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# Exposure to phthalates in children aged 5–7 years: Associations with thyroid function and insulin-like growth factors

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## HIGHLIGHTS

- We calculated urine mPAE concentrations separately for children from urban and rural areas.
- Phthalate exposure in children of 5–7 years has an adverse biological effect.
- Some phthalates might interfere with thyroid hormones and growth.

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## ABSTRACT

This study aimed to evaluate the associations between phthalate concentrations and thyroid function in preschool children. We collected demographic data and biological samples from 216 children aged 5–7 years. We calculated urinary concentrations of eight mono-phthalate metabolites (mPAEs) separately for children from urban and rural areas and investigated their associations with thyroid function and growth hormones. mPAE concentrations were higher in children from the urban area than in those from the rural area, and most mPAEs were positively associated with free triiodothyronine and free thyroxine. The insulin-like growth factor 1 (IGF-1) concentration decreased 0.082 ng/mL (95% confidence interval [CI]: −1.34, −0.113) with each 1 ng/mL increase in monomethyl phthalate (MMP) and 0.132 ng/mL (95% CI: −0.209, −0.055) with each 1 ng/mL increase in mono-*n*-butyl phthalate. The insulin-like growth factor binding protein 3 concentration decreased by 0.01 mg/L (95% CI: −0.001, −0.000) or 0.01 mg/L (95% CI: −0.003, −0.000) with each 1 ng/mL increase in MMP or monoethyl phthalate, respectively. Exposure to some phthalates at 5–7 years of age might interfere with thyroid hormones and growth.



## 1. Introduction

Phthalates, or phthalate esters (PAEs), are used as plasticizers in polyvinyl chloride plastics (Adibi et al., 2003). Specific members of this family of chemicals are present in many consumer products, including cosmetics, building materials, clothing, household furnishings, children's toys, modeling clay, automobiles, food packaging, and cleaning materials (Wittassek and Angerer, 2008). As PAEs are physically bound to polymer chains, they can be released into the environment by leaching, evaporation, and abrasion or through the application of phthalate-containing personal care products (Guo et al., 2012; Guo et al., 2014). Lifetime exposure in humans, even during intrauterine development, occurs through ingestion, inhalation, and the dermis (Wittassek et al., 2011; Wu et al., 2015). Phthalate exposure might affect neurodevelopment (Kobrosly et al., 2014), behavior (Tellez-Rojo et al., 2013), and asthma (Bertelsen et al., 2013), and monoethyl phthalate

*Abbreviations:* PAEs, phthalate esters; mPAEs, mono-phthalate metabolite; DMP, dimethyl phthalate; DEP, di-ethyl-phthalate; DBP, di-*n*-butyl phthalate; DnOP, di-*n*-octyl-phthalate; BBzP, butyl benzyl phthalate; DEHP, di(2-ethylhexyl)phthalate; MMP, monomethyl phthalate; MEP, monoethyl phthalate; MBP, mono-*n*-butyl phthalate; MOP, mono-isobutyl phthalate; MBzP, monobenzyl phthalate; MEHP, mono (2-ethylhexyl) phthalate; MEHHP, 2-ethyl-5-hydroxy-hexyl-phthalate; MEOHP, 2-ethyl-5-oxyhexyl phthalate; IGF-1, insulin-like growth factor 1; $FT_3$, free triiodothyronine; $TT_3$, total triiodothyronine; $TT_4$, total thyroxine; $FT_4$, free thyroxine; TSH, thyroid stimulating hormone; TG, thyroglobulin; IGFBP-3, insulin-like growth factor binding protein 3; TR, thyroid receptor; GH, growth hormone; HPLC-MS, high-performance liquid chromatography and mass spectrometry; RSD, relative standard deviation; CVs, coefficients of variation; LOD, limit of detection; ANOVA, analysis of variance; CI, confidence interval; SD, standard deviation; IQR, interquartile range; WGOC, Working Group on Obesity in China.

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(MEP) specifically has been associated with an increased risk of breast cancer (Lopez-Carrillo et al., 2010).

Normal thyroid function is important for growth and neurological development in children, and hypothyroidism in childhood is accompanied by growth retardation (Shi et al., 2012). Recent studies of animals and *in vitro* screening assays provide evidence that phthalate exposure might affect thyroid signaling through a number of potential mechanisms (Hu et al., 2013; Shi et al., 2012). Among 845 children aged 4–9 years, phthalate metabolites were negatively associated with serum free triiodothyronine ($FT_3$) and total triiodothyronine ($TT_3$) concentrations (Boas et al., 2010). In a sample of 1675 participants, urinary di(2-ethylhexyl)phthalate (DEHP) metabolite concentrations were inversely associated with total thyroxine ($TT_4$) and free thyroxine ($FT_4$) concentrations in adults (age, ≥20 years), but were positively associated with $TT_3$ and thyroid stimulating hormone (TSH) concentrations among adolescents (Axelsson et al., 2015).

Insulin growth factor (IGF)-I is the primary mediator of the growth effects of growth hormone and is thought to affect growth, health, and disease following fetal development (Boas et al., 2010). Insulin-like growth factor binding protein-3 (IGFBP-3) is the primary binding protein of IGF-1 and regulates the mitogenic and anti-apoptotic actions of IGFs. IGF-I and IGFBP levels are altered in patients with obesity and glucose intolerance, including diabetes mellitus (Shen et al., 2015). However, only one study conducted in Denmark investigated the relationship between phthalate exposure and IGFs in humans (Boas et al., 2010). Furthermore, children experience rapid growth and development and are more susceptible to external disturbances than adults.

Industrial activities can lead to elevated levels of PAEs in soil (Kong et al., 2012; Lan et al., 2012); however, few studies have analyzed PAE levels in humans in urban areas, compared with rural areas. In addition, environmental exposure in early development can adversely affect an individual's health long after childhood. Moreover, children are exposed to phthalates more extensively and at higher levels than adults (Guo et al., 2011b; Trasande et al., 2013). In this study, we aimed to assess phthalate metabolite levels in children from urban and rural areas. Furthermore, we investigated the associations with growth as well as thyroid hormone, IGF-1, and IGFBP-3 concentrations.

## 2. Methods

### 2.1. Study population

Our study was conducted in Xiangyang (population approximately 5.5 million), which covers 197,000 $km^2$ in western Hubei Province, central China and has a number of electronic manufacturing facilities. It was considered an urban area for the present study. For comparison, data were collected for 113 children from a rural area located 10 km from an urban area.

Children aged 5–7 years who underwent thorough clinical examinations between March 2013 and May 2013 were included when they were permanent residents of the region and attending school. Children with liver disease, a blood disorder, an inherited disease, or a disease requiring hormones were excluded. The Human Ethical Committee of the National Health Research Institutes in China approved the study. Each of the participants signed informed consent at enrollment.

### 2.2. Phthalate metabolite measurements

Each child collected a first morning urine sample in a polyethylene container on the day of examination. All specimens were collected with glass devices to avoid contamination and stored at −20 °C until analysis. We analyzed 8 metabolites from 6 different phthalate diesters: monomethyl phthalate (MMP) from dimethyl phthalate (DMP); MEP from di-ethyl-phthalate (DEP); mono-n-butyl phthalate (MBP) from di-n-butyl phthalate (DBP); mono-isobutyl phthalate (MOP) from di-n-octyl-phthalate (DnOP); monobenzyl phthalate (MBzP) from butyl benzyl phthalate (BBzP); and mono (2-ethylhexyl) phthalate (MEHP) from DEHP. Analyses also included the two secondary oxidized metabolites of DEHP: 2-ethyl-5-hydroxy-hexyl-phthalate (MEHHP) and 2-ethyl-5-oxyhexyl phthalate (MEOHP).

Metabolites were analyzed at the MOE Key Laboratory of Environment and Health, School of Public Health, Huazhong University of Science and Technology, Wuhan using a liquid chromatography-tandem mass spectrometry system (LC-MS/MS; Agilent, American) and the method for urinary phthalates described by Specht et al. (2015), with some modifications. In brief, the urine was added to labeled internal standards for all of the metabolites and glucoronidase to remove glucoronic acid. The proteins were precipitated using organic solvent, the samples centrifuged, and the supernatant injected into the LC-MS/MS equipment. For all analytes, good separation was obtained with a retention time on the column of 6.68–27.2 min. The calibration curve range was 0.100–200 ng/mL. The analysis quality was checked using chemical blank samples and an in-house quality control in all of the sample batches that were analyzed. Moreover, each sample was analyzed three times in three different analytical batches. The imprecision in the analyzed control sample was 8.17% for MMP, 11.0% for MEP, 15.9% for MEHHP, 13.2% for MEOHP, 11.4% for MBP, 6.68% for MBzP, 10.6% for MEHP, and 6.84% for MOP (Supplemental Table 1).

Urinary mPAE concentrations were corrected for urine dilution using specific gravity (SG): $Pc = P\,[(1.014–1)\,/\,(SG - 1)]$, where Pc is the specific gravity-corrected phthalate metabolite concentration (μg/L), P is the experimental phthalate metabolite concentration, 1.014 is the median SG value among the present study population, and SG is the specific gravity of the individual urine sample. SG was measured using a handheld refractometer (PAL10-S; Atago, Tokyo, Japan).

### 2.3. Hormone analyses

Nonfasting peripheral venous blood samples were drawn from an antecubital vein between 8:00 and 10:00 AM. They were clotted and centrifuged, and serum was stored at 20 °C until hormone analyses were performed. One batch of serum was used to measure thyroid hormone concentrations, including $FT_3$, $TT_3$, $FT_4$, $TT_4$, TSH, and thyroglobulin (TG), using various commercial immune enzymatic assay kits (AMEKO; Shanghai, China). The intraassay coefficients of variation (CVs) of these measures were all <6%, and the interassay CVs were all <10%. IGF-1 and IGFBP-3 were measured with solid-phase enzyme-labeled chemiluminescent immunometric assays (Immulite 2000, Siemens, Germany) and reference reagent (IMMULITE2000, IGF-1; IMMULITE2000, IGFBP-3). The intraassay CVs were <2.31% and 4.52%, respectively, and the interassay CVs were <7.32% and 7.83%, respectively.

### 2.4. Statistical analysis

The distributions of all 8 mono-phthalate metabolite ester (mPAE) concentrations were skewed: therefore, we report their values as medians and the 5th, 25th, 50th, 75th, and 95th percentiles. For mPAE concentrations below the limit of detection (LOD), we used an imputed value equal to LOD/√2. mPAE concentrations were compared between urban and rural areas using analysis of variance (ANOVA). Correlations between the individual mPAEs were evaluated using Spearman rank correlation coefficients ($r_S$) (Bertelsen et al., 2013).

The thyroid hormones and IGF-1 concentrations were also skewed. When analyzing the associations between mPAEs and other hormones, we combined the urban and rural areas.

In the linear regression models, thyroid hormone, IGF-1, and IGFBP-3 concentrations were the dependent variables, and mPAE concentration was the continuous independent variables. Thus, the linear coefficient (β) corresponds with a unit change in thyroid hormone, IGF-1, or IGFBP-3 concentration, with a 1-unit (ng/mL) increase in mPAE. We