

## PHENOLIC ENDOCRINE DISRUPTING COMPOUNDS IN URBAN RIVERS: MONITORING AND APPLICATION AS WASTEWATER TRACERS

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**Abstract** The research focused on a monitoring of phenolic endocrine disrupting compounds (alkylphenols and bisphenol A) in urban rivers of the Kharkiv region (Ukraine). The Lopan and Udy rivers have been sampled using POCIS passive sampling devices for the detection of degradation products of phenolic compounds and carbamazepine as a tracer of wastewater discharges. Principle component analysis was applied for the identification of the correlation between endocrine disruptors, wastewater tracer and general environmental parameters. Regional patterns of the occurrence and distribution of endocrine disruptors in urban rivers were discussed in the relation to industrial, domestic inputs and potential diffuse sources. The work discusses the perspectives of the application of passive sampling technique for the monitoring of time-varying organic pollutants. As the results, the POCIS-measured concentrations of alkylphenols were at maximum levels downstream of wastewater influences. Trace amounts of compounds were detected in sites upstream of wastewater discharges, i.e. in transboundary, rural and urban areas of the Kharkiv region, indicating additional inputs of alkylphenols and bisphenol A from non reported sources. Metabolites ratio of phenolic compounds have been applied to describe the contribution of potential sources of endocrine disruptors and to assess the wastewater treatment efficiency in the Kharkiv region. Ukrainian rivers were found significantly contaminated by the targeted phenolic endocrine disrupting compounds that can be explained by insufficient natural dilution of wastewaters, inefficient treatment processes at sewage plants and, possibly, inputs from uncontrolled sources.

**Keywords** phenolic endocrine disrupting compounds; urban waters; monitoring; Kharkiv; Ukraine

### INTRODUCTION

Endocrine disruptors are chemicals having a potential effect on the reproductive system of aquatic organisms and humans, resulting in hormonal system disturbance and formation of abnormalities (Cailleaud et al. 2011). Phenolic compounds, as alkylphenol polyethoxylates (AKP) and bisphenol A (BPA), are one of the growing concern endocrine disruptors, because of a high persistence, potential toxicological effects (Harman et al. 2008) and domination in wastewaters (Writer et al. 2010). Around 55% of world used AKP are consumed for industrial need and about 15% of AKP are expanded in household applications as nonionic surfactants in cleaning agents, disinfectants and pesticides formulations (Staples et al. 2001; Cailleaud et al. 2011). AKP are unstable in the aquatic environment, oxidizing to short-chain nonylphenol ethoxy-acetic acid (NP1EC), nonylphenoxy-acetic acid (NP2EC) and nonylphenols (NP) in aerobic conditions and hydrolyzing into various short chain metabolites as nonylphenol monoethoxylate (NP1EO) and nonylphenol diethoxylate (NP2EO) in anaerobic one (Cailleaud et al. 2007). Metabolites of NP and OP are known to exhibit high toxicity and able to mimic estrogens in the hormonal regulations (Cailleaud et al. 2007). Bisphenol A (BPA) is a monomer used in the production of polycarbonates and epoxy resins from

which a variety of products are generated and it is known by estrogenic properties, histopathological changes and multigenerational toxicity in aquatic organisms (Staples et al. 2011). European Union considers AKP as hazardous compounds that should be monitored and controlled in waters (EU 2008). But in some EU - neighboring countries, sharing transboundary water resources, i.e. Ukraine, Russia and Belarus, the information on phenolic endocrine disruptors in the environmental is very limited.

Taking into account the lack of regional studies on the distribution of phenolic compounds, but also the previous positive experience of the passive sampling application (Vystavna et al. 2012a,b), we focused our research on: (i) the investigation of phenolic endocrine disruptors in urban rivers of Eastern Ukraine using the time integrative passive sampling approach and (ii) the function of phenolic metabolites ratio as a tracer of wastewaters.

## METHODS AND MATERIALS

### Study area and sampling strategy

Two urban rivers, which are located in East Ukraine (Vystavna et al. 2012a,b) have been selected for this research. The study included 8 sites (Figure 1) on Udy and Lopan Rivers, which are parts of the Seversky Donets watershed (Vystavna et al. 2012a,b). The site selection was based on the proximity to the transboundary (Russia/Ukraine) areas (U01; L01), entrance in the Kharkiv city (U04; L03), upstream (U06; L08) and downstream (U07; L09) of mixed (domestic and industrial)

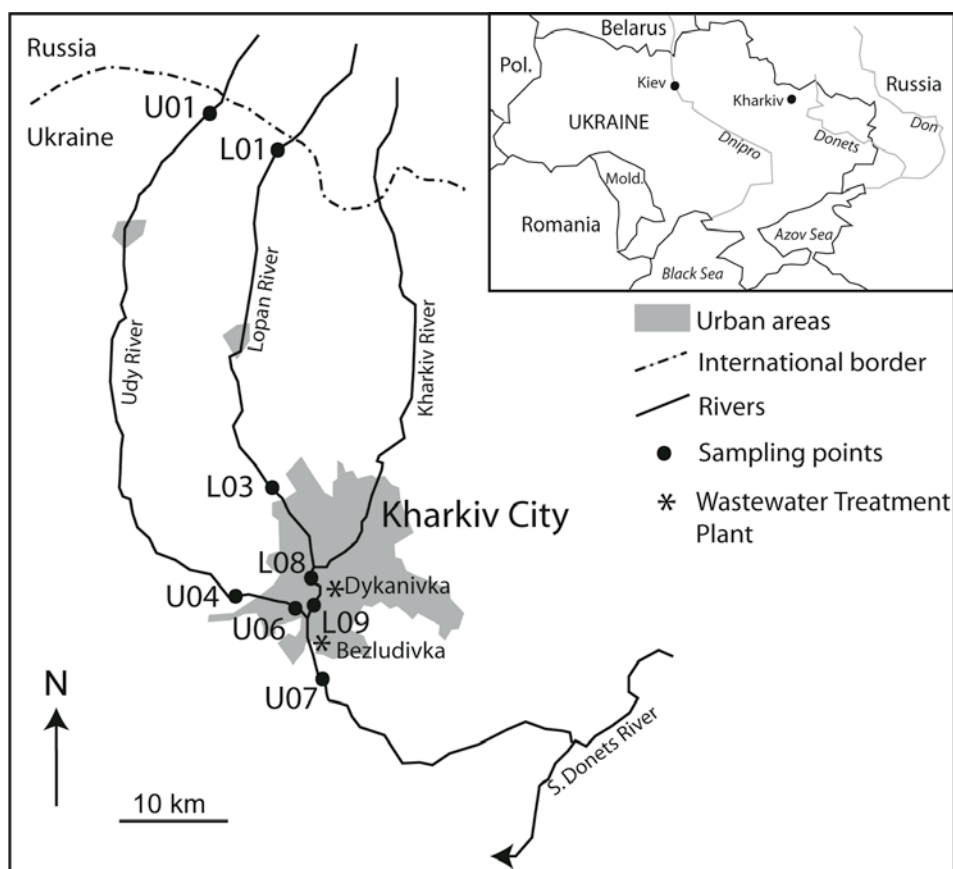


Figure 1. Location of the sampling sites

wastewaters discharges from 'Dykanivskiy' (c.a. 600,000 m<sup>3</sup>d<sup>-1</sup>) and 'Bezludivskiy' (c.a. 150,000 m<sup>3</sup>d<sup>-1</sup>) wastewater treatment plants (WWTP) (serve c.a. 1,000,000 inhabitants) (Suchkova et al. 2010; Vystavna et al. 2012a,b). The monitoring of Ukrainian rivers comprised 6 sampling campaigns in May, August and December, 2009 - 2010. WWTPs includes mechanical pre-treatment stage, primary and biological treatment with a following sludge rotation (Suchkova et al. 2010).

At the selected sites, replicated (n=2) POCIS - passive sampling devices have been fixed using a cotton net for the monitoring of phenolic compounds and wastewater tracer carbamazepine in studied watercourses (Vystavna et al. 2012a). The water temperature, pH and conductivity were measured with a WTW © Multiline P4 meter in the field before the installation and after the retrieval of the passive sampling devices. Water samples for chemical analysis of major ions were filtered in the field and stored in plastic polyethylene bottles before being analyzed by ion chromatography.

### Extraction and analysis

POCIS - pharmaceutical configuration with the Oasis HLB sorbent (Miege et al. 2011), exposure diameter of 54 mm and a sampling surface area of 45.8 cm, were purchased from Expometer (Tavelsjö, Sweden). After 3 weeks of exposure, each individual POCIS device was retrieved from the river, briefly rinsed with ultrapure water in order to remove any materials adhering to the membrane surface (biofilm, particles, etc.).

Extraction procedures for POCIS were adapted and validated (Vystavna et al. 2012a) from previously developed methods (Miege et al. 2011; Tapie et al. 2011). The POCIS sorbent was eluted using the following solution: methanol; methanol/dichloromethane mixture (50:50) and dichloromethane, and being spiked with internal standards (Miege et al. 2011). Extracts were finally evaporated to dryness using a nitrogen flux and transferred via methanol for phenolic compounds and acetonitrile for carbamazepine. Blanks (n=3) were performed in the laboratory concurrently with water samples. Degradation products of AKP: 4-tert-octylphenol (4OP), 4-n-nonylphenol (4NP), nonylphenol-ethoxy acetic acid (NP1EC), nonylphenol monoethoxylate (NP1EO) and diethoxylate (NP2EO), bisphenol A (BPA) and carbamazepine (CBZ), have been analyzed by LC-MS/MS. The residual standard deviation (RSD) of the spikes (n=3) values was in the range from 5 to 17 %. Blank levels were lower than the limit of quantification (1 ng per g of the OASIS sorbent).

### Determination of the concentration

Time-weighted average environmental concentrations of targeted phenolic compounds and CBZ have been estimated using the following equation (Tapie et al. 2011; Vystavna et al. 2012a) (Eq.1):

$$C_w = C_s M_s / (R_s t) \quad (1)$$

where  $C_w$  and  $C_s$  are concentrations of compounds in the water (ng L<sup>-1</sup>) and in the POCIS (ng g<sup>-1</sup>) respectively;  $M_s$  is the mass of the sorbent in the POCIS (g);  $t$  is the sampling period (days) and  $R_s$  is the sampling rate (L d<sup>-1</sup>).

We applied the sampling rates (Table 1) previously determined by Miege et al. (2011), considering the similarity of the devices configuration, flowing conditions, installation period and the water temperature variation.

**Table 1.** The sampling rates of phenolic compounds (PC)

PC	Sampling rate, Rs, l d <sup>-1</sup>
4OP	0.104
4NP	0.019
NP1EC	0.309
NP1EO	0.010
NP2EO	0.007
BPA	0.014
CBZ	0.29

Temperature dependent differences have not been taken into account (Vystavna et al. 2012a).

### The principle component analysis

The principle component analysis (PCA) has been applied for the estimation of the linear correlation between routine environmental parameters (TOC, pH, conductivity, HCO<sub>3</sub>, SO<sub>4</sub> and NO<sub>3</sub>), phenolic compounds (value is in ng of the contaminant per g of the OASIS sorbent of the sampler) and wastewater tracer - carbamazepine. The PCA allows us to describe the correlation between sampled fractions of organic compounds and other environmental parameters and find similar and divers patterns of their distribution in the Udy River (n=84 variables). On a representing PCA plot, the first axis (F1 and F2) describes the most significant correlation, the maximum attention is given to the point close to the circle centered at (0; 0) and whose radius is equal to 1. Points, which are close one to another, indicate the existence of a linear positive correlation between parameters and the case of the radical opposition between points shows the negative correlation of elements. Two points located on perpendicular diameters indicates an independency between values.

## RESULTS

### Accumulation

Phenolic compounds have been detected in two studied rivers, with the sum of mean values is up to 10 µg per g of the OASIS sorbent. The highest level is for NP1EC (up to 60 µg per g of the OASIS sorbent) and the lowest level for 4OP (up to 0.1 µg per of OASIS sorbent).

Comparison of the accumulation values in our study and previous research revealed that the presence of nonylphenols in Ukrainian rivers are significantly higher (more than in 10 times for some components) than in European waters (Table 2), but close to values detected in the natural water of USA.

**Table 2.** Endocrine disruptors (ED) in POCIS from some regional studies (maximum value, ng/POCIS)

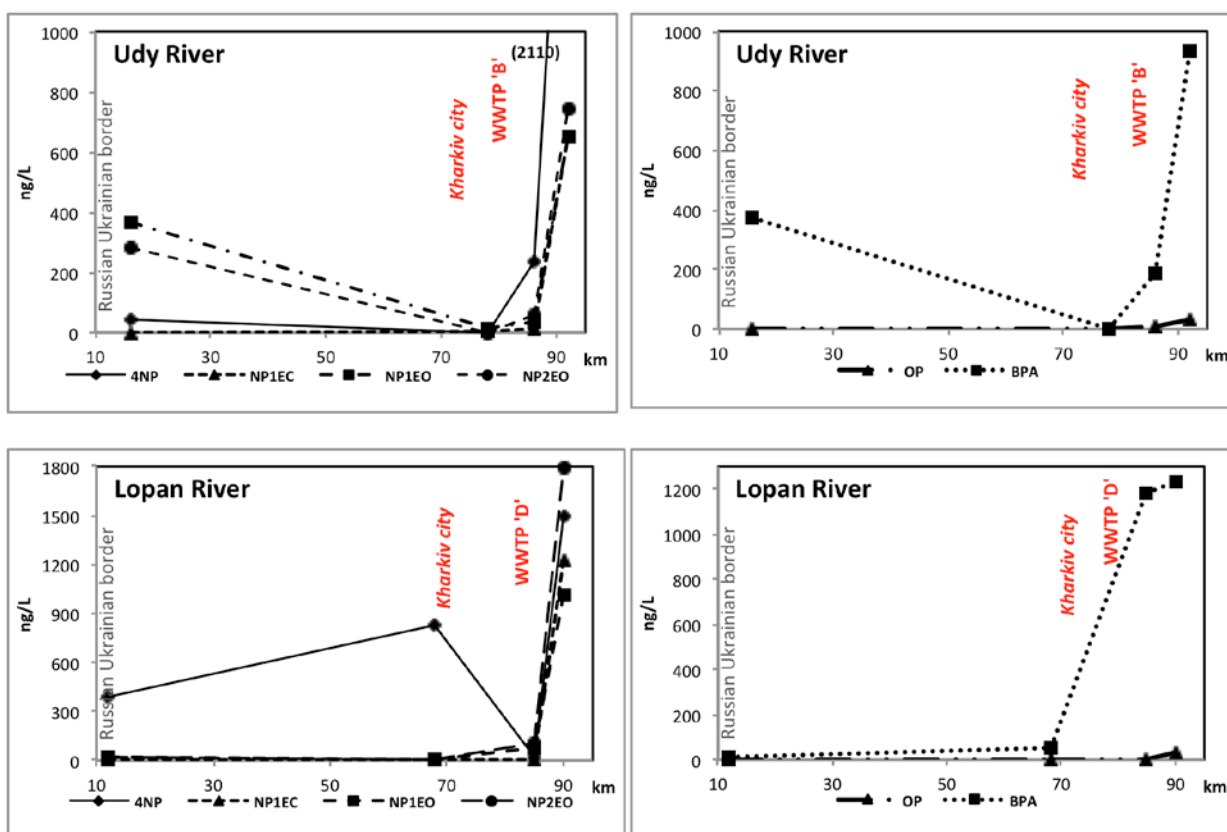
ED	Lopan R. Ukraine (this study)	Axios R. Greece <sup>a</sup>	Liguria, Italy <sup>b</sup>	New Jersey Stream USA <sup>c</sup>
4OP	109	11	nd	70
4NP	1203	119	25	nd
ΣNPEO	621	293	nd	1100
NP1EC	13718	nd	nd	nd
BPA	554	26	926	nd

<sup>a</sup> Arditoglou and Voutsas 2008a; <sup>b</sup> Di Carro et al. 2010; <sup>c</sup> Alvarez et al. 2005; nd – not determined in the study

The difference in the regional contamination can be explained by influences of both environmental (natural dilution and water hydro- and biochemistry) and socio-economic determinants (population density, economic activity, water and wastewater management) (Vystavna et al. 2012a,b).

### Concentration and distribution

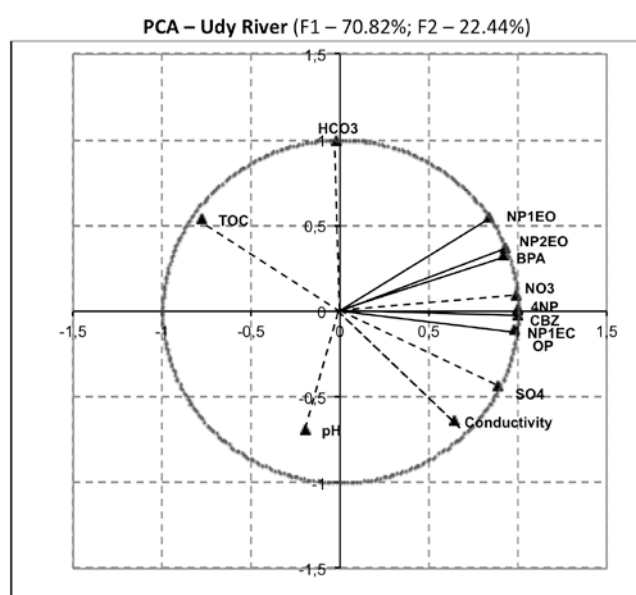
The spatial variation of estimated time –weighted average concentration of phenolic compounds (Eq.1) revealed different patterns in the upstream and downstream sampling sites (Figure 2).



**Figure 2.** The spatial distribution of phenolic compounds concentration (mean values) in water of Udy and Lopan Rivers

Thereby, the following order (by mean concentration in  $\mu\text{g L}^{-1}$ )  $(\text{NP1EO}+\text{NP2EO})0.66 \gg 4\text{NP}0.04 > \text{NP1EC}$  was observed in upstream sites. Here, AKP can be derived from untreated inputs and runoff (site U01) of nearest transboundary settlements. Additionally, the domination of NP1EO under 4NP characterized the influence of anaerobic processes on the degradation of polyethoxylates (Cailleaud et al. 2007) and the potential adsorption of hydrophobic products (4NP and 4OP) on the suspended matters (Xu et al. 2011). In contrast, the next order of phenolic compounds was described at the downstream:  $4\text{NP}2.34 \gg \{(\text{NP1EO}+\text{NP2EO})1.50 \gg \text{NP1EC}0.66\} \gg 4\text{OP}$ , with the growing of 4NP and NP1EC (more than in 50 times compare to upstream parts) from transboundary to urban areas.

Results of the PCA (Figure 3) indicated the strong association between NP1EC ( $r^2=0.99$ ), 4NP ( $r^2=0.99$ ) and wastewater tracer CBZ ( $r^2=0.99$ ) in Udy River.



**Figure 3.** Results of PCA

Additionally, these phenolic compounds are found to be significantly correlated with  $\text{NO}_3$  ( $r^2=0.98$ ), indicating the relation of AKP to municipal discharges. At the downstream, the growing of NP1EC shows the increasing of the influence of aerobic processes on the alkylphenols degradation (Cailleaud et al. 2007). The explanation can be the impact of the aeration of wastewater treatment processes on the pollutants distraction. The peak of 4NP concentration at the entrance of the Lopan River to the Kharkiv city (Figure 2) can be explained by the discharge of uncontrolled domestic and industrial wastewaters from the Derkachy settlement located in the upstream area.

In Lopan and Udy Rivers, the level of BPA is increasing in the urban area and downstream of municipal wastewater treatment inputs. The comparison of determined water concentrations with the available environmental quality standards revealed that 4NP, in sites located in the Kharkiv city, were significantly (up to 7 times) higher (Figure 2) than the value of nonylphenol in surface water ( $0.3 \mu\text{g L}^{-1}$ ) proposed by the European Union (EU 2006). Such situation indicates a potential environmental risk associated with the presence of endocrine disruptor in studied urban rivers of Ukraine.

### Application of phenols metabolites ratio as a tracer of industrial and municipal wastewaters

Phenolic metabolites ratio BPA/4NP have been analyzed as potential tracers of municipal and industrial wastewaters. The selection of these ratio was based on the results of the distribution of targeted compounds in studied rivers (Figure 3) and physical chemical characteristics of molecules. The BPA/4NP ration represents the relation between water soluble, rapidly degradable BPA (half life is about 1.2 to 3.4 days; Froehner et al. 2011) and persistent 4NP metabolites. In our study, the BPA/4NP ratio was significantly higher in upstream sites of Udy and Lopan Rivers, compared to the downstream part (Table 3).

The relation informs about the presence of continuous and untreated sources, what can be associated with the contaminated by phenolic compounds urban run-off (U06; L08) (Arditsoglou and Voutsas 2008) and with other unreported endocrine disruptor's inputs from residential areas (U01 and L03).

**Table 3.** The metabolites ratio of phenolic compounds (by mean concentration)

Sampling site	BPA/4NP
<i>Udy River</i>	
U01	9.3
U04	4NP<LOD
U06	0.8
U07	0.4
<i>Lopan River</i>	
L01	0.0
L03	0.1
L08	51.2
L09	0.8

It is interesting to note that 4NP has a good correlation with the wastewater tracer (Figure 3) – carbamazepine, indicating the potential application of phenolic metabolites as tracers of anthropogenic inputs.

### CONCLUSION

All targeted phenolic compounds were found in studied Ukrainian Rivers. The domination of NP1EO and NP2EO metabolites was observed in upstream sites and 4NP and NP1EC was detected in downstream sites. WWTPs were found to be major sources of the AKP in the rivers. Some phenolic metabolites (NP1EO, NP2EO, 4NP and BPA) were detected in upstream sites of rivers, indicating the existence of additional sources of endocrine disruptors in transboundary, rural and urban areas. The concentration of 4NP in Ukrainian rivers was significantly higher than the environmental quality standards recommended by European Union for NP. Pollution events on rivers have been studied using BPA/4NP metabolites ration. These ratios revealed the presence of untreated inputs in upstream sites and urban area in Kharkiv.

This research is a pilot survey on the presence of the endocrine disruptors in urban water of post-soviet East European countries. It was mainly focused on general patterns of the alkylphenols and bisphenols A in Ukrainian Rivers. The next step of the project will be an extension to the detailed study of the influence of environmental determinants on the passive monitoring of analytes, the comparison of the seasonal patterns of phenolic endocrine disruptors and the environmental risk assessment associated with the presence of pollutants in natural water used for the drinking supply purposes.

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