Variability in excretion of urinary metabolites of toxicants with short half-lives: Implications for results communication and interpretation

Asa Bradman, Ph.D.
Center for Environmental Research and Children's Health (CERCH)
School of Public Health
1995 University Avenue Suite 265
Berkeley, CA 94720

July 26, 2012
Today’s Talk

• Brief background on urinary metabolites as exposure biomarkers
• Examples of within and between subject variability
• Introduce discussion on
  o Implications for communicating results to study participants
  o Implications for results interpretation
Common Exposure Biomarkers

Measurements in:

• Urine
• Blood
• Breast milk
• Saliva
• Hair
• Meconium

• Urine is easy to collect, non-invasive, readily available, laboratory methods are commonly available. Especially useful for children.
What is a Metabolite?

Figure 1. The general metabolism of O,O-diethyl OP pesticides using diazinon as a model. The metabolites enclosed in boxes are excreted in urine.

Barr et al 2004
Example: Organophosphosphate (OP) Pesticides

- Account for about ½ of all insecticides used in U.S.
- Widely used in agriculture
- Some banned for home use
- Short half-lives in body (hrs-days)
- Excreted in urine as dialkyl phosphate (DAP) metabolites
- Acute neurotoxins
Urinary Biomarkers of Exposure

- **Dialkylphosphates (DAPs):** excretory products of OPs
  - DAPS = 3 dimethyls & 3 diethylls
  - ~75% of registered OPs metabolize to DAPs in urine
  - CLASS-specific NOT pesticide-specific

![Chemical Structures](diagram.png)
CHAMACOS Cohort Study

- Pregnancy (two visits)
- 6 mo
- 2 yr
- 5 yr
- 9 yr
- 12 yr
Prenatal and child OP metabolites in CHAMACOS and National Reference

* National Health and Nutrition Examination Survey
Fruit juice consumption could explain some variability in OP levels

Bradman and Kogut, et. al. in preparation
Prenatal DAPS related to WISC IQ at age 7

Adjusted for HOME score (6 months), maternal education and IQ, and language of testing

Bouchard et al., EHP, 2011
For OPs, urinary metabolites can provide valuable information about exposure and health effects.

However, there are limitations.
Variability

Sources:

• Intermittent exposures
• Short half-life in body
• Differences in metabolic capacity
• Differences in pharmacokinetic characteristics
Correlation of 24 hr samples collected three days apart (n=25, ages 3-6, Cr adj.)

<table>
<thead>
<tr>
<th></th>
<th>Correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total DAPS</td>
<td>0.35</td>
</tr>
<tr>
<td>Dimethyls</td>
<td>0.36</td>
</tr>
<tr>
<td>Diethyls</td>
<td>0.15</td>
</tr>
</tbody>
</table>

p>0.5

Bradman and Kogut et al, in final review. Do not cite.
Metabolites in 24 hr urine samples collected 3 days apart (n=25 pairs).

Estimated within and between variability (SD)

Between 35%
Within 65%

Do not cite.
Bradman and Kogut et al, in final review
Correlation of DAP metabolites in spot urine samples collected 1-6 days apart

<table>
<thead>
<tr>
<th>Days Elapsed Between Paired Samples</th>
<th>0 (Same Day)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spot Samples</td>
<td>(N=92(^a))</td>
<td>(303)</td>
<td>(248)</td>
<td>(203)</td>
<td>(154)</td>
<td>(77)</td>
<td>(25)</td>
</tr>
<tr>
<td>Total DAPs</td>
<td>0.46*</td>
<td>0.45*</td>
<td>0.25*</td>
<td>0.23*</td>
<td>0.17*</td>
<td>0.16</td>
<td>0.16</td>
</tr>
<tr>
<td>Total DMs</td>
<td>0.49*</td>
<td>0.48*</td>
<td>0.35*</td>
<td>0.30*</td>
<td>0.21*</td>
<td>0.18</td>
<td>0.13</td>
</tr>
<tr>
<td>Total DEs</td>
<td>0.25*</td>
<td>0.22*</td>
<td>-0.15</td>
<td>0.08</td>
<td>0.02</td>
<td>0.11</td>
<td>-0.27</td>
</tr>
</tbody>
</table>
## Variance apportionment for OP, BPA, and phthalate metabolites

<table>
<thead>
<tr>
<th>Variance</th>
<th>OP (%)</th>
<th>BPA (%)</th>
<th>Ph- DEHP (%)</th>
<th>Ph- DEP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between subject</td>
<td>31</td>
<td>9</td>
<td>17</td>
<td>77</td>
</tr>
<tr>
<td>Within-subject between-day</td>
<td>20</td>
<td>21</td>
<td>32</td>
<td>2</td>
</tr>
<tr>
<td>Within-subject within-day</td>
<td>49</td>
<td>70</td>
<td>51</td>
<td>21</td>
</tr>
</tbody>
</table>

Source: Bradman et al submitted (OP - children)
Source: Preau et al 2010; Ye et al 2010 (BPA and phthalates - adults)
BPA in adult urine over one week

Ye et al 2010
OP Exposure Classification in Children: Sensitivity and Specificity of Spot Samples vs One Week of Exposure

<table>
<thead>
<tr>
<th></th>
<th>High (Top 20%)</th>
<th>Elevated (Top 40%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity</td>
<td>Specificity</td>
</tr>
<tr>
<td>One Sample</td>
<td>0.46</td>
<td>0.87</td>
</tr>
<tr>
<td>Two Samples&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.60</td>
<td>0.90</td>
</tr>
<tr>
<td>Three Samples&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.64</td>
<td>0.91</td>
</tr>
</tbody>
</table>

Bradman and Kogut, in final review. Do not cite.
Implications for Returning Results

• Some metabolites show little intra-individual variability in spot samples.

• For others, high within-subject variability raises challenges:
  
  ➢ providing exposure information to participants (acute, vs. chronic)
  ➢ comparing to larger group or other populations
CHAMACOS: Approaches for Results Return

• Begins with consent
  ➢ Purpose of study
  ➢ Medical or non-medical utility of measurements
  ➢ Informed that results available
• Return results at each visit if requested
• One on one meeting
• Offer repeat testing – usually some tests low
• Provide education on reducing exposures

➢ To date: No problems or other concerns among participants.
Technical Challenges

- Urinary metabolites are a valuable tool to assess exposure to non-persistent pesticides:
  - Ease of collection, especially important for children
  - Laboratory methods often available

- Potential for exposure misclassification
Implications for epidemiology and risk assessment

• High intra-individual variability.

  ➢ Cross sectional sampling *may* give range of population exposure, but not indicator of individual chronic exposure.

  ➢ Single measurements may be relevant to acute exposures, but not chronic.

  ➢ Studies need to consider these factors to be adequately powered
Research Needs

• More research is needed to evaluate intra- and inter-person variability of exposure biomarkers;
Thanks to our funders

and Katie Kogut
Questions/Discussion