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# Does exposure to phthalates influence thyroid function and growth hormone homeostasis? The Taiwan Environmental Survey for Toxicants (TEST) 2013

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ABSTRACT

*Background:* Previous epidemiologic and toxicological studies provide some inconsistent evidence that exposure to phthalates may affect thyroid function and growth hormone homeostasis.
*Objective:* To assess the relations between exposure to phthalates and indicators of thyroid function and growth hormone homeostasis disturbances both among adults and minors.
*Methods:* We conducted a population-based cross-sectional study of 279 Taiwanese adults (≥18 years old) and 79 minors (< 18 years old) in 2013. Exposure assessment was based on urinary biomarkers, 11 phthalate metabolites measured by using online liquid chromatography/tandem mass spectrometry. Indicators of thyroid function included serum levels of thyroxine ($T_4$), free $T_4$, triiodothyronine, thyroid-stimulating hormone, and thyroxine-binding globulin (TBG). Growth hormone homeostasis was measured as the serum levels of insulin-like growth factor 1 (IGF-1) and insulin-like growth factor binding protein 3 (IGFBP3). We applied multivariate linear regression models to examine these associations after adjusting for covariates.
*Results:* Among adults, serum $T_4$ levels were negatively associated with urinary mono-(2-ethyl-5-hydroxyhexyl) phthalate ($\beta$=−0.028, $P$=0.043) and the sum of urinary di-(2-ethylhexyl) phthalate (DEHP) metabolite ($\beta$=−0.045, $P$=0.017) levels. Free $T_4$ levels were negatively associated with urinary mono-ethylhexyl phthalate (MEHP) ($\beta$=−0.013, $P$=0.042) and mono-(2-ethyl-5-oxohexyl) phthalate ($\beta$=−0.030, $P$=0.003) levels, but positively associated with urinary monoethyl phthalate ($\beta$=0.014, $P$=0.037) after adjustment for age, BMI, gender, urinary creatinine levels, and TBG levels. Postive associations between urinary MEHP levels and IGF-1 levels ($\beta$=0.033, $P$=0.006) were observed. Among minors, free $T_4$ was positively associated with urinary mono benzyl phthalate levels ($\beta$=0.044, $P$=0.001), and IGF-1 levels were negatively associated with the sum of urinary DEHP metabolite levels ($\beta$=−0.166, $P$=0.041) after adjustment for significant covariance and IGFBP3.
*Conclusions:* Our results are consistent with the hypothesis that exposure to phthalates influences thyroid function and growth hormone homeostasis.

## 1. Introduction

Phthalates are a family of industrial chemicals widely used as plasticizers and softeners in various commercial products including food packaging materials, medical equipment, toys, furniture, and cosmetics (Koch and Calafat, 2009). Routes of exposure to phthalates includes ingestion, dermal contact, and inhalation. Phthalates are rapidly metabolized to their respective monoesters and subsequent oxidative metabolites, which are excreted in the urine and feces (Dirven et al., 1993; Schmid and Schlatter, 1985). Urinary phthalate metabo-

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lites are broadly used as biomarkers of phthalate exposure in humans (Koch and Calafat, 2009; Wittassek et al., 2007). In 2011, a food scandal involving DEHP-tainted products (such as DEHP, DnBP and di-i-butyl phthalate (DiBP) in beverage, food and nutrition supplements) occurred in Taiwan (Wu et al., 2012). Most contaminant products were removed and immediate regulation was activated within two months. However, the phthalate exposure levels in Taiwanese people significantly decreased after this episode according to our biomonitoring survey (Huang et al., 2015). Whether actual phthalate exposure levels cause adverse health effects in the general Taiwanese population is still unknown.

Thyroid hormones are essential for many physiological processes, including fetal and child growth and development, energy balance, metabolism, and other functions of the nervous, cardiovascular, and reproductive systems of children and adults (Diamanti-Kandarakis et al., 2009; Miller et al., 2009). Recently, experimental studies have demonstrated that exposure to phthalates such as DEHP can affect thyroid signaling through numerous potential mechanisms such as sodium-iodide symporter (NIS) and growth (Boas et al., 2006; Liu et al., 2015). Information on phthalate exposure and thyroid function, as well as insulin-like growth factor 1 (IGF-1), in human studies is limited. Previous studies have indicated inverse relationships between urinary DEHP metabolites and serum thyroxine ($T_4$), free $T_4$, and triiodothyronine ($T_3$) in adults, despite a positive correlation with thyroid-stimulating hormone (TSH) (Meeker et al., 2007; Meeker and Ferguson, 2011). Some studies have revealed that urinary DEHP or DBP metabolites are negatively associated with serum $T_3$, free $T_3$, and IGF-1 in minors (Boas et al., 2010) as well as TSH in a DEHP-tainted child group (Wu et al., 2013); by contrast, other studies have reported a positive relationship between DEHP metabolites and $T_3$ (Meeker and Ferguson, 2011). However, the effects of exposure to phthalates on thyroid hormones and IGF-1 levels in adults and children are unclear, and the results remain inconsistent. Therefore, the purpose of the present study was to explore the relationships between exposure to phthalates and serum thyroid function and IGF-1 levels in Taiwanese adults and minors.

## 2. Methods

### 2.1. Study population

The source population comprised the general Taiwanese population. The study population was selected based on the sampling frame and procedures used in recruitment for the Nutrition and Health Survey in Taiwan (NAHSIT) (Pan et al., 2011). All detailed sampling procedures have been described in our previous studies (Huang et al., 2015). In brief, according to the population density and urbanization of each city in Taiwan, we selected seventeen townships of eleven cities or counties in the northern region (ex.: Taipei and New Taipei City), central region (ex.: Taichung and Chia-Yi City), southern region (ex.: Kaohsiung City), eastern region (ex.: Hua-Lien County) and remote island region (Peng-Hu County) of Taiwan between May 2013 and December 2013. All participants were required to be Taiwanese, aged 7 years or older, excluding pregnant and breast-feeding women, individuals with severe disease (ex.: cancer patients), foreigners, and citizens in hospitals or jails. A total of 500 participants were interviewed on the day of the health examination at a community center or elementary school; 394 individuals participated in this study (a response rate of nearly 78%). We excluded 36 participants who provided no blood samples. Ultimately, the study population comprised 279 adults and 79 minors. An interviewer-administered questionnaire was also used to obtain information regarding individual characteristics (age, gender, residence, and education), health, environmental exposures (cigarette smoking and insecticide usage), and lifestyle (plastic product and personal care products usage). This study was approved by the Research Ethics Committee of the National Health Research Institutes (No. EC1020206) in Taiwan. Written informed consent from each participant and additional signatures from the parents of minors were obtained prior to study enrollment.

### 2.2. Exposure assessment

Assessment of exposure to 7 commonly used phthalates, DEHP, DnBP, DiBP, DEP, di-isononyl phthalate (DiNP), benzyl butyl phthalates (BBzP), and dimethyl phthalate (DMP), were based on the levels of biomarkers i.e. metabolites of these phthalates in urine. A first-morning urine sample (20 mL) was collected from each participant by using a PP container, transferred to an acetonitrile-prewashed amber glass bottle and stored at −80 °C. We quantified 11 urinary phthalate metabolites including mono-ethylhexyl phthalate (MEHP), mono-(2-ethyl-5-oxo-hexyl) phthalate (MEOHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-(2-carboxymethylhexyl) phthalate (MCMHP), mono-*n*-butyl phthalate (MnBP), mono-iso-butyl phthalate (MiBP), mono-ethyl phthalate (MEP), mono-isononyl phthalate (MiNP), mono-benzyl phthalate (MBzP), and mono-methyl phthalate (MMP). The concentrations of the phthalate metabolites were determined using online liquid chromatography/tandem mass spectrometry (Agilent 1200/API 4000, Applied Biosystems, Foster City, CA, USA), as in the Taiwan Environmental Survey for Toxicants (TESTs) study (Huang et al., 2015). The urinary concentrations of the 11 phthalate metabolites were determined as ng/mL and μg/g creatinine. The molar sum (nmole/mL or μmole/g creatinine) of the DEHP metabolites (ΣDEHPm) was calculated by adding the molar concentrations of 5 metabolites: MEHP, MEHHP, MEOHP, MECPP, and MCMHP. The molar sum (nmole/mL or μmole/g creatinine) of the DBP metabolites (ΣDBPm) was calculated by adding the molar concentrations of 2 metabolites: MiBP and MnBP. The limit of detection (LOD) for MEHP, MEOHP, MEHHP, MECPP, MCMHP, MnBP, MiBP, MEP, MiNP, MBzP, and MMP were 0.7, 0.3, 0.3, 0.3, 0.1, 1, 1, 0.3, 0.1, 0.3, and 0.3 ng/mL, respectively. When the phthalate metabolite levels were lower than the LOD, we calculated our data as half of the LOD value. One blank, repeated quality control (QC) sample was included in each batch of analyzed samples. Concentrations of blank samples was to be less than 2 fold the method detection limit. The QC sample was spiked in pooled urine samples with a mixture of phthalate metabolite standards (20–50 ng/mL) in each sample. The relative percent difference for the repeated sample, as well as recovery of the QC sample, was to be less than ± 30%. Urinary creatinine levels were measured by spectrophotometric methods, with picric acid as a reactive compound for reading at 520 nm measurement (Beckman; DXC 800).

### 2.3. Outcome assessment

The outcomes of interest were thyroid function and growth hormone homeostatsis. Thyroid function was measured as the serum concentrations of thyroxine ($T_4$), free $T_4$, triiodothyronine ($T_3$), thyroid-stimulating hormone (TSH), and thyroxine-binding globulin (TBG). Growth hormone homeostasis was measured as the serum levels of insulin-like growth factor 1 (IGF-1) and insulin-like growth factor binding protein 3 (IGFBP3). The morning blood sample from each participant was collected and immediately centrifuged for 20 min at 4 °C, and then stored at −80 °C until analysis. All analyses were carried out blinded for the technician and in random order, and analyzed by a Taiwan Accreditation Foundation-certified laboratory (No. 1447 and 1673), recognized by the International Laboratory Accreditation Cooperation Mutual Recognition Arrangement (Huang et al., 2016a, 2016b). The serum levels of $T_4$, $T_3$, free $T_4$, and TSH were quantified using a chemiluminescent microparticle immunoassay on Beckman Coulter's UniCel (R) DxI 800 Immunoassay System (Beckman Coulter Inc., Brea, CA, USA). The assay sensitivities for $T_3$, $T_4$, free $T_4$, and TSH were 0.25 ng/mL, 1.0 μg/dL, 0.4 ng/dL, and