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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 ΣDEHP. Regarding daily intakes, the schoolchildren had significantly higher daily intakes of DnBP, BBzP, and ΣDEHP. 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 μg/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.

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 TAI, 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.

Abbreviations: BBzP, benzyl butyl phthalate; BSTFA, *N,O*-bis(trimethylsilyl)-trifluoro acetamide; BW, body weight; CE, creatinine clearance rate; DBP, dibutyl phthalate; DEHP, di(2-ethylhexyl) phthalate; DiBP, di-iso-butyl phthalate; DiNP, di-iso-nonyl phthalate; DMP, dimethyl phthalate; DnBP, di-*n*-butyl phthalate; DI, daily intake; EPA, Environmental Protection Agency; GC/MS, gas chromatography/mass spectrometry; ISAAC, International Studies of Asthma and Allergies on Childhood; LOD, limit of detection; LOQ, limit of quantification; MBzP, mono-benzyl phthalate; MCNP, mono(carboxynonyl) phthalate; MCPP, mono(3-carboxypropyl) phthalate; MECPP, mono(2-ethyl-5-carboxypentyl) phthalate; MEHP, mono(2-ethylhexyl) phthalate; MEHHP, mono(2-ethyl-5-hydroxyl-hexyl) phthalate; MEOHP, mono(2-ethyl-5-oxohexyl) phthalate; MiBP, mono-isobutyl phthalate; MnBP, mono-*n*-butyl phthalate; MTBSTFA, *N*-methyl-*N*-(*tert*-butyldimethylsilyl)-trifluoroacetamide; M_{W_p}, molecular weights of parent phthalate; NHANES, National Health and Nutrition Examination Survey; PCP, personal care product; PVC, polyvinyl chloride; RfD, reference dose; SIM, selective ion mode; TDI, tolerable daily intake.

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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; USCPS Commission, 2008; Japan Ministry of Health Law, 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. (2008a,b) 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.

Materials and methods

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., 2013; 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.

Phthalate metabolites in urine

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 fluoric-tape, wrapped with aluminium foil, and kept at −20 °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).

Standards and reagents

Mono-*n*-butyl phthalate (MnBP), MiBP, mono(3-carboxypropyl) phthalate (MCPP), mono-benzyl phthalate (MBzP), MEHP, mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), and mono(2-ethyl-5-carboxypentyl) phthalate (MECPP) standards and MEHP-d4 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. β-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.

Sample preparation

For the sample preparation, 50 μL of acetic acid (1 M; pH 4.8), buffer, and 50 μL of MEHP-d_4 (2 μg/mL) were added to 0.5 mL of sample urine. Solutions were incubated with 10 μL of β-glucuronidase/arylsulphatase (30/60 unit) at 36 °C for 24 h. The mixture was extracted with 100 μL 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 °C for 1 h. The adherence of sample to the tube was resolved with 30 μL of ethyl acetate. The derivatisation processes

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