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# Discharges of endocrine disrupting chemicals by combined sewer overflows into receiving water: case-study of the Paris conurbation

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## Abstract

Alkylphenol ethoxylate (APEOs) and bisphenol A (BPA) are well known as endocrine disrupting compounds. Among sources of these compounds within receiving waters, wastewater treatment plant effluents have been widely studied. However, Although APEOs and BPA are regularly quantified within wastewater and to a lesser extent in runoff, few studies deal with their discharges by combined sewer overflows. In this context, this study was launched to investigate the concentrations and the mass loads discharged per year by CSO in the Seine River. Therefore, discharges occurring at the one of the most important CSOs of Paris conurbation from June to November 2010 were sampled. During this period, eight events were collected by automatic samplers and even mean sample were analyzed in laboratory using liquid chromatography coupled to a tandem mass spectrometry. The first results highlight an important contamination of CSOs by BPA and levels of APEOs close to those reported in wastewaters. At the annually scale and according to our mass load calculation, CSOs do not appear as a relevant source of BPA and APEOs within the Seine River.

## Keywords

Bisphenol A; nonylphenol; octylphenol; combined sewer; overflows

## INTRODUCTION

During the last two decades, alkylphenol ethoxylates (APEOs), mainly composed of nonylphenol ethoxylates (NPEOs, 80%) and octylphenol ethoxylates (OPEOs, 20%), were regularly pointed out by the literature. APEOs are of concern because their biodegradation products, 4-nonylphenol (4-NP) and 4-tert-octylphenol (4-t-OP) have endocrine disrupting properties and can also affect aquatic wildlife (Brian et al. 2007). APEOs are used in a large amount of industrial, domestic and commercial applications such as detergents, lubricating agents or oil additives. Similarly, bisphenol A (BPA), recognized as endocrine disrupter (Wetherill et al. 2007), is mainly used as a monomer in the manufacture of polycarbonate plastics or epoxy resins. Owing to these different uses, the annual consumption of APEOs were evaluated about 500,000 tons per year in 2002 (Ying et al. 2002), and about 450,000 tons per year for BPA according to Vandenberg et al. (2007). Due to their endocrine disrupting properties, occurrence and behaviour of these pollutants within wastewater treatment plants (WWTP) and surface water are well documented (Ahel et al. 1994; Cailleaud et al. 2007; Giger et al. 2009).

Many old capital cities as Paris (London, New York city, Washington) are drained by combined sewer systems. During wet weather, wastewater and city runoff are combined and transferred to WWTP. However, during huge storm events, combined sewer flows could be higher than

treatment flow capacity of WWTP and lead to combined sewer overflows (CSOs). As a result, combined wastewater and runoff are directly discharged, generally without any treatment, into surface waters. To the best of our knowledge CSOs are known as an important source of pollutants into surface waters (Gasperi et al. 2010) and more especially for pollutants highly removed by WWTP such as APEOs and BPA (Weyrauch et al. 2010). Unfortunately, even if pollutants are regularly detected in wastewater and runoff water (Jackson and Sutton 2008; Björklund et al. 2009), few studies deal with concentrations and loads of these pollutants within CSOs and impact of these events on surface water quality (Gasperi et al. 2008; Musolff et al. 2010). To fulfill this lack, the present study, supported by the OPUR research program, focuses on concentrations and mass loads discharged by CSOs in the Seine River at one of the most important combined sewer outfall of Paris conurbation sited in Clichy. On a second step, the total mass loads discharged during small, medium or high CSO events were compared to the total daily loads of the major WWTP of Paris conurbation (account for 80% of treated water released into the river) and the daily loads exported by the river. Finally, annual loads of APEOs and BPA discharged during CSOs were estimated and then compared to annual loads of these pollutants exported by the Seine River to the downstream part of its basin.

## MATERIAL & METHODS

### Site characterization and sampling procedure

In order to study concentrations of pollutants within CSOs, 8 events were collected at Clichy outfall, sited downstream of Paris city (Figure 1) from June to November 2010. The volume discharged profile for these eight events are shown in Figure 1, and the main characteristics such as total volume discharged ( $\text{m}^3$ ), conductivity ( $\mu\text{S}\cdot\text{cm}^{-1}$ ) and the wastewater proportion based on the conductivity measurement are reported in Table 1.

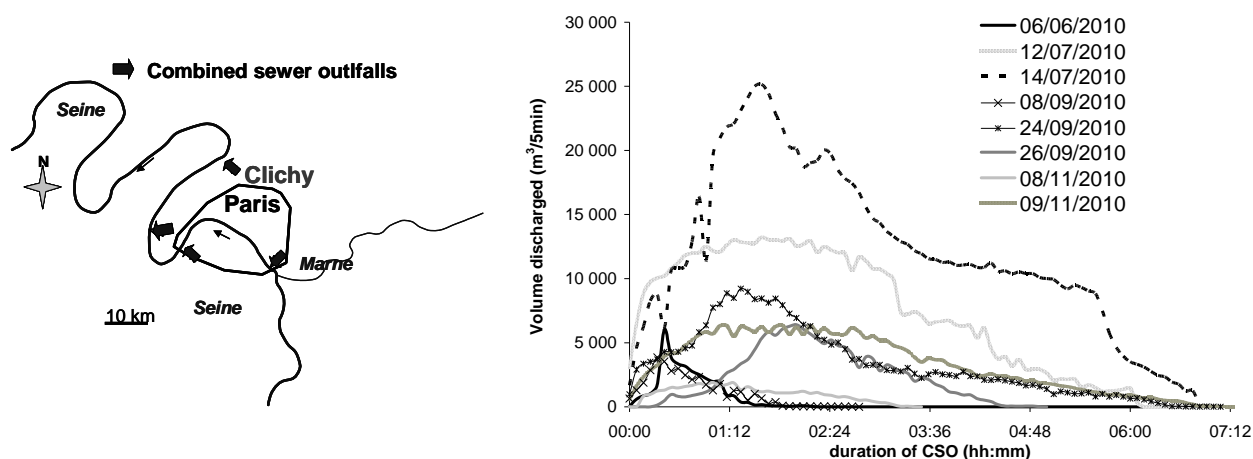


FIGURE 1: Sampling site and profile of CSO events sampled

Samples were collected by an automatic sampler linked to the trigger mechanism of the outfall pump, at fixed time step (five minutes) during a total sampling period of three hours. The automatic sampler was outfitted with twelve 1L glass bottles (fifteen minutes to fill one bottle). A manual mixing of the bottles proportionally to the flow volume was then performed to constitute a flow-weighted mean sample representative of the whole rain event.

**Table 1: Main characteristics for the eight CSO events sampled**

| Dates                               | 06/06   | 12/07   | 14/07     | 08/09   | 24/09   | 26/09   | 08/11   | 09/11   |
|-------------------------------------|---------|---------|-----------|---------|---------|---------|---------|---------|
| Volume (m <sup>3</sup> )            | 34,600  | 426,400 | 1,001,300 | 38,000  | 279,300 | 144,000 | 42,000  | 285,900 |
| Conductivity (μS.cm <sup>-1</sup> ) | 449     | 284     | 201       | 380     | 260     | 346     | 451     | 374     |
| % wastewater                        | 35 - 39 | 16 - 21 | 6 - 11    | 27 - 31 | 13 - 18 | 23 - 27 | 35 - 39 | 26 - 30 |

The total volume discharged, at Clichy outfall, during the eight CSO events sampled ranged from 34,596 m<sup>3</sup> on the 6<sup>th</sup> June to more than 1,000,000 m<sup>3</sup> on the 14<sup>th</sup> July 2010 (Table 1). The distribution of volumes discharged during these eight sampled events is in accordance with the distribution of volume discharged at Clichy outfall from the five last years. Indeed, during the five past years, 68% of CSO events were smaller than 150,000 m<sup>3</sup> discharged, 26% were between 150,000 and 500,000 m<sup>3</sup> and only 5% of events were higher than 500,000 m<sup>3</sup> of combined water released into receiving water.

Assuming that the conductivity of wastewater is close to 1,000 μS.cm<sup>-1</sup> and runoff conductivity between 100 - 200 μS.cm<sup>-1</sup>, High and low hypotheses on wastewater proportion were determined according to the conductivity of each flow-weighted water sample. Thus, the proportion of wastewater to CSOs ranged from 6-11% on the 14<sup>th</sup> July to 35-39% on the 8<sup>th</sup> November 2010 (Table 1). For the eight sampled CSOs the proportion of wastewater is fairly well negatively correlated to total volume discharged ( $R^2 = 0.72$ ). The higher the total volume discharged, the smaller the proportion of wastewater into combined sewer water.

### Dissolved phase extraction

Flow-weighted mean samples were filtered through a 0.45 μm porosity glass filter, in order to separate dissolved and particulate phases. After filtration, 100 ml of dissolved phase was spiked with 50 μl of an internal standards mixture (BPA-d6; n-OP-d17 and NP<sub>1</sub>EO-d2) for extraction follow-up and stored overnight at 4 °C to reach an equilibrium state. The extraction was performed on an Autotrace SPE Workstation (Caliper LifeScience) using an Oasis HLB (200 mg, 6 ml) cartridges. Firstly, the cartridges were conditioned with 10 ml of methanol and 10 ml of ultrapure water, and then samples were extracted at 5ml.min<sup>-1</sup> and neutral pH. After drying the cartridges under nitrogen stream during 35 minutes, elutions were performed with 12 ml of a mixture methanol/dichloromethane/ethylacetate (40:40:20, v/v). As method development of particulate extraction is not yet completed, results of this work will only focus on dissolved phase Pollutant analysis

### LC-MS-MS conditions

Additionally to BPA, nonylphenol mono and diethoxylate (NP<sub>1&2</sub>EO), octylphenol mono and diethoxylates (OP<sub>1&2</sub>EO), 4-NP, 4-t-OP and the nonylphenoxy acetic acid (NP<sub>1</sub>EC), resulting from aerobic biodegradation of APEOs, were analyzed (Table 2). Before analysis, extracts are spiked with internal standard for quantification (BPA-d16, n-NP, n-NP<sub>1</sub>EO and n-NP<sub>2</sub>EC). The analysis is performed by liquid chromatography coupled to a tandem mass spectrometry, LC-MS-MS (AQUITY UPLC / TQD, Waters). APEOs and BPA are separated on an AQUITY UPLC / BEH C<sub>18</sub> column, heated at 40 °C, with ultrapure water containing 4.5 mM NH<sub>4</sub>OH (A) and methanol containing 4.5 mM NH<sub>4</sub>OH (B) as mobile phase. Equilibration takes place with 50 % B at 0.4 ml.min<sup>-1</sup> and 10 μl are injected. The mass spectrometer is outfitted with an electrospray interface used in positive ionization mode (ESI+) for NP<sub>1-2</sub>EO and OP<sub>1-2</sub>EO and negative ionization mode (ESI-) for all other compounds. All compounds were detected in multiple reactions monitoring mode (MRM) with two mass transitions, for quantification and qualification. Cone voltage and collision energy were optimized for all compounds (Table 1).

**TABLE 2: compounds analyzed and optimized parameters for LC-MS-MS analysis**

| Compound           | M (g.mol <sup>-1</sup> ) | ESI Mode | Retention time (min) | Quantification   |                       | Qualification    |                       | Cone voltage (V) |
|--------------------|--------------------------|----------|----------------------|------------------|-----------------------|------------------|-----------------------|------------------|
|                    |                          |          |                      | Transition (m/z) | Collision energy (eV) | Transition (m/z) | Collision energy (eV) |                  |
| BPA                | 228                      | ESI-     | 1.82                 | 226.9 → 133.0    | 25                    | 226.9 → 212.2    | 25                    | 30               |
| 4-t-OP             | 206                      | ESI-     | 3.25                 | 205.5 → 134.2    | 17                    | 205.2 → 133.2    | 30                    | 45               |
| OP <sub>1</sub> EO | 250                      | ESI+     | 3.34                 | 268.2 → 113.4    | 10                    | 268.2 → 139.2    | 12                    | 20               |
| OP <sub>2</sub> EO | 294                      | ESI+     | 3.38                 | 312.3 → 183.3    | 10                    | 312.3 → 121.2    | 18                    | 20               |
| 4-NP               | 220                      | ESI-     | 3.52                 | 219.2 → 147.2    | 26                    | 219.2 → 133.2    | 26                    | 40               |
| NP <sub>1</sub> EC | 278                      | ESI-     | 2,47                 | 277.2 → 219.2    | 18                    | 277,2 → 133      | 38                    | 35               |
| NP <sub>1</sub> EO | 264                      | ESI+     | 3.58                 | 282.4 → 127.3    | 8                     | 282.4 → 85.4     | 15                    | 20               |
| NP <sub>2</sub> EO | 308                      | ESI+     | 3.61                 | 326.4 → 183.3    | 10                    | 326.4 → 71.2     | 20                    | 40               |

### Quantification and quality control

The quantification was performed using internal calibration based on areas relative to the internal standards. Internal standard n-NP was used for the quantification of NP, OP and n-OP-d17. Similarly, BPA-d16 was used for BPA and BPA-d6, n-NP<sub>1</sub>EO for OP<sub>1</sub>EO, OP<sub>2</sub>EO, NP<sub>1</sub>EO, NP<sub>2</sub>EO and n-NP<sub>1</sub>EO-d2 and n-NP<sub>2</sub>EC for NP<sub>1</sub>EC. For each compound, calibration curves were drawn and satisfactory determination coefficients were obtained ( $R^2 > 0.99$ ). Experimental blanks for SPE extractions, as well as mobile phase blanks were processed. No quantifiable contamination was observed. Finally, during a sequence, two calibration controls (lower and higher concentrations) were injected every 10 samples. A deviation between the control and the calibration curve lower than 20 % validates the quantification.

## RESULTS AND DISCUSSION

### Combined sewer overflow quality

Flow-weighted mean concentrations of APEOs and BPA are reported in Table 3. CSOs are featured by the predominance of BPA with concentrations ranging from 878 to 1,849 ng.l<sup>-1</sup> (median being 1,272 ng.l<sup>-1</sup>).

**TABLE 3: Concentrations (ng.l<sup>-1</sup>) of BPA and APEOs for the eight CSO events sampled**

| Dates              | 06/06 | 12/07 | 14/07 | 08/09 | 24/09 | 26/09 | 08/11 | 09/11 | Median |
|--------------------|-------|-------|-------|-------|-------|-------|-------|-------|--------|
| BPA                | 1,644 | 1,705 | 938   | 1,849 | 1,497 | 1,041 | 878   | 1,047 | 1,272  |
| 4-t-OP             | 61    | 88    | 64    | 117   | 71    | 38    | 45    | 63    | 63     |
| OP <sub>1</sub> EO | 66    | 56    | 44    | 138   | 68    | 21    | 117   | 8     | 61     |
| OP <sub>2</sub> EO | 13    | 14    | 14    | 24    | 17    | 14    | 14    | 13    | 14     |
| 4-NP               | 272   | 346   | 281   | 703   | 3,083 | 398   | 330   | 478   | 372    |
| NP <sub>1</sub> EC | 344   | 335   | 221   | 572   | 207   | 245   | 214   | 232   | 238    |
| NP <sub>1</sub> EO | 434   | 252   | 102   | 694   | 238   | 331   | 503   | 240   | 282    |
| NP <sub>2</sub> EO | 91    | 81    | 160   | 232   | 158   | 304   | 230   | 89    | 159    |
| Sum APEOs          | 1,280 | 1,172 | 886   | 2,482 | 3,843 | 1,331 | 1,452 | 1,123 | 1,305  |

The high concentrations of BPA in CSOs can be partially explained by high levels reported in wastewater (Hohne and Puttmann 2008). However, since no clear relation can be drawn between

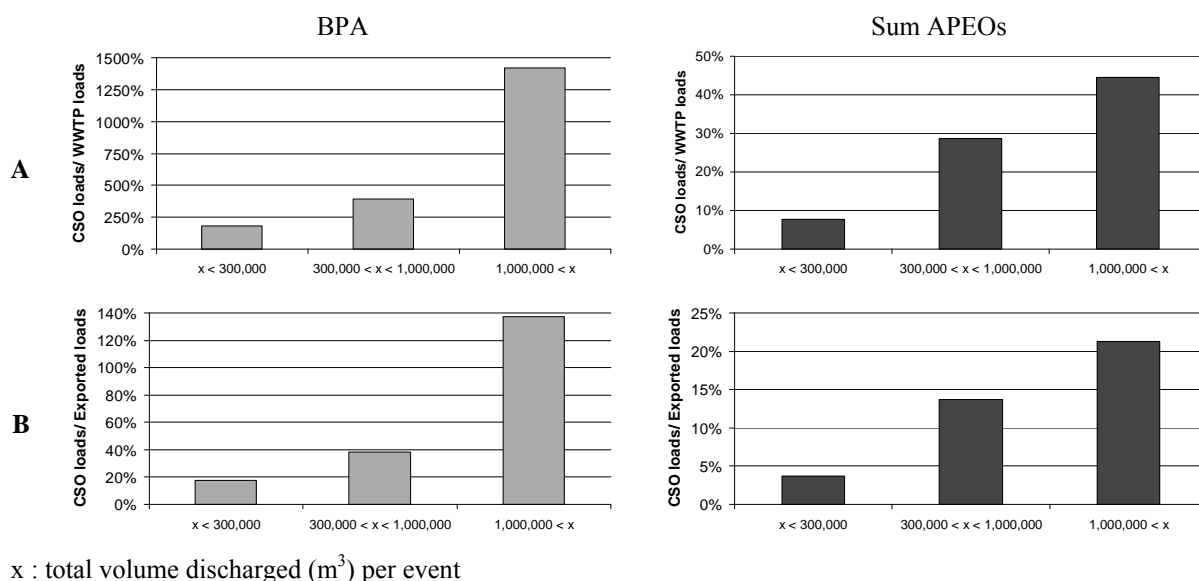
BPA concentrations and the wastewater proportion, contributions of other sources have also to be considered (sewer deposit erosion and runoff) (Gasperi et al. 2010). Considering that BPA is the main component of polycarbonate plastics widely used in building materials because of its properties and its high resistance (Kang et al. 2006), high level of BPA could be expected in runoff linked to the leaching of building materials. This hypothesis has to be confirmed since, currently, no study deals with occurrence of BPA in runoff.

For APEOs, total concentrations vary between 886 and 3,843 ng.l<sup>-1</sup>, median being 1,305 ng.l<sup>-1</sup> (Table 3). NPEOs are predominant (from 765 to 3,686 ng.l<sup>-1</sup>) and are approximately ten times more concentrated than OPEOs (from 73 to 176 ng.l<sup>-1</sup>). As for BPA, NPEOs and OPEOs concentrations are not correlated to proportion of wastewater meaning that other sources of contamination have to be considered. Among these sources, runoff can be probably considered as an important source since Bressy et al. (2011) reported high levels of 4-NP and 4-t-OP in runoff (470 and 38 ng.l<sup>-1</sup> respectively). According to the authors, these high levels may results from building and road leaching.

### Comparison of the CSO discharges to the daily WWTP and Seine River loads

This section aims to compare the CSO discharges at the scale of the rain events to the daily WWTP pollutant loads (Figure 2A), as well as, to the daily pollutants loads of the Seine River during dry weather periods (Figure 2B).

The pollutant loads discharged by CSOs were calculated based on CSO quality at Clichy outfall and total volume discharged during each event. Events can be classified as three categories, less than 300,000 m<sup>3</sup>, between 300,000 and 1,000,000 m<sup>3</sup> and more than 1,000,000 m<sup>3</sup> of combined water discharged per event.



**FIGURE 2: Comparison between loads (g.d<sup>-1</sup>) discharged during CSO event and A: WWTP daily loads; B: daily exported loads by the Seine River**

BPA loads discharged during small events, which are the most frequent in Paris conurbation, account for 184% of WWTP loads. Medium and high events could represent from 4 to more than 14 fold the total WWTP daily loads. These results highlight the importance of these kinds of event on total loads released into surface water, especially for highly removed pollutants (Weyrauch et al. 2010). During wet weather, BPA loads released by CSOs during small and medium events account respectively for 18 and 38% of dry weather exported loads by the Seine River, while high events represent more than 100% of these same exported loads. Thereby, small

and medium events could have a moderate impact on surface water loads, and high events may have a major impact on BPA loads exported by the Seine River during wet weather period.

For APEOs, the loads discharged by CSOs do not exceed 45% of WWTP loads even for high events, meaning that CSOs are not the main sources of APEOs during wet weather (Figure 2B). Moreover, along very high event, as 14<sup>th</sup> July 2010 which is the most important CSO event of the five past years, APEO loads represent less than 30% of exported loads by the Seine River. Smaller events are even less important. Medium and small events only account respectively for 14% and less than 5% of exported loads. These results clearly reveal that CSOs are not important point sources of APEOs, these events may have only low impact on receiving surface water quality

### Annual loads discharged into receiving water

Assuming that results found for Clichy outfall is representative of all CSOs of Paris conurbation, the median annual load discharged into receiving water by all CSO outfalls, at the scale of Paris conurbation, was determined (Table 4). Therefore, data about volume discharged from 2005 to 2010 were provided by the Interdepartmental Association for sewage Disposal in the Paris conurbation (SIAAP) and the Paris municipality. Table 4 reported the CSO annual loads of BPA and APEOs ( $\text{kg.y}^{-1}$ ) and compared them to the Seine River loads. For the Seine River pollutant loads, data were calculated according to twelve sampling campaigns performed during dry weather period on 2010.

Annually and according to our calculation, about  $22.8 \text{ kg.y}^{-1}$  of APEOS and  $17 \text{ kg.y}^{-1}$  BPA are discharged in the Seine River by CSOs (Table 4). Despite the high contamination of CSOs by BPA and APEOs, the contributions of these events to exported loads are rather low. Indeed, BPA discharged annually by CSOs only account for 3.7% of exported loads, while APEOs contributions do not exceed 1.3% for 4-NP. These low contributions of CSO to BPA and APEOs exported loads are probably a consequence of the small annual volume discharged by CSOs compared to annual water flow of the Seine River (only 0.2%).

**TABLE 4: annual loads discharged into receiving surface water ( $\text{kg.y}^{-1}$ )**

|                                       | $\Sigma$ Paris outfalls | $\Sigma$ WWTP effluents | Seine River   | %<br>$\Sigma$ Paris outfalls | %<br>$\Sigma$ WWTP effluents |
|---------------------------------------|-------------------------|-------------------------|---------------|------------------------------|------------------------------|
| Volume ( $\text{m}^3.\text{y}^{-1}$ ) | 14,443,482              | 772,705,000             | 9,486,374,400 | 0.2                          | 8.1                          |
| BPA                                   | 17.3                    | 33                      | 468           | 3.7                          | 7.1                          |
| 4-t-OP                                | 0.9                     | 28                      | 101           | 0.9                          | 27.6                         |
| OP <sub>1</sub> EO                    | 0.9                     | -                       | -             | -                            | -                            |
| OP <sub>2</sub> EO                    | 0.2                     | 33                      | 29            | 0.8                          | 115.4                        |
| 4-NP                                  | 9.8                     | 251                     | 777           | 1.3                          | 32.3                         |
| NP <sub>1</sub> EC                    | 4.0                     | 923                     | 1,007         | 0.4                          | 91.7                         |
| NP <sub>1</sub> EO                    | 4.4                     | 344                     | 469           | 1.0                          | 76.6                         |
| NP <sub>2</sub> EO                    | 2.9                     | 501                     | 449           | 0.5                          | 106.8                        |
| Sum APEOs                             | 22.8                    | 2,080                   | 2,831         | 0.8                          | 73.5                         |

- : no annual loads for the Seine River because of concentrations were regularly below quantification limit.

Finally, the annual loads released by WWTP into the Seine River were also estimated (Table 4). As reported in Table 4, the contributions of WWTP to exported loads by the Seine River are higher than contributions of CSOs. As APEOs studied are biodegradation products of alkylphenol polyethoxylates, processes occurring along WWTPs are sources of these compounds, especially for NP<sub>1</sub>EC (González et al. 2007). Indeed, while CSOs loads of OP<sub>2</sub>EO, NP<sub>1</sub>EC and NP<sub>1</sub>EO account respectively for 0.8%, 0.4% and 1.0% of exported loads, the annual loads released by WWTPs could explained the entire exported loads by the Seine River. Contrary, as BPA is highly biodegradable, annual loads of WWTP effluents are not much higher



than CSO loads, 7.1% for WWTP effluents against 3.7% for CSOs. At last, the sum of CSOs and WWTP effluents loads for BPA, 4-t-OP and 4-NP respectively account for 11%, 29% and 34% of exported loads, highlighting the existence of non urban sources. To the best of our knowledge, no clear comprehension of all pathways of introduction for these pollutants does exist. However, agricultural drain water and groundwater are pointed out by the literature as potential non-urban sources for APEOs and BPA (Zgola-Grzeskowiak et al. 2009; Musolff et al. 2010).

## CONCLUSIONS

As a conclusion, at the scale of a year, CSOs are not relevant sources of BPA and APEOs because of the low and discontinuous volumes discharged (0.2% of Seine River volume). Results have to be confirmed by integrating result on particulate fraction and by evaluating total mass loads. As distributions of APEOs and BPA between dissolved and particulate phases are link to the type of water, the influence of the particulate phase should not be the same for combined water and Seine river water.

However, CSO events could be considered as point sources of pollutants, during wet weather, more especially for pollutants highly removed by WWTP such as BPA. In order to improve our understanding of impacts of CSOs on surface water quality, sampling campaigns should be performed on the Seine River from the upstream of CSO outfalls to the outlet of Paris conurbation. These campaigns should enable us to follow the CSO pollution plumes into surface water, on the one hand, and evaluate the proportion of CSO loads on exported loads by the Seine River under wet weather conditions, on the other hand.

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