Supporting Information to:

Wastewater-based epidemiology as a new tool for estimating population exposure to phthalate plasticizers

Iria González-Mariño*, Rosario Rodil, Iván Barrio, Rafael Cela, José Benito Quintana*
Department of Analytical Chemistry, Nutrition and Food Sciences, IIAA – Institute for Food Analysis and Research, University of Santiago de Compostela, Constantino Candeira S/N, 15782 – Santiago de Compostela, Spain.

*Corresponding authors:
Iria González-Mariño:
phone: +34 881 816035; e-mail: iria.gonzalez@usc.es

José Benito Quintana:
phone: +34 881 814263 and +34 881 816035; e-mail: jb.quintana@usc.es

Summary: this file contains eleven SI pages with seven SI Tables and four SI Figures.
### Table S1. Characteristics of the WWTP sampled in the study.

<table>
<thead>
<tr>
<th>Location</th>
<th>Mean flow rate (m³ day⁻¹)</th>
<th>Population served by the plant</th>
<th>Sampling mode (volume and frequency of sampling)</th>
<th>Sampling date/period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Santiago de Compostela</td>
<td>53000</td>
<td>136500⁹</td>
<td>Grab sampling</td>
<td>15th March 2015 &amp; 15th April 2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24 h composite (120 mL every 10 min)</td>
<td>19 – 25th April 2016</td>
</tr>
<tr>
<td>Ares</td>
<td>13620</td>
<td>24490⁷</td>
<td>24 h composite (110 mL every 30 min)</td>
<td>2nd June 2016</td>
</tr>
<tr>
<td>Baiona</td>
<td>4881</td>
<td>12072⁸</td>
<td>24 h composite (480 mL every 60 min)</td>
<td>14th June 2016</td>
</tr>
<tr>
<td>Cambados</td>
<td>12334</td>
<td>13895⁹</td>
<td>24 h composite (500 mL every 60 min)</td>
<td>7th June 2016</td>
</tr>
<tr>
<td>Gondomar</td>
<td>12743</td>
<td>14056⁸</td>
<td>24 h composite (110 mL every 30 min)</td>
<td>1st June 2016</td>
</tr>
<tr>
<td>Nigrán</td>
<td>8954</td>
<td>17619⁹</td>
<td>24 h composite (100 mL every 30 min)</td>
<td>1st June 2016</td>
</tr>
</tbody>
</table>

³ Population calculated as 2.5 times the number of servers connected to the sewage system, according to the recommendations of the WWTP manager

⁷ Census data at 01/01/2015

² 24 h samples collected in time proportional mode, taking aliquots of the indicated volume at the indicated frequency
**Table S2.** Chemical formulae, retention time (RT), deuterated compound used as surrogate or internal standard (IS), transitions (precursor/product) used for quantification and confirmation and instrumental performance parameters for every analyte.

<table>
<thead>
<tr>
<th>Compound</th>
<th>[M-H] Formula</th>
<th>RT(^a)</th>
<th>Internal Standard</th>
<th>Precursor (m/z)</th>
<th>CV(^b)</th>
<th>Quantifier Product</th>
<th>Qualifier Product</th>
<th>Linearity R(^2d)</th>
<th>IQL(^e) μg L(^{-1})</th>
<th>Repeatability %RSD(^f)</th>
<th>Reproducibility %RSD(^g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP</td>
<td>C(_9)H(_7)O(_4)</td>
<td>4.4</td>
<td>MMP-D4</td>
<td>179</td>
<td>40</td>
<td>107</td>
<td>77</td>
<td>0.9995</td>
<td>0.31</td>
<td>2.2</td>
<td>2.3</td>
</tr>
<tr>
<td>MEP</td>
<td>C(_9)H(_7)O(_4)</td>
<td>5.1</td>
<td>MnBP-D4</td>
<td>193</td>
<td>44</td>
<td>77</td>
<td>121</td>
<td>0.9987</td>
<td>0.31</td>
<td>4.4</td>
<td>6.1</td>
</tr>
<tr>
<td>MiBP</td>
<td>C(<em>{12})H(</em>{13})O(_4)</td>
<td>7.6</td>
<td>MnBP-D4</td>
<td>221</td>
<td>48</td>
<td>77</td>
<td>134</td>
<td>0.9991</td>
<td>0.23</td>
<td>2.8</td>
<td>4.9</td>
</tr>
<tr>
<td>MnBP</td>
<td>C(<em>{12})H(</em>{13})O(_4)</td>
<td>7.9</td>
<td>MnBP-D4</td>
<td>221</td>
<td>48</td>
<td>77</td>
<td>177</td>
<td>0.9991</td>
<td>0.16</td>
<td>1.8</td>
<td>7.0</td>
</tr>
<tr>
<td>MBzP</td>
<td>C(<em>{15})H(</em>{13})O(_4)</td>
<td>8.8</td>
<td>MnBP-D4</td>
<td>255</td>
<td>48</td>
<td>77</td>
<td>183</td>
<td>0.9988</td>
<td>0.01</td>
<td>1.9</td>
<td>5.9</td>
</tr>
<tr>
<td>MEHHP</td>
<td>C(<em>{16})H(</em>{21})O(_5)</td>
<td>8.0</td>
<td>MEHHP-D4</td>
<td>293</td>
<td>60</td>
<td>145</td>
<td>121</td>
<td>0.9987</td>
<td>0.07</td>
<td>1.7</td>
<td>2.5</td>
</tr>
<tr>
<td>MEOHP</td>
<td>C(<em>{16})H(</em>{21})O(_5)</td>
<td>8.2</td>
<td>MEHHP-D4</td>
<td>291</td>
<td>44</td>
<td>143</td>
<td>121</td>
<td>0.9986</td>
<td>0.11</td>
<td>3.9</td>
<td>4.2</td>
</tr>
<tr>
<td>MECPP</td>
<td>C(<em>{16})H(</em>{21})O(_6)</td>
<td>8.0</td>
<td>MEHHP-D4</td>
<td>307</td>
<td>36</td>
<td>159</td>
<td>113</td>
<td>0.9991</td>
<td>0.09</td>
<td>3.0</td>
<td>4.7</td>
</tr>
<tr>
<td>MMP-D4</td>
<td>C(_{9})H(_7)D(_4)O(_4)</td>
<td>4.3</td>
<td>—</td>
<td>183</td>
<td>40</td>
<td>111</td>
<td>95</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>MnBP-D4</td>
<td>C(<em>{12})H(</em>{13})D(_4)O(_4)</td>
<td>7.8</td>
<td>—</td>
<td>225</td>
<td>48</td>
<td>81</td>
<td>17</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>MEHHP-D4</td>
<td>C(<em>{16})H(</em>{21})D(_4)O(_5)</td>
<td>7.9</td>
<td>—</td>
<td>297</td>
<td>60</td>
<td>125</td>
<td>18</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

\(^a\)Retention time
\(^b\)Capillary Voltage
\(^c\)Collision Energy
\(^d\)Determination Coefficient (linearity evaluated between 1 and 500 μg L\(^{-1}\))
\(^e\)Instrumental Quantification Limit
\(^f\)Relative Standard Deviation for consecutive injections of a 50 μg L\(^{-1}\) standard (n=5)
\(^g\)Relative Standard Deviation for injections of 50 μg L\(^{-1}\) standards in a nine-months period (n=5)
Table S3. Studies considered to calculate participant-weighted average excretion fractions for MnBP, MiBP, MBzP and DEHP metabolites (MEHHP, MEOHP and MCPP). To the best of authors’ knowledge, human excretion fractions for MMP and MEP are currently not available. Therefore, MnBP average excretion fraction of 0.69 was used due to its similar structure with linear side alkyl chains (Saravanabhavan et al., 2014; Koch et al., 2003).

<table>
<thead>
<tr>
<th>Reference</th>
<th>Number of participants</th>
<th>Phth. diester dose</th>
<th>Phth. diester</th>
<th>Phth. monoester</th>
<th>Molar excretion fraction (in 24 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson et al. 2001&lt;sup&gt;c&lt;/sup&gt;</td>
<td>8</td>
<td>Low</td>
<td>DnBP</td>
<td>MnBP</td>
<td>0.64</td>
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<tr>
<td></td>
<td>8</td>
<td>High</td>
<td>DnBP</td>
<td>MnBP</td>
<td>0.73</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>Low</td>
<td>BzBP</td>
<td>MBzP</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>High</td>
<td>BzBP</td>
<td>MBzP</td>
<td>0.78</td>
</tr>
<tr>
<td>Koch et al. 2012&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1</td>
<td>–</td>
<td>DnBP</td>
<td>MnBP</td>
<td>0.84</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>–</td>
<td>DIBP</td>
<td>MiBP</td>
<td>0.71</td>
</tr>
<tr>
<td>Anderson et al. 2011&lt;sup&gt;e&lt;/sup&gt;</td>
<td>20</td>
<td>Low</td>
<td>DEHP</td>
<td>MEHHP</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>High</td>
<td>DEHP</td>
<td>MEHHP</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Low</td>
<td>DEHP</td>
<td>MEOHP</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>High</td>
<td>DEHP</td>
<td>MEOHP</td>
<td>0.10</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>Low</td>
<td>DEHP</td>
<td>MECPP</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>High</td>
<td>DEHP</td>
<td>MECPP</td>
<td>0.12</td>
</tr>
<tr>
<td>Koch et al. 2005&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1</td>
<td>–</td>
<td>DEHP</td>
<td>MEHHP</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>–</td>
<td>DEHP</td>
<td>MEOHP</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>–</td>
<td>DEHP</td>
<td>MECPP</td>
<td>0.18</td>
</tr>
</tbody>
</table>


Table S4. Correction factors applied to estimate exposure to phthalate diesters from phthalate monoester mass loads in wastewater. Average excretion fractions were obtained by weighting molar excretion fractions provided in every study (Table S3) by the number of participants involved in every case.

<table>
<thead>
<tr>
<th>Phth. diester</th>
<th>Molecular weight</th>
<th>Phth. monoester</th>
<th>Molecular weight</th>
<th>Weighted average excretion fraction (in 24h)</th>
<th>Correction Factor (CF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DnBP</td>
<td>278.344</td>
<td>MnBP</td>
<td>222.237</td>
<td>0.69</td>
<td>1.80</td>
</tr>
<tr>
<td>DiBP</td>
<td>278.344</td>
<td>MiBP</td>
<td>222.237</td>
<td>0.71</td>
<td>1.76</td>
</tr>
<tr>
<td>BzBP</td>
<td>312.36</td>
<td>MBzP</td>
<td>256.253</td>
<td>0.73</td>
<td>1.68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MnBP</td>
<td>222.24</td>
<td>0.06</td>
<td>23.43</td>
</tr>
<tr>
<td>DEHP</td>
<td>390.556</td>
<td>MEHHP</td>
<td>294.343</td>
<td>0.16</td>
<td>8.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MEOHP</td>
<td>292.327</td>
<td>0.11</td>
<td>11.78</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MECPP</td>
<td>308.33</td>
<td>0.14</td>
<td>9.01</td>
</tr>
<tr>
<td>DEP</td>
<td>222.237</td>
<td>MEP</td>
<td>194.184</td>
<td>0.69</td>
<td>1.65</td>
</tr>
<tr>
<td>DMP</td>
<td>194.184</td>
<td>MMP</td>
<td>180.157</td>
<td>0.69</td>
<td>1.55</td>
</tr>
</tbody>
</table>
Table S5. Method quantification limits (MQL), percentages of recovery (%R) and relative standard deviations (%RSD) for the whole method.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MQL (ng L(^{-1}))</th>
<th>Internal standard corrected</th>
<th>%R (%RSD)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Effluent</td>
<td>Influent</td>
<td>Effluent (0.1 µg L(^{-1}))</td>
</tr>
<tr>
<td>MMP</td>
<td>31</td>
<td>32</td>
<td>105 (13)</td>
</tr>
<tr>
<td>MEP</td>
<td>7.5</td>
<td>8.1</td>
<td>100 (15)</td>
</tr>
<tr>
<td>MiBP</td>
<td>2.5</td>
<td>3.2</td>
<td>108 (9)</td>
</tr>
<tr>
<td>MnBP</td>
<td>2.8</td>
<td>3.9</td>
<td>107 (10)</td>
</tr>
<tr>
<td>MBzP</td>
<td>0.5</td>
<td>0.5</td>
<td>101 (9)</td>
</tr>
<tr>
<td>MEHHP</td>
<td>4.2</td>
<td>3.2</td>
<td>108 (11)</td>
</tr>
<tr>
<td>MEOHP</td>
<td>4.5</td>
<td>2.2</td>
<td>102 (13)</td>
</tr>
<tr>
<td>MECPP</td>
<td>4.0</td>
<td>1.7</td>
<td>107 (11)</td>
</tr>
</tbody>
</table>

Table S6. Regression coefficients (R) and p-values (p) for the pairwise correlation study between phthalate monoester excretion loads in 24 h influent samples. For Santiago, we used the average values of the seven days.

<table>
<thead>
<tr>
<th></th>
<th>MMP</th>
<th>MEP</th>
<th>MiBP</th>
<th>MnBP</th>
<th>MBzP</th>
<th>MEHHP</th>
<th>MEOHP</th>
<th>MECPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP</td>
<td>R</td>
<td>-0.213</td>
<td>-0.185</td>
<td>-0.3027</td>
<td>0.9527</td>
<td>-0.2248</td>
<td>-0.3607</td>
<td>-0.341</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.6847</td>
<td>0.7262</td>
<td>0.5598</td>
<td>0.0123</td>
<td>0.6685</td>
<td>0.4824</td>
<td>0.5084</td>
</tr>
<tr>
<td>MEP</td>
<td>R</td>
<td>-0.2134</td>
<td>-0.185</td>
<td>-0.3027</td>
<td>0.9527</td>
<td>-0.2248</td>
<td>-0.3607</td>
<td>-0.341</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.6847</td>
<td>0.7262</td>
<td>0.5598</td>
<td>0.0123</td>
<td>0.6685</td>
<td>0.4824</td>
<td>0.5084</td>
</tr>
<tr>
<td>MiBP</td>
<td>R</td>
<td>-0.1846</td>
<td>0.9975</td>
<td>0.9935</td>
<td>0.9342</td>
<td>0.494</td>
<td>0.3984</td>
<td>0.1959</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.7262</td>
<td>0.9975</td>
<td>0.9935</td>
<td>0.9342</td>
<td>0.494</td>
<td>0.3984</td>
<td>0.1959</td>
</tr>
<tr>
<td>MnBP</td>
<td>R</td>
<td>0.9527</td>
<td>0.9532</td>
<td>0.9342</td>
<td>0.945</td>
<td>0.45</td>
<td>0.3713</td>
<td>0.17</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.0123</td>
<td>0.0121</td>
<td>0.0201</td>
<td>0.0154</td>
<td>0.3705</td>
<td>0.4686</td>
<td>0.7474</td>
</tr>
<tr>
<td>MBzP</td>
<td>R</td>
<td>0.9527</td>
<td>0.9532</td>
<td>0.9342</td>
<td>0.945</td>
<td>0.3348</td>
<td>0.1558</td>
<td>-0.0347</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.0123</td>
<td>0.0121</td>
<td>0.0201</td>
<td>0.0154</td>
<td>0.3705</td>
<td>0.4686</td>
<td>0.7474</td>
</tr>
<tr>
<td>MEHHP</td>
<td>R</td>
<td>-0.2248</td>
<td>0.4844</td>
<td>0.494</td>
<td>0.45</td>
<td>0.3348</td>
<td>0.9766</td>
<td>0.9354</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.0685</td>
<td>0.3302</td>
<td>0.3193</td>
<td>0.3705</td>
<td>0.5818</td>
<td>0.0008</td>
<td>0.0061</td>
</tr>
<tr>
<td>MEOHP</td>
<td>R</td>
<td>-0.3607</td>
<td>0.3867</td>
<td>0.3984</td>
<td>0.3713</td>
<td>0.1558</td>
<td>0.9766</td>
<td>0.9762</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.4824</td>
<td>0.4489</td>
<td>0.434</td>
<td>0.4686</td>
<td>0.8025</td>
<td>0.0008</td>
<td>0.0008</td>
</tr>
<tr>
<td>MECPP</td>
<td>R</td>
<td>-0.341</td>
<td>0.1868</td>
<td>0.1959</td>
<td>0.17</td>
<td>-0.035</td>
<td>0.9354</td>
<td>0.9762</td>
</tr>
<tr>
<td></td>
<td>p</td>
<td>0.5084</td>
<td>0.723</td>
<td>0.7099</td>
<td>0.7474</td>
<td>0.9558</td>
<td>0.0061</td>
<td>0.0008</td>
</tr>
</tbody>
</table>
**Table S7.** Phthalate monoester estimated concentrations in urine (this study) versus median values of the concentrations found in 21 urine samples from the Spanish population (Herrero et al. 2015) and geometric mean of the concentrations measured in urine from 120 Spanish mothers (Cutanda et al. 2015).

<table>
<thead>
<tr>
<th>Average concentration (µg L⁻¹)</th>
<th>MMP</th>
<th>MEP</th>
<th>MiBP</th>
<th>MnBP</th>
<th>MBzP</th>
<th>MEHHP</th>
<th>MEOHP</th>
<th>MECPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ares</td>
<td>32.7</td>
<td>358.5</td>
<td>65.7</td>
<td>66.0</td>
<td>4.2</td>
<td>12.7</td>
<td>7.1</td>
<td>7.2</td>
</tr>
<tr>
<td>Baiona</td>
<td>37.6</td>
<td>396.4</td>
<td>71.6</td>
<td>70.6</td>
<td>2.6</td>
<td>8.0</td>
<td>5.4</td>
<td>3.7</td>
</tr>
<tr>
<td>Cambados</td>
<td>150.0</td>
<td>906.5</td>
<td>143.0</td>
<td>138.7</td>
<td>10.6</td>
<td>35.2</td>
<td>15.6</td>
<td>14.9</td>
</tr>
<tr>
<td>Gondomar</td>
<td>27.7</td>
<td>173.8</td>
<td>38.9</td>
<td>31.7</td>
<td>0.8</td>
<td>35.9</td>
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<td>34.2</td>
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<td>216.3</td>
<td>38.5</td>
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<tr>
<td><strong>Weighted average</strong></td>
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<td><strong>276.4</strong></td>
<td><strong>49.7</strong></td>
<td><strong>48.7</strong></td>
<td><strong>3.4</strong></td>
<td><strong>11.1</strong></td>
<td><strong>5.3</strong></td>
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<td><strong>Cutanda et al. 2015</strong></td>
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<td><strong>32.7</strong></td>
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<td><strong>21.4</strong></td>
<td><strong>13.8</strong></td>
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*In Nigrán, the estimated concentration in urine of MBzP was calculated considering a concentration in wastewater equal to LOD/2*
Figure S1. MEHP chromatogram of a procedural blank (green line) overlapped to a 10 ng mL$^{-1}$ standard (red line).
Figure S2. Isomer separation (MiBP and MnBP) on the C18 and the Phenyl-Hexyl column using water-MeOH and water-ACN as mobile phases.
**Figure S3.** Percentage of molar formation of MnBP and MBzP relative to the parent concentration in ultrapure water, at different pH and different temperatures (n=3).

MnBP

<table>
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<tr>
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<tr>
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MBzP

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<th>Temperature</th>
<th>% Formation from Parent Molar Concentration</th>
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<td>7</td>
<td>Room T</td>
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</table>
**Figure S4.** Recovery of the SPE process in wastewater extracted on Oasis HLB and Oasis MAX cartridges. Matrix effects for both sorbents, calculated by comparing the response of a spiked-after-SPE extract with that of a standard in MeOH (n=3).