Alkylphenolic compounds and bisphenol A contamination within a heavily urbanized area: case study of Paris

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Abstract This study evaluates the influence of a heavily urbanized area (Paris Metropolitan area), on receiving water contamination by both bisphenol A (BPA) and alkylphenol ethoxylate (APE) biodegradation product. The study began by investigating concentrations within urban sources. In addition to the more commonly studied wastewater treatment plant effluent, wet weather urban sources (including combined sewer overflows, urban runoff, and total atmospheric fallout) were considered. The initial results highlight a significant contamination of all urban sources (from a few nanograms per liter in atmospheric fallout to several micrograms per liter in the other sources) with clearly distinguishable distribution patterns. Secondly, concentration changes along the Seine River from upstream of the Paris Metropolitan area to downstream were investigated. While the concentrations of BPA and nonylphenoxy acetic acid (NP_1EC) increase substantially due to urban sources, the 4-nonylphenol concentrations remain homogeneous along the Seine. These results suggest a broad dissemination of 4-nonylphenol at the scale of the Seine River basin. Moreover, the relationship between pollutant concentrations and Seine River flow was assessed both upstream and downstream of the Paris conurbation.

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Consequently, a sharp decrease in dissolved NP_1EC concentrations relative to Seine River flow underscores the influence of single-point urban pollution on Seine River contamination. Conversely, dissolved 4-nonylphenol concentrations serve to reinforce the hypothesis of its widespread presence at the Seine River basin scale.

Introduction

Among the pollutants commonly cited in the literature for their endocrine-disrupting properties, alkylphenol ethoxylates (APE) and the 2,2-(4,4 hydroxydiphenyl) propane, commonly labeled bisphenol A (BPA), deserve special attention as a result of their ubiquity in the environment (Staples et al. 1998; Vethaak et al. 2005). APE, mainly composed of nonylphenol ethoxylate (NPE, 80 %) and octylphenol ethoxylate (OPE, 20 %), are widely used in industrial and domestic applications, such as lubrication, oil additives, detergents, and antistatic agents (Ying et al. 2002). A reasonable estimate of the world's annual production of NPE is around 500,000 tons (Ying et al. 2002). BPA is primarily used as a monomer in the manufacturing of polycarbonate plastics known for its high resistance to shocks and temperature (e.g., plastic windows, greenhouses, car bumpers, and baby bottles), as well as in epoxy resins designed as food contact lacquer for cans and tins. Recently, high levels of BPA were found in various types of papers (used as a color developer) such as thermal receipt papers, newspapers, napkins, or toilet papers (Liao and Kannan 2011). Due to these widespread uses, the worldwide

production of BPA has been evaluated at approximately 3,000,000 tons per year (Vandenberg et al. 2007).

While BPA is recognized as an endocrine-disrupting chemical (Wetherill et al. 2007), concerns over NPE and OPE have been on the rise due to their biodegradation products. In urban or natural environments, NPE can in fact be biodegraded through oxidative or non-oxidative pathways (John and White 1998; Jonkers et al. 2001). Under an oxidative biodegradation pathway, NPE transforms into carboxylic acids such as nonylphenol acetic acid (NP_1EC), while the non-oxidative biodegradation pathway leads to the production of short-chain ethoxylates such as nonylphenol diethoxylate (NP_2EO) and nonylphenol monoethoxylate (NP_1EO) (Giger et al. 2009). Lastly, NP_1EC and NP_1EO can be biodegraded into 4-nonylphenol (4-NP), known as the most toxic and persistent NPE biodegradation product within the aquatic environment (Servos 1999).

The occurrence of APE and BPA in the environment is closely correlated with anthropogenic activities (Bergé et al. 2012). Many studies suggest that these chemicals preferentially enter into the environment through urban sources (Sharma et al. 2009; Ying et al. 2002). Among the urban sources investigated in the literature, wastewater treatment plant (WWTP) effluent is frequently studied (Hohne and Puttmann 2008; Loyo-Rosales et al. 2007; Voutsa et al. 2006; Zhou et al. 2010). On the contrary, studies on wet weather urban sources such as combined sewer overflows (CSOs) and urban runoff are less readily available despite the high contamination levels of such sources (Björklund et al. 2009; Gasperi et al. 2008).

The Seine River basin, located in northwestern France, drains roughly a 32,000-km^2 area from its headwaters to Paris. It can be considered as representative of river basins exposed to the impacts of intense human activity (the Paris metropolitan area, 12 million residents, among the 30 most populous cities in the world and the third in Europe after Moscow and London) (Meybeck et al. 2007). This basin combines strong anthropogenic pressures with a very limited dilution factor in the Seine River, due to its low flow rate (the median Seine River flow within the Paris city limits, 350 m^3/s). No comprehensive data on the occurrence rates of APE and BPA are in fact currently available for the upper part of the Seine basin. To the best of our knowledge, only a few studies have dealt with the influence of such heavily urbanized conurbations (i.e., over 10 million inhabitants) on their environment (Isobe et al. 2001), and none of these studies have examined the watershed of a major European city.

This study therefore focuses on several objectives. The first is to assess the occurrence of APE and BPA concentrations and their relative significance in different urban sources throughout the Paris Metropolitan area, such as major WWTP effluent, the main CSO outfall, and urban runoff. The second objective of this study seeks to evaluate the influence of the Paris urban area on receiving water between upstream and downstream sampling sites. The third and last objective is to better analyze the concentration variation of target compounds on the basis of hydrological conditions at the upstream and downstream sites.

Materials and methods

Sampling sites/campaigns

Seine River water was collected at three locations ($n=11$ for each sampling location; see Table 1) from February 2010 to February 2011. The first site, Marnay, is located upstream of the Paris Metropolitan area (low-density urbanization), while the second site, Bougival, lies just downstream of the city of Paris (heavily urbanized), and the last site, Meulan, is situated downstream of all Paris wastewater effluent discharges (also heavily urbanized) (Fig. 1). Moreover, the Orgeval River running through an experimental agricultural catchment (Vilain et al. 2012) was also monitored in 2011 ($n=9$; Fig. 1). All samples were manually collected in 2-L amber glass bottles that had previously been heated at 500 °C.

The urban sources investigated include WWTP effluent, total atmospheric fallout (TAF), urban runoff, and CSOs. For WWTP effluent, all five major WWTPs (i.e., WWTP1 to WWTP5 in Fig. 1) in the Paris area were monitored on a monthly basis from January 2011 to November 2011 ($n=9$). These plants are supervised by the Paris Public Sanitation Authority (SIAAP) and treat more than 2 million m^3 of wastewater daily (70 % of the metro area population). Samples were manually collected in 2-L amber glass bottles. Among these five WWTPs, three distinct wastewater treatment processes are involved. First, the WWTP1 uses conventional activated sludge (AS) for carbon and nitrogen removal (AS-WWTP), whereas WWTP4 uses activated sludge for carbon removal and biofiltration (B) as well as nitrogen removal (ASB-WWTP) and, lastly, the more recent WWTP2, WWTP3, and WWTP5 use biofiltration units for both carbon and nitrogen removal (B-WWTP). The removal efficiencies on both organic matter (as evaluated with chemical oxygen demand (COD)) and nitrogen (nitrification, total Kjeldahl nitrogen (TKN); denitrification, total nitrogen (TN)) are listed in Table 1. According to these efficiency values, WWTPs 1, 2, 3, and 5 reveal high removal rates for organic matter and nitrogen. Conversely, WWTP4 (the largest WWTP within the Paris Metropolitan area and one of the largest in the world; over 1,600,000 m^3 of wastewater treated daily) exhibits lower efficiencies for organic matter (COD, 85 %), nitrogen (TKN, 70 %; TN, 20 %), and suspended solids (SS, 90 %). A modernization campaign of this WWTP is underway in order to improve its efficiency for carbon and nitrogen removal.

Table 1 Sampling site characteristics

Sample	No.	Period	Comments/details					
Orgeval River	9	Mar 2011–Nov 2011	Upstream site / agricultural watershed					
			Manually collected in 2-L glass bottles					
Seine River	11	Feb 2010–Feb 2011	3 stations (Marnay, Bougival, Meulan), increasing urbanization					
			Manually collected in 2-L glass bottles					
WWTP effluent	11	Jan 2011–Nov 2011			Removal efficiency			
			WWTP × (vol m^3/day)	Process	COD	TKN	TN	SS
			WWTP 1 (385,000)	AS	96 %	97 %	74 %	98 %
			WWTP 2 (48,000)	B	93 %	95 %	86 %	97 %
			WWTP 3 (233,000)	B	95 %	96 %	70 %	98 %
			WWTP 4 (1,612,000)	ASB	86 %	70 %	21 %	91 %
			WWTP 5 (92,000)	B	94 %	92 %	82 %	97 %
CSOs	8	June 2010–Nov 2010	Volume per event: 35,000 to 1,000,000 m^3					
			Automatic samplers: 1 L per 15 min / weighted mean samples					
Total atmospheric fallout	20	Feb 2011–Nov 2011	3 sites (Paris City, Lognes, Fontainebleau)					
			Total atmospheric fallout (dry + wet) (over 15 days)					
Urban runoff	4	July 2011–Oct 2011	Samples collected at the Sucy-en-Brie outfall, separate sewer					
			Automatic samplers: 1 L per 15 min / weighted mean samples					

In addition to WWTP effluent, urban sources during wet weather periods were also sampled, i.e., combined sewer overflows and urban runoff in separate sewers. Eight CSOs (with discharge volumes ranging from 35,000 to over 1,000,000 m^3) were sampled at the same outfall, one of the largest in the entire Paris Metropolitan area (Fig. 1). Urban runoff was sampled at the outlet of a small suburban catchment (Sucy-en-Brie 2.6 km^2) for four rain events (Fig. 1); for CSOs and runoff, sampling was performed by means of an automatic sampler (Bühler) at a rate of 1 L every 15 min. Flow-weighted composite samples representative of the complete discharge cycle were considered for analysis. Moreover, TAF were collected within the city limits of Paris city (heavily urbanized, $n=10$), Lognes (low-density urbanization, $n=5$), and the Fontainebleau forest ($n=5$) in 10-L glass bottles fitted with 1-m^2 aluminum funnels (Fig. 1) over seven periods (lasting 15 days) between January and November 2011.

Fig. 1 Sampling sites within the Paris Metropolitan area
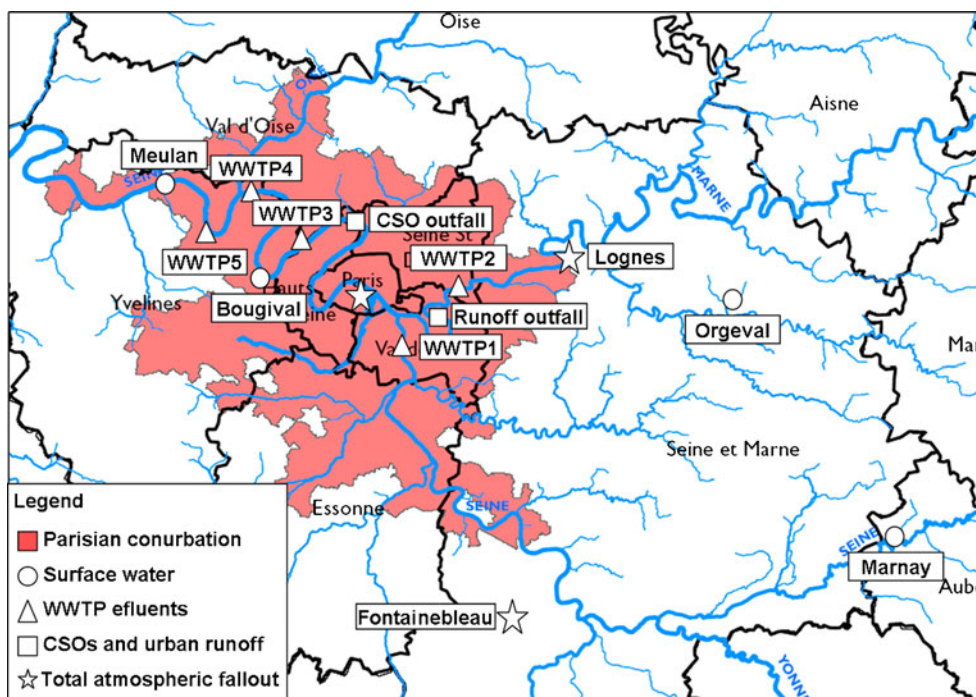
Analytical procedure

Sample preparation

Bisphenol A, 4-nonylphenol, nonylphenol monoethoxylate, nonylphenol diethoxylate, and nonylphenol acetic acid were all analyzed in both the dissolved phase and SS. Following filtration with glass fiber filters (0.7-μm porosity GF/F, Whatman), 100 mL of dissolved phase for CSOs or 250 mL for all other water samples were spiked with a surrogate standard mixture (BPA-d6, NP_1EO-d2, and OP-d17) before extraction.

For the dissolved phase, samples were extracted by solid phase extraction (Autotrace SPE Workstation, Caliper LifeScience) using OASIS® HLB cartridges (200 mg, 6 mL). After conditioning with 10 mL of methanol and 10 mL of ultra-pure water, the dissolved samples were extracted at 5 mL/min at a neutral pH. After drying, elutions were performed using 12 mL of a mixture of methanol (MeOH)/dichloromethane (DCM)/ethyl acetate (Etace; 40/40/20, v/v). Once freeze-dried, the SS were extracted via microwave-assisted extraction with a multiwave 3000 (Antonn Paar). The filters were extracted using 20 mL of a MeOH/DCM (60/40, v/v) mixture at 100 °C and 7 bar for a total of 30 min. After extraction, samples were cleaned by SPE using OASIS® HLB (200 mg, 6 mL). Following conditioning (3 mL of MeOH and 3 mL of $MeOH/H_2O$ (20/80 v/v), the samples dissolved in 500 μL of a $MeOH/H_2O$ mixture (50/50v/v) were deposited on top of the cartridges before they were washed with 3 mL of H_2O and then eluted with 9 mL of MeOH/DCM/Etace mixture (40/40/20, $v/v/v$).

In parallel of target organic compounds, the dissolved organic carbon (DOC) was analyzed in duplicate via a Shimadzu TOC-Vws. The DOC is transformed into CO_2 by a powerful oxidation using a combination of sodium persulfate and UV-oxidation at 80 °C. Thus, the CO_2 produced is analyzed by infrared. The measurement uncertainty of DOC analysis was calculated at 1 %.

UPLC-MS-MS analysis

Before analysis, both extracts were concentrated under nitrogen and spiked with an internal standard for quantification (BPA-d16, n-NP, and n-NP_1EO). This analysis was performed by use of liquid chromatography coupled with tandem mass spectrometry, UPLC-MS-MS (AQUITY UPLC/TQD, Waters). APEOs and BPA were separated on an AQUITY UPLC/BEH C_{18} column, heated to 40 °C with ultra-pure water (A) and methanol (B), each containing 4.5 mM NH_4OH as eluant. Equilibration took place with 50 % B at 0.4 mL/min, with 10 μL of the sample being injected. The mass spectrometer has been equipped with an electrospray interface used in positive ionization mode (ESI+) for NP_1EO and NP_2EO, along with a negative ionization (ESI−) mode for

all other compounds. These compounds have been detected in a multiple-reaction monitoring mode using two mass transitions. The optimized parameters for BPA and NPE analysis are displayed in Table 2. Our analytical protocol and the limits of quantification (LOQ) were validated according to French method-validation standard NF XPT 90-210 (1999).

Method validation

Both dissolved and particulate extraction recoveries were investigated through spiked matrix tests. For the dissolved phase, the recoveries of all target compounds were found above 80 %. In addition, no significant matrix effects were noticed during UPLC-MS-MS analysis. As regard the particulate matrices, the recoveries of BPA, NP, NP_1EO, and NP_2EO were found close to 100 % while the recoveries of NP_1EC were stable but do not exceed 55±1 % (median of nine tests). This low recovery was taken into account for calculation of LOQ and sample concentration, since the spiked matrix tests underscored a good reproducibility for this compound (<1 %). Once again, no significant matrix effects were noticed during UPLC-MS-MS analysis. Finally, the surrogate standard mixture (BPA-d6, NP_1EO-d2, and OP-d17) recoveries (always above 75 %) ensured that both dissolved and particulate extractions were correctly performed for every sample.

Measurement uncertainty

In order to assess the uncertainty of the analytical protocol, three surface water samples from the same sampling location (i.e., downstream of WWTP 4) were individually analyzed. This uncertainty was studied for just the dissolved phase extraction and UPLC-MS-MS analysis, but not for suspended solids. In receiving water, alkylphenols and BPA are in fact mainly present in the dissolved phase (4-NP, 80 %; 4-t-OP, 90 %; and BPA, 95 %; (Cladiere et al. 2010; Isobe et al. 2001). The dissolved phases of the three surface water samples were extracted in triplicate and each extraction was also analyzed in triplicate, meaning that nine concentration values were thus obtained for each sample and each target compound. These nine values were then compared to the median value in order to calculate relative deviations representative of the measurement uncertainty. As a final step, the absolute values of relative deviations generated for the three samples (9×3=27) were shown in Fig. 2 as histograms for BPA (median concentration, 20 ng/L), 4-NP (median, 57 ng/L), and NP_1EC (median, 125 ng/L).

For 4-NP, NP_1EC, and NP_2EO, the relative deviations generally lie within the 0–15 % range, while maximum values reach 20 % for 4-NP. The median value were selected in order to express the measurement uncertainty (±uncertainty) of 4-NP, NP_1EC, and NP_2EO and respectively estimated at 7,

Table 2 Optimized parameters for BPA and NPE for the UPLC-MS-MS analysis

Compounds	ESI mode	Retention time (min)	Quantification		Qualification		Cone voltage (ev)	LOQ[a] (ng/L)	LOQ[b] (ng/g)
			Transition (m/z)	Collision energy (eV)	Transition (m/z)	Collision energy (eV)			
BPA	ESI−	1.74	226.9→133.0	25	226.9→212.2	25	30	11	54
4-NP	ESI−	3.45	219.2→147.2	26	219.2→133.2	26	40	25	36
NP$_1$EO	ESI+	3.53	282.4→127.3	8	282.4→85.4	15	20	10	28
NP$_2$EO	ESI+	3.55	326.4→183.3	10	326.4→71.2	20	40	4	51
NP$_1$EC	ESI−	1.64	277.2→219.2	18	277.2→133.0	38	35	2	8

[a] Limit of quantification in dissolved phase

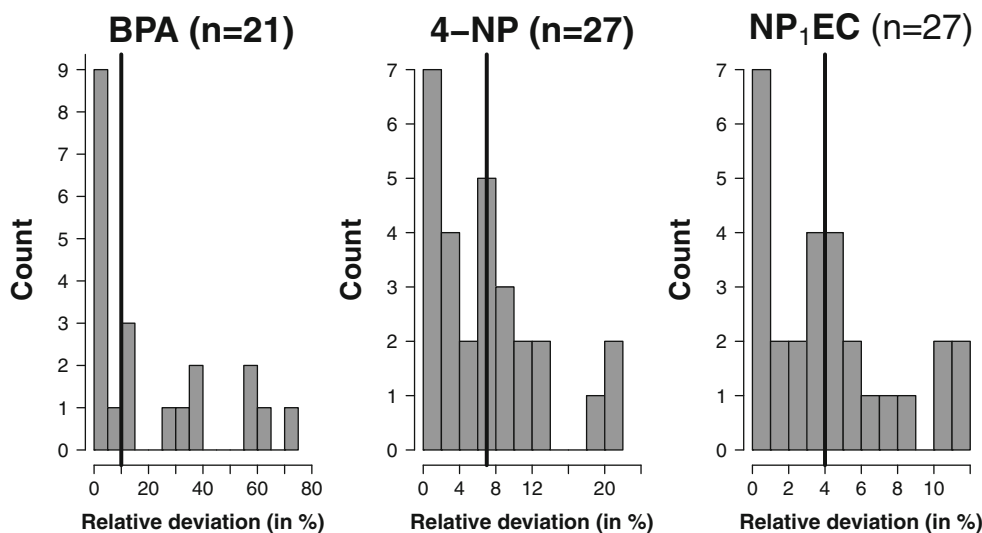[b] Limit of quantification in particulate phase

4, and 7 %. For BPA and NP$_1$EO, the relative deviations were calculated for only 21 analyses since their concentrations were close to their limits of quantification (BPA, 11 ng/L; NP$_1$EO, 10 ng/L). Similar to previous compounds and according to the median value (bold lines), the measurement uncertainty of BPA and NP$_1$EO were evaluated respectively at 10 and 14 %.

Data interpretation

The concentrations presented in this study are total concentrations (expressed in nanograms per liter), as calculated by summing dissolved concentrations (nanograms per liter), and SS concentrations (nanograms per liter). For the variations in concentrations with hydrological conditions, only the dissolved phase has been considered in order to better assess the origins of target compounds (whether point or diffuse sources) within the Seine River.

Results are displayed in box plots, which extend from the first quartile (Q1) to the third quartile (Q3), and thus contain the central half of the distribution score. The dotted lines indicate 1.5 times the interquartile range while outliers are denoted as points either above or below the dotted lines.

Since the concentration distributions of each sampling point cannot be assumed to be normally distributed, nonparametric tests were conducted to compare the concentrations found at different sites. The Wilcoxon signed-rank test was selected for this comparison at the Marnay, Bougival, and Meulan sites given that samples were collected on the same day on all three sites. For this same reason, the Wilcoxon signed-rank test was also used to compare the WWTP effluent. For all other matrices and sampling sites (TAF, CSO, runoff, and Orgeval), the Mann–Whitney U test was chosen to compare distributions of different sizes. In accordance with measurement uncertainty, the statistical significance was acceptable at $p < 0.05$ for all comparisons.

Results and discussion

Urban sources

Wastewater treatment plants

The results determined from WWTP effluent for BPA and NPE are shown in Fig. 3. Three groups, corresponding to

Fig. 2 Distribution histograms of relative deviations of BPA, 4-NP, and NP$_1$EC with respect to the median value. The *bold lines* represent the median of the relative deviations. The *y*-axis (count) represents the number of samples included in each category of relative deviation. Measurement uncertainties were determined for environmental samples at concentrations: BPA, 20 ng/L; 4-NP, 57 ng/L; and NP$_1$EC, 125 ng/L
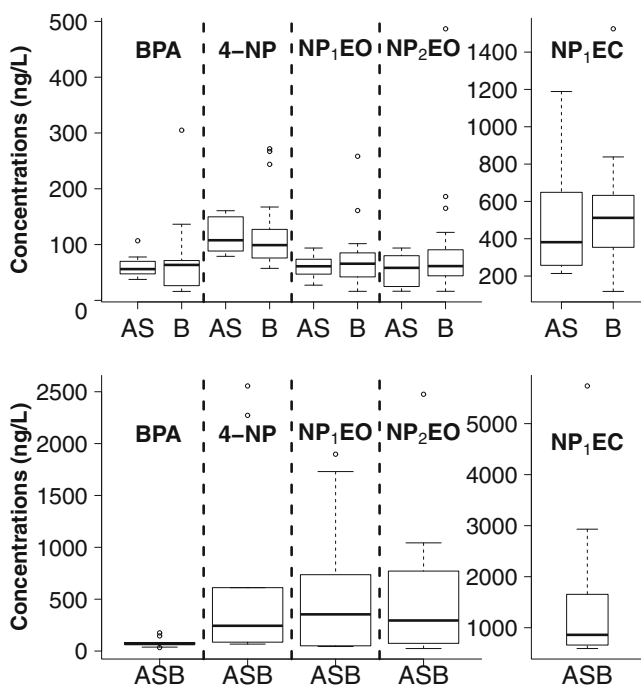
Fig. 3 Concentrations of target compounds (nanograms per liter) in WWTP effluent—*AS* activated sludge, *B* biofiltration, *ASB* activated sludge (carbon removal) + biofiltration (nitrogen removal)

AS-WWTP, ASB-WWTP, and B-WWTP (as described earlier), are considered herein.

For the AS-WWTP and B-WWTP considered, the median concentrations (±uncertainty) of effluent were evaluated at: 67 (±7) ng/L for BPA, 114(±8) ng/L for 4-NP, 74(±10) ng/L for NP_1EO, 74(±5) ng/L for NP_2EO, and 573(±23) ng/L for NP_1EC. For the ASB-WWTP (WWTP4), the median concentrations (±uncertainty) found in effluent were higher: 76(±8) ng/L for BPA, 244(±17) ng/L for 4-NP, 304(±43) ng/L for NP_1EO, 295(±21) ng/L for NP_2EO, and 842(±34) ng/L for NP_1EC. On the whole, these median concentrations are similar to those reported in the literature for Europe (Hohne and Puttmann 2008; Jonkers et al. 2009), the US (Loyo-Rosales et al. 2007), China (Zhou et al. 2010), and, in a previous study, the Paris Metropolitan area (Gilbert et al. 2012).

WWTP effluent has been characterized by the predominance of NPE (4-NP and NP_1EC), in comparison with BPA. The low BPA concentrations in WWTP effluent (with medians of 76 (±8) ng/L for ASB-WWTP, and 67(±7) ng/L for B-WWTP and AS-WWTP) stem from the high efficiency of wastewater treatment and high BPA biodegradation rate during wastewater treatment processes (approximately 90 % according to Zhou et al. (2010)). Concerning NPE, the main biodegradation product of the nonylphenol ethoxylate surfactants found in all WWTP effluent would be NP_1EC, with median concentrations of 842(±34) ng/L for ASB-WWTP and 573(±23) ng/L for B-WWTP and AS-WWTP. NP_1EC has already been identified as the major biodegradation product of NPE during wastewater

treatment processes and is essentially derived from aerobic biodegradation (Ahel et al. 1994). In contrast to results reported by Loyo-Rosales et al. (2007), yet still in agreement with those cited by Hohne and Puttmann (2008), no seasonal trend in concentrations could be drawn for all WWTP effluent from January 2011 to November 2011.

By comparing the AS-WWTP and B-WWTP effluent values (using the Wilcoxon signed-rank test), no significant differences in concentrations appear for any of the target compounds ($p > 0.05$). The ASB-WWTP effluent does not exhibit any statistical difference relative to either the AS-WWTP or B-WWTP effluent with respect to BPA and 4-NP ($p > 0.05$). Conversely, for the 4-NP precursors (NP_1EC, NP_1EO, and NP_2EO), significant differences were found between ASB-WWTP and AS-WWTP + B-WWTP effluent values ($p < 0.05$). The higher concentrations detected in ASB-WWTP effluent can certainly be explained by the lower biodegradation rate on organic and nitrogenous matter (COD, 86 %; NTK, 70 %; and TN, 21 %).

Urban sources during wet weather periods

The concentrations found in CSOs, runoff, and TAF are listed in Table 3.

The concentrations reported in Table 3 reveal significant differences between TAF, on the one hand, and CSOs and runoff on the other ($p < 0.05$). For example, while the concentrations of BPA range between 10(±1) and 180(±18) ng/L in TAF, concentrations vary between 917 and 2,098 ng/L (some 32 times higher) in CSOs and between 287 and 1,224 ng/L (13 times higher) in runoff. In addition, pattern changes are clearly noticeable in Table 3, while 4-NP displays the highest median concentration in TAF (80(±6) ng/L), BPA is the predominant compound in CSOs and runoff (CSO, 1,410(±141) ng/L; runoff, 635(±64) ng/L).

TAF concentrations are typically low. Minimum concentrations remain less than or close to the limits of quantification, whereas median and mean concentrations always lie below 100 ng/L (Table 3). The nonparametric Mann–Whitney U test, performed for all three sampling locations, points out that BPA concentrations are statistically higher in Paris (heavily urbanized) and Lognes (low-density urbanization) than in Fontainebleau (forest), while no significant difference is observed among the three sites for NPE.

Higher concentrations in CSOs and runoff compared with those measured in TAF highlight a clear concentration enrichment from atmosphere to catchment outlet. Such enrichments could originate from wastewater for CSOs or road and building leaching (Björklund et al. 2009; Bressy et al. 2011) for runoff since NPE and BPA are both used in building materials (concrete, plastics). For CSOs and runoff, the measured concentrations were similar to those reported for untreated wastewater (Zhou et al. 2010). These high

Table 3 Total atmospheric fallouts (TAF), combined sewer overflows (CSO), and runoff concentrations (ng/L)

Compound	TAF $n=20$ (ng/L)		CSO $n=8$ (ng/L)		Runoff $n=4$ (ng/L)	
	Min–max	Median–mean	Min–max	Median–mean	Min–max	Median–mean
BPA	10–180	52–66	917–2,098	1,410–1,466	287–1,224	510–635
4-NP	<LOQ–167	80–86	445–1,208	668–721	272–533	398–400
NP_1EO	<LOQ–65	21–24	357–1,257	598–768	47–401	144–184
NP_2EO	<LOQ–301	10–47	138–401	250–256	146–847	308–402
NP_1EC	2–63	15–17	220–591	251–312	193–426	225–267

<LOQ below the limit of quantification

concentration values underscore the need to treat wet weather effluent such as CSOs and runoff in order to prevent the short-term pollution of receiving water during wet weather periods, especially for BPA.

The comparison of CSOs and urban runoff, BPA, 4-NP, and NP_1EO has revealed significant differences in concentration, despite NP_1EC and NP_2EO concentrations showing statistical equivalence. Although these results suggest that the wastewater contained in CSOs may play a role in 4-NP, BPA, and NP_1EO concentrations, no correlation could be drawn between wastewater proportion and total concentrations of compounds in CSOs ($R^2 < 0.25$, $p > 0.05$ for all investigated compounds). However, a strong correlation between maximum flows discharged by CSOs (Q_{max} in cubic meters per second) and particulate contents could be determined for all target compounds ($R^2 \approx 0.80$, $p < 0.05$). The greater the Q_{max} value, the higher the particulate content. These results may confirm the existence of in-sewer sources, such as sewer deposit erosion during high-intensity events. For some events, Gasperi et al. (2010) demonstrated that in-sewer deposit erosion might actually play a major role in the polycyclic aromatic hydrocarbon or suspended solid contamination of CSOs.

Surface water

Concentration changes relative to the urbanization gradient

In order to track the influence of urban sources (WWTPs, CSOs, and runoff) on the Seine River, the dissolved organic carbon concentrations in Marnay, Bougival, and Meulan have been analyzed along with the target compounds. As expected, regardless of the measurement campaign, a significant increase in DOC concentrations was noticed between Marnay (median, 2.00(±0.02) mgC/L); Bougival (2.82(±0.03) mgC/L); and Meulan (3.34(±0.03) mgC/L; Wilcoxon signed-rank test, $p < 0.05$).

At the scale of all investigated sites, concentrations ranged from <11 ng/L to 154(±15) ng/L for BPA, from 28(±2) ng/L to 157(±11) ng/L for 4-NP, and from 2.1(±0.1) ng/L to 274(±11) ng/L for NP_1EC. These concentrations recorded in surface water inside the Paris Metropolitan area were similar to those

reported elsewhere in Europe (Arditsoglou and Voutsa 2010; Jonkers et al. 2010), but still lower than in the Seine River estuary (4-NP, 168 ng/L and NP_1EC, 544 ng/L; Cailleaud et al. 2007). Concerning the upstream sites at Orgeval (representative of rivers not influenced by urban activities) and Marnay (low-density urbanization), the concentrations of target compounds generally lie close to concentrations found in TAF. As a result, these sites could be considered representative of background pollution in the Seine River basin by target compounds.

Total concentrations with respect to the urbanization gradient (from agricultural site to heavily urbanized area) are depicted in Fig. 4.

Three compound groups can be distinguished.

In the first group, which includes BPA and NP_1EC, significant differences ($p < 0.05$) in concentrations between upstream sites (medians at Orgeval and Marnay: BPA, 20 (±2) ng/L; NP_1EC, 16(±1) ng/L) and downstream sites
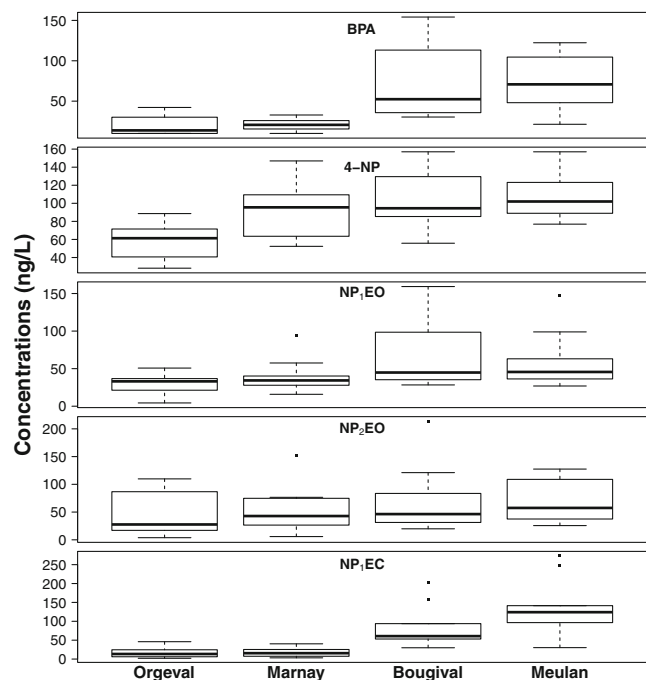

Fig. 4 Evolution of NPE and BPA concentrations relative to urbanization gradient

(Bougival and Meulan: BPA, 66($\pm$7)ng/L; NP_1EC, 124($\pm$5) ng/L) have been detected (Fig. 4). A similar evolution of concentrations from upstream to downstream stations of the Glatt River have been observed by Jonkers et al. (2009) for NP_1EC and by Voutsa et al. (2006) for BPA. These significant differences underscore the influence of urban sources on receiving water for BPA and NP_1EC. Whereas the Bougival and Meulan sites exhibit similar BPA concentrations, a significant difference could be noticed for NP_1EC between these two sites ($p < 0.05$). This difference is correlated with the large volume discharged by WWTP4, along with the high concentrations of NP_1EC (median, 842$\pm$ 34 ng/L). Though NP_1EC provided the least NPE biodegradation product at upstream sites, it became predominant at Meulan. Similarly, the prevalence of NP_1EC on a large range of organic pollutants in the Danube River has been reported by Loos et al. (2010).

The second group of compounds (NP_1EO and NP_2EO) reveals no significant difference in concentration across all sites (NP_1EO, 45($\pm$6)ng/L and NP_2EO, 50($\pm$4)ng/L). Concentration values found in the Orgeval River (agricultural basin) are statistically similar to those recorded at Meulan ($p > 0.05$). A wide variation in NP_1EO concentrations can be observed at Bougival (min, 20($\pm$3)ng/L; max, 213($\pm$30)ng/L; Fig. 4). The highest concentrations obtained at Bougival could be the result of activities ongoing at the Gennevilliers Port (the busiest in the entire Paris region), located upstream of the sampling site.

The last group (4-NP) reveals a contrasted pattern. A significant difference ($p < 0.05$) in concentrations between the Orgeval River (agricultural basin) and Seine River sites (Marnay, Bougival, and Meulan) is noticeable in Fig. 4. The occurrence of 4-NP in the Orgeval River (median, 61($\pm$4)ng/L) may be ascribed to either a mix of diffuse sources such as TAF (median, 80($\pm$6)ng/L) and groundwater (Latorre et al. 2003) or agricultural activities (Zgola-Grzeskowiak et al. 2009). On the other hand, the Marnay site may be partially influenced by urban sources and the biodegradation of precursors (NP_1EC, NP_1EO) into 4-NP along the Seine River. Nonetheless, no statistical difference between the Marnay (low-density urbanization), Bougival, and Meulan (heavily urbanized) sites could be determined ($p > 0.05$). This homogeneity of concentrations along the Seine River suggests that urban sources are probably not predominant for 4-NP in receiving water. Diffuse sources, such as TAF, groundwater or biodegraded 4-NP precursors, could thus play a key role in both the prevalence of 4-NP in the environment and the homogeneity of its concentrations along the Seine River. This observation is in agreement with a previous study on the Glatt River in Switzerland (Voutsa et al. 2006).

The concentrations of 4-NP found in the Seine River are lower than the environmental quality standard (EQS) established by Directive 2008/105/EC (i.e., 4-NP: 300 ng/L

based on the toxicity of single 4-NP) (Commission 2008). Actually, the highest concentration of 4-NP, recorded in Meulan (the most polluted site), was 151($\pm$11)ng/L. In spite of 4-NP concentrations lower than EQS and low concentrations of other EDC such as BPA (this study), OP (Cladiere et al. 2010), ethinylestradiol, and 17β-estradiol (Labadie and Budzinski 2005), Jugan et al. (2009) observed an estrogenic activity in the Seine River close to the Bougival site. Authors concluded that this estrogenic activity is strong enough to cause adverse effects on wildlife. This activity could be linked either to other endocrine disruptors or to the synergy of mentioned compounds, even at low concentrations (Brian et al. 2007).

Variation in pollutant concentrations depending on hydrological conditions

In Fig. 5, the dissolved concentrations of BPA, 4-NP, and NP_1EC measured at Marnay and Meulan have been compared to Seine River flow. During the sampling period, February 2010 to February 2011, Seine River flow varied from 13 to 228 m^3/s at Marnay and from 157 to 1,050 m^3/s at Meulan. Only the dissolved phase was investigated since, as previously mentioned, the distributions of BPA and NPE between dissolved and particulate phases mainly favor the dissolved phase. The prevalence of dissolved phase may be ascribed to the low SS concentrations in the Seine (6–20 mg/L at Meulan). Furthermore and depending on the specific compound group, the relationship between dissolved concentrations and Seine River flow enables a better understanding of the origins (point or diffuse sources) of NPE and BPA in the Seine River.

The first pattern, derived for NP_1EC, shows a sharp decrease in concentration as the Seine River flow increases, thereby suggesting that the NP_1EC stems from constant point sources diluted by the Seine River. This hypothesis is reinforced by the increase in NP_1EC concentration relative to the urbanization gradient, as mentioned above. For the most part therefore, NP_1EC probably originates from urban point sources such as WWTP effluent. While the NP_1EC found at Meulan could originate from Paris area urban sources, the concentrations at Marnay are likely to be caused by the city of Troyes (some 60,000 population), located about 50 km upstream of Marnay.

The second pattern, as reflected by the 4-NP and NP_2EO trends, features a decrease in concentration as Seine River flow is increasing. This decrease however is significantly less pronounced than the trend observed for NP_1EC. While the ratios between highest and lowest NP_1EC concentrations equal about 14 and 10 at Marnay and Meulan, respectively, the ratios for 4-NP do not exceed 3 at Marnay and 2 at Meulan. The influence of urban point sources might therefore be partially masked due to the broad contamination of

Fig. 5 Dissolved concentrations of target compounds (in nanograms per liter) vs. Seine River flow (cubic meters per second) at **a** Marnay and **b** Meulan
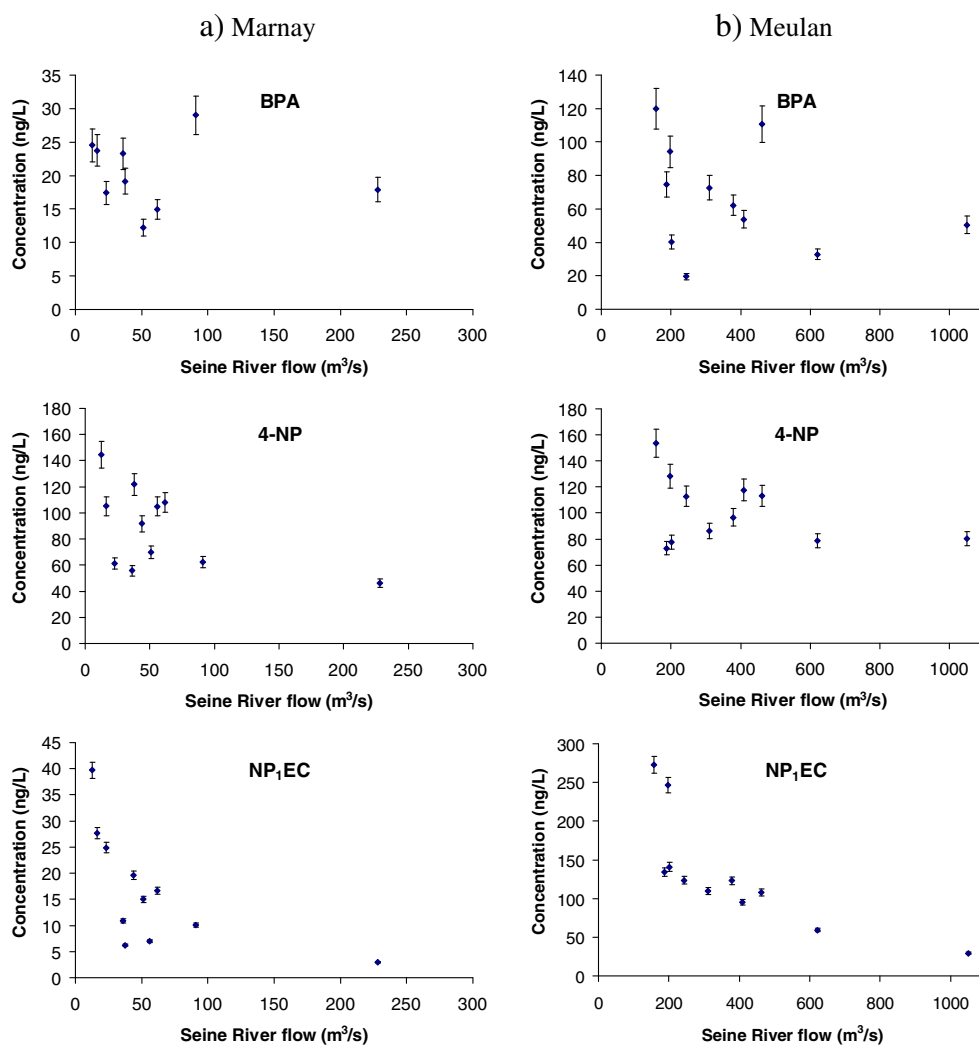
the Seine River basin by 4-NP. This broad contamination of the Seine River probably originates from the heavy use of NPE since 1960 as well as the persistence of 4-NP in the environment. Consequently, the diffuse sources mentioned above (namely TAF and groundwater) plus the biodegradation of precursors (NP_1EC, NP_1EO, and NP_2EO originate from urban sources) along the Seine River likely explain the avoidance of strong decreases in concentration with respect to hydrological conditions (lowest concentrations, 46(±3) ng/L at Marnay and 73(±5) ng/L at Meulan).

The final pattern, which includes BPA and NP_1EO, is difficult to interpret since the measurement uncertainties of these two compounds are significant and prevent drawing any relationship between dissolved concentrations and Seine River flow. It is impossible therefore to claim the predominance of either urban point sources or diffuse sources for BPA and NP_1EO. Nonetheless, in the case of BPA, the two highest concentrations at Meulan (111(±11) ng/L in January 2010 and 120(±12) ng/L in July 2010) were observed subsequent to rainy periods with considerable CSO

discharge (January, 250,000 m^3 dumped, July, 990,000 m^3). Consequently, wet weather urban sources, such as CSOs or urban runoff discharged into the Seine River, probably play a key role in the short-term contamination of the Seine River by BPA.

Conclusion

This study has investigated the presence of APE and BPA in urban sources and evaluated the impact of a heavily urbanized area, such as the Paris Metropolitan area, on receiving waters. Urban sources, i.e., WWTP effluent, combined sewer overflows, urban runoff, and total atmospheric fallout, were studied in detail. Depending on the urban source considered, differences in both concentrations and distribution patterns appear. WWTP effluent is characterized by the predominance of NPE, especially NP_1EC, due to the aerobic biological treatment of wastewater. No seasonal trend or significant difference in concentrations relative to the biological treatment of wastewater

could be noticed; only the effluent from WWTP4 (one of the world's largest WWTP) revealed higher concentrations of NPE and BPA, due to lower efficiencies on carbon and nitrogen removal than the area's other WWTPs. Conversely, wet weather urban sources (CSOs and runoff) are dominated by BPA, most likely as a result of wastewater contribution and the leaching of building materials.

This study has observed a significant influence from the Paris urban area on NP_1EC and BPA concentrations along the Seine River (respectively from $17(\pm1)$ to $124(\pm5)$ng/L and from $20(\pm2)$ to $66(\pm7)$ng/L). For all other NPE, no significant evolution of concentrations from upstream to downstream sites was noticed, hence suggesting that Paris' urban sources do not exert a major impact on receiving water and moreover underscoring the existence of diffuse sources at the scale of the Seine River basin. Also, the variation in dissolved concentrations vs. Seine River flow at the Meulan and Marnay sites indicates the importance of urban point sources for NP_1EC and reinforces the hypothesis of a widespread 4-NP dissemination at the Seine River basin scale. Unfortunately, the cases of BPA and NP_1EO could not be interpreted due to their measurement uncertainties.

To better understand the homogeneity of 4-NP concentrations along the Seine River as well as the river's NPE dynamics, a subsequent study should be carried out in order to asses the biodegradation rates of these compounds in receiving water. In closing, the diffuse sources most likely correlated with the origin of river flow must be clearly identified and better examined so as to understand the extent of NPE and BPA at the scale of the Seine River basin.

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