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Comparisons of urinary phthalate metabolites and daily phthalate intakes among  
Japanese families

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## Abstract

We measured urinary phthalate metabolites, including di-n-butyl phthalate (DnBP), di-isobutyl phthalate, benzyl butyl phthalate (BBzP), and di(2-ethylhexyl) phthalate (DEHP), from 178 school-aged children and their 284 family members using gas chromatography-mass spectrometry, and we calculated daily phthalate intakes. The highest median levels of phthalate metabolites were for mono-isobutyl phthalate in all participants except schoolchildren, where the highest levels were for mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP). Comparing the schoolchildren with their parents, the schoolchildren had significantly higher urinary metabolites for MEOHP, mono-(2-ethyl-5-carboxypentyl) phthalate, and  $\Sigma$ DEHP. Regarding daily intakes, the schoolchildren had significantly higher daily intakes of DnBP, BBzP, and  $\Sigma$ DEHP. All phthalate metabolite and sums of metabolite levels in the schoolchildren were positively correlated with their mothers' levels, except for MEHP, whereas fathers were less correlated with their children. The DEHP intake in this study was higher than that of most other studies. Moreover, 10% of the children and 3% of the adults exceeded the Reference Dose (RfD) value (20  $\mu\text{g/kg/day}$ ) of the U.S. Environmental Protection Agency, which indicates that it is important to focus on children's DEHP exposure because the children exceeded the RfD more than adults among the same families who shared similar exposure sources. Our results will contribute to considerations of the regulations for some phthalates and the actual phthalate exposure levels in the Japanese population.

1    *Keywords:*

- 2        • Children
- 3        • Phthalate metabolites
- 4        • Mother-child pairs
- 5        • Father-child pairs
- 6        • Phthalate exposure

- 1 Abbreviations:
- 2 BBzP, benzyl butyl phthalate
- 3 BSTFA, N,O-bis(trimethylsilyl)-trifluoro acetamide
- 4 BW, body weight
- 5 CE, creatinine clearance rate
- 6 DBP, dibutyl phthalate
- 7 DEHP, di(2-ethylhexyl) phthalate
- 8 DiBP, di-iso-butyl phthalate
- 9 DiNP, di-iso-nonyl phthalate
- 10 DMP, dimethyl phthalate
- 11 DnBP, di-n-butyl phthalate
- 12 DI, daily intake
- 13 EPA, Environmental Protection Agency
- 14 GC/MS, gas chromatography/mass spectrometry
- 15 ISAAC, International Studies of Asthma and Allergies on Childhood
- 16 LOD, limit of detection
- 17 LOQ, limit of quantification
- 18 MBzP, mono-benzyl phthalate
- 19 MCNP, mono(carboxynonyl) phthalate
- 20 MCPP, mono(3-carboxypropyl) phthalate
- 21 MECPP, mono(2-ethyl-5-carboxypentyl) phthalate
- 22 MEHP, mono(2-ethylhexyl) phthalate
- 23 MEHHP, mono(2-ethyl-5-hydroxyl-hexyl) phthalate
- 24 MEOHP, mono(2-ethyl-5-oxohexyl) phthalate
- 25 MiBP, mono-isobutyl phthalate
- 26 MnBP, mono-n-butyl phthalate

- 1 MTBSTFA, N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide
- 2 MW<sub>p</sub>, molecular weights of parent phthalate
- 3 NHANES, National Health and Nutrition Examination Survey
- 4 PCP, personal care product
- 5 PVC, polyvinyl chloride
- 6 RfD, Reference Dose
- 7 SIM, selective ion mode
- 8 TDI, Tolerable Daily Intake

## 1. Introduction

Phthalates are widely used as plasticisers for consumer products, such as toys, food containers, furniture, personal care products (PCPs), coatings of medications, and electric cables. The most commonly used phthalate is di(2-ethylhexyl) phthalate (DEHP) in Japan (Japan Plasticizer Industry Association and Ministry of Economy 2014), and its house dust level is higher in Japan than other countries (Ait Bamai et al. 2014). According to the Chemical Economics Handbook (Bizzari 2013), DEHP is still the dominant plasticizer/phthalate in Japan, while dibutyl phthalate (DBP) is consumed less in Japan than in Europe; DEHP consumption in Japan decreased slightly from 224 kilotons (kt) in 2000 to 161 kt in 2012. At the same time, consumption of DEHP decreased considerably in Europe and also in the US, from 395 kt in 2000 to 80 kt in 2012 and from 129 kt in 2000 to 70 kt in 2012, respectively. Therefore, higher DEHP dust levels in Japan reflect the characteristics of the Japanese market.

Humans are exposed to phthalates through food ingestion, inhalation, and dermal absorption throughout their lifetime beginning in foetal stages. In particular, the main source of exposure for high molecular weight phthalates is foodstuffs, while the source of exposure for low molecular weight phthalates seems to be very diffuse (Koch et al. 2013). Based on the endocrine-disrupting effects of phthalates in animal studies (Gray et al. 1982; Oishi 1993), the adverse effects of phthalates became a matter of international concern. In recent decades, DEHP and di-iso-nonyl phthalate (DiNP) were banned for use in toys and child-care products in the EU, USA, and Japan because of their reproductive toxicity (2005/84/EC 2005; Commission 2008; Japan Ministry of Health 2002). DBP and benzyl butyl phthalate (BBzP) are also banned for use in cosmetics in the EU (2004/93/EC 2004). However, phthalates, including DEHP, DBP, and BBzP, are still detected from human specimens, such as urine, serum, saliva, and breast milk (Hines et al. 2009; Koch et al. 2011; Silva et al. 2004).

Urinary phthalate metabolites are used as biomarkers of human exposure to phthalates as non-persistent chemicals with short half-lives (Calafat and McKee 2006). Currently, although

several epidemiological studies have reported urinary phthalate metabolite levels among mother-child pairs, only three studies have reported the associations of children's urinary phthalate metabolites and their mothers for the same urine sampling period (Kasper-Sonnenberg et al. 2012; Sathyanarayana et al. 2008a; Song et al. 2013). Kasper-Sonnenberg et al. (2012) measured urinary phthalate metabolite levels in mother/school-aged child pairs in Germany and found that metabolites of dimethyl phthalate (DMP), di-iso-butyl phthalate (DiBP), di-n-butyl phthalate (DnBP), DEHP, and DiNP were correlated between the mothers and children (Kasper-Sonnenberg et al. 2012). Conversely, Song et al. (2013) measured the urinary metabolite levels from mother/0–6-year-old child pairs in Korea and found that only mono-(2-ethylhexyl) phthalate (MEHP) was correlated between the mothers and children. Moreover, children had a faster relative metabolic rate of DEHP metabolism from MEHP to mono-(2-ethyl-5-hydroxyl-hexyl) phthalate (MEHHP) than mothers, especially younger children, who were the fastest (Song et al. 2013). Sathyanarayana et al. (2008) measured urinary metabolite levels from mother/1–37-month-old child pairs in the USA and found that correlation coefficients between mothers and their children increased with decreasing age of the children (Sathyanarayana et al. 2008a). However, to the best of our knowledge, there are no studies that measure urinary phthalate metabolite levels both in children and all of their family members, including their mother, father, and siblings, and that assess the differences of phthalate exposure levels among family members. Therefore, this study aimed to present the differences in phthalate exposure between children and adults among families that are thought to share lifestyle and home environments. We measured seven phthalate metabolite levels in urine samples from Japanese elementary schoolchildren and their family members. Next, we estimated daily phthalate intakes from urinary phthalate metabolite levels. In addition, we considered whether the phthalate exposure contributions among children were more correlated with their mothers or fathers.

## 2. Materials and Methods

### 2.1. Study population

This study was based on the second phase of a home environment and allergies study: a baseline questionnaire survey in 2008 and questionnaire and environmental measurements survey conducted between 2009 and 2010. The details of the baseline questionnaire survey in 2008 have been reported previously (Ukawa et al., 2012; Ait Bamai et al. 2014). Briefly, all 6393 schoolchildren from 12 public elementary schools in Sapporo were asked to participate in the study, of which 4408 children responded to the questionnaire (response rate 69.0%). A total of 951 children (832 households) agreed to allow a home visit to conduct environmental measurements. In 2009 and 2010, we contacted children who were still attending the same elementary school as in 2008, excluding those who left blanks on the baseline questionnaires regarding their gender, grade, and allergies for ISAAC (International Studies of Asthma and Allergies on Childhood). This selection procedure identified a total of 128 households who allowed home visits for environmental measurements, dust collection, spot urine collection, and questionnaire in October and November of 2009 and 2010. During the home visit survey, we collected 479 urine samples and questionnaires from the family members of the 128 households. The questionnaire included questions on gender, age, body height, weight, and time spent at home. We selected 471 participants who had data for the urine sample and data for their gender, age, body height, and weight. From these, we excluded grandparents (n=9). Finally, a total of 462 study participants from the 128 households were included in this study.

All participants provided their written informed consent. The parents provided informed consent for participation in this study if their children were under 12 years old. The study protocol was approved by the ethics board for epidemiological studies at Hokkaido University Graduate School of Medicine.

### 2.2. Phthalate metabolites in urine

### 2.2.1. Collection of urine samples

Parents were asked to collect the morning spot urine for the home visit and refrigerate the sample until our visit. Each urinary sample was dispensed into a stoppered glass test tube, which had been cleaned by acetone in our laboratory and sealed with fluoroc-tape, wrapped with aluminium foil, and kept at  $-20^{\circ}\text{C}$  until the day of analysis. All 462 urinary samples were assayed for creatinine using an enzyme-linked immunosorbent assay at SRL, Inc. (Tokyo, Japan).

### 2.2.2. Standards and reagents

Mono-n-butyl phthalate (MnBP), MiBP, mono(3-carboxypropyl) phthalate (MCP), mono-benzyl phthalate (MBzP), MEHP, mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono(2-ethyl-5-carboxypentyl) phthalate (MECPP) standards and MEHP-d<sub>4</sub> were purchased from Cambridge Isotope Laboratories, Inc., Massachusetts, USA. Acetonitrile and hydrochloric acid were purchased from Kanto Chemical Co., Inc., Tokyo, Japan. Acetic acid, ethyl acetate, and sodium sulphate were purchased from Wako Pure Chemical Industries, Ltd., Osaka, Japan.  $\beta$ -Glucuronidase/Arylsulfatase from *Helix pomatia* (30/60 unit) was purchased from Merck & Co., Inc., Darmstadt, Germany. N-methyl-N-(tert-butyldimethylsilyl)-trifluoroacetamide (MTBSTFA) was purchased from GL Sciences Inc., Tokyo, Japan.

### 2.2.3. Sample preparation

For the sample preparation, 50  $\mu\text{L}$  of acetic acid (1 M; pH 4.8), buffer, and 50  $\mu\text{L}$  of MEHP-d<sub>4</sub> (2  $\mu\text{g}/\text{mL}$ ) were added to 0.5 mL of sample urine. Solutions were incubated with 10  $\mu\text{L}$  of  $\beta$ -glucuronidase/arylsulphatase (30/60 unit) at  $36^{\circ}\text{C}$  for 24 h. The mixture was extracted with 100  $\mu\text{L}$  of hydrochloric acid (2 M). Ethyl acetate (2 mL) was added with vortexing for 30 s and then centrifuged (3000 rpm for 10 min). This extraction procedure was repeated twice. Supernatants were transferred into new tubes and dried at  $36^{\circ}\text{C}$  for 1 hour. The adherence of

sample to the tube was resolved with 30  $\mu$ L of ethyl acetate. The derivatisation processes for each metabolite were conducted using 30  $\mu$ L of MTBSTFA (Ito et al. 2005). After addition of MTBSTFA, the solutions were mixed by vortexing at 700 rpm for 30 min. All solutions were transferred into inserted vials, and 1  $\mu$ L was injected into GC/MS. MEHHP was not measurable because the derivatisation of MEHHP did not work well.

#### 2.2.4. Gas chromatography mass spectrometry (GC/MS)

Extracted solutions were analysed using GC/MS instrumentation. An HP GC6890 (Agilent Technologies Inc., Palo Alto, CA, USA) and Agilent 5973N MSD (Agilent Technologies Inc., Palo Alto, CA, USA) were used for analysis of 8 phthalate metabolites and MEHP-d<sub>4</sub>. The column was a DB-5MS (30 m  $\times$  0.25 mm i.d.  $\times$  0.25  $\mu$ m; Agilent Technologies, Inc., Santa Clarita, California, USA). Helium was used as the carrier gas (70 kPa, constant flow mode). The oven temperature was programmed as follows: 80  $^{\circ}$ C for 2 min, followed by 20 $^{\circ}$ C/min up to 300 $^{\circ}$ C for 20 min. The injector was operated in the split mode at 280 $^{\circ}$ C (1  $\mu$ L injection volume). The detector was operated in the selective ion mode (SIM) at a temperature of 230 $^{\circ}$ C.

#### 2.2.5. Quality control

The levels of MiBP, MnBP, MCPP, MBzP, MEHP, MEOHP, and MECPP were measured. Each calibration curve was prepared using 0.5 mL of standard pooled urine samples from healthy volunteers with added calibration standards that consisted of six concentrations (0, 0.01, 0.05, 0.1, 0.5, and 1.0  $\mu$ g/mL) prepared in 20% acetonitrile water. Each calibration standard also contained the internal standard (IS) (0.2  $\mu$ g/mL). Calibration curves were constructed to perform linear regressions of the peak area ratio between the standard and MEHP-d<sub>4</sub> versus six concentrations. Urine samples were quantified using calibration curves that presented good linearity and correlation coefficients ( $R^2$ ) > 0.995 for all compounds.

Quantification was performed using a relative-response ratio to an internal standard that most structurally matched the target analyte (Table 1).

Recoveries and relative standard deviations were evaluated using five replicate fortifications of a human urine sample with 0.01  $\mu\text{g/mL}$  of standard solution (Table 1). The procedural blank levels were determined using 1 mL of ultrapure water. In this study, the LOD values were determined as 0.005  $\mu\text{g/mL}$  (5  $\mu\text{g/L}$ ) for all metabolites. Because it was suspected that  $<0.01 \mu\text{g/mL}$  of quantitative assessment was acceptable, according to the chromatogram of the peaks and signal-to-noise ratios of each metabolite. In addition to this assessment,  $>0.005 \mu\text{g/mL}$  of quantitative assessment was acceptable in accordance with these ratios (Figure 1). However, the RSD value for MCPPE was too high (31.7%); therefore, we excluded MCPPE from further analysis. Positive correlations between primary DEHP metabolites of MEHP and secondary DEHP metabolites of MEOHP and MECPE were obtained ( $p<0.01$ ). For quantification of phthalate metabolites, the total area of the branched and linear isomer peaks was integrated. Calibration curves were constructed before and after each batch (approximately 50 samples) to maintain the quality of the analysis. All instruments for sample collection, preparation, and GC/MS analysis were washed with acetone and covered with aluminium foil until use to prevent phthalate contamination. To confirm that there was no phthalate contamination from the materials used for sampling, the sampling receptacles were extracted with acetone, and the blank values were examined. The blank value for MEHP was 0.0017  $\mu\text{g/mL}$  (range, 0.0016–0.0019  $\mu\text{g/mL}$ ). The blank values of other metabolites were not detected.

### 2.3. Estimated daily intake calculations

The daily intake of the target phthalates was calculated for each participant from the phthalate metabolite concentration in the urine. In the present paper, the calculations of the total daily intake of phthalates were estimated using the following model based on Koch et al. (2007)(Koch et al. 2007) and Wittassek et al. (2007):

$$DI = (C \times CE) / (F_{ue} \times BW \times MW_p)$$

where DI ( $\mu\text{g}/\text{kg}/\text{day}$ ) is the total daily intake of phthalate normalised for body weight; C ( $\mu\text{mol}/\text{gCr}$ ) is the urinary phthalate metabolite concentration; CE ( $\text{g}/\text{day}$ ) is the creatinine clearance rate; and  $F_{ue}$  is the urinary excretion factor, which describes the molar ratio between the excreted amount of a metabolite in relation to the intake of the parent phthalate: MiBP, MnBP, MBzP, MEHP, MEOHP, and MECPP, which were reported to be 0.69, 0.69, 0.73, 0.059, 0.15, and 0.185 (Koch et al. 2004a; Koch et al. 2005; Kohn et al. 2000), respectively. BW (kg) is body weight.  $MW_p$  (g/mol) is the molecular weights of the parent phthalate. The information on gender, age, body height, and weight for each participant was obtained from the self-reported questionnaire, except for preschoolers aged less than six years. We used the values of Japanese gender-age-specific body height and weight (Japan Ministry of Health 2013) for the preschoolers because the preschoolers were not asked to complete the questionnaire, and the calculations for the daily intake of phthalates required information regarding gender, age, body height, and weight. CE was estimated following the model based on Mage et al. (2004 and 2008)(Mage et al. 2004; Mage et al. 2008).

more than 18 years old

- $CE = 1.93 \times (140 - \text{Age}) \times BW^{1.5} \times ht^{0.5} \times 10^6 \dots \text{male}$
- $CE = 1.64 \times (140 - \text{Age}) \times BW^{1.5} \times ht^{0.5} \times 10^6 \dots \text{female}$

3–18 years old

- $CE = ht \times \{6.265 + 0.0564 \times (ht - 168)\} \times 10^3 \dots \dots ht < 168 \text{ cm} \text{ male}$
- $CE = ht \times \{6.265 + 0.2550 \times (ht - 168)\} \times 10^3 \dots \dots ht > 168 \text{ cm} \text{ male}$
- $CE = 2.045 \times ht \times \exp\{0.01552 \times (ht - 90)\} \times 10^3 \dots \dots \text{female}$

where Age (years old) is the participant's age. ht (cm) is height.

## 2.4. Data analysis and statistics

For values below the LOD, we assigned a value of the LOD divided by 2 (LOD/2). The metabolites of DBP and DEHP were combined into their sum of the individual metabolite concentrations as  $\Sigma$ DBP (MnBP and MiBP) and  $\Sigma$ DEHP (MEHP, MEOHP, and MECPP). To simplify the interpretations of the sums of the phthalate metabolite levels, urinary phthalate metabolite levels were converted to molecular concentration ( $\mu\text{mol/L}$ ).

We classified participants into five groups: “preschool siblings (3–6 years old)”, “schoolchildren (7–12 years old)”, “older siblings (13–24 years old)”, “mothers”, and “fathers”. Then, when comparing the differences in the phthalate metabolite and daily intake levels between family members, mothers and fathers were categorised using one variable, “parents”. The data for phthalate metabolite and daily intake levels were not normally distributed according to the Shapiro-Wilk W-test ( $p > 0.05$ ). The differences in the phthalate metabolite and daily intake levels with parents as a referent were analysed using the Steel test. For the multiple comparisons, the statistical significance of the p-value was  $p < 0.017$  based on Bonferroni’s correction. The other statistical significance of the p-value was  $p < 0.05$ . For statistical analyses, a two-tailed test and a 5% level of significance were used. All analyses were performed using JMP Pro 10 for Macintosh (SAS Institute Inc., Cary, NC).

### 3. Results

The present study is based on data from 462 participants (224 males and 238 females) aged from 3 to 56 years. The distribution of each age group was as follows: 29 preschool siblings, 178 schoolchildren, 40 older siblings, 125 mothers, and 90 fathers (total 215 parents) (Table 2).

The distributions of urinary phthalate metabolite levels (with non-creatinine-adjusted and creatinine-adjusted values) in the schoolchildren and their family members are shown in Table 2. All phthalate metabolites, except MnBP, were detected in more than 50% of the urine samples. The most frequently detected metabolite was MEOHP (98.9%), followed by MiBP,

MEHP, MBzP, MECPP, and MnBP. MECPP was detected in 93.1%, 92.7%, and 80.6% of urine samples among preschool siblings, schoolchildren, and older siblings, whereas they were detected in 56.7% and 57.6% of mothers and fathers, respectively. The highest median levels of phthalate metabolites were found for MiBP in all participants except for schoolchildren. Instead, MEOHP had the highest median level in schoolchildren. For most of the metabolites, the range between the minimum and maximum levels was 2-3 orders of magnitude.

The phthalate metabolite levels of schoolchildren and their family members are shown in Figure 2 for (a) non-creatinine-adjusted data and (b) creatinine-adjusted data. When comparing different groups with parents (mothers and fathers) as a control (a), preschool siblings had a significantly higher urinary metabolite level for MECPP. Schoolchildren had significantly higher urinary metabolite levels for MEOHP, MECPP, and  $\Sigma$ DEHP. When adjusting for creatinine values (b), the results showed stronger and clearer associations; preschool siblings had significantly higher urinary metabolite levels for MiBP, MBzP, MEOHP, MECPP, and  $\Sigma$ DEHP. Schoolchildren had significantly higher urinary metabolite levels for MBzP, MEOHP, MECPP, and  $\Sigma$ DEHP.

The median daily intakes of phthalate of schoolchildren and their family members are shown in Figure 3. Comparing different groups with parents as a control, preschoolers had significantly higher daily intakes of DiBP, BBzP,  $\Sigma$ DBP, and  $\Sigma$ DEHP. Schoolchildren had significantly higher daily intakes of DnBP, BBzP, and  $\Sigma$ DEHP. Taking into account the family relatedness, associations between phthalate metabolite and daily intake levels and family members were evaluated using multi-level analysis, and some of the significant values decreased; however, the tendencies did not change.

The correlations between schoolchildren and their mothers or fathers in terms of urinary phthalate metabolites and daily intake of phthalates are shown in Table 3. All phthalate metabolite and sums of metabolite levels in schoolchildren were positively correlated with their mothers' levels, except for MEHP. For fathers, only MiBP, MBzP, MECPP, and  $\Sigma$ DBP were

1 correlated with their schoolchildren. In addition, all daily intakes of phthalates were positively  
2 correlated with mothers, whereas for fathers, only DiBP, BBzP, and  $\Sigma$ DBP were correlated.  
3 After adjustment for creatinine, these results did not change.

#### 4 5 4. Discussion

6 We simultaneously measured seven urinary phthalate metabolite levels in Japanese  
7 elementary schoolchildren and their family members (siblings, mother, and father) using the  
8 GC/MS method. The present study revealed that preschool siblings and schoolchildren had  
9 higher levels of phthalate metabolites in their urine compared with their parents. Moreover,  
10 preschool siblings and schoolchildren also had higher levels of daily intake of most phthalates  
11 than their parents. All phthalate metabolite levels in schoolchildren were positively correlated  
12 with their mothers, except for MEHP, whereas the levels in fathers were less correlated with  
13 their children.

#### 14 15 *Distributions of urinary phthalate metabolites*

16 We measured the urinary levels of six phthalate monoesters using the GC/MS method in  
17 a population of elementary school children and their family members in Sapporo. However, we  
18 could not detect MEHHP using our method because derivatisation of MEHHP did not work  
19 well. The detection of MiBP, MEHP, and MEOHP was achieved for > 80% of samples  
20 measured in this population, and the detection of MBzP and MECPP was achieved for > 75%.  
21 MnBP was detected in 31.4% of the samples. The maximum and 75<sup>th</sup> percentile urinary  $\Sigma$ DBP  
22 levels were higher than  $\Sigma$ DEHP levels. The parent phthalate of MiBP, DiBP, is used for  
23 coatings of medications and cosmetics as a substitute for DnBP (Commission 2004; Koch et al.  
24 2012). Although DnBP and DiBP have been banned in cosmetics because of reproductive  
25 toxicity in Europe (Communities 1993), there are no regulations for cosmetics and medications  
26 for both DnBP and DiBP in Japan. It is possible that the high individual levels of  $\Sigma$ DBP may be

1 associated with medications or high frequencies of PCP use; the population of this study was  
2 based on a study of children's allergies, which had a high prevalence of asthma and allergies.  
3 However, this association cannot be ascertained in this study because the use of PCPs and  
4 medications was not assessed.

5 Exposure to phthalates occurs not just from diet, but also from other routes, such as  
6 medications, PCPs, cosmetics, and indoor air and dust. DEHP is the most widely used  
7 plasticiser in Japan, and we previously reported that DEHP had the highest phthalate level in  
8 dust among the same study population (Ait Bamai et al., 2014). The sum of the urinary DEHP  
9 metabolites ( $\Sigma$ DEHP: MEHP, MEOHP, and MECPP) in school children was positively  
10 correlated with DEHP in house dust (Supplementary Table 4). MiBP and/or MnBP were not  
11 correlated with their parent compounds in house dust. Both dust concentrations and detection  
12 frequencies of phthalates in house dust in this study are in line with our previous findings (Ait  
13 Bamai et al., 2014). The contribution of indoor exposure to DEHP is higher in dust than in the  
14 gas phase, whereas the contributions of DnBP and DiBP are higher in the gas phase than in dust  
15 (Beko et al. 2013). Therefore, DEHP in dust might also contribute to the sum of the DEHP  
16 urinary levels even though foods and other stuff are the main sources of exposure to DEHP.

#### 18 *Urinary phthalate metabolites and daily phthalate intakes among family members*

19 We observed that levels of urinary MEOHP, MECPP, and  $\Sigma$ DEHP, and daily intakes of  
20 DnBP, DiBP, BBzP,  $\Sigma$ DBP, and  $\Sigma$ DEHP were higher in preschool siblings and/or  
21 schoolchildren than in parents, suggesting that younger children have higher levels of exposure  
22 to these phthalates. When phthalate metabolite levels were adjusted for individual creatinine  
23 values, the results showed stronger and clearer associations because creatinine is strongly  
24 dependent on age and gender (Barr et al. 2005).

25 As for gender differences, there were no differences between mother and father for both  
26 phthalate metabolite levels and daily intake levels (Supplementary Table 2). Guo et al. (2011)

1 reported that no gender-specific differences were found in DEHP metabolites (Guo et al. 2011),  
2 which is consistent with our results. In contrast, studies from the German Environmental  
3 Survey IV and U.S. population of the National Health and Nutrition Examination Survey  
4 (NHANES) reported that levels of urinary MEP, MnBP, and MBzP among adults were higher  
5 in females than in males because of the use of PCPs such as cosmetic products and fragrances  
6 (Koch et al. 2003; Silva et al. 2004). As for children (preschoolers and schoolchildren), MBzP  
7 levels and daily BBzP intake in boys were higher than in girls ( $p = 0.007$  and  $p = 0.006$ ,  
8 respectively) (Supplementary Table 3). Several research groups have reported consistent results  
9 indicating that levels of DEHP metabolites, MBP, and MBzP were higher in boys than in girls  
10 (Becker et al. 2009; Boas et al. 2010; Frederiksen et al. 2011), and daily BBzP intake tended to  
11 be higher in boys than in girls (Frederiksen et al. 2011). In contrast, the NHANES reported that  
12 the median values tend to be slightly higher in girls than in boys for MEP, MBP, MBzP, and  
13 DEHP metabolites (MEHP, MEOHP, and MEHHP) (Hatch et al. 2008).

14 The percentage fraction of MEHP was significantly lower, and MECPP was higher in  
15 younger age groups (preschoolers and schoolchildren) than in parents. Our results are  
16 consistent with previous studies (Becker et al. 2004; Koch et al. 2004b; Song et al. 2013),  
17 which suggests the excretion of oxidative metabolites of DEHP is elevated in children  
18 compared with adults. However, as our analytic methods did not allow detection of the  
19 secondary DEHP metabolite of MEHHP, the fraction of MEHHP could not be assessed in this  
20 study.

#### 21 22 *Correlation of phthalate metabolites between schoolchildren and their mothers and fathers*

23 Most of the phthalate metabolites and daily intakes among schoolchildren were more  
24 strongly correlated with that of their mothers compared with their fathers. MBzP and  $\Sigma$ DBP  
25 among schoolchildren were correlated with both those of mothers and fathers, whereas  $\Sigma$ DEHP  
26 was correlated with that of mothers only. The main exposure routes of DEHP and DBP are diet

1 and PCPs, respectively (Colacino et al. 2010; Sathyanarayana et al. 2008b; Serrano et al. 2014).  
2 This may indicate that children share greater phthalate exposure with their mothers than with  
3 their fathers because of surrounding environment and lifestyle factors, such as the same diet  
4 and same PCP use. However, we did not assess diet and use of PCP. Therefore, further studies  
5 assessing daily diet and frequency of PCP use may contribute to knowledge of mother-child  
6 pair associations.

7 Several studies have reported urinary phthalate metabolite levels in mother/child pairs  
8 (Casas et al. 2011; Huang et al. 2009; Kasper-Sonnenberg et al. 2012; Lin et al. 2011;  
9 Sathyanarayana et al. 2008a; Song et al. 2013). However, there are only three studies that have  
10 sampled mother/child pair urine at the same time (Kasper-Sonnenberg et al. 2012; Song et al.  
11 2013), and there are no previous studies that have included all family members, including  
12 mothers, fathers, and siblings.

13 The percentage fractions of the DEHP metabolites of MEHP and MECPP were  
14 significantly higher in younger age groups (preschool siblings and schoolchildren) than in  
15 parents ( $p < 0.001$ ), which is consistent with previous studies (Kasper-Sonnenberg et al. 2012;  
16 Koch et al. 2006; Song et al. 2013).

#### 18 *Comparisons of urinary phthalate metabolite and daily phthalate intake levels with different* 19 *studies*

20 When comparing children's phthalate metabolite levels with those in previous studies  
21 (Bertelsen et al. 2013; CDC 2013; Cho et al. 2010; Hsu et al. 2012; Kasper-Sonnenberg et al.  
22 2012; Koch et al. 2011; Langer et al. 2013; Song et al. 2013), the levels of the metabolites of  
23 DEHP, MEHP, MEOHP, and MECPP were similar or higher than in other studies (Figure 4).  
24 The MBzP level was similar or higher than that reported in Germany, Denmark, USA, and  
25 Taiwan. We previously reported that the BBzP level in house dust among this study population  
26 was quite lower than that reported in other countries (Ait Bamai et al. 2014). However, the

1 urinary MBzP level was positively correlated with BBzP in house dust (Supplementary Table  
2 4), which suggests that BBzP in dust contributes to urinary MBzP levels despite low levels of  
3 BBzP in house dust. One previous study reported that indoor air BBzP levels were significantly  
4 correlated with urinary MBzP (Adibi et al. 2003), suggesting that inhalation may also be an  
5 important route of exposure to BBzP.

6 When comparing phthalate daily intakes among schoolchildren with other children's  
7 studies, the DiBP and BBzP intakes were similar, whereas the DnBP intake was quite lower  
8 than that reported in other children's studies (Beko et al. 2013; Frederiksen et al. 2011; Koch et  
9 al. 2007; Koch et al. 2011; Lin et al. 2011; Wittassek et al. 2007), as shown in Figure 5. On the  
10 other hand, despite our underestimated daily DEHP intake because of a lack of the value of  
11 MEHHP, our DEHP intake was higher than in other studies except for a study in Taiwan (Lin  
12 et al., 2011). We previously reported that high levels of DEHP in house dust were detected  
13 from this study population with polyvinyl chloride (PVC) flooring, and the DEHP level in dust  
14 was remarkably higher than in other studies (Ait Bamai et al. 2014). In addition, our data  
15 showed positive correlations between DEHP in house dust and daily DEHP intake and  $\Sigma$ DEHP  
16 metabolites in schoolchildren's urine, but not correlated with mothers/fathers urine  
17 (Supplementary Table 4). It again may suggest that for the children in this study population,  
18 dust much contributes to total DEHP exposure than foods and other exposure sources. In  
19 contrast, it has been reported that DEHP levels in house dust (Becker et al. 2004) and indoor air  
20 (Adibi et al. 2008) are not correlated with DEHP metabolites in urine. Koch et al., 2013 also  
21 concluded that house dust/air does not seem to be a significant route of DEHP exposure.  
22 Therefore, these inconsistent results may be caused by high level of DEHP in house dust.  
23 When comparing the daily DEHP intake in this population to the Tolerable Daily Intake (TDI)  
24 value ( $50 \mu\text{g/kg/day}$ ) of the EU and the Reference Dose (RfD) value ( $20 \mu\text{g/kg/day}$ ) of the U.S.  
25 Environmental Protection Agency (US EPA), 2 (0.4%) and 27 (5.8%) of the 462 participants

1 exceed the TDI and the RfD, respectively. Of that number, 1 and 20 were children  
2 (preschoolers plus schoolchildren), which means approximately 0.5% and 10% of children and  
3 0.4% and 3% of adults exceed the TDI and RfD values, respectively. Therefore, it is important  
4 to examine children's DEHP exposure because exceedance of the RfD is occurring more in  
5 children than in adults in the same families sharing similar exposure sources.

6 Interpretation of these comparisons should be cautious because the methods for collecting  
7 urine differ in each of the studies. Although production and use of DEHP, BBzP, and DnBP are  
8 temporally decreasing in recent years due to government regulations, leading to temporal  
9 decline of these urinary metabolite levels (CDC 2013; Silva et al. 2004; Zota et al. 2014), the  
10 level in 10% of children in this study exceeded the RfD of the US EPA. Therefore, it is  
11 necessary to continue monitoring exposure to phthalates and to consider RfD with the health  
12 effects for children in mind.

### 14 *Limitations*

15 There are several limitations to this study. First, we collected urine samples only once  
16 from the first morning void. Moreover, we did not analyse samples taken from individuals over  
17 time. Therefore, it is possible that phthalate metabolite levels in this study were high or low by  
18 chance. Second, the distributions of age groups in this study were not even, with the number of  
19 schoolchildren and adults being 178 and 219, respectively, whereas the number of preschoolers  
20 and adolescents was 29 and 36, respectively. Therefore, statistical comparisons between age  
21 groups may be affected by this imbalance. Participation in this study was based on a study  
22 assessing the associations between elementary schoolchildren's allergies and their indoor  
23 environment, which means that children aged 6–12 years and their parents and siblings were  
24 the main participants. This study population has a propensity to have an interest in indoor air  
25 quality and their health (Ait Bamai et al. 2014), which may result in differences from the  
26 general Japanese population phthalate levels. Third, our GC/MS analysis procedures could not

1 achieve detection of MEHHP, which accounts for a large portion of DEHP metabolites, from  
2 urine samples because the derivatisation of MEHHP did not work well. Thus, we may have  
3 underestimated our  $\Sigma$ DEHP and daily DEHP intake. Comparisons of our urinary phthalate  
4 metabolite levels and daily intakes to other studies should be made cautiously. We used  
5 MTBSTFA as a derivatisation reagent. Since Kim et al. (2014) recently reported that the  
6 GC/MS method using N,O-bis(trimethylsilyl)-trifluoro acetamide (BSTFA) as a derivatisation  
7 reagent allowed detection of MEHHP (Kim et al. 2014), although our study did not use BSTFA,  
8 further study needs to use BSTFA as a derivatisation reagent to detect MEHHP. However,  
9 using this method also allowed detection of seven phthalate monoesters in human urine  
10 samples of a large sample size with practical analysis cost. Fourth, the use of PCPs and  
11 medications, food consumption, and other behavioural patterns were not assessed in this study.  
12 Therefore, we could not ascertain the exposure source. Further studies are needed to advance  
13 our understanding of phthalate exposure.

## 15 5. Conclusions

16 We measured six urinary phthalate metabolite levels using a GC/MS method in Japanese  
17 (Sapporo) elementary schoolchildren and their family members. Most phthalate metabolite  
18 levels in children were similar or higher than in other studies. The daily intake of DEHP was  
19 higher, whereas the DnBP intake was quite lower than in other children's studies. DEHP  
20 metabolites and the daily intake of DEHP were especially high in preschool siblings compared  
21 with their parents. All phthalate metabolite levels in schoolchildren were positively correlated  
22 with the levels of their mothers except for MEHP, whereas the levels in fathers were less  
23 correlated with that of their children. Although there is decreasing production and use of  
24 phthalates, 10% of children and 3% of adults still exceeded the RfD value for DEHP, which  
25 indicates an important need to focus on children's DEHP exposure. Active endocrine phthalates  
26 act in a common fashion. However, the Japanese population is probably exposed to other

1 phthalates that were not measured in the present study, e.g., diethyl phthalate, DMP, DiNP, and  
2 DiDP. Therefore, the cumulative exposure has to be taken into account in future studies. Our  
3 results will contribute to considerations of the regulations for some phthalates and the actual  
4 phthalate exposure levels in the Japanese population.

5

## References

- 2004/93/EC D. 2004. Directive 2004/93/ec of the european parliament and of the council of 21 september 2004 amending council directive 76/768/eec for the purpose of adapting its annexes ii and iii to technical progress.
- 2005/84/EC D. 2005. Directive 2005/84/ec of the european parliament and of the council of 14 december 2005.
- Adibi JJ, Perera FP, Jedrychowski W, Camann DE, Barr D, Jacek R, et al. 2003. Prenatal exposures to phthalates among women in new york city and krakow, poland. *Environ Health Perspect* 111:1719-1722.
- Adibi JJ, Whyatt RM, Williams PL, Calafat AM, Camann D, Herrick R, et al. 2008. Characterization of phthalate exposure among pregnant women assessed by repeat air and urine samples. *Environ Health Perspect* 116:467-473.
- Ait Bamai Y, Araki A, Kawai T, Tsuboi T, Saito I, Yoshioka E, et al. 2014. Associations of phthalate concentrations in floor dust and multi-surface dust with the interior materials in japanese dwellings. *Sci Total Environ* 468:147-157.
- Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. 2005. Urinary creatinine concentrations in the u.S. Population: Implications for urinary biologic monitoring measurements. *Environ Health Perspect* 113:192-200.
- Becker K, Seiwert M, Angerer J, Heger W, Koch HM, Nagorka R, et al. 2004. Dehp metabolites in urine of children and dehp in house dust. *Int J Hyg Environ Health* 207:409-417.
- Becker K, Goen T, Seiwert M, Conrad A, Pick-Fuss H, Muller J, et al. 2009. Geres iv: Phthalate metabolites and bisphenol a in urine of german children. *Int J Hyg Environ Health* 212:685-692.
- Beko G, Weschler CJ, Langer S, Callesen M, Toftum J, Clausen G. 2013. Children's phthalate intakes and resultant cumulative exposures estimated from urine compared with estimates

from dust ingestion, inhalation and dermal absorption in their homes and daycare centers. PLoS One 8:e62442.

Bertelsen RJ, Carlsen KC, Calafat AM, Hoppin JA, Haland G, Mowinckel P, et al. 2013. Urinary biomarkers for phthalates associated with asthma in norwegian children. Environ Health Perspect 121:251-256.

Bizzari SN. 2013. Chemical economics handbook plasticizers.

Boas M, Frederiksen H, Feldt-Rasmussen U, Skakkebaek NE, Hegedus L, Hilsted L, et al. 2010. Childhood exposure to phthalates: Associations with thyroid function, insulin-like growth factor i, and growth. Environ Health Perspect 118:1458-1464.

Calafat AM, McKee RH. 2006. Integrating biomonitoring exposure data into the risk assessment process: Phthalates [diethyl phthalate and di(2-ethylhexyl) phthalate] as a case study. Environ Health Perspect 114:1783-1789.

Casas L, Fernandez MF, Llop S, Guxens M, Ballester F, Olea N, et al. 2011. Urinary concentrations of phthalates and phenols in a population of spanish pregnant women and children. Environ Int 37:858-866.

CDC. 2013. The fourth national report on human exposure to environmental chemicals, updated tables, september 2013.CDC.

Cho SC, Bhang SY, Hong YC, Shin MS, Kim BN, Kim JW, et al. 2010. Relationship between environmental phthalate exposure and the intelligence of school-age children. Environ Health Perspect 118:1027-1032.

Colacino JA, Harris TR, Schecter A. 2010. Dietary intake is associated with phthalate body burden in a nationally representative sample. Environ Health Perspect 118:998-1003.

Commission E. 2004. European union risk assesment report. Di-butyl phthalate.Institute for Health and Consumer Protection

European Chemicals Bureau.

1 Commission USCPS. 2008. Public law 110-314-aug. 14, 2008 consumer product safety  
2 important act of 2008. PUBLIC LAW 110-314.

3 Communities E. 1993. Council regulation 793/93/eec.

4 Frederiksen H, Aksglaede L, Sorensen K, Skakkebaek NE, Juul A, Andersson AM. 2011.  
5 Urinary excretion of phthalate metabolites in 129 healthy danish children and  
6 adolescents: Estimation of daily phthalate intake. *Environ Res* 111:656-663.

7 Gray TJ, Rowland IR, Foster PM, Gangolli SD. 1982. Species differences in the testicular  
8 toxicity of phthalate esters. *Toxicol Lett* 11:141-147.

9 Guo Y, Wu Q, Kannan K. 2011. Phthalate metabolites in urine from china, and implications for  
10 human exposures. *Environ Int* 37:893-898.

11 Hatch EE, Nelson JW, Qureshi MM, Weinberg J, Moore LL, Singer M, et al. 2008. Association  
12 of urinary phthalate metabolite concentrations with body mass index and waist  
13 circumference: A cross-sectional study of nhanes data, 1999-2002. *Environ Health* 7:27.

14 Hines EP, Calafat AM, Silva MJ, Mendola P, Fenton SE. 2009. Concentrations of phthalate  
15 metabolites in milk, urine, saliva, and serum of lactating north carolina women. *Environ*  
16 *Health Perspect* 117:86-92.

17 Hsu NY, Lee CC, Wang JY, Li YC, Chang HW, Chen CY, et al. 2012. Predicted risk of  
18 childhood allergy, asthma, and reported symptoms using measured phthalate exposure in  
19 dust and urine. *Indoor Air* 22:186-199.

20 Huang PC, Kuo PL, Chou YY, Lin SJ, Lee CC. 2009. Association between prenatal exposure  
21 to phthalates and the health of newborns. *Environ Int* 35:14-20.

22 Ito Y, Yokota H, Wang R, Yamanoshita O, Ichihara G, Wang H, et al. 2005. Species  
23 differences in the metabolism of di(2-ethylhexyl) phthalate (dehp) in several organs of  
24 mice, rats, and marmosets. *Arch Toxicol* 79:147-154.

25 Japan Ministry of Health LaW. 2002. Ministerial notification no. 267 of mhlw.

1 Japan Ministry of Health LaW. 2013. Table 2-6. Average body weight and height. Available:  
2 [http://www.mhlw.go.jp/toukei/youran/indexyk\\_2\\_1.html](http://www.mhlw.go.jp/toukei/youran/indexyk_2_1.html) [accessed 06.24 2014].

3 Japan Plasticizer Industry Association and Ministry of Economy TaI. 2014. Information of  
4 japan plasticizer industries.

5 Kasper-Sonnenberg M, Koch HM, Wittsiepe J, Wilhelm M. 2012. Levels of phthalate  
6 metabolites in urine among mother-child-pairs - results from the duisburg birth cohort  
7 study, germany. *Int J Hyg Environ Health* 215:373-382.

8 Kim M, Song NR, Choi JH, Lee J, Pyo H. 2014. Simultaneous analysis of urinary phthalate  
9 metabolites of residents in korea using isotope dilution gas chromatography-mass  
10 spectrometry. *Sci Total Environ* 470-471:1408-1413.

11 Koch HM, Drexler H, Angerer J. 2003. An estimation of the daily intake of di(2-  
12 ethylhexyl)phthalate (dehp) and other phthalates in the general population. *Int J Hyg*  
13 *Environ Health* 206:77-83.

14 Koch HM, Bolt HM, Angerer J. 2004a. Di(2-ethylhexyl)phthalate (dehp) metabolites in human  
15 urine and serum after a single oral dose of deuterium-labelled dehp. *Arch Toxicol*  
16 78:123-130.

17 Koch HM, Drexler H, Angerer J. 2004b. Internal exposure of nursery-school children and their  
18 parents and teachers to di(2-ethylhexyl)phthalate (dehp). *Int J Hyg Environ Health*  
19 207:15-22.

20 Koch HM, Bolt HM, Preuss R, Angerer J. 2005. New metabolites of di(2-ethylhexyl)phthalate  
21 (dehp) in human urine and serum after single oral doses of deuterium-labelled dehp. *Arch*  
22 *Toxicol* 79:367-376.

23 Koch HM, Preuss R, Angerer J. 2006. Di(2-ethylhexyl)phthalate (dehp): Human metabolism  
24 and internal exposure-- an update and latest results. *Int J Androl* 29:155-165; discussion  
25 181-155.

- 1 Koch HM, Becker K, Wittassek M, Seiwert M, Angerer J, Kolossa-Gehring M. 2007. Di-n-  
2 butylphthalate and butylbenzylphthalate - urinary metabolite levels and estimated daily  
3 intakes: Pilot study for the german environmental survey on children. *Journal of*  
4 *Exposure Science and Environmental Epidemiology* 17:378-387.
- 5 Koch HM, Wittassek M, Bruning T, Angerer J, Heudorf U. 2011. Exposure to phthalates in 5-6  
6 years old primary school starters in germany--a human biomonitoring study and a  
7 cumulative risk assessment. *Int J Hyg Environ Health* 214:188-195.
- 8 Koch HM, Christensen KL, Harth V, Lorber M, Bruning T. 2012. Di-n-butyl phthalate (dnbp)  
9 and diisobutyl phthalate (dibp) metabolism in a human volunteer after single oral doses.  
10 *Arch Toxicol* 86:1829-1839.
- 11 Koch HM, Lorber M, Christensen KL, Palmke C, Koslitz S, Bruning T. 2013. Identifying  
12 sources of phthalate exposure with human biomonitoring: Results of a 48h fasting study  
13 with urine collection and personal activity patterns. *Int J Hyg Environ Health* 216:672-  
14 681.
- 15 Kohn MC, Parham F, Masten SA, Portier CJ, Shelby MD, Brock JW, et al. 2000. Human  
16 exposure estimates for phthalates. *Environ Health Perspect* 108:A440-442.
- 17 Langer S, Beko G, Weschler CJ, Brive LM, Toftum J, Callesen M, et al. 2013. Phthalate  
18 metabolites in urine samples from danish children and correlations with phthalates in dust  
19 samples from their homes and daycare centers. *Int J Hyg Environ Health*.
- 20 Lin S, Ku HY, Su PH, Chen JW, Huang PC, Angerer J, et al. 2011. Phthalate exposure in  
21 pregnant women and their children in central taiwan. *Chemosphere* 82:947-955.
- 22 Mage DT, Allen RH, Gondy G, Smith W, Barr DB, Needham LL. 2004. Estimating pesticide  
23 dose from urinary pesticide concentration data by creatinine correction in the third  
24 national health and nutrition examination survey (nhanes-iii). *J Expo Anal Environ*  
25 *Epidemiol* 14:457-465.

1 Mage DT, Allen RH, Kodali A. 2008. Creatinine corrections for estimating children's and  
2 adult's pesticide intake doses in equilibrium with urinary pesticide and creatinine  
3 concentrations. *J Expo Sci Environ Epidemiol* 18:360-368.

4 Oishi S. 1993. Strain differences in susceptibility to di-2-ethylhexyl phthalate-induced  
5 testicular atrophy in mice. *Toxicol Lett* 66:47-52.

6 Sathyanarayana S, Calafat AM, Liu F, Swan SH. 2008a. Maternal and infant urinary phthalate  
7 metabolite concentrations: Are they related? *Environ Res* 108:413-418.

8 Sathyanarayana S, Karr CJ, Lozano P, Brown E, Calafat AM, Liu F, et al. 2008b. Baby care  
9 products: Possible sources of infant phthalate exposure. *Pediatrics* 121:e260-268.

10 Serrano SE, Karr CJ, Seixas NS, Nguyen RH, Barrett ES, Janssen S, et al. 2014. Dietary  
11 phthalate exposure in pregnant women and the impact of consumer practices.  
12 *International journal of environmental research and public health* 11:6193-6215.

13 Silva MJ, Barr DB, Reidy JA, Malek NA, Hodge CC, Caudill SP, et al. 2004. Urinary levels of  
14 seven phthalate metabolites in the u.S. Population from the national health and nutrition  
15 examination survey (nhanes) 1999-2000. *Environ Health Perspect* 112:331-338.

16 Song NR, On JW, Lee J, Park JD, Kwon HJ, Yoon HJ, et al. 2013. Biomonitoring of urinary  
17 di(2-ethylhexyl) phthalate metabolites of mother and child pairs in south korea. *Environ*  
18 *Int* 54:65-73.

19 Wittassek M, Heger W, Koch HM, Becker K, Angerer J, Kolossa-Gehring M. 2007. Daily  
20 intake of di(2-ethylhexyl)phthalate (dehp) by german children -- a comparison of two  
21 estimation models based on urinary dehp metabolite levels. *Int J Hyg Environ Health*  
22 210:35-42.

23 Zota AR, Calafat AM, Woodruff TJ. 2014. Temporal trends in phthalate exposures: Findings  
24 from the national health and nutrition examination survey, 2001-2010. *Environ Health*  
25 *Perspect* 122:235-241.

Figure 1

Chromatograms of each phthalate metabolite.

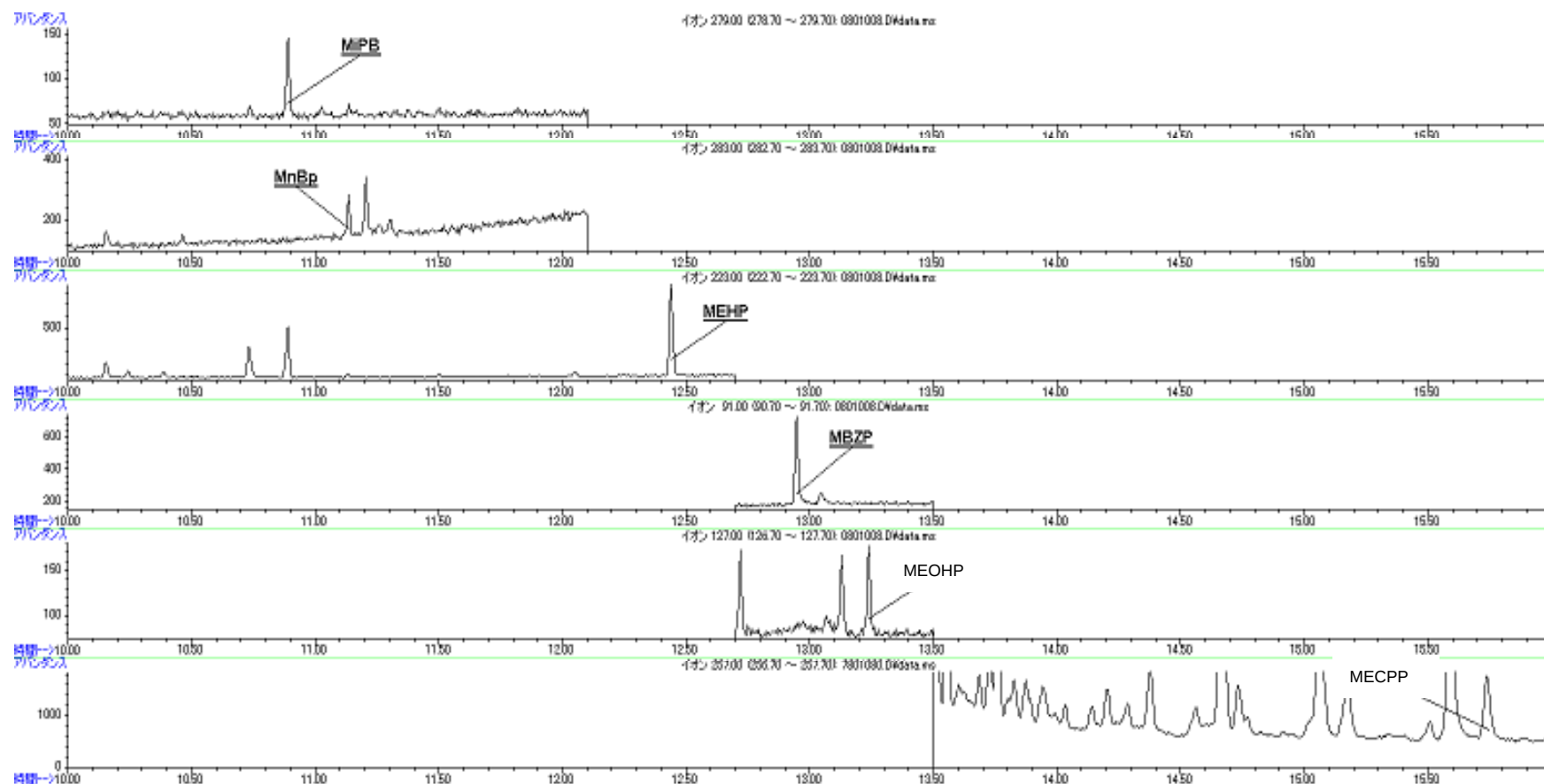
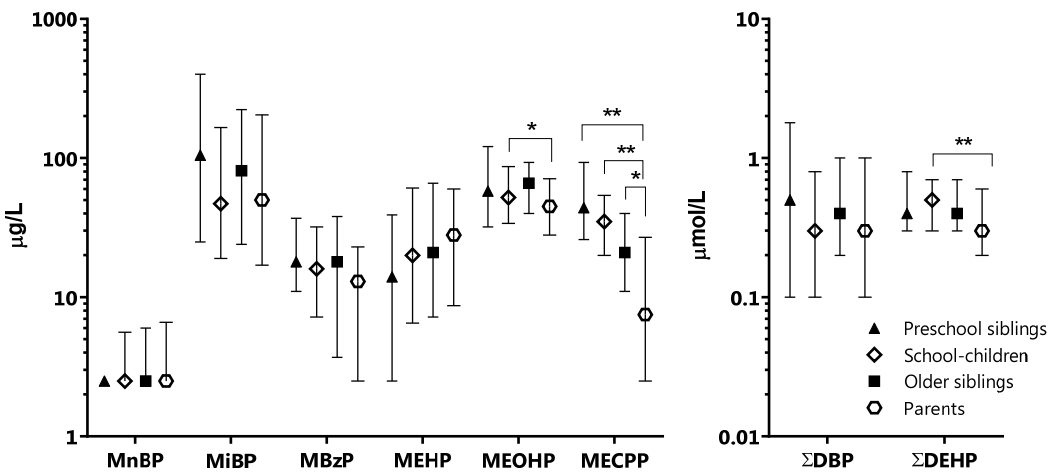


Figure 2

Phthalate metabolite levels in different age groups.

(a) Non-creatinine-adjusted



(b) Creatinine-adjusted

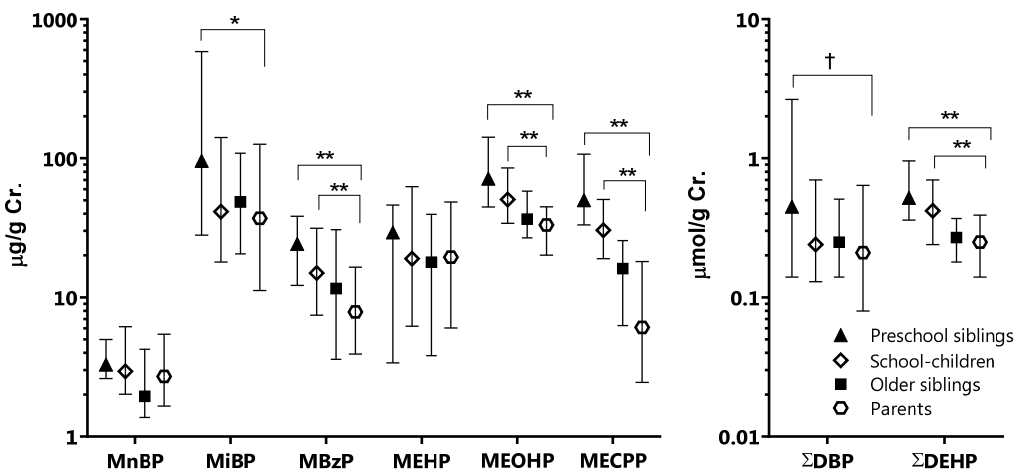


Figure 3.

Median daily intake of phthalates in different age groups.

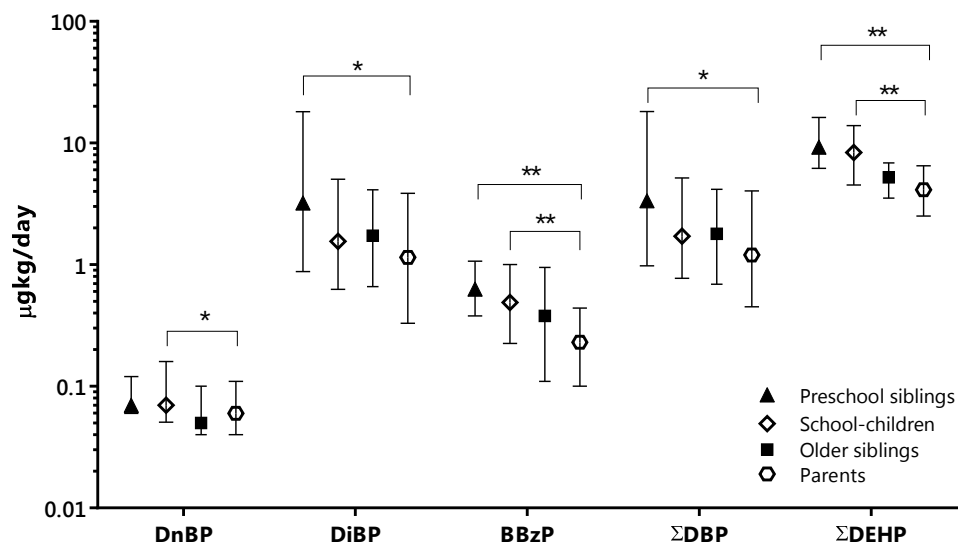
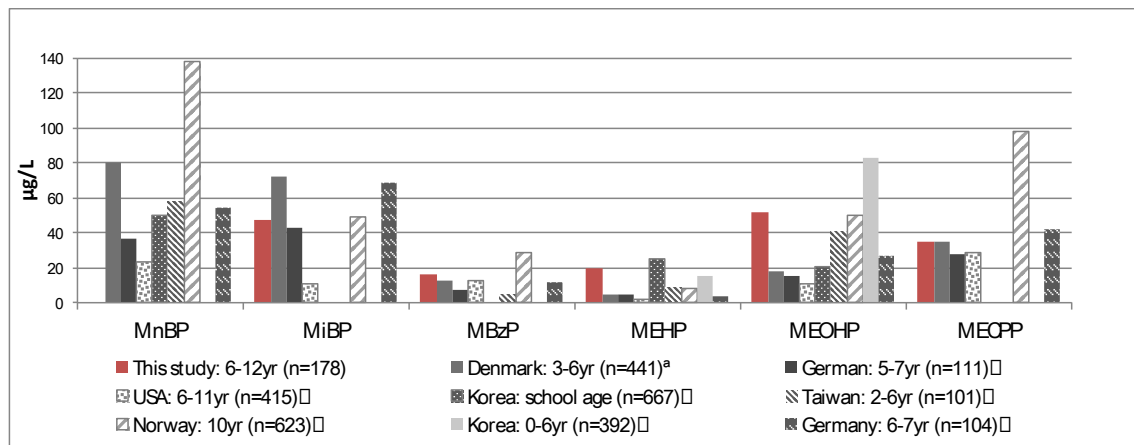


Figure 4.

Levels of urinary phthalate metabolites ( $\mu\text{g/L}$ ) in this study, compared with several previous studies.



a: Langer et al., 2013

b: Koch et al., 2011

c: CDC, 2013

d: Cho et al., 2010

e: Hsu et al., 2010

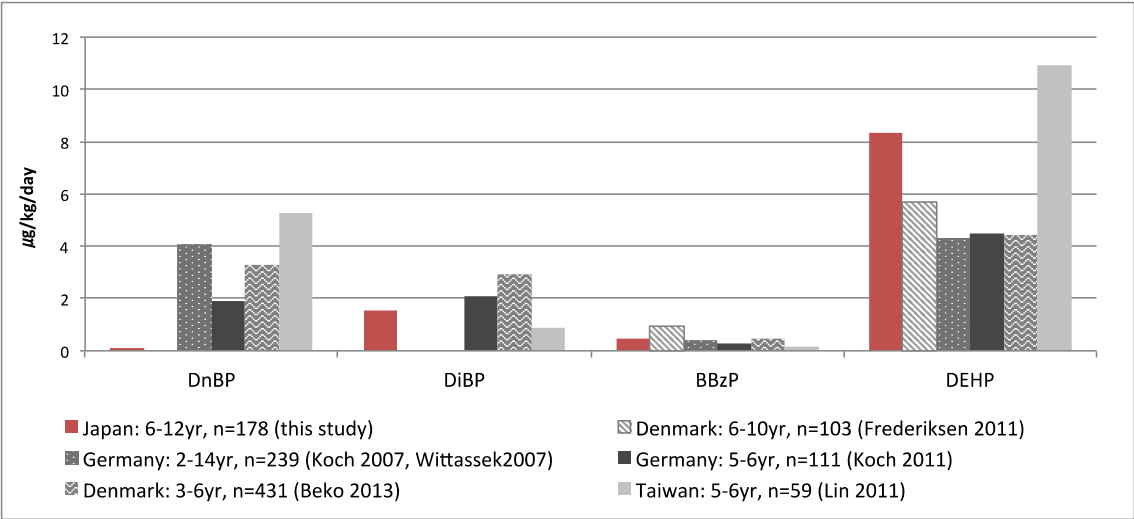
f: Bertelsen et al., 2013

g: Song et al., 2013

h: Kasper-Sonnenberg et al., 2012

Figure 5.

Levels of daily phthalate intake ( $\mu\text{g}/\text{kg}/\text{day}$ ) in this study, compared with several previous studies.



## Figure legends

Fig. 1. Chromatograms of each phthalate metabolite.

X-axis shows the retention time. Y-axis shows the peak level.

Fig. 2. Creatinine-adjusted phthalate metabolite levels (a) and sums of metabolites (b) in different age groups.

Lower and upper error bars indicate the 25% and 75% percentiles, and the median is indicated by the symbol. The comparisons between the parents and different age groups were analysed using the Kruskal-Wallis test, and p values were adjusted using Bonferroni's correction.

†:  $p < 0.017$ ; \*:  $p < 0.01$ ; \*\*:  $p < 0.001$

$\Sigma$ DBP: sum of MnBP and MiBP;  $\Sigma$ DEHP: sum of MEHP, MEOHP, and MECPP.

Fig. 3. Median daily intake of phthalates in different age groups.

Lower and upper error bars indicate the 25% and 75% percentiles, and the median is indicated by the symbol. The comparisons between the parents and different age groups were analysed using the Kruskal-Wallis test, and p values were adjusted using Bonferroni's correction.

\*:  $p < 0.01$ ; \*\*:  $p < 0.001$

$\Sigma$ DBP: sum of MnBP and MiBP;  $\Sigma$ DEHP: sum of MEHP, MEOHP, and MECPP.

Fig. 4. Levels of urinary phthalate metabolites ( $\mu\text{g/L}$ ) in this study, compared with several previous studies.

X-axis shows the phthalate metabolites. Y-axis shows the urinary phthalate metabolite levels ( $\mu\text{g/L}$ ).

Fig. 5. Levels of daily phthalate intake ( $\mu\text{g/kg/day}$ ) in this study, compared with several previous studies.

X-axis shows the phthalate metabolites. Y-axis shows the daily phthalate intake levels ( $\mu\text{g/kg/day}$ ).

Table 1

Mass transitions and recovery for each phthalate metabolite and internal standard (n=5).

| Compounds | Quantitation<br>(m/z) | Confirmation<br>(m/z) | Retention<br>times<br>(min) | Recovery<br>(%) | RSD (%) |
|-----------|-----------------------|-----------------------|-----------------------------|-----------------|---------|
| MnBP      | 283                   | 227                   | 11.13                       | 88.6            | 26.3    |
| MiBP      | 279                   | 223                   | 10.89                       | 86.2            | 8.6     |
| MBzP      | 91                    | 166                   | 12.95                       | 116.3           | 26.8    |
| MEHP      | 223                   | 335                   | 12.44                       | 99.2            | 22.1    |
| MEOHP     | 127                   | 201                   | 13.24                       | 86.8            | 16.7    |
| MECPP     | 337                   | 147                   | 15.75                       | 136.2           | 9.3     |
| MCPP      | 201                   | 267                   | 14.04                       | 115.1           | 31.7    |
| MEHP-d4   | 227                   | 167                   | 12.43                       | -               | -       |

RSD: relative standard deviation.

Table 2.

Distribution of urinary phthalate metabolite levels (with non-creatinine-adjusted values) in schoolchildren and their family members.

|                              | Preschool siblings (n=29) |                         |          |            |           | Schoolchildren (n=178) |                         |          |            |             | Older siblings (n=40) |                     |          |            |           | Mothers (n=125) |                         |          |            |           | Fathers (n=90) |                         |          |            |           |
|------------------------------|---------------------------|-------------------------|----------|------------|-----------|------------------------|-------------------------|----------|------------|-------------|-----------------------|---------------------|----------|------------|-----------|-----------------|-------------------------|----------|------------|-----------|----------------|-------------------------|----------|------------|-----------|
|                              | >LO<br>D%                 | Ran<br>ge               | 25th     | Med<br>ian | 75th      | >LO<br>D%              | Ran<br>ge               | 25th     | Med<br>ian | 75th        | >LO<br>D%             | Rang<br>e           | 25th     | Med<br>ian | 75th      | >LO<br>D%       | Ran<br>ge               | 25th     | Med<br>ian | 75th      | >LO<br>D%      | Ran<br>ge               | 25th     | Med<br>ian | 75th      |
| Phthalate metabolites (µg/L) |                           |                         |          |            |           |                        |                         |          |            |             |                       |                     |          |            |           |                 |                         |          |            |           |                |                         |          |            |           |
| MnBP                         | 10.3                      | <LO<br>D-<br>74<br>9.90 | <L<br>OD | <LO<br>D   | <LOD      | 29.2                   | <LO<br>D -<br>36<br><LO | <L<br>OD | <LO<br>D   | 5.62<br>021 | 30.6                  | <LO<br>D -13        | <L<br>OD | <LO<br>D   | 6.0       | 33.9            | <LO<br>D -<br>37<br><LO | <L<br>OD | <LO<br>D   | 6.0       | 39.1           | <LO<br>D -<br>57<br><LO | <L<br>OD | <LO<br>D   | 8.3       |
| MiBP                         | 100                       | -<br>830<br>2           | 24.6     | 105.<br>0  | 40<br>1.3 | 96.6                   | D-<br>699<br>5          | 18.9     | 47.0       | 166.<br>5   | 97.2                  | <LO<br>D -<br>4008  | 24.1     | 81.3       | 22<br>2.7 | 95.3            | D-<br>694<br>6          | 17.3     | 47.3       | 16<br>9.7 | 92.4           | D-<br>208<br>4          | 14.6     | 52.6       | 26<br>3.4 |
| MBzP                         | 86.2                      | <LO<br>D -<br>327       | 11.0     | 17.8       | 36.<br>9  | 80.3                   | D-<br>161<br>6          | 7.2      | 16.3       | 32.5        | 75                    | <LO<br>D -<br>981   | 3.7      | 18.3       | 38.<br>0  | 74.0            | <LO<br>D -<br>445       | <L<br>OD | 11.6       | 23.<br>1  | 73.9           | <LO<br>D -<br>269       | <L<br>OD | 13.4       | 22.<br>7  |
| MEHP                         | 65.5                      | <LO<br>D -<br>144       | <L<br>OD | 14.1       | 39.<br>4  | 81.5                   | <LO<br>D -<br>522       | 6.5      | 19.7       | 60.9        | 83.3                  | <LO<br>D -<br>450   | 7.2      | 20.5       | 65.<br>9  | 85.8            | <LO<br>D -<br>416       | 10.3     | 28.6       | 59.<br>9  | 73.9           | <LO<br>D -<br>597       | <L<br>OD | 24.6       | 62.<br>0  |
| MEOHP                        | 96.6                      | <LO<br>D -<br>316       | 32.0     | 58.4       | 12<br>1.5 | 99.4                   | <LO<br>D-<br>300<br>0   | 33.9     | 51.5       | 87.4        | 100                   | 5.839<br>13-<br>193 | 39.8     | 66.4       | 93.<br>1  | 98.4            | <LO<br>D -<br>199       | 27.4     | 47.3       | 71.<br>9  | 98.9           | <LO<br>D -<br>205       | 28.4     | 44.6       | 66.<br>0  |
| MECPP                        | 93.1                      | <LO<br>D -<br>245       | 25.5     | 44.1       | 93.<br>2  | 92.7                   | <LO<br>D-<br>535<br>7   | 19.6     | 34.9       | 54.2        | 80.6                  | <LO<br>D -<br>127   | 10.7     | 20.9       | 39.<br>8  | 56.7            | <LO<br>D -<br>182       | <L<br>OD | 7.5        | 26.<br>7  | 57.6           | <LO<br>D -<br>419       | <L<br>OD | 7.5        | 27.<br>5  |
| Phthalate sums (µmol/L)      |                           |                         |          |            |           |                        |                         |          |            |             |                       |                     |          |            |           |                 |                         |          |            |           |                |                         |          |            |           |
| ΣDBP                         |                           | 0.02<br>-37             | 0.1      | 0.5        | 1.4       |                        | 0.02<br>-32             | 0.1      | 0.2        | 0.8         |                       | 0.02-<br>3          | 0.1      | 0.4        | 1.1       |                 | 0.02<br>-31             | 0.1      | 0.2        | 0.8       |                | 0.02<br>-9              | 0.1      | 0.3        | 1.0       |
| ΣDEHP                        |                           | 0.1-<br>2               | 0.3      | 0.5        | 0.7       |                        | 0.08<br>-29             | 0.3      | 0.4        | 0.7         |                       | 0.09-<br>3          | 0.3      | 0.4        | 0.7       |                 | 0.04<br>-2              | 0.2      | 0.3        | 0.6       |                | 0.06<br>-2              | 0.2      | 0.3        | 0.6       |

LOD: limit of detection

ΣDBP: sum of MnBP and MiBP; ΣDEHP: sum of MEHP, MEOHP, and MECPP

Table 3  
Basic characteristics of schoolchildren and their family members.

|                                | Preschool siblings<br>n=29 |          | School-children<br>n=178 |          | Older siblings<br>n=40 |          | Mothers<br>n=125 |          | Fathers<br>n=90 |          |
|--------------------------------|----------------------------|----------|--------------------------|----------|------------------------|----------|------------------|----------|-----------------|----------|
|                                | Median                     | Range    | Median                   | Range    | Median                 | Range    | Median           | Range    | Median          | Range    |
| Gender (male/ female)          | 15/14                      |          | 100/78                   |          | 19/21                  |          | 0/126            |          | 90/0            |          |
| Age                            | 5                          | 3-6      | 9                        | 7-12     | 15                     | 13-24    | 41               | 27-51    | 42              | 31-56    |
| Height (cm)                    | 108.6*                     | 94.8-121 | 135                      | 119-167  | 158                    | 147-170  | 158              | 147-170  | 170             | 155-187  |
| Weight (kg)                    | 18*                        | 14-24    | 30                       | 19-56    | 47.5                   | 34-87    | 52               | 41-82    | 68              | 52-90    |
| Creatinine in urine<br>(µg/mL) | 794                        | 283-1765 | 1031                     | 307-2754 | 1518                   | 274-4392 | 1300             | 344-3434 | 1662            | 630-3349 |

\*: values were obtained from data of the Ministry of Health, Labour and Welfare (2013). Table 2-6. Average body weight and height,  
<[http://www.mhlw.go.jp/toukei/youran/indexyk\\_2\\_1.html](http://www.mhlw.go.jp/toukei/youran/indexyk_2_1.html)>

Table 4

Spearman coefficients of the correlations of urinary phthalate metabolites and daily intake of phthalates between schoolchildren and their mothers or fathers.

| Phthalate metabolite (µg/L) |                   |                  | Daily intake of phthalate (µg/kg/day) |                   |                  |
|-----------------------------|-------------------|------------------|---------------------------------------|-------------------|------------------|
|                             | Mother<br>(n=125) | Father<br>(n=90) |                                       | Mother<br>(n=125) | Father<br>(n=90) |
| MnBP                        | 0.23**            | 0.08             | DnBP                                  | 0.21*             | 0.11             |
| MiBP                        | 0.25**            | 0.29**           | DiBP                                  | 0.27**            | 0.22*            |
| MBzP                        | 0.17*             | 0.40**           | BBzP                                  | 0.29**            | 0.41**           |
| MEHP                        | 0.60              | -0.04            |                                       |                   |                  |
| MEOHP                       | 0.25**            | 0.02             |                                       |                   |                  |
| MECPP                       | 0.38**            | 0.28**           |                                       |                   |                  |
| ΣDBP                        | 0.25**            | 0.29**           | ΣDBP                                  | 0.29**            | 0.22*            |
| ΣDEHP                       | 0.32**            | 0.05             | ΣDEHP                                 | 0.30**            | 0.02             |

Spearman's ρ

\*:  $p < 0.05$ ; \*\*:  $p < 0.01$

ΣDBP: sum of MnBP and MiBP; ΣDEHP: sum of MEHP, MEOHP, and MECPP.

Supplementary Table 1.

Distribution of urinary phthalate metabolite levels (creatinine-adjusted values) in schoolchildren and their family members.

|                                                           | Preschool siblings (n=29) |      |        |       | Schoolchildren (n=178) |      |        |       | Older siblings (n=40) |      |        |       | Mothers (n=125) |      |        |       | Fathers (n=90) |      |        |       |
|-----------------------------------------------------------|---------------------------|------|--------|-------|------------------------|------|--------|-------|-----------------------|------|--------|-------|-----------------|------|--------|-------|----------------|------|--------|-------|
|                                                           | Range                     | 25th | Median | 75th  | Range                  | 25th | Median | 75th  | Range                 | 25th | Median | 75th  | Range           | 25th | Median | 75th  | Range          | 25th | Median | 75th  |
| <i>Creatinine-adjusted phthalate metabolites (µg/gCr)</i> |                           |      |        |       |                        |      |        |       |                       |      |        |       |                 |      |        |       |                |      |        |       |
| MnBP                                                      | <LOD-105                  | <LOD | <LOD   | <LOD  | <LOD-38                | <LOD | <LOD   | 6.2   | <LOD-14               | <LOD | <LOD   | 4.3   | <LOD-31         | <LOD | <LOD   | 5.4   | <LOD-37        | <LOD | <LOD   | 6.3   |
| MiBP                                                      | 11.5-10636                | 28.1 | 95.8   | 586.3 | <LOD-1317              | 18.0 | 41.4   | 141.0 | <LOD-5958             | 20.6 | 48.6   | 108.6 | <LOD-8264       | 12.5 | 37.9   | 120.4 | <LOD-1281      | 8.1  | 36.1   | 161.4 |
| MBzP                                                      | <LOD-881                  | 12.2 | 24.3   | 38.3  | <LOD-860               | 7.5  | 15.0   | 31.5  | <LOD-223              | 3.6  | 11.6   | 30.6  | <LOD-309        | <LOD | 9.4    | 17.3  | <LOD-103       | <LOD | 6.9    | 14.5  |
| MEHP                                                      | <LOD-181                  | <LOD | 29.3   | 46.2  | <LOD-484               | 6.2  | 19.0   | 62.5  | <LOD-313              | 3.8  | 17.9   | 39.6  | <LOD-615        | 9.4  | 22.8   | 52.7  | <LOD-306       | <LOD | 12.7   | 47.2  |
| MEOHP                                                     | <LOD-524                  | 44.6 | 71.7   | 142.3 | <LOD-2147              | 34.1 | 50.5   | 85.4  | 10.3457-120           | 26.8 | 36.6   | 58.1  | <LOD-466        | 21.1 | 35.9   | 48.5  | <LOD-168       | 19.4 | 26.4   | 41.7  |
| MECPP                                                     | <LOD-251                  | 33.2 | 50.0   | 107.3 | <LOD-3833              | 19.0 | 30.4   | 50.5  | <LOD-73               | 6.3  | 16.1   | 25.6  | <LOD-151        | <LOD | 7.1    | 21.0  | <LOD-207       | <LOD | 5.1    | 15.5  |
| <i>Creatinine-adjusted sum of phthalates (µmol/gCr)</i>   |                           |      |        |       |                        |      |        |       |                       |      |        |       |                 |      |        |       |                |      |        |       |
| ΣDBP                                                      | 0.06-48                   | 0.1  | 0.5    | 2.6   | 0.03-59                | 0.1  | 0.2    | 0.7   | 0.03-27               | 0.1  | 0.3    | 0.5   | 0.02-37         | 0.1  | 0.2    | 0.6   | 0.02-6         | 0.1  | 0.2    | 0.7   |
| ΣDEHP                                                     | 0.14-3                    | 0.4  | 0.5    | 1.0   | 0.06-21                | 0.2  | 0.4    | 0.7   | 0.06-2                | 0.2  | 0.3    | 0.4   | 0.05-4          | 0.2  | 0.3    | 0.4   | 0.03-1         | 0.1  | 0.2    | 0.3   |

Cr: creatinine; LOD: limit of detection

ΣDBP: sum of MnBP and MiBP; ΣDEHP: sum of MEHP, MEOHP, and MECPP

Supplementary Table 2.

Comparisons of urinary phthalate metabolites ( $\mu\text{g/L}$  or  $\mu\text{mol/L}$ ) and daily phthalate intakes ( $\mu\text{g/kg/day}$ ) between mothers and fathers.

|                              | Mothers (n=125) |        |        | Fathers (n=90) |        |        | p value |
|------------------------------|-----------------|--------|--------|----------------|--------|--------|---------|
|                              | 25th            | Median | 75th   | 25th           | Median | 75th   |         |
| <i>Phthalate metabolites</i> |                 |        |        |                |        |        |         |
| MnBP                         | <LOD            | <LOD   | 6.20   | <LOD           | <LOD   | 8.13   | n.s     |
| MiBP                         | 16.86           | 44.87  | 172.04 | 13.94          | 52.56  | 271.99 | n.s     |
| MBzP                         | <LOD            | 11.41  | 23.57  | <LOD           | 13.38  | 22.70  | n.s     |
| MEHP                         | 10.43           | 28.60  | 60.82  | <LOD           | 24.80  | 63.28  | n.s     |
| MEOHP                        | 27.79           | 47.26  | 71.57  | 27.79          | 44.19  | 65.07  | n.s     |
| MECPP                        | <LOD            | 7.48   | 26.34  | <LOD           | 7.41   | 26.14  | n.s     |
| ΣDBP                         | 0.09            | 0.21   | 0.79   | 0.09           | 0.25   | 1.24   | n.s     |
| ΣDEHP                        | 0.20            | 0.34   | 0.59   | 0.22           | 0.32   | 0.55   | n.s     |
| <i>Daily intakes</i>         |                 |        |        |                |        |        |         |
| DnBP                         | 0.04            | 0.05   | 0.10   | 0.04           | 0.06   | 0.17   | n.s     |
| DiBP                         | 0.34            | 1.06   | 3.19   | 0.31           | 1.52   | 6.21   | n.s     |
| BBzP                         | 0.10            | 0.25   | 0.40   | 0.10           | 0.22   | 0.53   | n.s     |
| ΣDBP                         | 0.43            | 1.10   | 3.29   | 0.48           | 1.67   | 6.42   | n.s     |
| ΣDEHP                        | 2.41            | 4.10   | 5.99   | 2.60           | 4.16   | 7.10   | n.s     |

Mann-Whitney U-test

LOD: limit of detection

$\Sigma\text{DBP}$ : sum of MnBP and MiBP;  $\Sigma\text{DEHP}$ : sum of MEHP, MEOHP, and MECPP

Supplementary Table 3.

Comparisons of urinary phthalate metabolites ( $\mu\text{g/L}$  or  $\mu\text{mol/L}$ ) and daily phthalate intakes ( $\mu\text{g/kg/day}$ ) between boys and girls.

|                              | Boys (n=115) |        |        | Girls (n=92) |        |        | p value |
|------------------------------|--------------|--------|--------|--------------|--------|--------|---------|
|                              | 25th         | Median | 75th   | 25th         | Median | 75th   |         |
| <i>Phthalate metabolites</i> |              |        |        |              |        |        |         |
| MnBP                         | <LOD         | <LOD   | 6.86   | <LOD         | <LOD   | <LOD   | n.s     |
| MiBP                         | 20.38        | 46.57  | 148.20 | 17.97        | 53.37  | 187.31 | n.s     |
| MBzP                         | 10.23        | 19.14  | 39.54  | <LOD         | 14.94  | 26.87  | 0.007   |
| MEHP                         | 5.40         | 18.49  | 58.98  | 7.90         | 21.53  | 66.10  | n.s     |
| MEOHP                        | 38.13        | 63.57  | 90.33  | 33.12        | 48.13  | 81.83  | n.s     |
| MECPP                        | 19.16        | 35.15  | 55.51  | 15.22        | 28.17  | 49.95  | n.s     |
| Σ.sP                         | 0.11         | 0.23   | 0.69   | 0.09         | 0.25   | 0.85   | n.s     |
| Σ.sHP                        | 0.28         | 0.46   | 0.67   | 0.25         | 0.39   | 0.70   | n.s     |
| <i>Daily intakes</i>         |              |        |        |              |        |        |         |
| DnBP                         | 0.08         | 0.05   | 0.16   | 0.07         | 0.04   | 0.17   | n.s     |
| DiBP                         | 1.53         | 0.70   | 4.96   | 1.62         | 0.56   | 5.15   | n.s     |
| BBzP                         | 0.55         | 0.27   | 1.15   | 0.38         | 0.11   | 0.79   | 0.006   |
| Σ.00                         | 1.66         | 0.80   | 5.10   | 1.73         | 0.65   | 5.23   | n.s     |
| Σ.sHP                        | 8.90         | 4.86   | 14.34  | 7.12         | 4.23   | 12.42  | n.s     |

Mann-Whitney U-test

LOD: limit of detection

$\Sigma$ DBP: sum of MnBP and MiBP;  $\Sigma$ DEHP: sum of MEHP, MEOHP, and MECPP

Supplementary Table 4.

Spearman's correlations between levels of phthalates in house dust and levels of phthalate metabolites in urine.

| Dust                          | Metabolite/daily intake | School children | Mothers       | Fathers      |
|-------------------------------|-------------------------|-----------------|---------------|--------------|
| DnBP <sub>floor</sub>         | MnBP (µg/L)             | -0.03           | -0.12         | -0.02        |
|                               | ΣDBP (µmol/L)           | 0.04            | 0.00          | -0.03        |
|                               | DI MnBP (µg/kg/day)     | 0.03            | -0.02         | 0.04         |
|                               | DI ΣDBP (µg/kg/day)     | 0.05            | 0.05          | 0.00         |
| DnBP <sub>multi-surface</sub> | MnBP (µg/L)             | 0.03            | -0.04         | 0.14         |
|                               | ΣDBP (µmol/L)           | -0.04           | 0.05          | 0.13         |
|                               | DI MnBP (µg/kg/day)     | 0.03            | 0.06          | 0.09         |
|                               | DI ΣDBP (µg/kg/day)     | -0.03           | 0.10          | 0.12         |
| DiBP <sub>floor</sub>         | MiBP (µg/L)             | -0.08           | 0.00          | -0.01        |
|                               | ΣDBP (µmol/L)           | -0.09           | 0.02          | 0.00         |
|                               | DI MiBP (µg/kg/day)     | -0.04           | -0.03         | 0.06         |
|                               | DI ΣDBP (µg/kg/day)     | -0.04           | -0.02         | 0.08         |
| DiBP <sub>multi-surface</sub> | MiBP (µg/L)             | -0.10           | 0.01          | -0.07        |
|                               | ΣDBP (µmol/L)           | -0.11           | 0.01          | -0.08        |
|                               | DI MiBP (µg/kg/day)     | -0.09           | -0.01         | -0.04        |
|                               | DI ΣDBP (µg/kg/day)     | -0.10           | -0.01         | -0.04        |
| BBzP <sub>floor</sub>         | MBzP (µg/L)             | <b>0.30**</b>   | <b>0.22*</b>  | <b>0.22*</b> |
|                               | DI MBzP (µg/kg/day)     | <b>0.27**</b>   | <b>0.23**</b> | 0.17         |
| BBzP <sub>multi-surface</sub> | MBzP (µg/L)             | <b>0.17*</b>    | 0.11          | 0.07         |
|                               | DI MBzP (µg/kg/day)     | <b>0.19*</b>    | <b>0.18*</b>  | 0.04         |
| DEHP <sub>floor</sub>         | MEHP (µg/L)             | 0.14            | 0.13          | 0.14         |
|                               | MEOHP (µg/L)            | 0.08            | 0.01          | -0.04        |
|                               | MECPP (µg/L)            | <b>0.16*</b>    | 0.14          | 0.12         |
|                               | ΣDEHP (µmol/L)          | <b>0.17*</b>    | 0.10          | 0.05         |
|                               | DI ΣDEHP (µg/kg/day)    | <b>0.24**</b>   | 0.11          | 0.16         |
| DEHP <sub>multi-surface</sub> | MEHP (µg/L)             | 0.07            | -0.03         | 0.07         |
|                               | MEOHP (µg/L)            | 0.13            | 0.06          | 0.01         |
|                               | MECPP (µg/L)            | <b>0.23**</b>   | <b>0.21*</b>  | 0.01         |
|                               | ΣDEHP (µmol/L)          | <b>0.17*</b>    | 0.07          | -0.02        |
|                               | DI ΣDEHP (µg/kg/day)    | 0.11            | 0.03          | 0.07         |

Spearman's correlation; \*: p<0.05; \*\*: p<0.01

Σ DBP: sum of MnBP and MiBP; ΣDEHP: sum of MEHP, MEOHP, and MECPP.

Dust data has already reported in Ait Bamai et al., 2014

Preschool and older siblings are not shown here because of number of participants are no enough to calculate correlations.