Assessing population exposure to phthalate plasticizers in thirteen Spanish cities through the analysis of wastewater

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Abstract

Phthalates are widely used plasticizers that produce endocrine-disrupting disorders. Quantifying exposure is crucial to perform risk assessments and to develop proper health measures. Herein, a wastewater-based epidemiology approach has been applied to estimate human exposure to six of the mostly used phthalates within the Spanish population. Wastewater samples were collected over four weekdays from seventeen wastewater treatment plants serving thirteen cities and ca. 6 million people (12.8 % of the Spanish population). Phthalate metabolite loads in wastewater were transformed into metabolite concentrations in urine and into daily exposure levels to the parent phthalates. Considering all the sampled sites, population-weighted overall means of the estimated concentrations in urine varied between 0.7 ng/mL and 520 ng/mL. Very high levels, compared to human biomonitoring data, were estimated for monomethyl phthalate, metabolite of dimethyl phthalate. This, together with literature data pointing to other sources of this metabolite in sewage led to its exclusion for exposure assessments. For the remaining metabolites, estimated concentrations were closer to those found in urine. Their 4-days average exposure levels ranged from 2 to 1347 μg/(day·inh), exceeding in some sites the daily exposure thresholds set for di-iso-butyl phthalate and di-n-buthyl phthalate by the European Food Safety Authority.

Keywords: phthalic acid esters; wastewater-based epidemiology; environmental human exposure; risk assessment; Spain

1. Introduction

Phthalate esters (PAEs, dialkyl or alkyl aryl esters of the *o*-phthalic acid) have been used as plasticizers for more than 80 years. They are added to plastic polymers to increase their flexibility, transparency, durability, and/or toughness. Di-(2-ethylhexyl) phthalate (DEHP) has been primarily applied to polyvinyl chloride (PVC), and consequently can be found in a wide variety of consumer and industrial goods such as building and furnishing materials, wires, textiles, medical devices, toys, or food containers (Wittassek et al., 2011). Short-chain PAEs, i.e. dimethyl phthalate (DMP), diethyl phthalate (DEP), di-iso-butyl phthalate (DiBP), di-n-butyl phthalate (DnBP), and butyl benzyl phthalate (BzBP), are also used in personal care products, detergents, paints, and adhesives (Shu et al., 2019; Wittassek et al., 2011). Since PAEs are not chemically bound to the containing material, they are easily released into the surrounding environment through various routes such as direct migration, leaching and even evaporation in the case of the most volatile and low-molecular-weight derivatives (Gong et al., 2016). As a result, the human population is continuously exposed to PAEs by inhalation, dermal absorption, ingestion of contaminated foods and water, or accidental ingestion of dust and soil (Gong et al., 2016; Wittassek et al., 2011). PAEs are classified as endocrine disruptors and, therefore, interfere with the biosynthesis, secretion or metabolism of naturally occurring hormones, affecting reproductive health and sexual development, the production of insulin-like factor 3, and the abdominal obesity (Katsikantami et al., 2016; Zarean et al., 2016). Exposure to these chemicals has also been associated with attention-deficit and hyperactivity disorders, allergic symptoms, asthma, hypertension, and even thyroid cancer (Engel et al., 2010; Katsikantami et al., 2016; Liu et al., 2020; Zarean et al., 2016). Quantifying exposure levels to PAEs is crucial to guarantee the adoption of control measures and to perform risk assessments based on established safety limits, such as Oral

Reference Doses (RfD) and Tolerable Daily Intakes (TDI), set by the U.S. Environmental Protection Agency (US EPA, 1987a, 1987b, 1987c, 1988) and the European Food Safety Authority (EFSA, 2005a, 2005b, 2005c), respectively.

Characterization of the human exposome is usually performed by human biomonitoring (HBM), i.e. by measuring parent chemicals and/or their metabolites in biological matrices (tissues, blood, serum, urine) (Dennis et al., 2017). Although undoubtedly useful, this approach is limited to a reduced number of individuals, affected by ethical issues and requires a large amount of human and economic resources to extrapolate the results to the population level. The analysis of wastewater to measure human excretion products, usually termed as wastewater-based epidemiology (WBE), has arisen in recent years as a promising tool to complement HBM studies (Gracia-Lor et al., 2018). Based on the concept that wastewater is a largely diluted and integrated sample of urine of an entire community, this approach has been successfully applied to estimate the consumption of illicit drugs (Gonzalez-Mariño et al., 2020), caffeine (Senta et al., 2015), nicotine (Castiglioni et al., 2015; Rodríguez-Álvarez et al., 2014; Senta et al., 2015), alcohol (Rodríguez-Álvarez et al., 2015; Ryu et al., 2016), and pharmaceuticals (Baz-Lomba et al., 2016; van Nuijs et al., 2015). This concept has been extended to estimate the exposure to environmental contaminants and the associated potential side-effects, including pesticides (Rousis et al., 2017a, 2017b), organophosphate flame retardants (Been et al., 2017; Castro et al., 2019), mycotoxins (Gracia-Lor et al., 2020), bisphenol A (Lopardo et al., 2019), and PAEs (Du et al., 2018; González-Mariño et al., 2017). In a very recent study, Tang et al. (2020) compared the levels of PAE metabolites in pooled urine with the levels measured in wastewater from Southeast Queensland, Australia. They concluded that the contribution of urinary excretion to the per capita mass load measured in wastewater was <10% for MMP, MiBP and MnBP, postulating that there are additional sources of such PAE metabolites in wastewater. On the other hand, for DEHP oxidation metabolites, the urinary contribution would be much higher indicating that, in these cases, WBE could provide useful information to monitor exposure trends (Tang et al., 2020). Compared to HBM, WBE provides population instead of individual-level measurements, and analyses are faster, less expensive, less affected by ethical considerations and not biased by individual excretion profiles. Alternatively, WBE studies must be performed following a best practice protocol to minimize the sources of uncertainty (Castiglioni et al., 2013) and require a careful selection of the target exposure biomarkers (Gracia-Lor et al., 2017).

The objectives of this study were (i) to determine the levels of seven PAE metabolites (the corresponding monoesters of DMP, DEP, DiBP, DnBP and BzBP, and two oxidized forms of DEHP) in wastewater from different Spanish regions; (ii) to estimate PAE metabolite levels in urine and compare them with the levels found in other studies performed in Spain; and (iii) to estimate PAE human exposure levels for the Spanish population and to compare them with established human health safety limits. In a previous study, the WBE approach was applied to estimate PAE exposure levels and validated with few samples from NW Spain (Gonzalez-Mariño et al., 2017). In this study, we extended our sampling domain to seventeen wastewater treatment plants (WWTPs) located in thirteen cities in seven Spanish regions, covering 12.8% of the Spanish population. Daily composite samples were collected over four days, and PAE metabolite levels determined in them were used to estimate (i) metabolite concentrations in urine; and (ii) daily exposure levels to PAEs. To the best of our knowledge, this is the largest study performed in Spain to estimate human exposure to chemicals by WBE.

2. Materials and methods

2.1. Chemicals and reagents

For low molecular weight PAEs, the metabolites selected as biomarkers of exposure were the monoesters: monomethyl phthalate (MMP), monoethyl phthalate (MEP), mono-*n*-butyl phthalate (MnBP), mono-*i*-butyl phthalate (MiBP) and monobenzyl phthalate (MBzP). For DEHP, the oxidized forms of its monoesters, mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP) were selected instead. In general, both types of metabolites (monoesters and oxidized forms) are excreted in urine as glucuronide complexes, which are hydrolyzed in wastewater by β-glucuronidase enzymes produced by fecal bacteria (D'Ascenzo et al., 2003).

Standards of MMP, MEP, MnBP, and MBzP were supplied by AccuStandard (New Haven, CT, USA). MiBP, MEHHP, and MEOHP were supplied by Toronto Research Chemicals (TRC, Toronto, ON, Canada). The deuterated analogs monomethyl phthalate-D_4 (MMP-D_4), mono-*n*-butyl phthalate-D_4 (MnBP-D_4), and mono-(2-ethyl-5-hydroxyhexyl) phthalate-D_4 (MEHHP-D_4) were also supplied by TRC. Individual stock standard solutions were prepared in methanol (MeOH) at a concentration of 1000 μg/mL. Mixed stock solutions containing 10 μg/mL of all the analytes or deuterated analogs (used as surrogate or internal standards, IS) were prepared in MeOH and stored in the dark at -20 °C until use.

HPLC-grade MeOH, acetic acid (100%), and hydrochloric acid (HCl, 37%) were supplied by Merck KGaA (Darmstadt, Germany). Ultrapure water was obtained by purifying demineralized water in a Milli-Q Gradient A-10 system (Merck-Millipore, Bedford, MA, USA).

2.2. Sampling

Wastewater samples were collected at seventeen WWTPs located in thirteen cities in seven Spanish regions (Figure 1): Santiago de Compostela (Galicia, Northwest of Spain), Bilbao and its metropolitan area (Basque Country, North), Madrid and Móstoles (Madrid region, Centre of Spain), Toledo and Guadalajara (Castilla La Mancha, Centre), Lleida, Barcelona, Tarragona and Reus (Cataluña, Northeast), Castellón and Valencia (Valencia region, East), and Palma de Mallorca (Balearic Islands, Mediterranean sea). Three WWTPs were sampled in Valencia (covering the whole city and its metropolitan area), two WWTPs in the city of Madrid, and two WWTPs in Palma de Mallorca (one receiving wastewater from the other, so combined as a single WWTP for load and exposure calculations). Individually, these WWTPs serve between 48,000 and 1,163,000 inhabitants, covering altogether a population of ca. 6 million people (Table S1), i.e. 12.8% of the Spanish population in 2018 (INE, 2019). With the exception of Barcelona, Madrid, and Móstoles, where participating WWTPs serve 35%, 30% and 90% of the total population, respectively, the WWTPs sampled give service to the whole municipality where they are located. Composite samples of raw wastewater integrated over 24 h were collected at the entrance of each WWTP over four consecutive weekdays (Mo-Th in all cases but Reus and Tarragona, Tu-Fr) in spring or the early summer of 2018. Time-proportional or flow-proportional sampling modes were applied depending on the automatic samplers available at every WWTP. Aliquots of 0.3 L were transferred into glass bottles and frozen immediately after collection. They were shipped frozen to the University of Santiago de Compostela within one week of their collection, and processed upon arrival to the laboratory. The number of inhabitants served by each WWTP, the methodology used to estimate the population served (which was selected after discussion with the WWTP managers to achieve the most realistic estimate), the daily wastewater flow rates, and further sampling details (sampling mode, time and dates) are provided in the Supplementary Material, Table S1.

2.3. Sample treatment, analysis and quality control

Wastewater samples were treated following a previously optimized solid-phase extraction (SPE) procedure (Gonzalez-Mariño et al., 2017). Briefly, 100 mL aliquots were filtered through 0.7 µm glass microfiber filters GF/A (Whatman, Kent, U.K.) followed by 0.45 µm cellulose filters (Millipore, Bedford, MA, USA). After acidification to pH 2.0 with HCl and addition of 50 ng of IS, samples were extracted onto Oasis HLB-60 mg cartridges (Waters Corp., Milford, MA, USA) previously rinsed with MeOH and pH 2.0 ultrapure water. Sorbents were dried under nitrogen for 30 min, analytes recovered with 5 mL of MeOH, and eluates concentrated down to 1.0 mL under nitrogen.

The optimized separation and detection conditions (Gonzalez-Mariño et al., 2017) were transferred from the original Varian liquid chromatography-mass spectrometry (LC-MS) system to a newer Acquity UPLC® H class system interfaced to a Xevo TQD triple quadrupole mass spectrometer (Waters, Milford, MA, USA). LC separation was performed at 40 °C with a Luna Phenyl-Hexyl column (150×2 mm I.D., particle size 3 µm) from Phenomenex (Torrance, CA, USA). A dual eluent system consisting of (A) 0.1% acetic acid in ultrapure water and (B) 0.1% acetic acid in MeOH was used, at a flow rate of 0.2 mL/min. The chromatographic gradient was as follows: 0 min (60% B), 10 min (80% B), 10.5 min (100% B), 15.5 min (100% B), 15.6 min (60% B), 20 min (60% B). Injection volume was set at 10 µL.

Working parameters of the electrospray ionization source of the Xevo TQD were: negative ionization, 3 kV (capillary voltage), 150 °C (source temperature), 600 L/h (desolvation gas flow), 200 °C (desolvation temperature), and 10 L/h (cone gas flow).

Nitrogen was used as desolvation and cone gas, and argon as collision gas. Analytes were recorded in Selected Reaction Monitoring (SRM) mode by acquiring one (for IS) or two (for analytes) precursor→product ion transitions per compound. The detection of the two SRM transitions and the compliance with their corresponding retention time and SRM ratio were the minimal criteria set to confirm the identity of a substance, in accordance with the guidelines of the 2002/657/EC Decision (European Commission, 2002). Transitions, retention times, cone voltage values and collision energies are displayed in Table S2 of the Supplementary Material.

Quantification was performed by the IS method using MMP-D_4 as IS for MMP; MnBP-D_4 for MEP, MiBP, MnBP, and MBzP; and MEHHP-D_4 for MEHHP and MEOHP. Percentages of relative recovery in influent wastewater varied between 76% and 100%, with RSD values $\leq$ 15% (Gonzalez-Mariño et al., 2017).

Procedural blanks consisting of 100 mL of ultrapure water spiked with 50 ng of IS were processed together with every set of samples. Instrumental blanks (clean solvent injections) were run repeatedly along the injection sequence. The repeated detection of mono-(2-ethyl-5-carboxypentyl) phthalate, another DEHP oxidation metabolite initially included in the method, in the procedural blanks led us to exclude it from the group of analytes. For the remaining compounds, method detection (MDL) and quantification (MQL) limits were estimated from the less concentrated real samples as the levels providing a signal-to-noise ratio of 3 and 10, respectively. These limits ranged from 0.69 to 10 ng/L and from 2.9 to 32 ng/L, respectively (Table S2).

2.4. Estimation of metabolite average concentrations in urine and daily exposure levels to PAEs

Metabolite concentrations in 24 h composite wastewater samples (C_{ww}, ng/L) were multiplied by wastewater daily flow rates ($Flow_{ww}$, m^3/day) and divided by the population served by each WWTP (no. of inh) to get population-normalized metabolite loads in µg/(day·inh):

$$Load_{metab}\left({}^{\mu g}/_{(day \cdot inh)}\right) = C_{ww}\left({}^{ng}/_{L}\right) \times \frac{Flow_{ww}\ \left({}^{m^3}/_{day}\right)}{n.\ of\ inh}$$

These loads were further used to estimate:

(i) <u>metabolite concentrations in urine</u> (C_{urine}, µg/L), by simply dividing metabolite loads by 1.57 L, considered the average volume of urine excreted per person and day (González-Mariño et al., 2017):

$$C_{urine}\left({}^{\mu g}/_{L}\right) = {Load_{metab}\left({}^{\mu g}/_{(day \cdot inh)}\right)}\Big/{1.57\left({}^{L}/_{(day \cdot inh)}\right)}$$

(ii) <u>daily exposure levels to PAEs</u>, by multiplying individual metabolite loads by a correction factor (CF) that takes into account the molar fraction of the parent PAE excreted as a specific metabolite and the ratio between their molecular weights (MW) (to convert excreted amounts into intake):

$$Exposure_{PAE}\left({}^{\mu g}/_{(day \cdot inh)}\right) = \mathrm{Load}_{metab}\left({}^{\mu g}/_{(day \cdot inh)}\right) \times CF$$

$$CF = \frac{MW_{PAE}/MW_{metabolite}}{Molar\ Excr.Fraction}$$

These CFs were previously calculated by González-Mariño et al., (2017) considering the human metabolism studies published up to then (four) and the number of participants involved in every study (from one to 20). Their values are 1.65 for DEP, 1.76 for DiBP, 1.80 for DnBP, 1.68 for BzBP, 11.8 for DEHP when using MEOHP loads, and 8.40 for DEHP when using MEHHP loads. Estimated exposure levels were compared to the TDI values set by the EFSA (EFSA, 2005a, 2005b, 2005c) and the RfDs set by the US EPA

(US EPA, 1987a, 1987b, 1987c, 1988). To this end, Safe Reference Values (SRV) were calculated considering an average body weight of 70.8 kg for adults (average European body weight according to Walpole et al. (2012)) and 11.5 kg for toddlers (according to the World Health Organization, 18-month toddler body weight is 11.8 kg for boys and 11.1 kg for girls, percentile 75%, WHO, 2006). SRVs are compiled in Table S3.

2.5. Data treatment

For both sets of estimations (i.e, metabolite concentrations in urine and daily PAE exposure levels), 4-day average values were calculated for single WWTPs and population-weighted overall means for the seventeen WWTPs altogether (all WWTPs, all days). According to the US EPA Guidance for Quality Assessment (EPA, 2015) data below the MDL are usually substituted by a value between zero and the MDL to perform data analysis (EPA, 2015). However, this substitution is not recommended if more than 50% of the values are below the MDL. Considering that this was the case for MBzP, MEHHP, and MEOHP in this study, two different scenarios were assessed for these compounds:

- Underestimating scenario, in which data below the MDL were replaced by zero, and data falling between the MDL and the MQL by the MDL, biasing the results to lower estimates
- Overestimating scenario, in which data below the MDL were replaced by the MDL, and data between the MDL and the MQL by the MQL, biasing the results to higher estimates

3. Results and discussion

3.1. PAE metabolites in wastewater: concentrations and population-normalized loads

MMP, MEP, MiBP, and MnBP were identified in all samples. MEP was the substance found at the highest concentrations (335-12700 ng/L), followed by MMP (72-3828 ng/L), MiBP (39-1974 ng/L), and MnBP (7-867 ng/L). MBzP, MEOHP, and MEHHP were found (>MDL) in 35%, 41% and 28% of the samples, respectively, with concentrations varying between 6.7 and 45 ng/L (MBzP), 10 and 128 ng/L (MEOHP), and 18 and 170 ng/L (MEHHP) (Figure 2). In terms of ranges, the order of relative abundance matches the one previously observed in raw wastewater samples (n=14) from the NW of Spain (González-Mariño et al., 2017). However, maximum levels were considerably lower in that study for some metabolites, i.e. 1599 ng/L for MEP or 277 ng/L for MiBP. Also, maximum levels for MBzP, MEOHP, and MEHHP were lower. These differences may be attributed to both geographical and population diversities. In the former study, only six WWTPs located within a radius of 100 km in the same Spanish region (Galicia, Northwest of Spain) were monitored. They served small and medium-size cities (between 12,000 and 136,500 inhabitants) with an economy based on services, administration, and the tourism sector. In the current study, we extended the analysis to seventeen WWTPs located in seven different Spanish regions of the Northwest (Galicia), North (Basque Country), East (Cataluña and Valencia region), and Centre of the country (Madrid and Castilla La Mancha), including also the Balearic Islands in the Mediterranean sea. Both medium-size and large cities (between 48,000 and 1,163,000 inhabitants) were considered. They comprised a wide variety of populations with great economic diversities: industrial-based economy, tourism-based economy, etc. Thus, differences in the use of PAEs and, consequently, in the average levels of their metabolites in wastewater are expected between (i) the current and the previous WBE study; and (ii) the different locations addressed in the current study. Actually, when considering the only city that was monitored in both cases (Santiago de Compostela) daily concentrations were rather similar for all metabolites but for the butylated derivatives on the first sampling date of the current study (remarkably higher).

Du et al. (2018) reported a different order of relative abundance of PAE metabolites after analysing wastewater from 27 Chinese cities, some of them with millions of inhabitants. In their study, MnBP presented the highest concentrations (ca. 7000 ng/L), followed by its isomer MiBP (ca. 2600 ng/L), then MMP (2670 ng/L), and MEP (1581 ng/L) (Du et al., 2018). In Australia, Tang et al. (Tang et al., 2020) analysed wastewater from three WWTPs over six years and found the highest median values for MMP (2900 ng/L), followed by MEP (1900 ng/L), MnBP (1400 ng/L) and MiBP (1000 ng/L). Note that the monoester metabolite of DEHP is excluded in this comparison.

Metabolite concentrations in 24 h composite samples were converted into population-normalized metabolite loads in µg/(day·inh) considering wastewater flow rates and the number of people served by each WWTP. As explained in section 2.2., the two WWTPs in Palma de Mallorca were considered as a single plant serving the sum of both populations, and in consequence the number of sites was reduced to sixteen. In those cases where metabolite concentrations were below the MDL or fell between the MDL and the MQL, the two scenarios explained in section 2.5 were considered (hence, two values are provided in Table S4). Pairwise correlation studies performed with 4-days average loads of MMP, MEP, MiBP, and MnBP (i.e. analytes found in all samples) showed statistically significant correlations at the 95% of confidence level (p-value<0.05) only between the two butylated metabolites (MiBP and MnBP, see Table S5 for regression coefficients and p-values). This suggests a potential common source of exposure to DiBP and DnBP all around Spain.

3.2. Estimation of urinary concentrations of PAE metabolites

Population-normalized metabolite loads were used to estimate metabolite concentrations in urine, considering an average volume of urine of 1.57 L excreted per person and day (González-Mariño et al., 2017) (Table S6). Average concentrations of 4 days varied between 70 and 773 ng/mL for MEP, between 33 and 608 ng/mL for MMP, between 9.2 and 317 ng/mL for MiBP, and between 2.2 and 156 ng/mL for MnBP. For MBzP and MEOHP, they were below 5 ng/mL at all sampled sites even in the overestimating scenario, and for MEHHP, they were below 7 ng/mL in all cases. For the compound found at the highest levels (MEP), estimated 4-day average urine concentrations were higher in large touristic cities (Barcelona, Valencia and Palma de Mallorca). Only Guadalajara (centre of Spain, ca. 95,000 inhabitants, 613 ng/mL) was an exception. For MMP, MiBP, and MnBP (the other metabolites positively quantified in all samples), no clear trend could be observed. MMP 4-day average was remarkably high in Bilbao (608 ng/mL versus 33-165 ng/mL in the remaining sites), and MiBP and MnBP were also very high in Santiago de Compostela (317 and 156 ng/mL, respectively, versus 9.2-85 ng/mL and 2.2-53 ng/mL in the remaining sites).

Population-weighted overall means for each metabolite considering all the sampled sites (Table 1) were compared to their median and geometric mean urine concentrations calculated in several biomonitoring studies performed in Spain (Casas et al., 2011; Casas et al., 2016; Cutanda et al., 2015; Herrero et al., 2015; Valvi et al., 2015), as well as to the levels estimated in our previous WBE study (González-Mariño et al., 2017). Except for wastewater-derived calculations, MMP was determined only in one study involving 21 participants from Madrid (Herrero et al., 2015). The median of MMP concentrations found in urine in that case (7 ng/mL) was remarkably lower than the population-weighted overall mean estimated in the current study (162 ng/mL), and also lower than the overall mean estimated in our previous wastewater-based study focused on the NW of Spain (88 ng/mL, González Mariño et al., 2017). The high value observed here is highly affected by the MMP urine concentration estimated in Bilbao (605 ng/mL). Excluding this site, the population-weighted overall mean is 74 ng/mL, much closer to the previously estimated value of 88 ng/mL, but still 10 times higher than the MMP concentrations measured in urine by Herrero et al. (Herrero et al., 2015). In the study conducted by Tang et al. (2020) to assess the contribution of urinary excretion to the mass loads of PAE metabolites in wastewater, a remarkably low contribution (<1%) of urinary MMP to wastewater loads was inferred. Consequently, authors concluded that there may be sources, still unknown, other than urine for the occurrence of some PAE metabolites in wastewater. Our results support this conclusion for MMP, but not for the other metabolites for which the concordance between estimated levels (by wastewater analysis) and measured levels in urine of the Spanish population is higher. Further research combining wastewater analyses and human biomonitoring on the same sampled area is needed to confirm/discard this hypothesis and to discern which PAE metabolites cannot be used as biomarkers of exposure in WBE.

The overall mean concentration estimated for MEP (520 ng/mL) using WBE was higher than the geometrical means and median values measured in urine from adults (69-336 ng/mL, Casas et al., 2011; Casas et al., 2016; Cutanda et al., 2015; Herrero et al., 2015; Valvi et al., 2015), but lower than the median of the concentrations reported in urine from 4-years children (755 ng/mL, Casas et al., 2011). Children are more exposed to PAEs and, thus, overall means calculated considering all the population (wastewater analyses) may lead to intermediate values between the exposure levels undergone by children and adults (differentiated by urine analyses). This profile, however, was not kept in the case of MiBP and MnBP: population-weighted overall means estimated by WBE (57 ng/mL and 40 ng/mL, respectively) were higher than the geometric means and median values of the concentrations measured in real urine from both adults and children, though at the same order of magnitude, a behaviour also observed in our previous wastewater study in the NW of Spain (González Mariño et al., 2017). This result is biased by the high urinary levels estimated in Santiago de Compostela, also located in the NW of Spain (Table S6). The exclusion of these levels leads to overall means of 50 ng/mL for MiBP and 36 ng/mL for MnBP, which are closer to the median of the concentrations of these PAE metabolites measured in 4-years children urine (42 ng/L for MiBP and 30 ng/L for MnBP, Casas et al., 2011). Population-weighted overall means for MBzP, MEOHP, and MEHHP were between 3 and 40 times lower than the concentrations measured in real urine, but in the same order of magnitude than the levels estimated by González-Mariño et al. (2017). However, the low detection frequency of these metabolites makes the comparison with levels measured in real urine even more difficult.

Except for MMP, WBE estimations of urine levels of PAE metabolites and real urine concentrations agreed on the pattern of abundance, with MEP being the metabolite detected at the highest level, followed by the butylated derivatives, DEHP metabolites and, finally, MBzP.

3.3. Estimation of daily exposure to PAEs

Metabolite loads in wastewater were used to estimate daily exposure levels to PAEs (Table 2) under the assumption that their occurrence in sewage is primarily due to human excretion. Since both Tang et al. (2020) and our results in section 3.2. point to the likely existence of other sources contributing to MMP loads in wastewater, DMP exposure data is not included in this discussion. As for Table S4 and Table S6, two values (corresponding to the two scenarios described in section 2.5) are provided in Table 2 in those cases where concentrations in wastewater were below the MDL or fell between the MDL and the MQL. Average exposure values of 4 days are displayed in Figure 3 (overestimating scenario).

At all sites but Santiago de Compostela, the highest values were estimated for DEP: ~2000 µg/(day·inh) in Barcelona, Valencia PII, Valencia QB, and Palma de Mallorca, and between 181 and ca. 1600 µg/(day·inh) in the remaining sites. In Santiago, the highest exposure level was estimated for DiBP (879 µg/(day·inh)), followed closely by DEP (717 µg/(day·inh)) and accompanied by high exposure to DnBP (441 µg/(day·inh)). This result is affected by the high MiBP and MnBP loads measured in this location on the first sampling day (Table S4). Levels measured on the following days were between 4 and 24 times lower, but no explanation could be provided and so all days were considered for 4-day average and population-weighted overall mean calculations. For DEHP and BzBP, average exposure values were remarkably lower than the levels obtained for the other four PAEs. The exposure profile found at most of the sites of this study (DEP >DiBP>DnBP>DEHP>BzBP) differs from the one observed in Chinese cities by Du et al (Du et al., 2018). There, the highest exposure levels were found for DnBP and DiBP, suggesting a different profile of PAE exposure between both countries: people in China are more exposed to butylated phthalates (DnBP and DiBP), whereas Spanish people are more exposed to the shorter ester chain derivative DEP. This observation is in agreement with the results derived from urine analysis (Casas et al., 2011; Casas et al., 2016; Cutanda et al., 2015; Gao et al., 2016; Guo et al., 2011; Herrero et al., 2015; Valvi et al., 2015).

Average exposure values of 4 days were compared to the SRVs calculated using TDIs and RfDs as daily exposure thresholds (US EPA, 1987a, 1987b, 1987c, 1988), and considering an average body weight of 70.8 kg for adults and 11.5 kg for toddlers (WHO,

2006) (Table 2). Exposure to butylated PAEs were equal to or surpassed the SRVs derived for toddlers according to the TDI set by the EFSA (115 µg/(day·toddler)) in ten out of the sixteen sites (Table 2): Barcelona (DiBP), Bilbao (both DiBP and DnBP), Castellón (DnBP), Guadalajara (both), Lleida (DiBP), Madrid North (both), Santiago de Compostela (both), Valencia PI, PII and QB (both). Average exposure values for the remaining five PAEs were below SRVs in all cases.

Population-weighted overall means in this study (Table 2) were: 1347 µg/(day·inh) for DEP, 158 µg/(day·inh) for DiBP, 112 µg/(day·inh) for DnBP, 2 µg/(day·inh) for BzBP, and varied between 26 and 44 µg/(day·inh) for DEHP (depending on the metabolite and scenario selected for the calculation). Thus, overall means exceeded the SRVs for toddlers in the case of DiBP (SRV derived from TDI: 115 µg/(day·toddler)) and was very close to it in the case of DnBP (SRV derived from TDI: 115 µg/(day·toddler)). Considering that the analysis of wastewater does not allow for differentiation between exposure undergone by adults and children, but it assumes identical amounts of PAE metabolites excreted by ones and others (Du et al., 2018), toddlers exposure may be underestimated and the derived risk may be even higher to that reported here.

To quantify the contribution of the five PAEs (DEP, DiBP, DnBP, BzBP, and DEHP, excluding DMP) to PAEs exposure total risk, the concept of toxic equivalents (Tox Eq_{PAE}) was used. Toxic equivalents describe the individual toxicity of a single PAE relative to the most toxic derivative(s), namely, DEHP. They were calculated based on the RfDs provided by EPA (Tox $Eq_{PAE} = RfD_{lowest} / RfD_{PAE}$) and are displayed in Table S3. TDIs could not be used since they are not available for DEP. DiBP contributed to almost 50% of the total risk in Madrid North and Santiago de Compostela, whereas DEHP was responsible of >50% in Castellón, Móstoles and Valencia PI, and DEP accounted for 59% of the total risk in Reus (Figure 4). These three compounds represented the major contributors to the total risk at all assessed sites. DnBP accounted for less than 25%, and BzBP for less than 1%. On a national scale, considering population-weighed values, DEHP would be the phthalate which poses most concern (30% of the total risk) followed closely by DEP (27%) and DiBP (25%).

4. Conclusions

WBE was applied to assess the overall exposure to different PAEs within the Spanish population. Following a recent study (Tang et al., 2020) and comparing PAE metabolite levels in urine estimated from our wastewater analyses with previous human biomonitoring data, MMP occurrence in wastewater is suspected to have other sources than human urine, and so DMP risk assessment was not performed. Among the remaining PAEs, results obtained on a local scale pointed to the butylated derivatives as the ones posing the higher concern, particularly for toddlers. On a national scale, including all the sampled sites, DEHP accounted for the higher percentage of total risk, 30%, but followed closely by DEP and DiBP. Further studies combining wastewater and urine analyses within the same population (same sampled area) are highly recommended to (i) compare the results of WBE and human biomonitoring in order to validate WBE data; and (ii) discern whether human excretion is the only source of these PAE metabolites in sewage or, as suggested by Tang et al. (Tang et al., 2020), additional sources are contributing to their occurrence in this matrix, aiming also to track which these sources are. In this regard, in-sewer stability tests involving the joint quantification of the parent low molecular weight PAEs and their hydrolytic metabolites will help to understand if there are any biotic or abiotic processes promoting phthalate diesters hydrolysis towards the corresponding monoesters. It is well known that oxidative metabolites are less prone to be formed exogenously, so these tests are less crucial for DEHP and the remaining high molecular weight PAEs.

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Supplementary material: Supplementary material is provided at the journal site

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Table 1. Metabolite concentrations in urine: population-weighted overall means of the values estimated in this study considering all locations, all days, versus median and geometric mean values reported in several biomonitoring studies performed in Spain. For wastewater-based derived concentrations, two scenarios were assessed. Underestimating scenario: values<MDL replaced by zero; MDL<values<MQL replaced by MDL; overestimating scenario: values<MDL replaced by MDL; MDL<values<MQL replaced by MQL.

Concentrations (μg/L)	MMP	MEP	MiBP	MnBP	MBzP	MEOHP	MEHHP
This study: several Spanish regions, population-weighted overall mean underestimating scenario – overestimating scenario	162	520	57	40	0.71-0.79	2.1-2.4	2.0-2.5
Gonzalez-Mariño et al., 2017: NW of Spain, population-weighted overall mean	88	276	50	49	3.4	5.3	11
Herrero et al., 2015: 21 participants, Madrid, median	7.0	69	23	19	2.6	6.2	5.3
Cutanda et al., 2015: 120 mothers, Toledo, geometric mean	NA	161	37	33	8.5	14	21
Casas et al., 2011: 120 pregnant women, Asturias-Guipuzcoa-Sabadell-Valencia, median	NA	324	30	28	11	16	17
Casas et al., 2011: 30 children (4 years) Granada, median	NA	755	42	30	33	45	57
Valvi et al., 2015: 657 pregnant women, 1st trimester, Sabadell, geometric mean	NA	213	24	23	9.2	15	22
Valvi et al., 2015: 657 pregnant women, 3rd trimester, Sabadell, geometric mean	NA	329	26	25	9.0	17	21
Casas et al., 2016: 657 pregnat women, Sabadell, geometric mean	NA	336	29	29	11	19	26

NA: not applicable (not reported)

Table 2. Daily exposure to PAEs: average exposure values of 4 days (in µg/(day·inh)) versus safe reference values derived from EPA Reference Doses (RfD) and EFSA Tolerable Daily Intakes (TDI). Two values are provided for BzBP and DEHP in those cases where at least one concentration in sewage fell <MDL or between the MDL and the MQL: daily exposure derived from the underestimating scenario **-** daily exposure derived from the overestimating scenario.

Average exposure (±SD) µg/(day·inh)	**DEP**	**DiBP**	**DnBP**	**BzBP**	**DEHP**	
					based on MEOHP	**based on MEHHP**
Barcelona	1972 (±1034)	173 (±62)	98 (±28)	0.00 - 0.29 (±0.07)	20 (±26) - 25 (±21)	0.00 - 8 (±2)
Bilbao	1025 (±222)	175 (±35)	130 (±47)	2 (±3)	24 (±29) - 30 (±23)	14 (±28) - 22 (±23)
Castellón	690 (±512)	82 (±33)	115 (±63)	2 (±4)	72 (±62) - 75 (±58)	39 (±45) - 43 (±41)
Guadalajara	1587 (±215)	236 (±102)	146 (±14)	9 (±5)	68 (±58) - 71 (±54)	56 (±39) - 59 (±34)
Lleida	788 (±137)	141 (±66)	89 (±24)	2 (±3)	0.00 - 10 (±1)	0.00 - 9 (±1)
Madrid Centre	690 (±189)	89 (±25)	56 (±15)	1 (±2)	0.00 - 5.1 (±0.1)	0.00 - 5.0 (±0.1)
Madrid North	921 (±184)	216 (±77)	119 (±85)	0.00 - 0.23 (±0.01)	0.00 - 6.7 (±0.4)	0.00 - 6.6 (±0.4)
Móstoles	645 (±267)	111 (±81)	101 (±103)	4 (±6)	79 (±113) - 81 (±110)	79 (±107) - 81 (±104)
Reus	674 (±174)	26 (±17)	6 (±4)	0.00 - 0.180 (±0.002)	0.00 - 5.31 (±0.06)	0.00 - 5.21 (±0.05)
Santiago de Compostela	717 (±345)	879 (±1127)	441 (±473)	12 (±2)	27 (±3) - 90 (±8)	27 (±3) - 90 (±8)
Tarragona	484 (±121)	41 (±45)	25 (±39)	1 (±2)	0.00 - 5.9 (±0.2)	15 (±29) - 19 (±26)
Toledo	181 (±35)	29 (±10)	12 (±6)	0.00 - 0.21 (±0.01)	0.00 - 6.2 (±0.4)	0.00 - 6.1 (±0.4)
Valencia PI	1315 (±340)	136 (±57)	118 (±79)	2 (±2)	83 (±78) - 85 (±76)	87 (±88) - 89 (±85)
Valencia PII	2002 (±609)	165 (±60)	150 (±80)	2 (±2)	77 (±55) - 80 (±49)	82 (±74) - 85 (±70)
Valencia QB	1918 (±1217)	127 (±68)	114 (±88)	2 (±3)	59 (±118) - 64 (115)	0.00 - 7 (±1)
Palma de Mallorca	1822 (±522)	111 (±26)	105 (±19)	5 (±3)	71 (±35) - 74 (±35)	0.00 - 7.1 (±0.2)
Population-weighted overall mean	1347 (±490)	158 (±74)	112 (±56)	2 (±2)	38 (±36) - 44 (±33)	26 (±27) - 33 (±26)
SRV (µg/(day.adult)) from RfD-EPA	56640	7080	7080	14160	1416	1416
SRV (µg/(day.adult)) from TDI-EFSA	NA	708	708	35400	3540	3540
SRV (µg/(day.toddler)) from RfD-EPA	9200	1150	1150	2300	230	230
SRV (µg/(day.toddler)) from TDI-EFSA	NA	115	115	5750	575	575

NA: Not applicable (No TDI available)

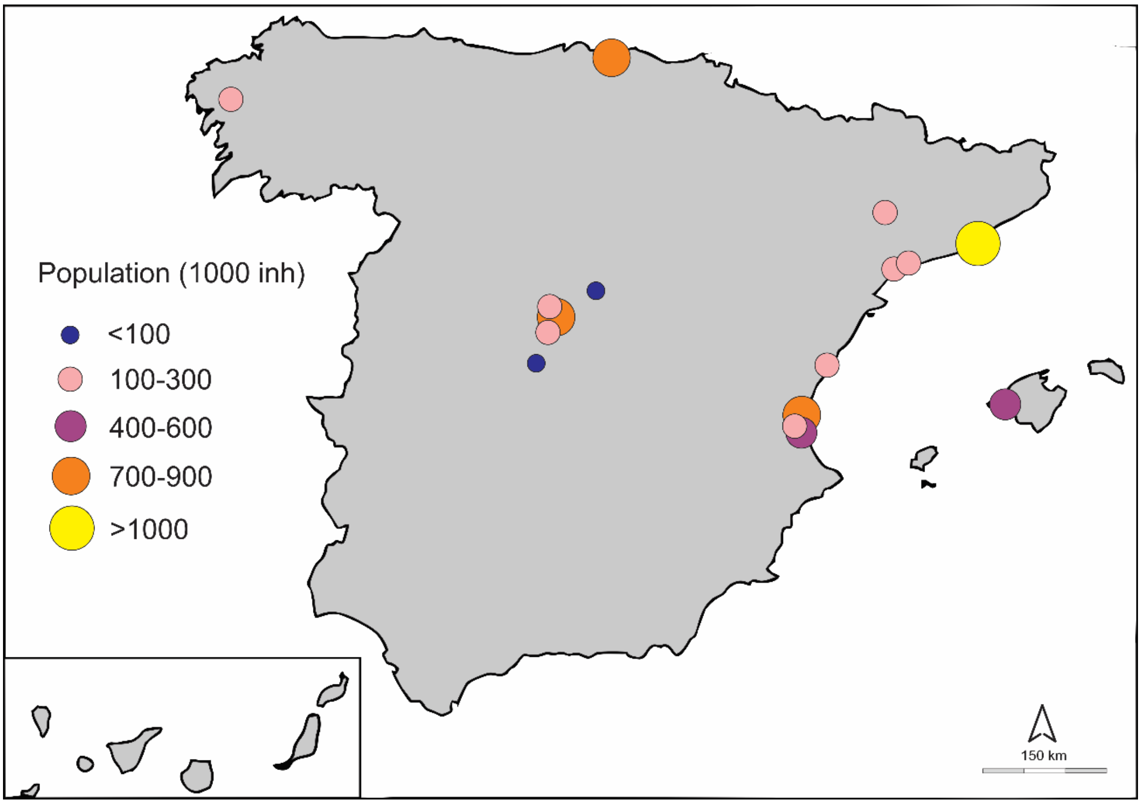


Figure 1. Location of the wastewater treatment plants included in this study. The size of the semicircles is directly proportional to the number of inhabitants served by each plant. Source of the map for its elaboration:

https://d-maps.com/carte.php?num_car=2193&lang=es.

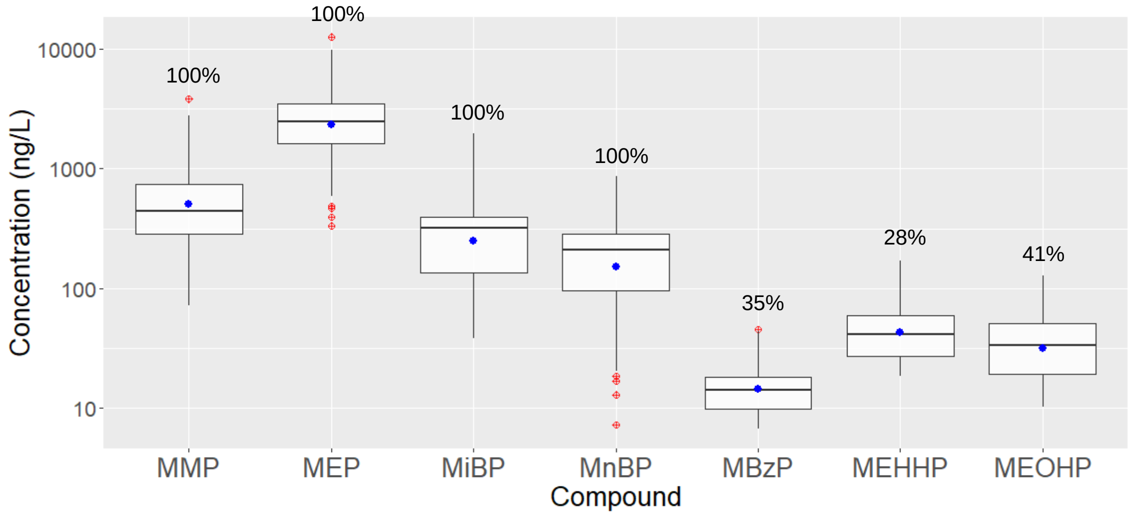


Figure 2. Range of metabolite concentrations in wastewater (in ng/L, logarithm scale, all locations considered). Only concentrations >MQL are reflected in the Box-Whisker plots. Detection frequency (% samples > MDL) is indicated above every Box-Whisker.

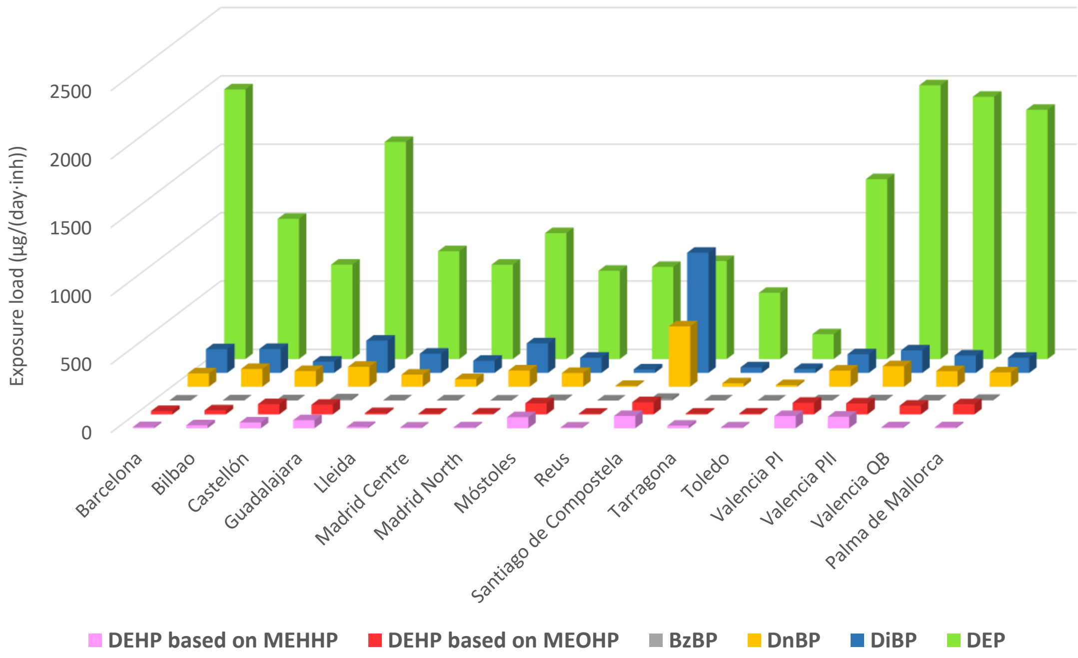


Figure 3. Average exposure values of 4 days (in µg/(day·inh)) in the different locations assessed. In those cases where concentrations were below the MDL or between the MDL and the MQL, the overestimating scenario was applied (concentrations<MDL replaced by MDL; MDL<concentrations<MQL replaced by MQL).

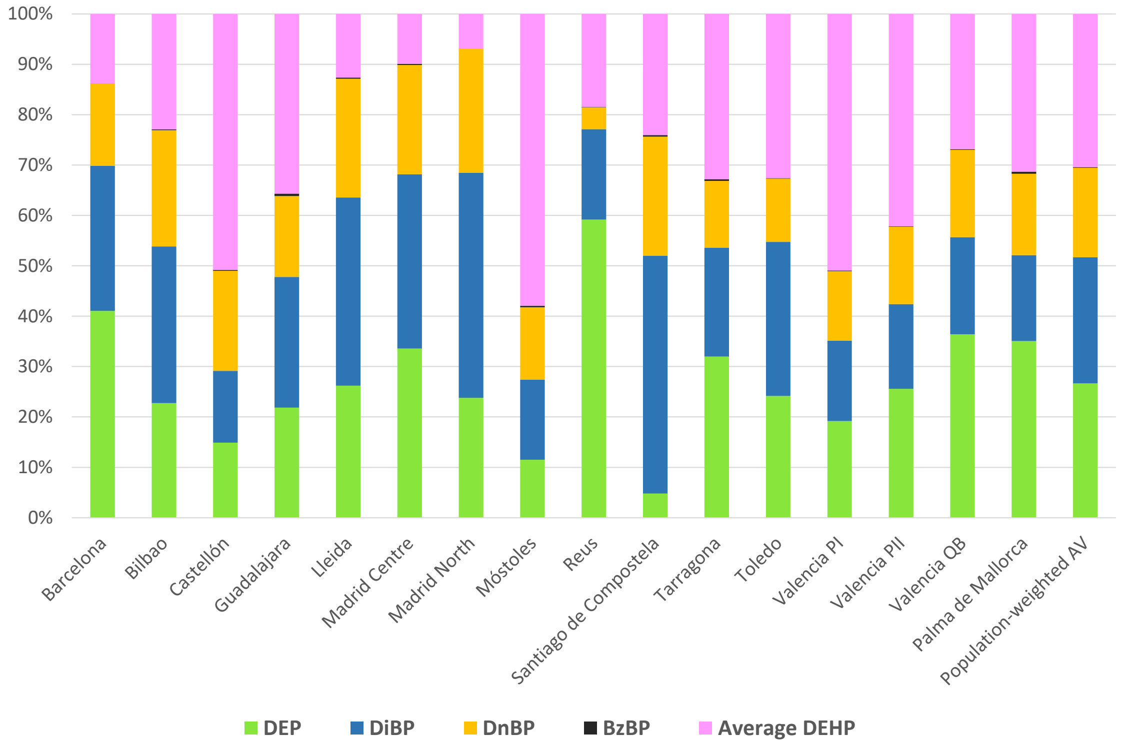
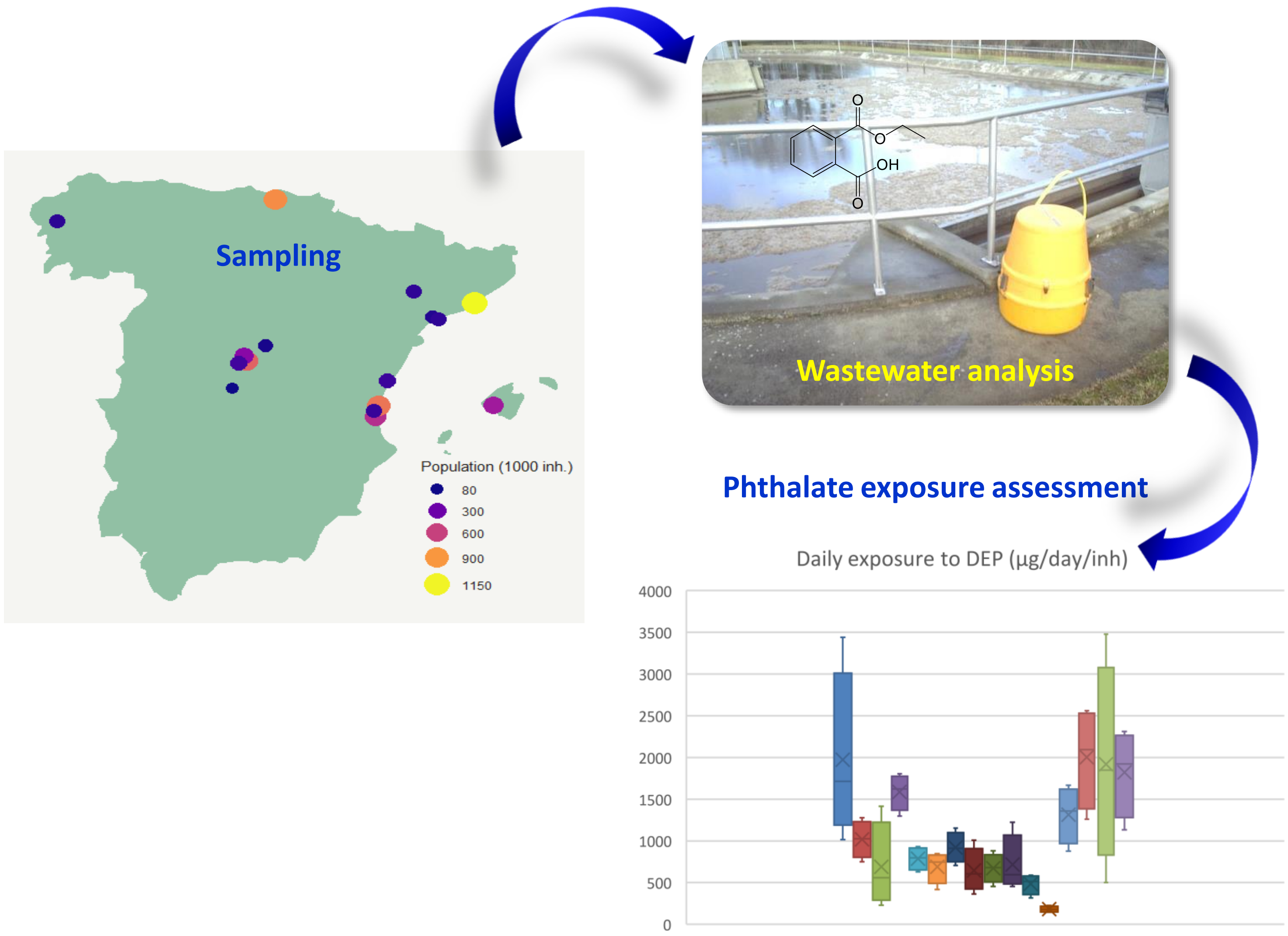


Figure 4. Contribution of every individual PAE to PAEs exposure total risk. Calculations were performed considering toxic equivalents provided in Table S3 and applying the overestimating scenario in those cases where concentrations were below the MDL or between the MDL and the MQL (concentrations<MDL replaced by MDL; MDL<concentrations<MQL replaced by MQL). For DEHP, average exposure of the two metabolites was obtained.

Highlights:

- First nationwide study in Spain assessing exposure to phthalates by analysing sewage
- 17 sewage treatment plants serving 13 cities and ca. 6 million people sampled
- Estimated levels in urine close to levels reported in literature for 5 metabolites
- Average exposure levels for parent phthalates ranged from 2 to 1347 μg/(day·inh)
- Safe reference values of butylated phthalates exceeded in some sites


Sampling
Population (1000 inh.)
80
300
600
900
1150
O
O
OH
O
Wastewater analysis
Phthalate exposure assessment
Daily exposure to DEP (µg/day/inh)
4000
3500
3000
2500
2000
1500
1000
500
0

Supplementary Material

Assessing population exposure to phthalate plasticizers in thirteen Spanish cities through the analysis of wastewater

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List of tables

Table S1. Details of the sampled wastewater treatment plants (WWTPs): name, population served, method used to estimate this population, locations/districts served, percentage of the main city population covered by the WWTP, sampling details and daily flow rates.

City[1]	**Barcelona**	**Bilbao**	**Castellón**	**Guadalajara**	**Lleida**	**Madrid**	**Madrid**	**Móstoles**	**Palma de Mallorca**	**Palma de Mallorca**	**Reus**	**Santiago de Compostela**	**Tarragona**	**Toledo**	**Valencia**	**Valencia**	**Valencia**
WWTP name (code)	Prat de Llobregat	Galindo	Castellón De La Plana	Guadalajara	Lleida	Madrid Centre	Madrid North	El Soto	Palma I	Palma II	Reus	Silvouta	Tarragona	Estiviel	Pinedo I (Valencia PI)	Pinedo II (Valencia PII)	Quart Benager (Valencia QB)
Population served by the WWTP	1163154	860237	171669	94755	143612	727176	227869	187281	406492	47961	115000	136500	142635	79793	527222	788242	162249
Method used to estimate the population served[2]	C (2017)	C (2016)	C (2015)	Average BOD Jan-April 2018	C (2017)	Average COD sampling period	Average BOD 2016 (with 60g BOD/d)	H x 3.5 (WWTP recommendation)	C (2017)	C (2017)	C (2017)	H x 2.5 (WWTP recommendation)	C (2017)	Average BOD April-May 2018	COD	COD	COD
Locations/districts served by the WWTP	Barcelona, Cervelló, Cornellà de Llobregat, Esplugues de Llobregat, Hospitalet de Llobregat, El Prat de Llobregat, Sant Boi de Llobregat, San Joan Despí, San Just Desvern	Abanto-Zierbena, Alonsotegi, Arrigorriaga, Barakaldo, Barrika, Basauri, Berango, Bilbao, Derio, Erandio, Etxebarri, Galdakao, Getxo, Leioa, Lezama, Loiu, Ortuella, Portugalete, Santurtzi, Sestao, Sondika, Sopelana, Trapagaran, Ugao-Miravalles, Urduliz,Zamudio, Zaratamo, Zeberio	Castellón De La Plana	Guadalajara	Lleida, Alpicat	Chamartín, Tetuán, Moncloa-Aravaca, Chamberí, Centro, Arganzuela, Retiro, Ciudad Lineal, Salamanca, Moratalaz, Puente de Vallecas	Chamartín, Tetuán, Moncloa-Aravaca, Fuencarral-El Pardo, Pozuelo de Alarcón, Las Rozas, Majadahonda	Móstoles, Alcorcón, Fuenlabrada (all served also by other WWTPs)	Palma beach, Sant Jordi, El Pil·lari, Son Sant Joan airport, part of Palma city	Main part of Palma city, Son Castelló, Can Valero and Son Rosinyol Industrial States, Marratxí, Esporles and Bunyola	Reus, Castellvell, Almoster	Santiago de Compostela	Tarragona, La Canonja, els Pallaresos	Toledo	Valencia	Albal, Alcasser, Alfafar, Benetusser, Beniparrell, Burjassot, Catarroja, Llocnou de la Corona, Massanassa, Mislata, Paiporta, Paterna, Picanya, Picassent, Sedaví, Silla, Torrent, Valencia,	Alaquàs, Aldaia, Manises, Mislata, Quart de Poblet, Xirivella
% of main city population covered by WWTP(s)[3]	35%	100%	100%	100%	100%	30%		90%	100%		100%	100%	100%	100%	100%		
Location of autosampler	Mechanical bar screens	After coarse screens and pumping	Before fine screen	Before fine screen	Before fine screen	After sieving	After fine screen	After fine screen	After fine screen	After fine screen	After fine screen	After fine screen	Before fine screen	After sieving	After fine screen	After fine screen	After fine screen
Autosampler refrigerated?	Yes	No	No	No	No	Yes	Yes	Yes	No	No	No	No	No	No	Yes	Yes	No
Time of beginning of the sampling	9:00	8:00	8:30	10:00	6:00	8:00	8:00	8:00	10:00	10:00	20:00	9:00	8:00-9:00	8:00	8:00	8:00	8:00
Sampling mode[4]	T (50 mL/10 min)	T (100 mL/60 min)	T (100 mL/15 min)	T (200 mL/60 min)	T (200 mL/60 min)	T (400 mL/30 min)	T (100 mL/60 min)	T (100 mL/60 min)	T (100 mL/15 min)	T (100 mL/15 min)	F	T (150 mL/10 min)	T (450 mL/60 min)	T (100 mL/15 min)	T (100 mL/60 min)	T (100 mL/60 min)	F

City[1]	Barcelona	Bilbao	Castellón	Guadalajara	Lleida	Madrid	Madrid	Móstoles	Palma de Mallorca	Palma de Mallorca	Reus	Santiago de Compostela	Tarragona	Toledo	Valencia	Valencia	Valencia
Sampling date[5] - 1	2018.03.19	2018.04.23	2018.04.16	2018.05.07	2018.03.12	2018.05.21	2018.06.25	2018.05.21	2018.04.16	2018.04.23	2018.04.17	2018.03.19	2018.04.17	2018.04.23	2018.04.16	2018.04.16	2018.04.16
Sampling date - 2	2018.03.20	2018.04.17	2018.04.17	2018.05.08	2018.03.13	2018.05.22	2018.06.26	2018.05.22	2018.04.10	2018.04.24	2018.04.18	2018.03.13	2018.04.18	2018.04.17	2018.04.10	2018.04.10	2018.04.10
Sampling date - 3	2018.03.14	2018.04.18	2018.04.11	2018.05.02	2018.03.07	2018.05.16	2018.06.20	2018.05.23	2018.04.11	2018.04.18	2018.04.19	2018.03.14	2018.04.19	2018.04.18	2018.04.11	2018.04.11	2018.04.11
Sampling date - 4	2018.03.15	2018.04.19	2018.04.12	2018.05.03	2018.03.08	2018.05.17	2018.06.21	2018.05.17	2018.04.12	2018.04.19	2018.04.20	2018.03.15	2018.04.20	2018.04.19	2018.04.12	2018.04.12	2018.04.12
Flow (m^3/day) - 1	253120	312461	29597	29849	34500	107309	47045	29649	44849	50180	17980	99917	25713	15450	118934	272420	38055
Flow (m^3/day) - 2	400037	287205	33363	30342	44720	109814	46726	25420	45739	48840	17720	102672	24426	14935	131003	213799	36143
Flow (m^3/day) - 3	255858	268352	46317	30246	40700	108173	41622	27738	49850	51905	18100	111999	24673	13713	136262	248374	29789
Flow (m^3/day) - 4	244204	256858	37625	29853	41410	112666	42978	26379	44579	50979	17742	122643	23748	13627	121017	205501	26932

[1] Name of the main city served by the WWTP (some WWTPs receive wastewater from other towns included in the capital metropolitan area)
[2] C: census (year); BOD: biochemical oxygen demand; COD: chemical oxygen demand; H: number of homes connected to the sewage system
[3] WWTPs serving parts of the same main city were considered all together for this calculation
[4] T: time proportional (volume sampled/frequency of sampling); F: flow proportional
[5] Sampling date/day 1-4: Mo-Th for all cities but Reus and Tarragona (Tu-Fri)

Table S2. Analytes and deuterated analogs used as surrogate/internal standards (IS), retention times (RT), cone voltage (CV) and collision energy (CE) values, quantifier (Q) and qualifier (q) transitions, ratio between them, method detection limits (MDL) and method quantification limits (MQL).

Compounds	$[M\text{-}H]^-$ Formula	RT (min)	IS	Precursor *m/z*	CV (V)	Quantifier (Q)		qualifier (q)		Ratio q/Q	MDL (ng/L)	MQL (ng/L)
						m/z	CE	*m/z*	CE			
MMP	$C_9H_7O_4$	3.3	MMP-D_4	179	27	107	8	77	17	1.33	10	32
MEP	$C_{10}H_9O_4$	3.8	MBP-D_4	193	22	77	15	121	10	1.28	1.3	4.2
MiBP	$C_{12}H_{13}O_4$	5.9	MBP-D_4	221	27	77	16	134	12	0.81	1.5	4.9
MnBP	$C12H_{13}O_4$	6.2	MBP-D_4	221	23	77	17	177	9	0.38	1.1	3.5
MBzP	$C_{15}H_{11}O_4$	7.0	MBP-D_4	255	27	77	20	183	10	0.86	0.69	2.3
MEOHP	$C_{16}H_{19}O_5$	6.4	MEHHP-D_4	291	27	143	12	121	16	0.84	2.9	9.5
MEHHP	$C_{16}H_{21}O_5$	6.2	MEHHP-D_4	293	32	145	13	121	20	1.03	4.0	13
MMP-D_4	$C_9H_3D_4O_4$	3.3	—	183	27	111	8	—	—	—	—	—
MBP-D_4	$C_{12}H_9D_4O_4$	6.1	—	225	23	81	17	—	—	—	—	—
MEHHP-D_4	$C_{16}H_{17}D_4O_5$	6.1	—	297	32	125	20	—	—	—	—	—

Table S3. Oral Reference Doses (RfD) and Tolerable Daily Intakes (TDI) set by the U.S. Environmental Protection Agency (EPA) and the European Food Safety Authority (EFSA) for the five phthalate esters considered for risk assessment. Safe Reference Values (SRV) calculated from RfD and TDI considering average body weights of 70.8 kg for adults and 11.5 kg for toddlers (Walpole et al., 2012, WHO et al., 2006). Toxic Equivalents (Tox EqPAE), i.e. factors expressing the individual toxicity of a single phthalate relative to the most toxic derivative, calculated based on the RfDs provided by the EPA.

Phthalate esters	**RfD-EPA (μg/(kg·day))**	**TDI-EFSA (μg/(kg·day))**	**SRV (μg/(day·adult)) 70.8 kg adult**		**SRV (μg/(day·toddler)) 11.5 kg toddler**		**Tox EqPAE**
			RfD-EPA	**TDI-EFSA**	**RfD-EPA**	**TDI-EFSA**	
DEP	800	—	56640	—	9200	—	0.025
DiBP	100	10	7080	708	1150	115	0.2
DnBP	100	10	7080	708	1150	115	0.2
BzBP	200	500	14160	35400	2300	5750	0.1
DEHP	20	50	1416	3540	230	575	1

Walpole, S.C., Prieto-Merino, D., Edwards, P., Cleland, J., Stevens, G., Roberts, I., 2012. The weight of nations: an estimation of adult human biomass. BMC Public Health, 12, 439-444.

World Health Organization (WHO 2006). WHO Child Growth Standards Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development.

Table S4. Population-normalized metabolite loads (in μg/(day·inh)) for every location and day. Simple average, standard deviation (SD), median, 5th and 95th percentiles.

Compounds -day[1]	Barcelona	Bilbao	Castellón	Guadalajara	Lleida	Madrid Centre	Madrid North	Móstoles	Reus	Santiago de Compostela	Tarragona	Toledo	Valencia PI	Valencia PII	Valencia QB	Palma de Mallorca[3]	Average (μg/(day·inh))	SD (μg/(day·inh))	Median (μg/(day·inh))	5th percentile	95th percentile
MMP - 1	100	1013	40	117	171	82	153	64	58	203	73	83	66	69	55	225	175	220	91	42	717
MMP - 2	224	784	170	44	238	76	282	79	48	54	42	44	69	134	269	151					
MMP - 3	295	867	76	235	119	55	92	61	53	86	35	59	58	90	98	333					
MMP - 4	197	1137	294	188	182	35	223	172	55	74	59	42	48	159	121	326					
MEP - 1	1041	585	139	953	527	514	699	611	534	742	192	138	533	765	304	1287	660	439	536	138	1465
MEP - 2	616	776	394	787	565	473	539	221	407	367	356	87	1011	1476	1101	686					
MEP - 3	2087	455	284	1015	439	432	568	366	418	275	338	115	895	1062	1139	1403					
MEP - 4	1039	658	857	1095	381	254	427	367	275	355	288	101	751	1553	2108	1044					
MiBP - 1	70	118	23	106	83	52	114	122	29	1445	6.9	25	51	42	22	79	97	180	69	11	188
MiBP - 2	96	108	52	90	128	70	141	12	11	295	61	14	119	112	103	48					
MiBP - 3	148	72	43	121	69	38	169	68	11	60	9.5	12	87	110	61	70					
MiBP - 4	78	97	68	218	39	41	67	51	6.8	192	15	15	52	109	101	54					
MnBP - 1	32	89	33	70	46	35	132	135	6.2	635	3.4	11	22	20	6.9	65	63	82	47	3.1	131
MnBP - 2	63	74	44	82	68	39	67	3.6	3.1	153	46	7.1	124	120	120	48					
MnBP - 3	66	34	66	88	44	29	41	57	3.2	73	4.2	4.9	70	88	44	70					
MnBP - 4	55	90	112	83	38	20	24	27	1.1	117	2.2	2.9	46	105	83	51					
MBzP - 1[2]	0.00-0.15	0.00-0.25	0.00-0.12	9.2	0.00-0.17	0.00-0.10	0.00-0.14	7.0	0.00-0.11	6.9	0.00-0.12	0.00-0.13	0.00-0.16	0.00-0.24	0.00-0.16	1.7-1.8	1.6-1.7	2.5-2.4	–	–	–
MBzP - 2[2]	0.00-0.24	3.7	0.00-0.13	3.1	0.00-0.21	2.4	0.00-0.14	0.00-0.094	0.00-0.11	7.4	2.6	0.00-0.13	3.1	0.00-0.19	0.00-0.15	1.9-2.0					
MBzP - 3[2]	0.00-0.15	0.00-0.22	0.00-0.19	5.3	0.00-0.20	0.00-0.10	0.00-0.13	2.1	0.00-0.11	8.9	0.00-0.12	0.00-0.12	1.8	2.3	0.00-0.13	3.2-3.3					
MBzP - 4[2]	0.00-0.14	0.00-0.21	4.3	3.4	4.1	0.00-0.11	0.00-0.13	0.00-0.10	0.00-0.11	6.0	0.00-0.11	0.00-0.12	0.00-0.16	2.6	3.8	5.1-5.1					
MEOHP - 1[2]	0.00-0.63	5.0	0.50-1.6	9.6	0.00-0.70	0.00-0.43	0.00-0.60	20	0.00-0.45	2.1-7.0	0.00-0.52	0.00-0.56	0.00-0.65	0.00-1.0	0.00-0.68	4.4-4.7	3.1-3.8	4.9-4.8	–	–	–
MEOHP - 2[2]	0.00-1.0	0.00-0.97	6.7	10	0.00-0.90	0.00-0.44	0.00-0.59	0.00-0.39	0.00-0.45	2.2-7.1	0.00-0.50	0.00-0.54	16	6.8	20	3.0-3.3					
MEOHP - 3[2]	4.6	0.00-0.90	4.1	3.3	0.00-0.82	0.00-0.43	0.00-0.53	6.6	0.00-0.46	2.4-7.8	0.00-0.50	0.00-0.50	8.7	11	0.00-0.53	7.1-7.4					
MEOHP - 4[2]	2.2	3.3	13	0.00-0.91	0.00-0.84	0.00-0.45	0.00-0.55	0.00-0.41	0.00-0.45	2.6-8.5	0.00-0.48	0.00-0.50	4.1	8.9	0.00-0.48	9.6-9.9					
MEHHP - 1[2]	00.00-0.87	6.7	0.00-0.69	8.9	0.00-0.96	0.00-0.59	0.00-0.82	27	0.00-0.62	2.9-9.7	0.00-0.72	0.00-0.77	0.00-0.90	0.00-1.4	0.00-0.94	0.00-0.83	3.0-4.0	5.9-5.9	–	–	–
MEHHP - 2[2]	0.00-1.4	0.00-1.3	9.0	11	0.00-1.2	0.00-0.60	0.00-0.82	0.00-0.54	0.00-0.61	3.0-10	6.9	0.00-0.75	13	7.1	0.00-0.89	0.00-0.83					
MEHHP - 3[2]	0.00-0.88	0.00-1.2	0.00-1.1	0.00-1.3	0.00-1.1	0.00-0.59	0.00-0.73	10.6	0.00-0.63	3.3-11	0.00-0.69	0.00-0.69	24	21	0.00-0.73	0.00-0.89					
MEHHP - 4[2]	0.00-0.84	0.00-1.2	9.5	7.3	0.00-1.2	0.00-0.62	0.00-0.75	0.00-0.56	0.00-0.62	3.6-12	0.00-0.66	0.00-0.68	4.8	11	0.00-0.66	0.00-0.84					

[1] Day 1-4: Mo-Th for all cities but Reus and Tarragona (Tu-Fri)

[2] Two values provided in those cases where concentrations were <MDL or fell between the MDL and the MQL: load obtained from the underestimating scenario - load obtained from the overestimating scenario. Median and percentiles were not calculated due to detection frequency (% samples > MDL) < 50%

[3] WWTPs at Palma de Mallorca (two): combined and considered as a single one

Table S5. Regression coefficients (R), sample size (n) and p-values of the Pearson pairwise correlation study performed between 4-days average loads for MMP, MEP, MiBP, and MnBP.

		MMP	MEP	MiBP	MnBP
MMP	R		0.1315	0.0311	0.0881
	n		16	16	16
	p-value		0.6273	0.9088	0.7457
MEP	R	0.1315		0.0236	0.1513
	n	16		16	16
	p-value	0.6273		0.9308	0.5758
MiBP	R	0.0311	0.0236		0.9658
	n	16	16		16
	p-value	0.9088	0.9308		<0.0001
MnBP	R	0.0881	0.1513	0.9658	
	n	16	16	16	
	p-value	0.7457	0.5758	<0.0001	

Table S6. Estimated metabolite concentrations in urine (in ng/mL). Average values of 4 days in the different locations assessed, simple average for all locations and all days, standard deviation (SD), median, 5th and 95th percentiles.

Compounds	Barcelona	Bilbao	Castellón	Guadalajara	Lleida	Madrid Centre	Madrid North	Móstoles	Reus	Santiago de Compostela	Tarragona	Toledo	Valencia PI	Valencia PII	Valencia QB	Palma de Mallorca	Average (μg/(day·inh))	SD (μg/(day·inh))	Median (μg/(day·inh))	5th percentile	95th percentile
MMP	130	608	92	93	113	40	119	60	34	66	33	36	38	72	86	165	112	141	58	27	459
MEP	762	396	267	613	305	267	356	249	260	277	187	70	508	773	741	704	421	280	342	88	933
MiBP	62	63	30	85	51	32	78	40	9.2	317	15	10	49	59	46	40	62	114	44	7.0	120
MnBP	34	46	41	52	31	20	42	36	2.2	156	8.9	4.2	42	53	40	37	40	52	30	2.0	83
MBzP[1]	0.00-0.11	0.59-0.69	0.69-0.76	3.3	0.65-0.74	0.39-0.44	0.00-0.09	1.5	0.00-0.07	4.7	0.42-0.48	0.00-0.08	0.78-0.83	0.78-0.85	0.61-0.68	1.9	1.0-1.1	1.6-1.5	–	–	–
MEOHP[1]	1.1-1.3	1.3-1.6	3.9-4.1	3.7-3.8	0.00-0.52	0.00-0.28	0.00-0.36	4.3-4.4	0.00-0.29	1.5-4.8	0.00-0.32	0.00-0.33	4.5-4.6	4.2-4.3	3.2-3.5	3.8-4.0	2.0-2.4	3.1-3.0	–	–	–
MEHHP[1]	0.00-0.63	1.1-1.7	2.9-3.2	4.3-4.5	0.00-0.71	0.00-0.38	0.00-0.50	6.0-6.1	0.00-0.40	2.0-6.8	1.1-1.4	0.00-0.46	6.6-6.8	6.2-6.4	0.00-0.51	0.00-0.54	1.9-2.6	3.8-3.7	–	–	–

[1] Two values provided in those cases where concentrations were <MDL or fell between the MDL and the MQL: value from the underestimating scenario - value from the overestimating scenario. Median and percentiles were not calculated due to detection frequency < 50%