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Diversity of the molecular responses to separate wastewater effluents in freshwater mussels

Halina I. Falfushynska [a], Lesya L. Gnatyshyna [a], Olesya Y. Osadchuk [a], Anna Farkas [c], Agnes Vehovszky [c], David O. Carpenter [b], Janos Gyori [c,1], Oksana B. Stoliar [a,*,1]

[a] Research Laboratory of Comparative Biochemistry and Molecular Biology, Ternopil National Pedagogical University, M. Kryvonosa Str., 2, Ternopil, 46027, Ukraine
[b] Institute for Health and the Environment, University at Albany, Rensselaer, NY 12144, USA
[c] Balaton Limnological Institute, Centre for Ecological Research, Hungarian Academy of Sciences, Klebelsberg str. 3, Tihany 8237, Hungary

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ABSTRACT

The environmental safety of pharmaceutical and personal care products (PPCPs) requires a crucial examination. The aim of this study was to evaluate the responses of biomarkers of stress and toxicity in freshwater mussels to the effect of commonly found PPCPs in wastewater. We treated male mussels *Unio tumidus*, from an undisturbed site with ibuprofen (IBU, 250 ng L^{-1}), triclosan (TCS, 500 ng L^{-1}), or estrone (E1, 100 ng L^{-1}) for 14 days. Untreated mussels from this site (C) and mussels inhabiting a polluted area (P) were also examined after a similar time of being kept in the laboratory. The consequences of chronic exposure of the mussels in the P-group were reflected in elevated concentrations of oxyradicals (1.4 times), oxidized glutathione (4.3 times), lipofuscin (2.2 times), and DNA-strand breaks in the digestive gland (DG) in comparison to the C-group, higher levels of caspase-3 activity in the DG, and vitellogenin-like proteins in gonads among all studied groups. Exposed mussels demonstrated some common responses with mussels in the P-group: elevated levels of lactate/pyruvate ratio, lipofuscin (IBU and E1), DNA fragmentation (TCS and E1), and caspase-3 activity (TCS and E1). Exposed to PPCPs mussels also showed elevation of ethoxyresorufin-*O*-deethylase and/or glutathione-*S*-transferase activity in the DG and a decrease in lysosomal stability in hemocytes (TCS and E1). The TCS group was distinguished by having the highest level of DNA-fragmentation and the lowest concentrations of total glutathione, oxyradicals, lipofuscin, pyruvate, and lactate, reflecting total metabolic depression. These results show that selected PPCPs at low concentrations alter a variety of physiological processes in this animal model system.

1. Introduction

Pharmaceuticals and personal care products (PPCPs) are substances of concern. Even modern wastewater treatment plants are generally not very effective in removing these compounds, and as a result, PPCPs are usually discharged with effluent into receiving waters. The situation is particularly serious in Central and Eastern Europe, including Ukraine. Small, widely scattered villages of fewer than 2,000 people constitute almost 30% of the total population in these countries. This amounts to over 40 million people who utilize PPCPs in large amounts but usually are deprived of communal services (http://www.gwp.org/en/GWP-CEE/about). Urban wastewater stations frequently are old-fashioned and decaying due to the problems associated with the economic transition, and their facilities are overwhelmed. According to the European Environmental Agency in 2003 (http://www.ooskanews.com), wastewater treatment is a major problem in Ukraine due to poor quality and inefficiency of wastewater and sludge treatment as a result of technical deficiencies, poor maintenance and overload of existing installations.

While most PPCPs are not persistent pollutants, they are continually introduced to surface waters from human wastes at low (nM) concentrations (Wagner and Oehlmann, 2009). Nevertheless, evaluation of PPCP safety is frequently based on the assessment of their acute toxicity at μM and even mM concentrations (Orvos et al., 2002; Heckmann et al., 2007; Pounds et al., 2008). Recent research has demonstrated the toxicity of PPCPs. Some PPCPs cause significant elevation of stress markers, acting primarily through oxidation pathways (Quinn et al., 2011). Others alter the physical state of lipid bilayers and alter membrane transport and ion permeability (Cherednichenko et al., 2012). The action of greatest concern is that PPCPs are suspected to be “endocrine disruptors” (ED) (Gagné and Blaise, 2003; Janer et al., 2005; Oehlmann et al., 2007; Segner, 2009; Jonkers et al., 2010). Unfortunately in Ukraine, data concerning the effects of PPCPs and particularly EDs in surface waters are absent, except of some sporadic signs of toxicity in the indigenous aquatic animals from urban and rural areas (Unionid mollusks, Cyprinid fish, and ranid amphibians) (Falfushinska et al., 2008; Falfushynska et al., 2011, 2012.

* Corresponding author. Tel.: +380 352 52 24 48; fax: +380 352 436055.
E-mail address: Oksana.Stolyar@gmail.com (O.B. Stoliar).
[1] The authors contributed equally in this work.

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Bivalve mollusks have proven to be suitable sentinel organisms to study aquatic toxicity due to their sedentary nature, filter-feeding behavior, and ability to accumulate pollutants (including persistent organic pollutants) (Hayer and Pihan, 1996; Viarengo et al., 2007). They respond with markers of general toxicity, such as oxidative stress, genotoxicity, cytotoxicity, and metabolic arrest (Viarengo et al., 2007) and are sensitive to certain wastewater substances (Gagné et al., 2004; Binelli et al., 2006; Falfushynska et al., 2009, 2010). They show responses in detoxifying enzymes ethoxyresorufin-*O*-deethylase (EROD) and glutathione-S-transferase (GST) and elevate levels of vitellogenin-like proteins (Vtg-LP) in gonads of males in response to xenoestrogens (Gagné et al., 2004; Binelli et al., 2006). The study of caspase-3 activity in mollusks is of particular interest due to the high activity of apoptosis in bivalves (Romero et al., 2011).

The aim of this study was to evaluate the responses of molecular biomarkers of stress and toxicity in freshwater mussels to the effect of typical PPCPs at concentrations typically found in the environment in comparison with the responses of mussels inhabiting a chronically polluted environment. Substances selected for this study are PPCPs of growing concern to human and environmental health (Jonkers et al., 2010). The anti-inflammatory agent ibuprofen (IBU) has been detected in surface waters worldwide (e.g. 345 t in Germany in 2001) (Fent et al., 2006), especially at sites very close to the outflow of wastewater treatment plants, with concentrations ranging up to 85000 $ng \cdot L^{-1}$ (Blaise et al., 2006). The synthetic antimicrobial agent, triclosan (TCS), has been incorporated into more than 700 different industrial and personal care products. These products, including deodorants, soaps, toothpastes, and various plastic products, contain 0.1–0.3% TCS and are discharged into wastewater (Dann and Hontela, 2011). TCS is among the top 10 most commonly detected organic wastewater compounds and has been detected in 56.8% of surface water samples with a median concentration of about 50 $ng \cdot L^{-1}$ (Brausch and Rand, 2011). TCS level in the Tamiraparani River in India reached as high as 3800–5160 $ng \cdot L^{-1}$ at two sites (Ramaswamy et al., 2011). According to the information of Environment Canada, concentrations of triclosan in the effluent of certain Wastewater Treatment Plants were as high as 315 – 411 $ng \cdot L^{-1}$ (http://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=6EF68BEC-1). Native estrogen, estrone (E1), had been reported at concentrations of 74.2 $ng \cdot L^{-1}$ even though the mean elimination efficiency of wastewater treatment plants is about 83.2% (Zhou et al., 2011).

2. Materials and methods

2.1. Experimental groups

Specimens of bivalve mollusks *Unio tumidus* (8 ± 1 cm length, 42 ± 5 g mass, about 6 years old) were collected at depths from 0.5 to 1 m at two sites in the basin of Dnister River (Western part of Ukraine) in early autumn (September) (Fig. 1). The forestry site is located in the upstream portion of the Seret River (49°49′ N, 25°23′ E) where no industrial contamination was expected. Animals from this site were used as referent controls. Another site (P) is situated in the lower portion of the Nichlava River near the boroughs of the city of Borshchiv (48°48′N, 26°00′E). This river receives effluents from the region with intense agricultural activity and also from the city, which has no wastewater treatment plant. The Public Administration of the Environmental Protection in the Ternopil region (personal communication) confirmed that this site appeared to be the most polluted site in the region. However, the range of indexes determined is limited and official information is lacking. Our recent studies have shown that the levels of nitrites (1.4 ± 0.1 mg $NO_2^- \cdot L^{-1}$), phosphates (24.5 ± 2.5 mM), NH_4^+ (2.8 ± 0.3 $mg \cdot L^{-1}$), Cu (6.3 ± 0.6 $mg \cdot L^{-1}$), and phenol (4.9 ± 0.5 $mg \cdot L{-}1$) here are above the acceptable limits for human consumption (Falfushynska et al., 2010).

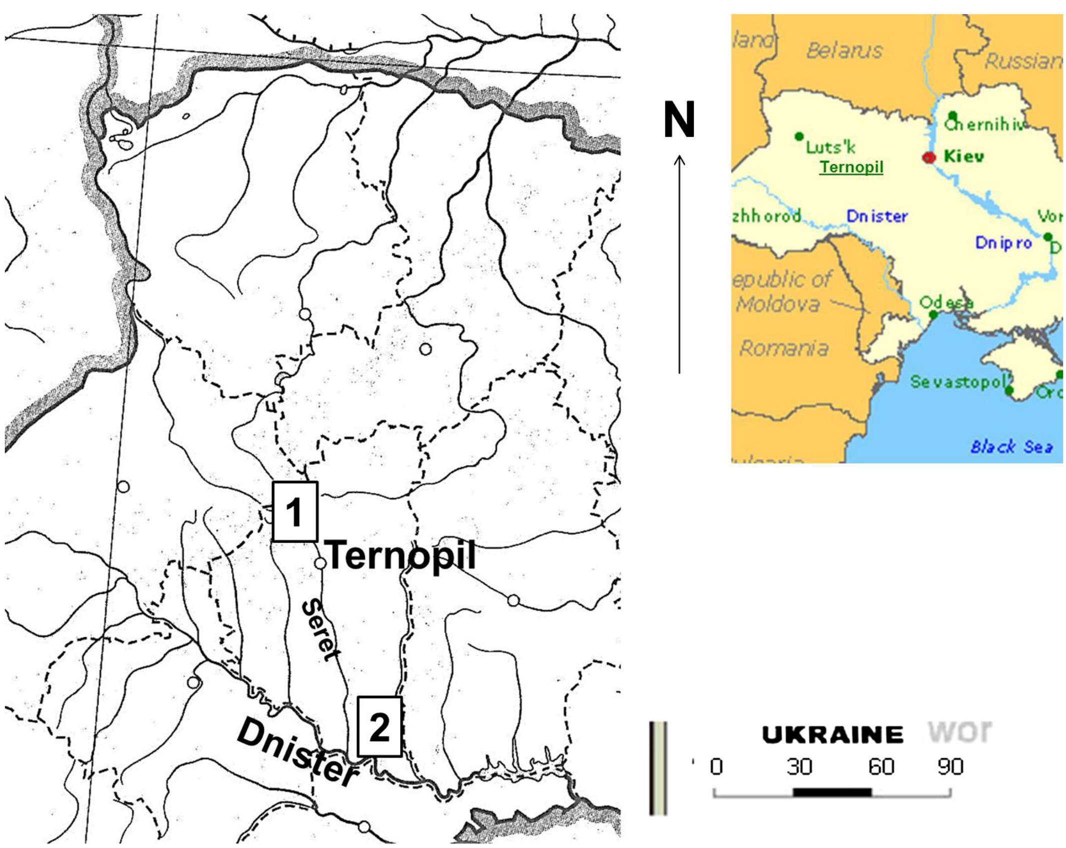

Fig. 1. Map of locations where samples were collected. The map from http://worldmap.org.ua/Pages/Europe/Ukraine/Map_of_Ukraine_eng_0022.html was utilized. 1, forestry (control) site; 2, polluted site. (For interpretation of the references to colour in this figure, the reader is referred to the web version of this article.)