Does Exposure to Phthalates Influence Thyroid Function and Growth Hormone Homeostasis? The Taiwan Environmental Survey for Toxicants (TEST) 2013

Han-Bin Huang[1], Wen-Harn Pan[2,3], Wan-Ting Chang [4], Yue-Liang Guo[4,5], Jouni J. K. Jaakkola[6,7], Po-Chin Huang[4,8,*]

[1] School of Public Health, National Defense Medical Center, Taipei, Taiwan;

[2] Institute of Biomedical Sciences, Academia Sinica, Taipei, Taiwan;

[3]Division of Preventive Medicine and Health Service Research, Institute of Population Health Sciences, National Health Research Institutes, Miaoli, Taiwan;

[4] National Environmental Health Research Center, National Institute of Environmental Health Science, National Health Research Institutes, Miaoli, Taiwan;

[5] Institution of Occupational Medicine and Industrial Hygiene, National Taiwan University College of Public Health, Taipei, Taiwan;

[6]Center for Environmental and Respiratory Health Research, Faculty of Medicine, University of Oulu, Oulu, Finland;

[7]Medical Research Center, University of Oulu and Oulu University Hospital, Oulu, Finland;

[8]Research Center for Environmental Medicine, Kaohsiung Medical University, Kaohsiung, Taiwan.

Correspondence author (*): Dr. Po-Chin Huang, Ph.D., 35 Keyan Road, Zhunan, Miaoli County 35035, Taiwan, Tel: +886-37-246166 ext. 38507, Fax: +886-37-584-730, E-mail: pchuang@nhri.org.tw

Running Title: Phthalates may alter thyroid and growth hormone.

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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. 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. 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 and the sum of urinary di-(2-ethylhexyl) phthalate (DEHP) metabolite levels. Free T_4 levels were negatively associated with

urinary mono-ethylhexyl phthalate (MEHP) and mono-(2-ethyl-5-oxohexyl) phthalate levels, but positively associated with urinary monoethyl phthalate and mono-*n*-butyl phthalate (MnBP) after adjustment for age, BMI, gender, urinary creatinine levels, and TBG levels. Postive associations between urinary MEHP levels and IGF-1 levels were observed. Among minors, free T_4 was positively associated with urinary MBzP levels, and IGF-1 levels were negatively associated with the sum of urinary DEHP metabolite levels 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.

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 metabolites 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) occured 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.

Methods

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 (Kaohsiung City), eastern region (ex.: Hua-Lien County) and remote island region (Peng-Hu County) of Taiwan. All participants were required to be Taiwanese, aged 7 years or older, excluding pregnant and breast-feeding women, individuals with severe disease (eg, cancer patients), foreigners, and citizens in hospitals or jails. We selected 17 townships in 11 cities or counties in the northern, central, southern, eastern, and remote island regions of Taiwan between May 2013 and December 2013. 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.

Exposure assessment. Assessment of exposure to 7 commonly used phthalates, DEHP, DnBP, DiBP, DEP, di-iso-nonyl 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-iso-nonyl 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. The molar sum (nmole/mL) 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) 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%.

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 minutes 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., 2016). 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 0.0025 μIU/mL, respectively. Serum TBG was measured using the immunoenzymometric assay with ACCUBIND ELISA Microwells (Monobind Inc., Product Code 3525-300). Serum IGF-1 was measured using an immunoradiometric assay (IRMA) kit (IGF-IRIACT, Cis Bio, Gif-sur-Yvette, France), and serum IGFBP3

was measured using an IRMA kit (ACTIVE IGFBP3 IRMA, Diagnostic Systems Laboratories Inc., Webster, TX, USA). The assay sensitivities for TBG, IGF-1, and IGFBP3 were 1.0 μg/mL, 1 ng/mL, and 0.5 ng/mL, respectively. The laboratory reference ranges for TSH, T_3, T_4, Free T_4, and TBG were 0.35 - 4.94 μIU/mL, 58 - 159 ng/dL, 4.87 - 11.72 μg/dL, 0.70 - 1.48 ng/dL, and 15.8-25.4 μg/mL, respectively. Most of thyroid hormones in the study population were within reference range.

Statistical methods. Descriptive statistics on the participant demographics were tabulated with the distributions of phthalate metabolite concentrations, thyroid hormones, and IGF-1 levels. Because of significant differences in some thyroid hormone and IGF-1 levels in minors compared with adults, we separated our data set into 2 groups in subsequent analyses. All levels of hormones and phthalate metabolites were transformed using the natural logarithm (Ln) to meet the normality assumption. Principal component analysis (PCA) was applied to assess the potential sources of exposure to different phthalates. Physiological factors or variables significantly correlated with urinary phthalate metabolites or thyroid hormones were included in the multiple regression model. We then constructed full multivariable linear regression models with serum thyroid hormones or IGF-1 levels as the dependent variable and individual ln-transformed urinary phthalate metabolite

concentrations as a predictor, with age (continuous variable), gender (dichotomous), BMI (continuous), urinary creatinine levels (continuous), and ln-transformed serum TBG or IGFBP3 (continuous) as covariates. These covariates were selected based on previous studies (Boas et al., 2010; Meeker and Ferguson, 2011) and a 10% change-in-estimated criterion (Rothman et al., 2008). In addition, we utilized the quartile of urinary phthalate metabolite levels to assess the nonlinear relationship between phthalate exposure and hormone levels. A subgroup analysis was conducted to exclude 45 adults with a self-reported history of other endocrine diseases. No differences were observed between adults with and without including 45 adults with a self-reported history of other endocrine diseases. Finally, we concluded these participants to present the results of this study. A *P* value of < .05 was considered statistically significant. SAS Version 9.1.3 was used for all statistical analyses.

Results

Study population. The study population included 376 subjects, 279 (74.2%) adults and 97 (25.8%) minors. The mean age and BMI in adults were 53.4 years (SD 17.3) and 24.8 (SD 4.4), respectively. Approximately half of them were women (53.8%). A high percentage of participants were non-smokers (74.8%), did not consume alcohol (86.9%), or chew betel nuts (93.2%), or did not use pesticides at home (74.9%).

Approximately half of the minors were boys (59.6%). The mean age and BMI in minors were 12.6 years (SD 3.2) and 20.6 (SD 5.3), respectively. The detailed characteristics of all participants are presented in Table 1.

Distributions of the biomarkers of exposure and measures of outcome. Distributions of urinary phthalate metabolite and hormone levels among adults and minors are compared in Table 2. The geometric mean (GM) levels of urinary MBzP, MEHHP, MEOHP and ΣDEHPm in adults were significantly lower than those found in minors. No significant differences in the GM levels of other urinary phthalate metabolites were observed between adults and minors. The GM levels of serum T_3, IGF-1, and IGFBP3 in adults were significantly lower than those in minors (103.6 vs 132.8 ng/dL for T_3; 173.6 vs 398.5 ng/dL for IGF-1; 3591 vs 4360 ng/mL for IGFBP3), whereas the serum TSH levels in adults were significantly higher than those in minors (1.54 vs 1.41 μIU/mL). The GM levels of serum T_4, free T_4, and TBG were comparable between adults and minors.

Relationships between biomarkers of phthalate exposure and levels of thyroid hormones. Table 3 and Table 4 present the relations between biomarkers of exposure and measures of thyroid function and growth hormone homeostasis separately for adults and minors, adjusted for core covariates in linear regression. In minors, the serum T_3 levels were significantly related to urinary MEHP levels (β = .029, P = .029).

None of the concentrations of urinary phthalate metabolites explained T_3 levels in adults. In adults, the serum T_4 levels were inversely related to both the levels of urinary MEHHP and ΣDEHPm (β = -.030, P = .032 for MEHHP; β = -.050, P = .009 for ΣDEHPm). In minors, no associations between levels of any urinary phthalate metabolites or serum T_4 in minors were observed. In adults, the levels of serum-free T_4 were positively related to the levels of both urinary MEP and MnBP (β = .014, P = .039 for MEP; β = .017, P = .005 for MnBP), and inversely to the levels of urinary MEHP and MEOHP (β = -.013, P = .036 for MEHP; β = -.028, P = .004 for MEOHP). Positive associations between levels of urinary MBzP and serum-free T_4 also in minors were found (β = .044, P = .001). We found no relations between the levels of any urinary phthalate metabolites or serum TSH among adults and minors.

Relationships between biomarkers of phthalate exposure and levels of growth hormones. We found a positive association between levels of urinary MEHP and IGF-1 in adults (β = .033, P = .006), but no such association was present in minors.

In minors, the levels of IGF-1 were related to the levels of urinary ΣDEHPm (β = -.166, P = .041). Figures 1 and 2 show the nonlinear relationships between phthalate metabolites and hormone levels according to the quartiles of urinary phthalate metabolite levels in Taiwanese adults and minors for the detection rate of urinary phthalate metabolites of more than 50%. For adults, we found significant differences

in the fourth (Q4) quartile of MEHHP (β = -.125, P = .003) and Q4 of ΣDEHPm (β = -.120, P = .004) compared with the first (Q1) quartile for the T_4 levels in adults. Compared with Q1 for the free T_4 levels in adults, significant differences in the third (Q3) and the second (Q2) quartile of MEHP (β = -.083, P = .01 for Q3 of MEHP; β = -.078, P = .014 for Q2 of MEHP) were observed. Serum-free T_4 levels in Q4 of MnBP (β = .082, P = .018) were higher than those in Q1. Serum-free T_4 levels in Q4 and Q3 of MEP (β = .091, P = .007 for Q4 of MEP; β = .08, P = .015 for Q3 of MEP) were higher than those in Q1. We observed significant differences in Q4, Q3 and Q2 of MEHP (β = .125, P = .045 for Q4 of MEHP; β = .176, P = .003 for Q3 of MEHP; β = .151, P= .01 for Q2 of MEHP) compared with Q1 for the IGF-1 levels. For minors, significant differences in Q4 of ΣDEHPm were found compared with Q1 for the IGF-1 levels (β = -.403, P = .033).

Discussion

This is the first study to evaluate systematically the effects of phthalate exposure on measures of thyroid function and growth hormone homeostasis in the general Taiwanese adult and minor populations. We found evidence of negative associations between urinary MEHHP or ΣDEHPm levels and serum T_4, and between urinary MEHP or MEOHP and serum free T_4 in Taiwanese adults, whereas we found a positive association between urinary MEP and serum free T_4. Some of our results are

consistent with those of relevant studies. Meeker et al (2007) first reported a significantly inverse relationship between MEHP and free T_4 levels in 408 American men (Meeker et al., 2007). Meeker and Ferguson (2011) studied 1364 adults aged ≥ 20 years from the 2007–2008 National Health and Nutrition Examination Survey (NHANES) and found inverse relationships between DEHP metabolites and T_4 levels (Meeker and Ferguson, 2011). DEHP acts as a thyroid antagonist (Shen et al., 2009; Shi et al., 2011) and causes a change in iodine uptake by altering the sodium-iodine symporter in vitro studies (Wenzel et al., 2005). A previous study of zebrafish (*Danio rerio*) embryos treated with diverse concentrations of MEHP demonstrated that whole-body T_4 levels were significantly decreased, probably through the upregulation of genes related to thyroid hormone metabolism (Zhai et al., 2014). Liu et al showed that DEHP could reduce thyroid hormones by influencing biosynthesis through biotransformation, bio-transport, receptor levels, and metabolism of thyroid hormones in male rats (Liu et al., 2015). However, we also found positive relationships between urinary MEP and free T_4 levels, which were inconsistent with previous studies (Brown et al., 1978; Pereira et al., 2007). Although personal care products and cosmetics are potential sources of DEP exposure in Chinese adults (Gao et al., 2016), little information is available on whether frequent DEP exposure alters thyroid hormones. Mechanistic studies are necessary to elucidate our findings.

The results for the participants aged 7–18 years are inconsistent with those for the adults. Although the sample size of this age group was relatively small, we derived evidence of significantly positive associations between urinary MEHP levels and serum levels of T_3. Meeker and Ferguson (2011) also reported positive associations between DEHP metabolites and T_3 and TSH levels in 329 adolescents aged 12–19 years from the 2007–2008 NHANES (Meeker and Ferguson, 2011). However, Boas et al (2008) found that T_3 and free T_3 were inversely correlated with crude phthalate concentrations, including MEP and DEHP metabolites among 845 children (aged 4-9 y) in Denmark (Boas et al., 2010). Recently, Wu et al (2013) studied 60 children in Taiwan aged younger than 10 years (Wu et al., 2013). They reported that TSH and T_3 levels decreased when children were exposed to high levels of phthalate-tainted foodstuffs. In addition, de Cock et al (2014) found no significant associations between DEHP metabolites assessed in cold blood and serum T_4 levels in a Dutch prospective cohort study of 86 infants (de Cock et al., 2014). Therefore, differences in study design, age group, sample size, exposure profiles, and covariates could result in inconsistent findings.

Our results also showed that urinary ΣDEHPm levels were negatively associated with IGF-1 levels in minors, but urinary MEHP levels were positively associated with IGF-1 levels in adults. Boas et al (2010) reported inverse relationships between

urinary ΣDEHP levels and IGF-1 levels in 845 children aged 4–9 years (Boas et al., 2010), which was consistent with our findings. We also found different effects on IGF-1 levels regarding phthalate exposure in adults and minors. Meeker and Ferguson (2011) observed different effects on thyroid function with regard to phthalate exposure, such as DEHP in adults and adolescents (Meeker and Ferguson, 2011). Recently, Chang et al (2015a) demonstrated that urinary MEHP percentages were negatively associated with insulin-like factor 3 (INSL3) in 176 men (Chang et al., 2015a). In experimental studies, increasing exposure to DEHP in adult rats has decreased the expression levels of INSL3 (Guo et al., 2013; Li et al., 2012), and exposure to MEHP and MBP appeared to suppress INSL3 gene expression (Pathirana et al., 2011). However, previous studies of IGF-1 effects have indicated that prenatal exposure to low-dose DEHP may increase levels of the mRNA for IGF-1 (Lin et al., 2008). Furthermore, phthalates could potentially interact with other endocrine pathways such as the hypothalamic-pituitary axis or androgen biosynthesis. Such complex effects—in addition to the age group—might contribute to differences in effects. Understanding whether phthalate exposure, such as IGF-1 in adults and minors, could have different effects on growth requires future studies to elucidate these associations and potential biological mechanisms.

Based on our PCA results, we found 2 components extracted in adults and minors that accounted for 51.7% and 55.4% of the variability, respectively (Supplemental Figure S1). This indicated 2 major potential exposure sources of phthalate for Taiwanese adults and minors. MEHP, MEHHP, MEOHP, MECCP, and MCMHP were highly correlated with component 1 (PC1), whereas MiBP, MnBP, MBzP, and MiNP were moderately/ highly correlated with component 2 (PC2). These results indicate that PC1 is dominated by the group of DEHP metabolites and PC2 by the group of MiBP, MnBP, MBzP, and MiNP. Our findings are similar to those of the previous study of young Chinese adults (Gao et al., 2016). DEHP is the most abundant phthalate in foods (Guo et al., 2012). Foods may be a major exposure source of phthalates for Taiwanese adults and minors. The sources of MiBP, MnBP, MBzP, and MiNP could be lacquers, varnishes, coatings, and food-packaging materials (Calafat and McKee, 2006; Cao, 2010). For other metabolites, the major sources of MEP could be personal care products (Bao et al., 2015; Guo et al., 2014). Regarding the source of MMP, a previous study suggested that the levels of DMP in milk products, instant noodles, cakes, cookies, and salted eggs were higher than those in other foods (Guo et al., 2012). However, there may still be other unknown sources of exposure.

Most of the thyroid hormone levels in our study population were within the reference range, although we found that T_4, free T_4, TBG, and IGF1 levels were significantly

different between the regions of residence (Supplemental Table B) as compared with those of the participants living in Northern Taiwan. Further stratified analysis revealed no significantly decreased levels of T_4 or free T_4 varied according to insecticide use in our participants (Supplemental Table C). Furthermore, we found no differences between urinary phthalate metabolites levels and insecticide use at home (Supplemental Table D) in our participants. Thus, insecticide use is less likely to have affected our results. The consumption of iodine intake in our subjects could be another crucial confounder. From 1966 to 2004, the Taiwan government required salt manufacturers to add iodine to their products. A NAHSIT survey reported no significant changes in iodine consumption, but sodium consumption decreased in the general Taiwanese population from 1993 to 2008 (Pan et al., 2011). In addition, other early regional data in Taipei revealed that only 3% of participants excreted less than 50 μg of iodine/g creatinine (mild deficiency), but 42.7% of the population excreted more than 300 μg of iodine/g creatinine (Lin et al., 1991). According to a more recent report from NAHSIT (funded by the Taiwan Ministry of Health and Welfare), the median level of urinary iodine in the Taiwanese general population (NAHSIT 2004-2008) was 100 μg of iodine/g creatinine (WHO optimal: 100-199 μg), at the lowest value suggested by the WHO (Chang, 2015b). Moreover, previous studies have indicated that iodine excretion had a negligible impact on the relationships between

phthalate metabolites and thyroid hormone levels (Mendez and Eftim, 2012). Thus, the consumption of iodine in our participants is less likely to have biased our results. There were several limitations to this study. First, the cross-sectional study design limited the causal inference from the observed associations between phthalate exposure, thyroid function, and IGF-1 measures. Second, only one urine sample per participant was assessed for phthalate metabolite concentrations, which may not be representative of the average body burden of the participant because of the short half-lives of these chemicals. Nevertheless, previous studies have shown that single-spot urine samples of phthalate levels may be moderately representative of long-term average exposure (Hauser et al., 2004; Teitelbaum et al., 2008). Similarly, we collected only one serum sample per participant to be analyzed for thyroid hormones and IGF-1 levels. These hormone levels may vary in an individual over time. However, Andersen et al (2002) reported that thyroid hormone measures in an individual are retained within relatively narrow limits over time (Andersen et al., 2002). Finally, we did not collect information concerning the iodine or selenium status of our participants, which may be critical because deficiencies in these trace elements can impair normal thyroid hormone function (Zimmermann and Kohrle, 2002). However, no subjects had a goiter or thyroid-related diseases, according to their self-reported questionnaire. We have no reason to expect that an iodine or selenium

deficiency would be associated with phthalate exposure or that any deficiencies in these substances would affect the precision of the estimated effects rather than the estimates themselves (Schisterman et al., 2009). Serum-free T_4 levels measured using chemiluminescent immunoassays in the present study could have been influenced by binding protein concentrations. Because we explored the associations between urinary phthalate metabolites levels and free T_4 levels adjusted for TBG levels, the effects from binding protein concentrations in the present study could be limited.

Conclusions

Overall, our results provide evidence that exposure to environmental phthalates could influence the thyroid hormones and growth homeostasis in both adults and minors. More detailed research of different populations with various lifestyles is necessary to verify the specific findings and establish the temporal relationships between markers of exposure and effects, and actuate the potential clinical and public health implications of these associations.

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Table1. Characteristics of the study population (N = 358).

Variables	Adults (≧18 years, N = 279)		Minors (<18 years, N = 79)	
	N	%	N	%
Gender				
Male	129	46.2	47	59.5
Female	150	53.8	32	40.5
Age (years, Mean ± SD)	279	53.4 ± 17.3	79	12.6 ± 3.2
BMI (Mean ± SD)	279	24.8 ± 4.4	79	20.6 ± 5.3
Region				
Northern Taiwan	84	30.1	24	30.4
Central Taiwan	69	24.7	25	31.6
Southern Taiwan	50	17.9	10	12.7
Eastern Taiwan	49	17.6	9	11.4
Remote islands	27	9.7	11	13.9
Marital status				
Single	48	17.2	78	98.7
Married	203	72.8	1	1.3
Divorce/ widowed	28	10.0	0	0
Education				
≦Elementary school	76	27.3	37	46.8
Junior high school	40	14.3	24	30.4
Senior high school	66	23.7	18	22.8

≧College/ Graduates	97	76.3	0	0
Cigarette smoking[a]				
Yes	70	25.2	2	2.5
Alcohol consumption[b]				
Yes	36	13.1	1	1.3
Tea drinking [c]				
Yes	162	58.3	41	51.9
Coffee drinking[c]				
Yes	117	41.9	6	7.6
Betel nut chewing[d]				
Yes	19	6.8	1	1.3
Insecticide use at home				
Yes	70	25.1	21	26.6

[a] Subjects consuming at least one cigarette per day.

[b] Subject consuming at least one bottle of alcohol drink per week.

[c] Subjects consuming at least one cup of tea or coffee per week.

[d] Subject chewing at least one betel nut per week.

Table 2 Median and geometric mean levels (ng/ mL) of urinary phthalate metabolites and thyroid and growth hormones among Taiwanese adults and minors.

Variables	Adults (>=18 years) (N = 279)				Minors (<18 years) (N = 79)				*p-value*[a]
	<LOD%	N	Median (Interquartile range)	GM (95% CI)	<LOD%	N	Median (Interquartile range)	GM (95% CI)	
Phthalate metabolites									
MMP	3.7	273	24.09 (11.66, 53.88)	24.32 (20.14, 29.37)	2.6	78	43.45 (15.86, 84.09)	37.11 (26.70, 51.57)	0.718
MEP	9.2	273	12.10 (5.11, 28.56)	10.77 (8.64, 13.43)	5.1	78	15.49 (7.33, 31.39)	13.47 (9.46, 19.18)	0.463
MiBP	28.9	273	7.17 (0.15, 17.26)	3.55 (2.75, 4.60)	19.2	78	13.89 (4.65, 23.98)	6.71 (4.26, 10.58)	0.194
MnBP	13.2	273	15.60 (5.93, 29.17)	9.86 (7.82, 12.43)	9.0	78	21.473 (13.66, 45.57)	16.82 (11.46, 24.67)	0.605
MBzP	78.8	273	0.15 (0.15, 0.15)	0.30 (0.26, 0.36)	70.5	78	0.15 (0.15, 2.22)	0.43 (0.29, 0.63)	0.040
MEHP	23.1	273	6.67 (2.53, 12.11)	3.56 (2.85, 4.44)	20.5	78	7.37 (2.41, 12.64)	4.00 (2.67, 6.01)	0.948
MEHHP	2.2	273	16.37 (9.79, 30.05)	15.54 (13.67, 17.66)	3.8	78	25.48 (13.57, 39.37)	21.95 (16.36, 29.46)	0.022
MEOHP	6.6	273	10.16 (5.60, 16.98)	8.38 (7.18, 9.77)	1.3	78	19.55 (9.33, 32.34)	17.40 (13.81, 21.92)	0.001
MECPP	4.4	273	20.22 (10.90, 31.98)	16.91 (14.47, 19.75)	3.8	78	34.68 (18.42, 63.61)	28.29 (20.92, 38.25)	0.076
MCMHP	36.6	273	3.19 (0.15, 6.57)	1.52 (1.22, 1.90)	21.8	78	5.77 (2.49, 11.37)	3.32 (2.23, 4.95)	0.203
MiNP	89.0	273	0.15 (0.15, 0.15)	0.21 (0.19, 0.24)	88.5	78	0.15 (0.15, 0.15)	0.23 (0.18, 0.31)	0.207
ΣDEHPm (nmole/mL)[b]		273	0.20 (0.12, 0.32)	0.20 (0.18, 0.22)		78	0.32 (0.16, 0.50)	0.30 (0.25, 0.37)	0.032

ΣDBPm (nmole/mL)[b]	273	0.11 (0.05, 0.20)	0.10 (0.08, 0.11)	78	0.16 (0.09, 0.31)	0.16 (0.13, 0.20)	0.676
Hormones[c]							
TSH (μIU/mL)	279	1.57 (1.10, 2.43)	1.54 (1.41, 1.68)	79	1.46 (1.04, 1.95)	1.41 (1.25, 1.58)	0.006
T_3 (ng/dL)	279	107 (92, 120)	104 (101, 107)	79	132 (116, 151)	133 (126, 140)	<0.001
T_4 (μg/dL)	279	7.47 (6.26, 8.60)	7.30 (7.11, 7.50)	79	7.38 (6.47, 8.52)	7.34 (6.99, 7.70)	0.931
Free T_4 (ng/dL)	279	0.93 (0.83, 1.07)	0.93 (0.91, 0.95)	79	0.98 (0.85, 1.11)	0.96 (0.92, 1.00)	0.210
TBG (μg/mL)	272	21.8 (18.9, 24.5)	21.2 (20.6, 21.7)	76	22.4 (17.3, 24.7)	21.2 (20.0, 22.4)	0.774
IGF1 (ng/mL)	274	170 (128, 241)	174 (164, 183)	76	451 (285, 577)	399 (355, 447)	<0.001
IGFBP-3 (ng/mL)	272	3750 (3101, 4296)	3591 (3469, 3719)	76	4427 (3885, 5182)	4360 (4144, 4588)	<0.001

Abbreviations: geometric mean (GM), insulin-like growth factor 1 (IGF-1), insulin-like growth factor binding protein 3(IGFBP3), limit of detection (LOD), mono-methyl phthalate (MMP), mono-ethyl phthalate (MEP), mono-iso-butyl phthalate (MiBP), mono-n-butyl phthalate (MnBP), mono-benzyl phthalate (MBzP), mono-ethylhexyl phthalate (MEHP), mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), mono-(2-ethyl-5-oxo-hexyl) phthalate (MEOHP), mono-(2-ethyl-5-carboxypentyl) phthalate (MECPP), mono-(2-carboxymethylhexyl) phthalate (MCMHP), mono-iso-nonyl phthalate(MiNP), thyroxine (T_4), free T_4, triiodothyronine (T_3), thyroid-stimulating hormone (TSH), thyroxine-binding globulin (TBG).

[a] Student's t test calculated for the difference in means between adults and minors.

[b] ΣDEHPm=sum molar concentrations of MEHP+MEHHP+MEOHP+MECPP+MCMHP; ΣDBPm = sum molar concentrations of MiBP+MnBP.

[c] The laboratory reference ranges for TSH, T_3, T_4, Free T_4, and TBG were 0.35 - 4.94 μIU/mL, 58 - 159 ng/dL, 4.87 - 11.72 μg/dL, 0.70 - 1.48 ng/dL, and 15.8-25.4 μg/mL, respectively.

Table 3. Adjusted regression coefficient and 95% CI for change in serum thyroid and growth hormones in relation to unit-increased in Ln-phthalate metabolites (ng/mL) in Taiwanese adults and minors.

Variables	Ln T3[c]					
	Adults (>=18 years) (N = 266)			Minors (<18 years) (N = 75)		
	Beta	95%CI	*p*-value	Beta	95%CI	*p*-value
MMP	0.005	-0.012, 0.022	0.528	0.013	-0.026, 0.051	0.509
MEP	-0.010	-0.025, 0.005	0.174	-0.010	-0.042, 0.022	0.540
MiBP	-0.006	-0.019, 0.006	0.327	-0.003	-0.027, 0.020	0.786
MnBP	-0.004	-0.018, 0.009	0.524	0.011	-0.015, 0.037	0.404
MBzP	0.001	-0.017, 0.019	0.904	-0.010	-0.039, 0.018	0.459
MEHP	0.005	-0.009, 0.019	0.509	**0.029**	**0.003, 0.056**	**0.029**[*]
MEHHP	-0.003	-0.030, 0.024	0.811	0.000	-0.039, 0.037	0.961
MEOHP	-0.020	-0.041, 0.002	0.075[#]	0.045	-0.012, 0.102	0.119
MECPP	0.000	-0.021, 0.021	0.993	-0.033	-0.067, 0.002	0.062[#]
MCMHP	-0.014	-0.028, 0.001	0.059[#]	0.020	-0.008, 0.047	0.156
MiNP	-0.002	-0.027, 0.022	0.850	0.030	-0.004, 0.064	0.086[#]
ΣDBPm[a] (nmole/mL)	-0.018	-0.037, 0.002	0.072[#]	-0.001	-0.049, 0.046	0.953
ΣDEHPm[b] (nmole/mL)	-0.018	-0.056, 0.020	0.347	0.038	-0.035, 0.111	0.302

Table 3. (cont.)

Variables	Ln T_4[c]					
	Adults (>=18 years) (N = 266)			Minors (<18 years) (N = 75)		
	Beta	95%CI	*p*-value	Beta	95%CI	*p*-value
MMP	0.006	-0.011, 0.023	0.514	0.030	-0.002, 0.062	0.070[#]
MEP	-0.013	-0.028, 0.002	0.100[#]	0.010	-0.017, 0.038	0.454
MiBP	-0.011	-0.024, 0.001	0.079[#]	-0.017	-0.036, 0.003	0.094[#]
MnBP	-0.012	-0.025, 0.002	0.095[#]	0.016	-0.006, 0.038	0.151
MBzP	-0.004	-0.023, 0.014	0.645	-0.013	-0.037, 0.011	0.300
MEHP	0.004	-0.010, 0.018	0.573	0.010	-0.013, 0.033	0.402
MEHHP	**-0.030**	**-0.057, -0.003**	**0.032**[*]	0.006	-0.027, 0.038	0.734
MEOHP	-0.012	-0.034, 0.010	0.289	0.004	-0.045, 0.054	0.866
MECPP	0.015	-0.006, 0.036	0.152	-0.009	-0.040, 0.021	0.541
MCMHP	-0.014	-0.029, 0.001	0.060[#]	-0.021	-0.045, 0.002	0.070[#]
MiNP	-0.019	-0.044, 0.006	0.131	-0.008	-0.038, 0.022	0.596
ΣDBPm[a] (nmole/mL)	-0.011	-0.031, 0.009	0.264	0.015	-0.026, 0.055	0.469
ΣDEHPm[b] (nmole/mL)	**-0.050**	**-0.088, -0.013**	**0.009**[*]	-0.024	-0.087, 0.039	0.444

Table 3. (cont.)

Variables	Ln Free T_4[c]					
	Adults (>=18 years) (N = 266)			Minors (<18 years) (N = 75)		
	Beta	95%CI	*p*-value	Beta	95%CI	*p*-value
MMP	0.003	-0.013, 0.018	0.729	-0.009	-0.047, 0.030	0.655
MEP	**0.014**	**0.001, 0.027**	**0.039**[*]	0.004	-0.027, 0.036	0.786
MiBP	-0.004	-0.015, 0.007	0.484	0.002	-0.021, 0.025	0.874
MnBP	**0.017**	**0.005, 0.029**	**0.005**[*]	0.019	-0.006, 0.044	0.140
MBzP	0.012	-0.004, 0.028	0.132	**0.044**	**0.018, 0.070**	**0.001**[*]
MEHP	**-0.013**	**-0.026, 0.000**	**0.036**[*]	-0.023	-0.049, 0.004	0.088[#]
MEHHP	-0.001	-0.025, 0.023	0.933	0.002	-0.036, 0.040	0.923
MEOHP	**-0.028**	**-0.047, -0.009**	**0.004**[*]	0.000	-0.058, 0.057	0.992
MECPP	-0.007	-0.026, 0.011	0.447	-0.003	-0.038, 0.032	0.869
MCMHP	-0.011	-0.023, 0.002	0.109	0.003	-0.024, 0.031	0.807
MiNP	-0.001	-0.023, 0.021	0.926	0.004	-0.031, 0.039	0.828
ΣDBPm (nmole/mL)[a]	0.007	-0.010, 0.025	0.414	0.006	-0.041, 0.053	0.796
ΣDEHPm (nmole/mL)[b]	0.001	-0.032, 0.035	0.935	0.030	-0.043, 0.103	0.411

Table 3. (cont.)

Variables	Ln TSH[c]					
	Adults (>=18 years) (N = 266)			Minors (<18 years) (N = 75)		
	Beta	95%CI	*p*-value	Beta	95%CI	*p*-value
MMP	0.002	-0.062, 0.066	0.954	0.008	-0.102, 0.118	0.891
MEP	0.042	-0.014, 0.098	0.142	0.014	-0.078, 0.105	0.767
MiBP	-0.005	-0.052, 0.042	0.835	-0.022	-0.089, 0.044	0.505
MnBP	0.032	-0.019, 0.083	0.212	0.041	-0.032, 0.115	0.266
MBzP	0.037	-0.031, 0.106	0.280	-0.025	-0.106, 0.055	0.532
MEHP	-0.007	-0.060, 0.046	0.796	-0.048	-0.125, 0.028	0.212
MEHHP	0.013	-0.090, 0.115	0.806	-0.085	-0.192, 0.023	0.122
MEOHP	0.010	-0.072, 0.092	0.809	0.083	-0.081, 0.246	0.319
MECPP	0.006	-0.072, 0.085	0.871	0.035	-0.065, 0.136	0.485
MCMHP	0.043	-0.012, 0.097	0.126	0.068	-0.010, 0.146	0.085[#]
MiNP	0.054	-0.039, 0.148	0.254	-0.041	-0.141, 0.059	0.412
ΣDBPm (nmole/mL)[a]	0.006	-0.068, 0.079	0.883	0.009	-0.126, 0.144	0.898
ΣDEHPm (nmole/mL)[b]	0.041	-0.102, 0.184	0.575	0.027	-0.183, 0.238	0.796

Table 3. (cont.)

Variables	Ln IGF-1[d]					
	Adults (>=18 years) (N = 266)			Minors (<18 years) (N = 75)		
	Beta	95%CI	*p*-value	Beta	95%CI	*p*-value
MMP	-0.015	-0.043, 0.013	0.282	0.023	-0.063, 0.109	0.591
MEP	0.000	-0.025, 0.024	0.959	-0.039	-0.109, 0.032	0.277
MiBP	-0.011	-0.032, 0.009	0.282	-0.012	-0.065, 0.041	0.646
MnBP	0.000	-0.022, 0.022	0.996	-0.003	-0.061, 0.054	0.905
MBzP	-0.020	-0.050, 0.009	0.182	-0.024	-0.087, 0.038	0.441
MEHP	**0.033**	**0.010, 0.056**	**0.006**[*]	0.052	-0.007, 0.111	0.085[#]
MEHHP	-0.007	-0.051, 0.037	0.751	-0.040	-0.127, 0.047	0.366
MEOHP	0.013	-0.023, 0.049	0.479	-0.113	-0.239, 0.013	0.077[#]
MECPP	-0.006	-0.040, 0.028	0.715	-0.060	-0.136, 0.017	0.123
MCMHP	0.001	-0.023, 0.026	0.910	-0.018	-0.081, 0.045	0.574
MiNP	-0.013	-0.053, 0.028	0.542	0.010	-0.067, 0.087	0.800
ΣDBPm (nmole/mL)[a]	-0.006	-0.038, 0.026	0.702	0.023	-0.082, 0.128	0.664
ΣDEHPm (nmole/mL)[b]	-0.033	-0.094, 0.029	0.294	**-0.166**	**-0.325, -0.000**	**0.041**[*]

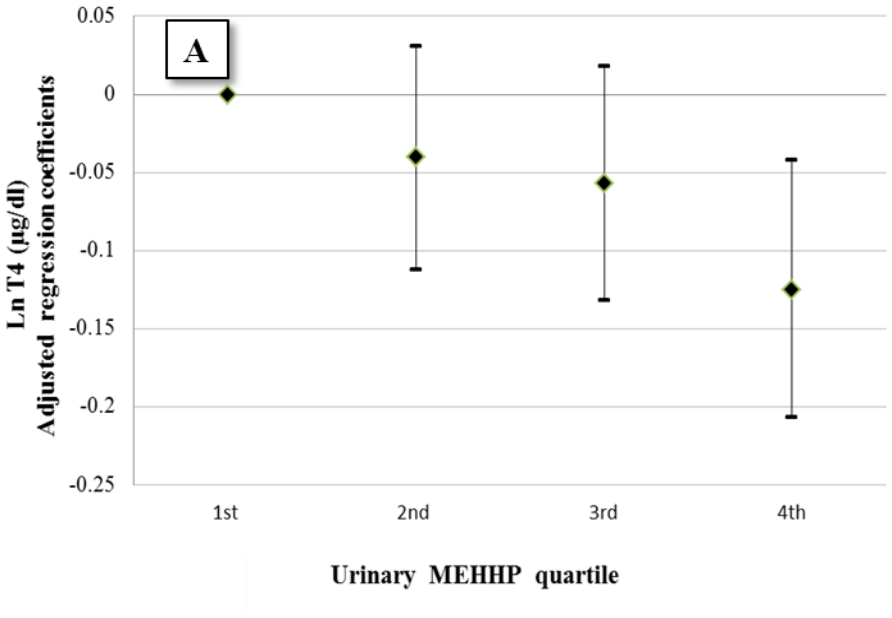
Abbreviations were shown in the footnote of Table 2.

[a] ΣDBPm =sum molar concentrations of MiBP+MnBP.

[b] ΣDEHPm=sum molar concentrations of MEHP+MEHHP+MEOHP+MECPP+MCMHP.

[c] Adjustment for age, BMI, gender, urinary creatinine levels, and TBG levels; * indicates $P<0.05$; # indicates $P<0.10$.

[d] Adjustment for age, BMI, gender, urinary creatinine levels, and IGFBP-3; * indicates $P<0.05$; # indicates $P<0.10$.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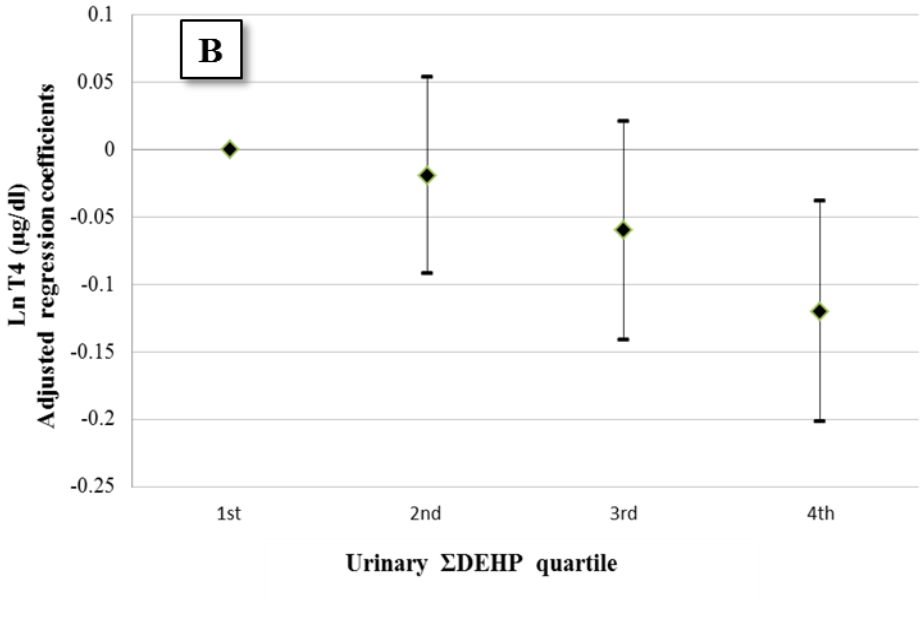
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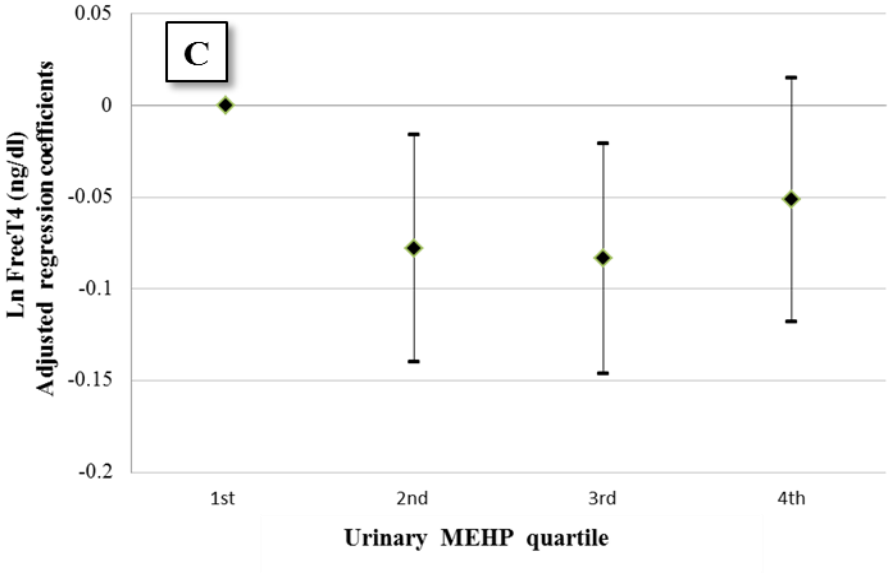
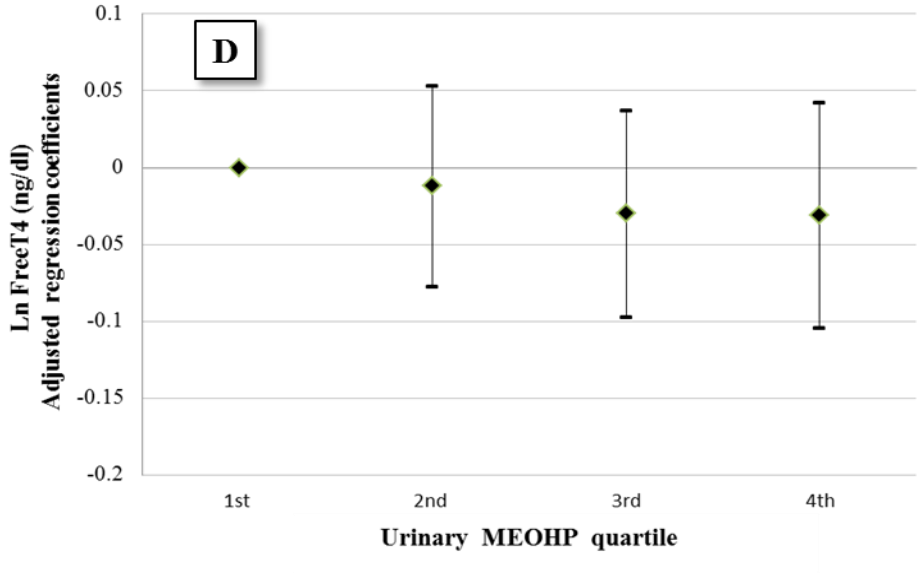
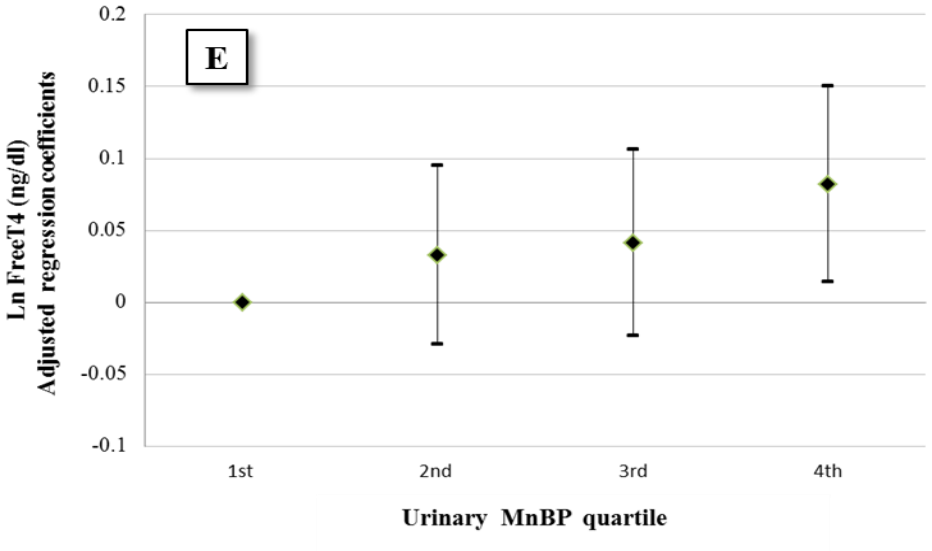
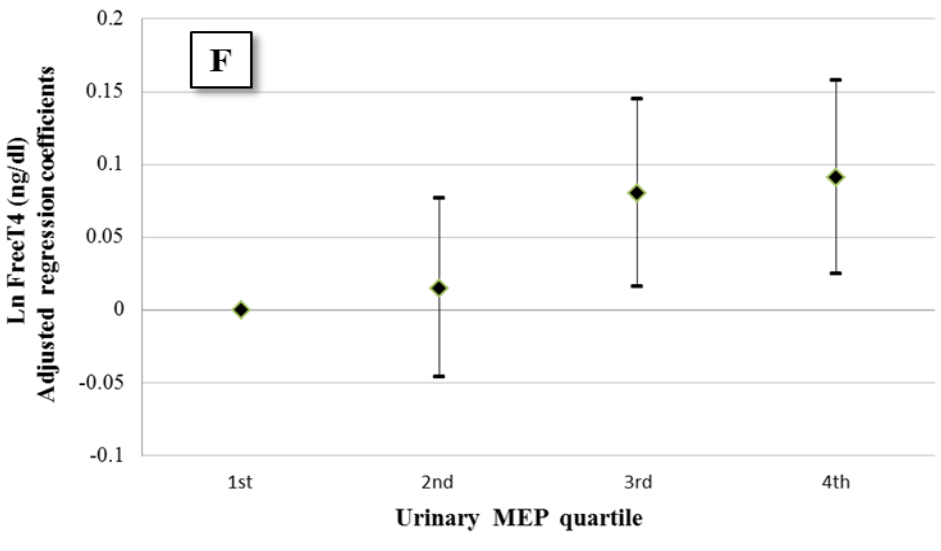
4th
Urinary ΣDEHP quartile


C
0.05
0
-0.05
-0.1
-0.15
-0.2
Ln FreeT4 (ng/dl)
Adjusted regression coefficients
1st
2nd
3rd
4th
Urinary MEHP quartile


D
0.1
0.05
0
-0.05
-0.1
-0.15
-0.2
Ln FreeT4 (ng/dl)
Adjusted regression coefficients
1st
2nd
3rd
4th
Urinary MEOHP quartile


E
0.2
0.15
0.1
0.05
0
-0.05
-0.1
Ln FreeT4 (ng/dl)
Adjusted regression coefficients
1st
2nd
3rd
4th
Urinary MnBP quartile


F
0.2
0.15
0.1
0.05
0
-0.05
-0.1
Ln FreeT4 (ng/dl)
Adjusted regression coefficients
1st
2nd
3rd
4th
Urinary MEP quartile

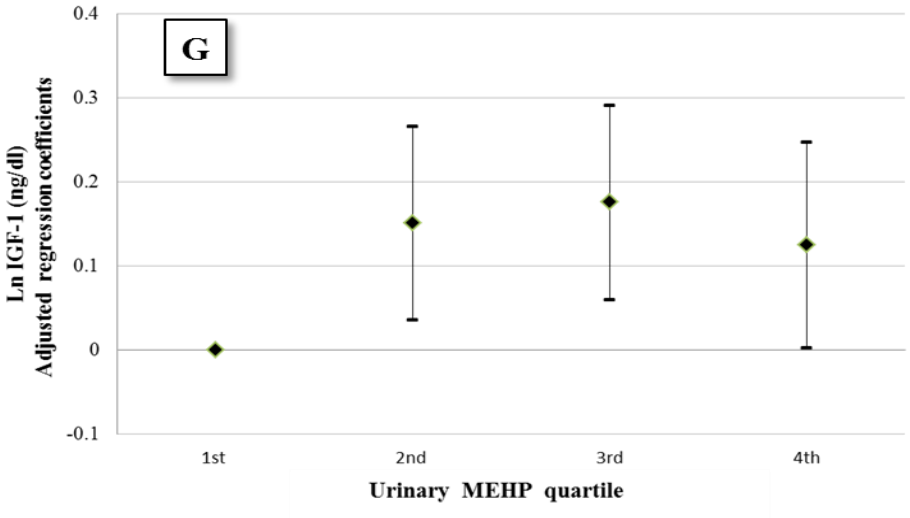
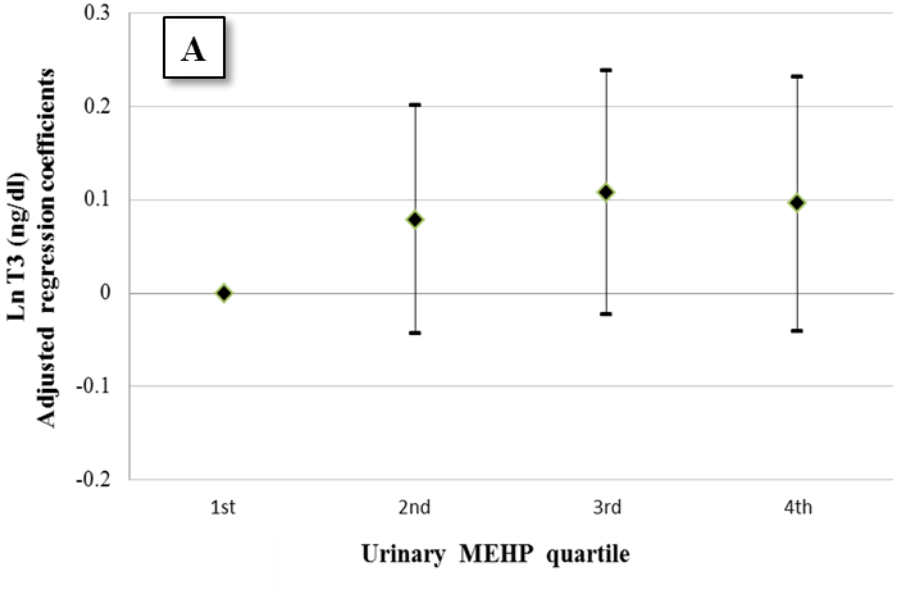

G
0.4
0.3
0.2
0.1
0
-0.1
Ln IGF-1 (ng/dl)
Adjusted regression coefficients
1st
2nd
3rd
4th
Urinary MEHP quartile

Figure 1. Regression coefficients (95% confidence intervals) for a change in T_4 associated with quartiles of urinary MEHHP (A) and ΣDEHPm (B) concentrations and free T_4 associated with quartiles of urinary MEHP (C), MEOHP (D), MnBP (E) and MEP (F) concentrations, as well as IGF-1 associated with quartiles of urinary MEHP (G) concentrations in adults. All models were adjusted for age, gender, BMI, urinary creatinine levels, and serum TBG levels or IGFBP-3 levels.


A
0.3
0.2
0.1
0
-0.1
-0.2
Ln T3 (ng/dl)
Adjusted regression coefficients
1st
2nd
3rd
4th
Urinary MEHP quartile

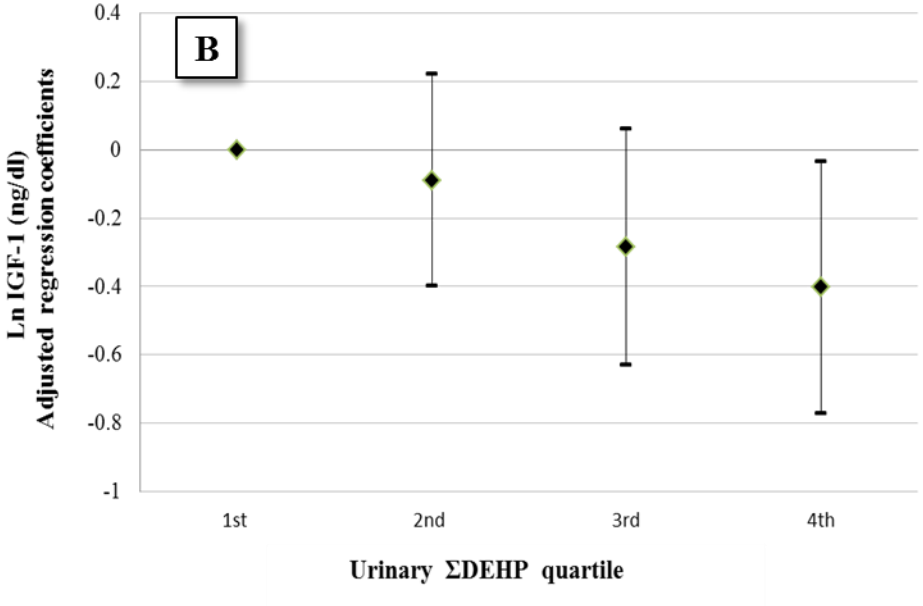

B
0.4
0.2
0
-0.2
-0.4
-0.6
-0.8
-1
Ln IGF-1 (ng/dl)
Adjusted regression coefficients
1st
2nd
3rd
4th
Urinary ΣDEHP quartile

Figure 2. Regression coefficients (95% confidence intervals) for a change in T_3 associated with quartiles of urinary MEHP (A) concentrations and IGF-1 associated with quartiles of urinary ΣDEHPm (B) concentrations in minors. All models were adjusted for age, gender, BMI, urinary creatinine levels, and serum TBG levels or IGFBP-3 levels.