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Investigation of phthalate metabolites in urine and daily phthalate intakes among three age groups in Beijing, China☆

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ABSTRACT

Phthalates are widely used as binders and plasticizers in industrial and consumer products but show diverse toxicity. We investigated the level of human exposure to phthalates in Beijing, one of the most densely populated cities in the world. In this study, 12 metabolites of phthalates were measured in 70 spot urine samples collected from Beijing residents from August 2017 to April 2018 using ultra high-performance liquid chromatography tandem mass spectrometry. We found that metabolites of phthalates were ubiquitous in all urine samples. Total concentrations of phthalate metabolites ranged from 39.6 to 1931 ng mL^{-1}, with median concentrations were in decreasing order of children (371 ng mL^{-1})> younger adults (332 ng mL^{-1})> older adults (276 ng mL^{-1}). Mono-n-butyl phthalate (MnBP) was the predominant compound, and occurred at concentrations greater than those reported for people in other countries. The mean values of estimated daily intakes (EDIs) of ∑phthalate were 35.2, 10.3 and 10.9 ng $(kg\text{-}bm)^{-1}$ d^{-1} for children, younger adults and older adults, respectively. EDIs of di-n-butyl phthalate (DnBP), di-iso-butyl phthalate (DiBP) and di-(2-ethylhexyl) phthalate (DEHP) exceeded reference values suggested by the US Environmental Protection Agency and the European Food Safety Authority. When concentrations were normalized to volume or creatinine-adjusted, hazard quotients (HQs) for 40 of 70 participants exhibited larger HQs >1 for individual phthalates, which was indicative of potential for adverse effects. Thus, exposure to phthalates might be a critical factor contributing to adverse health effects in Beijing residents. To the best of our knowledge, this is the first study to establish a pre-baseline level of urinary phthalate metabolites among residents in Beijing.

1. Introduction

Phthalates (PAEs) are a class of artificial, organic chemicals used primarily as binders and plasticizers in industrial and consumer products (Lü et al., 2018). Phthalates with a high-molecular weight including di-(2-ethylhexyl) phthalate (DEHP), butyl benzyl phthalate (BBzP), di-isononyl phthalate (DiNP), dicyclohexyl phthalate (DCHP), di-n-decyl phthalate (DDP) and dioctyl phthalate (DOP) are widely used in the production of polyvinyl chloride, resins, toys, food packages and pharmaceutical products. Other phthalates with lower formula masses, including dimethyl phthalate (DMP), diethyl phthalate (DEP), di-iso-butyl phthalate (DiBP), and di-n-butyl phthalate (DnBP) are added to cosmetics and personal care products (Schettler et al., 2010; Wang et al., 2015). Phthalates are not fully bound to polymer matrixes, and can therefore be easily released into the surrounding environment (Ying et al., 2018). To date, phthalates have been detected in indoor air and dust, drinking water, lake water, the atmosphere, and, with high concentrations of up to part-per-million (ppm), in the indoor air of private cars (Chi et al., 2017; Gao et al., 2019; Li et al., 2019; Zhang et al., 2014). Thus, the general population is continuously exposed to phthalates via multiple routes of inhalation, food intake, dust ingestion and dermal absorption (Huang et al., 2018; Shi et al., 2017; Shi et al.,

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2012; Zhang et al., 2019).

Phthalates have been identified as endocrine disruptors, leading to reproductive toxicity and genotoxic effects on laboratory animals (Ghisari and Jorgensen, 2009; Yin et al., 2018), exposure of humans to phthalates has become a major public health concern (Mankidy et al., 2013; Xi et al., 2012). Subsequent epidemiological studies have reported a positive relationship between phthalate exposure and impact on reproductive diseases or endocrine system, including malformation genital, precocious puberty and obesity (Liao et al., 2018; Luísa et al., 2018; Sathyanarayana et al., 2016; Srilanchakon et al., 2017). Exposure to phthalates has been suggested to cause a decrease in systolic or diastolic blood pressure in pregnant women and was positively associated with coronary heart disease among elderly people (Su et al., 2019). As a result, some countries have added phthalates to lists of chemicals of concern to be regulated and or monitored (Wang et al., 2018).

Phthalates are rapidly metabolized by phase I enzymes and conjugated. Metabolites of phthalates in urine have proven to be useful biomarkers of exposure to assess internal doses of parent phthalates (Guo et al., 2011b). Monitoring of the occurrence of phthalate metabolites in human urine has been conducted previously in international studies (Becker et al., 2009; Blount et al., 2000; Frederiksen et al., 2011; Lee et al., 2018). The highest concentration of total phthalate metabolites was reported in Kuwait, with a concentration of up to 19,300 ng mL^{-1}, indicating widespread intake of phthalates (Guo et al., 2011a). China has a large demand for phthalates, accounting for approximately 90% of plasticizer consumption worldwide. The ubiquitous use and potential exposure to phthalates warrants even more attention in China than in other countries. To date, several studies have examined the urinary concentrations of metabolites of phthalates in China, confirming widespread human exposures (Gao et al., 2016; Wang et al., 2015; Zhang et al., 2018). However, to the best of our knowledge, no previous studies have compared urinary concentrations of metabolites of phthalates in people of different age groups in urban areas of China. Given that Beijing is one of the most densely populated cities in the world, it is deemed appropriate to investigated the occurrence of phthalates in the urine of residents of various age groups in Beijing.

In the present study, concentrations of 12 metabolites of phthalates were measured in the urine of 70 individuals in three age groups: children (<18 years), younger adults (18–50 years), and older adults (>50 years) in Beijing, China. The aims of current study were to: 1) develop a pre-baseline by quantifying phthalates metabolites in the urine of Beijing residents; 2) characterize profiles of absolute and relative concentrations of phthalates from which daily exposures and internal doses of phthalates from which hazard quotients (HQs) could be calculated for residents of Beijing; and 3) describe differences in exposure to phthalates between different age groups.

2. Materials and methods

2.1. Sample collection

This study was approved by the Ethics Committee of National Institute of Environmental Health, Chinese Center for Disease Control and Prevention (NIEH, China CDC). Participants were recruited from authors' colleagues, friends and their families who worked and/or living in different district of Beijing. All 70 healthy participants were informed of the purposes, policies and procedures governing the study and their permissions were obtained. Demographic information of participants were given in Supplementary Material Table S1. A total of 70 spot urine samples were collected, between August 2017 and April 2018, from individuals stratified by sex and classified into three groups: children (<18 years, $N = 20$), younger adults (18–50 years, $N = 30$), and older adults (>50 years, $N = 20$). Urine (4 mL) was collected into 5 mL polypropylene tubes, transported to the laboratory on ice, and then stored at −70 °C until analysis.

2.2. Materials

Twelve target compounds including monomethyl phthalate (MMP), monoethyl phthalate (MEP), monoisobutyl phthalate (MiBP), mono-n-butyl phthalate (MnBP), monocyclohexyl phthalate (MCHP), mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono(2-ethyl-5- oxohexyl) phthalate (MEOHP), mono(2-ethylhexyl) phthalate (MEHP), monobenzyl phthalate (MBzP), monooctyl phthalate (MOP), monoisononyl phthalate (MiNP), monoisodecyl phthalate (MiDP) and their isotopically-labeled internal were purchased from the Cambridge Isotope Laboratories (Andover, MA, USA). Creatinine was purchased from CDN isotopes (Quebec, Canada). Methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). Ammonia hydroxide solution (25%–28% solution in water) and β-glucuronidase (from *helix pomatia*, 85,000 U mL^{-1}) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ammonium acetate, formic acid and acetic acid (LC-MS grade) were purchased from Fisher Scientific. Standard reference materials (SRM 3672) were obtained from the National Institute of Standards and Technology (Gaithersburg, USA).

2.3. Quantification of metabolites of phthalates

Concentrations of target compounds were determined by use of an ultra-high performance liquid chromatography (I Class, Waters, USA) system, which was interfaced with a AB SCIEX 6500 (Applied Biosystems, USA) triple quadrupole mass spectrometer in multiple-reaction monitoring (MRM) mode. The procedure about sample preparation and instrumental analysis were stated in the supplementary material.

2.4. Quality assurance and quality control

For each batch of 25 samples, including 8 standard curve points, 14 urine samples, 1 procedural blank, 1 SRM 3672 sample and 1 matrix spiked samples with the concentration of 20 ng mL^{-1}, were processed by use of identical procedures. Artificial urine was used to prepare the calibration curve, and the procedural blank was prepared by adding 2 mL of ultrapure water instead of samples of urine. Method detection limits (MDLs) and method quantification limits (MQLs) were determined according to methods specified by the U.S. EPA (EPA, 2016), and ranged from 0.07 to 0.82 ng mL^{-1} (Table S4). Calibration curves based on eight concentrations, ranging from 0.5 to 100 ng mL^{-1}, were prepared for each batch of samples of urine and correlation coefficients (r^2) of all standard curves were greater than 0.999. The average recoveries rate and relative standard deviation of target compounds ranged from 90% to 109% and 4.5%–13%, respectively. The reference and detected values of SRM 3672 are reported (Table S5). Furthermore, reliabilities of quantifications of metabolites and urine creatinine were verified by successfully passed the program of German External Quality Assessment Scheme (G-EQUAS) in 2018.

2.5. Estimated daily intakes of PAEs

Both normalizations to volumes of urine and concentrations of creatinine were used to report concentrations of metabolites of PAEs in urine, that were subsequently used to estimate daily intakes of parent PAEs (Wang et al., 2015). Volume-based and

creatinine-based models were described by Equations in supplementary material.

2.6. Hazards posed by PAEs

Exposure to PAEs can cause adverse effects on humans. The tolerable daily intakes (TDI) and the reference doses (RfD) of PAEs were established by EFSA and the U.S. EPA for protection of public health (Wang et al., 2015). TDI and RfD values of individual PAEs were obtained from the literature (Gao et al., 2016). In this study, HQ, which is defined as the EDI divided by TDI or RfD (Equation (1)), was used to assess the hazards posed by exposures of people to individual phthalate. The HI (Equation (2)) is the sum of HQ. Values of HQ or HI greater than 1 indicate that intakes of PAEs were greater than reference limits.

$$HQ_{PAEs}=\frac{EDI}{TDI\ or\ RfD} \quad (1)$$

$$HI_{PAEs}=\sum HQ_{PAEs} \quad (2)$$

2.7. Statistical analyses

SPSS (version 17.0) was used for data statistical analyses. Concentrations less than MDLs were set as MDLs divided by 2 for further analyses. The Kolmogorov-Smirnov test was used to test for normality of distributions of concentrations of metabolites of PAEs in urine. When concentrations of metabolites of PAEs were non-normally distributed, the nonparametric Mann-Whitney *U* test and Kruskal-Wallis test was used to test for significant differences between male and female and among the three age groups, respectively. $P < 0.05$ was defined as statistically significant.

3. Results and discussion

3.1. Concentrations of phthalate metabolites

Rates of detection and concentrations of detectable phthalate metabolites in urine stratified by age are presented in Fig. 1 and Table S6. In this study, more than one metabolite was found in all urine samples, suggesting that residents of Beijing are ubiquitously exposed to phthalates. The detected rates of MMP, MEP, MiBP, MnBP, MEOHP, and MEHHP were 100%, greater than the rates of MEHP (94.3%) and MBzP (52.9%). MCHP, MOP, MiNP and MiDP were less than MDLs in any of the samples, and were excluded from further analyses. Our results were similar to those of previous studies in China, reporting that MOP, MiNP and MCHP were less frequently detected than other metabolites (Gao et al., 2016; Guo et al., 2011b). This finding could be explained by the following factors: 1) exposure to parent phthalates of these metabolites (DiOP, DiNP, DCHP and DDP) rarely occurred; or 2) some phthalates are first metabolized to form primary metabolites, then metabolized again to form secondary metabolites. The primary monoester metabolites of parent phthalates are not the major metabolites. According to the Fourth National Report on Human Exposure to Environmental Chemicals (US.CDC, 2015), secondary metabolites, including momo-(carboxyoctyl) phthalate (MCOP) and mono(3-carboxypropyl) phthalate (MCPP), have been identified as biomarkers of exposure to DiNP and DiOP, respectively. Hence, for assessing exposure to DiNP and DiOP, monitoring MCOP and MCPP should be considered in future studies.

No significant gender differences were found between concentrations of metabolites of phthalates when normalized to volume or creatinine-adjusted, in all three age groups. However, greater concentrations of phthalates metabolites were generally found in children compared with younger adults or older adults (Fig. 1a and b). In the volume model, total concentrations of the eight detectable metabolites ranged from 39.6 to 1931 ng mL^{-1}, with concentrations in decreasing order with age: children > younger adults > older adults, with concentrations of 110–1864 (median: 371) ng mL^{-1}, 39.6–1282 (median: 332) ng mL^{-1}, and 68.1–1931 (median: 276) ng mL^{-1}, respectively. Similar results of concentrations of ∑phthalate metabolites were found in creatinine adjusted data. Ranges (median) of total creatinine-adjusted concentrations of metabolites in urine (μg g^{-1}) in three age groups were 138–3602 (1113) in children, 42.4–739 (257) in younger adults, and 79.8–854 (283) in older adults, respectively. The relatively high exposure to phthalates in children might have been caused by two factors. First, children require more energy per kilogram of body mass than adults, resulting in greater exposure to phthalates via the diet (WHO, 2011). Second, exposure pathways might be different between children and adults due to the unique hand-to-mouth behaviors, including pica. For example, children's toys made of soft polyvinyl chloride are often plasticized with phthalates. Children

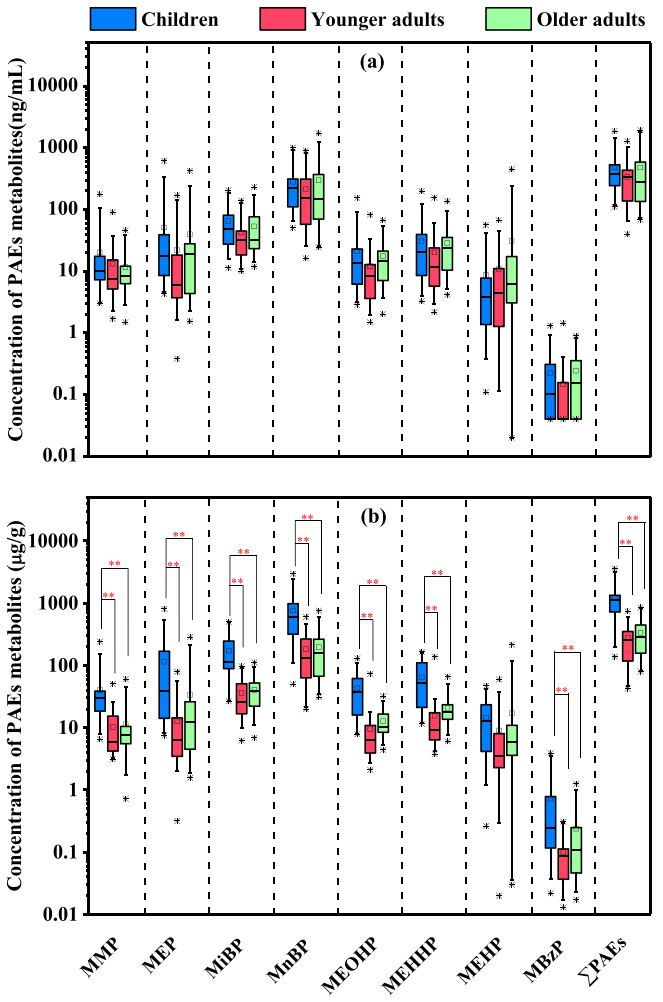

Fig. 1. Concentrations of metabolites of phthalates in urine (individual and ∑phthalates of detectable) from three age groups of Beijing: (a) ng mL^{-1} (volume-model); and (b) μg g^{-1} (creatinine-model). Lower and upper edges of boxes are 25% and 75% centiles. The whiskers represent 5th and 95th centiles. The horizontal line means the median concentration. The symbols of □ and * represent the mean and maximum/minimum values, respectively. ** represents a significant difference in three age groups with $p < 0.01$.

might extract and ingest certain quantities of phthalates when they suck or chew on toys or hands (Healy et al., 2015). Interestingly, except for MEHP, significant differences ($p < 0.01$) were observed in the other individual phthalates and ∑phthalate among three age groups in creatinine-adjusted concentrations (Fig. 1b). The significantly greater creatinine-adjusted concentrations of metabolites of phthalates were related to lower concentrations of creatinine in children than that in younger adults and older adults. These results suggest that children seemed more likely to be exposed to phthalates than adults in Beijing.

A comparison with international findings was conducted by examining the total concentrations for identical measured metabolites. The sum of concentrations of eight detectable metabolites in urine of Beijing residents (mean: 417 ng mL^{-1}) were approximately 1.5- to 3.7-fold greater than those in urine of people from Guangzhou, China (112 ng mL^{-1}), Malaysia (267 ng mL^{-1}), Japan (148 ng mL^{-1}), Korea (113 ng mL^{-1}) (Guo et al., 2011a; Rocha et al., 2017; Zhang et al., 2018), but less than those in India (485 ng mL^{-1}) (Guo et al., 2011a), Denmark (470 ng mL^{-1}) (Mieritz et al., 2012), Brazil (613 ng mL^{-1}) (Rocha et al., 2017), the United States (853 ng mL^{-1}) (Hernández-Díaz et al., 2008) and Saudi Arabia (1240 ng mL^{-1}) (Lee et al., 2019). These results indicate that exposure concentrations of phthalates in humans vary widely between countries, suggesting that there might be different sources or/and pathways of phthalates in different areas.

3.2. Profiles and comparison of metabolites of phthalates

Similar concentration profiles with minor differences in phthalate metabolites were found between age groups. As shown in Table S6, for children and older adults, the concentrations of metabolites of phthalates varied widely in decreasing order, as follows:
MnBP > MiBP > MEHHP > MEP > MEOHP > MMP > MEHP > MBzP. For younger adults, MnBP, MiBP and MEHHP were also the top three ranked compounds, but the concentration of MEP was lower than that of MEOHP and MMP. These results appear to suggest similar applications of phthalate as well as exposure sources and pathways in different age groups in Beijing residents.

MnBP is the predominant phthalate, with a median concentration in urine samples of children: 225 ng mL^{-1} (creatinine model: 606 μg g^{-1}), younger adults: 152 ng mL^{-1} (creatinine model: 134 μg g^{-1}) and older adults: 146 ng mL^{-1} (creatinine model: 160 μg g^{-1}), respectively, consistent with previous reports (Guo et al., 2011b; Zhang et al., 2018). Significant differences in urinary MnBP concentration were found in children compared with younger adults and older adults, reflecting a high level of exposure of children in Beijing to DnBP, the parent phthalate to MnBP. Concentrations of urinary metabolites of phthalates in human studies in various countries, are summarized in Table S7. Median levels of MnBP in children, younger adults and older adults in China were approximately 1.3- to 13- fold higher than those in American (children: 23.3, younger adults: 25.9, older adults: 11.4 ng mL^{-1}) (Kim et al., 2016; US.CDC, 2015), German (children: 93.4, younger adults: 49.8 ng mL^{-1}) (Becker et al., 2009; Fromme et al., 2007), Saudi Arabian (children: 179, younger adults: 96.4 ng mL^{-1}) (Iman et al., 2019; Lee et al., 2019), Mexican (younger adults, 72.4 ng mL^{-1}) (Romero et al., 2011), Brazilian (children, 42.4 ng mL^{-1}) (Rocha et al., 2017) and Korean (older adults, median: 33.3 ng mL^{-1}) (Lee et al., 2018). All of these results suggest higher exposure of people in China to DnBP compared with other countries. DnBP is a high-yield compound, with annual production in 2004 estimated to be more than 60,000 tons in China (Guo et al., 2011b). The high production and ubiquitous use of DnBP might lead to more exposure of Chinese people to this compound, compared with people in other countries. Moreover, should be noted that urinary concentrations of MnBP in Chinese people have been increasing from 2014 to 2018, from 34.7 to 225 ng mL^{-1}. In 2018, concentrations of MnBP in the urine of children and younger adults were approximately 6.5- and 2.5- fold greater than that in children in 2014 (Wu et al., 2017) and younger adults in 2010 (Guo et al., 2011b), respectively. The increased urinary MnBP concentration reflects the phenomenon of excessive use of phthalate, leading to high exposure of the Chinese population to DnBP, especially for children. However, in view of our study design, which included a relatively small number of participants, the current results have several intrinsic limitations. Thus, the increasing exposure profiles of MnBP should be validated with a lager sample size in future.

As the initial monoester metabolite of DiBP, MiBP is a critical pollutant. Median concentrations of MiBP were 48.1, 32.1, and 31.5 ng mL^{-1} in children, younger adults and older adults, respectively. Similar to MnBP, children also exhibited a higher concentration of MiBP compared with concentrations in other age groups. This result indicated that exposure to DiBP among children was higher than that of adults in Beijing. Concentrations of MiBP observed in the three age groups were less than those found in children (5–9 year-olds) in Beijing (median: 54.1 ng mL^{-1}) (Gong et al., 2015), Germany (median: 88.1 ng mL^{-1}) (Becker et al., 2009) and Saudi Arabia (median: 155 ng mL^{-1}) (Lee et al., 2019), in younger adults in Nanjing (median: 47.8 ng mL^{-1}) (Pan et al., 2015) and young adults (about 20 years old) in urban areas across China (median: 55.6 ng mL^{-1}) (Guo et al., 2011b), or in older adults in Shanghai (median: 99 ng mL^{-1}) (Dong et al., 2018). However, the urinary concentrations of MiBP observed in this study were greater than those reported in children, younger adults and older adults in the United States, Mexico and Brazil (Rocha et al., 2017; Romero et al., 2011). One possible explanation is that with the ban of DiBP worldwide (Loganathan, 2016), the use of this compound in these countries is significantly lower than in China (Wang et al., 2018). Comparisons of concentrations of urinary MiBP in people in China appear to reflect continuous exposure to DiBP, potentially indicating that exposure to DnBP and DiBP constitutes a health risk for Chinese people, that warrants concern.

The median concentration of MEP in the urine was found to occur in decreasing order with older adults (19.1 ng mL^{-1}), children (17.4 ng mL^{-1}) and younger adults (5.9 ng mL^{-1}). Concentrations of MEP in older adults and children were nearly 3- fold greater than those in younger adults. Several studies have reported that MEP is excreted at greater concentrations in adults than children because adults are more likely to use personal care products (Silva et al., 2004). In the current study, urinary concentrations of MEP in younger adults were lower than those in children and older adults in Beijing. High concentrations of MEP have been observed in various populations worldwide, and concentrations in urine of the Beijing residents were lower than those in reported among people in Brazil (children: 57.3 ng mL^{-1}) (Rocha et al., 2017), the United States (children: 33 ng mL^{-1}, younger adults: 208 ng mL^{-1}, older adults: 70.6 ng mL^{-1}) (Kim et al., 2016), Mexico (younger adults: 83.2 ng mL^{-1}) (Romero et al., 2011), Denmark (younger adults, 28 ng mL^{-1}) (Frederiksen et al., 2013) and Belgium (younger adults: 74 ng mL^{-1}) (Dirtu et al., 2013). DEP has been reported to occur at high levels (5486–38663 μg mL^{-1}) in perfume (Just et al., 2010), and people in Europe and America are more likely to use perfume than people in China. Hence, differences in the use of perfume products in various countries may explain some of the regional differences reported. In brief, lower concentrations of MEP in urine suggested a relatively low level exposure to DEP among people living in Beijing.

Concentrations of urinary MMP in children (median:

10.1 ng mL^{-1}) were slightly higher than those in younger adults (median: 7.4 ng mL^{-1}) or older adults (median: 8.3 ng mL^{-1}). This result indicates that the levels of exposure of various age groups to DMP were comparable. Internationally, there have been few reports of urinary MMP levels, but concentrations of MMP in urine of residents of Beijing were greater than those reported in the United States, Germany and Australia (Kasper Sonnenberg et al., 2012; Ramos et al., 2016), indicating a greater exposure to DMP in China than elsewhere. However, in China, a trend of decreasing exposure to DMP has been observed in recent years, with median concentrations of its metabolite, MMP of 40%–80% less in 2018 compared with the concentrations observed between 2010 and 2017. These findings suggest that the use of DMP might have decreased in China in recent years.

Widespread use of DEHP in commercial products results in substantial exposure to humans. In the current study, MEOHP, MEHHP and MEHP were used as biomarkers to assess exposure to the parent material, DEHP. Median concentrations of MEHHP, MEOHP and MEHP in children, younger adults and older adults were 16.7, 10.0, and 4.6 ng mL^{-1}, respectively. Secondary oxidative metabolites of DEHP, such as MEHHP and MEOHP, are excreted in amounts 2- to 4- fold greater than concentrations of the initial metabolite of MEHP. Compared with MEOHP and MEHP, MEHHP was found to be a better biomarker for assessing exposure to DEHP. Metabolism of DEHP in humans is complex and can yield many oxidative metabolites including mono (2-ethyl-5-carboxypentyl) phthalate (MECPP), which generally occur at greater concentrations than urinary MEHHP in Chinese children and young adults (Gao et al., 2016; Wang et al., 2015). However, information regarding the concentrations of urinary MECPP in elderly people in China is currently limited. Age, sex, health status, and routes of exposure can influence concentrations of oxidative metabolites in urine. Therefore, the concentration of MECPP in elderly Chinese people should be measured in further investigations. In this current study, the median concentration of $\sum$DEHP metabolites was 43.3 ng mL^{-1} in older adults followed by children (median: 38.5 ng mL^{-1}) and younger adults (median: 28.1 ng mL^{-1}). These results indicate that exposure to DEHP was greater among older adults and children compared with younger adults. Given that DEHP has carcinogenic potency (Junaid et al., 2018), it is important to elucidate the level of human exposure to DEHP, particularly for susceptible populations such as children, elderly people, and/or pregnant women.

The greatest concentration of MBzP was 1.4 ng mL^{-1}, which was 10- to 100- fold less than the concentration of other phthalate metabolites. The concentration of MBzP (median: 0.1 ng mL^{-1}) in Beijing residents was 2- and 3- fold less than that in residents of Shanghai (median: 0.2 ng mL^{-1}) (Dong et al., 2018) and Guangzhou (0.3 ng mL^{-1}) (Zhang et al., 2018), respectively, and 100- fold less than concentrations reported in the United States (Kim et al., 2016) and Germany (Becker et al., 2009). Furthermore, MBzP exhibited the greatest rate of detection in urine of people in more developed countries such as the United States, Sweden, Japan, and Germany (Högberg et al., 2008; Itoh et al., 2007), whereas it was observed in only half of the samples of urine from people in Beijing, Shanghai, and Guangzhou (Dong et al., 2018; Zhang et al., 2018). These results suggested that the use of BzBP in China is relatively limited, and exposure of the Chinese people to BzBP is less than that in other countries.

3.3. Composition profile of metabolites

As shown in Fig. S2, MnBP, MiBP, and three DEHP metabolites, MEP, MMP, and MBzP, accounted for 58.9%–64.8%, 11.1%–14.9%, 10%–16.1%, 4.5%–10.7%, 2.4%–4.2% and less than 0.1% in the urine of people in Beijing in various age groups. The sum of MnBP and MiBP contributed over 70% of the total metabolites of phthalates, while other metabolites including MMP, MEP, MBzP, and metabolites of DEHP accounted for less than 30%. These results were consistent with the previous studies, providing evidence that MnBP and MiBP were the dominant compounds (Guo et al., 2011b; Wu et al., 2017; Zhang et al., 2018). This is likely due to the relatively high level of exposure to DnBP and DiBP (Gao et al., 2016). However, in the United States (Johns et al., 2016), Mexico (Romero et al., 2011), Brazil (Rocha et al., 2017), Japan (Guo et al., 2011a), Korea (Lee et al., 2018), India (Guo et al., 2011a), Malaysia (Guo et al., 2011a), Norway (Engel et al., 2018), Egypt (Colacino et al., 2011) and Saudi Arabia (Lee et al., 2019), the major metabolite of phthalates in human urine was MEP, which contributed 31%–70% of the total metabolites of phthalates. The current results further suggest that the profile of urinary phthalate metabolites in China is unique. Considering that people could be exposed continuously to phthalates through dermal absorption, inhalation, and ingestion, the different composition profiles of metabolites of phthalates may be caused by different exposure sources from environmental media, food and consumer products.

3.4. Estimated daily intakes (EDIs) of phthalates

EDIs of individual and total phthalates were calculated for all participants using two models (Table 1). For the volume model, median EDIs of total phthalates for children, younger adults, older adults, and the general population were 15.9, 19.9, 17.5, and 17.5 μg (kg-bm)$^{-1}$ d^{-1}, respectively. Among the three age groups, no significant differences were observed for EDIs of individual phthalates or combined phthalates. Generally, EDIs (μg (kg-bm)$^{-1}$ d^{-1}) of DnBP were greatest (median: 8.5, ranged 0.7–81.8), and were significantly greater than those of DEHP (3.3, 0.4–124), DiBP (1.9, 0.4–13.1), DEP (0.5, 2.0 × 10^{-2}-22.9), DMP (0.4, 0.1–6.1), and BBzP (0.4 × 10^{-2}, 0.2 × 10^{-2}–0.1). Relative profiles of EDIs were similar when concentrations of phthalates were adjusted by normalizing to concentrations of creatinine, for which EDIs of DnBP, DEHP, DiBP, DEP, DMP, and BBzP for all participants, ranging from 0.6 to 56.2, 0.2–30.0, 0.2–11.6, 1.0 × 10^{-2}–27.5, 2.0 × 10^{-2}–7.3, and 0.1 × 10^{-2}–0.1 μg (kg-bm)$^{-1}$ d^{-1}, with median values of 6.3, 1.9, 1.3, 0.3, 0.2 and 0.3 × 10^{-2}μg (kg-bm)$^{-1}$ d^{-1}, respectively. The contributions of DnBP to total EDIs of phthalates ranged from 49.4% to 57.4%, which was greater than the contributions of DEHP (17.3%–34.2%) when normalized to volume or creatinine (Fig. 2). These results suggested that DnBP and DEHP were the dominant compounds, and that exposure to these phthalates are of particular concern for residents of Beijing.

Compared with the results of previous studies, EDIs of DnBP in children in the current study were more than four times greater than those of children (median: 1.9 μg (kg-bm)$^{-1}$ d^{-1}) in mid-eastern China and university students (Geometric mean: 1.4 μg (kg-bm)$^{-1}$ d^{-1}) in southern China, while EDIs for DEP, DMP, DiBP, and DEHP were comparable (median: 0.7, 0.3, 1.5, and 3.7 μg (kg-bm)$^{-1}$ d^{-1}) (Wang et al., 2015; Zhang et al., 2018). In addition, EDIs for DEP and BBzP in this study were less than those reported for children and younger adults women in Saudi Arabia (median: 49.8 and 0.6 μg (kg-bm)$^{-1}$ d^{-1}) (Lee et al., 2019) and France (median: 1.0 and 0.4 μg (kg-bm)$^{-1}$ d^{-1}) (Zeman et al., 2013), respectively. EDIs of DMP in Beijing residents was less than those reported in Austria (median: 1.3 μg (kg-bm)$^{-1}$ d^{-1}). EDIs of DnBP were one order of magnitude greater than the value reported in NHANES (2005–2008) in the United States (0.5 μg (kg-bm)$^{-1}$ d^{-1}), Austria (0.4 μg (kg-bm)$^{-1}$ d^{-1}) (Christina et al., 2015), and Denmark (0.7 μg (kg-bm)$^{-1}$ d^{-1}) (Frederiksen et al., 2013). EDIs for DiBP were less than those reported in Saudi Arabia (28 μg (kg-bm)$^{-1}$ d^{-1}) (Lee et al., 2019) or Belgium (median: 2.3 μg (kg-bm)$^{-1}$ d^{-1})

Table 1
The EDIs of parent phthalates for different age groups of people in Beijing.

Phthalates	Category	Volume-model				Creatinine-model				TDIs μg (kg bm)$^{-1}$ d^{-1}	RfDs μg (kg bm)$^{-1}$ d^{-1}
		GM	Mean	Median	Range	GM	Mean	Median	Range		
DMP	Children	0.4	0.7	0.4	0.1–6.1	0.7	1.1	0.7	0.2–7.3	–	–
	Younger adults	0.4	0.7	0.3	0.1–3.5	0.2	0.3	0.2	0.1–1.8		
	Older adults	0.4	0.6	0.4	0.1–2.6	0.2	0.3	0.2	2.0×10^{-2}-1.2		
	General	0.4	0.6	0.4	0.1–6.1	0.3	0.5	0.2	2.0×10^{-2}-7.3		
DEP	Children	0.7	1.9	0.7	0.2–22.9	1.3	3.2	1.1	0.3–27.5	–	800
	Younger adults	0.4	1.2	0.4	2.0×10^{-2}-11.3	0.2	0.4	0.2	1.0×10^{-2}-1.8		
	Older adults	0.7	1.7	0.9	2.0×10^{-2}-11.2	0.3	0.9	0.3	4.0×10^{-2}-8.8		
	General	0.6	1.6	0.5	2.0×10^{-2}-22.9	0.4	1.3	0.3	1.0×10^{-2}-27.5		
DiBP	Children	2.0	2.6	1.9	0.5–8.2	3.6	4.7	3.8	0.8–11.6	10	100
	Younger adults	1.9	2.5	2.0	0.4–7.6	1.0	1.2	1.0	0.3–3.6		
	Older adults	2.1	2.9	1.8	0.7–13.1	0.9	1.1	1.2	0.2–3.1		
	General	2.0	2.6	1.9	0.4–13.1	1.4	2.2	1.3	0.2–11.6		
DnBP	Children	8.4	11.3	9.0	2.0–40.3	15.3	20.2	16.1	2.0–56.2	10	100
	Younger adults	8.1	12.9	8.7	0.7–64.4	4.1	5.9	4.2	0.6–18.5		
	Older adults	8.5	15.6	6.7	1.5–81.8	3.7	5.4	3.7	0.7–22.5		
	General	8.3	13.2	8.5	0.7–81.8	5.8	9.8	6.3	0.6–56.2		
BBzP	Children	0.4×10^{-2}	0.8×10^{-2}	0.4×10^{-2}	0.1×10^{-2}-0.1	0.8×10^{-2}	2.0×10^{-2}	0.7×10^{-2}	0.1×10^{-2}-0.1	500	200
	Younger adults	0.5×10^{-2}	0.8×10^{-2}	0.3×10^{-2}	0.2×10^{-2}-0.1	0.2×10^{-2}	0.3×10^{-2}	0.3×10^{-2}	0.1×10^{-2}-1.0×10^{-2}		
	Older adults	0.7×10^{-2}	1.2×10^{-2}	0.9×10^{-2}	0.2×10^{-2}-0.1	0.3×10^{-2}	0.6×10^{-2}	0.3×10^{-2}	0.1×10^{-2}-3.0×10^{-2}		
	General	0.5×10^{-2}	0.9×10^{-2}	0.4×10^{-2}	0.2×10^{-2}-0.1	0.4×10^{-2}	0.9×10^{-2}	0.3×10^{-2}	0.1×10^{-2}-0.1		
DEHP	Children	2.4	4.3	2.6	0.4–28.7	4.4	6.1	4.9	0.8–20.9	50	20
	Younger adults	2.9	5.3	2.8	0.4–33.2	1.5	2.5	1.1	0.2–21.0		
	Older adults	4.5	10.8	3.6	1.0–127	1.9	3.3	2.0	0.6–30.0		
	General	3.1	6.6	3.3	0.4–124	2.2	3.8	1.9	0.2–30.0		
∑Phthalate	Children	16.2	20.7	15.9	4.5–77.4	29.6	35.2	34.8	7.8–93.0		
	Younger adults	16.7	22.6	19.9	1.8–102	8.4	10.3	10.0	1.2–24.2		
	Older adults	18.6	31.6	17.5	4.9–186	8.1	10.9	7.9	2.0–44.1		
	General	17.1	24.6	17.5	1.8–186	11.9	17.6	12.5	1.2–93.0		

AbbreviationsTDIs: Tolerable daily intakes derived by EFSA; RfDs: Reference doses by the U.S. EPA.

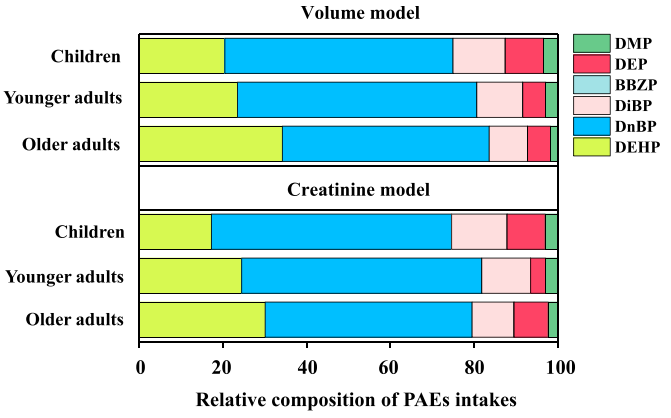

Fig. 2. Composition profiles of PAEs intakes in different age groups in volume-model and creatinine-model.

(Dewalque et al., 2014), but greater than those reported in Austria (1.1 μg (kg-bm)$^{-1}$ d^{-1}) (Christina et al., 2015). Overall, comparison of the present EDI findings with international studies indicates that people in Beijing are exposed to relatively high concentrations of DnBP, but lower levels of exposure to BBzP and DEP.

EDIs based on concentrations normalized to creatinine among children were 1.6- to 5-fold greater than those calculated based on concentrations normalized to volume. However, for younger adults and older adults, the pattern of results indicated that EDI doses of phthalates were 1.5- to 2-fold greater based on volume than those normalized to creatinine. This scenario may have been caused by differences in urinary creatinine excretion rates among children, younger adults, and older adults. In the present study, the mean concentration of creatinine in children was 0.5 g L^{-1} (ranging from 0.2 to 1.8 g L^{-1}), while concentrations of creatinine for younger adults and older adults were 1.4 and 1.3 g L^{-1}, respectively. The lower concentration of creatinine in children could affect EDIs for phthalates when normalizing to creatinine. This is a possible explanation for the significantly higher levels of EDIs of phthalates among children compared with younger adults and older adults when the data were normalized to creatinine.

3.5. Hazards posed by phthalates

In the volume model, the EDIs results showed that one elderly person exhibited a level exceeding the TDI of 10 μg (kg-bm)$^{-1}$ d^{-1} for DiBP, 31 participants (nine children, 13 young adults, and nine elderly people) exceeded the TDI of 10 μg (kg-bm)$^{-1}$ d^{-1} for DnBP,

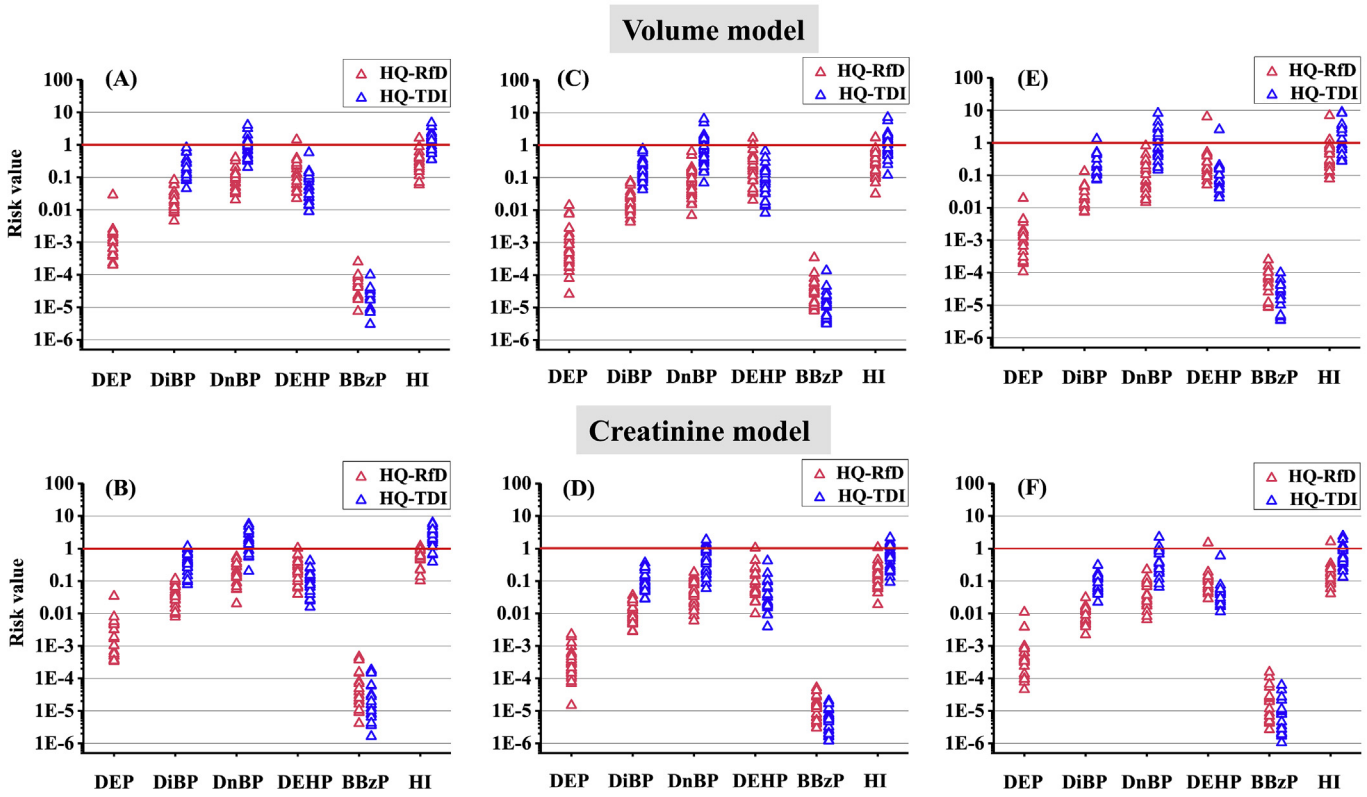

Fig. 3. Hazard quotients (HQs) and hazard index (HI) for reference people based on tolerable daily intake (TDI) and reference doses (RfD). (A) and (B) represent for children; (C) and (D) represent for younger adults; (E) and (F) represent for older adults.

one elderly person exceeded the TDI for DEHP, and four participants (one child, two young adults, and one elderly person) exceeded the RfDs value for DEHP. These results indicated that exposure of Beijing residents to DnBP resulted in a greater risk. After adjustment for concentrations of creatinine, two children exceeded the TDI of DiBP, and 23 participants (14 children, six young adults, and three elderly people) exceeded the TDIs for DnBP. For DEHP, three participants (one children, one young adult, and one elderly person) had greater EDIs than the RfDs value recommend by the US EPA. When EDIs were adjusted to creatinine, the results indicated even more clearly that children were more likely to be affected by exposure to phthalates than adults. Since the f_{ue} value for children is unknown and the f_{ue} value for adults was used for calculation of children's EDIs, the accuracy of EDIs for children is uncertain and equivocal. The results of a previous study demonstrated that, children are more likely to produce oxidative metabolites rather than monoesters (Wang et al., 2015). Hence, the actual exposure of DnBP and DiBP to children might be underestimated.

Values of HQ for DnBP, DiBP and DEHP exceeded the recommended values based on TDIs, while the HQ of DEHP exceeded the threshold based on RfDs (Fig. 3). These results indicate that DnBP, DiBP, and DEHP constitute the main risks of phthalate exposure. HQ and HI values of less than 1 indicate that exposure to phthalates is unlikely to cause measurable adverse effects. HQ or HI values exceeding 1 suggest a potential risk of adverse effects. In the present study, 40 of 70 participants exhibited high HQ values for a single phthalate in both the volume- and creatinine-adjusted models. The highest HI values in children, younger adults and older adults were 6.2, 7.2, and 8.8, respectively. 85% of children had high HI values compared with 70% in younger adults and 45% in older adults, suggesting that most people in Beijing might exhibit adverse effects caused by phthalates. Several other studies reported that nearly half of Chinese younger adults and 20% of Chinese children exhibited HI values exceeding 1.0 (Gao et al., 2016; Wang et al., 2015). Therefore, there is a risk that these compounds could result in a range of negative health impacts in China, including endocrine disruption, reproductive toxicity, and carcinogenesis.

4. Conclusions

This is the first study to establish a pre-baseline level of phthalate metabolites in the urine of Beijing residents. Concentrations, profiles, daily intakes, and hazards posed by metabolites of phthalates and their parent phthalates were assessed in three age groups of people living in Beijing. MMP, MEP, MiBP, MnBP, MEHHP, MEOHP, and MEHP were ubiquitous in the urine, and MnBP was the dominant compound. Total concentrations of selected metabolites in children were greater than those in younger adults and older adults, when normalized for both volume and concentrations of creatinine. Compared with other countries, people from Beijing exhibited a unique profile of phthalate metabolites. EDIs for DiBP, DnBP, and DEHP exceeded the reference values suggested by the US EPA and EFSA. Most participants exhibited HIs and HQs that exceeded the threshold of 1. Exposure to and effects of DnBP and DEHP are of particular concern for Beijing residents.

Author contribution section

Conception and design of study: Ying Zhu. Sample collection: Xiaojian Hu, Feng Zhao, Peng Du. Acquisition of data: Xu Zhang, Tian Qiu, Yifu Lu. Analysis and/or interpretation of data: Xu Zhang, Linna Xie, Yanwei Yang. Drafting the manuscript: Xu Zhang, Song Tang, John P. Giesy. Critical revision: Xu Zhang, Ying Zhu, Song Tang.

Declaration of competing interest

None declared.

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Appendix A. Supplementary data

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Supplementary Material

Investigation of phthalate metabolites in urine and daily phthalate intakes among three age groups in Beijing, China

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Target compounds analysis

Isotope-dilution was used to determine concentrations of targeted metabolites of PAEs in human urine. In brief, an aliquot of 2 mL of urine was transferred into a 5 mL tube. Then 0.5 mL of ammonium acetate solution (1 mol/L, pH=6.8), 20 ng of isotopically labeled internal standards, and 20 μL *β*-glucuronidase were added. The mixture was incubated, overnight at 37℃. After the urine sample was enzymatically hydrolyzed, ammonia hydroxide solution (200 μL) was added and vortexed for 30 seconds. Sample were centrifuged at 1500 g for 10 min. Oasis MAX (60 mg/3 mL) cartridges were conditioned with 3 mL of methanol followed by 3 mL of water. The supernatant was passed through this conditioned cartridge with a flow rate of 0.5 mL/min. The cartridge was then washed with 3 mL of water and 3 mL of methanol. Targeted analytes were eluted from the cartridge using 2 mL of 2% formic acid in methanol (V/V). An aliquot of 0.2 mL eluate was added to 0.8 mL of 5% acetonitrile in water (V/V), and then went through a filter membrane (0.22 μm, GHP) before identification and quantification by use of UPLC-MS/MS.

Concentrations of target compounds were determined by use of an ultra-high performance liquid chromatography (I Class, Waters, USA) system, which was interfaced with a AB SCIEX 6500 (Applied Biosystems, USA) triple quadrupole mass spectrometer in multiple-reaction monitoring (MRM) mode. Chromatographic separation (Fig. S1) was achieved using a BEH phenyl column (100 mm× 2.1 mm, 1.7 μm, Waters). Mobile phases, A and B for chromatographic separation of target compounds were acetonitrile and water with 0.1% acetic acid, respectively (Table S2). Ions used for quantification as well as instrumental parameters and conditions for quantification of metabolites of PAEs are given in Table S3. Concentrations of creatinine in urine were determined by use of high performance liquid chromatography (1200 serials, Agilent, USA) by use of previously described methods (Zhang et al., 2018) (Table S1).

Estimated Daily Intakes of PAEs

$$EDI(\mu g/kg\ bm/day) = \frac{U_c(\mu g/L) \times UV(L/day) \times MM_P}{f_{ue} \times bm \times MM_M} \quad (1)$$

$$EDI(\mu g/kg\ bm/day) = \frac{U_{cr}(\mu g/g) \times CE(g/day) \times MM_P}{f_{ue} \times bm \times MM_M} \quad (2)$$

In equation (1), U_C is the concentrations of metabolite of PAEs in urine. UV is the daily excretion volume of urine. MM_P and MM_M are molecular masses of parent PAEs and their respective metabolites. f_{ue} is the mole fraction of metabolites of PAEs excreted in relation to PAEs, ingested with values of 0.69, 0.7, 0.69, 0.69, 0.23, 0.15 or 0.059 for MMP, MEP, MiBP, MnBP, MEHHP, MEOHP and MEHP, respectively (Guo et al., 2011; Wang et al., 2015). Bm is the body mass (kg). U_{CC} is the urinary monoester metabolites concentration adjusted to concentrations of creatinine. CE is the rate of excretion of creatinine (CE) and values of CE for various ages of people have been reported previously (Mage et al., 2008).

Table S1. Basic information and creatinine concentration of collected urine.

Sample numbers	Age (year)	Gender	Body mass (kg)	Length (cm)	Sample collection date	Creatinine concentration (g/L)
Children						
1	13	Male	48	150	September, 2017	0.42
2	4	Male	18	110	September, 2017	0.17
3	1	Male	11	80	October, 2017	0.49
4	5	Male	20	113	October, 2017	0.57
5	6	Male	22	120	October, 2017	1.19
6	5.5	Male	22	120	March, 2018	0.73
7	1	Male	11	76	March, 2018	0.18
8	1	Male	11	81	April, 2018	0.38
9	2	Male	16	95	April, 2018	0.26
10	4	Male	16	100	April, 2018	0.79
11	4	Female	15	110	September, 2017	0.76
12	2	Female	12	80	September, 2017	0.31
13	2	Female	14	86	September, 2017	0.34
14	3.5	Female	13	108	September, 2017	0.30
15	5	Female	16	106	November, 2017	0.32
16	3	Female	14	91	November, 2017	0.15
17	6	Female	23	129	April, 2018	0.25
18	2.4	Female	16	82	April, 2018	0.41
19	3.8	Female	15	98	April, 2018	0.32
20	12	Female	50	167	April, 2018	1.81
Younger Adults						
21	38	Male	80	175	August, 2017	1.79
22	32	Male	82	173	August, 2017	1.94
23	35	Male	74	175	August, 2017	0.94
24	34	Male	84	178	October, 2017	0.46
25	36	Male	68	175	October, 2017	1.40
26	39	Male	70	170	October, 2017	1.78
27	33	Male	60	165	October, 2017	2.36
28	36	Male	65	169	November, 2017	1.17
29	37	Male	65	170	November, 2017	1.27
30	28	Male	65	172	November, 2017	3.37
31	45	Male	70	170	November, 2017	1.13
32	35	Male	85	178	November, 2017	4.62
33	42	Male	65	175	March, 2018	0.49
34	27	Male	62	173	March, 2018	1.43
35	40	Male	76	170	April, 2018	1.21
36	36	Female	50	160	August, 2017	1.24
37	33	Female	58	168	August, 2017	0.36
38	32	Female	58	164	September, 2017	0.72
39	33	Female	55	165	September, 2017	1.09
40	36	Female	59	160	October, 2017	1.87

41	39	Female	64	162	October, 2017	0.82
42	32	Female	42	156	October, 2017	3.08
43	33	Female	60	160	November, 2017	1.37
44	45	Female	49	160	November, 2017	0.30
45	42	Female	60	159	March, 2018	1.09
46	36	Female	47	150	March, 2018	0.41
47	43	Female	52	160	March, 2018	1.39
48	30	Female	52	160	April, 2018	1.69
49	45	Female	50	160	April, 2018	2.14
50	31	Female	48	156	April, 2018	0.38
Older adults						
51	54	Male	77	165	August, 2017	1.03
52	52	Male	80	170	August, 2017	2.41
53	63	Male	75	165	August, 2017	2.26
54	58	Male	78	170	August, 2017	2.31
55	54	Male	78	178	November, 2017	0.76
56	64	Male	90	180	November, 2017	1.49
57	52	Male	64	169	March, 2018	1.40
58	66	Male	75	168	March, 2018	1.01
59	61	Male	63	165	April, 2018	2.07
60	71	Male	65	167	April, 2018	1.71
61	52	Female	60	160	August, 2017	0.95
62	61	Female	55	160	August, 2017	0.97
63	54	Female	58	157	August, 2017	0.66
64	50	Female	60	167	September, 2017	0.71
65	64	Female	60	148	September, 2017	0.79
66	53	Female	60	160	November, 2017	0.98
67	70	Female	69	158	November, 2017	1.33
68	55	Female	55	154	March, 2018	0.16
69	55	Female	65	152	April, 2018	1.64
70	71	Female	55	165	April, 2018	1.58

Table S2. Mobile phase gradient used for the determination of target compounds.

Time	Mobile A	Mobile B
0 min	95%	5%
14.5 min	50%	50%
18 min	10%	90%
19 min	10%	90%
19.5 min	95%	5%
23 min	95%	5%

Table S3. MRM scan model for target compounds and their optimized mass spectrometric parameters. MRM detection window for each compound was set at 50 msec.

Compounds	RT (min)	Precursor Ion (m/z)	Product Ion (m/z)	CE (eV)	DP (V)
MMP	6.73	179.0	77.0*	-20	-30
			107.0	-18	-30
MEP	8.43	192.9	77.0*	-32	-38
			121.0	-20	-38
MiBP	11.93	221.0	77.0*	-23	-55
			134.0	-23	-55
MEHHP	11.93	293.0	121.0*	-23	-50
			145.0	-23	-50
MnBP	12.17	221.1	76.9*	-20	-46
			71.0	-21	-46
MEOHP	12.40	291.0	121.0*	-25	-45
			143.0	-25	-45
MBzP	13.21	255.0	77.0*	-26	-43
			183.0	-19	-50
MCHP	13.50	247.0	77.0*	-28	-43
			97.1	-28	-43
MEHP	17.12	277.0	134.0*	-24	-50
			77.0	-42	-50
MOP	17.43	277.1	77.0*	-32	-55
			127.0	-30	-55
MNP	17.44	291.0	141.0*	-29	-42
			77.0	-39	-42
MDP	18.14	305.0	154.9*	-26	-51
			77.0	-30	-45
MMP-IS	6.74	183.0	79.0	-27	-29
MEP-IS	8.43	197.0	79.0	-26	-42
MIBP-IS	11.93	225.0	81.1	-27	-28
MEHHP-IS	11.93	297.1	124.2	-25	-26
MNBP-IS	12.17	225.0	79.0	-30	-29
MEOHP-IS	12.40	295.2	123.9	-23	-28
MBZP-IS	13.21	259.0	76.9	-28	-25
MCHP-IS	13.50	251.1	79.0	-29	-27
MEHP-IS	17.12	281.0	136.9	-25	-26
MOP-IS	17.43	280.9	127.0	-25	-33

MNP-IS	17.44	295.1	79.2	-32	-28
MDP-IS	18.14	309.1	79.1	-31	-35

RT: Retention time in minutes; CE: Collision energy; DP: De-clustering potential; MRM: Multiple reaction monitoring; *: Quantitative ion.

Table S4. Validation and performance data of the developed method.

Compounds	Recovery (%)	RSD (%)	Matrix effect (%)	MDLs (ng/mL)	MQLs (ng/mL)
MMP	101	4.5	96	0.30	1.00
MEP	104	4.7	92	0.60	1.98
MIBP	95.2	5.0	87	0.37	1.22
MEHHP	94	7.0	89	0.13	0.45
MNBP	95	13	94	0.82	2.70
MEOHP	96	5.2	99	0.10	0.35
MBZP	101	14	90	0.08	0.27
MCHP	103	8.9	92	0.15	0.50
MEHP	109	12	97	0.13	0.45
MOP	103	10	95	0.07	0.25
MNP	90	13	86	0.09	0.30
MDP	107	9.7	93	0.20	0.70

Table S5. Expected (ng/mL) and experimental (ng/mL) values of target compounds in SRM 3672 (n=5).

	MEP	**MIBP**	**MNBP**	**MEHHP**	**MEOHP**	**MEHP**	**MBZP**
Expected value	94.5	6.40	10.6	24.8	14.9	4.13	8.37
Experimental value	97.4±2.9	5.96±0.6	9.89±0.6	22.3±1.2	13.8±0.7	4.63±0.2	8.19±0.4

Table S6. The concentrations of selected phthalate metabolites (in ng/mL and in μg/g creatinine) in urine samples from children (*N* = 20), younger adults (*N* = 30), and older adults (*N* = 20).

Phthalates	Metabolites	Category	DF	Volume-model			Creatinine-model		
				GM	Median	Range	GM	Median	Range
DMP	MMP	Children	100	11.2	10.1	3.0-177	28.4	30.2	6.5-243
		Younger adults	100	8.7	7.4	1.7-90.3	7.6	6.0	3.1-50.5
		Older adults	100	8.9	8.3	1.5-45.9	7.8	7.7	0.7-60.1
		General	100	9.4	8.8	1.5-177	11.2	9.1	0.7-243
DEP	MEP	Children	100	19.0	17.4	4.3-615	48.2	38.4	7.5-809
		Younger adults	100	8.1	5.9	0.4-170	7.1	6.3	0.3-79.2
		Older adults	100	14.6	19.1	1.5-424	12.8	12.4	1.6-285
		General	100	12.2	10.5	0.4-615	14.5	12.7	0.3-809
DiBP	MiBP	Children	100	49.0	48.1	11.3-205	124	116	26.6-502
		Younger adults	100	32.7	32.1	10.1-138	28.6	26.3	6.2-97.4
		Older adults	100	39.0	31.5	11.9-231	34.3	39.0	6.9-112
		General	100	38.6	34.7	10.1-231	45.8	43.0	6.2-502
DnBP	MnBP	Children	100	208	225	50.7-1005	529	606	50.1-2971
		Younger adults	100	140	152	16.1-900	122	134	19.7-602
		Older adults	100	157	146	24.5-1715	138	160	31.0-759
		General	100	162	164	16.1-1715	192	188	19.7-2971
BBzP	MBzP	Children	55.0	0.1	0.1	ND -1.3	0.3	0.2	ND -3.9
		Younger adults	46.7	0.1	ND	ND -1.4	0.1	ND	ND -0.3
		Older adults	60.0	0.1	0.2	ND -0.9	0.1	0.1	ND -1.3
		General	52.9	0.1	0.1	ND -1.4	0.1	0.1	ND -3.9
DEHP	MEOHP	Children	100	12.4	13.7	2.8-155	31.5	37.0	7.9-130
		Younger adults	100	7.6	8.3	1.5-82.5	6.7	6.3	2.1-73.0
		Older adults	100	13.0	14.5	2.0-66.8	11.4	10.4	4.4-32.3
		General	100	10.2	10.0	1.5-155	12.1	10.7	2.1-130
	MEHHP	Children	100	18.1	20.3	3.3-197	46.0	51.7	11.5-166
		Younger adults	100	12.0	11.8	2.2-155	10.5	9.3	3.8-137
		Older adults	100	20.5	23.5	4.2-136	18.0	18.2	6.0-65.7
		General	100	15.7	16.7	2.2-197	18.7	17.8	3.8-166
	MEHP	Children	100	3.4	3.8	0.1-56.2	8.7	11.7	0.3-47.2
		Younger adults	93.3	4.0	4.5	ND -67.5	3.5	3.6	ND-59.7
		Older adults	90.0	5.0	6.2	ND -446	4.5	5.9	ND -216
		General	94.3	4.1	4.6	ND -446	4.9	4.9	ND -216

Abbreviations: DF, detected rate (%); GM, geometric mean; ND, not detected (< MDLs).

Table S7. Summary of urinary phthalate metabolites concentrations (in median concentration, ng mL^{-1}) in different age groups globally.

Location	Year	MnBP	MiBP	MMP	MEP	MEOHP	MEHHP	MEHP	MBzP	MCHP	MOP	MiNP	MiDP	Reference
Children														
Beijing, China	2018	225	48.1	10.1	17.4	13.7	20.3	3.88	0.10	ND	ND	ND	ND	This study
Guangxi, China	2016	172	—	18.3	12.4	14.9	30.2	6.72	0.12	—	4.00×10^{-2}	—	—	(Hu et al., 2017)
Beijing, China	2015	120	54.1	—	15.3	28.2	47.9	9.30	ND	—	—	—	—	(Gong et al., 2015)
Hubei, China	2014	34.7	10.6	18.5	14.0	7.30	17.7	4.40	2.7	ND	ND	0.600	—	(Wu et al., 2017)
Brazil	2013	42.4	43.8	8.30	57.3	16.7	23.8	19.2	1.91	0.880	1.15	ND	1.90	(Rocha et al., 2017)
Denmark	2011	32.0	54.0	—	20.0	12.0	23.0	2.00	7.00	—	ND	ND	ND	(Frederiksen et al., 2013)
German	2006	93.4	88.1	—	—	46.0	36.3	6.70	18.1	—	—	—	—	(Becker et al., 2009)
U.S.	2010	23.3	10.9	2.36	33.0	11.1	17.0	1.71	11.6	ND	ND	ND	—	(US.CDC, 2015)
Saudi Arabia	2017	179	155	14.0	259	41.3	75.0	8.90	4.10	0.600	ND	ND	2.10	(Lee et al., 2019)
Younger adults														
Beijing, China	2018	152	32.1	7.38	5.90	8.28	11.8	4.47	ND	ND	ND	ND	ND	This study
Shenzhen, China	2015	139	-	4.59	4.87	4.31	5.45	2.86	0.240	ND	ND	ND	ND	(Chen et al., 2019)
Nanjing China	2015	77.6	47.8	19.8	13.4	8.40	13.1	4.60	0.100	—	ND	0.12	—	(Pan et al., 2015)
China	2010	71.7	55.6	35.0	31.7	9.32	14.2	1.84	ND	ND	ND	ND	ND	(Guo et al., 2011)
U.S.	2010	25.9	4.90	1.40	208	15.9	23.1	2.10	17.4	—	—	—	—	(US.CDC, 2015)
Mexico	2007	72.4	8.36	—	83.2	31.8	45.8	5.16	4.37	—	—	—	—	(Romero Franco et al., 2011)
German	2007	49.8	36.1	—	—	14.4	17.5	4.00	8.00	—	—	3.10	—	(Fromme et al., 2007)
Saudi Arabia	2017	96.4	47.9	—	290	16.2	11.1	14.3	1.14	—	—	—	—	(Iman et al., 2019)
Older adults														
Beijing China	2018	146	31.5	8.26	19.1	14.5	23.3	6.18	0.150	ND	ND	ND	ND	This study
Shanghai, China	2017	69.2	99.0	13.1	35.0	3.45	7.78	6.55	0.89	—	—	—	—	(Dong et al., 2018)
Korea	2014	33.3	—	—	—	11.6	16.1	—	2.22	—	—	—	—	(Lee et al., 2018)
U.S.	2012	11.4	4.50	—	70.6	7.22	11.5	—	4.31	—	—	—	—	(Kim et al., 2016)

Abbreviations:“—” mean not analyzed.

Figure S1. Chromatogram maps of target PAEs metabolites in UPLC-MS/MS.

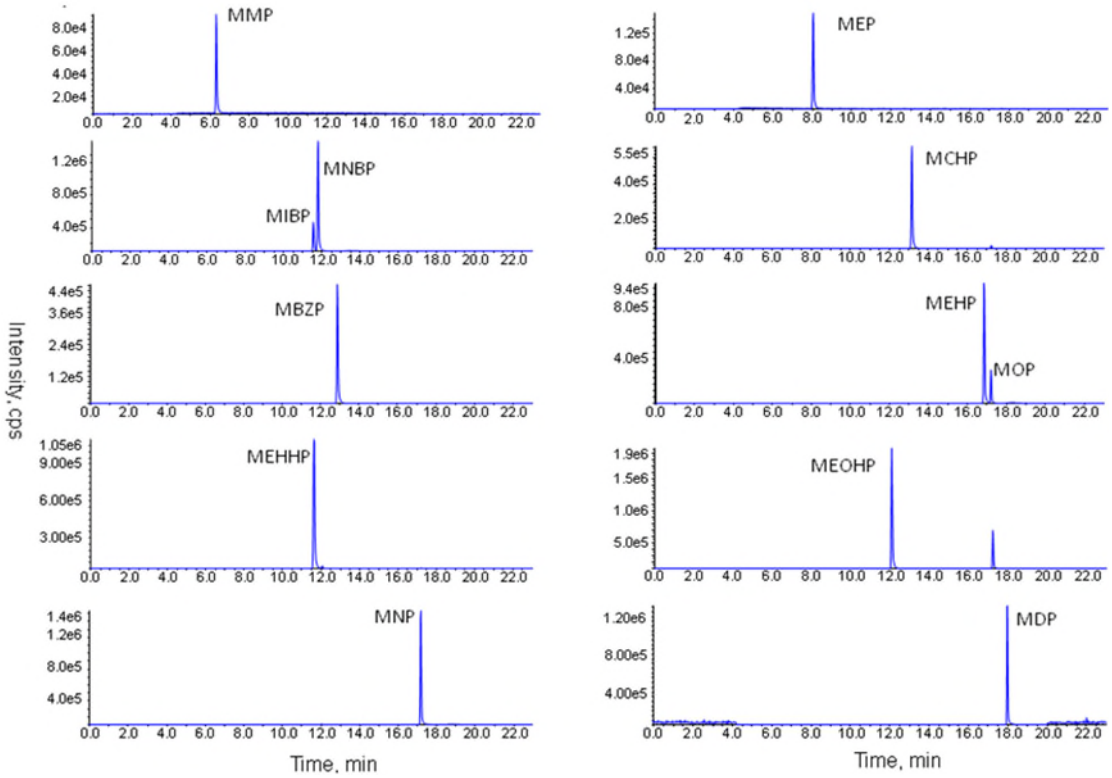
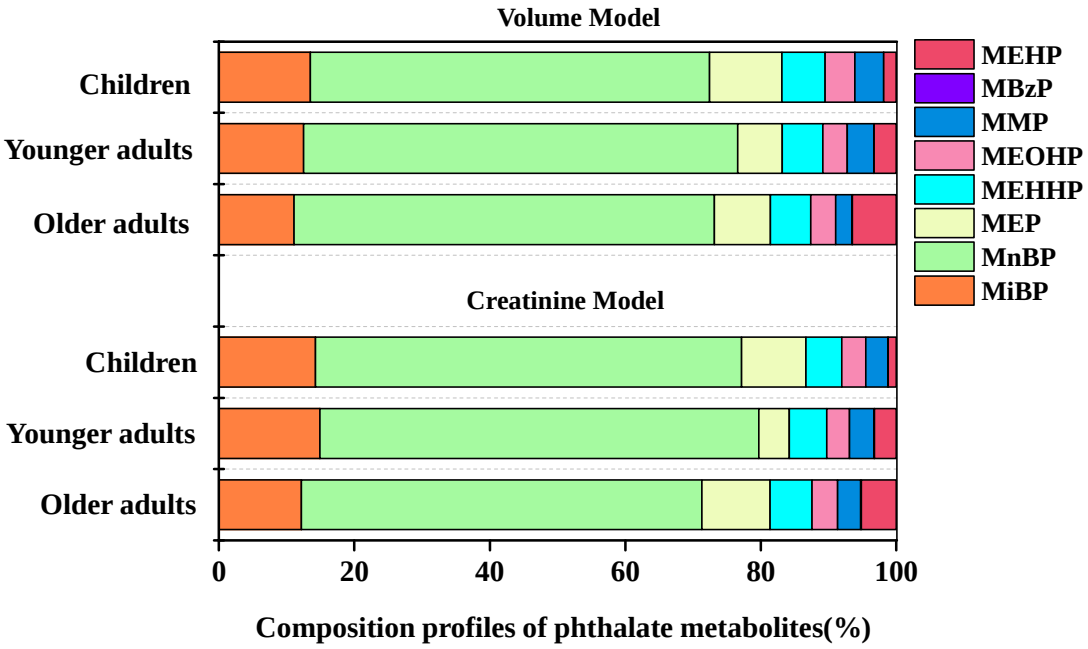


Figure S2. Relative compositions of metabolites of PAEs in urine of various age groups.



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