Effects of Phthalates on the Female Reproductive System

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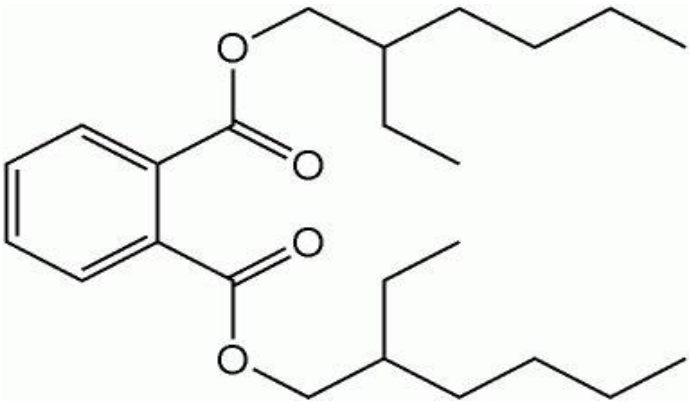
Overview

- 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

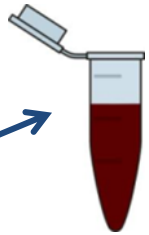
Prenatal DEHP exposure affects reproductive outcomes in female offspring

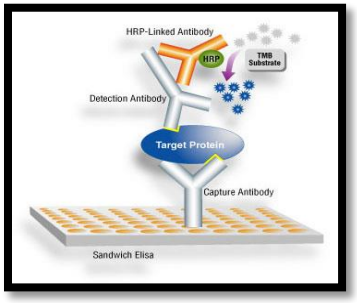
Prenatal DEHP exposure has transgenerational effects on reproduction in female offspring

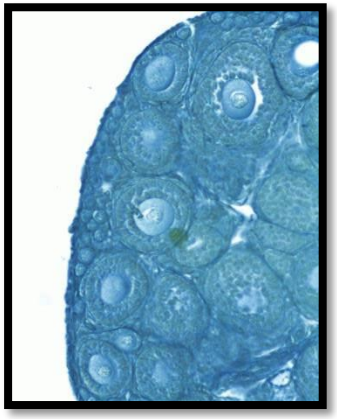
Study Design

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

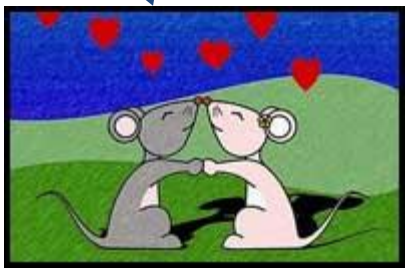
20µg/kg/day

200µg/kg/day

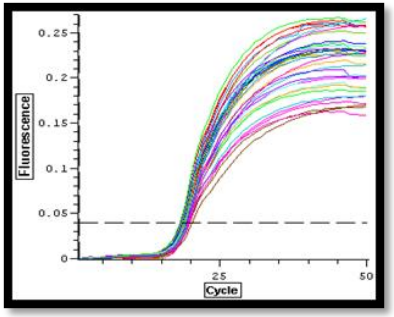
200mg/kg/day

500mg/kg/day

750mg/kg/day



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

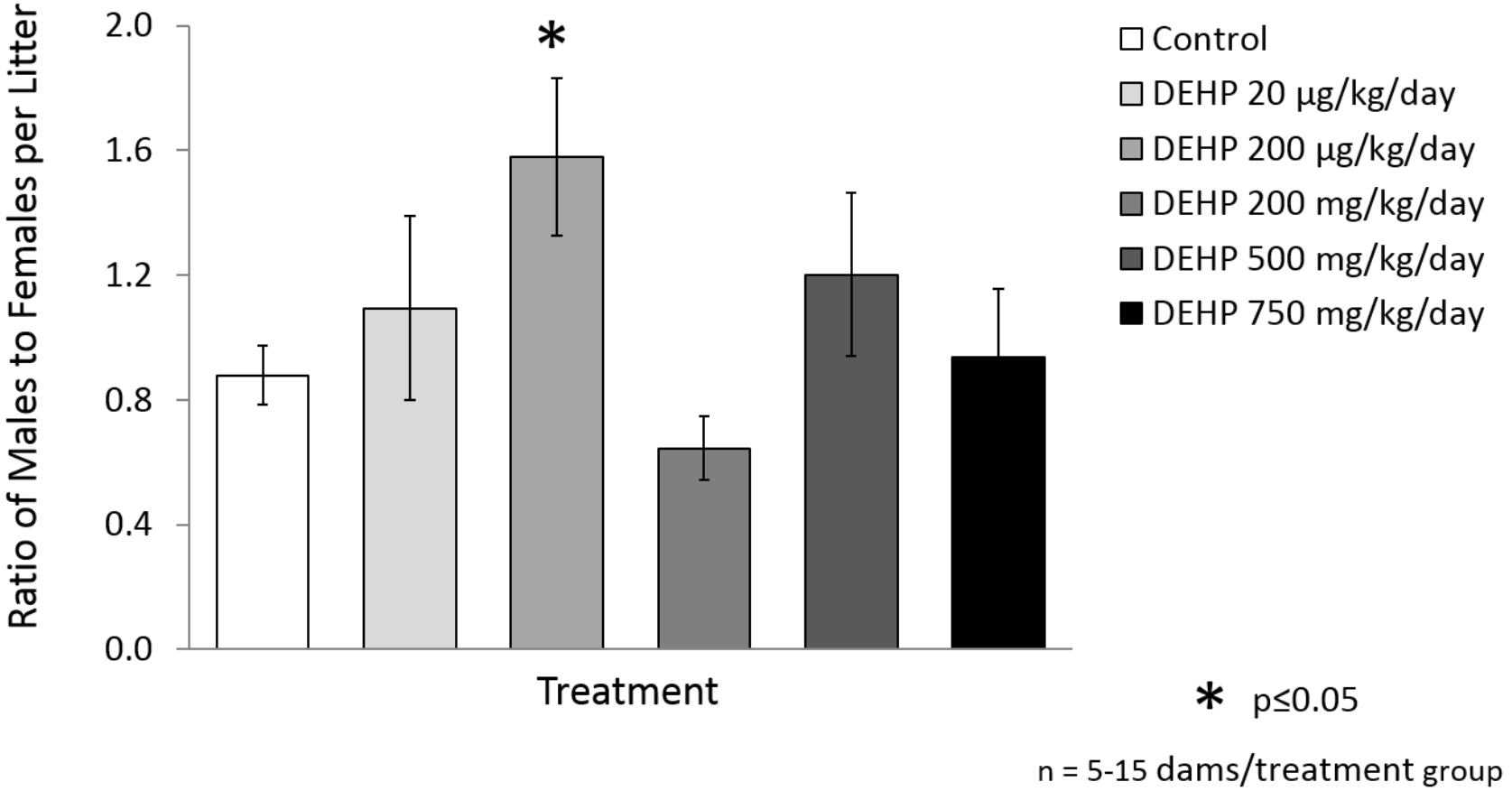


Results (F1 Generation)

- 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)

Niermann, et al. 2015

Effect of DEHP on sex ratio



Effect of DEHP on Fertility (3 months)

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

* $p < 0.05$

Effect of DEHP on Fertility (6 months)

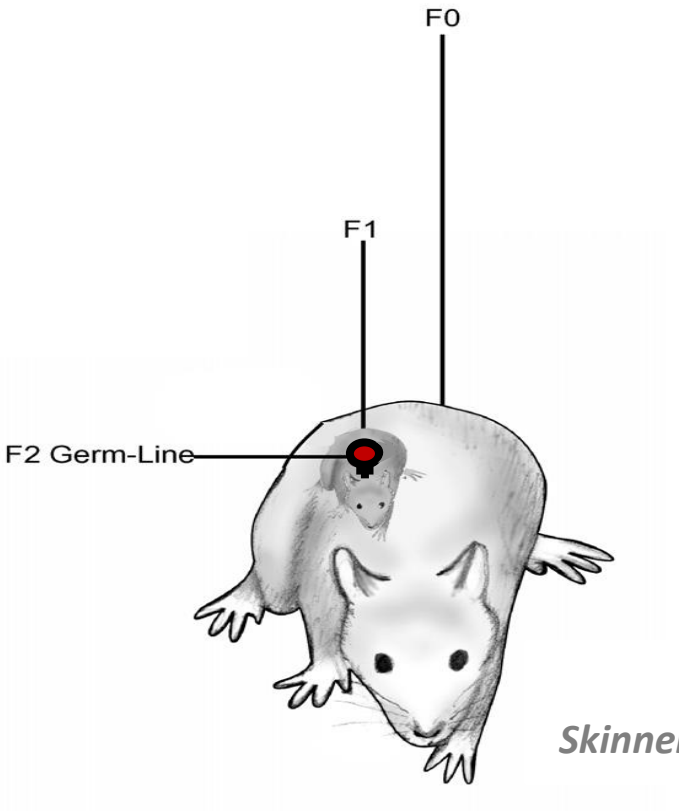
Treatment	No litter produced	>5 days to get pregnant	Lost some pups (2 or less)	Lost all pups
control	16.7 (n=12)	25.0 (n=12)	0.0 (n=10)	10.0 (n=10)
20 μg/kg/day	11.1 (n=9)	25.0 (n=8)	25.0 (n=8)^	25.0 (n=8)
200 μg/kg/day	27.3 (n=11)	0.0 (n=11)*	0.0 (n=8)	12.5 (n=8)
500 mg/kg/day	0.0 (n=4)	0.0 (n=4)	0.0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

*$p < 0.05$
^ $p < 0.06$

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)

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

Conclusion

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

F1 Generation	**F2 Generation**
↑ uterine weight	↓ uterine weight
↓ ovarian weight	↓ ovarian weight
↓ fertility	↓ fertility

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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