Mining the chemical information on urban wastewater : monitoring human exposure to phosphorus flame retardants and plasticizers

Environmental Measurements Methods

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Frederic Been, Michiel Bastiaensen, Foon Yin Lai, Katerina Libousi, Nikolaos S. Thomaidis, Lisa Benaglia, Pierre Esseiva, Olivier Delémont, Alexander L.N. van Nuijs, and Adrian Covaci

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[1]*Frederic Been, [1]Michiel Bastiaensen, [1]Foon Yin Lai, [2]Katerina Libousi, [2]Nikolaos S. Thomaidis, [3]Lisa Benaglia, [3]Pierre Esseiva, [3]Olivier Delémont, [1]Alexander L. N. van Nuijs, [1]Adrian Covaci

1) Toxicological Centre, University of Antwerp, Universiteitsplein 1, 2610 Wilrijk, Belgium
2) Laboratory of Analytical Chemistry, Department of Chemistry, University of Athens, Panepistimiopolis Zografou, 15771 Athens, Greece
3) Ecole des Sciences Criminelles, University of Lausanne, 1015 Lausanne-Dorigny, Switzerland

* corresponding author. Email: frederic.been@uantwerpen.be, Tel: +32 3 265 27 43

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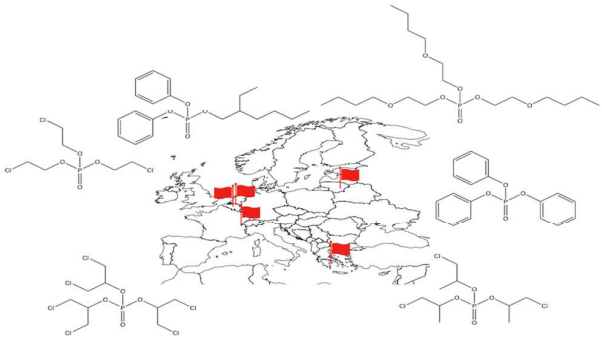

Abstract

At the individual level, exposure to contaminants is generally assessed by biomonitoring through the analysis of specific biomarkers in biological matrices. Yet, these studies are costly and logistically demanding, limiting their applicability to monitor population-wide exposure over time and space. By focusing on a selection of exposure biomarkers to phosphorous flame retardants and plasticizers (PFRs), this study aims at exploring the possibility of using wastewater as a complementary source of information about exposure. Wastewater samples were collected from five cities in Europe and analysed using a previously established method. Substantial differences in biomarker levels were observed between the investigated catchments, suggesting exposure differences. Time trends in the PFR biomarkers between 2013 and 2016 were found to agree with results from human biomonitoring studies and reports about production volumes. Using Monte Carlo simulations, average urinary concentrations were estimated. These were generally higher compared to results from human biomonitoring surveys. Various explanations for these differences (i.e., other excretion routes, external sources and different sampling approaches) were formulated. We provide evidence that wastewater analysis provides unique information about geographical and temporal differences in exposure, which would be difficult to gather using other monitoring tools.

Introduction

Phosphorous flame retardants and plasticizers (PFRs) are commonly used in consumer goods such as textiles, electronics, furniture, paints, polyvinyl chloride plastics, polyurethane foams and lubricants to hinder combustion and to comply with existing safety standards[1–3]. Since the use of polybrominated diphenyl esters has been restricted, the use of PFRs has been increasing, with an estimated global consumption of 680,000 tonnes in 2015[3]. Generally, two groups of PFRs are used, namely halogenated (generally used as flame retardants) and non-halogenated PFRs (generally used as plasticizers)[4]. However, because these chemicals are added to products rather than being chemically bonded, they can be released during usage by volatilization, leaching and abrasion[5]. Substantial levels have been found in indoor environments (i.e., dust and air), due to their release from building materials, furniture, textiles and electronics, as well as outdoor environments (e.g., waste- and surface water, sediments, biota and soil)[1,3]. Owing to their ubiquitous presence, humans are potentially exposed to these chemicals *via* various routes. Air, dust inhalation, dietary intake (i.e., food and drinks), dermal and hand-to-mouth contact have been mentioned as potential pathways[6–8]. Various studies have investigated the toxicity of PFRs, highlighting potential negative health endpoints. For instance, tris(chloroethyl) phosphate (TCEP), tris(2-chloroisopropyl) phosphate (TCIPP), tris(1,3-dichloroisopropyl) phosphate (TDCIPP) and tris(2-butoxyethyl) phosphate (TBOEP) have either been shown to be carcinogenic in animals or are suspected carcinogens for humans[3,9]. Neurotoxic effects were linked to tri-n-butyl phosphate (TNBP) and triphenyl phosphate (TPHP)[10,11]. Endocrine disrupting effects were associated to exposure to TDCIPP[12], whereas tris(2-chloroisopropyl) phosphate (TCIPP) and TDCIPP were linked to dermatitis[12].

Analysis of biomarkers, i.e. parent compounds and/or phase I or phase II metabolites, in biological matrices, particularly urine, is currently the most common approach to monitor

human exposure to PFRs. These studies generally target specific cohorts of the population (e.g., children and pregnant women) and aim at providing estimates of exposure. Whilst being essential to relate exposure to health endpoints and other epidemiological/demographic factors, in particular for vulnerable populations, these studies are impractical when seeking to monitor spatial and temporal exposure on a broader scale. In particular, logistic (i.e., collection of samples from countless individuals), ethical and economical (i.e., large monitoring programs incur in high costs related to sampling and analysis) aspects limit their applicability. Furthermore, inclusion of a temporal dimension is often difficult as it requires longitudinal sampling schemes. Yet, obtaining spatial and temporal data about exposure in a timely manner is crucial to monitoring the wellbeing of communities and to detect potential threats. Planning of strategies to mitigate negative health effects as well as resources allocation would greatly benefit from the generated information. In this light, mining the chemical information available in wastewater could substantially facilitate the collection of spatio-temporal data about human exposure to contaminants[13]. For more than a decade, wastewater analysis, also referred to as "wastewater-based epidemiology" (WBE), has been successfully implemented to gather information about the consumption of illicit drugs in communities[14]. The approach relies on the analysis of licit and illicit drug metabolites in wastewater samples collected at the influent of wastewater treatment plants (WWTPs). Being an aggregate of human excretions, wastewater potentially contains a much larger number of relevant biomarkers, both endogenous and exogenous[15]. The possibility of obtaining relevant data about community-wide health from wastewater, such as exposure to environmental contaminants, has recently been recognised[15].

Owing to the abundance of potentially relevant biomarkers present in wastewater, this study aims at investigating the possibility of using wastewater analysis as an innovative tool to monitor, at the population level, exposure to PFRs. The approach provides complementary

data which could be used to rapidly identify risks for human health, guide the planning of direct measurements (e.g., targeted biomonitoring campaigns), improve resources allocation and help plan and evaluate adequate responses (e.g., mitigation campaigns, policy changes). To achieve this, exposure biomarkers of PFRs were measured in influent wastewater samples using a previously established analytical protocol[16]. Four goals were set in this study: (i) Geographical features in exposure were assessed by analysing samples collected in five different WWTPs located in Europe; (ii) Temporal trends were investigated by analysing historical samples, covering the period 2013-2016, collected at one WWTP; (iii) Spatial and temporal features were investigated statistically and compared to both human biomonitoring data and production volumes; (iv) Finally, an attempt was made to estimate per capita average urinary concentrations based on mass loads of chemicals measured in wastewater, which were then compared to results from human biomonitoring studies.

Materials and methods

Target compounds

Table 1 provides a list of the target analytes (i.e., metabolites) and their corresponding parent compounds. Only PFR metabolites were analysed in the context of this study.

Table 1: Target PFR metabolites and their corresponding parent compounds. a) DPHP has been shown to be only a minor metabolite of EHDPHP[17]. However, it can also be used as a plasticizer, although at lower volumes compared to TPHP[18]. Furthermore, it can be formed from PFRs other than TPHP and should thus be considered a biomarker of exposure to aryl-PFRs[17]. b) In vitro liver metabolism studies suggested a low clearance of TCEP. Therefore, it is recommended to add TCEP as a target in biomonitoring studies[19,20].

Parent compound	Metabolite
2-ethylhexyldiphenyl phosphate (EHDPHP)	2-ethyl-5-hydroxyhexyl diphenyl phosphate (HO-EHDPHP) 2-ethylhexyl phenyl phosphate (EHPHP) Diphenyl phosphate ([a]DPHP)
Tris(2-butoxyethyl) phosphate	Bis(2-butoxyethyl) phosphate (BBOEP)

(TBOEP)	Bis(2-butoxyethyl) 3′-hydroxy-2-butoxyethyl phosphate (HO-TBOEP) 2-hydroxyethyl bis(2-butoxyethyl) phosphate (BBOEHEP)
Tris(2-chloroisopropyl) phosphate (TCIPP)	bis(2-chloro-isopropyl) phosphate (BCIPP) 1-hydroxy-2-propyl bis(1-chloro-2-propyl) phosphate (BCIPHIPP)
Triphenyl phosphate (TPHP)	Diphenyl phosphate ([a]DPHP) Hydroxyphenyl phenyl phosphate (HO-DPHP)
Tris(1,3-dichloroisopropyl) phosphate (TDCIPP)	Bis(1,3-dichloroisopropyl) phosphate (BDCIPP)
Tris(chloroethyl) phosphate (TCEP)	Tris(chloroethyl) phosphate ([b]TCEP)

107

108 Wastewater samples

109 Wastewater samples were collected from the influent of WWTPs in Antwerp (BE), Brussels
110 (BE), Athens (GR), Geneva (CH) and Vilnius (LT) in 2016 or 2017 (see Table S1 for further
111 details). For Antwerp, additional historical samples collected between 2013 and 2015 were
112 included. All samples were 24-hour composites, which were frozen immediately after
113 collection and shipped as such to our laboratory for analysis. The estimated number of
114 inhabitants served by each WWTP (census-based) and flow measurements were provided by
115 the WWTP personnel.

116

117 Sample preparation and chemical analysis

118 Wastewater samples were processed and analysed following the procedure established by
119 Been et al.[16], with the addition of three compounds (i.e., BCIPP, BDCIPP and BBOEP)
120 which were not included in the original method. Briefly, wastewater samples (100 mL) were
121 thawed, immediately spiked with mass labelled reference standards (final concentration 50 ng
122 L^{-1}) and then centrifuged at 3000 RCF for 20 min. The supernatant was further filtered
123 (GF/A, 1.6 μ m, Whatman, Sigma-Aldrich) and acidified to pH 4-5 using HCl (37%). The

124 acidified samples were then extracted using solid-phase extraction (SPE, Bond-Elut C18
125 cartridges, 3 mL, 200 mg, Agilent) and analysed using a validated liquid chromatography-
126 tandem mass spectrometry (LC-MS/MS) method[16]. The analytical protocol was validated
127 also for the newly added compounds (see Supporting Information, Sections 1-3) and the
128 stability of the latter compounds in raw wastewater was confirmed (see Supporting
129 Information, Section 4).
130
131 Data analysis
132 Population-normalised loads for each analyte (in mg day^{-1} 1000 $inhabitants^{-1}$) were obtained
133 by multiplying measured concentrations by the corresponding flows and then further dividing
134 these by the size of the population served by the WWTP. Spearman correlations between
135 measured analyte concentrations (only 2016 and 2017 data) were computed as the data were
136 not normally distributed.
137 Pairwise differences in concentrations between analytes and between locations were tested
138 using Wilcoxon rank sum test. Temporal trends in analyte concentrations were investigated
139 using Mann-Kendall trend test (α = 0.05). Monte Carlo simulations were used to estimate
140 average urinary concentrations of target analytes based on measured analyte concentrations.
141 In particular, daily urine production per person (in L day^{-1}) was estimated using a gamma
142 distribution as determined by Rauch et al.[21]. Measured concentrations, daily flows and
143 population estimates were modelled using normal distributions (Table 2). Average urinary
144 concentrations were computed as follows:

$$\overline{U_i} = \left(\frac{WW_i \times Q}{Pop}\right) \Big/ V_u$$

145 where $\overline{U_i}$ is the estimated urinary concentration of analyte *i* per void (in ng mL^{-1}); WW_i is the
146 concentration of analyte *i* measured in wastewater (in ng L^{-1}); Q is the daily wastewater flow

147 (in L day^{-1}); *Pop* is the size of the served population and V_u is the daily urine production per
148 person (in mL day^{-1}). The latter term is used to estimate the total amount of urine produced
149 per day by the population served by the WWTP. All calculations and statistical tests were
150 performed using *R* software[22].

151

152 Table 2: Parameters used to estimate average urinary concentrations using Monte Carlo simulations. μ = mean,
153 SE = standard error.

Variable	Distribution	Parameters	Description
Analyte concentration (WW_i)	Normal, N (μ, SE^2)	μ = measured concentration (wastewater) SE = 20% of measured concentration	SE was estimated based on method validation results[1]
Flow (Q)	Normal, N (μ, SE^2)	μ = daily flow SE = 20% of daily flow	SE of 20% was based on previous findings by Ort et al.[23,24]
Daily urine production (V_u)	Gamma, Γ (α, β, Δ)	α = 5.315 β = 0.25 Δ = 0.5 (offset)	Parameters (α and β) and the offset (Δ) were determined by Rauch et al.[21]
Catchment population (*Pop*)	Normal, N (μ, SE^2)	μ = inhabitants provided by WWTP personnel SE = 30% of the population	SE of 30% was chosen based on expected fluctuations in the size of the population

154

155

156 **Results and discussion**

157 Occurrence of PFR exposure biomarkers

158 Concentrations of selected PFR exposure biomarkers measured in 24-h composite wastewater
159 samples are reported in Table S4. In agreement with our previous study[16], HO-DPHP
160 (method quantification limit (MQL) 2.3 ng L^{-1} [16]) could not be detected in any of the
161 samples. BCIPP was also not detected in the collected samples (MQL 15.4 ng L^{-1}, Table S3).
162 Highest concentrations were measured for EHPHP (range 284-2124 ng L^{-1}), followed by

DPHP (range 86-1037 ng L^{-1}) and TCEP (range 213-323 ng L^{-1}). Concentrations of DPHP and TCEP measured in wastewater most likely do not originate exclusively from human excretions. In fact, these compounds could be washed off from consumer goods in indoor and outdoor environments, as they are being used as flame retardants and plasticizers[19,20,25,26]. For TCEP, the parent compound is directly measured and contributions from non-human sources cannot be excluded. Apart from being used as a plasticizer, although substantially less than TPHP[25], DPHP can also be formed from other aryl-PFRs[17]. As such, it is not a specific biomarker for TPHP. Regarding EHPHP, to the best of our knowledge there is currently no information about its use as flame retardant (FR) or plasticizer that could explain the very high concentrations measured. In future studies, it would be thus interesting to determine if EHPHP can be detected in consumer goods, as well as indoor and outdoor environments. Concentrations of all other analytes were between < MQL and 165 ng L^{-1}. Overall, measured concentrations are in line with results from a preliminary study conducted in four WWTPs in Belgium[16]. In the latter study[16], concentrations of selected PFR exposure biomarkers were measured in wastewater samples and, in particular, their stability was assessed by conducting preliminary experiments in raw wastewater. Findings from this study suggested that the targeted analytes, including the additional ones added in this work (see Supporting Information, Section 4), are stable and are not formed in wastewater in presence of parent compounds.

Strong positive correlations were obtained between BBOEP, BBOEHEP and HO-TBOEP (Figure 1), as these are all metabolites of TBOEP, one of the most abundant PFRs found in indoor environments and a suspected carcinogen[19,27,28]. Highest concentrations were measured for BBOEP, followed by HO-TBOEP and BBOEHEP. Results from urine analysis, however, vary substantially across studies. In a recent study of pooled urine samples, TBOEP metabolites could be detected in all samples and highest concentrations were reported for

188 BBOEP, followed by BBOEHEP and HO-TBOEP[29]. Similarly, all TBOEP metabolites could
189 be detected after administration of a single dose of TBOEP to six volunteers[30]. Yet, highest
190 concentrations were measured for BBOEHEP, followed by BBOEP and HO-TBOEP. In
191 another study, BBOEP and HO-TBOEP were either not detected or only at low frequency (<
192 6%)[31]. These differences could be at least partly linked to the multiphasic excretion of
193 BBOEP. In fact, while BBOEHEP and HO-TBOEP show a classic excretion profile with a
194 half-life of < 6 h in urine, BBOEP concentrations decrease less quickly[30]. Since wastewater
195 samples are collected over 24 h, thus providing a composite (or aggregated) average
196 concentration, this could further amplify the difference in excretion profiles. Microbial
197 transformation of TBOEP into its metabolites in wastewater could potentially also contribute
198 to the measured concentrations. Nevertheless, hydrolysis of TBOEP into BBOEP in
199 wastewater has been reported to be very slow[32]. Preliminary experiments also indicated that
200 HO-TBOEP is not readily formed from TBOEP in wastewater[16]. Yet, in the latter case,
201 experiments were carried out without biofilms, which have been shown to have potential
202 impacts on stability in sewers[33]. Finally, because of the high concentrations of TBOEP found
203 in wastewater (i.e., 3-4 μg L^{-1}) [20,34,35], even if a minor fraction of the parent is transformed, it
204 could substantially influence measured biomarker concentrations.
205 Interestingly, moderate positive correlations were observed between BCIPHIPP and
206 BDCIPP, which are metabolites of two different PFRs, namely TCIPP and TDCIPP. This
207 could be linked to their occurrence in similar consumer goods. For instance, results from dust
208 analyses suggest that TCIPP and TDCIPP are present at higher levels in carpeted houses[36]
209 and foamed chairs[37]. Moreover, BCIPHIPP and BDCIPP levels found in wastewater were in
210 the same order of magnitude, similarly to results from urine analysis[31]. A moderate
211 correlation was also observed between DPHP and EHPHP. Again, this could be linked to the
212 compounds having common sources, as DPHP can also be excreted after exposure to

213 EHDPHP[17], one of the main PFRs found in food[38]. Yet, DPHP itself can be used as
214 plasticizer, or originate also from hydrolysis of bisphenol-A bis(diphenyl phosphate) (BDP)
215 and resorcinol bis(diphenyl)phosphate (RDP)[17,39]. On the contrary, negative correlations were
216 observed between EHPHP, BDCIPP and the metabolites of TBOEP (i.e., BBOEP,
217 BBOEHEP and HO-TBOEP), which appear to be linked to wastewater flows (see Table S5).
218 In fact, correlation analysis indicates that EHPHP, and DPHP, concentrations increase with
219 higher flows (Spearman $\rho = 0.53$ and 0.51, respectively, p-value < 0.01).
220 Contribution from precipitation and/or mobilisation from outdoor surfaces[40,41] during rain
221 could explain these results. For DPHP, this is in line with its reported use as plasticizer,
222 whereas for EHPHP, these findings strengthen the hypothesis that the chemical is being
223 employed as flame retardant and/or plasticizer. Nonetheless, the increase in concentration
224 could also be due to a re-release from particulate matter deposited in sewers which could
225 occur during higher flows. On the other hand, BDCIPP, BBOEP and HO-TBOEP appear to
226 decrease with high flows (Spearman $\rho = -0.60$, -0.29 and -0.52, respectively, p-value < 0.01),
227 suggesting that dilution occurs during rain events and that there is likely no contribution from
228 non-human sources and/or no re-release from particulate matter. BBOEHEP was also found
229 to have a negative correlation with flows (Spearman $\rho = -0.18$), yet this was not statistically
230 significant (p-value = 0.12). For the remaining compounds, no significant correlation with
231 flow rates could be observed. Catchment specific correlations between analytes were
232 computed (see Figure S2), however in most cases these were not significant due to the
233 limited number of samples analysed per individual catchment. Thus, no particular inference
234 can be drawn for the latter.

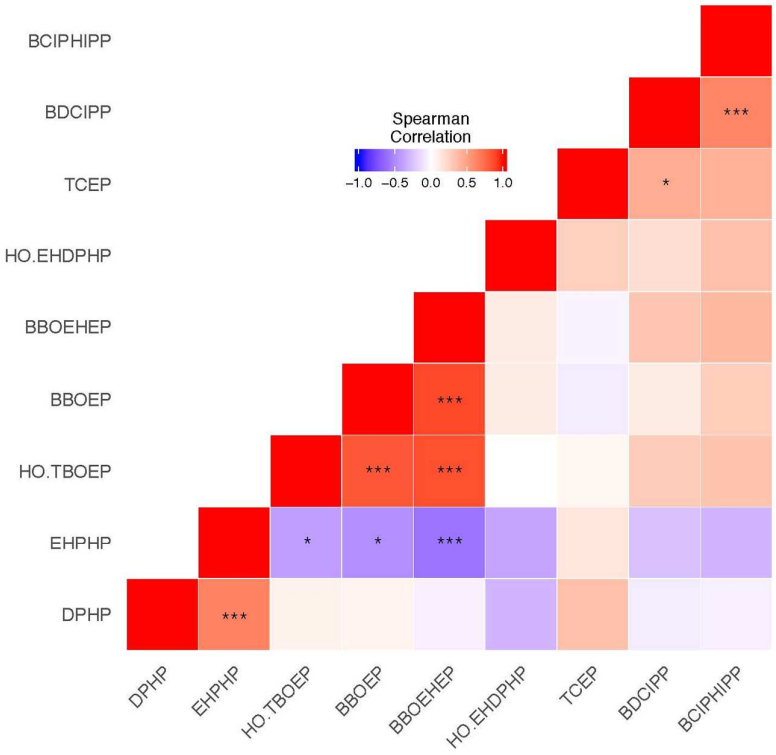

Figure 1: Heatmap of spearman correlations of analyte concentrations. Only samples collected in 2016 and 2017 were considered (no temporal data). * = *p-value* < 0.01, ** = *p-value* < 0.001, *** = *p-value* < 0.0001

Geographical comparisons

To allow comparisons between different locations, population-normalised loads (in mg day^{-1} 1000 inhabitants^{-1}) were computed as described above. For comparison purposes, only samples collected in 2016 and 2017 were considered (Figure 2). Population-normalised loads of target analytes ranged from < 1 mg day^{-1} 1000 inhabitants^{-1} for HO-EHDPHP, to up to almost 800 mg day^{-1} 1000 inhabitants^{-1} for EHPHP. Antwerp and Brussels, the two largest Belgian cities, had similar population-normalised loads for most of the targeted compounds. Nonetheless, differences were observed for BDCIPP, which was significantly higher in Antwerp compared to Brussels (Wilcoxon rank sum, *p*-value = 0.001). Geneva (CH) was also characterised by loads similar to those measured in the two Belgian cities. Interestingly, Athens (GR) presented the lowest figures for all compounds, except EHPHP, which was

measured at similar loads compared to Brussels. Vilnius (LT) was characterised by particularly high EHPHP loads, whilst normalised loads of other target compounds were similar to the other locations or lower.

Owing to the ubiquitous presence of PFRs in our environment, one would have expected levels to be relatively homogenous, at least across Europe. The results however, suggest that there are substantial differences in exposure across communities. These findings are in line with results from a preliminary investigation[16], which showed that there can be substantial differences between and within countries. Nonetheless, additional locations should be included in future monitoring campaigns to obtain a broader picture and samples should be collected over longer periods of time to investigate the existence of seasonal variations. Moreover, catchment characteristics and sampling approaches can have an impact on estimated population normalised loads[23,42]. Similarly, population estimates used here were based on static figures provided by WWTP personnel and do not allow to account for fluctuations which might take place within and between days[43]. Consequently, minor differences in population normalised loads between sampled locations should be interpreted with caution.

Currently, only few countries organise extensive human biomonitoring on a regular basis due to logistic and economical limitations and in such circumstances, exposure data gathered through wastewater analysis could be highly compelling to either complement existing schemes or to plan specific investigations (e.g., targeted biomonitoring studies). For instance, from a public health perspective it would be interesting to further investigate the results obtained for EHPHP in Vilnius, as the population might be at risk of being highly exposed to EHDPHP, one of the most abundant PFRs found in food[38], and/or to EHPHP, as measured concentrations seem to suggest that the compound is being used directly as a plasticizer. On a broader scale, data collected from international wastewater sampling campaigns could also be

used to complement ongoing monitoring schemes, such as the Human Biomonitoring for the European Union program (HBM4EU)[44].

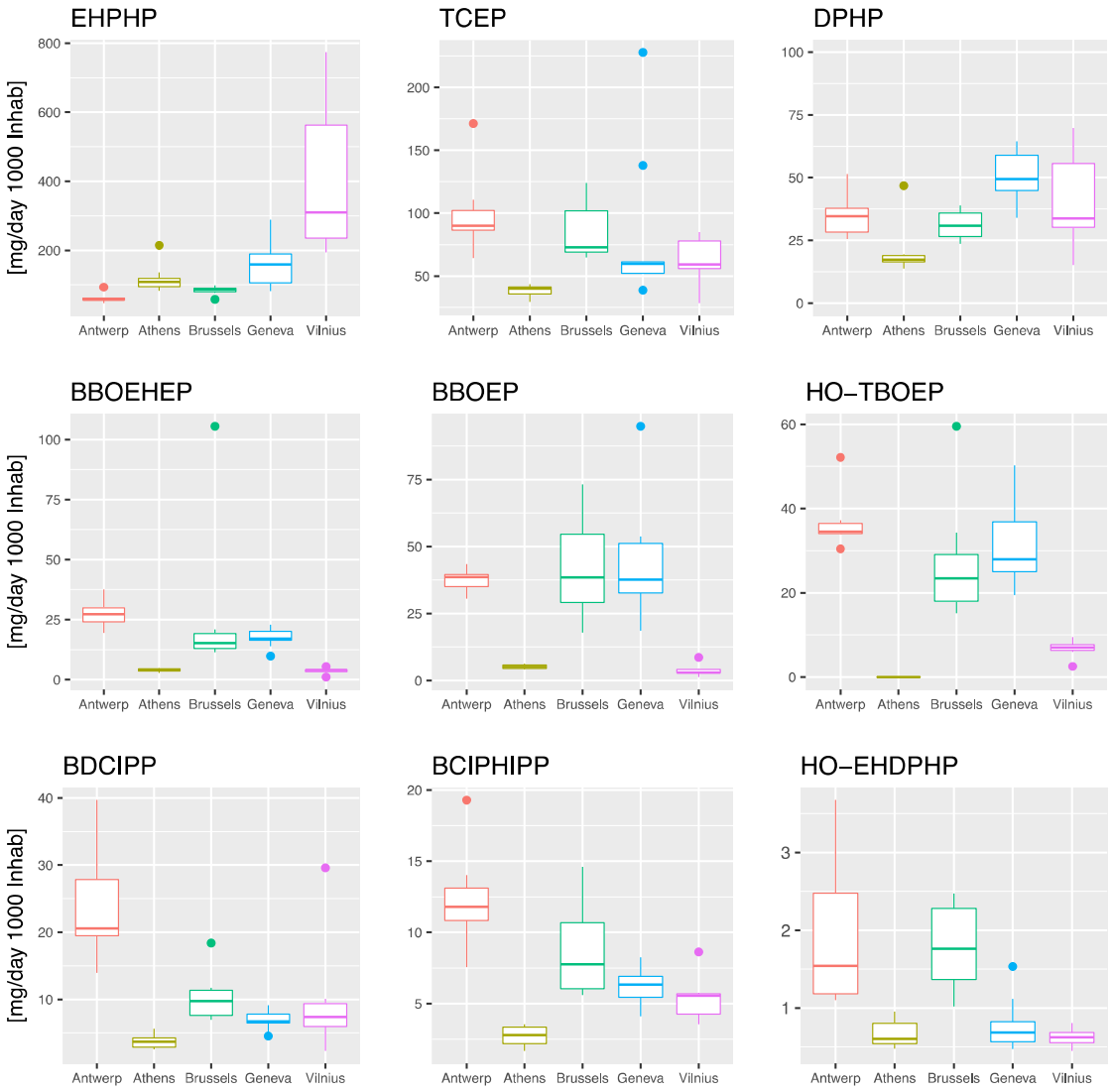

Figure 2: Boxplots of population-normalised loads of target analytes measured in the five WWTPs. Only data from 2016 and 2017 were considered. Y-axis of population normalised loads differs per compound.

Temporal trends

Temporal trends were investigated in wastewater samples collected between 2013 and 2016 from the WWTP of the city of Antwerp. Population-normalised loads were computed as

285 described previously and results are shown in Figure 3 (see Figure S2 for the corresponding
286 flows). Overall, substantial changes in levels of target chemicals could be observed over the
287 investigated period. Since 2013, a decreasing trend in population-normalised loads was
288 observed for DPHP, EHPHP and HO-EHDPHP, however this was statistically significant
289 only for the latter two compounds (Mann-Kendall test, p-value < 0.05). A significant
290 decrease over the monitoring period was also observed for TCEP (Mann-Kendall test, p-
291 value = 0.001). A steady increase in loads was on the other hand observed for BCIPHIPP,
292 BDCIPP and BBOEHEP, yet these were significant only for BCIPHIPP and BBOEHEP
293 (Mann-Kendall test, p-value < 0.01). Remarkably, BBOEHEP, BBOEP and HO-TBOEP,
294 which all originate from the same parent compound (i.e., TBOEP) show different trends in
295 the available data, although positive correlations were observed previously. As discussed
296 above, various hypotheses were formulated which could explain these diverging observations
297 (i.e., different excretion profiles, 24h composite versus spot sampling and, although less
298 likely, potential transformation of TBOEP to its metabolites in wastewater).
299 Comparison of the observed trends with data regarding production volumes, environmental
300 and human exposure levels to PFRs is highly compelling. However, to the best of our
301 knowledge, both long- (i.e., years) and short-term (i.e., weeks and months) temporal data are
302 limited. In an attempt to obtain an overview about the use and exposure to PFRs, a
303 comparison with existing data was made. However, it should be noted that countries and
304 periods considered in available human biomonitoring and production volume figures, do not
305 necessarily coincide with those considered here.
306 In terms of production volumes, the limited data available indicates that use of TCEP in
307 Norway and Finland has been declining since 2003 due to the fact that it is no longer
308 produced in Europe[45]. Although conducted in another country, results from wastewater
309 analysis seem to confirm this, as TCEP decreased from 163 to 100 mg day^{-1}

1000 inhabitants^{-1} (7-day mean) between 2013 and 2016 (Kruskal-Wallis rank sum test, p-value = 0.006). Production and use of TCIPP has, on the other hand, been increasing, because it is being used as a replacement for TCEP[3,9,46]. Findings from indoor analysis indicate that TCIPP, together with TBOEP, is one of the main PFRs found in dust samples[6,47]. The increase in measured levels of BCIPHIPP and BBOEHEP are in line with these reports and suggest that, at least in the monitored catchment, exposure to these PFRs is still on the rise.

Reports about production volumes of TPHP are less clear. A substantial drop in DPHP loads was observed in this study after 2013, yet levels appear to be stable since. As discussed previously however, DPHP should not be regarded as a specific biomarker of TPHP[17,18]. Regarding trends in human exposure, latest figures from the United States indicate an increase from 2004 to 2016 in levels of DPHP and BDCIPP measured in urine[48]. Whilst focused only on the period 2013-2016, results obtained here indicate that levels of BDCIPP have been increasing since 2014. Based on a study conducted in Shandong (China) between 2011 and 2015, changes in levels of selected PFRs could be highlighted[49]. Particularly, levels of TCEP were shown to have increased, whilst TPHP was shown to have decreased. As discussed previously, measured levels of TCEP were shown to have decreased in this study.

Evidence from dust analysis suggests that levels of PFRs vary between seasons, with higher levels measured in winter compared to summer months[50]. Changes in urinary concentrations over time from human biomonitoring studies are not well documented, but seasonal trends have been observed by Hoffman et al.[8,48]. In particular, compared to winter, samples collected in summer had significantly higher concentrations of BDCIPP, DPHP and BCIPHIPP. Potential explanations for this pattern include seasonal changes in behaviour (e.g. differences in time spent indoors or changes in ventilation) and increased exposure through inhalation, as PFRs might shift more from dust to air in warmer months[51]. In the context of

this study, samples were collected always between March and April to minimize potential seasonal effects. Considering the limited data about seasonal changes in exposure, it would be interesting to conduct longer sampling campaigns to determine if these exist and what their magnitude is. Such unique data would be otherwise extremely difficult to obtain through conventional studies.

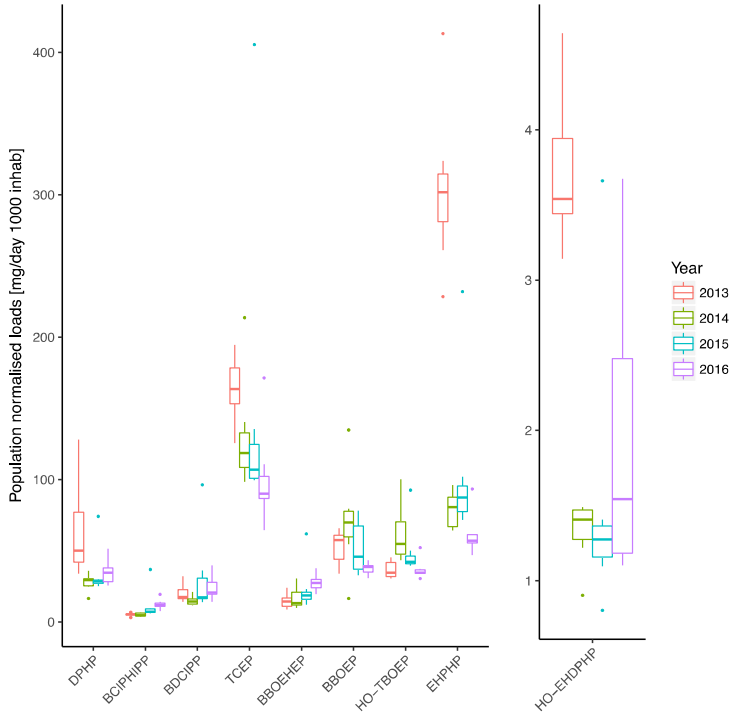

Figure 3: Population-normalised loads [mg day^{-1} 1000 inhbitants^{-1}] measured in Antwerp between 2013 and 2016.

Average urinary concentration and exposure estimates

Using Monte Carlo simulations, average urinary concentrations of target analytes were computed based on measured mass loads, the estimated number of inhabitants connected to the WWTP and the average daily urinary production model. For estimates, only data from 2016 and 2017 were used. Furthermore, those compounds for which use, or suspected use, as flame retardants and plasticizers has been reported, were not included in the calculations (i.e., EHPHP, TCEP and DPHP). Results are reported in Table 3. Estimated BBOEHEP

concentrations ranged between 5 ng mL^{-1} in Brussels to 22 ng mL^{-1} in Athens. Currently, very little data are available concerning levels of this compound except for results recently reported by Völkel et al.[30]. In their study, the authors detected the compound in 78% of the analysed samples (i.e., young children), at median levels of 0.18 ng mL^{-1} (mean and 95th percentile of 0.33 and 1.29 ng mL^{-1}). These figures are one order of magnitude lower compared to levels estimated in this context from wastewater. Similarly, the authors measured also levels of BBOEP (median, mean and 95th percentile of 0.16, 0.36 and 1.54 ng mL^{-1}, respectively) and comparable levels were reported by Van den Eede et al.[31] (< 0.35 – 0.53 ng mL^{-1}). Maximum concentrations of 7 ng mL^{-1} were reported in another study[52]. Estimates from wastewater analysis ranged between 7.0 and 30 ng mL^{-1}. Concentrations of HO-TBOEP ranging from 0.016 to 0.063 ng mL^{-1} in pooled urine samples (100% detection frequency) were recently reported by He et al.[29], whilst concentration estimated here ranged from < MQL to 30 ng mL^{-1}. Similarly, BDCIPP concentrations estimated in the present study ranged from 4.7 to 19.8 ng mL^{-1}, whereas concentrations in biomonitoring studies were in the 0.2-1 ng mL^{-1} range[31,53], with maxima up to 15 ng mL^{-1} [54]. Urinary concentrations of BCIPHIPP reported in the literature were also lower compared to estimates based on wastewater. For instance, mean concentrations of BCIPHIPP in pooled urine samples of 1.7 and 1.9 ng mL^{-1} were reported, with maxima of up to 9.4 ng mL^{-1} [31]. No figures from the literature were available about HO-EHDPHP and EHPHP which could be compared to findings from this study.

Overall, urinary concentrations estimated from wastewater analysis were higher compared to mean figures found in the literature. It is interesting to notice that for various compounds (i.e., BBOEHEP, BDCIPP and BCIPHIPP), wastewater estimates were in the same order of magnitude as maximum concentrations reported from human exposure studies. Multiple factors could explain the observed differences. First, other excretion routes, in particular

faeces, could contribute to measured levels of target compounds in wastewater specimens. In fact, if chemicals adsorbed in faecal matter are partly or completely re-dissolved in wastewater during in-sewer transportation due to turbulence, there could be a substantial increase in concentrations. However, although there is likely substantial inter-individual variability in excretion rates, these will tend to average values when a large number of people is considered (which is the case for WWTP catchments). This will thus not influence spatial (i.e., between catchments) nor temporal (i.e., monitoring the same catchment over time) comparisons.

Second, the contribution from non-human sources cannot be excluded. For instance, some of the measured biomarkers might be found in consumer goods and thus enter sewer systems through leaching and washing off. However, as it is the case for TCEP, DPHP and potentially EHPHP, such situations would lead to particularly high concentrations which could be easily detected. Furthermore, the fact that high concentrations of these compounds are measured in wastewater could potentially still be highly relevant from a public health perspective (e.g., communities being exposed to particularly high levels).

Third, in-sewer stability of both parent and metabolites needs to be further investigated in the presence of biofilms. Due to the substantial levels found in wastewater for some parent PFRs (e.g., TBOEP[34]), in-sewer transformation could impact measured biomarker loads, although stability studies conducted so far suggest that the targeted compounds are stable in wastewater.

Fourth, uncertainties in the calculation approach could also affect the estimates. In particular, the lack of precise and dynamic population data could bias the estimates. Moreover, the approach used to calculate urinary concentrations from wastewater implicitly assumes that analyte concentrations in urine remain constant across voids, which is most likely not the case. However, these uncertainties only affect estimates of average urinary concentrations

and do not prevent from using population normalised loads to monitor community-wide exposure over space and time. Finally, wastewater analysis relies on the collection of 24 h composite samples, whilst biomonitoring studies generally use spot urine samples. In this perspective WBE would provide a more complete picture since measured concentration are an aggregate of daily excretion, while substantial variation can be expected between spot urine samples.

The results obtained here illustrate that wastewater analysis can be used as an innovative and complementary tool to human biomonitoring studies to gather unique data about spatial and temporal trends in exposure, which could hardly be obtained with other approaches[55]. Although various issues currently limit the comparability of urinary concentrations derived from wastewater analysis and results from human biomonitoring studies, results obtained from wastewater can still be complemented with other figures to further investigate the relationship between exposure to specific contaminants and epidemiological and socio-economical covariates. Moreover, the effect of changes in policies and/or manufacturing practices can be easily monitored and the collected data could help assessing risk for population as well as guide the planning of targeted interventions (e.g., biomonitoring, mitigation campaigns).

Table 3: Estimated average urinary concentration. SE = standard error.

	Antwerp	**Athens**	**Brussels**	**Geneva**	**Vilnius**
	Estimated urinary concentration [ng mL^{-1} person^{-1}] (± SE)				
BBOEHEP	11.0 ± 0.9	21.8 ± 6.5	5.0 ± 1.9	17.0 ± 7.2	12.0 ± 1.1
BBOEP	29.0 ± 4.8	29.1 ± 7.3	7.0 ± 3.6	27.0 ± 5.6	30.2 ± 6
HO-TBOEP	30.0 ± 1.6	26.7 ± 7.2	< MQL	19.0 ± 4.5	19.5 ± 2
BDCIPP	13.0 ± 2.2	19.8 ± 7.2	9.2 ± 6.7	6.6 ± 0.9	4.7 ± 0.4

BCIPHIPP	5.0 ± 0.4	10.1 ± 3.3	3.3 ± 1.4	5.4 ± 0.8	4.4 ± 0.3
HO-EHDPHP	0.8 ± 0.1	1.6 ± 0.62	1.0 ± 0.6	1.1 ± 0.2	0.5 ± 0.1

421

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