concentration of 1 mg/ml and stored at −20 °C.

Nitrogen used for drying from Multax was of 99.999 percent purity.

2.2. Sampling sites and sample collection

River waters upstream (*V*1, *U*1) and downstream (*V*2, *V*3, *U*3) of two wastewater treatment plants (WWTP) as well as tap waters were monitored. In Warsaw points of water supply and discharge from WWTP are located in relatively close proximity to each other, the point of the discharge of the effluents is 0.5 km downstream from the *V*1, which corresponds to the *V*2 water sampling point. The points *V*3 and *U*3 are the points located 0.5 km downstream from the WWTPs, therefore at those points effluent should be completely mixed with river water of the Vistula and Utrata, respectively. The characteristics of the WWTP studied as well as the detailed description of water collection sites are presented in Tables 1 and 2, respectively.

Samples were collected twice on 18th July 2013 (1°) and 7th August 3013 (2°) in 1 l glass bottles. Once collected, samples were kept at 4 °C in darkness until arrival to the laboratory and processed within 12 h.

2.3. Sample preparation

The analyses of antidepressants in the collected samples were performed following a previously developed and validated method based on HPLC–MS/MS (to be published elsewhere).

Briefly, aqueous samples were filtered through glass fibre filters (GF/C, Whatman), and membrane filters (0.2 μm, Sartorius). The quality control (QC) samples were prepared by spiking the Milli-Q water with the analytes. The volumes of filtrate/QC of 500 ml were collected for extraction and spiked with isotopically-labeled fluoxetine (internal standard). Target compounds were extracted by solid-phase extraction (SPE) (Oasis HLB cartridges, 3 cm^3, 400 mg) using a Phenomenex vacuum system (Torrance, CA, U.S.). The three first elutions were performed with pure methanol (3 × 2 ml), while the forth with a mixture of methanol and acetone (1:1, 1 × 2 ml). The eluents were evaporated under a stream of nitrogen at 40 °C and reconstituted in a methanol–water mixture (10:90) (1 ml).

2.4. Instrumental analysis

Instrumental analysis of all samples was done by high performance liquid chromatography coupled to mass spectrometry with hybrid triple quadrupole/

Table 1
WWTPs characteristics.

WWTP	Czajka	Pruszkow
Population equivalent [mln]	2.1	0.16
Average flow [m^3/s]	4.6	0.46
Receiving river water	Vistula	Utrata
Primary treatment	Primary settling	Primary settling
Secondary treatment	Activated sludge	Activated sludge