Application of PBPK Modeling and *In Vitro* Assays to Conduct Cumulative Risk Assessments for Environmental Exposures

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Relationship of Exposure, Biomonitoring Data, and Health Effects

EXPOSURE

TARGET TISSUE DOSE

HEALTH EFFECTS

PBPK Modeling

In Vitro Data

Biomonitoring data alone do not fully reflect the nature of exposure (i.e. routes, activities)

Biomonitoring data may be complexly related to the biological effective dose at the site of action

Biomonitoring data alone do not provide information on health effects or risk

from all possible sources at a given time
Linking Exposure to Health Effects

Chemical concentrations in human blood from biomonitoring studies

Chemical concentrations in animal blood in toxicity studies

Pharmacokinetic Modeling

Reverse dosimetry

Forward dosimetry

Human exposures
(Chemical concentrations in environment)

Animal exposures
(Administered doses in toxicity studies)

Margin of safety

Traditional risk assessment

Pharmacokinetic modeling
Linking Exposure to Health Effects
Problems Vary with the Nature of the Chemical

- **Volatiles**
  - Complex household exposures
  - Rapid clearance
    - Blood levels highly sensitive to recent exposures

- **Highly persistent compounds**
  - Slow approach to steady state
  - Apparent clearance confounded by changes in body weight, fat content

- **Intermediate persistence compounds**
  - Interpretation depends on rate of clearance
    - Need to consider timing of exposures vs. sampling
  - May need to deal with active metabolites
Linking Exposure to Health Effects
Problems Vary with the Nature of the Biomarker

- **Active parent chemical / metabolite in blood**
  - Often a good surrogate for target tissue dose
  - Directly comparable to blood levels at NOAEL/LOAEL
  - Estimation of exposure requires PK modeling

- **Inactive parent chemical / metabolite in blood**
  - Any use requires PK modeling
  - Not directly comparable to blood levels at NOAEL/LOAEL

- **Parent chemical or metabolite in urine**
  - Estimation of exposure (uptake) depends on representativeness of sample
  - Estimation of blood levels requires PBPK modeling
Exposure Reconstruction: An “ILL-Posed Problem”
(Many possible solutions)

Comparison of PBBK Predicted Blood Concentrations with Experimental Data

Comparison of Reconstructed Exposure Conditions with Actual Exposure Conditions

Requires population-level, probabilistic approach

(Sohn et al., 2004)
Monte Carlo Analysis to Simulate Population Exposures
(Liao et al. 2007)

<table>
<thead>
<tr>
<th>Component</th>
<th>Exposure Parameters</th>
<th>Physiological Parameters</th>
<th>Partition Coefficients</th>
<th>Kinetic Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Gut Tissue</td>
<td></td>
<td></td>
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<tr>
<td>Lung</td>
<td></td>
<td></td>
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<tr>
<td>Fat Tissue</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Rapidly Perfused Tissues</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Slowly Perfused Tissues</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Duodenum</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Liver Tissue</td>
<td></td>
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</tr>
<tr>
<td>Surface</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Time of the day

QSAR

In Vitro
Monte Carlo Simulation for TCE Concentrations in Blood Across a Population (Liao et al., 2007)

Impact of Uncertainty in QSAR-derived PBPK Model

![Histogram of ln(TCE in water, ug/L) with bars for QSAR-based and published model-based parameters]
Probabilistic Reverse Dosimetry Approach
(Tan et al., 2006, 2007)

- Chloroform level in blood (ppt)
- Probability

Monte Carlo analysis

- ppb of chemical in air or μg/L of chemical in water
- Estimated distribution of chemical in blood
- "Exposure Conversion Factor" distribution
- Convolute Distributions
- Estimated population exposure distribution
- Distribution of measured blood concentrations

Invert Distribution
Predicted Distribution of Exposures to Chloroform in the Population Reported in NHANES III (mg/kg/day)
Cumulative Risk Assessment for Phthalates Using *In Vitro* Potency Estimates

- **Goal:** Cumulative risk for combined exposures to all phthalates, including any active metabolites

- **Problem:** *In vivo* data not available on all phthalates / metabolites reported in NHANES

- **Risk assessment approach**
  1. Estimate daily intake (Di) of each phthalate ester from NHANES urinary excretion data
  2. Estimate relative potency (Pi) of phthalate monoesters and oxidative metabolites using *in vitro* assay for testosterone inhibition, using MBP as the basis for comparison
  3. Calculate cumulative risk: \( R_{cum} = \sum (Pi \times Di) \)
  4. Determine MoE vs. RfD (0.1 mg/kg/d)
In Vitro Assay for Testosterone Inhibition (R2C rat Leydig cells)

Comparison of in vitro and in vivo IC₅₀ values.

<table>
<thead>
<tr>
<th></th>
<th>In vivo (fetal rat testes)ᵃ</th>
<th>In vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>MEHP</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>MEP</td>
<td>&gt;306ᵇ</td>
<td>205</td>
</tr>
<tr>
<td>MMP</td>
<td>&gt;&gt;409ᵇ</td>
<td>713.9</td>
</tr>
</tbody>
</table>

ᵃCalculated from PBPK models (Clewell et al., 2008; Gentry et al., 2011) and data in rat fetal testes (Clewell et al., 2009; Kurata et al., 2012).
ᵇNo testosterone reduction measured at highest tested doses (750 mg/kg/day) in vivo. Fetal testes monoester concentrations at 750 mg/kg/day DEP and DMP maternal dose (Clewell et al., 2010).
## Estimating Cumulative Phthalate Risk

<table>
<thead>
<tr>
<th>COMPOUND</th>
<th>Percentile</th>
<th>Concentration in Urine (μg/g creatinine)</th>
<th>Daily Intake (μMol/day)</th>
<th>Relative potency factor</th>
<th>MBP Equivalent Intake (μMol/day)</th>
<th>DBP Equivalent Intake (mg/kg/day)</th>
<th>% Total Risk that results directly from DBP</th>
<th>MoE vs RfD = 0.1 (mg/kg/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mono butyl phthalate (MBP)</td>
<td>50th %</td>
<td>21.5</td>
<td>0.1016</td>
<td>1</td>
<td>0.1016</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>95th %</td>
<td>91.5</td>
<td>0.4323</td>
<td>1</td>
<td>0.4323</td>
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<td></td>
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<tr>
<td>Mono-2-ethylhexyl phthalate (MEHP)</td>
<td>50th %</td>
<td>4.43</td>
<td>0.0167</td>
<td>0.53</td>
<td>0.0089</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>95th %</td>
<td>35.1</td>
<td>0.1324</td>
<td>0.53</td>
<td>0.0702</td>
<td></td>
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<tr>
<td>5-hydroxy MEHP</td>
<td>50th %</td>
<td>17.6</td>
<td>0.0628</td>
<td>0.194</td>
<td>0.0122</td>
<td></td>
<td></td>
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</tr>
<tr>
<td></td>
<td>95th %</td>
<td>160</td>
<td>0.5708</td>
<td>0.194</td>
<td>0.1107</td>
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<tr>
<td>5-oxo MEHP</td>
<td>50th %</td>
<td>12.5</td>
<td>0.0449</td>
<td>0.029</td>
<td>0.0013</td>
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<tr>
<td></td>
<td>95th %</td>
<td>92.3</td>
<td>0.3315</td>
<td>0.029</td>
<td>0.0096</td>
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<tr>
<td>Mono-ethyl phthalate (MEP)</td>
<td>50th %</td>
<td>171</td>
<td>0.9255</td>
<td>0.0145</td>
<td>0.0134</td>
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<tr>
<td></td>
<td>95th %</td>
<td>1430</td>
<td>7.7397</td>
<td>0.0145</td>
<td>0.1122</td>
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<tr>
<td>Mono-methyl phthalate (MMP)</td>
<td>50th %</td>
<td>1.45</td>
<td>0.0085</td>
<td>0.0042</td>
<td>0.0000</td>
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<tr>
<td></td>
<td>95th %</td>
<td>10</td>
<td>0.0583</td>
<td>0.0042</td>
<td>0.0002</td>
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<tr>
<td>Mono (2-octyl) phthalate (MnOP)</td>
<td>50th %</td>
<td>&lt; LOD</td>
<td></td>
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<td></td>
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<td></td>
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<tr>
<td></td>
<td>95th %</td>
<td>3.1</td>
<td>0.0117</td>
<td>0.125</td>
<td>0.0015</td>
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<tr>
<td>Mono benzyl phthalate (MBzP)</td>
<td>50th %</td>
<td>15.1</td>
<td>0.0714</td>
<td>0.109</td>
<td>0.0078</td>
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</tr>
<tr>
<td></td>
<td>95th %</td>
<td>95.8</td>
<td>0.4531</td>
<td>0.109</td>
<td>0.0494</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total:</td>
<td>50th %ile</td>
<td>0.1452</td>
<td>0.00067</td>
<td>70%</td>
<td>148</td>
<td></td>
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<tr>
<td></td>
<td>95th %ile</td>
<td>0.7861</td>
<td>0.00365</td>
<td>55%</td>
<td>28</td>
<td></td>
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</tbody>
</table>
Goal: Cumulative risk for exposures to all phthalates, including any active metabolites

Difficulty:
- Exposure estimates are based on urinary concentrations of total monoester (free plus glucuronide)
- Appropriate dose metric for health effects is free blood concentrations of all active compounds (monoesters and their active metabolites)

Role of PBPK model:
- Relate of total monoester urine concentrations to blood concentrations of free monoester
- Estimate maternal exposure from NHANES data (reverse dosimetry) and predict associated fetal exposure (forward dosimetry)
Estimation of parameters for human phthalate model

Physiological parameters
(literature, e.g.: Brown et al., 1997)

Partitioning parameters
(in vitro: rodent tissue)

Metabolic parameters
(in vitro: human tissue)

Pharmacodynamic potency
(in vitro: rodent cells)

PBPK model

PD model

In vivo kinetic data

Validation
Metabolism of MEHP by 23 selected human CYP isoforms (Choi et al., 2011)

Percentage of human UGT isoforms involved in phase II conjugation. (Joo et al., 2011)
Reverse Dosimetry

- Simulations of 60 exposure concentrations - 0.01 to 10 µg/kg
- Exposures - oral bolus (i.e., ingestion of food) occurring over 15 min
- Urine and amount of MBP accumulated for random lengths of time beginning just prior to ingestion of meal to 3 h post exposure
- Sampling times varied from 0.5 to 3 h post last elimination event
- 1,000 iterations were accumulated at each exposure concentration
### Exposure Reconstruction for NHANES III Data on Di-n-Butyl Phthalate

<table>
<thead>
<tr>
<th>Percentile</th>
<th>NHANES III Concentration in Urine (µg/L)</th>
<th>Predicted Intermittent Exposure* (µg/kg/day)</th>
<th>Continuous Exposure (µg/kg/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>-</td>
<td>0.30</td>
<td>0.25</td>
</tr>
<tr>
<td>25%</td>
<td>-</td>
<td>0.45</td>
<td>0.25</td>
</tr>
<tr>
<td>50%</td>
<td>20.4</td>
<td>1.17</td>
<td>0.8</td>
</tr>
<tr>
<td>75%</td>
<td>40.4</td>
<td>3.78</td>
<td>1.4</td>
</tr>
<tr>
<td>90%</td>
<td>73.6</td>
<td>5.46</td>
<td>3.0</td>
</tr>
<tr>
<td>95%</td>
<td>108</td>
<td>7.74</td>
<td>4.2</td>
</tr>
</tbody>
</table>

*Assumed to be sum of three ingestion events per day

Compared well with 24-hour urine data in German study (Wittassek et al., 2007): average = 1.9 µg/kg/day, 95% = 5.3 µg/kg/day
Forward dosimetry analyses with the human pregnancy PBPK model for DBP

- Based on 100,000 iterations using the reverse dosimetry output

<table>
<thead>
<tr>
<th></th>
<th>Daily Exposure (µg/kg/day)</th>
<th>Maternal Blood</th>
<th>Maternal Blood Total</th>
<th>Fetal Blood</th>
<th>Fetal Testes</th>
<th>Amniotic Fluid</th>
<th>Urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1.885</td>
<td>0.32</td>
<td>0.52</td>
<td>0.21</td>
<td>0.073</td>
<td>2.02</td>
<td>44.8</td>
</tr>
<tr>
<td>Std. Deviation</td>
<td>2.038</td>
<td>0.34</td>
<td>0.55</td>
<td>0.27</td>
<td>0.096</td>
<td>6.87</td>
<td>61.7</td>
</tr>
<tr>
<td>CV %</td>
<td>108.1%</td>
<td>108.2%</td>
<td>106.8%</td>
<td>125.8%</td>
<td>131.2%</td>
<td>340.2%</td>
<td>137.7%</td>
</tr>
</tbody>
</table>

**Confidence Interval of the Mean**

<table>
<thead>
<tr>
<th></th>
<th>Lower 95%</th>
<th>Upper 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily Exposure</td>
<td>1.872</td>
<td>1.897</td>
</tr>
<tr>
<td>Maternal Blood</td>
<td>0.316</td>
<td>0.32</td>
</tr>
<tr>
<td>Maternal Blood Total</td>
<td>0.51</td>
<td>0.52</td>
</tr>
<tr>
<td>Fetal Blood</td>
<td>0.21</td>
<td>0.22</td>
</tr>
<tr>
<td>Fetal Testes</td>
<td>0.073</td>
<td>0.074</td>
</tr>
<tr>
<td>Amniotic Fluid</td>
<td>1.98</td>
<td>2.06</td>
</tr>
<tr>
<td>Urine</td>
<td>44.5</td>
<td>45.2</td>
</tr>
</tbody>
</table>
Comparison of mono-butyl phthalate concentration in maternal and fetal tissues in the rodent at the NOAEL (10 mg/kg/day, Lehmann et al., 2004) and the human at 95th percentile determined in the forward dosimetry.
Acknowledgements

Hamner colleagues

Rebecca Clewell – phthalate PBPK model development
  – phthalate in vitro assay development
Jerry Campbell – human phthalate PBPK modeling
Pergentino Balbuena – phthalate in vitro assay
Cecilia Tan – reverse dosimetry approach (now at EPA)

Funding

ACC – reverse dosimetry, PBPK model for DBP, in vitro phthalate assay
CCC – trihalomethane reverse dosimetry
EPA NCER STAR Grant – cumulative risk assessment for phthalates