Associations between urinary phthalate monoesters and thyroid hormones in pregnant women

Po-Chin Huang\(^1\), Pao-Lin Kuo\(^2\), Yue-Liang Guo\(^3\), Pao-Chi Liao\(^1\) and Ching-Chang Lee\(^{1,4}\)

\(^1\)Department of Environmental and Occupational Health, Medical College, National Cheng Kung University, Tainan, Taiwan, Republic of China; \(^2\)Department of Obstetrics and Gynecology, National Cheng Kung University Hospital, Tainan, Taiwan, Republic of China; \(^3\)Department of Environmental and Occupational Medicine, College of Medicine, National Taiwan University, Taiwan, Republic of China; \(^4\)Correspondence address. Tel: +886-6-274-4412; Fax: +886-6-274-3748; E-mail: cclee@mail.ncku.edu.tw

BACKGROUND: Maternal hypothyroidism during pregnancy can cause adverse effects in the fetus. Scientific evidence has shown that probable thyroid-like function of some phthalates in vitro and in vivo, and phthalates exposure, can begin in utero. This study investigated the association between phthalate exposure and thyroid hormones in pregnant women. METHODS: Serum and spot urine samples were collected from 76 Taiwanese pregnant women at second trimester. Thyroid hormones, including thyroid-stimulating hormone (TSH), triiodothyronine (T\(_3\)), thyroxine (T\(_4\)) and free T\(_4\) (FT\(_4\)) were analysed in serum samples, and five urinary phthalate monoesters, including mono butyl phthalate (MBP), monoethyl phthalate (MEP) and mono ethylhexyl phthalate (MEHP), were measured. RESULTS: Urinary MBP, MEP and MEHP, the median levels of which were 81.8, 27.7 and 20.6 ng/ml, respectively, were the predominant substances in the urinary phthalate monoesters. Significant mild negative correlations were found between T\(_4\) and urinary MBP (\(R = -0.248\), \(P < 0.05\)), and between FT\(_4\) and urinary MBP (\(R = -0.368\), \(P < 0.05\)). After adjusting for age, BMI and gestation, urinary MBP levels showed negative associations with FT\(_4\) and T\(_4\) (FT\(_4\): \(\beta = -0.110\), \(P < 0.001\); T\(_4\): \(\beta = -0.112\), \(P = 0.003\)). CONCLUSIONS: Exposure to di-n-butyl phthalate (DBP) may affect thyroid activity in pregnant women, but how DBP affects thyroid function is unclear. Further studies are needed to elucidate the mechanism of action and to investigate whether any other factors related to DBP exposure alter the thyroid function.

Keywords: pregnant women; urinary phthalate monoesters; thyroid hormones.

Introduction
Thyroid hormone is essential for fetal development of the brain, neurons, heart and other organs during critical points of gestation (Cunningham et al., 2006). The prevalence of subclinical hypothyroidism is 2–5% in pregnant women. Maternal hypothyroidism during pregnancy causes preterm birth and low birth weight, and it impairs post-natal mental development in infants (Haddow et al., 1999; Poppe et al., 1999; Poppe and Glinoer, 2003). Therefore, fetal thyroid function crucially depends on the supply of maternal thyroid hormones during pregnancy (Morreale de Escobar et al., 2004).

Some environmental toxicants, such as perchlorate, polychlorinated biphenyls (PCBs) and inorganic mercury (Hg), potentially alter human thyroid function during pregnancy by inhibiting iodide transport, inhibiting thyroglobulin iodination, or competitively inhibiting thyroid hormone binding receptors (Wolff, 1998; Takser et al., 2005; Wang et al., 2005). Phthalates, including butyl benzyl phthalate (BBP), di-n-butyl phthalate (DBP), di-(2-ethylhexyl) phthalate (DEHP) and di-ethyl phthalate (DEP) are reproductive and developmental toxicants in animal models (Api, 2001; Kavlock et al., 2002a,b, 2006). DBP and DEHP are considered anti-androgenic endocrine disruptors because of their possible effect on animal gonads and reproduction (Harris et al., 1997; Ema and Miyawaki, 2001). In addition, some studies (Hinton et al., 1986; Price et al., 1988; Poon et al., 1997; Sugiyama et al., 2005; Pereira et al., 2007) have reported possible antagonistic effects of phthalates on the thyroid gland in vivo and thyroid tissue in vitro. However, little is known about this issue in humans.

Phthalates are added to plastics to make them soft and flexible, to cosmetics as a vehicle for fragrance, and many other daily products, such as building materials, paints, children’s toys and medical devices (Api, 2001; ATSDR, 1995, 2001, 2002; Kavlock et al., 2002a). Because phthalates are released from these products, humans are exposed to them by food consumption, inhalation and dermal absorption. Phthalates are metabolized to their monoesters within a few hours or
Materials and Methods

Participants
Our participants were pregnant women for whom abnormal blood biochemical levels of alpha fetal protein and free β-hCG or advanced maternal age (>35 years old) suggested the need to undergo amniocentesis by the clinical suggestion from gynaecologists. After they signed the informed consent for amniocentesis (for medical purposes), we interviewed them and explained the benefits and risks of participating in this longitudinal project during 2005–2006. After signing the informed consent form for this study, 76 (86.3%) of the women initially recruited were followed up in their second trimester. There were 12 women excluded either because they were carrying a fetus with abnormal genetic defects or because they miscarried. The protocol and informed consent form were approved by the Institutional Review Board of National Cheng Kung University Hospital.

Sample collection
We drew 8-ml blood samples via venipuncture into chemically clean glass tubes containing no anti-coagulant. After the blood had been centrifuged, serum samples were obtained for thyroid hormone analysis. Urine samples were collected in 250-ml glass vessels and immediately transferred into 12-ml amber glass bottles for phthalate monoester analysis. To prevent possible contamination of the urine samples, all the glassware was washed in methanol (MeOH), acetonitrile (ACN) and acetone, and then sealed with aluminum foil. All the serum and urine samples were collected at the same time and stored at −70 and −20°C, respectively, until they were analysed.

Urinary phthalate monoester analysis
We used a slightly modified version of a previously published method of measuring urinary phthalate monoester levels using high performance liquid chromatography electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS), offline solid-phase extraction (SPE) and isotope dilution (Blount et al., 2000; Silva et al., 2004). MBP, MBzP, MEHP, MEP, MMP (all >99.9%) and their 13C-labelled internal standard (>99.9%) were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Ammonium acetate (>98%) was purchased from Sigma Aldrich Laboratories, Inc. (St. Louis, MO, USA). Ammonium hydroxide (30%) and ethyl acetate (>99.9%) were purchased from J. T. Baker (Phillipsburg, NJ, USA). MeOH, ACN, formic acid (all >98%) and water (HPLC-grade) were purchased from Merck (Darmstadt, Germany). β-glucuronidase (Escherichia coli-K12) was purchased from Roche Biomedical (Mannheim, Germany). The Mightysil RP-18 GP (L) (100 mm x 2.0 mm, 5 μm) analytical column and Mightysil RP-18 GP (5 mm x 2.0 mm, 5 μm) guard column were purchased from Kanto Chemical Industries (Tokyo, Japan).

Each urine sample (1 ml) was thawed, sonicated for 6 min and poured into a glass culture tube (75 x 125 mm) (Kimax borosilicate glass tubes; Kimble/Kontes, Vineland, NJ, USA). Samples were then buffered with ammonium acetate (250 μl, 1M pH 6.5) and then spiked with a mixture of isotope phthalate monoester standards (100 ng, 0.1 ng/μl) and β-glucuronidase enzyme (5 μl, 200 U/ml). The sample was incubated at 37°C for 90 min to deconjugate the glucuronidated phthalate metabolites. After the sample was incubated, it was loaded into a SPE cartridge (Nexus; Varian, Inc., Palo Alto, CA, USA). Aliquots of 1 ml each of formic acid and H2O were eluted to remove hydrophilic compounds. Then, 2 ml of acetonitrile and 2 ml of ethyl acetate were added to collect phthalate monoesters. The extract was dried with nitrogen gas and reconstituted with 1 ml of HPLC-grade H2O in a 2-ml glass vial. One blank and one quality control (QC) sample were included in each batch of samples analysed. The QC sample was spiked in pooled urine with a mixture of phthalate monoester standards (100 ng/ml). The Mightysil RP-18 GP (L) analytical column with a 5-mm guard column was used for chromatographic fractionation. The chromatographic fractionation was done using a linear gradient program with an organic solvent (acetonitrile and 0.1% formic acid) and an aqueous solvent (H2O/MeOH (9:1, v/v)) at a flow rate of 0.6 ml/min. The effluent was then directly analysed using tandem mass spectrometry (API 365; Applied Biosystems, Foster City, CA, USA) with electro-spray ionization. The limits of detection (LOD) for five urinary phthalate monoesters were 5.0 ng/ml (MBP), 1.8 ng/ml (MBzP), 0.9 ng/ml (MEHP), 1.4 ng/ml (MEP) and 1.8 ng/ml (MMP). The calibration ranges of urinary MBP and other urinary phthalate monoesters were 10–1000 and 5–1000 ppb, respectively. The correlation coefficient (R2) and relative SD (RSD) of the calibration curve should be >0.995 and <15%, respectively. The recoveries of 13C12-labelled internal-standard and native-standard of each phthalate monoester in samples should be >50 and 75%, respectively. The phthalate monoesters level of the blank sample should be lower than twice of the minimum detectable limit in each batch. The SPE recoveries of five phthalate monoesters ranged from 74 to 88%, and the RSD of spiked QCs ranged from 9 to 23%.

Creatinine and thyroid hormones examination
Creatinine in urine and thyroid hormones in serum samples was measured by our hospital’s pathology department. Samples of 8 ml of urine that had been stored at −20°C were analysed using combined clinical chemistry and immunoassay tests (Modular Analytics Serum Work Area; Roche Diagnostics). Samples of 2 ml of serum were analysed for estradiol (E2), FSH, progesterone (PG), triiodothyronine (T3), thyroxin (T4), free T4 (FT4) and thyroid-stimulating hormone (TSH) using an electrochemoluminescence immunoassay (ECLIA) (Elecsys 2010 and Modular Analytics E170; Roche Diagnostics).
When the level of creatinine exceeded the reference range, it was analysed again for confirmation.

**Questionnaire**

An interview questionnaire was designed to obtain information about the general phthalate-exposure scenarios of pregnant women. Participants were asked to provide information about personal characteristics (age, height, weight, education, occupational history, medical care status, pregnancy history, etc.) and life-style habits (alcohol intake and tobacco use) to adjust for other confounding factors. Trained interviewers administered the questionnaires according to standard operating procedures prepared in advance.

**Statistical analysis**

Commercially available statistical software (JMP version 5.01; SAS Institute, Cary, NC, USA) was used for statistical analysis. Outcomes were evaluated for normal distribution and outliers. Levels of T3, T4 and FT4 were normal, so these data did not need to be log transformed. On the contrary, the concentrations of E2, FSH, PG and TSH were log adjusted for significant covariates.

The mean age of the participants was 33.6 ± 3.3 years (range: 26–43 years). The average duration of gestation when recruited and BMI were 27.9 ± 2.3 weeks and 20.9 ± 2.5, respectively. The average number of pregnancies and child-births per participant were 1.9 ± 1.0 and 1.5 ± 0.6, respectively. All our participants were non-smokers, but 14 participants had been exposed to passive smoke (18.4%). None of the participants were ‘alcohol drinkers’, which was defined as ‘someone who consumed any alcohol at all during pregnancy’. About 25% of them had been in a newly decorated home or workplace within one year before the study. Less than 5% of them had received medical care, such as a blood transfusion or intravenous drip. The detectable rates of MBP, MEHP, MEP, MMP and MBzP in all urine samples were 96, 100, 100, 63 and 17%, respectively (Table 2). Median levels with (and without) creatinine adjustments for five urinary phthalate monoesters were 195 (81.8 ng/ml) for MBP, 68.0 (27.7 ng/ml) for MEHP, 60.8 (20.6 ng/ml) for MEP, 10.8 (4.3 ng/ml) for MMP and 3.7 (0.9 ng/ml) for MBzP (Table 2). Levels of urinary MBP, MEP and MEHP were the highest of the five metabolites measured, which indicated that the participants were exposed predominantly to the phthalates DBP, DEP and DEHP. Although median urinary MBP levels were three times higher than median urinary MEP levels, the 95th percentile level of urinary MEP was seven times higher than that of urinary MBP, which showed the large variation of DEP exposure in pregnant women. The creatinine-adjusted levels of urinary phthalate monoesters were at least twice those which were not creatinine-adjusted (Table 2). Therefore, we used creatinine-unadjusted phthalate monoester levels for further analysis.

**Results**

**Demographic characteristics of participants**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.6 ± 3.3</td>
</tr>
<tr>
<td>BMI</td>
<td>20.9 ± 2.5</td>
</tr>
<tr>
<td>Duration of gestation (weeks)</td>
<td>27.9 ± 2.3</td>
</tr>
<tr>
<td>Pregnancies and births</td>
<td></td>
</tr>
<tr>
<td>Number of current pregnancy</td>
<td>1.9 ± 1.0</td>
</tr>
<tr>
<td>Number of current birth</td>
<td>1.5 ± 0.6</td>
</tr>
<tr>
<td>Smoking status (n/%)</td>
<td></td>
</tr>
<tr>
<td>Active smoker</td>
<td>0/0</td>
</tr>
<tr>
<td>Passive smoker</td>
<td>14/18.4</td>
</tr>
<tr>
<td>Non-smoker</td>
<td>62/81.6</td>
</tr>
<tr>
<td>Alcohol drinker (n/%)</td>
<td>0/0</td>
</tr>
<tr>
<td>New decoration of living or working place during previous 1 year (n/%)</td>
<td></td>
</tr>
<tr>
<td>Home</td>
<td></td>
</tr>
<tr>
<td>Moving to a new house</td>
<td>7/9.2</td>
</tr>
<tr>
<td>Just decorated</td>
<td>4/5.3</td>
</tr>
<tr>
<td>Workplace</td>
<td></td>
</tr>
<tr>
<td>Moving to a new workplace</td>
<td>2/2.6</td>
</tr>
<tr>
<td>Just decorated</td>
<td>6/7.9</td>
</tr>
<tr>
<td>Medical care during previous 3 months (n/%)</td>
<td></td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>3/3.9</td>
</tr>
<tr>
<td>Intravenous drip</td>
<td>3/3.9</td>
</tr>
<tr>
<td>Oxygen mask</td>
<td>1/1.3</td>
</tr>
</tbody>
</table>

"Moving to new house or workplace’ means moved into a new building. 'Just decorated’ means the place lived or worked in was recently decorated, e.g. painted or the flooring was changed.

**Urinary phthalate monoesters**

The detectable rates of MBP, MEHP, MEP, MMP and MBzP in all urine samples were 96, 100, 100, 63 and 17%, respectively (Table 2). Median levels with (and without) creatinine adjustments for five urinary phthalate monoesters were 195 (81.8 ng/ml) for MBP, 68.0 (27.7 ng/ml) for MEHP, 60.8 (20.6 ng/ml) for MEP, 10.8 (4.3 ng/ml) for MMP and 3.7 (0.9 ng/ml) for MBzP (Table 2). Levels of urinary MBP, MEP and MEHP were the highest of the five metabolites measured, which indicated that the participants were exposed predominantly to the phthalates DBP, DEP and DEHP. Although median urinary MBP levels were three times higher than median urinary MEP levels, the 95th percentile level of urinary MEP was seven times higher than that of urinary MBP, which showed the large variation of DEP exposure in pregnant women. The creatinine-adjusted levels of urinary phthalate monoesters were at least twice those which were not creatinine-adjusted (Table 2). Therefore, we used creatinine-unadjusted phthalate monoester levels for further analysis.

**E2 and thyroid-related hormone levels**

There is no reference range for E2 or thyroid hormones during pregnancy. However, our data still provided some clues by comparing participants’ levels with those of the general population in Taiwan. More than 90% of the levels of the thyroid hormones T3, T4 and TSH were within the reference values of the general population (Table 3). The median level (0.93 ng/dl) of FT4, however, merely matched the lowest level for the general population, which indicated that half of our participants might have had a mild insufficiency of T4 (i.e. hypothyroidism). In addition, the median creatinine level
Increasing age was correlated with lower T₃ and T₄ levels, whereas older (older) were found between T₄ and urinary MBP (P = 0.05) and FT₄ and urinary MBP (P = 0.05) (Table 4). Significantly mild negative correlations were found between T₄ and MBP (R = −0.248, P < 0.05) and FT₄ and MBP (R = −0.368, P < 0.05). Increasing age was correlated with lower T₃ and T₄ levels, and increasing BMI was correlated with higher urinary MBP levels (creatinine-adjusted and not creatinine-adjusted). After adjusting for age, BMI, gestational age and other phthalate monoesters of the participants, urinary MBP levels showed a negative association with FT₄ and T₄ (FT₄; β = −0.110, P < 0.001; T₄; β = −0.112, P = 0.003).

### Discussion

We found a correlation between high-exposure levels of certain phthalates and alterations of some thyroid hormones in the pregnant women in the present study. High urinary MBP exposure was associated with lower serum T₄ and FT₄ in these women during the second trimester. Although our sample size was small, the association between the concentrations of urinary MBP and FT₄ existed after a multivariate analysis. Few toxicological data are available on phthalate exposure, monoesters in pregnant women may have obscured the correlation. The original concentration of each phthalate monoester is suggested for creatinine-sensitive individuals such as pregnant women. To identify the major factors contributing to serum T₄ and FT₄ concentrations, we used a multivariate regression model to examine the association between thyroid hormone levels and urinary phthalate monoesters (Table 5).

### Table 3: Estrogenic and thyroid hormone distribution of pregnant women (n = 76)

| Hormone (pg/ml) | Percentile | Min | 5th | 25th | 50th | 75th | 95th | Max | Reference range

| E₂ (pg/ml) | 4856.0 | 7549.0 | 11 748.0 | 16 670.0 | 22 145.0 | 34 820.0 | 47 820.0 | 5.1–4250.0
| FSH (mIU/ml) | 0.1 | 0.1 | 0.1 | 0.1 | 0.11 | 0.14 | 0.16 | 0.11–198.0
| Progesterone (ng/ml) | 45.0 | 49.0 | 81.0 | 115.0 | 160.0 | 309.0 | 441.9 | 0.035–59.0
| T₃ (ng/dl) | 72.6 | 86.3 | 114.0 | 132.0 | 152.0 | 209.0 | 246.0 | 84.6–202.0
| T₄ (ng/dl) | 4.39 | 5.31 | 7.53 | 8.85 | 9.97 | 11.2 | 13.6 | 5.13–14.1
| Free T₄ (ng/dl) | 0.46 | 0.69 | 0.80 | 0.93 | 1.04 | 1.25 | 1.35 | 0.93–1.7
| TSH (mIU/ml) | 0.22 | 0.31 | 0.74 | 1.1 | 1.6 | 3.4 | 5.19 | 0.27–4.2
| Creatinine (mg/dl) | 3.9 | 6.4 | 17.3 | 39.4 | 75.5 | 158.6 | 185.6 | 30.0–125.0

### Table 2: Unadjusted and adjusted-creatinine concentrations of urinary phthalate monoesters in our study and compared with other studies

| Urinary phthalate monoesters | Percentile | Median (range) | US pregnant women | US female popula
d

| MBP | 76 | 13.2 | 21.6 | 40.6 | 81.8 | 131.0 | 368.0 | 580.0 | 3.0 (5.8–167)
| MBzP | 76 | 0.9 | 0.9 | 0.9 | 0.9 | 33.4 | 35.3 |
| MEP | 76 | 2.2 | 3.2 | 5.2 | 7.4 | 124.0 | 140.0 | 271.0 | 174.0 (28–2230)
| MEHP | 76 | 5.85 | 7.21 | 13.1 | 20.6 | 38.6 | 273.0 | 381.0 |
| MMP | 76 | 0.7 | 0.7 | 0.7 | 4.3 | 14.7 | 87.8 | 237.2 |

**Note:** MBP, monobutyl phthalate; MBzP, monobenzyl phthalate; MEP, monoethyl phthalate; MEHP, mono-2-ethylhexyl phthalate and MMP, monomethyl phthalate.

**Reference range for the general population in Taiwan. The analytic sensitivities of T₃, T₄, free T₄ and TSH were 19.5 ng/dl, 0.42 pg/μl, 0.023 ng/dl and 0.014 μIU/ml, respectively; those of creatinine, E₂, FSH and progesterone were 0.05 mg/dl, 5.0 pg/μl, 0.1 mIU/ml and 0.03 ng/ml, respectively. The coefficient variations of T₃, T₄, free T₄ and TSH were 2.9, 4.2, 3.1 and 3.0%, respectively; those of creatinine, E₂, FSH and progesterone were 1.8, 4.1, 3.4 and 4.0%, respectively.
studies (Blount et al., 2000; Koch et al., 2003; Silva et al., 2004a). Fourth, although phthalate monoester levels vary within days, it has been suggested (Hoppin et al. 2002) that a single urine sample is a good indicator for phthalate monoester measurement. Good sensitivity and specificity for predicting human exposure to urinary MEP, MBP, MBzP, MMP and MEHP has been reported to range between 0.56 and 0.74 and 0.83 and 0.9, respectively, (Hoppin et al. 2002). Therefore, temporal variability of phthalate metabolites may also reduce the correlation between thyroid hormone and urinary phthalate monoester levels. Fifth, using creatinine to adjust the phthalate monoester levels in pregnant women may be not appropriate. Creatinine is influenced by muscle mass, racial differences and dietary intake of meat. In addition, by the second trimester, the glomerular filtration rate and renal blood flow in pregnant women were ~50 and 70% higher, respectively, than in age-matched healthy women who were not pregnant (James et al., 2005). The serum creatinine levels of pregnant women dropped about 10% in the second trimester and increased to normal values post-partum (Kuhlback Widholm, 1966). Therefore, urinary creatinine level may be unusually diluted or concentrated during pregnancy. Urinary MBP, MEP and MEHP levels in US pregnant women were ~1.5 times higher than in age-matched healthy US women who were not pregnant (Adibi et al., 2003). Finally, although creatinine-adjusted phthalate monoester levels may be affected by using cosmetics or personal care products, they are ultimately determined by the physiological change of creatinine during pregnancy.

The highest urinary MEP level (5466 ppb) was found in a participant who was a cosmetologist and had worked for >10 years before she became pregnant. Urinary MEP is a major phthalate metabolite associated with people who use personal care products and with pregnant women exposed through inhalation (Adibi et al. 2003; Duty et al. 2005). Therefore, cosmetologists may be exposed to higher levels of DEP through direct skin contact and inhalation of DEP evaporation from

\[ \text{FSH}^b \quad \text{E2}^b \quad \text{PG}^b \quad \text{TSH}^b \quad \text{T3} \quad \text{T4} \quad \text{FT4} \quad \text{BMI} \quad \text{Gestation} \quad \text{MBP} \quad \text{MEP} \quad \text{MEHP} \quad \text{MBzP} \quad \text{MMP} \]

\[ \begin{array}{cccccccccccccc}
\text{FSH} & 1.0 \\
\text{E2} & -0.192 & 1.0 \\
\text{PG} & -0.252^* & 0.566^* & 1.0 \\
\text{TSH} & 0.287^* & -0.171 & -0.102 & 1.0 \\
\text{T3} & -0.051 & -0.033 & 0.078 & 0.145 & 1.0 \\
\text{T4} & -0.006 & -0.039 & 0.054 & 0.291^* & 0.709^* & 1.0 \\
\text{FT4} & -0.095 & -0.007 & 0.061 & 0.172 & 0.299^* & 0.761^* & 1.0 \\
\text{Age} & 0.077 & -0.009 & 0.025 & 0.203 & -0.307^* & -0.218^* & -0.008 & 1.0 \\
\text{BMI} & -0.120 & -0.087 & -0.174 & -0.044 & 0.309 & 0.137 & -0.010 & -0.106 & 1.0 \\
\text{Gestation} & -0.302^* & 0.419^* & 0.524^* & -0.106 & -0.018 & 0.038 & 0.057 & -0.029^* & -0.131 & 1.0 \\
\text{MBP} & 0.204 & 0.034 & -0.046 & 0.079 & -0.234 & -0.248^* & -0.368^* & -0.082 & 0.081^* & -0.098 & 1.0 \\
\text{MEP} & -0.016 & -0.178 & -0.132 & -0.020 & -0.212^* & -0.292^* & -0.191^* & 0.156 & -0.133 & -0.116 \\
\text{MEHP} & -0.007 & 0.127 & -0.015 & -0.066 & -0.100 & -0.090 & 0.166 & -0.030 & 0.030^* & 0.070^* & 0.466 & 1.0 \\
\text{MBzP} & -0.031 & 0.067 & 0.043 & -0.009 & -0.007 & -0.059 & -0.056 & 0.156 & -0.114^* & 0.026 & 0.194^* & 0.130 \\
\text{MMP} & -0.055 & 0.207 & 0.065 & -0.082 & -0.019 & -0.039 & 0.017 & 0.080 & 0.095^* & 0.199 & 0.183^* & 1.0 \\
\text{MEHP} & -0.056 & 0.217 & 0.109 & -0.070 & -0.008 & -0.021 & 0.041 & 0.142 & 0.064 & 0.234 & 0.208^* \\
\text{MBzP} & -0.013 & -0.010 & -0.161 & -0.078 & -0.259 & -0.089 & 0.007 & -0.038 & -0.006 & -0.004 & 0.190 & -0.031 & 0.184 & 0.182 & 1.0 \\
\text{MBP} & -0.015 & 0.027 & -0.094 & 0.181 & -0.223 & -0.199 & -0.037 & 0.118 & -0.129 & 0.034 & 0.387^* & 0.394^* & 0.082 & 0.060 \\
\end{array} \]

*aSpearman correlation coefficients: *P < 0.05. One outlier was excluded because of hypothyroidism (n = 75). Correlation coefficient of using creatinine-adjusted urinary phthalate monoesters are given in parentheses.
cosmetic products. In addition, a recent study reported the synergistic effect of DEP and PCB on rat thyroids (Pereira et al., 2007). We suggest that risk assessment of occupational phthalate exposure in cosmetologists, hairdressers, cosmetics saleswomen, etc. at reproductive age is worthy of further investigation.

This is the first report that shows an association between phthalate exposure and thyroid hormones in pregnant women. Although maternal thyroid hormone changes are linked to phthalate monoester levels in urine, we believe that our data may have missed the most critical period of fetal development. Fetal thyroid is not functional before the 12th week of gestation, which indicates that the fetus depends entirely on maternal thyroid hormones during the first trimester (Morreale de Escobar and Rove, 2004; Zoeller et al., 2004). However, a maternal surge of free \( T_4 \) concentrations occurs in the first trimester (between 6 and 12 weeks) in healthy pregnant women, and free \( T_4 \) concentrations gradually decrease and become steady after the middle of the second trimester, a suitable clinical point for determining the variation of thyroid hormones in pregnant women, and are maintained until term (Glinoer, 1997; Hume et al., 2004; Morreale de Escobar et al., 2004). Some longitudinal studies of large numbers of pregnant women without iodine deficiency reported that serum \( FT_4 \) levels were an average of 10–15% lower at delivery than in non-pregnant women. However, free hormone levels are maintained within the non-pregnant reference range in most pregnant women (Ball et al., 1989;
Burrow et al., 1993; Sieiro Netto et al., 2004). Based on the scientific evidence above, concentrations of urinary phthalate monoesters might be an alternation of maternal phthalates exposure in early pregnancy if the exposure profiles of phthalates in pregnant women were consistent. Since food consumption is the main exposure route of DBP in the general population (Chan and Meek, 1994), accounting for over 90% of DBP exposure. In addition, MBP has been detected in amniotic fluid, which revealed a possible indicator of thyroid function in the fetus (Silva et al. 2004b). Therefore, our data may be a proxy of maternal phthalate exposure in early pregnancy.

Conclusions

We found that levels of T₄ and FT₄ in pregnant women were significantly negatively associated with urinary MBP levels after adjusting for age, BMI and gestation time, whereas we found no significant association between E₂, FSH, or PG and urinary phthalate monoester levels. The fall in T₄ and FT₄ levels during pregnancy may be potentially harmful to fetal development. Hence, several questions require further investigation. Do phthalates affect thyroid hormones beginning in early pregnancy? What mechanisms do phthalates and their metabolites use to regulate thyroid hormones?

Acknowledgements

We are grateful for the pregnant women who participated in this study. We are also greatly in debt to our colleagues at the Research Center of Environmental Trace Toxic Substances, National Cheng Kung University, Tainan, Taiwan, for instrument support. This work was supported by grant (NSC 93-2621-Z-006-005) from the National Science Council in Taiwan.

References


Table 5: Multivariate linear regression between serum FT₄ and T₄ levels, and their corresponding urinary phthalate monoesters (FT₄: R² = 0.240; T₄: R² = 0.187)*

<table>
<thead>
<tr>
<th>Variables</th>
<th>FT₄ (pmole/l)</th>
<th>T₄ (nmole/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estimate</td>
<td>Prob&gt;</td>
</tr>
<tr>
<td>Intercept</td>
<td>1.270</td>
<td>0.013</td>
</tr>
<tr>
<td>Age</td>
<td>0.024</td>
<td>0.886</td>
</tr>
<tr>
<td>BMI</td>
<td>0.088</td>
<td>0.579</td>
</tr>
<tr>
<td>Gestational age</td>
<td>−0.117</td>
<td>0.598</td>
</tr>
<tr>
<td>MBP</td>
<td>−0.110</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>MEP</td>
<td>0.026</td>
<td>0.124</td>
</tr>
<tr>
<td>MEHP</td>
<td>−0.015</td>
<td>0.474</td>
</tr>
<tr>
<td>MBzP</td>
<td>0.022</td>
<td>0.232</td>
</tr>
<tr>
<td>MMP</td>
<td>0.016</td>
<td>0.165</td>
</tr>
</tbody>
</table>

*One outlier was excluded because of hypothyroidism (n = 75). All the parameters were log transformed.


James DK, Steer PJ, Weiner CP, Gonik B. High Risk Pregnancy: Management of Environmental Trace Toxic Substances, National Cheng Kung University, Tainan, Taiwan, for instrument support. This work was supported by grant (NSC 93-2621-Z-006-005) from the National Science Council in Taiwan.
Huang et al.


