Prenatal exposure to an environmentally relevant phthalate mixture accelerates reproductive aging in multiple generations of female mice.
Introduction
Phthalates are ubiquitously used in many consumer products including medical bag and IV tubing, PVC pipes, food storage containers, and personal care products. Daily exposure via ingestion, inhalation, and dermal contact.
Phthalates are known endocrine disrupting chemicals

- Males
  - Decreased sperm quality
  - Delayed preputial separation
  - Reduced anogenital distance
  - Reduced anogenital distance
  - Inhibited ovarian steroidogenesis
  - Accelerated primordial follicle recruitment
  - Disrupted estrous cyclicity

- Females
  - Reduced estrous cyclicity
  - Disrupted estrous cyclicity

- Males
  - Decreased sperm quality
  - Decreased sperm quantity
Many studies focus on single phthalate exposure vs. phthalate mixtures, including phthalates. Humans are exposed to mixtures of chemicals.
Phthalate Mixture

- DEP: Diethyl Phthalate
- DEHP: Di(2-ethylhexyl) Phthalate
- DBP: Dibutyl Phthalate
- DiNP: Diisononyl Phthalate
- DiBP: Diisobutyl Phthalate
- BzBP: Benzylbutyl Phthalate

Based on levels detected in the iKids study.
Reproductive Aging in Females

- Normal reproductive aging
  - Depletion of the follicle pool
  - Dysregulation of the hypothalamic-pituitary-gonadal (HPG) axis
  - Acyclicity
  - Decreased fertility
  - Decreased ovulation
  - Increased inflammation
  - Increased fibrosis
  - Increased reactive oxygen species
  - Increased cysts (rodents)

- Ovarian aging
  - Normal reproductive aging

Reproductive Aging in Females
Transgenerational Exposure

Transgenerational

F3

Not exposed

Multigenerational

F2

F1

F0

Direct Exposure

Modified photo courtesy of Katie Chiang
Hypothesis

Prenatal exposure to a mixture of phthalates accelerates reproductive aging in multiple generations of female mice.
Experimental Design

Control
- Mixture 20 µg/kg/day
- Mixture 200 µg/kg/day
- Mixture 200 mg/kg/day
- Mixture 500 mg/kg/day

M1
- Count follicle numbers, measure hormones involved in the HPG axis, and monitor estrous cyclicity at 13 months of age

F0
- GD 10.5 - Birth

F1
- P1

F2
- P2

F3
- P3

Micrographs: Image 1 - Image 2 - Image 3
Phthalate Mixture Caused Irregular Cyclicity in the F3 Generation

* \( p \leq 0.05, \) = \( 0.05 < p < 0.1 \)
Phthalate Mixture May Not Affect Follicle Numbers in the F1 Generation

Numbers in the F1 Generation
### Treatment Percent Ovaries with Cysts

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent Ovaries with Cysts</th>
</tr>
</thead>
<tbody>
<tr>
<td>500 mg/kg/day</td>
<td>55.6</td>
</tr>
<tr>
<td>200 mg/kg/day</td>
<td>77.8</td>
</tr>
<tr>
<td>20 mg/kg/day</td>
<td>62.5</td>
</tr>
<tr>
<td>Control</td>
<td>33.3</td>
</tr>
</tbody>
</table>

Phthalate Mixture May Increase the Occurrence of Cysts in the F1 Generation at 13 months of age.

Cystic ovaries from mouse prenatally exposed to the phthalate mixture in the F1 generation.
Phthalate Mixture Altered Folliculogenesis in the F2 and F3 Generations

F3 Generation

F2 Generation

* p ≤ 0.05, ^ = 0.05 < p < 0.1

n=3-4
Phthalate Mixture Decreased Sex Steroids in the F1 and F2 Generations

Testosterone

Progestrone

Mixture 500 mg/kg/day
Mixture 200 mg/kg/day
Mixture 100 mg/kg/day
Control

* p < 0.05

n=4-11
Phthalate Mixture Altered Gonadotropins in the F1 and F3 Generations

- F1
- F2
- F3

ng/mL

LH

- Mixture 500 mg/kg/day
- Mixture 200 mg/kg/day
- Mixture 100 mg/kg/day
- Control

FSH

n=4

* p ≤ 0.05, ^ = 0.05 < p < 0.1
Overall Summary

- Phthalate mixture accelerates some biomarkers of reproductive aging:
  - Increased time spent in metestrus/diestrus
  - Altered follicle pool
  - Dysregulation of the HPG axis

Overall:

- 8% DBP
- 15% DnBP
- 15% DEP
- 35% DiBP
- 21% DEHP
Conclusion

Prenatal exposure to an environmentally relevant phthalate mixture accelerates some biomarkers of reproductive aging in a multi- and transgenerational manner in female mice.
Future Directions

- Determine if phthalates:
  - Accelerate the aging of the ovary by increasing fibrosis, reactive oxygen species, and inflammation
  - Accelerate the decline in reproductive capacity by causing acyclicity and decreasing fertility quicker than controls
  - Accelerate the decline in reproductive capacity by increasing acyclicity and decreasing fertility quicker than controls
Acknowledgments

Committee members
- Dr. Jodi Flaws
- Dr. Indrani Bagchi
- Dr. Romana Nowak
- Dr. Prabu Reddi

Lab members not pictured
- Dr. Mary Laws
- Dr. Changqing Zhou
- Dr. Prabhu Reddi
- Dr. Romana Nowak
- Dr. Indrani Bagchi
- Dr. Jodi Flaws

Past lab member
- Dr. Changqing Zhou

Funding
- Billie A. Field Fellowship
- NIH P01 ES022848
- EPA RD-83449301
- EPA RD-83449301
- EPA RD-83449301
- EPA RD-83449301