Association between Prenatal Phthalate Exposure and Birth Outcomes

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Abstract. Objective: To understand the association between exposure in pregnant women to phthalic acid esters (PAEs) and birth outcomes. Method: The study was conducted from April to June 2013 in Xiamen, China. A total 1020 pregnant women (gestational age ≤16 weeks) met the inclusion criteria and were included in the cohort. Finally, 25 women with premature delivery and 15 with low birth weight were defined as case groups. According to the study criteria, we selected 100 healthy participants as a control group. Participant information was collected by questionnaires and urine samples were collected, and detect five PAEs (MMP, MEP, MBP, MBzP, and MEHP) levels in urine samples. Single and multi-factor logistic analyses were used to analyze the association between PAE concentration and birth outcomes. Results: Medians of the five PAEs above in the premature delivery group were 36.13 ng/ml, 19.02 ng/ml, 13.64 ng/ml, 2.11 ng/ml, 43.47 ng/ml, respectively. In the control group, these were 35.48 ng/ml, 17.62 ng/ml, 10.15 ng/ml, 2.34 ng/ml, 35.62 ng/ml, respectively. The result of multi-factor logistic analysis indicated that MBP and MEHP were associated with premature delivery: OR 1.629, 95% CI (1.067, 2.488), OR 1.675, 95% CI (1.028, 2.729), respectively. Medians of the five PAEs in the low birth weight group were 37.28 ng/ml, 21.36 ng/ml, 14.60 ng/ml, 2.63 ng/ml, 45.72 ng/ml, respectively. In the control group, these were 35.48 ng/ml, 17.62 ng/ml, 10.15 ng/ml, 2.34 ng/ml, 35.62 ng/ml respectively. MEP, MBP, and MEHP were associated with low birth weight: OR 1.699, 95% CI (1.074, 2.688), OR 1.621, 95% CI (1.061, 2.475), and OR 1.817, 95% CI (1.005, 3.284), respectively. Conclusion: MBP and MEHP exposure in pregnant women were risk factors of premature delivery and MEP, MBP, MEHP exposure were risk factors of low birth weight. Preventing or reducing environmental exposure of pregnant women to these PAEs is of utmost importance.

Introduction

Phthalic acid esters (PAEs), also called phthalates, have been widely used as plasticizer in the production all kinds of goods, pharmaceuticals, and personal care products. Common phthalates include dimethyl phthalate (DMP), diethyl phthalate (DEP), dibutyl phthalate (DBP), diocyl phthalate, (DOP), di-2-ethylhexyl phthalate (DEHP), and benzyl butyl phthalate (BBP). Pregnant women and children are more sensitive to PAE exposure than the general population. Population studies have shown that women of childbearing age are a group at high risk of PAE exposure. Blount have found that more than 75% of the population had a detectable presence of phthalate monoester metabolites in the body, and metabolism levels in women of childbearing age are higher than those of men and women at other ages[1]. Prenatal PAE exposure not only has an impact on pregnant women’s health but also influences growth of the fetus and can cause serious consequences. Preterm birth and low birth weight (LBW) are the greatest concerns. Premature birth not only can lead to postpartum death, it can also affect newborn growth and development and can seriously harm infant health. Studies have shown that exposure to DEHP during pregnancy is associated with reduced pregnancy period, although this association has not yet been clarified. Birth
weight is an important indicator of whether a newborn is healthy and is considered abnormal when the value is too low or too high. LBW is an important cause of death in children under 5 years old. Increased incidence of adult cardiovascular disease, insulin resistance, and metabolic diseases are also associated with LBW, as demonstrated[2]. There are variable factors that affect birth weight, mainly women’s education level, vocational and social factors[3], pre-pregnancy BMI, nutrition during pregnancy[4], comorbid diseases, and others. Epidemiological research in recent years has shown that environmental endocrine disruptors also play a very important role in the occurrence of LBW[5]. Through analysis of the correlation between PAE exposure levels and preterm birth and LBW in pregnant women, we aimed to provide data supporting further research on these relationships.

Participants and Methods

According to our inclusion and exclusion criteria, a cohort of 1020 pregnant women (gestational age ≤16 weeks) at Maternal and Child Health Hospital in Xiamen, China were selected as participants in this study, which was conducted from April to June 2013. Participant information, such as basic personal information, lifestyle during pregnancy, and antenatal care information were collected using questionnaires, and urine samples were collected from participants. From the time of delivery, we collected information for a total 998 pregnant women, however, 22 participants were lost to follow-up. Thus, a final total of 998 pregnant women were enrolled in the study.

Inclusion criteria: (1) resident in Xiamen for more than 1 year, (2) natural pregnancy. (3) single birth, and (4) gestational age ≤ 16 weeks at the time maternal health card was issued.

Exclusion criteria: (1) history of endocrine or metabolic diseases, liver or kidney disease, blood system diseases, genetic diseases, and occupational contact, (2) spouse has a genetic disease history, (3) receiving hormone therapy, and (4) use of assisted reproductive technologies, such as in vitro fertilization.

Finally, 25 women with premature delivery and 15 with LBW were defined as case groups. According to the criteria for case-cohort studies, we selected 100 healthy participants as a control group.

Detection of PAE Monoesters

Sample Collection and Detection

After issuing maternal and child health cards and obtaining their written informed consent, we collected midstream clean-catch urine samples from all participants. Samples were collected into two sterile glass beakers. One sample was used for detecting urinary creatinine, the other sample was stored in a glass bottle at –20 °C, for subsequent PAE ester detection. Urinary creatinine testing was conducted within 2 hours after collection, using an automated urine chemistry analyzer(Olympic AU5400,Japan) in the clinical laboratory of Maternal and Child Health Hospital, Xiamen.

PAE Monoester Testing

Monoester metabolite levels of the five PAEs (MMP, MEP, MBP, MBzP and MEHP) were detected by solid phase extraction and high performance liquid chromatography tandem mass spectrometry (SPE-HPLC-ESI-MS/MS).

Statistical Analysis

We used Epi Data 2 to create the participant questionnaire, and the information was collected independently by two investigators. The data were entered into SPSS software Version 17.0 (SPSS, Inc., Chicago, IL, USA) for statistical analysis. Continuous variables were analyzed using a t-test and categorical variables using the $\chi^2$ test. For single factor analysis, PB or LBW cases were selected as the dependent variable (yes=1, no=0). Maternal demographics, pregnancy information,
and concentrations of the five PAEs were established as independent variables ($p<0.05$). Subsequently, suspected risk factors identified in single factor analysis were selected (PAE monoester levels were transformed into four categorical variables on the basis of the four quantiles) as independent variables, and PB or LBW as the dependent variable for multi-factor logistic regression analysis ($\alpha=0.05$).

**Results**

**Analysis of the Relationship between PB and PAE Levels**

We listed main relative factors about premature birth and PAE levels, and formed the valuation method (Tab. 1). According to the questionnaire, we collected the participants’ basic information, like age, BMI, education level, history of fertility, and so on (Tab. 2).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Valuation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years] [X1]</td>
<td>17+ = 1  25+ = 2  35+ = 3</td>
</tr>
<tr>
<td>Education level [X2]</td>
<td>Junior high school and below=1</td>
</tr>
<tr>
<td></td>
<td>High school, technical, secondary school=2</td>
</tr>
<tr>
<td></td>
<td>Diploma, bachelor’s degree and above=3</td>
</tr>
<tr>
<td>Family income [RMB thousand/year] [X3]</td>
<td>&lt; 5 = 1  5+ = 2  10+ = 3  20+ = 4</td>
</tr>
<tr>
<td>Folic acid supplements [X4]</td>
<td>never=0  after pregnant=1  before pregnant=2</td>
</tr>
<tr>
<td>Calcium supplements [X5]</td>
<td>never=0  ever=1</td>
</tr>
<tr>
<td>Plastic cup use [X6]</td>
<td>no=0  yes=1</td>
</tr>
<tr>
<td>Hair perms, dryer [X7]</td>
<td>no=0  yes=1</td>
</tr>
<tr>
<td>Make-up use [X8]</td>
<td>no=0  yes=1</td>
</tr>
<tr>
<td>Drinking water type [X9]</td>
<td>tap water=1  bottled water=2</td>
</tr>
<tr>
<td>Home decor [X10]</td>
<td>no=0  yes=1</td>
</tr>
<tr>
<td>Passive smoking [X11]</td>
<td>no=0  yes=1</td>
</tr>
<tr>
<td>Medication [X12]</td>
<td>no=0  yes=1</td>
</tr>
</tbody>
</table>
Table 2. Basic Statistical Data of the 25 Premature Birth Cases, 15 Low Birth Weight Cases and 100 Controls.

<table>
<thead>
<tr>
<th>Basic characteristic</th>
<th>Control (n=100)</th>
<th>PB (n=25)</th>
<th>LBW (n=15)</th>
<th>p-value(PB)</th>
<th>p-value(LBW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>28.15±3.28</td>
<td>29.73±3.78</td>
<td>28.07±3.58</td>
<td>0.039*</td>
<td>0.931</td>
</tr>
<tr>
<td>Pre-pregnancy BMI</td>
<td>20.67±2.32</td>
<td>19.01±2.54</td>
<td>18.56±3.01</td>
<td>0.002*</td>
<td>0.002*</td>
</tr>
<tr>
<td>Age at menarche [years]</td>
<td>13.65±1.36</td>
<td>13.43±1.56</td>
<td>13.79±1.41</td>
<td>0.484</td>
<td>0.712</td>
</tr>
<tr>
<td>Duration of menstruation [days]</td>
<td>5.84±1.15</td>
<td>5.61±1.24</td>
<td>5.20±1.36</td>
<td>0.380</td>
<td>0.052</td>
</tr>
<tr>
<td>Period between menstruation [days]</td>
<td>30.52±2.11</td>
<td>29.57±2.9</td>
<td>31.21±2.18</td>
<td>0.066</td>
<td>0.242</td>
</tr>
<tr>
<td>Passive smoking</td>
<td>23</td>
<td>10</td>
<td>8</td>
<td>0.085</td>
<td>0.025*</td>
</tr>
<tr>
<td>Folic acid supplements</td>
<td>86</td>
<td>18</td>
<td>10</td>
<td>0.132</td>
<td>0.127</td>
</tr>
<tr>
<td>Calcium supplements</td>
<td>74</td>
<td>15</td>
<td>7</td>
<td>0.167</td>
<td>0.064</td>
</tr>
<tr>
<td>Pregnancy complications</td>
<td>12</td>
<td>8</td>
<td>6</td>
<td>0.028*</td>
<td>0.013*</td>
</tr>
<tr>
<td>Medication during pregnancy</td>
<td>13</td>
<td>0</td>
<td>0</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Education level</td>
<td></td>
<td></td>
<td></td>
<td>0.909</td>
<td>0.421</td>
</tr>
<tr>
<td>Junior high school and below</td>
<td>15</td>
<td>4</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High school, technical secondary</td>
<td>52</td>
<td>14</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diploma, bachelor’s and above</td>
<td>33</td>
<td>7</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family income [10,000/year]</td>
<td></td>
<td></td>
<td></td>
<td>0.324</td>
<td>0.017*</td>
</tr>
<tr>
<td>&lt;5</td>
<td>25</td>
<td>4</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5–10</td>
<td>43</td>
<td>12</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10–20</td>
<td>19</td>
<td>8</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 20</td>
<td>13</td>
<td>1</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gravidity</td>
<td></td>
<td></td>
<td></td>
<td>0.026*</td>
<td>0.573</td>
</tr>
<tr>
<td>1</td>
<td>62</td>
<td>10</td>
<td>8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>31</td>
<td>9</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 3</td>
<td>7</td>
<td>6</td>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
<td>0.234</td>
<td>0.694</td>
</tr>
<tr>
<td>0</td>
<td>72</td>
<td>19</td>
<td>10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>23</td>
<td>3</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 2</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Term [gestational weeks]</td>
<td>39.12±2.79</td>
<td>35.89±3.21</td>
<td>36.10±3.01</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
<tr>
<td>Birth weight [g]</td>
<td>3118.19±222.89</td>
<td>2653.17±26</td>
<td>2464.36±25</td>
<td>0.000*</td>
<td>0.000*</td>
</tr>
<tr>
<td>Neonatal length [cm]</td>
<td>50.44±2.11</td>
<td>48.95±2.34</td>
<td>48.37±2.26</td>
<td>0.003*</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

Notes: Numeric data, means±SD or frequency (%). BMI=weight [kg] / height [m]². *p-value < 0.05.
All five PAEs monoesters could be detected in most samples, and the detection rate of the case groups were significantly higher than that of the control group (Tab. 3, Tab.4).

### Table 3. LEVEL of Five PAEs and Detection Rates in Urine Samples of 25 Premature Birth Cases.

<table>
<thead>
<tr>
<th>PAE monoester</th>
<th>Percentile</th>
<th>Detection rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P&lt;sub&gt;25&lt;/sub&gt;</td>
<td>P&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td>Unadjusted [ng/ml]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary creatinine, adjusted [μg/g Cr]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP</td>
<td>19.02(28.53)</td>
<td>36.13(54.20)</td>
</tr>
<tr>
<td>MEP</td>
<td>3.92(6.39)</td>
<td>19.02(31.00)</td>
</tr>
<tr>
<td>MBP</td>
<td>3.82(6.53)</td>
<td>13.64(23.33)</td>
</tr>
<tr>
<td>MBzP</td>
<td>1.45(2.38)</td>
<td>2.11(3.46)</td>
</tr>
<tr>
<td>MEHP</td>
<td>11.34(17.01)</td>
<td>43.47(65.21)</td>
</tr>
</tbody>
</table>

### Table 4. PAE Levels and Detection Rate in Urine Samples of 100 Controls.

<table>
<thead>
<tr>
<th>PAE monoester</th>
<th>Percentile</th>
<th>Detection rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P&lt;sub&gt;25&lt;/sub&gt;</td>
<td>P&lt;sub&gt;50&lt;/sub&gt;</td>
</tr>
<tr>
<td>Unadjusted [ng/ml]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary creatinine, adjusted [μg/g Cr]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MMP</td>
<td>18.32(28.21)</td>
<td>35.48(54.64)</td>
</tr>
<tr>
<td>MEP</td>
<td>3.87(6.42)</td>
<td>17.62(29.25)</td>
</tr>
<tr>
<td>MBP</td>
<td>0.33†(0.57)</td>
<td>10.15(17.56)</td>
</tr>
<tr>
<td>MBzP</td>
<td>0.12†(0.20)</td>
<td>2.34(3.91)</td>
</tr>
<tr>
<td>MEHP</td>
<td>9.82(14.82)</td>
<td>35.62(53.79)</td>
</tr>
</tbody>
</table>

Note: Not detected, expressed as L0D/2. Abbreviation: Cr, urinary creatinine.

The t-test results showed that MBP and MEHP levels in the premature birth case group were higher than those in the control group (Tab. 5). We selected premature delivery as the dependent variable (yes=1, no =0), used maternal demographic and pregnancy information, and concentrations of the five PAE monoesters as independent variables. The results are given in Tab. 6. From the table we can see that maternal age, pre-pregnancy BMI, complications during pregnancy, gravidity, and the five PAE monoesters were associated with premature delivery (p value < 0.05). We then applied the multi-factor analysis model.

### Table 5. Comparison of Levels of Five PAEs between PB, LBW Cases and Controls.

<table>
<thead>
<tr>
<th>PAE monoester</th>
<th>PB (n=25)</th>
<th>Cases (n=15)</th>
<th>controls (n=100)</th>
<th>t-test(PB)</th>
<th>p-value(PB)</th>
<th>t-test (LBW)</th>
<th>p-value (LBW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP</td>
<td>1.73(0.17)</td>
<td>1.75(0.18)</td>
<td>1.70(0.15)</td>
<td>−0.871</td>
<td>0.386</td>
<td>−1.172</td>
<td>0.244</td>
</tr>
<tr>
<td>MEP</td>
<td>1.49(0.16)</td>
<td>1.53(0.18)</td>
<td>1.45(0.13)</td>
<td>−1.312</td>
<td>0.192</td>
<td>−2.106</td>
<td>0.037†</td>
</tr>
<tr>
<td>MBP</td>
<td>1.32(0.15)</td>
<td>1.34(0.15)</td>
<td>1.25(0.11)</td>
<td>−2.634</td>
<td>0.010*</td>
<td>−2.809</td>
<td>0.006*</td>
</tr>
<tr>
<td>MBzP</td>
<td>0.59(0.07)</td>
<td>0.58(0.07)</td>
<td>0.57(0.04)</td>
<td>−1.888</td>
<td>0.061</td>
<td>−0.806</td>
<td>0.422</td>
</tr>
<tr>
<td>MEHP</td>
<td>1.82(0.18)</td>
<td>1.83(0.17)</td>
<td>1.74(0.14)</td>
<td>−2.407</td>
<td>0.018*</td>
<td>−2.256</td>
<td>0.026*</td>
</tr>
</tbody>
</table>

Note: PAE monoester values in case and control groups were means of each distribution after logarithmic transformation (standard deviation).
Table 6. Single Factor Logistic Regression Analysis of Influencing Factors for PB.

<table>
<thead>
<tr>
<th>Factors</th>
<th>β value</th>
<th>Standard deviation</th>
<th>( \chi^2 ) value</th>
<th>( p )-value</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>0.538</td>
<td>0.214</td>
<td>6.320</td>
<td>0.012*</td>
<td>1.713</td>
<td>(1.126, 2.605)</td>
</tr>
<tr>
<td>Pre-pregnancy BMI</td>
<td>-0.679</td>
<td>0.299</td>
<td>5.157</td>
<td>0.023*</td>
<td>0.507</td>
<td>(0.282, 0.911)</td>
</tr>
<tr>
<td>Age menarche [years]</td>
<td>-0.114</td>
<td>0.278</td>
<td>0.168</td>
<td>0.682</td>
<td>0.892</td>
<td>(0.517, 1.539)</td>
</tr>
<tr>
<td>Menstruation duration [days]</td>
<td>0.217</td>
<td>0.220</td>
<td>0.973</td>
<td>0.324</td>
<td>1.242</td>
<td>(0.807, 1.912)</td>
</tr>
<tr>
<td>Period between menstruation [days]</td>
<td>0.288</td>
<td>0.301</td>
<td>0.915</td>
<td>0.339</td>
<td>1.334</td>
<td>(0.739, 2.406)</td>
</tr>
<tr>
<td>Passive smoking</td>
<td>0.318</td>
<td>0.236</td>
<td>1.816</td>
<td>0.178</td>
<td>0.892</td>
<td>(0.425, 1.437)</td>
</tr>
<tr>
<td>Folic acid supplements</td>
<td>-0.276</td>
<td>0.213</td>
<td>1.679</td>
<td>0.195</td>
<td>0.759</td>
<td>(0.500, 1.152)</td>
</tr>
<tr>
<td>Calcium supplements</td>
<td>-0.247</td>
<td>0.311</td>
<td>0.631</td>
<td>0.427</td>
<td>0.781</td>
<td>(0.425, 1.437)</td>
</tr>
<tr>
<td>Pregnancy complications</td>
<td>0.947</td>
<td>0.325</td>
<td>8.490</td>
<td>0.004*</td>
<td>2.578</td>
<td>(1.363, 4.874)</td>
</tr>
<tr>
<td>Education level</td>
<td>-0.381</td>
<td>0.211</td>
<td>3.261</td>
<td>0.071</td>
<td>0.683</td>
<td>(0.452, 1.033)</td>
</tr>
<tr>
<td>Annual family income</td>
<td>-0.470</td>
<td>0.248</td>
<td>3.592</td>
<td>0.058</td>
<td>0.625</td>
<td>(0.384, 1.016)</td>
</tr>
<tr>
<td>Gravidiy</td>
<td>0.645</td>
<td>0.271</td>
<td>5.665</td>
<td>0.017*</td>
<td>1.906</td>
<td>(1.121, 3.242)</td>
</tr>
<tr>
<td>Parity</td>
<td>0.428</td>
<td>0.241</td>
<td>3.154</td>
<td>0.076</td>
<td>1.534</td>
<td>(0.957, 2.460)</td>
</tr>
<tr>
<td>MMP</td>
<td>0.632</td>
<td>0.316</td>
<td>4.000</td>
<td>0.046*</td>
<td>1.881</td>
<td>(1.013, 3.495)</td>
</tr>
<tr>
<td>MEP</td>
<td>0.597</td>
<td>0.246</td>
<td>5.890</td>
<td>0.015*</td>
<td>1.817</td>
<td>(1.122, 2.942)</td>
</tr>
<tr>
<td>MBP</td>
<td>0.871</td>
<td>0.258</td>
<td>11.397</td>
<td>0.001*</td>
<td>2.389</td>
<td>(1.441, 3.962)</td>
</tr>
<tr>
<td>MBzP</td>
<td>0.499</td>
<td>0.219</td>
<td>5.192</td>
<td>0.023*</td>
<td>1.647</td>
<td>(1.072, 2.530)</td>
</tr>
<tr>
<td>MEHP</td>
<td>0.716</td>
<td>0.325</td>
<td>4.854</td>
<td>0.028*</td>
<td>2.046</td>
<td>(1.082, 3.869)</td>
</tr>
</tbody>
</table>

Note: Abbreviations: OR, odds ratio, CI, confidence interval.

After adjustment for maternal age, pre-pregnant BMI, complications during pregnancy, and gravidity, the multivariate logistic regression analysis showed that MBP, MEHP concentrations in pregnant women were associated with premature delivery. However, no relationship was found between MEP, MMP, and MBzP concentrations and premature birth.( Tab. 7)

Table 7. Multi-factor Logistic Regression Analysis of Influencing Factors for PB.

<table>
<thead>
<tr>
<th>Variables</th>
<th>β value</th>
<th>Standard deviation</th>
<th>( \chi^2 ) value</th>
<th>( p )-value</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>0.589</td>
<td>0.254</td>
<td>5.377</td>
<td>0.020*</td>
<td>1.802</td>
<td>(1.095, 2.965)</td>
</tr>
<tr>
<td>Pre-pregnancy BMI</td>
<td>-0.239</td>
<td>0.246</td>
<td>0.944</td>
<td>0.331</td>
<td>0.787</td>
<td>(0.486, 1.275)</td>
</tr>
<tr>
<td>Pregnancy complications</td>
<td>0.577</td>
<td>0.276</td>
<td>4.371</td>
<td>0.037*</td>
<td>1.781</td>
<td>(1.037, 3.059)</td>
</tr>
<tr>
<td>Gravidiy</td>
<td>0.659</td>
<td>0.241</td>
<td>7.477</td>
<td>0.006*</td>
<td>1.933</td>
<td>(1.205, 3.100)</td>
</tr>
<tr>
<td>MMP</td>
<td>0.255</td>
<td>0.226</td>
<td>1.273</td>
<td>0.259</td>
<td>1.290</td>
<td>(0.829, 2.010)</td>
</tr>
<tr>
<td>MEP</td>
<td>0.237</td>
<td>0.234</td>
<td>1.026</td>
<td>0.311</td>
<td>1.267</td>
<td>(0.801, 2.005)</td>
</tr>
<tr>
<td>MBP</td>
<td>0.488</td>
<td>0.216</td>
<td>5.104</td>
<td>0.024*</td>
<td>1.629</td>
<td>(1.067, 2.488)</td>
</tr>
<tr>
<td>MBzP</td>
<td>0.228</td>
<td>0.268</td>
<td>0.724</td>
<td>0.395</td>
<td>1.256</td>
<td>(0.743, 2.124)</td>
</tr>
<tr>
<td>MEHP</td>
<td>0.516</td>
<td>0.249</td>
<td>4.294</td>
<td>0.038*</td>
<td>1.675</td>
<td>(1.028, 2.729)</td>
</tr>
</tbody>
</table>
Relationship between PAE Exposure during Pregnancy and LBW

We listed basic information about low birth weight cases, and compared it to controls (Tab. 2). The \( t \)-test results showed that MEP, MBP, and MEHP levels in the LBW case group were higher than those in the control group (Tab. 8). The results of the control group may be found in Tab. 4. The \( t \)-test results showed that MEP, MBP and MEHP levels in the low birth weight group were higher than those in the control group (Tab. 5).

Table 8. Levels of Five PAEs and Detection Rate in Urine Samples of 15 Low Birth Weight Cases.

<table>
<thead>
<tr>
<th>PAE monoesters</th>
<th>Percentile Unadjusted [ng/ml]</th>
<th>Urinary creatinine, adjusted [μg/g Cr]</th>
<th>Detection rate (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( P_{25} )</td>
<td>( P_{50} )</td>
<td>( P_{75} )</td>
</tr>
<tr>
<td>MMP</td>
<td>18.92(28.76)</td>
<td>37.28(56.67)</td>
<td>90.32(137.29)</td>
</tr>
<tr>
<td>MEP</td>
<td>3.99(6.54)</td>
<td>21.36(35.03)</td>
<td>40.78(66.88)</td>
</tr>
<tr>
<td>MBP</td>
<td>1.21(2.08)</td>
<td>14.60(25.11)</td>
<td>40.65(69.92)</td>
</tr>
<tr>
<td>MBzP</td>
<td>1.35(2.21)</td>
<td>2.63(4.31)</td>
<td>4.84(7.94)</td>
</tr>
<tr>
<td>MEHP</td>
<td>11.40(16.99)</td>
<td>45.72(68.12)</td>
<td>82.10(122.33)</td>
</tr>
</tbody>
</table>

We selected LBW as the dependent variable (yes=1, no =0), and used maternal demographic and situation during pregnancy, as well as concentrations of the five PAE monoesters as independent variables. The results are given in Tab. 9. From the table we can see that maternal age, pre-pregnancy BMI, passive smoking, complications during pregnancy, family income, gestational weeks of pregnancy, and the five PAE monoesters were associated with LBW \( (p \text{ value}<0.05) \), using the multi-factor analysis model.

Table 9. Single Factor Logistic Regression Analysis of Influencing Factors for LBW.

<table>
<thead>
<tr>
<th>Influencing factors</th>
<th>( \beta ) value</th>
<th>Standard deviation</th>
<th>( \chi^2 ) value</th>
<th>( P )-value</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>0.438</td>
<td>0.183</td>
<td>5.729</td>
<td>0.017*</td>
<td>1.550</td>
<td>(1.083, 2.218)</td>
</tr>
<tr>
<td>pre-pregnancy BMI</td>
<td>-0.632</td>
<td>0.212</td>
<td>8.887</td>
<td>0.003*</td>
<td>0.532</td>
<td>(0.351, 0.805)</td>
</tr>
<tr>
<td>Age at menarche [years]</td>
<td>-0.105</td>
<td>0.278</td>
<td>0.143</td>
<td>0.706</td>
<td>0.900</td>
<td>(0.522, 1.553)</td>
</tr>
<tr>
<td>Menstruation duration [days]</td>
<td>0.246</td>
<td>0.221</td>
<td>1.239</td>
<td>0.266</td>
<td>1.279</td>
<td>(0.829, 1.972)</td>
</tr>
<tr>
<td>Days between menstruation</td>
<td>0.372</td>
<td>0.302</td>
<td>1.517</td>
<td>0.218</td>
<td>1.451</td>
<td>(0.803, 2.622)</td>
</tr>
<tr>
<td>Passive smoking</td>
<td>0.567</td>
<td>0.241</td>
<td>5.535</td>
<td>0.019*</td>
<td>1.763</td>
<td>(1.099, 2.827)</td>
</tr>
<tr>
<td>Folic acid supplements</td>
<td>-0.356</td>
<td>0.215</td>
<td>2.742</td>
<td>0.098</td>
<td>0.700</td>
<td>(0.460, 1.068)</td>
</tr>
<tr>
<td>Calcium supplement</td>
<td>-0.211</td>
<td>0.306</td>
<td>0.475</td>
<td>0.490</td>
<td>0.810</td>
<td>(0.445, 1.475)</td>
</tr>
<tr>
<td>Pregnancy complications</td>
<td>1.247</td>
<td>0.325</td>
<td>14.722</td>
<td>0.000*</td>
<td>3.480</td>
<td>(1.840, 6.580)</td>
</tr>
<tr>
<td>Education level</td>
<td>-0.522</td>
<td>0.272</td>
<td>3.683</td>
<td>0.055</td>
<td>0.593</td>
<td>(0.348, 1.011)</td>
</tr>
<tr>
<td>Family annual income</td>
<td>-0.469</td>
<td>0.203</td>
<td>5.338</td>
<td>0.021*</td>
<td>0.626</td>
<td>(0.420, 0.931)</td>
</tr>
<tr>
<td>Gravidity</td>
<td>0.309</td>
<td>0.198</td>
<td>2.435</td>
<td>0.119</td>
<td>1.362</td>
<td>(0.924, 2.008)</td>
</tr>
<tr>
<td>Parity</td>
<td>0.180</td>
<td>0.413</td>
<td>0.190</td>
<td>0.663</td>
<td>1.197</td>
<td>(0.533, 2.690)</td>
</tr>
<tr>
<td>Term (gestational weeks)</td>
<td>-0.635</td>
<td>0.231</td>
<td>7.557</td>
<td>0.006*</td>
<td>0.530</td>
<td>(0.337, 0.833)</td>
</tr>
<tr>
<td>MMP</td>
<td>0.598</td>
<td>0.269</td>
<td>4.942</td>
<td>0.026*</td>
<td>1.818</td>
<td>(1.073, 3.081)</td>
</tr>
<tr>
<td>MEP</td>
<td>0.630</td>
<td>0.297</td>
<td>4.500</td>
<td>0.034*</td>
<td>1.878</td>
<td>(1.049, 3.361)</td>
</tr>
</tbody>
</table>
To control for confounding factors, we selected the six suspected risk factors and PAE monoesters as independent variables, and LBW as the dependent variable for multi-factor logistic regression analysis (Tab. 10).

<table>
<thead>
<tr>
<th>Variables</th>
<th>β value</th>
<th>Standard deviation</th>
<th>$\chi^2$ value</th>
<th>$p$-value</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age [years]</td>
<td>0.307</td>
<td>0.185</td>
<td>2.754</td>
<td>0.097</td>
<td>1.359</td>
<td>(0.946, 1.953)</td>
</tr>
<tr>
<td>Pre-pregnancy BMI</td>
<td>-0.598</td>
<td>0.246</td>
<td>5.909</td>
<td>0.0015*</td>
<td>0.550</td>
<td>(0.340, 0.891)</td>
</tr>
<tr>
<td>Passive smoking</td>
<td>0.401</td>
<td>0.289</td>
<td>1.925</td>
<td>0.165</td>
<td>1.493</td>
<td>(0.848, 2.631)</td>
</tr>
<tr>
<td>Pregnancy complications</td>
<td>0.367</td>
<td>0.360</td>
<td>1.039</td>
<td>0.308</td>
<td>1.443</td>
<td>(0.713, 2.923)</td>
</tr>
<tr>
<td>Annual family income</td>
<td>-0.269</td>
<td>0.217</td>
<td>1.537</td>
<td>0.215</td>
<td>0.764</td>
<td>(0.499, 1.169)</td>
</tr>
<tr>
<td>Term[gestational Weeks]</td>
<td>-0.708</td>
<td>0.203</td>
<td>12.164</td>
<td>0.0001*</td>
<td>0.493</td>
<td>(0.331, 0.733)</td>
</tr>
<tr>
<td>MMP</td>
<td>0.355</td>
<td>0.226</td>
<td>2.467</td>
<td>0.116</td>
<td>1.426</td>
<td>(0.916, 2.221)</td>
</tr>
<tr>
<td>MEP</td>
<td>0.530</td>
<td>0.234</td>
<td>5.130</td>
<td>0.024*</td>
<td>1.699</td>
<td>(1.074, 2.688)</td>
</tr>
<tr>
<td>MBP</td>
<td>0.483</td>
<td>0.216</td>
<td>5.000</td>
<td>0.025*</td>
<td>1.621</td>
<td>(1.061, 2.475)</td>
</tr>
<tr>
<td>MBzP</td>
<td>0.328</td>
<td>0.268</td>
<td>1.498</td>
<td>0.221</td>
<td>1.388</td>
<td>(0.821, 2.347)</td>
</tr>
<tr>
<td>MEHP</td>
<td>0.597</td>
<td>0.302</td>
<td>3.908</td>
<td>0.048*</td>
<td>1.817</td>
<td>(1.005, 3.284)</td>
</tr>
</tbody>
</table>

After adjustment for maternal age, pre-pregnancy BMI, passive smoking, complications during pregnancy, annual family income, and gestational weeks of pregnancy, the multivariate logistic regression analysis showed that MEP, MBP, and MEHP concentrations in pregnant women were associated with LBW. However, no relationship was found between MMP and MBzP concentrations and LBW.

**Discussion**

This study found that urine sample levels of the five PAEs investigated in women who had premature delivery were higher than in those of women with a normal pregnancy term. Levels of MMP, MEP and MEHP between the case and control groups were significantly different ($p$ value < 0.05).

The five PAE monoesters tested could be detected in most of the samples, and the detection rate in the case groups were significantly higher than in the control group. PAEs exist widely in the
environment and these substances can cross the placental barrier, thereby exposing the fetus in utero to common PAEs. High maternal MEHP in preterm infants was found in a study of 331 African American and Dominican mothers and newborns born in New York City[6]. In that study, for women in their third trimester with urine specific gravity-corrected MEHP concentrations that were in the highest quartile, infant gestational age was decreased by 5.1 days (95% CI 2.1–8.4), which was significantly less than for women in the lowest quartiles (p < 0.001). Our study was not consistent with research in New York City, which showed that total exposure concentrations of MEHP, MBP, and MEP may be related to increased pregnancy periods[6]. Our results are different from the above study, mostly likely owing to different experimental design and population characteristics (age, ethnicity, education level, and quality of care), as well as exclusion criteria and exposure sources and levels. In our cohort, PAE levels in pregnant women with gestational age 37 weeks differed from the urine screening results of the 2001–2002 National Health and Nutrition Survey (NHANES) [6]. In our study, higher concentrations of MMP and MEHP, and corrected and uncorrected MBzP concentrations, were lower than that of the general population in the USA[7].

In our LBW group, urine MEP, MBP and MEHP levels were higher than in the control group (p < 0.05), suggesting that intrauterine DEP, DBP and DEHP exposure in LBW group was significantly higher than in the control group. To exclude the influence of confounding factors, multi-factor logistic regression analysis showed that MEP, MBP, and MEHP levels in pregnant women were related to LBW, suggesting that prenatal PAE exposure can cause adverse effects to neonatal health. No relationship was found here between MMP and MBzP levels and LBW. Wen found that high MEP levels in urine were associated with LBW, but found no relationship between MMP and MBP levels and LBW[8]. Zhang found that neonatal DBP and MBP levels in cord blood and meconium were correlated with LBW, and there were dose-response relationships between DBP, MBP or MEP exposure and LBW, which suggests that in utero exposure to MBP or MEP are important risk factors for LBW[9]. The difference in our findings may be related to the different samples tested. Analysis in our research was of PAE monoester concentrations in urine, Zhang analyzed PAE concentrations in meconium and umbilical cord blood[10]. In addition, their study sample was larger (231 women). Jiang found that together with increased MBP and MEHP levels in meconium, birth weight decreased gradually[11]. This indicates that we should expand the sample size and collect a variety of biological samples, for further comprehensive analysis. In summary, although samples types and sizes differ between studies, the overall trend is consistent with respect to PAE exposure in pregnant women and associations with PB and LBW.

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References


