International Journal of Hygiene and Environmental Health 220 (2017) 869–879

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

Exposure sources and their relative contributions to urinary phthalate metabolites among children in Taiwan

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ARTICLE INFO

Article history:
Received 6 January 2017
Received in revised form 15 April 2017
Accepted 15 April 2017

Keywords:
Indoor environment
Lifestyle behavior
Mixed model
Plastic container or packaging
RAPIT

ABSTRACT

Phthalate exposure is omnipresent and known to have developmental and reproductive effects in children. The aim of this study was to determine the phthalate exposure sources and their relative contributions among children in Taiwan. During the first wave of the Risk Assessment of Phthalate Incident in Taiwan (RAPIT), in 2012, we measured 8 urinary phthalate metabolites in 226 children aged 1–11 years old and in 181 children from the same cohort for the wave 2 study in 2014. A two-stage statistical analysis approach was adopted. First, a stepwise regression model was used to screen 80 questions that explored the exposure frequency and lifestyle for potential associations. Second, the remaining questions with positive regression coefficients were grouped into the following 6 exposure categories: plastic container/packaging, food, indoor environment, personal care products, toys, and eating out. A mixed model was then applied to assess the relative contributions of these categories for each metabolite. The use of plastic container or food packaging were dominant exposure sources for mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), and mono-*n*-butyl phthalate (MnBP). The indoor environment was a major exposure source of mono-methyl phthalate (MMP), mono-benzyl phthalate (MBzP), and mono-isobutyl phthalate (MiBP). The consumption of seafood showed a significant correlation with MEHP. The children's modified dietary behavior and improved living environment in the second study wave were associated with lower phthalate metabolite levels, showing that phthalate exposures can be effectively reduced.

1. Introduction

Phthalates, a family of chemicals, are used as plasticizers in numerous commercial products that are ubiquitous in our daily living environment. Among these compounds, di-2-ethylhexyl phthalate (DEHP) and benzyl butyl phthalate (BzBP), which both have a high molecular weight, are the most frequently used plasticizers in polyvinyl chloride (PVC) production (Colacino et al., 2011; Guo et al., 2011). Other lower molecular weight phthalates, such as diethyl phthalate (DEP), dimethyl phthalate (DMP), and di-isobutyl phthalate (DiBP), are used in cosmetics, insecticides, and pharmaceutical products (Colacino et al., 2011; Guo et al., 2011; Watkins et al., 2014).

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http://dx.doi.org/10.1016/j.ijheh.2017.04.002
1438-4639/

Exposure to phthalates may occur through food ingestion, air inhalation, and direct dermal contact with various types of products, such as personal care products (Chen et al., 2008; Larsson et al., 2014; Schecter et al., 2013). The diet is reported to be a predominant exposure pathway (Chen et al., 2008), followed by dust ingestion and indoor air inhalation (Van Holderbeke, 2014). Phthalates can migrate into food through production, packaging, and preparation (Chen et al., 2008; Fierens et al., 2012; Schecter et al., 2013; Tsumura et al., 2001). To assess the exposure risk through dietary intake, the phthalate concentrations in various food samples from consumer markets have been widely studied (Chang et al., 2014; Sakhi et al., 2014; Schecter et al., 2013; Van Holderbeke et al., 2014). Phthalate migration due to food packaging and preparation were also reported (Chen et al., 2008; Fierens et al., 2012; Tsumura et al., 2001). The phthalate concentrations acquired through other exposure routes, such as indoor dust, have also been investigated (Bornehag et al., 2005; Hsu et al., 2011; Kolarik et al., 2008).

Measuring phthalate metabolites in voided urine illustrates the integrated exposure from various routes, and it is a well-accepted practice that is used to compare the extent of phthalate exposures in different populations across the US and European and Asian countries (Černá et al., 2015; Guo et al., 2011; Lorber et al., 2010; Wittassek et al., 2007; Wu et al., 2013). To determine the exposure sources of phthalate metabolites in urine, especially in children, questionnaires were used to assess the associations between food consumption habits, cooking practices, the use of personal care products, and the indoor living environment with urinary phthalate metabolites (Colacino et al., 2011; Larsson et al., 2014; Lewis et al., 2013; Watkins et al., 2014). However, while these studies identified significant associations between the dietary consumption and lifestyle behaviors with certain phthalate metabolites, the relative contributions and the variations in different exposure sources to specific phthalates is still unclear. Using repeated questionnaires on food intake, lifestyles, and living environments, we intended to identify the variability and relative contributions of different phthalate exposure sources among children in Taiwan.

2. Materials and methods

2.1. Study participants

The participating children were recruited for a follow-up prospective study known as the Risk Assessment of the Phthalate Incident in Taiwan (RAPIT) after an incident in 2011 (Yen et al., 2011; Wu et al., 2012). In May 2011, the phthalate DEHP and, to a lesser extent, di-isononyl phthalate (DiNP) were found to have been illegally used for many years as clouding agents in Taiwan. Numerous foods including sports drinks, juice beverages, tea drinks, fruit jam/nectar/jelly, and health or nutrient supplements were contaminated by the phthalate adulterants. Subjects who were suspected to have been highly exposed to the DEHP-contaminated foods were recruited for the RAPIT study from 3 participating hospitals located in northern, central, and southern Taiwan after the 2011 incident (Chen et al., 2016). A total of 226 children under 12 years of age underwent an exposure assessment questionnaire interview, blood and urine collection, and a physical examination during a clinical visit between August 2012 and February 2013 for the first wave of the study. Among the subjects, 181 (including one new recruit) participated in the second wave study of the RAPIT between July 2014 and February 2015. Because phthalates have a short half-life, and because a previous record of exposure factors may not appropriately contribute to the current metabolite levels, a cross-sectional method was adopted for the study design. Written informed consent on behalf of the participating children was obtained from their parents or caregivers. The study was approved by the Research Ethics Committee of the National Health Research Institutes of Taiwan (No. EC1000903).

2.2. Urinary sample collection and creatinine analysis

After informed consent was obtained, spot urine samples (50 ml) were collected using glass containers at the clinic, and the samples were transferred into an amber glass bottle, and stored at −80 °C. All the glass containers were prewashed with acetonitrile (ACN) and methanol and sealed with aluminum foil before sampling.

2.3. Analysis of urinary phthalate metabolites

We used a published method (Huang et al., 2015, 2016) to analyze the levels of urinary phthalate metabolites. The method involved the use of an isotope dilution standard and an online solid phase extraction coupled with liquid chromatography-electrospray ionization tandem mass spectrometry (LC-ESI–MS/MS) (Agilent 1200/API4000, Applied Biosystems, Foster City, CA, USA). Eight phthalate metabolites in the urine samples were analyzed to assess each child's current exposure status, namely mono-2-ethylhexyl phthalate (MEHP), mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP), mono-2-ethyl-5-oxohexyl phthalate (MEOHP), mono-methyl phthalate (MMP), mono-ethyl phthalate (MEP), mono-isobutyl phthalate (MiBP), mono-benzyl phthalate (MBzP), and mono-*n*-butyl phthalate (MnBP). After thawing and sonicating them for 10–15 min, the urine samples (100 μl) were briefly loaded into a glass vial (2 ml) that contained ammonium acetate (20 μl, Sigma Aldrich Laboratories, Inc., St. Louis, MO, USA), β-glucuronidase (10 μl, Escherichia coli-K12, Roche Biomedical, Mannheim, Germany), 11 mixed phthalate metabolite standards, and a mixture of ten isotopic ($^{13}C_4$) phthalate metabolite standards as the internal standards (100 μl, Cambridge Isotope Laboratories, Inc., Andover, MA, USA). After each sample was incubated (at 37 °C for 90 min), 270 μl of a solution (5% ACN, Merck, Darmstadt, Germany) containing 0.1% formic acid (FA, Merck, Darmstadt, Germany) was added, and the samples were vortexed and sealed with a PTFE cap for analysis. Two columns were used in our on-line system as follows: one C18 column (Inertsil ODS-3, 33*4.6 mm, 5 μm, GL Science, Tokyo, Japan) for the extraction and clean-up of the collected samples, and one analytical column (Inertsil Ph, 150 *4.6 mm, 5 μm, GL Science, Tokyo, Japan) for separating the different phthalate metabolites. We used a negative, multiple reaction monitoring model for mass detection. The ion pairs of each phthalate metabolite are listed as follows: MMP (179/107), MEP (193/121), MnBP (221/71), MiBP (221/71), MBzP (255/183), MEHP (277/134), MEHHP (293/121), and MEOHP (291/143), with a limit of detection (LOD) for the MMP, MEP, MnBP, MiBP, MBzP, MEHP, MEHHP, and MEOHP of 0.12, 0.12, 0.12, 0.24, 0.12, 0.24, 0.12, and 0.24 ng/mL, respectively. One blank, one repeat and one quality control (QC) sample were included in each batch of analyzed samples. The concentration of blank samples was required to be less than twice the method detection limit. The QC sample was spiked in pooled urine samples, which contained a mixture of phthalate metabolite standards (20–50 ng/mL) in each sample. The relative percent difference for the repeat sample and the recovery of each QC sample was below ±30%. In addition, the quality of the MEHHP, MEOHP, MnBP, MiBP and MBzP measures was certified by an international laboratory comparison program (The German External Quality Assessment Scheme, G-EQUAS 52, Erlangen, Germany).