Study of Phthalates in Pregnant Women and Children

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STAR PROGRESS REVIEW WORKSHOP
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• A large, coherent body of animal studies demonstrates reproductive toxicity of several phthalates

• Phthalate exposure is prevalent in the US population (CDC 2002, 2003)

• Urine assays are now sensitive, reliable and (relatively) inexpensive

No human studies of prenatal phthalate exposure prior to ours
Phthalates and their metabolites

**Parent Compound**
- Dibutyl phthalate (DBP)
- Benzyl butyl phthalate (BzBP)
- Di-isobutyl phthalate (DiBP)
- Diethyl phthalate (DEP)
- Di-n-octyl phthalate (DnOP)
- Dimethyl phthalate (DMP)
- Di-2-ethylhexyl phthalate (DEHP)

**Primary Metabolites**
- Mono-n-butyl phthalate (MBP)
- Mono-benzyl phthalate (MBzP)
- Mono-isobutyl phthalate (MiBP)
- Mono-ethyl phthalate (MEP)
- Mono-3-carboxypropyl phthalate (MCPP)
- Mono-methyl phthalate (MMP)
- Mono-2-ethylhexyl phthalate (MEHP)
- Mono-2-ethyl-5-hydroxyhexyl phthalate (MEHHP)
- Mono-2-ethyl-5-oxohexyl phthalate (MEOHP)
**Di-2-Ethylhexyl Phthalate (DEHP)**

- US production >1 mill lbs/yr
- >95% used as a plasticizer in PVC
- Not chemically bound: migrates from plastic
- Multiple urinary metabolites
  - **MEHHP (52.7%)**
  - **MEOHP (31.8%)**
  - **MEHP (15.5%)**

(Koch 2004)
Dibutyl Phthalate (DBP)

- **US production:**
  >1 million lbs/yr
- **Occupational exposure:**
  (e.g. nail salons)
- **In:**
  - Cellulose acetate plastics
  - Lacquer, varnish, adhesives
  - Medical coatings and patches
  - Cosmetics and nail polish
Overview of Phthalate Toxicology

- Anti-androgenic
- Decrease AGD
- Phthalate Syndrome
Phthalates decrease fetal testosterone

DEHP-induced reduction in fetal testosterone is age dependent

(Parks et al, 2000)
Anogenital Distance (AGD)

- Sexually dimorphic: In rodents, AGD is about twice as long in males as in females
- In rodents, male AGD is shortened by prenatal exposure to anti-androgens (including: phthalates, flutamide and vinclozolin)
DEHP and AGD (Moore, 2001)

Male:female ratio = 2.0

Male:female ratio = 1.6
Antiandrogen-treated rats with reduced AGD at birth have reduced AGD as adults.
Hotchkiss et al. 2004

“AGD is forever”
(Gray 2006)
Phthalate Syndrome

Malformations of:
- Perineum: Reduced AGD
- Epididymis
- Vas deferens
- Seminal vesicles
- Prostate
- External genitalia:
  - Hypospadias
  - Cryptorchidism

Downregulation of:
- Fetal testicular testosterone
- Insl-3

Significant ↓ in testosterone and Leydig cell differentiation @ doses below the NOAEL for DBP
The question we addressed:

Does prenatal phthalate exposure alter male sexual development in humans?
Study of Phthalates in Pregnant Women and Children

Designed to assess infant genital development in relation to prenatal phthalate exposure
Study Population

Mothers who:

• Were recruited at a prenatal visit in: Columbia MO, Minneapolis MN, and Los Angeles CA
• Agreed to follow-up study
• Provided a prenatal urine sample
  – Urine was not requested in first study year
Boys’ Physical Exam

- Anthropometry
- Male genital exam
  - Anogenital distance
  - Testicular descent
  - Penile length and width
  - Scrotal size and condition
Measuring Anogenital Distance

This is similar to toxicological measure

AGD is repeatable (CV = 7.2%)
• AGD rarely measured in Humans

• In girls: AGD is distance A to C

AGD by Sex

If male:female ratio = 2.0

Male:female ratio = 1.5
Analysis of Male Anogenital Distance (AGD)

- AGD increases with both age and weight
- These are strongly correlated
- We used standard growth curves to adjust for body size (CDC, 2000)
- Weight percentile (WT%) calculated for each boy at each visit
Analysis of AGD (Continuous)

- **Expected AGD** modeled:
  - Using all visits (mixed model)
  - WT% and age were the only significant predictors

- **AGD and phthalates**
  - Using all visits (mixed model)
  - Log transformed phthalate metabolite concentration used (to normalize data)
Phthalate Exposure Assessment

- Samples were collected mid-pregnancy (median 28.6 weeks gestation)
- Concentration of nine phthalate metabolites measured by CDC (blinded to identify of individuals or outcomes)
- Analyzed as continuous and categorical variables
  - low (<25\textsuperscript{th}%), medium, high(\geq 75\textsuperscript{th}%)
- Creatinine (and square-root creatinine) not significant covariates
Concentration of four metabolites in prenatal samples (N=85, ng/mL)

<table>
<thead>
<tr>
<th></th>
<th>25th P</th>
<th>Median</th>
<th>75th P</th>
<th>% &gt; LOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>MBP</td>
<td>7.2</td>
<td>13.5</td>
<td>30.9</td>
<td>97</td>
</tr>
<tr>
<td>MBzP</td>
<td>3.5</td>
<td>8.3</td>
<td>23.5</td>
<td>94</td>
</tr>
<tr>
<td>MiBP</td>
<td>0.7</td>
<td>2.5</td>
<td>5.1</td>
<td>74</td>
</tr>
<tr>
<td>MEP</td>
<td>53.3</td>
<td>128.4</td>
<td>436.9</td>
<td>98</td>
</tr>
</tbody>
</table>

Levels somewhat lower than those measured in US female population
AGI by Metabolite Concentration

Metabolite concentration (ng/mL)

Long  Medium  Short

AGI
Blue: Long
Red: Medium
Yellow: Short

MBP  MBzp  MEP/10  MiBP x 10
*Odds ratio is relative to low concentration for that analyte (< 25th percentile). Bars represent 95% confidence interval.

Abbreviations: MBP = Mono-n-butyl phthalate, MBzP = Mono-benzyl phthalate, MEP = Mono-ethyl phthalate, MiBP = Mono-isobutyl phthalate
Categorical Analysis of AGD

- **Residual AGD** = Observed AGD - Expected AGD
- Categorized AGD by size of the residual:
  - "**Shorter**" < 25\textsuperscript{th} Percentile
  - 25\textsuperscript{th}% ≤ "**Intermediate**" < 75\textsuperscript{th}%
  - 75\textsuperscript{th} percentile ≤ "**Longer**" AGD:
## Prenatal MBP and Male AGI

<table>
<thead>
<tr>
<th>MBP</th>
<th>AGI*</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Smaller</td>
<td>Not</td>
</tr>
<tr>
<td>Low</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Med</td>
<td>24</td>
<td>19</td>
</tr>
<tr>
<td>High</td>
<td>17</td>
<td>5</td>
</tr>
</tbody>
</table>

*Smaller = Less than age-adjusted expected value, Not smaller = at least as large as age-adjusted expected value
The problem of mixtures

• Until recently, toxicology examined one phthalate at a time
• But that is not how people are exposed: most people are exposed to multiple phthalates (CDC, 2005)
• New toxicology suggests: 
  – Dose Additivity
Cumulative Toxicity of Phthalates

- **Mixtures**
  - DBP plus BBP
  - DEHP plus DBP
  - Procymidone plus DBP
  - Linuron plus BBP
  - Seven antiandrogens, including 3 phthalates
  - These mixtures all produced cumulative effects

Gray, Personal communication
Joint exposure to four “High Risk” phthalates and AGI

<table>
<thead>
<tr>
<th>Joint Score</th>
<th>AGI* Smaller</th>
<th>Larger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highest</td>
<td>9</td>
<td>1</td>
</tr>
<tr>
<td>Lowest</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>11</td>
</tr>
</tbody>
</table>

*Highest = All (or all but one) of 4 phthalates in top 25%; Lowest = All (or all but one) in lowest 25%

**Odd ratio unstable but very high:**
Lower 95% confidence interval > 5.5
Other findings

• Significantly correlated with AGD:
  - Degree of testicular descent
  - Penile volume
  - Scrotal size

This cluster of outcomes consistent with “phthalate syndrome” in rodents
Comparing animal and human studies

<table>
<thead>
<tr>
<th>Factor</th>
<th>Rodent</th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td>Route</td>
<td>Oral</td>
<td>Oral, inhalation, dermal, parenteral</td>
</tr>
<tr>
<td>Dose</td>
<td>High, medium</td>
<td>Low</td>
</tr>
<tr>
<td>Agent</td>
<td>Single</td>
<td>Mixture</td>
</tr>
</tbody>
</table>
Routes of Exposure

- Percutaneous
- Ingestion
- Dermal Absorption
- Inhalation

*Measurable in urine, serum, breast milk, amniotic fluid*
Were these changes seen at very high phthalate levels?

- “High” level ($\geq 75^{\text{th}}$ percentile) was compared to levels in NHANES samples
- “High” levels in our study seen in about 25% of adult US females
- How do these compare to EPA reference dose?
## Estimated exposure and EPA reference dose (µg/ kg/ day)

<table>
<thead>
<tr>
<th>Phthalate</th>
<th>Median</th>
<th>95th%</th>
<th>Reference dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>DEHP</td>
<td>1.32</td>
<td>9.32</td>
<td>20</td>
</tr>
<tr>
<td>DEP</td>
<td>6.64</td>
<td>112.3</td>
<td>800</td>
</tr>
<tr>
<td>BBzP</td>
<td>0.50</td>
<td>2.47</td>
<td>200</td>
</tr>
<tr>
<td>DBP</td>
<td>0.99</td>
<td>2.68</td>
<td>100</td>
</tr>
</tbody>
</table>

(Marsee, et al, 2006)
Clinical implications of the phthalate syndrome??

- **In rodents:**
  - At birth: Shorter AGD, impaired testicular descent, hypospadias
  - Later: Low sperm count, rarely testicular tumors

- **Our study of humans suggests:**
  - At birth: Shorter AGD (some, but most NS, decrease in testicular descent, smaller penile volume)

*Future studies needed to determine clinical correlates in humans*
U MO
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Sara Stewart, Lynn Teague

UCLA
Christina Wang, Cathy Mao

U MN
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U IA
Amy Sparks, Shannon Sullivan

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