Assessment of Di (2-ethylhexyl) Phthalate Exposure by Urinary Metabolites as a Function of Sampling Time

Moon-seo Park1, Yun-jung Yang1, Yeon-pyo Hong1, Sang-yon Kim1, Yong-pil Lee2

1Department of Preventive Medicine, College of Medicine; 2Graduate School of Social Development, Chung-Ang University

Objectives: In most DEHP exposure assessment studies, single spot urine sample was used. It could not compare the exposure level among studies. Therefore, we are going to represent the necessity of selection of proper sampling time of spot urine for assessing the environmental DEHP exposure, and the association urinary DEHP metabolites with steroid hormones.

Methods: We collected urine and plasma from 25 men. The urine sampling times were at the end of the shift (post-shift) and the next morning before the beginning of the shift (pre-shift). Three metabolites of DEHP (mono(2-ethylhexyl) phthalate [MEHP], mono-(2-ethyl-5-hydroxyhexyl)phthalate [MEHHP], and mono(2-ethyl-5-oxohexyl)phthalate [MEOHP]) in urine were analyzed by HPLC/MS/MS. Plasma luteinizing hormone, follicle stimulating hormone, testosterone, and 17β-estradiol were measured at pre-shift using a ELISA kit. A log-transformed creatinine-adjusted urinary MEHP, MEHHP, and MEOHP concentration were compared between the post- and pre-shift. The Pearson’s correlation was calculated to assess the relationships between log-transformed urinary MEHP concentrations in pre-shift urine and hormone levels.

Results: The three urinary metabolite concentrations at post-shift were significantly higher than the concentrations in the pre-shift (p<0.0001). The plasma hormones were not significantly correlated with log-transformed creatinine-adjusted DEHP metabolites.

Conclusions: To assess the environmental DEHP exposure, it is necessary to select the urine sampling time according to the study object. There were no correlation between the concentration of urinary DEHP metabolites and serum hormone levels.

Key words: DEHP metabolites, Exposure, Sampling time, Steroid hormones, Urine, HPLC/MS/MS

INTRODUCTION

Phthalate is a common man-made chemical to which humans are exposed. In particular, di(2-ethylhexyl) phthalate (DEHP) is one of the most widespread phthalate plasticizers, and is extensively used in the preparation of flexible polyvinylchloride (PVC) plastics [1]. DEHP is used in a variety of applications, including wire and cable insulation, wallpaper, vinyl upholstery, car seats, footwear, raincoats, packaging, children’s toys, and medical devices (tubing and blood storage bags) [2-4]. The total production volume of DEHP in Western Europe was 2 hundred thousand tons in 2004, and approximately 7 million tons worldwide [5]. In Korea, DEHP accounts for 86% of phthalate production approximately 125 tons [6].

Toxicology studies have reported that MEHP is toxic to Leydig cells and Sertoli cells, which play crucial roles in spermatogenesis and testosterone production in the testis [7-10]. The MEHP-induced inhibition of testosterone production in Leydig cells is thought to be associated with decreased pituitary luteinizing hormone secretion and reduced steroidogenic enzyme activity [7]. In experimental studies, DEHP has anti-androgenic activity and male reproductive toxicity [8,10-12]. The correlations between urinary phthalate metabolites in pregnant women and subtle genital changes in their infant males [13], and breast-milk phthalate metabolites and steroid hormone in male infant [14] have been reported. Even if fetuses and infants are considered more susceptible to environmental contaminants than adult, the association between urinary phthalate metabolites in adult male and reproductive function was investigated [15-19].

The urinary metabolites are used in biological monitoring of general human population for DEHP exposure in the USA and Europe [20,21]. The levels of urinary DEHP metabolites have been reported in Korean children and adult Korean women [22,23] and the plasma levels of DEHP have been reported in adult Korean males [24]. DEHP is rapidly metabolized to its monoester, mono(2-ethylhexyl) phthalate (MEHP), which is believed to be the active molecule. It can be further metabolized to oxidative products such as mono-
(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and metabolized into mono(2-ethyl-5-oxohexyl)phthalate (MEOHP) [25-27]. Monoesters and the oxidative metabolites of phthalates may be conjugated as the glucuronide (phase II biotransformation), and both free and conjugated metabolites can be excreted in the urine and feces [19]. Due to the ubiquitously present of DEHP in environment and laboratory equipment, the metabolites were used to assess the DEHP exposure.

In most studies, the single spot urine samples were collected on the day of monitoring [18,20,28-30]. However, urinary metabolites levels could have temporal variability [28], because the half life time of urinary metabolites after a single DEHP dose in a man was about 5 to 10 hours [31]. Therefore, the appropriate urine collection time would be considered for assessment of DEHP exposure.

This study was going to represent the necessity of selection of proper sampling time of spot urine for assessing the environmental DEHP exposure, and the association urinary DEHP metabolites- MEHP, MEHHP and MEOHP- with steroid hormones which were luteinizing hormone, follicle-stimulating hormone, testosterone and estradiol.

METHODS

I. Subjects

The investigation was conducted on 25 adult males who work in dental laboratories. They were exposed to DEHP during the work hours by dermal contact and through dust inhalation. They resided in Seoul, Korea. The study participants were between 30 and 50 years of age (median, 38.5 years). The subjects were provided a questionnaire about general characteristics (alcohol consumption, smoking amount, and activities) and phthalate exposure (use of coating materials, hemodialysis, and hospital admissions). All protocols were approved by the Ethics Committee for the Protection of Persons in Biochemical Research at the Institute of Medical Science of Chung-Ang University in Seoul, Korea. All subjects volunteered to participate in the study and gave written informed consent.

II. Sample Collection

For comparison of DEHP exposure during the work hours, each participant collected spot urine samples on two consecutive days using sterile polyethylene specimen cups. Urine samples were collected at the end of the shift (post-shift) and the next morning prior to the beginning of the shift (pre-shift). The post- and pre-shift urine samples were collected at 5-6 PM and 8-10 AM, respectively. None of the materials used were contaminated with detectable amounts of the target analytes. All specimen bottles were immediately frozen at -80°C until analysis. The creatinine content of each urine sample was recorded.

Blood samples were obtained between 8 AM and 10 AM after a 12-hour overnight fast for measuring sex steroid hormones. Each sample was collected into heparinized glass vacutainer tubes by a nurse via venipuncture of the median cubital vein. After blood collection, the blood samples were centrifuged at 3000 rpm for 15 min at room temperature and the plasma was collected. Plasma was frozen at -80°C until analysis.

III. DEHP Metabolite Analysis

1) Chemicals

MEHP, MEHHP, and MEOHP were purchased from Wako (Wako Pure Chemical Inc., Osaka, Japan), and 13C4-MEHP, 13C4-MEHHP, and 13C4-MEOHP were purchased for internal standards from Cambridge Isotopes Laboratories, Inc. (Andover, MA, USA). HPLC-grade acetonitrile and water were obtained from Merck (Darmstadt, Germany) and JT Baker (Phillipsburg, NJ, USA), respectively. Ammonium acetate (purity, 98.2%) and β-glucuronidase (from Escherichi. coli-K12) were purchased from Sigma (St. Louis, MO, USA) and Roche Diagnostics GmbH (Mannheim, Germany), respectively. To minimize the risk of contamination with DEHP during sample handling and analysis, all glassware used in the study was washed previously, sonicated with ethanol for 10 min, rinsed with methanol, then dried in oven for 2 hrs (> 180°C).

2) Analytical Procedure

The analytical method used to quantify the urinary DEHP metabolites has been described previously [22]. Each urine sample was analyzed by high-performance liquid chromatography with tandem mass spectrometry (HPLC/MS/MS), on-line enrichment, and column switching techniques.

3) Sample Preparation

Frozen urine samples were allowed to equilibrate to
room temperature. The samples were vortex-mixed and 490 μL aliquots were then transferred to 1.5 mL brown glass screw-cap vials. Then, 200 μL of ammonium acetate (1M, pH 6.5) and 10 μL of β-glucuronidase for releasing the monoester metabolites from their conjugated form were added to the samples. The samples were incubated for 2 hr at 37°C in a drying oven. After hydrolysis, each sample was sonicated for 10 min and 1% acetic acid of an acetonitrile solution (v/v %) was added for extraction (1:6 [v/v %]). The mixture was centrifuged (3000 rpm for 10 min), and the supernatant was transferred into another 1.5 mL glass screw-cap vial, then 10 μL of the supernatant was injected into the LC-MS/MS system for quantitative analysis.

4) Analytical Validation

Using the liquid chromatography-MS/MS conditions described above, the retention times for MEHP, MEHHP, and MEOHP were 14.4, 19.4, and 14.9 min, respectively. The calibration graphs obtained for each metabolite were linear (r² > 0.999) over the calibration range from 0.5-200 ng/mL. The limits of quantification (LOQ) were 2.5 ng/mL for MEHP, 2.3 ng/mL for MEHHP, and 1.5 ng/mL for MEOHP, and the limits of detection (LOD) were 0.8 ng/mL for MEHP, 0.9 ng/mL for MEHHP, and 0.5 ng/mL for MEOHP. The average recovery of MEHP, MEHHP, and MEOHP in urine samples ranged from 99%-111% (RSD <4.2 %, n=6).

IV. Determination of Plasma Hormones

Plasma levels of luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone (T), and 17β-estradiol (E2) were measured using a commercial IBL enzyme-linked immunosorbent assay (ELISA) kit (IBL International GMBH, Hamburg, Germany).

V. Statistical Analyses

Creatinine-adjusted urinary MEHP, MEHHP, and MEOHP concentrations were calculated using geometric means (GMs) and distribution percentiles. The urinary levels of MEHP, MEHHP, and MEOHP were skewed right and were transformed by the natural logarithm for statistical analysis. Plasma hormone concentrations of LH, FSH, T, and E2 closely approximated normality and were not transformed. Log-transformed creatinine-adjusted urinary MEHP, MEHHP, and MEOHP concentrations were compared between the post- and pre-shifts by paired t-tests. The Pearson’s correlation was calculated to assess the relationships between log-transformed urinary MEHP concentrations and hormone levels. All statistical analyses were carried out with STATA version 10 (StataCorp, Texas, USA). The statistically significant level was considered at a p<0.05.

RESULTS

I. General Characteristics

The general characteristics of the subjects are shown in Table 1. The median age and BMI were 38.9 years and 25.4 kg/m², respectively. The duration of residence was 25.3 ± 24.3 years. Forty percent of the workers used varnish during work hours.

II. Urinary DEHP Metabolites Concentrations

Urine samples were collected on 2 different days for 25 subjects. In the 50 samples, DEHP metabolites were detected in 100% of the samples. The unadjusted and creatinine-adjusted median concentrations of the three DEHP metabolites (MEHP, MEHHP, and MEOHP) were represent Table 2 and 3. The creatinine-adjusted log-transformed urinary DEHP metabolite concentrations (μg/g creatinine) were compared between the post- and pre-shift samples (Figure 1). The GMs were 3.10 μg/g Cr for MEHP, 4.37 μg/g Cr for MEHHP, and 3.40 μg/g Cr for MEOHP in the post-shift urine samples, and 2.23 μg/g Cr for MEHP, 3.54 μg/g Cr for MEHHP, and 2.65 μg/g Cr for MEOHP in the pre-shift urine samples. The three urinary metabolite concentrations at the end of the work day (post-shift) were significantly higher than the concentrations in the next morning samples (pre-shift)(p<0.0001).

### Table 1. General characteristics of the subjects (n=25)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (y)</td>
<td>38.9</td>
</tr>
<tr>
<td>Median BMI (kg/m²)</td>
<td>25.4</td>
</tr>
<tr>
<td>Residence duration (y)</td>
<td>25.3 ± 24.3</td>
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<td>Using varnish during working time</td>
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</tr>
<tr>
<td>No</td>
<td>15 (60.0)</td>
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<tr>
<td>Yes</td>
<td>10 (40.0)</td>
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</table>

The association between DEHP metabolites with steroid hormones. The urinary DEHP metabolite concentrations in the post-shift samples were significantly higher than pre-shift samples.

The concentrations of the three DEHP metabolites (MEHP, MEHHP, and MEOHP) were presented skewed right and were transformed by the natural logarithm for statistical analysis. The urinary MEHP, MEHHP and MEOHP concentrations in pre-shift were significantly lower than the post-shift, regardless of the creatinine adjustment (Table 3 and Figure 1). The GMs of urinary MEHP, MEHHP and MEOHP concentrations (µg/g creatinine) were 3.10 µg/g Cr, 4.37 µg/g Cr and 3.40 µg/g Cr in post-shift and 2.23 µg/g Cr, 3.54 µg/g Cr and 2.65 µg/g Cr in pre-shift (Table 3). The urinary DEHP metabolites of workers were not significantly different between varnish during working duration and not using (data was not shown). In Korea, the urinary level of MEHP was 9.6 µg/g Cr in children and 39.6 µg/g Cr in adult women [23]. The GMs of urinary MEHP were 19.5 µg/g Cr in boys and 15.3 µg/g Cr in girls [22]. The levels of urinary DEHP metabolites in our study were higher than in Korean children and lower than in adult Korean women. The median concentrations of urinary

### Table 2. The unadjusted MEHP, MEHHP and MEOHP (µg/L) concentration in dental technicians (n=25) in post- and pre-shift urine samples

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Sampling time</th>
<th>GM (µg/L)</th>
<th>GSD</th>
<th>Min (µg/L)</th>
<th>5th (µg/L)</th>
<th>25th (µg/L)</th>
<th>50th (µg/L)</th>
<th>75th (µg/L)</th>
<th>95th (µg/L)</th>
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<tbody>
<tr>
<td>MEHP</td>
<td>Post-shift</td>
<td>2.53</td>
<td>0.93</td>
<td>2.50</td>
<td>2.66</td>
<td>6.74</td>
<td>10.38</td>
<td>25.10</td>
<td>29.50</td>
</tr>
<tr>
<td></td>
<td>Pre-shift</td>
<td>2.14</td>
<td>0.97</td>
<td>1.03</td>
<td>2.11</td>
<td>4.99</td>
<td>7.51</td>
<td>15.00</td>
<td>48.20</td>
</tr>
<tr>
<td>MEHHP</td>
<td>Post-shift</td>
<td>3.81</td>
<td>0.69</td>
<td>15.10</td>
<td>18.70</td>
<td>27.20</td>
<td>43.35</td>
<td>70.00</td>
<td>137.00</td>
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<tr>
<td></td>
<td>Pre-shift</td>
<td>3.45</td>
<td>0.70</td>
<td>7.14</td>
<td>10.60</td>
<td>19.40</td>
<td>33.00</td>
<td>52.70</td>
<td>93.20</td>
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<tr>
<td>MEOHP</td>
<td>Post-shift</td>
<td>2.84</td>
<td>0.69</td>
<td>6.27</td>
<td>6.45</td>
<td>10.10</td>
<td>15.15</td>
<td>26.00</td>
<td>51.20</td>
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<tr>
<td></td>
<td>Pre-shift</td>
<td>2.56</td>
<td>0.71</td>
<td>2.72</td>
<td>4.02</td>
<td>9.15</td>
<td>13.70</td>
<td>19.40</td>
<td>33.60</td>
</tr>
</tbody>
</table>


### Table 3. The creatinine-adjusted MEHP, MEHHP and MEOHP (µg/g creatinine) concentration in dental technicians (n=25) in post- and pre-shift urine samples

<table>
<thead>
<tr>
<th>Metabolites</th>
<th>Sampling time</th>
<th>GM (µg/g creatinine)</th>
<th>GSD</th>
<th>Min (µg/g creatinine)</th>
<th>5th (µg/g creatinine)</th>
<th>25th (µg/g creatinine)</th>
<th>50th (µg/g creatinine)</th>
<th>75th (µg/g creatinine)</th>
<th>95th (µg/g creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEHP</td>
<td>Post-shift</td>
<td>3.10</td>
<td>0.84</td>
<td>5.06</td>
<td>6.69</td>
<td>14.56</td>
<td>22.20</td>
<td>38.33</td>
<td>76.67</td>
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<tr>
<td></td>
<td>Pre-shift</td>
<td>2.23</td>
<td>0.80</td>
<td>2.42</td>
<td>3.69</td>
<td>4.40</td>
<td>8.00</td>
<td>18.39</td>
<td>23.83</td>
</tr>
<tr>
<td>MEHHP</td>
<td>Post-shift</td>
<td>4.37</td>
<td>0.66</td>
<td>20.90</td>
<td>23.34</td>
<td>55.52</td>
<td>86.17</td>
<td>122.40</td>
<td>204.29</td>
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<tr>
<td></td>
<td>Pre-shift</td>
<td>3.54</td>
<td>0.62</td>
<td>9.69</td>
<td>16.33</td>
<td>25.18</td>
<td>31.07</td>
<td>54.74</td>
<td>114.63</td>
</tr>
<tr>
<td>MEOHP</td>
<td>Post-shift</td>
<td>3.40</td>
<td>0.67</td>
<td>7.59</td>
<td>7.86</td>
<td>12.63</td>
<td>33.04</td>
<td>46.68</td>
<td>87.26</td>
</tr>
<tr>
<td></td>
<td>Pre-shift</td>
<td>2.65</td>
<td>0.59</td>
<td>6.14</td>
<td>6.40</td>
<td>9.34</td>
<td>13.49</td>
<td>20.85</td>
<td>41.33</td>
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</table>


### DISCUSSION

We would like to suggest the proper sampling time of spot urine for assessing the environmental DEHP exposure, and observe the association urinary DEHP metabolites with steroid hormones. The urinary DEHP metabolite concentrations in the post-shift samples were significantly higher than pre-shift samples.

The association between log-transformed creatinine-adjusted DEHP metabolites concentrations and sex steroid hormones in post- and pre-shift are shown in Table 4. The correlation coefficients of urinary MEHP and MEHHP, MEOHP were 0.74 and 0.78 in post-shift and 0.83 and 0.84 in pre-shift (p<0.0001). The correlation coefficients of urinary MEHHP and MEOHP were 0.96 in post-shift and 0.99 in pre-shift (p<0.0001). The plasma hormones (LH, FSH, E2, and T) were not significantly correlated with log-transformed creatinine-adjusted DEHP metabolites in post- and pre-shift samples.
MEHP, MEHHP, and MEOHP in healthy German adults were 4.35 μg/L, 12.66 μg/L, and 9.02 μg/L [32], and 5.1 μg/L, 15.9 μg/L, and 22.7 μg/L, respectively [28]. The median urinary MEHP concentration in US adult population was 3.2 μg/L [20]. Also the GMs of urinary MEHP, MEHHP, and MEOHP were 3.75 μg/g Cr, 25.4 μg/g Cr, and 16.8 μg/g Cr in male phthalate manufacturers, respectively [30]. The urinary DEHP metabolites in our study were higher than healthy German and US adult males but lower than adult phthalate manufacturers in the USA. The GM of urinary MEHP, MEHHP and MEOHP concentration at post-shift in ours were 8.9-, 34.6- and 26.9-fold lower than PVC film manufacturers [30]. Also, median MEHP concentration (μg/g creatinine) among workers preparing and using DEHP-containing plastisols in a glass coating factory was 1.8-fold higher than our result [33]. These differences might be explained by the differences of the workplace. Thus, for assessing the DEHP exposure, occupational history should be

| Table 4. Pearson’s correlation coefficients between log-transformed urinary creatinine-adjusted MEHP, MEHHP and MEOHP concentrations and sex steroid hormones concentrations sampled in pre-shift (next day) (n=25) |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
|                  | Post-shift       | Pre-shift (next day) |
|                  | MEHP             | MEHHP            | MEOHP            | MEHP             | MEHHP            | MEOHP            | LH               | FSH              | E2               | T               |
| p-value          |                  |                  |                  |                  |                  |                  |                  |                  |                  |                  |
| MEHP             | 1.0000           | 0.7403           | 0.7793           | 1.0000           | 0.8251           | 0.8410           | 0.1231           | 0.0970           | -0.2774          | -0.0839          |
| MEHHP            |                  | (<0.0001)        | (<0.0001)        | (<0.0001)        | (<0.0001)        | (0.5576)         | (0.6446)         | (0.1795)         | (0.6902)         |                  |
| MEOHP            |                  |                  |                  | (<0.0001)        | (<0.0001)        | (0.5914)         | (0.3666)         | (0.0899)         | (0.4326)         |                  |
| p-value          |                  |                  |                  |                  |                  |                  |                  |                  |                  |                  |
| LH               | 0.1819           | 0.2614           | 0.2868           | 1.0000           | 0.3671           | 0.0925           | 0.2411           | 0.2457           | 0.8408           |                  |
| p-value          | (0.3842)         | (0.2068)         | (0.1646)         | (0.0711)         | (0.9226)         | (0.0925)         | (0.8408)         | (0.0025)         | (0.0423)         |                  |
| FSH              | -0.0706          | 0.0666           | 0.0922           | 1.0000           | 0.0926           | 0.1320           | 0.9423           | 0.8408           |                  |                  |
| p-value          | (0.7377)         | (0.7518)         | (0.8612)         | (0.6599)         |                  |                  |                  |                  |                  |                  |
| E2               | -0.1947          | -0.2186          | -0.2027          | 1.0000           |                  |                  |                  |                  |                  |                  |
| p-value          | (0.3511)         | (0.2939)         | (0.3113)         | (0.6599)         |                  |                  |                  |                  |                  |                  |
| T                | -0.3642          | -0.2987          | -0.3518          | 1.0000           |                  |                  |                  |                  |                  |                  |
| p-value          | (0.0735)         | (0.1470)         | (0.1052)         |                  |                  |                  |                  |                  |                  |                  |


**Figure 1.** The comparison of log-transformed creatinine adjusted MEHP, MEHHP and MEOHP concentration between post-shift and pre-shift (next day) using paired t-test.

MEHP, MEHHP, and MEOHP in healthy German adults were 4.35 μg/L, 12.66 μg/L, and 9.02 μg/L [32], and 5.1 μg/L, 15.9 μg/L, and 22.7 μg/L, respectively [28]. The median urinary MEHP concentration in US adult population was showed 3.2 μg/L [20]. Also the GMs of urinary MEHP, MEHHP, and MEOHP were 3.75 μg/g Cr, 25.4 μg/g Cr, and 16.8 μg/g Cr in male phthalate manufacturers, respectively [30]. The urinary DEHP metabolites in our study were higher than healthy German and US adult males but lower than adult phthalate manufacturers in the USA. The GM of urinary MEHP, MEHHP and MEOHP concentration at post-shift in ours were 8.9-, 34.6- and 26.9-fold lower than a PVC film manufacturers [30]. Also, median MEHP concentration (μg/g creatinine) among workers preparing and using DEHP-containing plastisols in a glass coating factory was 1.8-fold higher than our result [33]. These differences might be explained by the differences of the workplace. Thus, for assessing the DEHP exposure, occupational history should be
considered.

The excretion ratio of urinary MEHP to MEHHP to MEOHP in the post- and pre-shift was 1 to 1.4 to 1.1, and 1 to 1.6 to 1.2, respectively (Table 3). In other studies, the ratios of the mean concentration of MEHP to MEHHP to MEOHP were 1 to 5.1 to 3.6 [34], and 1 to 4.3 to 3.3 [21]. The excretion ratio was quite different, but it suggests that MEHHP and MEOHP could appear suitable for the assessment of human DEHP exposure than MEHP. In some studies, DEHP exposure assessment in humans relied on urinary MEHP concentration [16,31]. However, urinary MEHHP and MEOHP concentrations have been used to assess the DEHP exposure in recent studies [20,23,35]. The urinary MEHHP and MEOHP could be more appropriate for assessing the DEHP exposure because the excretion ratio of urinary MEHP to MEHHP to MEOHP showed larger than 1.0 in the results, the MEHHP and MEOHP are not subject to external contamination like MEHP or DEHP and the half-lives of these metabolites were longer than MEHP [31].

Urinary MEHP, MEHHP, and MEOHP concentrations were significantly correlated with each other in the post- and pre-shift samples (Table 4). This relationship had been observed in previous studies [30,32,36,37]. Especially, the correlation coefficient between MEHHP and MEOHP showed higher than those of MEHP, and MEHHP and MEOHP. It would be resulted from shorter half life time of MEHP than MEHHP and MEOHP [31].

In this study, the relationship between the level of urinary DEHP metabolites and plasma steroid hormones was not showed. Toxicological studies have been reported that DEHP possess antiandrogenic activity and reduce testosterone and estradiol levels [3,10]. Recent study was reported that phthalates may inhibit the gene or protein expression related to steroidogenesis such as steroidogeneic acute regulated protein, peripheral benzodiazepine receptor, and P450 side chain cleavage in Leydig cells [38]. Some human studies have shown a negative relationship between human phthalate exposure and serum steroid hormone levels or hormone indicators [18,19,39,40], but a young Swedish population had no association between urinary MEHP and testosterone [40]. Antiandrogenic activity in experimental animal could not reflect what occurs in adult humans [18]. It seems to be that the DEHP exposure level in the study did not cause an antiandrogenic effect. Further hormonal research is needed on reproductive effects of DEHP exposure in adult men.

In our results, the post-shift urinary DEHP metabolite concentrations were quite different from the pre-shift concentrations. It suggests that the spot urine samples could not reflect the real exposure and may cause a lack of comparability because of the short half-lives of DEHP. Therefore, sampling time of spot urine for assessing the environmental DEHP exposure is to be determined according to study purpose.

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CONFLICT OF INTEREST

The authors have no conflict of interest to declare on this study.

REFERENCES


