Seasonal variations of bisphenol A in the Danube River by the municipality of Novi Sad, Serbia

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Abstract: Seasonal variations of bisphenol A (BPA, IUPAC: 4,4′-(propane-2,2-diyldiphenol) were investigated in the Danube River along the Novi Sad bank, Serbia using solid-phase extraction followed by the gas chromatographic mass spectrometric method. The obtained results confirmed the presence of BPA above the limit of quantification (6 ng L⁻¹) in 22 out of 32 the water samples at all eight sampling sites. The BPA concentration varied from <6 to 693 ng L⁻¹. The mean BPA concentration for summer (220 ng L⁻¹) significantly differed from those obtained for autumn (39 ng L⁻¹), winter (6 ng L⁻¹) and spring (41 ng L⁻¹). The risk quotients, expressed as the ratio of the 95th percentile of the measured environmental concentrations for each season and the predicted no-effect concentration were higher than one (>1) for autumn, spring and summer. The high potential risk that is attributed to the elevated summer concentrations is probably the result of increased human activities and weather conditions.

Keywords: bisphenol A; Danube River; endocrine disrupter; GC–MS; surface water.

INTRODUCTION

Bisphenol A (BPA, 4,4′-(propane-2,2-diyl)diphenol) is one of the most investigated and discussed endocrine disrupting compounds (EDCs), and has received much attention as an emerging chemical of concern in order to protect wildlife and human health.¹ Endocrine disrupters (EDs) can interfere with hormonal action and with human cell functions mainly through interaction with specific receptors and can induce numerous adverse health effects. Therefore, the occurrence of BPA in the environment could have potential harmful effects on

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the reproductive system, growth, digestion and metabolic processes of aquatic organisms, such as fish or mussels. Moreover, its effects on the human endocrine system through direct exposure via drinking water (BPA was used in polycarbonate plastic bottles) and its function have been discussed. BPA is also believed to pose a potential risk in the development of various types of cancers.

BPA is an organic compound extensively used as an intermediate in the production of polycarbonate plastics and epoxy resins, unsaturated polyester–styrene resins and flame-retardants. It is used in a broad range of products for the modern consumer society, including reusable baby bottles, toys, food and drink storage containers, protective coatings for metal cans, adhesives and paints, electronic components, personal computers, CDs and DVDs, sports equipment, medical devices, eyeglass lenses, dental sealants and dental bonding agents, etc. More than 3.6 million tons of BPA are synthesized worldwide annually and its production is constantly increasing owing to a growing demand for plastic products.

The first country that recognized the importance and necessity to legislate and regulate the environmental issue of BPA was Canada. Although, the European Commission and the US government have banned BPA use in baby bottles and packaging items for children aged 0–3, BPA is still under discussion to be prioritized for regulation by the European Union and the United States because of its possible adverse impact on human health and the environment. Various declarations and statements have been issued (the Berlaymont declaration, the Ramazzini statement, European Environmental Bureau, World Health Organization, etc.) on this challenging topic, and the debate is still ongoing on how to regulate and reduce the BPA concentrations effectively in consumer products and aquatic systems in order to protect humans and wildlife.

Recent studies confirmed the presence of BPA in different human biological matrices, such as urine, breast milk and hair. The presence of BPA in food, at levels up to 1858 µg kg⁻¹, is associated with the manufacture of various polycarbonate plastic products (packages or food contact materials) or it is leached from the final products as a result of the conducted hydrolysis of polymers at high temperature, or under acid/basic conditions. The main source of the BPA occurrence in the aquatic environment is municipal and industrial wastewater (mainly from chemical plants). BPA concentration in surface water is reported to be between <0.001 and 92 µg L⁻¹. Despite its rapid degradation, favored by aerobic conditions, with a half-life of about 0.5–5 days in the aquatic environment, the constant release into the surface water makes BPA a pseudo-persistent pollutant in the environment.

Although, European legislation set environmental quality standards for priority and certain other pollutants, BPA permitted levels, together with some pharmaceuticals, personal care products, algal toxins and various endocrine disrupters
have not been regulated by any legislative bodies yet because of the insufficient reliable data on their safety or risks.16,19

In the Western Balkan Region, few extensive multinational monitoring programs have been realized in the last decade. First comprehensive information on the occurrence of different emerging contaminants including EDCs along the Danube River was conducted during the Joint Danube Survey 1 (in 2001) and the Joint Danube Survey 2 (in 2007), organized by the International Commission for the Protection of the Danube River (ICPDR). During the Joint Danube Survey 2, 34 organic compounds were screened along the entire course and the highest concentrations of emerging contaminants were recorded around the Budapest area. BPA was detected in only few samples (15 out of 52) in concentration up to 68 ng L\(^{-1}\).20 Moreover, within the EMCO project, some classes of emerging contaminants were determined in municipal and industrial wastewater derived from Bosnia and Hercegovina, Croatia and Serbia.21 This study confirmed the occurrence of BPA in municipal wastewater in the Western Balkan Region and the mean concentration of BPA was 510 ng L\(^{-1}\).21 Besides these findings, monitoring data on water status in Serbia are still scarce and limited in terms of sampling sites and number of compounds.

Recently, screening analyses of the wastewater discharged into the Danube along the Novi Sad municipality were performed and more than 130 chemical contaminants were detected.22 Furthermore, non-target screening analysis of Danube water samples in the area of Belgrade confirmed the presence of a wide range of organic contaminants.23

The primary goal of this study, conducted for the first time, was to examine the occurrence of BPA along the Danube River in the area of Novi Sad (Serbia). The obtained results were also used to evaluate the seasonal variations of this endocrine disrupter and to assess the potential risk of the determined BPA concentrations.

**EXPERIMENTAL**

**Chemicals**

The standard bisphenol A, IUPAC: 2,2-bis-(4-hydroxyphenyl)propane, was obtained from Sigma Aldrich (Deisenhofen, Germany). Methanol, dichloromethane and ethyl acetate were of HPLC grade (LGC Promochem, Wesel, Germany). The silylation agent N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA, IUPAC: trimethylsilyl(1E)-2,2,2-trifluoro-N-trimethylsilylethanimidate) was obtained from Fluka (Sigma Aldrich, Deisenhofen, Germany) and ultra pure water was used. The regenerated cellulose filters (0.45 µm) and cartridges were obtained from Agilent Technologies. All glassware was rinsed with methanol and dried at 100 °C for 1 h before use.
Collection of water samples

A map of the showing the sampling sites with code for the samples is given in Fig. S-1 of the Supplementary material to this paper. Details about the collection of the samples and the main characteristics of the studied area are also given in the Supplementary material.

Sample preparation

The samples were prepared using the method previously developed by Ballesteros et al.24 A solid-phase extraction procedure (SPE) with Agilent Bond Elut Plexa (200 mg per 6 mL) type of cartridges was used for sample pre-concentration and removal of the existing interferents. SPE was performed on an Agilent vacuum manifold for 12 columns connected to a water vacuum pump. The pH values of filtered water samples were adjusted to pH 3 with 1 M hydrochloric acid to increase the extraction efficiency.24,25 The cartridges were activated with 5 mL mixture of dichloromethane and methanol (50:50, V/V) followed by 5 mL of pure methanol and 15 mL of ultra pure water (pH 3). The final extraction volume of each sample was 500 mL. The analytes were eluted with 5 mL of methanol. After isolation, the eluate was gently evaporated to dryness at 40 °C under a nitrogen stream (5.0 purity) and then the residue of each sample was reconstituted in 50 µL of ethyl acetate. A silylation step was performed using 50 µL of BSTFA prior to the gas chromatography-mass spectrometry (GC–MS) analysis following the method obtained by Schönfelder et al. (Fig. 1).26

A fully automated derivatization procedure using amber glass GC vials was performed in order to avoid degradation of the reagent and derivatives by light and humidity. Just before the GC-MS analysis, the BSTFA reagent was injected into the vial with an analyte and the mixture was heated for 20 min at 50 °C. After cooling of the mixture, the derivatized form of the analyte was immediately injected into the GC–MS.

GC–MS analysis

The analyses were realized using an Agilent GC 7890A coupled with 5975C VL mass detector employing the method developed by Zafra et al.27 The system was used in the SCAN (complete spectrum) and in selected ion monitoring (SIM) modes. The separation was realized on a fused silica capillary column DB-5 MS (60 m, 0.25 mm i.d., 0.25 µm film thickness; J & W Scientific, Folsom, CA, USA). The column stationary phase was the low polarity phenyl arylene polymer, virtually equivalent to (5 %-phenyl)-methylpolysiloxane. The MS quadrupole temperature was set at 150 °C and the electron impact (EI) ion source temperature of the MS was at 230 °C. A 2-µL aliquot of the silylated samples were automatically injected using the split-
less injection mode at 250 °C. A programmed GC oven temperature was applied. The initial oven temperature was set at 50 °C and held for 1 min. Then, the temperature was increased to 150 °C (held 1 min) and 270 °C via ramps of 25 and 10 °C min⁻¹, respectively, and maintained at 270 °C for 2 min. High purity helium (99.99 %) was used as the carrier gas at a flow rate of 1.0916 mL min⁻¹. The characteristic ions listed in Table I for the SIM mode operation were used for quantification of BPA in the water samples.

TABLE I. SIM mode characterization

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Monitored ions (m/z)</th>
<th>Retention time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Quantifier</td>
<td>Qualifier</td>
</tr>
<tr>
<td>BPA</td>
<td>357</td>
<td>373 and 207</td>
</tr>
</tbody>
</table>

The BPA in the water samples was quantified with the external standard bis(trimethylsilyl)bisphenol A. A stock solution for calibration standards and quality control was prepared by dissolving an appropriate amount of BPA in methanol. The spiked blank water samples with appropriate BPA concentrations were extracted and silylated following the previously described sample treatment procedure. The linearity was examined over the range 10–100 ng mL⁻¹ of BPA. Each concentration level was injected in triplicate, and the calibration curves were constructed by considering the relative abundance as a function of the BPA concentration. A new set of BPA calibration standards was prepared for each season (in November 2012, March 2013, May 2013 and September 2013). The appropriate calibration curve was applied to all samples analyzed within the same season. The correlation coefficient ($R^2$) of all calibration curves was more than 0.998 and each curve was checked (and corrected if required) at a concentration level near the determined BPA concentrations in samples (6–50 ng mL⁻¹). Quality control samples that were run in each season were prepared in the same manner. The relative standard deviation of the response factors of the calibration curves for the same standard concentration was less than 10 %, which is in accordance with the requirements of Commission Decision 2002/657/EC (European Commission, 2002)²⁸ and the slope of the calibration curves did not differ significantly from zero. The limit of quantification (LOQ) was set at $S/N$ ratios ≥10. All instrumental and procedural blanks were far below the LOQ of 6 ng L⁻¹. The identification of BPA was confirmed using Fiehn and NIST08 mass spectral libraries. A characteristic chromatogram of the examined water samples containing BPA, after extraction and the derivatization procedure, is presented in Fig. S-2 of the Supplementary material.

**Statistical analyses**

Statistical analyses were performed using OriginPro 8 SRO (OriginLab Corporation, Northampton, MA, USA). The comparison of the mean values of the measured concentrations during different seasons was performed by the one-way analysis of variance (ANOVA) using the Tukey test and the results were considered significant at $p < 0.05$.

**RESULTS AND DISCUSSION**

**Occurrence and seasonal variations of BPA in the surface water**

During the one-year trial, contamination of the Novi Sad municipality by BPA was confirmed at each sampling site. BPA was found with a high frequency of the detection (68.75 %) in 22 out of 32 water samples above the LOQ (6 ng L⁻¹).
The BPA concentration varied from 11 to 693 ng L$^{-1}$ and the mean BPA concentration was 77 ng L$^{-1}$.

The obtained BPA levels in the surface water varied depending on the sampling sites, possible sources and routes of pollution during the four seasons (Table II). The seasonal occurrence of BPA in the surface water at the chosen sites along the Novi Sad section of the Danube is shown in Fig. 2.

TABLE II. BPA concentrations in the Danube samples in the municipality of Novi Sad; n.d.: $<\text{LOQ}$

<table>
<thead>
<tr>
<th>Sample</th>
<th>Range, ng L$^{-1}$</th>
<th>Mean, ng L$^{-1}$</th>
<th>Frequency of detection, % ($N=4$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RI</td>
<td>n.d.–77</td>
<td>29</td>
<td>75</td>
</tr>
<tr>
<td>GC</td>
<td>n.d.–304</td>
<td>90</td>
<td>50</td>
</tr>
<tr>
<td>GC2</td>
<td>n.d.–177</td>
<td>73</td>
<td>75</td>
</tr>
<tr>
<td>RO</td>
<td>n.d.–693</td>
<td>186</td>
<td>50</td>
</tr>
<tr>
<td>RP</td>
<td>n.d.–140</td>
<td>63</td>
<td>75</td>
</tr>
<tr>
<td>DM</td>
<td>n.d.–41</td>
<td>21</td>
<td>75</td>
</tr>
<tr>
<td>DL</td>
<td>n.d.–130</td>
<td>42</td>
<td>75</td>
</tr>
<tr>
<td>DR</td>
<td>n.d.–383</td>
<td>108</td>
<td>75</td>
</tr>
</tbody>
</table>

Fig. 2. Seasonal variations of the BPA concentration in the Danube surface water.
In autumn, BPA was detected above the $LOQ$ (6 ng L$^{-1}$) in 7 out of 8 collected samples at concentration levels up to 98 ng L$^{-1}$. The maximum detected concentration (98 ng L$^{-1}$) was in the RP sample, while the lowest concentration was recorded at the RI sampling site (13 ng L$^{-1}$). The high detection rate of 87.5% during the first campaign confirmed the presence of BPA in the Danube surface water in the Novi Sad area.

In the winter period, BPA was only detected in two samples (out of eight), RP and DM, at concentrations of 13 and 33 ng L$^{-1}$, respectively. The low detection rate and extensive reduction of the BPA concentration in winter were assumed to be due to the dilution effect because of the weather conditions and high river flow rate. However, the presence of BPA at the DM sampling site away from direct anthropogenic impact was unexpected during the winter. Probably, it was the result of the specific water flow and is attributed to the pollution effect of the DTD Canal.

On contrary, BPA was detected in almost all spring samples (7 out of 8). Again, as in the autumn, the maximum detected BPA concentration was at the RP site (140 ng L$^{-1}$), while the lowest concentration was in the DM sample (11 ng L$^{-1}$). The major contributor to the high concentration of BPA in autumn and in spring at the locality $RP$ could be the more intense surface runoff over waste disposal sites due to the increased volume of rainfall, as well as frequent hospital procedures during these periods.

During the summer, the detected BPA levels were above the $LOQ$ in six samples (out of eight) at high concentrations, ranging from 77 ng L$^{-1}$ in the RI sample to 693 ng L$^{-1}$ in the RO sample. Although, BPA has a short half-life in surface water due to its rapid photo and microbial degradation,16 which was favored in the summer, due to the higher temperatures, stronger sunlight and increased activity of microorganisms, the overload of BPA contamination tended to resist degradation in the surface water and was transported much further along the Danube. Unexpectedly high BPA concentration at RI site (77 ng/L) was probably influenced by increased anthropogenic activity in upstream weekend settlements. Similar to the autumn and spring, septic tanks and unauthorized discharges from upstream settlements contributed to the presence of BPA at the RI site in the summer. The highest concentration in the summer was observed at the RO site (693 ng L$^{-1}$). At the same site in autumn and winter, the BPA levels were below the $LOQ$, demonstrating that the determined levels in spring and summer were probably attributed to occasional discharges. One of the possible sources of BPA contamination at the RO site could be overhaul of the Thermal Power Plant, such as cleaning of the equipment, replacement of working fluids, painting, etc., which is common during the summer. The elevated BPA levels detected along the Danube banks (at the DL and DR sites) in comparison to the middle of the river (at the DM site) are in accordance with the water flow regime.
In the middle of the river, due to intensive horizontal and vertical turbulent flow, the degree of BPA dilution is significant. On the other hand, in the laminar flow along the coast, diffusion of pollutants is limited and the degree of dilution is considerably smaller.

The mean BPA concentration for the summer (220 ng L\(^{-1}\)) significantly differed from the mean BPA levels for autumn (39 ng L\(^{-1}\)), winter (6 ng L\(^{-1}\)) and spring (41 ng L\(^{-1}\)). There was no significant difference between the mean BPA concentrations detected for autumn, winter and spring. These seasonal differences could be the result of variations in the environmental conditions, such as rainfall and temperature, and/or different usage patterns of BPA-related products. During the summer period, traffic, sports and entertainment activities are intensified and among these, the consumption of beverages in plastic bottles is increased as well as the use of BPA-containing paints for boats. Additionally, the summer is the typical low water level season. All of this probably contributed to direct contamination of the Danube by solid waste and excretion, which significantly affected the water quality in the surface layers.

In a study performed in Brazil, similar results regarding the seasonal variation of BPA concentrations were obtained.\(^{32}\) Considering the 26 samples collected at the eight sampling sites along the Atibaia River (São Paulo State, Brazil), BPA was found in 56 % of samples in the four campaigns and the highest concentrations were recorded in the dry period. On the contrary, in a study performed in China, the highest concentration of BPA in the surface water was found in the winter.\(^{33}\) A possible explanation for the different seasonal occurrence of BPA might be the low water level of the Huangpu River from November to April and the flooding period that occurs during the summer in China.\(^{33}\) However, the obtained mean BPA concentration for the five campaigns in China (95 ng L\(^{-1}\))\(^{33}\) was similar to the mean BPA value (77 ng L\(^{-1}\)) found in the present study.

The BPA levels obtained in the present study were also compared with available data related to European rivers. The detected concentrations of BPA in this investigation were in the range of <6 to 693 ng L\(^{-1}\) (the mean BPA concentration was 77 ng L\(^{-1}\)). Although, the levels found in freshwater streams of Germany were higher (up to 1927 ng L\(^{-1}\)).\(^{34}\) BPA only appeared in samples from October, while no BPA was detected in December, April and September. However, BPA was quantified in Dutch surface waters at levels up to 330 ng L\(^{-1}\), with the highest concentration being found in the summer, similar to the present study.\(^{35}\) In the Seine River and in the Rhine surface water, BPA was found in a lower range (11–154\(^{36}\) and 42 to 229 ng L\(^{-1}\),\(^{25}\) respectively). The maximum BPA concentration in the present study (693 ng L\(^{-1}\)) was higher than the previously recorded maximum BPA concentration in the Danube surface water (68 ng L\(^{-1}\)) measured during the Joint Danube Survey 2.\(^{20}\) However, in the same
study, BPA was detected in the Danube tributaries at concentrations up to 490 ng L\(^{-1}\).\(^{20}\)

The relatively high BPA concentrations found in the investigated Danube River stretch in Serbia could be explained by the fact that Novi Sad still has no urban wastewater treatment plant and that most of the industries around Novi Sad municipality are without wastewater treatment facilities.

**Possible environmental implication**

The octanol–water partition coefficient, log \(K_{ow}\), for BPA is 3.64, which suggests that BPA has modest capacity for bioaccumulation.\(^{37}\) The presence of the BPA endocrine disrupter might have adverse effects on aquatic organisms\(^{38}\) and the consumption of BPA contaminated freshwater fish\(^{35}\) is probably one of the main routes of contamination of humans.\(^{16}\)

Following the European Union guidelines, the environmental risk assessment is evaluated by comparing the predicted environmental concentration (\(PEC\)) or by measuring the environmental concentration (\(MEC\)) with the sensitivity of the aquatic ecosystem for BPA (predicted no-effect concentration, \(PNEC\)). The ratio \(PEC/PNEC\) (or \(MEC/PNEC\)), known as the risk quotient (\(RQ\)), should not exceed 1, otherwise, a risk to the aquatic environment is assumed.\(^{39}\) The \(PNEC\) values are based on eco-toxicity data from single species laboratory tests combined with safety factors or even on derived provisional \(PNEC\) values.\(^{40}\) Based on literature data, the aquatic \(PNEC\) value for BPA varies from 0.06 µg L\(^{-1}\) estimated by Wright-Walters et al.\(^{18}\) using a non-parametric methodology to 0.175 µg L\(^{-1}\) in Canada,\(^{41}\) 1.5 µg L\(^{-1}\) in the European Union according to the European Union risk assessment report,\(^{42}\) 1.6 µg L\(^{-1}\) in Japan\(^{43}\) and even to 64 µg L\(^{-1}\) derived by Staples et al.\(^{44}\)

The possible negative implications of BPA on the Danube surface water were evaluated using the 95\(^{th}\) percentile of the measured BPA concentrations along the Danube section by the Novi Sad municipality together with a \(PNEC\) value to calculate the \(RQ\) for each season. All sampling points were considered as one locality due to the relatively small distance between the sites. The strictest standard, i.e., the lowest \(PNEC\) value of water based on the Wright-Walters and co-workers methodology\(^{18}\) was applied in order to reduce the uncertainties of standardized acute toxicity tests in risk assessment approach.

The calculated ratio ranged from 0.44 in winter to 9.73 in summer. The \(MEC/PNEC\) ratio was below 1 only in winter (Fig. 3). The high \(RQ\) in summer is attributed to the elevated BPA level at the RO site (693 ng L\(^{-1}\)). Considering this value as an exception, probably due to an occasional discharge at this site, the \(RQ\) was recalculated using the mean summer BPA concentration (220 ng L\(^{-1}\)). However, the obtained \(MEC/PNEC\) ratio was still above 1 (3.67). Moreover, the \(RQ\) calculated by dividing the mean BPA concentration derived in this study (77 ng
L$^{-1}$) with the $PNEC$ value was above one, indicating that potential risk of BPA in this Danube section exists.

This risk assessment approach was based on an evaluation of the toxic potential of a single plasticizer to the aquatic environment without taking into account the possibility and effects of BPA acting additively with other EDs.

CONCLUSIONS

This research investigated the occurrence and seasonal variations of BPA as an endocrine disrupter in the Danube surface water of the Novi Sad section, Serbia using SPE followed by the GC–MS method. BPA was found at all 8 sampling sites during the one-year trial. In 22 out of 32 water samples, BPA was detected above the $LOQ$ (6 ng L$^{-1}$), in concentrations varying from 11 to 693 ng L$^{-1}$.

The highest concentrations of this compound were observed in the summer and significantly differed from the determined mean BPA levels for autumn, winter and spring. These seasonal variations might have resulted from environmental factors, such as the low water level, and increased anthropogenic activities. The calculated risk quotient was below one only in the winter. The obtained results indicate that the aquatic environment of the Danube River by Novi Sad municipality is not sufficiently protected from possible adverse effects to BPA exposure and that attention should be paid to BPA pollution control in order to protect drinking water supply sources, which are very close to the river-banks.

SUPPLEMENTARY MATERIAL

Details about samples collection and the main characteristics of the studied area, as well as a representative GC–MS chromatogram are available electronically from http://www.shd.org.rs/JSCS/, or from the corresponding author on request.
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ИЗВОД
СЕЗОНСКЕ ВАРИЈАЦИЈЕ БИСФЕНОЛА А У ПОВРШИНСКОЈ ВОДИ ДУНАВА, НА ПОДРУЧЈУ НОВОГ САДА

МАЈА МИЛАНОВИЋ1, ЈАН СУБИЋ1, НЕВЕНА ГРУНИЋ ЛЕТИЋ1, ЈЕЛЕНА РАДОНИЋ2, МАЈА ТУРК СЕКУЛИЋ2, МИРИЈАНА БОЈИНОВИЋ МИЛОРАДОВ Прецаи и НАТАША МИЛИЋ1

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Сезонске промене концентрација бисфенола А у површинској води Дунава, на територији Новог Сада, анализирани су применом гасне хроматографије са масеном спектрометријом. Бисфенол А детектован је изнад лимитата квалитације (6 ng L⁻¹) у 22 узорка воде (од укупно 32 узорка) у опсегу концентрација 11–693 ng L⁻¹. Средња вредност концентрације бисфенола А у летњем периоду (220 ng L⁻¹) статистички је значајни виша од средњих концентрација измерених у јесен (39 ng L⁻¹), зиму (6 ng L⁻¹) и пролеће (41 ng L⁻¹) и укажу на већу контаминацију Дунава током летњег периода. Провећен риск по животну средину, изражен као однос 95. перцентила измерених концентрација бисфенола А у површинској води током четири годишња доба и концентрације за коју је предвиђено да нема биолошке ефekте, био је мањи од јединице само током зиме. Високе концентрације бисфенола А у летњем периоду које су последица повећане људске активности и временских услова представљају потенцијални ризик по животну средину на подручју Новог Сада.

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REFERENCES
SUPPLEMENTARY MATERIAL TO
Seasonal variations of bisphenol A in the Danube River by the municipality of Novi Sad, Serbia

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COLLECTION OF WATER SAMPLES

The surface water samples (1 L) were collected in autumn (November 2012), winter (March 2013), spring (May 2013) and summer (September 2013), in amber glass bottles at eight representative locations (RI, GC, GC2, RP, RO, DM, DL, DR) along the Danube River of the Novi Sad municipality, Serbia and stored at 4 °C until the analysis were performed. Samples were collected on the same day in every season and the meteorological conditions were the same for all localities within a particular season. All 32 water samples were taken at a depth of 50 cm. The position of sampling points in relation to the flow of the river could be seen in Fig. S-1.

The sampling sites were selected according to the recommendations of the ICPDR’s experts (The International Commission for the Protection of the Danube-ICPDR, www.icpdr.org), in order to estimate the level of violation of the ecological status of the Danube by the municipality of Novi Sad. Location RI was chosen in order to determine the status of the Danube prior to any local urban and canal tributary impact. Sampling points GC, GC2, RO and RP were positioned 100 m downstream, i.e., transversely from the four sewage discharges of the city. The locations GC and GC2 were selected to determine the influence of two biggest untreated municipal wastewater discharges (with capacity of 400 L s⁻¹ during the dry period). The location RO was chosen to determine the influence of untreated wastewater from the oil refinery and the rain and urban runoff water from the industrial (thermal-heating plant, oil refinery) and suburban underdeveloped area. Sampling point RP was selected in order to explore the

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impact of untreated municipal wastewater from numerous illegal settlements with waste disposal sites and unauthorized traffic, as well as the influence of wastewater coming from two clinical centers. The sampling locations DM (central part of the river) and DL and DR (along the bank) were chosen as control sites in order to estimate the possible existence and impact intensity of the municipality of Novi Sad on the river water quality after some period (estimation of the impact of dilution, sorption and sedimentation processes).

Fig. S-1. Map of the Danube River sampling sites.

All the samples were filtered through regenerated cellulose 0.45 μm membrane filters (Agilent Technologies, Germany) and extracted within 24 h in the laboratory.

MAIN CHARACTERISTICS OF THE STUDIED AREA

The Danube flows through ten countries from the west to the east, passing through four capital cities before emptying into the Black Sea, and its drainage basin extends into the territories of nine more nations. Constantly, it is influenced by nutrients and other polluting substances. Therefore, continuous water quality monitoring of the Danube followed by pollution reduction steps is obligatory for all countries of the Danube Basin.

Novi Sad, the second largest town in Serbia, is located on the banks of the Danube and the DTD Canal. During the NATO bombing in 1999, Novi Sad was faced with the destruction of all bridges and the Oil Refinery and suffered an economic downturn. However, during the last decade, the industry has been recovering.
The river pollution in the area of Novi Sad is caused by direct and indirect discharge of urban and industrial wastes and run-off. The acute problem is the emission of more than 95% of the total industrial wastewater without any treatment. The dominating industries in this area are chemical and food industries and building materials production. The biggest polluters such as the Oil Refinery, meat processing plants and the production of chemicals and cosmetics are situated on the Danube and DTD Canal banks. Additionally, Novi Sad has joint collector for both industrial and municipal wastewaters and no Urban Wastewater Treatment Plant.

The GC and GC2 sampling point are influenced by discharges of the two main sewers of the town, Cepelin and Beogradski kej. Furthermore, the Municipality Water Supply System uses mainly drinking water abstraction points Petrovaradinska ada (Petrovaradin fluvial island) and Ratno ostrvo (War island), that are positioned only a few kilometers downstream from these main municipal wastewater discharges. The RO sampling site is located near the main water supply source Ratno ostrvo and is 100 m downstream from the discharge site of the sewage collector for collecting industrial wastewater from the big local oil refinery complex as well as rain and urban run-off water from the industrial zone and the underdeveloped suburban area with septic tanks. The most dangerous potential pollution sources near RO are the Oil Refinery and a Thermal Power Plant that are situated only a few hundred meters upstream. Additionally, the influence of the extensive agricultural production in the backland cannot be ignored. The RP location is situated on the right riverbank near the water source Petrovaradinska ada. In the vicinity, there are numerous illegal settlements with septic tanks and waste disposal sites, unauthorized traffic and an old industrial complex. Sampling points DL, DM and DR are outside direct anthropogenic influence. The intensity impact of the municipality and the possible harmful effect of the DTD Canal on the river water quality were examined at these sites. Previously, it was concluded that the DTD Canal has the worse water quality in Novi Sad, where increased concentrations of organic compounds, mineral forms of nitrogen phenols as well as mercury, manganese, copper and iron were recorded. The RI sampling site is located upstream of the city before municipal discharge points. However, suburban and weekend settlements without a sewage system, together with septic tanks and an old industrial complex nearby could influence the surface water quality in this area.

According to all, the sampling sites (Fig. S-1) were chosen for seasonal analysis of BPA at kilometer 1255 of the Danube River in order to identify the primary source of the BPA emission (GC, GC2, RO, RP), the possible influence of incoming pollution (RI), the global impact of the city on the river status (DM, DR, DL) as well as the seasonal fluctuation of the BPA concentration. The pos-
Possible pollution by the DTD Canal was examined at the DL, DM and DR sampling points.

**REPRESENTATIVE GC–MS CHROMATOGRAM OF A SAMPLE**

Fig. S-2. Representative GC–MS chromatogram of a Danube water sample extract after clean up and the derivatization procedure (SIM mode).