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Estimation of community-wide exposure to bisphenol A via water fingerprinting

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ABSTRACT

Molecular epidemiology in human biomonitoring allows for verification of public exposure to chemical substances. Unfortunately, due to logistical difficulties and high cost, it evaluates only small study groups and as a result does not provide comprehensive large scale community-wide exposure data. Wastewater fingerprinting utilizing metabolic biomarkers of exposure that are excreted collectively by studied populations into urine and ultimately into the community's wastewater, provides a timely alternative to traditional approaches. This study aimed to provide comprehensive spatiotemporal community-wide exposure to bisphenol A (BPA, including BPA intake) using wastewater fingerprinting. Wastewater fingerprinting was undertaken using high resolution mass spectrometry retrospective data mining of characteristic BPA human metabolism marker (bisphenol A sulphate), applied to a large geographical area of 2000 km^2 and a population of ~1.5 million served by 5 WWTPs (wastewater treatment plants) accounting for > 75% of the overall population in the studied catchment. Community-wide BPA intake was found to be below temporary tolerable daily intake (t-TDI) level of 4 $\mu g\,kg^{-1}\,day^{-1}$ set by the European Food Safety Agency (EFSA) suggesting overall low exposure at 3 WWTPs serving residential areas with low industrial/commercial presence. However, at two WWTPs serving communities with higher industrial/commercial presence, higher BPA sulphate loads corresponding to higher (up to 14 times) BPA intakes (exceeding 10 $\mu g\,kg^{-1}\,day^{-1}$ at one WWTP and reaching 50 $\mu g\,kg^{-1}\,day^{-1}$ at the second WWTP) were observed and they are likely linked with occupational exposure. Characteristic temporal variations of BPA intake were noted in most studied WWTPs with the lowest intake occurring during weekends and the highest during weekdays.

1. Introduction

BPA belongs to the group of endocrine disruptors (EDCs). EDCs are exogenous chemicals with the potential to interfere with the hormonal regulation, hence with the endocrine system, consequently affecting health and reproduction in animals and humans. In addition to developmental and reproductive effects, their potential for contributing to metabolic disorders such as obesity is drawing more and more attention (Casals-Casas and Desvergne, 2011). In the recent EU document '*State of the art assessment of endocrine disrupters*' (Kortenkamp et al., 2011) there is an urgent call for new approaches to establish further evidence for humans' exposure to EDCs, especially those chemicals which are still not regulated (such as many suspected EDCs in personal care and consumer products). Regulatory decisions about endocrine disruptors will have to rely on weight-of-evidence procedures which are yet to be established (Kortenkamp et al., 2011). It is therefore important to develop new tools which will allow for long term real-time monitoring of collective community-wide exposure to and effects from EDCs.

BPA is one of the most extensively studied EDCs because of its ubiquity and its suspected effects including hormonal activity (BPA acts as a weak oestrogen), effects on physical, neurological and behavioural development and potential carcinogenicity (Joint Fao Oms Expert Committee On Food Additives, 2010; Rochester, 2013). Because of a lack of evidence, the EFSA (European Food Safety Agency) has temporary lowered in 2015 the tolerable daily intake (TDI) from 50 $\mu g\,kg^{-1}\,day^{-1}$ (set originally in 2006 by using the no observed adverse effect level (NOAEL) of 5 mg/kg/day and an uncertainty factor of 100) to temporary t-TDI of 4 $\mu g\,kg^{-1}\,day^{-1}$ (until the outcome of a

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long-term study will help reducing uncertainties about potential health effects (European Food Safety Authority (EFSA), 2015a). Even though BPA is not intentionally added as ingredient to personal care products or in the food production process, its presence might be due to migration from polycarbonate containers, epoxy resins coating or to the degradation of the BPA-containing material (Hartle et al., 2016; Poustka et al., 2007). Geens et al. (2012) summarised all the dietary (generally considered to be the main source of BPA) and non-dietary exposure pathways (e.g. dust, thermal paper, dental materials, etc.). Christensen et al. (Christensen et al., 2012) concluded that the non-dietary exposure to be one-third of the cohort median exposure. Currently, public exposure to BPA is generally assessed using two approaches. The first one entails the monitoring of concentrations of contaminants in exposure media with exposure media contact rates (Lu et al., 2018). Interestingly, the European Union (Aschberger et al., 2010) and EFSA (European Food Safety Authority (EFSA), 2015b) found the dietary intake to vary significantly with age, indicating major exposure risk for infants (up to 13 $\mu g\,kg^{-1}\,day^{-1}$) and 1.5 $\mu g\,kg^{-1}\,day^{-1}$ for adults. The second approach relies on human biomonitoring. This approach measures the concentration of BPA and metabolites in biological fluids to back-calculate the overall exposure including both known and unknown sources (Dekant and Völkel, 2008). However, the main limitation of this approach, due to logistical difficulties and high cost, is the restricted size of study groups and inability to gather comprehensive information on the complexity of spatial and temporal exposure to BPA (Bonassi and Au, 2002). Therefore, the community lacks robust measures that can be used to gather real-time information on community-wide exposure to BPA.

Wastewater based epidemiology (WBE) utilizing water fingerprinting has the potential to overcome some of the above difficulties and is able to assess both internal and external combined exposure to EDCs. Exposure assessment can be undertaken through the analysis of both parent EDCs and their metabolites in wastewater and in the environment. There are several points of verification. For example, the presence of characteristic metabolites in wastewater is an indication that EDCs have found their way into the body (internal exposure resulting from e.g. consumption of EDCs with food, accidental ingestion due to EDCs absorbed onto indoor dust, or absorption through skin of EDCs used in PCPs) (Fig. 1).

The key concept of WBE is that human biotransformation products of both endogenous or exogenous compounds resulting from exposure to xenobiotic agents such as drugs, food toxicants and pollutants are collected and pooled by the wastewater system, and are transported to WWTPs, providing evidence of the extent of the exposure for the population (Fig. 1). As WWTPs serve well-defined populations, estimation of exposure to EDCs in a given period can be made based on the analysis of wastewater (usually with sensitive and selective mass spectrometry techniques) after taking into account human metabolism patterns (and possible transformation in sewers) of target analytes. WBE is currently utilised to determine community-wide illicit drug use (Ort et al., 2014; Thomas et al., 2012; Castrignanò et al., 2018). Earlier work with therapeutic drugs has demonstrated the close correspondence between known amounts consumed by the population and the amounts estimated from concentrations of metabolic drug residues in wastewater (Heberer and Feldmann, 2005; Kasprzyk-Hordern et al., 2009) or in surface waters (ter Laak et al., 2010). Table S1 gathers examples of WBE application in public health and lifestyle assessment. For example, Ryu et al. (2016) reported a Europe-wide monitoring of an oxidative stress biomarker, 8-iso-PGF2α and found that increased levels of 8-iso-PGF2α were observed at the inner-city level correlating with the degree of urbanization and levels of nicotine use. Rousis et al. (2017) studied community-wide exposure to pyrethroid pesticides in Italian cities. (Gracia-Lor et al. (2017) undertook Europe-wide profiling of caffeine use. Lopardo et al. (2017) identified new biomarkers of internal exposure to endocrine disruptors. González-Mariño et al. (2017) investigated community-wide exposure to phthalate plasticizers in Spain.

This is the first study aimed to estimate community-wide exposure to BPA (including BPA intake) using wastewater fingerprinting for BPA sulphate, a metabolic biomarker of BPA intake. This study covers a large geographical area of 2000 km^2 (including several rural and urban settlements) and a population of ~1.5 million accounting for > 75% of the overall population in the studied catchment.

2. Experimental

2.1. Materials and chemicals

Bisphenol A sulphate (BPA sulphate, CAS 847696-37-1) was purchased from Toronto Research chemicals (TRC, Canada) (Table S2). The internal standards used were: 4-chloro-3-methylphenol-d2 (QMX (UK)) and BPA-d16 (Sigma-Aldrich (Gillingham, UK)). Water was purified using a Milli-Q purification system from Millipore (Nottingham, UK). Methanol, formic acid (> 95%), HCl (concentrated), 1 M NaOH, 1 M NH_4OH, NH_4F, 2-propanol and bisphenol A (free BPA) were purchased from Sigma (UK) and Fisher (UK). All solvents used were of LC grade or higher. All the glassware was deactivated using 5% DMDCS (Sigma,

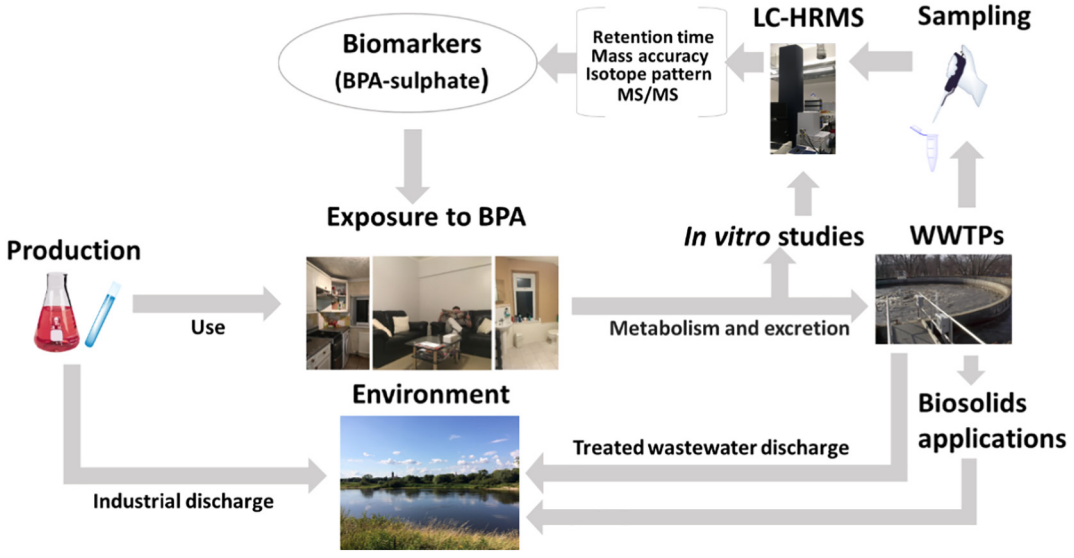

Fig. 1. Schematic representation of exposure to EDCs (BPA) in the household environment.

Table 1
Information about WWTPs investigated.

Site	A	B	C	D	E
Sewer residence time[a] (h)	< 0.5–4	< 0.5–4	< 0.5–9	< 0.5–2	< 1–24
Population served	37,000	67,870	105,847	17,638	909,617
Industrial contribution (%)	< 1	19	1	< 1	5
Mean flow ($m^3 d^{-1}$)	8242 ± 3085	11,202 ± 3202	24,875 ± 2167	2924 ± 199	153,061 ± 12,245

[a] Under summer (dry weather) flow.

UK) to prevent losses from analyte adsorption. The deactivation procedure consisted of washing the glassware once with 5% DMDCS followed by two washes with toluene and lastly three washes with methanol.

2.2. Sampling and sample collection

Wastewater (untreated, after physical screening) was collected between June and October 2015 from 5 major WWTPs (Table 1) contributing to one river catchment in the South-West UK and covering an area of approximately 2000 km^2 and the population of ~1.5 million (this constitutes > 75% of the overall population in the catchment).

Wastewater was collected on 7 consecutive days at each WWTP as volume proportional 24 h composites with sample collection frequencies of 10 min using ISCO 3700 portable samplers packed with ice (RS Hydro, Worcestershire, UK) (Petrie et al., 2015). All samples were transported on ice to the laboratory where they were frozen (−20 °C) awaiting further processing.

2.3. Biomarker stability in wastewater

Biomarker stability in unfiltered wastewater was performed in PVC bottles in aerobic conditions. Two wastewater aliquots were investigated in duplicate (4° and 17°) to simulate respectively sampler and room temperature. Change in concentration was monitored over 24 h, which is the maximum period of time a sample can remain in the sampler awaiting collection. Samples (100 mL each) were taken at the following interval times (0, 3, 12 and 24 h) and prepared for analysis as described below.

2.4. Sample preparation and analysis

All samples were analysed with two analytical methods. Targeted analysis of BPA was undertaken with UPLC-XevoTQD. Retrospective identification and quantification of BPA sulphate was undertaken with UHPLC-MaXis QTOF mass spectrometer. All details are provided below.

2.4.1. Analysis of free (unconjugated) BPA

Aqueous samples (50 mL) were filtered using GF/F glass microfibre filter 0.75 µm (Fisher Scientific, UK), adjusted to pH 7.5 ± 0.1 and spiked with 50 ng of internal standard (50 µL of a 1 µg mL^{-1} methanolic solution, BPA-D16) according to the procedure described by Petrie et al. (Petrie et al., 2015). Solid phase extraction (SPE) was performed using Oasis HLB sorbents (Waters, UK), which were conditioned using 2 mL MeOH followed by 2 mL H_2O at 1 mL min^{-1}. Samples were then loaded at 5 mL min^{-1} and dried under vacuum. Elution was performed using 4 mL MeOH at a rate of 1 mL min^{-1}. Methanolic extracts were subsequently dried under nitrogen using a TurboVap evaporator (Caliper, UK, 40 °C, N^2, < 5 psi). Dried extracts were reconstituted in 500 µL 80:20 H_2O:MeOH ready for analysis.

Suspended particulate matter (SPM) and sludge samples were obtained as a result of wastewater filtration through the GF/F filter. Treated by using microwave assisted extraction (MAE) as described by Petrie et al. (2015). Briefly samples were frozen, freeze-dried and 0.25 g samples were spiked with 50 ng of BPA-D16. Extraction was achieved by heating the samples at 110 °C using a 800 W MARS 6 microwave (CEM, UK) and 25 mL of 50:50 MeOH:H_2O (pH 2). MAE methanolic extracts were adjusted to < 5% of MeOH using H_2O (pH 2). SPE was performed using Oasis MCX cartridges (Waters, UK) conditioned with 2 mL MeOH followed by 2 mL H_2O (pH 2). Samples were loaded and dried as described previously. Elution was performed using 2 mL 0.6% HCOOH in MeOH. Once dried, extracts were reconstituted in 500 µL 80:20 H_2O:MeOH and filtered using pre-LCMS 0.2 µm PTFE filters (Whatman, Puradisc).

Analysis of all samples was undertaken using a Waters Acquity UPLC system (Waters, Manchester, UK) coupled with a Xevo TQD Triple Quadrupole Mass Spectrometer (Waters, Manchester, UK). A reversed-phase BEH C18 column (150 × 1.0 mm, 1.7 µm particle size) (Waters, Manchester, UK) was used with a 0.2 µm, 2.1 mm in-line column filter. Separation of analytes was achieved using 80:20 H_2O:MeOH mobile phase containing 1 mM NH_4F (mobile phase A) and 5:95 H_2O:MeOH also containing 1 mM NH_4F (mobile phase B) at 25 °C and flow rate of 0.04 mL min^{-1}. Mobile phase gradient was as follows: starting conditions: 100% A maintained for 0.5 min, reduced to 40% A over 2 min and to 0% A over a further 5.5 min and then maintained for 6 min before returning to starting conditions (maintained for further 8.4 min to allow re-equilibration; total run time was 22.5 min). The injection volume was 15 µL. The Xevo TQD Triple Quadrupole Mass Spectrometer was equipped with an electrospray ionisation source. Nitrogen was used as the nebulising and desolvation gas, and argon as the collision gas. Analysis was performed in negative ionisation mode with a capillary voltage of 3.20 kV, the desolvation temperature of 400 °C and the source temperature of 150 °C. The cone gas flow was 100 L $h^{-1}$and the desolvation gas flow was 550 L h^{-1}. The multiple reaction monitoring (MRM) transitions for BPA were 227.3 > 212.1 *m/z* (quantifier, 22 eV collision energy) and 227.3 > 132.7 m/z (qualifier, 25 eV collision energy). The MRM transition for BPA-D16 was 241.1 > 223.1 m/z (20 eV collision energy). The cone voltage for both BPA and BPA-D16 was 40 V. Detailed discussion regarding the method and its performance can be found elsewhere (Petrie et al., 2015) and in Tables S3A–S5A.

2.4.2. Analysis of BPA sulphate

Liquid samples were spiked with internal standard (25 µL of a solution 1 µg mL^{-1}) and filtered using GF/F glass microfibre filter 0.75 µm (Fisher Scientific, UK) and solid phase extraction (SPE) was performed using Oasis HLB (Waters, UK) according to the procedure described elsewhere (Lopardo et al., n.d.) and above (Section 2.4.1). The SPE extraction recovery was evaluated at two different concentrations in duplicate.

Samples were analysed using Dionex Ultimate 3000 HPLC coupled with a Bruker Maxis HD Q-TOF according to (Lopardo et al., n.d.). Briefly, a multi-step gradient was used to separate the analytes at a flow rate of 0.4 mL min^{-1} on a BEH C18 column (50 × 2.1 mm, 1.7 µM, Waters UK) using mobile phase A (1 mM ammonium fluoride in water) and mobile phase B (methanol). The mass spectrometer was equipped with an ESI source and was operated in both positive and negative ionisation mode. The source settings were as follows: capillary voltage was set at 4.5 kV, the end plate offset was set to 500 V, a pressure of 3 Bar was used for the nebulizer gas, the drying gas (nitrogen) flow was

11 L min^{-1} and the drying temperature was set at 220 °C. Analysis was run in both full scan mode (MS) and broadband collision induced dissociation (bbCID) mode. Calibrant solution was injected before each run. Quality control samples were run every 10 samples. For details see (Lopardo et al., n.d.) and Table S3B.

BPA sulphate was identified using post-acquisition data mining of analysed wastewater samples as discussed in Lopardo et al. (2018). Commercially available BPA sulphate standard was used to confirm the presence of BPA sulphate in wastewater samples as well as to establish a calibration curve for subsequent quantification of BPA sulphate. 4-chloro-3-methylphenol-d2, already present in analysed samples, was used as an internal standard for BPA-sulphate quantification.

Method performance was verified as follows. Linearity was established by triplicate injection of a 13-point calibration curve ranging in concentration from 0.01 to 100 ng mL^{-1}. Instrumental detection limits (IDLs) and instrument quantitation limits (IQLs) were calculated according to the lowest concentration which gave a signal to noise ratio of ≥3 and ≥10 respectively. Recovery of target chemicals was determined by spiking crude wastewater at a concentration of 4 and 50 ng L^{-1}. BPA and BPA sulphate validation parameters are listed in Tables S4B and S5B.

2.5. Daily mass loads and BPA intake

Daily mass loads of BPA sulphate (mg day^{-1}) were calculated by multiplying the concentrations (mg L^{-1}) found in a 24 h composite raw wastewater sample by the daily wastewater flow rate (L day^{-1}). Total free BPA concentrations in raw wastewater were calculated after taking into account both the amount of free BPA adsorbed onto solid particulate matter (SPM) and the amount present in the liquid fraction. Mass loads (mg day^{-1}) were then normalised to the number of people served by each WWTP (mg day^{-1} 1000 inhabitants^{-1}), in order to compare results between different WWTPs. Population-wide BPA intakes (mg day^{-1} 1000 inh^{-1}) were calculated using the following equation:

$$BPA_{intake}[mg\ day^{-1}1000inh^{-1}] = \frac{(conc.\ x\ V)x\ CF}{P}$$

where: conc. is the concentration of BPA sulphate (mg L^{-1}) in influent wastewater, V is the volume of wastewater received by the WWTP per day (L day^{-1}), P stands for the population served by the WWTP and CF is the correction factor. CF was calculated taking into account the molecular mass ratio between free BPA and BPA sulphate and BPA sulphate excretion ratio. There are published studies that have investigated the presence of BPA sulphate in urine. Three papers were considered in this study. Ye et al. (2005) found that BPA sulphate represented 21% of BPA metabolites in urine (n = 30). Ho et al. (2017) analysed 140 urine samples finding that BPA sulphate represented 6.25% of BPA metabolites. Thayer et al. (Thayer et al., 2015) instead observed that only 3% of the total BPA-d6 ingested by 14 volunteers was excreted in urine as BPA sulphate and that the total administered dose recovered in urine was 84–109%, with > 90% of BPA-d6 excreted as metabolites within 24 h from consumption. Study by Thayer et al. (2015) is the only one providing truly representative excretion factors. However, it focussed on a very limited dataset (only 14 volunteers contributed). CF for BPA sulphate was therefore calculated as 0.16, using 8.41% as BPA sulphate excretion factor (weighed mean of the percentage of BPA sulphate excreted against the number of urine samples in all three studies). However, as we acknowledge uncertainties linked with studies by Ye et al. (2005) and Ho et al. (2017), we also calculated daily intake using CF of 0.45 corresponding with 3% excretion as reported by Thayer et al. (Thayer et al., 2015) only.Population-wide BPA intakes were then normalised to the weighted mean of the population weight (mg/ kg day^{-1}) using the following formula:

$$BPA_{intake}[mg\ kg^{-1}day^{-1}] = \frac{BPA_{intake}[mg\ day^{-1}1000inh^{-1}]}{56.3}$$

where 56.3 is the weighted mean of the UK population weight in kg after taking into account as follows: 4.5 kg for babies at 1–2 months, 7 kg for babies at 4–6 months, 11 kg for children 1.5–4.5 years old, and 60 kg for adults (Mørck, 2011). Percentage of people n years old in the UK was sourced from UK Office for National Statistics (ONS).

Daily BPA intake was evaluated for weekdays (Tuesday-Friday) and weekends (Sat-Sun). Monday measurements were not taken into account in weekday vs. weekend comparison due to metabolism of BPA requiring up to 24 h after exposure for metabolite excretion in urine (Thayer et al., 2015). Statistical analysis was undertaken using F-Test Two-Sample for Variances followed by either *t*-Test: Two-Sample Assuming Equal Variances or *t*-Test: Two-Sample Assuming Unequal Variances.

3. Results and discussion

3.1. BPA sulphate as a biomarker of BPA intake

Based on our previous work (Lopardo et al., 2018), BPA sulphate was selected as a characteristic metabolic biomarker of human internal exposure to BPA. A literature search revealed no known non-metabolic sources of BPA sulphate in the environment. Analysis of solid and liquid fractions of wastewater collected from an activated sludge tank revealed that only a minor fraction of BPA sulphate adsorbs onto solid matter (< 7%), which indicates that the analysis of only aqueous fraction of wastewater for BPA sulphate is required in order to estimate BPA intake. Low sorption of BPA sulphate was expected given the greater hydrophilicity of BPA sulphate compared to free (unconjugated) BPA. However, to ensure high accuracy of measurements, internal standards were added to wastewater samples before filtration through GF/F filters, which is in the presence of suspended particulate matter (SPM), in order to correct for possible sorption of analytes to SPM. In addition, a study of BPA sulphate stability confirmed its high stability in wastewater over 24 h sampling time at 4 °C (Fig. S1), validating its applicability as a biomarker of public BPA exposure via wastewater fingerprinting.

3.2. Daily mass loads of free BPA and BPA sulphate in wastewater

Free BPA and BPA sulphate were identified and quantified (Table S6) in all collected samples (Fig. 2 and Table 1). BPA sulphate was found at higher concentrations than free BPA. This is, as discussed above, due to extensive metabolism of BPA which leads to excretion of BPA mostly as conjugated metabolic residues. Its total concentrations ranged from 0.7 to 1.5 μg L^{-1} for WWTPs A, C and D and from 1.5 to 60 μg L^{-1} for WWTPs B and E. Concentrations of free BPA ranged from 0.5 to 0.9 μg L^{-1} in WWTPs A and C, while in WWTPs B, D and E they ranged from 1.3 to 100 μg L^{-1}. Recorded SPM concentrations ranged from 0.03 to 0.27 μg g^{-1} in WWTPs A, C and D while in WWTPs B, and E they ranged from 0.1 to 15 μg g^{-1}.

Due to sampling being conducted in WWTPs located in 5 different geographical locations (Fig. 2) within the same river catchment, considerable spatial variability in daily free BPA and BPA sulphate loads could be observed, and because sampling at each WWTP was operated over 7 consecutive days (Wednesday to Tuesday), it was possible to gather information about both spatial and temporal variability for a full week.

Free BPA loads ranged from 179 to 597 mg day^{-1} 1000 inh^{-1} at WWTPs A, C and D. Statistical analysis revealed that temporal (week vs weekend) free BPA changes at these WWTPs are not statistically significant (p = 0.2897, 0.2737 and 0.3639 for WWTPs A, C and D respectively). At WWTP E, free BPA loads were on average two orders of magnitude greater during weekdays when compared to weekends. Weekday vs. weekend loads denoted 9897 and 1517 mg day^{-1} 1000 inh^{-1} respectively (p = 0.0452). WWTP B showed the greatest, statistically significant (p = 0.0325), variability with free BPA loads

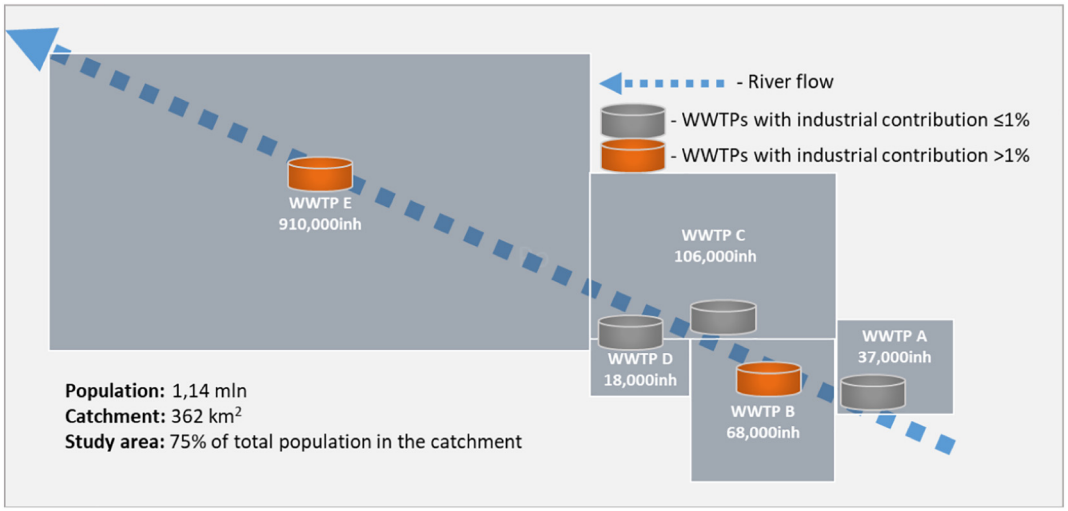

Fig. 2. Studied catchment area and 5 major WWTPs serving 5 cities.

averaging at 3913 mg day^{-1} 1000 inh^{-1} during weekday and 539 mg day^{-1} 1000 inh^{-1} during weekend (Fig. 3). These loads correspond to concentrations of free BPA in wastewater up to 100 µg L^{-1} which is considerably more than what is generally observed since free BPA can be usually found in urban wastewater at concentrations around 1 µg L^{-1}. The reason why such high concentrations of free BPA were observed at WWTPs B and E might be the contribution of industrial wastewater as suggested by Petrie et al. (Petrie et al., 2019). Higher free BPA loads were in fact observed downstream from a paper producing plant from Fuerhacker in 2003 (Fuerhacker, 2003). As expected, high loads of free BPA at WWTPs B and E corresponded, due to elevated exposure, with high loads of BPA sulphate as discussed below (Fig. 3).

BPA sulphate loads ranged from 147 to 916 mg day^{-1} 1000 inh^{-1} at WWTPs A, C and D (Fig. 3) whereas average loads at WWTPs B and E were an order of magnitude greater (from 495 to 17,003 mg/day 1000 inh^{-1}, Fig. 3). BPA sulphate loads recorded during weekly sampling campaigns were relatively constant (RSD % ≤ 40%) at WWTP A and C (Fig. 3, p = 0.2931 and 0.4266 respectively). On the other hand, statistically significant temporal BPA sulphate loads variability was observed at WWTPs D and E (RSD% ≥ 50%, 0.0140 and 0.0160 for WWTPs D and E respectively), indicating generally lower exposure during weekends compared to weekdays (Fig. 3). WWTP B showed the greatest variability in BPA sulphate due to loads being more than an order of magnitude greater on two weekdays. However, the weekday pattern vs weekend was found to be irregular and statistically insignificant (p = 0.0746), possibly due to accidental release of free BPA leading to elevated exposure levels on two weekdays only, as explained later.

3.3. Estimation of public BPA intake via wastewater fingerprinting

The community-wide BPA intake (internal exposure) was estimated using the WBE approach as described in the Experimental section. Calculated loads of BPA sulphate at WWTPs A, C and D corresponded to an estimated BPA intake (Fig. 3 and Table S8) which was consistently below TDI level set by EFSA (at 4 µg kg^{-1} day^{-1}) suggesting overall low exposure.

Weekly average population A, C and D intake was consistent and accounted for: 1.9, 1.8 and 1.3 µg kg^{-1} day^{-1} respectively which is in line, albeit at the higher end, with published literature focussing on estimated BPA intake through dietary exposure, e.g. 0.08–1.5 µg kg^{-1} day^{-1} (NTP, 2008), indicating other exposure sources. On the other hand, WWTPs B and E were characterised by higher loads corresponding to higher, up to 14 times, intakes that were above the TDI threshold for several days during the sampling period. WWTP B results (48 µg kg^{-1} day^{-1} intake on Wednesday) indicate that accidental release of free BPA linked with elevated exposure occurred on or just before Wednesday. Interestingly, WWTP E shows overall high exposure (on average exceeding 10 µg kg^{-1} day^{-1}), possibly as a result of occupational exposure.

Diet is considered the primary source of exposure to BPA for the general population (NTP, 2008), although non-dietary contributions should not be ignored as they can provide a significant contribution to the overall daily intake, e.g. infant and child exposure can reach 14 µg kg^{-1} day^{-1}, and occupational exposure could be as high as 100 µg kg^{-1} day^{-1} as reported by NTP in powder paint workers (NTP, 2008). Indeed, it can be seen from Table 1 that WWTPs A, C and D serve smaller, more residential communities and receive very low industry derived wastewater accounting for ≤1%. This could explain lower daily loads of both free BPA and BPA sulphate. On the other hand, WWTP B and E have higher contribution from industrial wastewater accounting for 19 and 5%. Additionally, WWP E is a large treatment works serving almost 1mln people. Higher free BPA levels and resultant higher exposure and high free BPA loads were therefore expected in the communities served by WWTP B and E.

It is also interesting to note that characteristic temporal variations of BPA intake were observed in three out of five studied WWTPs, namely WWTP B, D and E with the lowest intake occurring during weekends and the highest during weekdays. Population B served by WWTP B showed average intake on Wed–Thur denoting 38.5 µg kg^{-1} day^{-1} and only 1.4 µg kg^{-1} day^{-1} on Sat–Sun (p = 0.0745). Population E served by WWTP E showed statistically significant increase in intake during weekdays averaging at 17.7 µg kg^{-1} day^{-1} when compared to average weekend intake of 5.4 µg kg^{-1} day^{-1} (p = 0.0160). A similar pattern was observed in WWTP D with average BPA intake accounting for 2.1 µg kg^{-1} day^{-1} during weekdays and only 0.4 µg kg^{-1} day^{-1} during weekend (p = 0.0181). The population contributing to WWTP A and C showed much lower, or no significant difference in BPA intake (WWTP A: 1.7 vs 1.6 µg kg^{-1} day^{-1}, p = 0.2447 and WWTP C: 1.7 vs 1.7 µg kg^{-1} day^{-1}, p = 0.4265). This is an important observation indicating that public exposure to BPA is much higher during working days. There are several possible reasons for this including healthier diet during weekends vs higher exposure of workers in industrial settings during weekdays. Indeed, temporal variation in BPA exposure in sites B and E confirms yet again our hypothesis that higher levels of BPA intake in these locations are due to occupational exposure.

The above results indicate that only under certain scenarios daily BPA intake exceeds EFSA TDI levels. However, one needs to note that in this manuscript weighted mean average excretion values sourced from

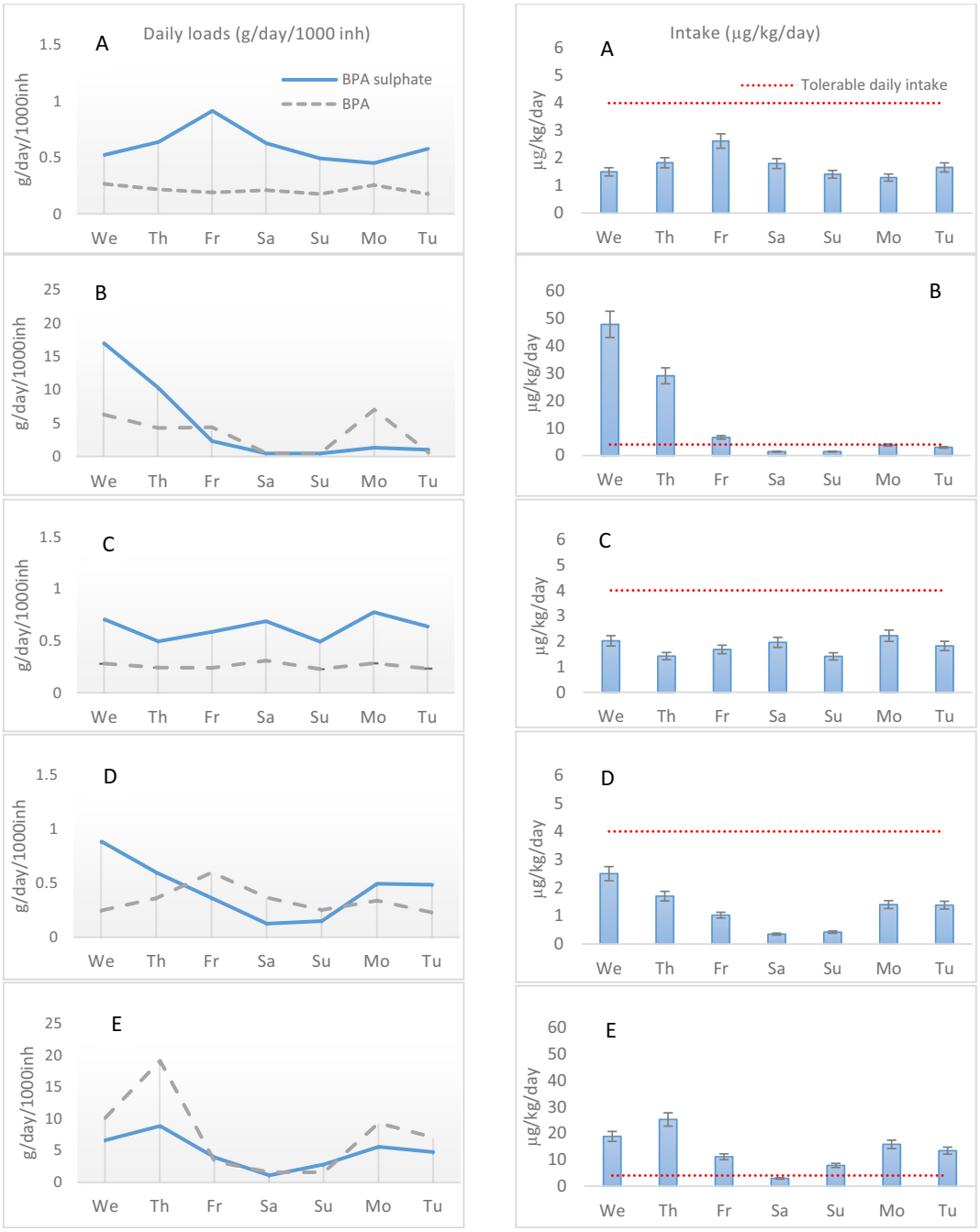

Fig. 3. Temporal variability of population normalised daily loads of BPA and BPA sulphate (left) and estimated BPA daily intake (right) in the 5 WWTPs investigated during 7 consecutive days. Note: different Y-axis scales showing the extent of spatial variability in BPA/BPA sulphate levels/exposure represented by different WWTPs. Average %RSD, < 10%.

all three published studies on BPA sulphate biomonitoring were used (Ye et al., 2005) (Ho et al., 2017) (Thayer et al., 2015). These studies reported different BPA sulphate excretion levels ranging from 21% (n = 30) (Ye et al., 2005) and 6.25% (n = 140) (Ho et al., 2017) to only 3% (n = 14) (Thayer et al., 2015). It is also important to note that only Thayer et al. (2015) undertook metabolite profiling after administration of BPA-d6. The other two studies focussed on the analysis of a large pool of urine samples without providing any knowledge of actual exposure levels and therefore probably overestimated the percentage of BPA sulphate excretion. In order to understand uncertainties of our calculations, we also calculated daily BPA intake using only 3% excretion (CF factor of 0.45). The results are presented in Table S7. The results clearly indicate significantly elevated BPA exposure levels exceeding the safe TDI level of 4 µg kg^{-1} day^{-1}. Such a result is concerning and warrants further study which will be undertaken in due course mainly in the context of refining CF values followed by a larger scale human biomonitoring study.

3.4. Cumulative BPA loads versus daily BPA intake

Thayer et al. (2015) reported in the most recent study on BPA metabolism in 14 volunteers using BPA-d6 (to eliminate other sources of BPA than consumed for the study) that 95% ± 7.1% of total BPA-d6 (dose administered) was excreted in urine in 24 h with only 0.11% ± 0.19% (of total d6-BPA) as unconjugated d6-BPA and the remaining dose as conjugated dBPA: 87% ± 6.9% as BPA-d6

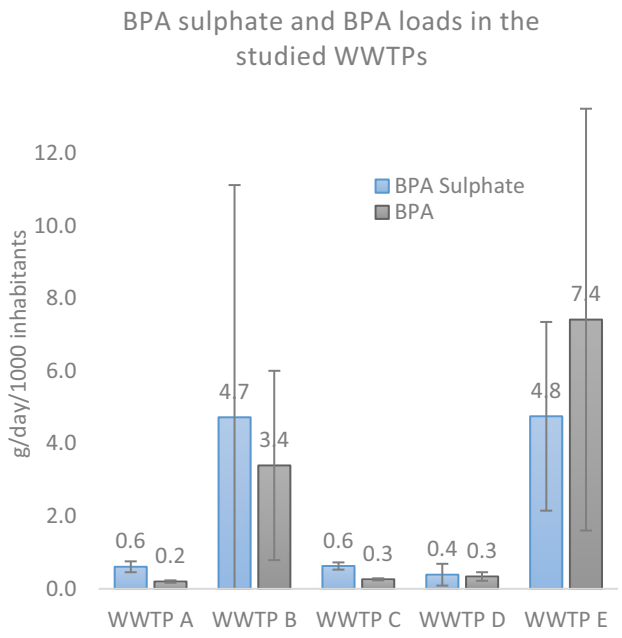

Fig. 4. Comparison between weekly average BPA and BPA sulphate loads in the 5 WWTPs investigated.

glucuronide and 3 ± 2.3 as BPA-d6 sulphate. In this study we used 8.41% as BPA sulphate excretion factor (based on weighed mean of the percentage of BPA sulphated excreted against the number of urine samples in the different studies) (see Section 2.5). Therefore, it could be assumed that only a small fraction (< 1%) of the total BPA load would be present in wastewater as free BPA with < 10% as BPA sulphate and > 90% as BPA glucuronide. BPA glucuronide was not measured in this study neither was hydrolysis of BPA glucuronide undertaken during sample preparation. As can be seen in Fig. 4, free BPA constitutes larger percentage of the overall BPA load than expected from metabolism studies. There are two main reasons for this: (1) direct disposal of unconsumed BPA leading to increased loads of free BPA, (2) possible cleavage of free BPA from BPA glucuronide due to microbial hydrolysis in wastewater; this aspect will require further studies. Interestingly, comparison of population normalised daily free BPA loads (accounting for liquid and solid particulate matter) and BPA sulphate loads revealed that, despite higher levels of free BPA than expected due to metabolism only, the presence of free BPA corresponds to its actual intake (measure via BPA sulphate). For example, as can be seen in Fig. 4, average weekly loads of BPA are higher in WWTP B and E than WWTP A, C and D. This is also the case with BPA sulphate loads. Interestingly, higher external exposure and subsequent intake in populations B and E, is likely linked with higher industrial wastewater contribution at WWTPs B and E. WWTP B receives the highest contribution reaching 19%, including food manufacture, toiletry manufacture and paint stripping (Table 1). Site E receives 5% of its wastewater from several industrial contributors including commercial laundrette, food manufacturing and vehicle washing, packaging industry, food warehousing and distribution. Overall, site B, as the most industrialised of all five sites studied, indicates, yet again, the highest public exposure to BPA and is likely linked with occupational exposure. This indicates that both free BPA and BPA sulphate could serve as good indicators of intake. Simply, the higher the external exposure (free BPA) the higher the intake (BPA sulphate).

This study reports the strong potential of using wastewater fingerprinting as a tool to estimate public exposure to industry and household derived chemicals with the aim of evaluating community-wide risks, verifying spatiotemporal trends in exposure and indicate possible sources of exposure (e.g. link occupational exposure with industrial activity in the studied area). This cannot be currently achieved with the available epidemiology, risk assessment and human biomonitoring tools due to their limitations including: small study groups resulting from the high cost of biomonitoring and significant lag time in obtaining data. On the other hand, the main limitation of this approach is that it cannot, and it is not intended to, estimate individual exposure to chemicals for example in children vs adults or man vs woman. It can however provide estimates at a community level, and as a result, is has the potential to become a powerful tool for large scale screening studies identifying communities at risk and leading to further more comprehensive work of at a more localised scale.

4. Conclusions

This study has provided the first comprehensive spatiotemporal community-wide exposure assessment to BPA (including BPA intake) using wastewater fingerprinting. This study was also the first to verify spatiotemporal changes in BPA intake including occupational exposure. Community-wide BPA intake was found to be on average 1.6 μg kg^{-1} day^{-1} in more residential areas (WWTPs A, C and D) with low industrial contribution, which is consistently below the t-TDI level set by EFSA (at 4 μg kg^{-1} day^{-1}), and suggesting overall low exposure in these locations. However, at two WWTPs (B and E), higher BPA sulphate loads corresponding to higher intakes (exceeding 10 μg kg^{-1} day^{-1} in the population served by WWTP E and reaching 50 μg kg^{-1} day^{-1} in the population served by at WWTP B), that were well above (up to 14 times higher) the TDI threshold were observed and they are likely linked with occupational exposure. Characteristic temporal variations of BPA intake were observed in WWTPs B, D and E with the lowest intake occurring during weekends and the highest during weekdays. This is an important observation indicating that public exposure to BPA is much higher during working days. There are several possible reasons for this including healthier diet during weekends vs higher exposure of workers in industrial settings during weekdays (especially in more industrialised communities' B and E). Further work will need to be undertaken to fully understand sources of free BPA and BPA sulphate that might be contributing to the biomarker concentration levels in the studied catchment. Furthermore, additional studies are required to fully understand human metabolism of BPA with the aim of providing a robust correction factor for BPA intake calculation. Finally, better tools for estimating population size are critically needed to reduce uncertainties linked with population size/movement in studied catchment areas.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2018.12.048.

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Supplementary material

Estimation of community-wide exposure to bisphenol A via water fingerprinting

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Tables

Figures

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Table S1. WBE for public health and lifestyle assessment

Compounds	Biomarker	Health status - comments	References
Illicit and abused drugs	Drugs and urinary metabolites Enantiomeric enrichment	Lifestyle	(Andrés-Costa et al., 2017; Castrignanò et al., 2018, 2017; Jones et al., 2014; Kasprzyk-Hordern et al., 2008; van Nuijs et al., 2011)
Alcohol	Ethyl sulphate, Isoxanthohumol, Resveratrol Metabolites	Lifestyle	(Reid et al., 2011)(Ryu et al., 2016a)
Caffeine	1-methylxanthine, 7-methylxanthine	Lifestyle/population biomarker	(Gracia-Lor et al., 2017; Senta et al., 2015)
Tobacco	cotinine, tobacco specific nitrosamines, menthol, 8-iso-prostaglandin F2α	Lifestyle	(Lai et al., 2017)(Ryu et al., 2016b)
Pharmaceuticals	Specific pharmaceuticals and their metabolites Enantiomeric enrichment	Health	(Camacho-Muñoz et al., 2016; Jones et al., 2014; Kasprzyk-Hordern et al., 2008; Petrie et al., 2015)
Endocrine disruptors	Urinary metabolites	Health	(Lopardo et al., 2018)
Pesticides	Specific pesticides and their metabolites Enantiomeric enrichment	Health	(Rousis et al., 2017a, 2017b, 2016)
Phthalates	Urinary metabolites	Health	(Andrés-Costa et al., 2017; Castrignanò et al., 2018, 2017; Jones et al., 2014; Kasprzyk-Hordern et al., 2008; van Nuijs et al., 2011)
Oxidative stress	8-iso-prostaglandin F2alpha	Health	(Reid et al., 2011)(Ryu et al., 2016a)
Cancer	mtDNA	Health	(Gracia-Lor et al., 2017; Senta et al., 2015)

Table S2 General information about BPA and BPA sulphate

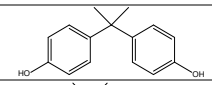
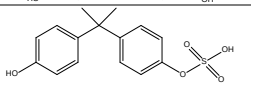
Chemical	CAS number	Molecular Weight	**Log Kow**	Formula	Structure
Bisphenol A	80-05-7	228.3	3.32	$C_{15}H_{16}O_2$	
Bisphenol A sulphate	847696-37-1	308.1		$C_{15}H_{16}O_5S$	

Table S3A UHPLC-QqQ parameters used in the determination of BPA

Chemical	Rt (min)	Precursor ion	Product ion 1	CV(V)/ CE(eV)	Product ion 2	CV(V)/ CE(eV)	Ion ratio	Internal standard
Bisphenol A sulphate	8.96	227.3	212.1	40/22	132.7	40/25	1.60±0.08	Bisphenol A-D16

Table S3B UHPLC-QTOF parameters used in the determination of BPA sulphate

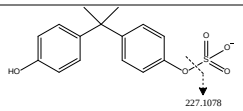
Chemical	Rt (min)	m/z $[M-H]^-$	Mass error (ppm)	bbCID	Fragment Structure
Bisphenol A sulphate	6.8	307.0646	<10	307.0646 > 227.1078	

Table S4A UHPLC-QqQ instrument performance parameters

Analyte	IS	Linearity Range [µg L^{-1}]	R^2	Accuracy* [%]	Precision* [%]	IDL [µg L^{-1}]	IQL [µg L^{-1}]
Bisphenol A	Bisphenol A-D16	0.1-600	0.997	104.6	1.3	0.03	0.10

**concentration levels: 10,100, 500 ng/mL used for precision and accuracy*

Table S4B UHPLC-QTOF instrument performance parameters

Analyte	IS	Linearity Range [µg L^{-1}]	R^2	Accuracy* **[%]**	**Precision* [%]**	IDL [µg L^{-1}]	IQL [µg L^{-1}]
Bisphenol A Sulphate	4-chloro-3-methylphenol-D2	1.39 - 103.4	0.9972	98.3	2.1	0.41	1.39

**concentration levels: 0.1, 5 and 100 ng/mL used for precision and accuracy*

Table S5A SPE-UHPLC-QqQ and MAE-SPE-UHPLC-QqQ method performance parameters

Analyte	Wastewater			SPM/Sludge		
	SPE recovery [%]*	MDL [ng L^{-1}]	MQL [ng L^{-1}]	MAE recovery [%]*	MDL [ng g^{-1}]	MQL [ng g^{-1}]
BPA	112.2	0.85	2.79	95.7	0.27	0.88

** concentration levels: 100, 1000 ng/L and 50, 100 ng/g used for SPE and MAE recovery*

Table S5B SPE-UHPLC-QTOF method performance parameters

Analyte	Wastewater		
	SPE recovery [%]*	MDL [ng L^{-1}]	MQL [ng L^{-1}]
BPA sulphate	63.7±6.3	1.6	5.5

** based on duplicate extractions at two concentration levels*

Table S6 BPA and BPA sulphate concentrations in wastewater (weekly averages and daily means).

Analyte			Concentration				
			WWTPA	WWTPB	WWTPC	WWTP D	WWTP E
BPA	Weekly average	Aqueous [µg L^{-1}]	0.2±0.06	1.5±1.3	0.1±0.07	0.4±0.6	6.2±5.3
		SPM [µg g^{-1}]	0.9±0.2	20.4±15.7	0.98±0.1	1.7±0.2	37.2±29.3
	Wed	Aqueous [µg L^{-1}]	1.3	41.2	1.0	1.6	51.9
		SPM [µg g^{-1}]	0.3	3.6	0.2	0.1	7.8
	Thu	Aqueous [µg L^{-1}]	1.1	27.0	1.0	2.0	99.3
		SPM [µg g^{-1}]	0.21	2.22	0.12	0.2	15.5
	Fri	Aqueous [µg L^{-1}]	0.9	29.7	1	1.62	19.41
		SPM [µg g^{-1}]	0.2	2.3	0.1	1.9	0.2
	Sat	Aqueous [µg L^{-1}]	0.9	3.6	1.2	1.9	8.6
		SPM [µg g^{-1}]	0.2	0.3	0.3	0.1	1.1
	Sun	Aqueous [µg L^{-1}]	0.8	2.8	1.1	1.4	8.6
		SPM [µg g^{-1}]	0.2	1.5	0.03	0.1	1
	Mon	Aqueous [µg L^{-1}]	0.5	36.2	0.9	1.8	36.8
		SPM [µg g^{-1}]	0.1	0.1	0.1	0.1	9.9
	Tue	Aqueous [µg L^{-1}]	0.6	2.3	0.8	1.4	35.5
		SPM [µg g^{-1}]	0.1	0.1	0.1	0.1	7.2
BPA sulphate	Weekly average	Total [µg L^{-1}]	3.1±1.2	32.4±42.5	2.7±0.4	2.8±1.8	28.0±13.8
	Wed	Total [µg L^{-1}]	3.1	121.1	2.9	6.0	38.4
	Thu	Total [µg L^{-1}]	3.8	70.3	2.2	3.6	53.0
	Fri	Total [µg L^{-1}]	5.1	17.0	2.6	2.2	23.8
	Sat	Total [µg L^{-1}]	3.3	3.5	3.1	0.7	6.5
	Sun	Total [µg L^{-1}]	2.7	4.0	2.3	0.8	17.5
	Mon	Total [µg L^{-1}]	1.1	6.9	3.1	2.9	27.8
	Tue	Total [µg L^{-1}]	2.3	3.9	2.5	2.8	28.8

Table S7 Estimated BPA intake using a correction factor (CF) of 0.45 based only on Thayer et al. BPA sulphate excretion percentage (Thayer et al., 2015).

		WWTP A	WWTP B	WWTP C	WWTP D	WWTP E
BPA intake (µg/day/kg)	Wed	5.43±0.5	157.56±16	5.36±0.5	8.05±0.8	50.94±6
	Thu	6.82±0.6	91.44±9.1	4.14±0.4	4.82±0.5	70.24±7
	Fri	9.16±0.9	22.13±2.2	4.95±0.5	2.90±0.3	31.49±3.1
	Sat	5.90±0.6	4.57±0.5	5.79±0.6	0.97±0.1	8.59±0.9
	Sun	4.87±0.5	5.22±0.5	4.33±0.4	1.10±0.1	23.19±2.3
	Mon	1.94±0.2	9.00±0.9	5.73±0.6	3.86±0.4	36.79±3.6
	Tue	4.06±0.4	5.08±0.5	4.75±0.5	3.71±0.4	38.17±3.8

Table S8 Estimated BPA intake using a correction factor (CF) of 0.16 based on Thayer et al.; Ho et al.; and Ye et al., BPA sulphate excretion percentage (Thayer et al., 2015); (Ho et al., 2017); (Ye et al., 2005).

		WWTP A	WWTP B	WWTP C	WWTP D	WWTP E
BPA intake (µg/day/kg)	Wed	1.94±0.2	46.27±5.6	1.92±0.2	2.87±0.3	18.19±1.8
	Thu	2.43±0.2	30.66±3.1	1.48±0.1	2.67±0.3	25.08±2.5
	Fri	3.27±0.3	7.90±0.8	1.77±0.2	1.72±0.2	11.25±1.1
	Sat	2.11±0.2	1.63±0.2	2.07±0.2	0.35±0.03	3.07±0.3
	Sun	1.74±0.2	1.86±0.2	1.55±0.2	0.39±0.04	8.28±0.8
	Mon	0.69±0.7	3.21±0.3	2.05±0.2	1.38±0.1	14.14±1.4
	Tue	1.45±0.1	1.81±0.2	1.70±0.2	1.33±0.1	13.63±1.3

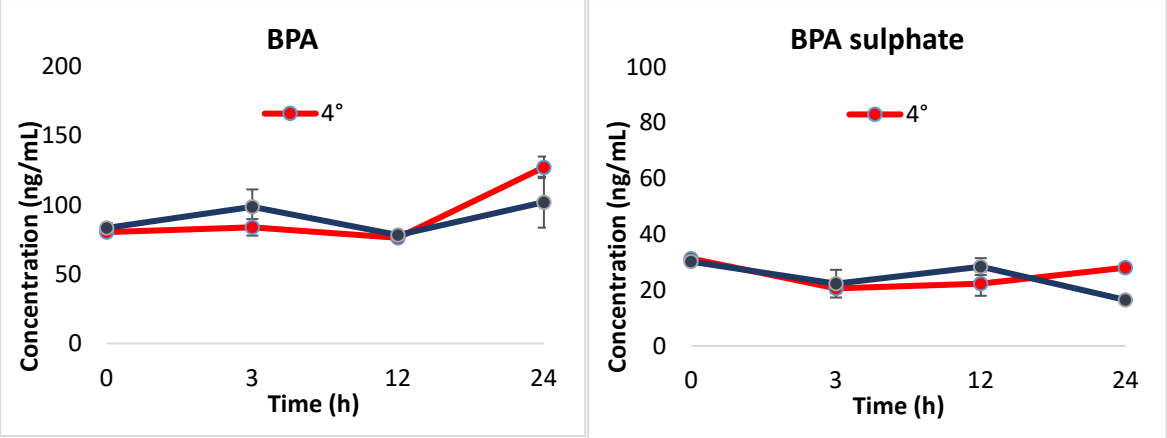


Figure S2 BPA and BPA sulphate concentration change over a 24h stability study