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# Phthalate levels and related factors in children aged 6–12 years☆

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ABSTRACT

Although previous studies showed that children are widely exposed to phthalates, the sources of phthalate exposure for school-aged children in China are not well understood. This study aimed to assess phthalate metabolite levels and explore the factors influencing exposure in children. We collected demographic data and biological samples from 336 children aged 6–12 years. We calculated urinary concentrations of 14 mono-phthalate metabolites and conducted chi-square ($\chi^2$) tests and logistic regression analysis to determine the variables associated with phthalate levels. Mono-*n*-butyl phthalate (MnBP) and mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) were the most abundant urinary phthalate metabolites. In addition, housing type, decorating materials in the home, and frequency of canned food consumption were associated with exposure to low molecular weight phthalates. Water source, duration of time spent playing with toys, residential area, and frequency of canned food consumption were associated with exposure to high molecular weight phthalates. Based on these results, potential strategies to reduce exposure to phthalates include avoiding plastic food containers and chemical fragrances as well as eating fewer processed foods, especially canned foods, and foods in plastic packaging.



## 1. Introduction

Phthalate esters (PAEs), which are synthetic chemicals, are ubiquitous environmental contaminants (Myridakis et al., 2015). As such, they have been identified as global pollutants and have received increasing public attention in recent years (Liu et al., 2016). Many consumer products contain specific members of this family of chemicals (Zhang et al., 2014), including cosmetics, clothing, nutritional supplements, pharmaceuticals, dentures, medical devices, children's toys, glow sticks, modeling clay, automobiles, food packaging, cleaning materials, waxes, insecticides, and lubricants (Johns et al., 2015; Kim et al., 2011). In general, high molecular weight phthalates (HMWP; metabolites >250 Da), such as di-2-ethylhexyl phthalate (DEHP), benzylbutyl phthalate (BzBP), di-isononyl phthalate (DiNP), and di-*n*-octyl phthalate (DnOP), are primarily used in the production of polyvinyl chloride (PVC) (Smarr et al., 2015; Tellez-Rojo et al., 2013). Low molecular weight phthalates (LMWP), such as dimethyl phthalate (DMP), diethyl phthalate (DEP), and dibutyl phthalates (DBP), are often used in personal care products (Specht et al., 2014).

Because phthalate plasticizers are not chemically bound to PVC, they can leach, migrate, or evaporate into indoor air and the atmosphere, foodstuffs, and other materials (Upson et al., 2013; Van Holderbeke et al., 2014). Therefore, phthalates are detected not only in consumer products but also in food and the indoor environment, including the air and dust (Van Holderbeke et al., 2014; Wang et al., 2013). As a result, humans are exposed through ingestion, inhalation, and dermal exposure throughout their entire lifetime, including during intrauterine development (Sakhi et al., 2014). Children, especially those of school age, are exposed to phthalates more extensively and at higher levels than adults (Shi et al., 2015).

Phthalate diesters are rapidly metabolized after exposure, and metabolites of phthalate compounds are found almost universally in human urine and have been detected in amniotic fluid (Zhu et al., 2016). Higher urinary phthalate metabolite levels are generally measured in children than in adolescents and adults, which could

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be related with more frequent exposure to toys and canned food containing phthalates (Gao et al., 2016). In addition, the mouthing behaviors of infants and children can lead to additional phthalate intake. Past human studies have linked early-life phthalate exposure with altered neurological development, childhood allergies, and decreased anogenital distance in boys (Shi et al., 2012). Several phthalate metabolites exhibit anti-androgenic activity, and there is evidence that some developmental endpoints vary by sex (Chen et al., 2013). Phthalate exposure in early development and even preconception can adversely affect an individual's health long after childhood.

Therefore, exposure to PAEs during childhood development affects the health of not only children but also the entire society (Mieritz et al., 2012; Mouritsen et al., 2013). However, there has been very little research on phthalate levels and related factors in China, and research is urgently needed. The specific aims of the present study were to assess the exposure to PAEs, by measuring their metabolites in urine samples from children aged 6–12 years, and to investigate the factors that influence phthalate exposure in children.

## 2. Materials and methods

### 2.1. Study population

In this cross-sectional study, data were collected from January 2014 to July 2014 in Shiyan (101° 79′ N, 32° 65′ E) western Hubei Province, central China. Children aged 6–12 years, who were residents of the region and attended school, underwent thorough clinical examinations. Children with liver disease, blood disorders, inherited diseases, or hormone diseases were excluded. The Human Ethical Committee of the National Health Research Institutes in China approved the study. Each of the participants' parents/guardians provided written, informed consent at enrollment.

### 2.2. Urine sampling

Each child collected the total volume of their first morning urinary void in 750 mL polyethylene containers, which were prewashed in 10% nitric acid (>3 h) and rinsed twice in purified water. At the schools, field workers stored the urine samples in a cooler (4 °C) and checked the questionnaires for missing answers. The filled urine container was weighed in the laboratory, and two 2-mL urine samples were transanalytes. None of the containers showed contamination during the washing procedure or from excretion from the container material.

### 2.3. Survey instrument

We used a self-administered questionnaire, which was developed based on a previous questionnaire used in the US, Peru, and Congo and further adapted to the setting in China (Bai et al., 2016). Prior to use, the questionnaire was reviewed by a team of six child health experts to assess the relevance and wording of the questions as well as accuracy of the translation into Chinese. Then, the questionnaire was pilot tested with 20 children to ensure that the questions were clear and understandable to all participants. A strict and standardized quality assurance/quality control procedure was used during the entire process.

The questionnaire was divided into three sections: socio-demographic information, living conditions, and children's lifestyle. Socio-demographic information included gender (boy or girl), residential area (urban or rural), age, maternal education (college degree, high school, or middle school or below), paternal education, maternal occupation (farmer, worker, professional), paternal occupation (farmer, worker, professional), maternal use of hair dye in the home (yes or no), parental smoking inside (none, 1–10, or >10 cigarettes), and total annual household income (<$3,000, $3000–8,000, or >$8000). Living conditions included housing type (roughcast houses, brick-wood structure, or reinforced concrete), domestic fuel type (wood, coal, or gas), decorating materials (bricks, wall paints, or tiles), residence close to the road (yes or no), and average housing area (<10, ≥10 to <30, or ≥30 $m^2$). Children's lifestyle information included outside activity duration (<1, ≥1 to <2, or ≥2 h), duration of time playing with toys (<1, ≥1 to <2, or ≥ 2 h), frequency of dairy product consumption (1–2 times a month, 1–2 times a week, or 1–2 times a day), frequency of puffed food consumption (1–2 times a month, 1–2 times a week, or 1–2 times a day), frequency of canned food consumption (1–2 times a month, 1–2 times a week, or 1–2 times a day), frequency of wrapping food in plastic (none, sometimes, or frequently), and water source (tap water, well water, or pond water).

Considering that children stay with their parents, we went to each selected home several times to ensure that all eligible children from the school had the opportunity to be invited to participate in the survey, with the aim of collecting more representative data. Questionnaires were distributed to the children by postgraduate students. To accurately assess the factors related with children's phthalate levels, participants and their parents were asked to respond without referring to the literature or consulting others. Additionally, children and their parents were asked to provide written commitment not to disclose the questions to their neighbors when they signed the written informed consent.

### 2.4. Exposure assessment

Urinary phthalate and creatinine concentrations were measured at the Department of Institute of Clinical Medicine, Renmin Hospital, Hubei University of Medicine, Shiyan.

The following phthalate metabolites were analyzed using liquid chromatography tandem mass spectrometry (LC-MS/MS): monomethyl phthalate (MMP), monoethyl phthalate (MEP), monoisobutyl phthalate (MiBP), mono-*n*-butyl phthalate (MnBP), monobenzyl phthalate (MBzP), mono(carboxyoctyl) phthalate (MCOP), mono-(3-carboxypropyl) phthalate (MCPP), mono-*n*-octyl phthalate (MOP), mono-cyclohexyl phthalate (MCHP), mono-isononyl phthalate (MiNP), mono (2-ethylhexyl) phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), and mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP). Mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP) was analyzed using isotope dilution LC-MS/MS with preceding enzymatic deconjugation followed by automatic solid-phase extraction. The following solvents were used for LC separation: A, 0.1% acetic acid in acetonitrile and B, 0.1% acetic acid in water. Solvent programming involved 0.0–2.0 min, 10% B; 17.0 min, 25% B; 21 min, 30% B; 23 min, 60% B; 25 min, 70% B; 27 min, 90% B; and 32 min, 10% B. For all analytes, good separation was obtained with a retention time on the column of 6.68–27.22 min. The analysis quality was checked using chemical blank samples and an in-house quality control in all of the sample batches that were analyzed. The inter-day variation, expressed as the relative standard deviation (RSD), was <10.0% for all analytes except MOP (15.2%) and MiNP (13.8%), and the recovery of spiked samples was >90.0% for all analytes except MiNP (85.6%), MOP (80.6%), and MCHP (78.6%). Values for metabolites at levels less than the limit of detection (LOD) were replaced with the LOD divided by the square root of two (Wolff et al., 2010).

Urinary creatinine was determined with a creatinine kit (UCR ELISA Kit, Shanghai, China) using an automatic biochemical analyzer (ClinitekStatus, SIEMENS, Germany). Individual and summed metabolite concentrations were divided by urinary creatinine

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