Effects of Phthalates on the Female Reproductive System

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Overview

• Background
  – Phthalates
    • Di(2-ethylhexyl) phthalate (DEHP)
  – Known effects of phthalates on reproduction
• Our Children’s Center Study (Project 2)
  – Hypothesis
  – Study design
  – Data
  – Conclusions
• Future directions
What is di-(2-ethylhexyl) phthalate?
Di-(2-ethylhexyl) phthalate (DEHP)

- DEHP is a widely used plasticizer in polyvinyl-chloride (PVC) products
- DEHP levels range from 1-40% of total weight in most (PVC) products, up to 80% in some medical equipment
Why the concern?
DEHP is in many products

DEHP can leach from products and expose the general population through ingestion, inhalation, and dermal contact.
DEHP is present in human tissues

- Human blood samples
- Maternal plasma samples
- Urine samples (metabolites)
- Breast milk samples
- Ovarian follicular fluid samples
- Cord blood samples
- Amniotic fluid samples
Prenatal DEHP Exposure Affects Reproductive Outcomes

• Epidemiological studies
  – Prenatal exposure is associated with reduced anogenital distance and testosterone levels in boys (Swan et al. 2005)

• Rodent models
  – Prenatal exposure alters reproductive tract development and sexual differentiation in male offspring (Gray et al. 2000)
  – Little is known about prenatal effects on reproduction in female offspring
What are the effects of prenatal DEHP exposure on the reproductive system of female offspring?

Are these effects transgenerational?
Hypotheses

Prenatal DEHP exposure affects reproductive outcomes in female offspring

Prenatal DEHP exposure has transgenerational effects on reproduction in female offspring
Study Design

Pregnant CD-1 mice orally dosed daily from GD11-birth

Female pups collected from each litter at PNDs 1, 8, 21, and 60

Treatment groups

Vehicle control
20µg/kg/day
200µg/kg/day
200mg/kg/day
500mg/kg/day
750mg/kg/day

Female pups used to monitor estrous cyclicity and fertility (F1, F2, and F3)
Results (F1 Generation)

• Prenatal DEHP exposure does not affect:
  – Anogenital distance
  – Age of pubertal onset
  – Estrous cyclicity

• Prenatal DEHP exposure affects:
  – Uterine weight (increases)
  – Ovarian weight (decreases)
  – Male to female sex ratio (more males)
  – Fertility (decreases)
Effect of DEHP on sex ratio

![Bar graph showing the effect of DEHP on sex ratio.](Image)

- Control
- DEHP 20 µg/kg/day
- DEHP 200 µg/kg/day
- DEHP 200 mg/kg/day
- DEHP 500 mg/kg/day
- DEHP 750 mg/kg/day

* p≤0.05

n = 5-15 dams/treatment group
## Effect of DEHP on Fertility (3 months)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>no litter produced</th>
<th>&gt;5 days to get pregnant</th>
<th>Lost some pups (2 or less)</th>
<th>Lost all pups</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>5.3 (n=19)</td>
<td>0.0 (n=19)</td>
<td>11.1 (n=18)</td>
<td>11.1 (n=18)</td>
</tr>
<tr>
<td>20 µg/kg/day</td>
<td>11.1 (n=9)</td>
<td>22.2 (n=9)*</td>
<td>0.0 (n=8)</td>
<td>0.0 (n=8)</td>
</tr>
<tr>
<td>200 µg/kg/day</td>
<td>18.2 (n=11)</td>
<td>9.1 (n=11)</td>
<td>11.1 (n=9)</td>
<td>11.1 (n=9)</td>
</tr>
<tr>
<td>200 mg/kg/day</td>
<td>11.1 (n=9)</td>
<td>11.1 (n=9)</td>
<td>12.5 (n=8)</td>
<td>0.0 (n=8)</td>
</tr>
<tr>
<td>500 mg/kg/day</td>
<td>25.0 (n=4)</td>
<td>0.0 (n=3)</td>
<td>0.0 (n=3)</td>
<td>0.0 (n=3)</td>
</tr>
<tr>
<td>750 mg/kg/day</td>
<td>10.0 (n=10)</td>
<td>0.0 (n=10)</td>
<td>11.1 (n=9)</td>
<td>22.2 (n=9)</td>
</tr>
</tbody>
</table>

* p < 0.05
### Effect of DEHP on Fertility (6 months)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No litter produced</th>
<th>&gt;5 days to get pregnant</th>
<th>Lost some pups (2 or less)</th>
<th>Lost all pups</th>
</tr>
</thead>
<tbody>
<tr>
<td>control</td>
<td>16.7 (n=12)</td>
<td>25.0 (n=12)</td>
<td>0.0 (n=10)</td>
<td>10.0 (n=10)</td>
</tr>
<tr>
<td>20 µg/kg/day</td>
<td>11.1 (n=9)</td>
<td>25.0 (n=8)</td>
<td>25.0 (n=8)^</td>
<td>25.0 (n=8)</td>
</tr>
<tr>
<td>200 µg/kg/day</td>
<td>27.3 (n=11)</td>
<td>0.0 (n=11)*</td>
<td>0.0 (n=8)</td>
<td>12.5 (n=8)</td>
</tr>
<tr>
<td>500 mg/kg/day</td>
<td>0.0 (n=4)</td>
<td>0.0 (n=4)</td>
<td>0.0 (n=4)</td>
<td>25.0 (n=4)</td>
</tr>
<tr>
<td>750 mg/kg/day</td>
<td>30.0 (n=10)</td>
<td>20.0 (n=10)</td>
<td>28.6 (n=7)^*</td>
<td>0.0 (n=7)</td>
</tr>
</tbody>
</table>

* *p < 0.05
^ *p < 0.06
Are these effects transgenerational?
Hypothesis

Prenatal DEHP exposure has transgenerational effects on reproduction in female offspring

Skinner, 2008
Results (F2 generation)

• DEHP does not affect:
  – sex ratio

• DEHP significantly decreases:
  – Anogenital distance
  – Uterine weight
  – Ovarian weight
  – Fertility
### Effect of DEHP on Fertility (3 months)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Never became pregnant</th>
<th>Took ≥ 5 days to pregnant</th>
<th>Lost some pups (2 or less)</th>
<th>Lost all pups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0% (n=10)</td>
<td>10% (n=10)</td>
<td>20% (n=10)</td>
<td>10% (n=10)</td>
</tr>
<tr>
<td>20µg/kg/day</td>
<td>12.5% (n=8)</td>
<td>14.3% (n=7)</td>
<td>0% (n =7)</td>
<td>0% (n=7)</td>
</tr>
<tr>
<td>200µg/kg/day</td>
<td>12.5% (n=8)</td>
<td>14.3% (n=7)</td>
<td>28.6% (n=7)</td>
<td>42.9% (n=7)</td>
</tr>
<tr>
<td>500mg/kg/day</td>
<td>16.7% (n=6)</td>
<td>20% (n=5)</td>
<td>20% (n=5)</td>
<td>20% (n=5)</td>
</tr>
<tr>
<td>750mg/kg/day</td>
<td>0% (n=7)</td>
<td>14.3% (n=7)</td>
<td>14.3% (n=7)</td>
<td>0% (n=7)</td>
</tr>
</tbody>
</table>
Conclusion

Prenatal DEHP exposure may cause some two-generational effects in female offspring

<table>
<thead>
<tr>
<th>F1 Generation</th>
<th>F2 Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>↑ uterine weight</td>
<td>↓ uterine weight</td>
</tr>
<tr>
<td>↓ ovarian weight</td>
<td>↓ ovarian weight</td>
</tr>
<tr>
<td>↓ fertility</td>
<td>↓ fertility</td>
</tr>
</tbody>
</table>
Future Directions

• Determine if prenatal DEHP exposure affects the F3 generation
• Determine if prenatal DEHP exposure affects hormone levels in the F1, F2, and F3 generations
• Determine if prenatal DEHP exposure affects ovarian structure/function in the F1, F2, and F3 generations
Acknowledgements

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