

The Effects of a Phthalate Metabolite Mixture on Antral Follicle Growth and Sex Steroid Synthesis in Mice

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Overview

- Background
 - Phthalates
 - Phthalate metabolite mixture
 - Ovary
- Effects of the phthalate metabolite mixture on the ovary
 - Antral follicle growth
 - Steroidogenesis
- Conclusions

What are phthalates?



Phthalates

Commonly used as plasticizers and additives



Why the concern?



Phthalates

- **Detected in human fluids and tissues**
- **High exposure in children and women**
- **Racial disparities in exposure**
- **Exposure estimates:**
 - > 13 phthalates (NHANES)
 - Serum levels up to 450 ng/ml (single phthalate)
 - Up to 250 $\mu\text{g}/\text{kg}$ bw/day (single phthalate)

Phthalates

- **Associated with human health risks**
 - High blood pressure
 - Increased insulin resistance
 - Pregnancy loss
 - Preterm birth
 - Decreased sex steroid hormone levels
 - Fertility problems

Phthalates

- **Cause adverse effects in animal models**
 - Affect body weight
 - Disrupt development of reproductive organs
 - Disrupt puberty onset
 - Reduce fertility
 - Induce reproductive diseases

Single Phthalates vs. Phthalate Mixture

- Previous studies focus on single phthalates
- Humans are exposed to a mixture of phthalates

Phthalate Mixtures

- Limited information available
- Previous mixtures not relevant to human exposure
- Very high doses
- Lack of information on ovarian effects

Why focus on a
metabolite mixture?

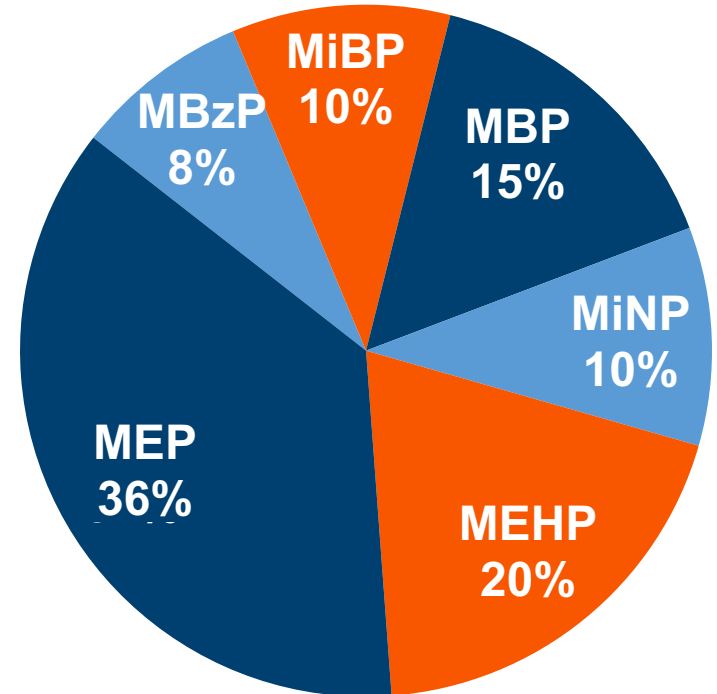


Phthalate Metabolite Mixture

- Parent phthalates are quickly metabolized in the body
- Metabolites reach the ovary
 - Present in ovarian follicular fluid
- Metabolites may be more toxic than parent compounds
- Information on the direct effects of mixtures of phthalate metabolites on the ovary is limited

Phthalate Metabolite Mixture

- MEP - monoethyl phthalate
- MiBP - monoisobutyl phthalate
- MBP - monobutyl phthalate
- MBzP - monobenzyl phthalate
- MEHP - mono(2-ethylhexyl) phthalate
- MiNP - monoisononyl phthalate



Based on levels detected in the iKids study

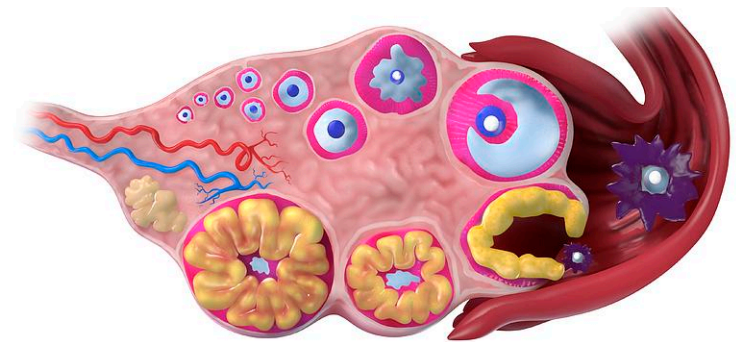
Calculated by summing detected Phase I and Phase II metabolites

Why study the effects of the mixture on the ovary?

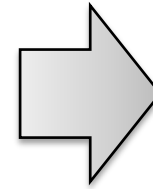
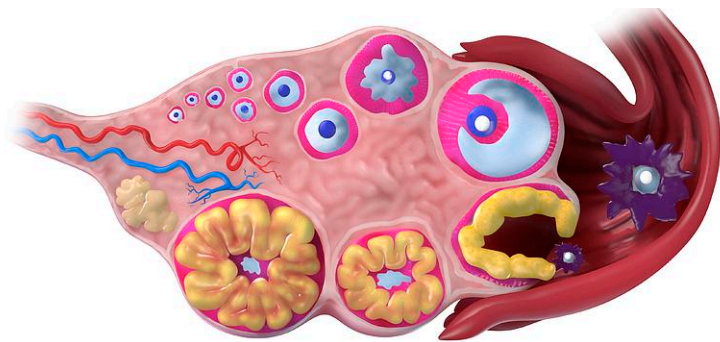
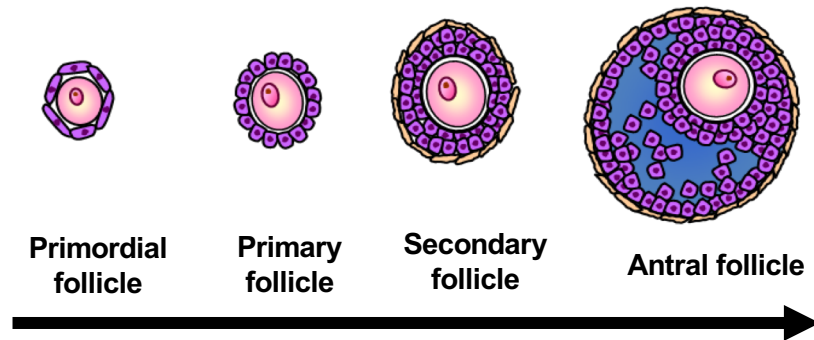


Functions of the Ovary

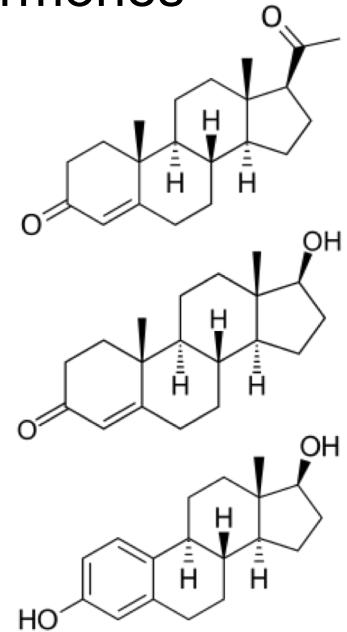
- Ovulation (oocytes)
 - Fertility
- Synthesize and secrete hormones
 - Development of ova
 - Implantation
 - Menstrual/estrous cyclicity
 - Maintenance of the reproductive tract
 - Fertility



Folliculogenesis



sex steroid
hormones

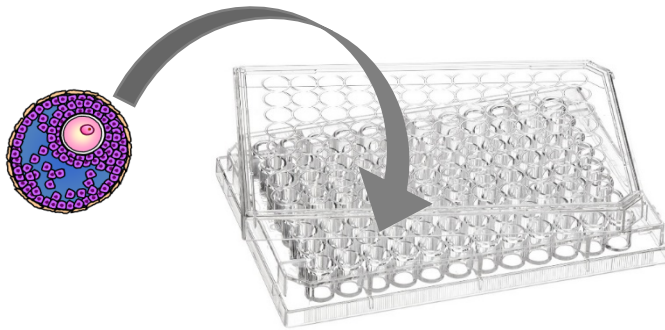


Hypothesis

Exposure to a mixture of phthalate metabolites decreases growth and alters sex steroid synthesis in antral follicles.

Experimental Design

**Mouse antral follicles
(32-42 days old)**



24-96 hours



Growth
measurements

Snap-frozen
follicles

Frozen follicle
media

qPCR

Steroid synthesis

Phthalate metabolite
mixture treatments:

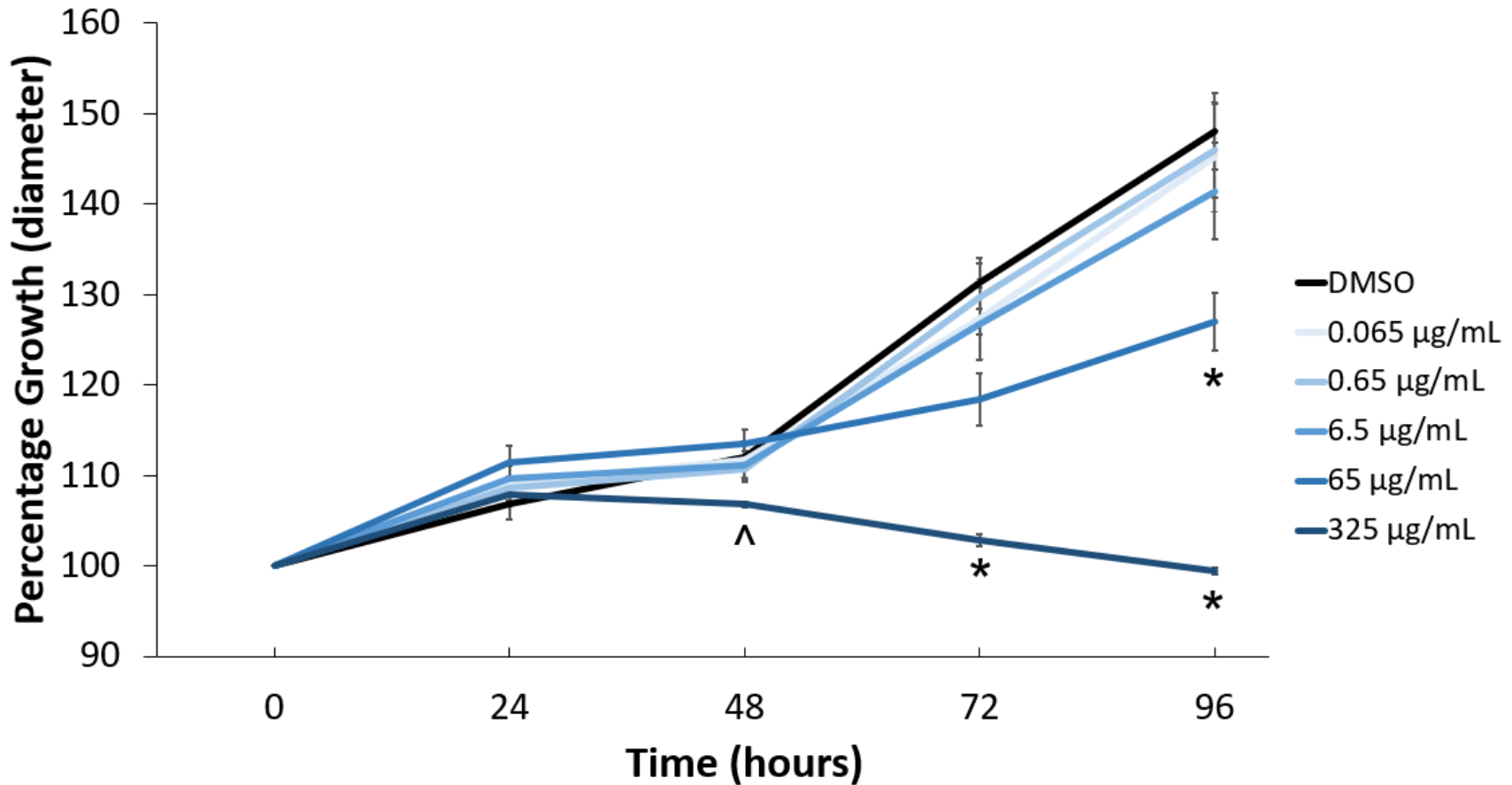
- DMSO (vehicle control)
- 0.065 $\mu\text{g}/\text{mL}$
- 0.65 $\mu\text{g}/\text{mL}$
- 6.5 $\mu\text{g}/\text{mL}$
- 65 $\mu\text{g}/\text{mL}$
- 325 $\mu\text{g}/\text{mL}$

ELISA

Pregnenolone
Progesterone
Androstenedione
Testosterone
Estradiol

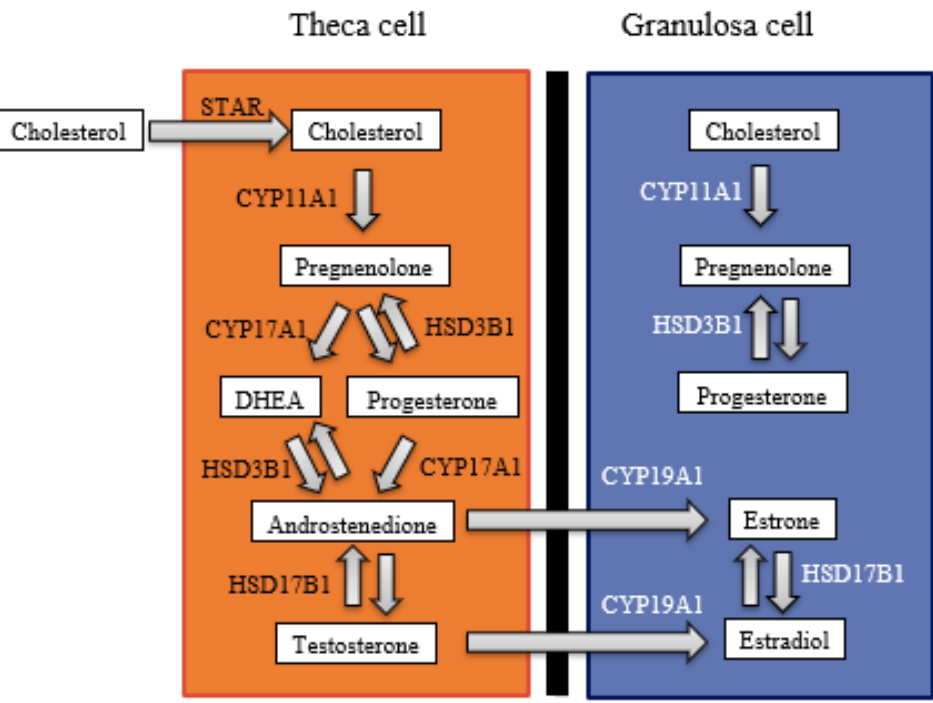
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Effect of the Metabolite Mixture on Antral Follicle Growth



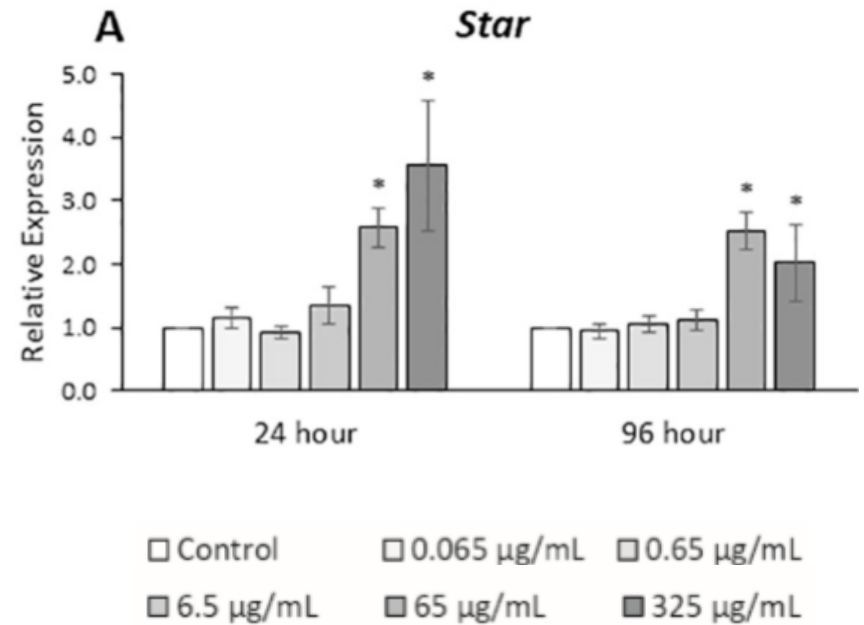
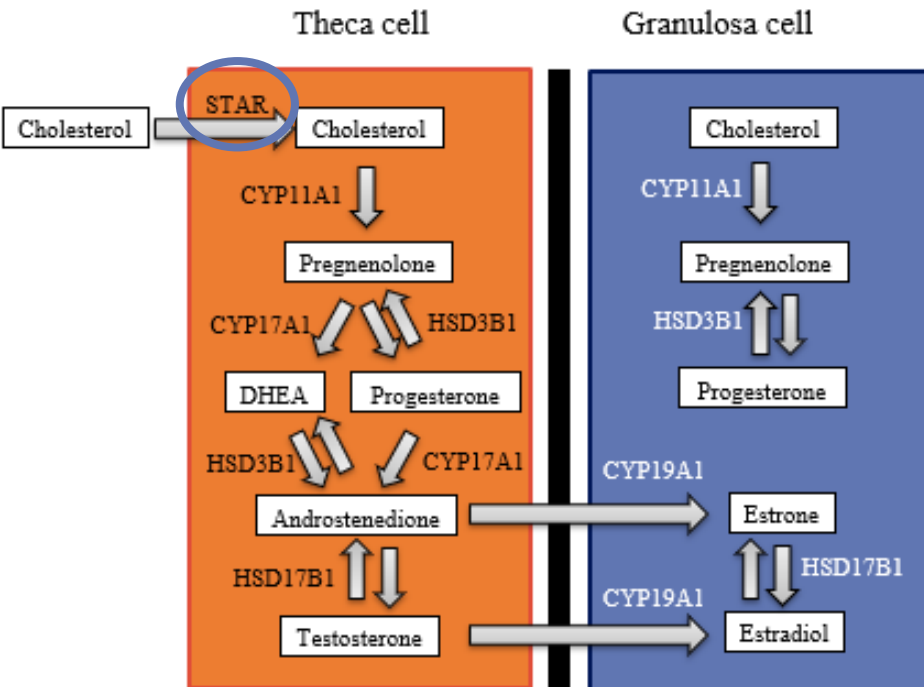
* $p \leq 0.05$, ^ $0.05 < p \leq 0.10$

Effect of the Mixture on Regulators of Steroidogenesis



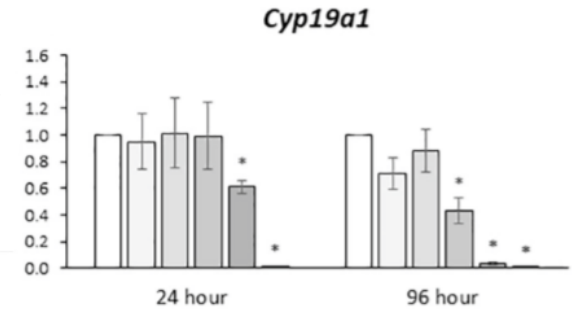
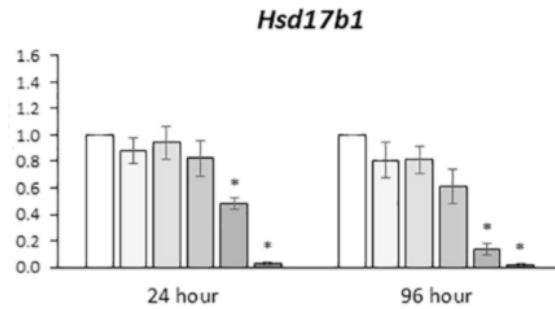
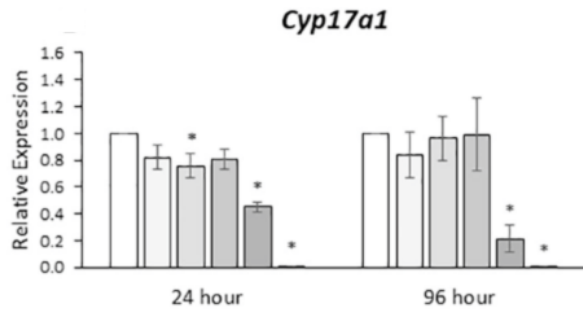
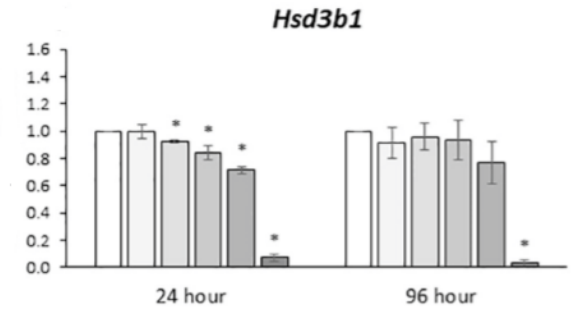
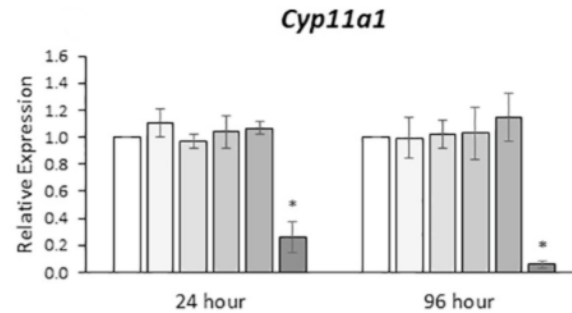
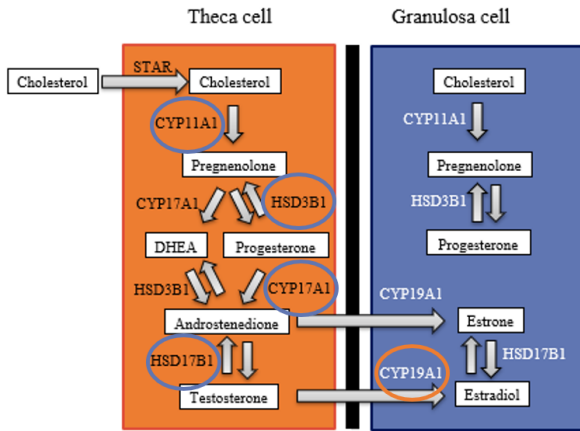
- STAR: steroidogenic acute regulatory protein
- CYP11A1: cytochrome-P450 cholesterol side-chain cleavage
- HSD3B1: 3 β -hydroxysteroid dehydrogenase
- CYP17A1: 17 α -hydroxylase
- HSD17B1: 17 β -hydroxysteroid dehydrogenase
- CYP19A1: aromatase

The Metabolite Mixture Increases *Star* Expression



* $p \leq 0.05$

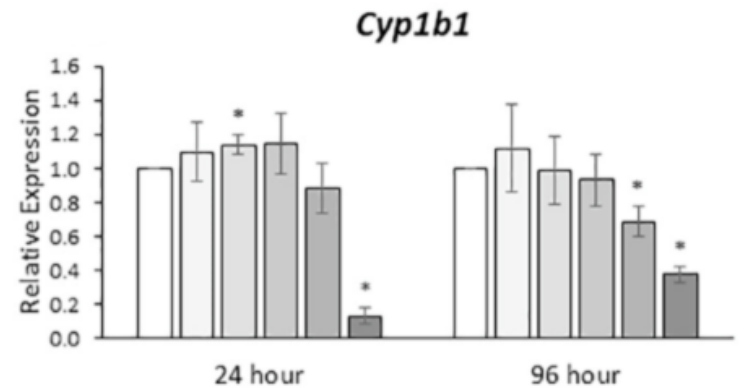
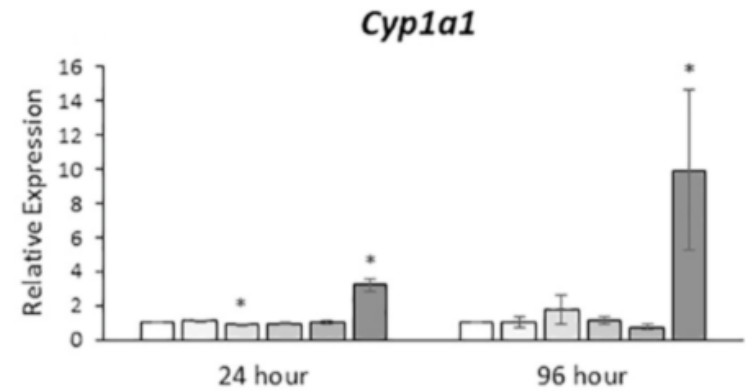
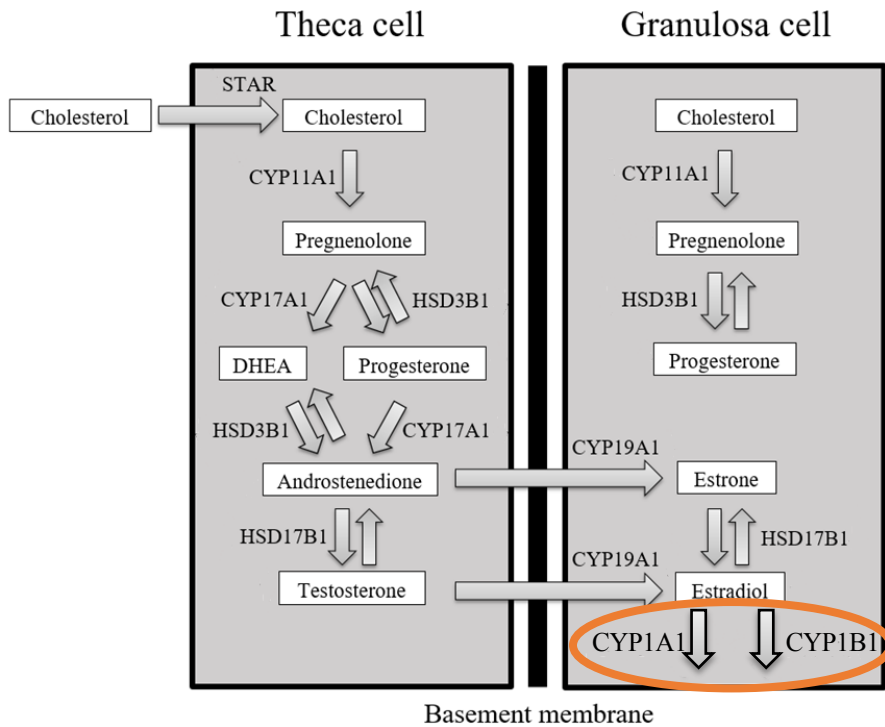
The Metabolite Mixture Decreases Steroidogenic Enzymes



□ Control □ 0.065 µg/mL □ 0.65 µg/mL □ 6.5 µg/mL □ 65 µg/mL ■ 325 µg/mL

* $p \leq 0.05$

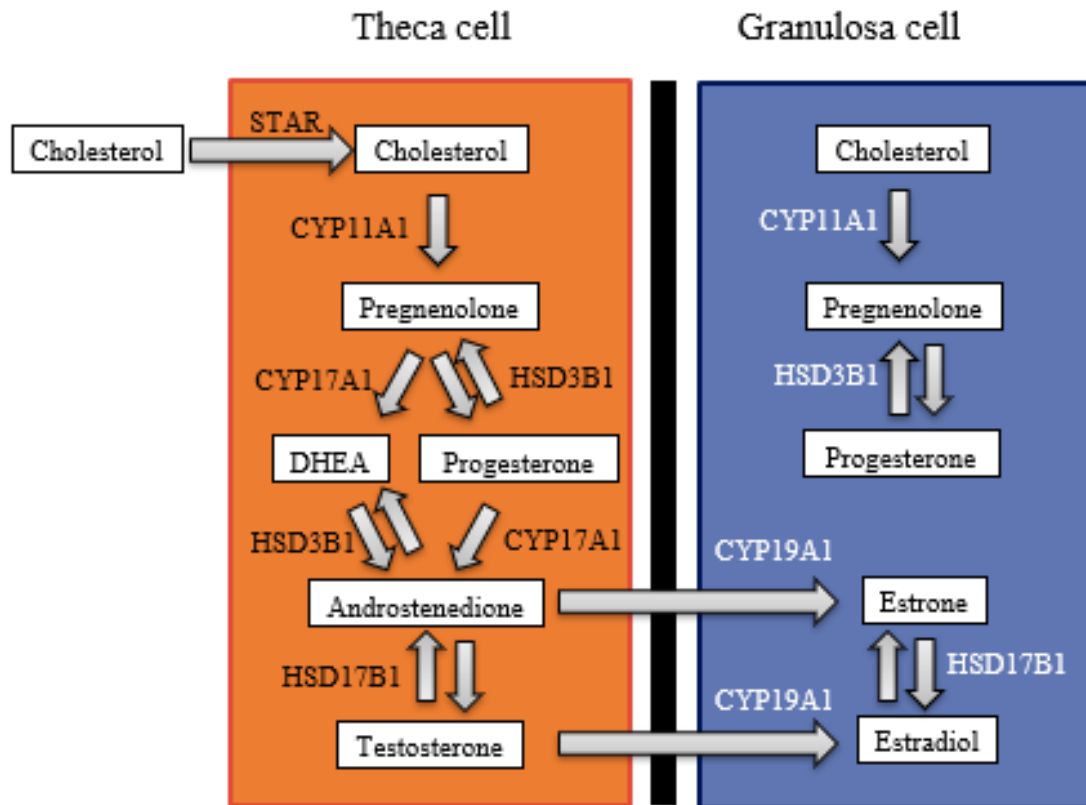
The Metabolite Mixture Alters Estradiol Degradation



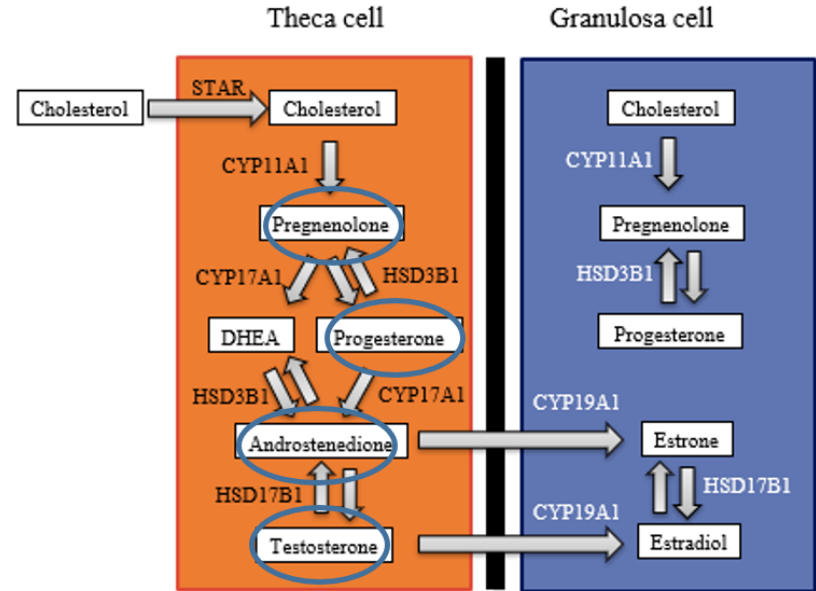
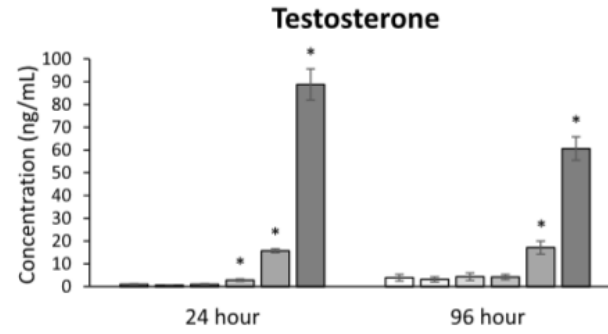
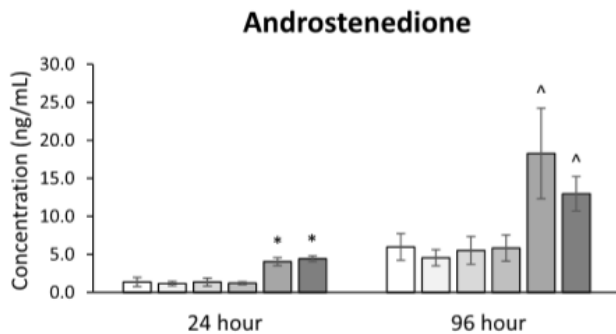
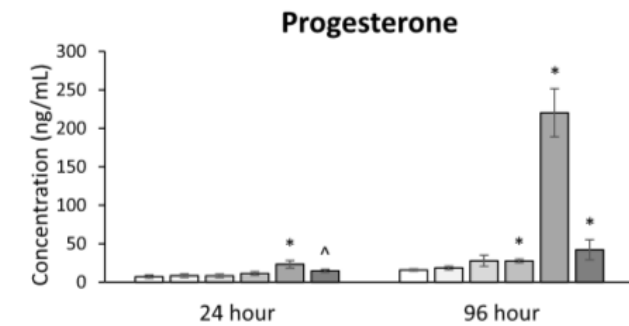
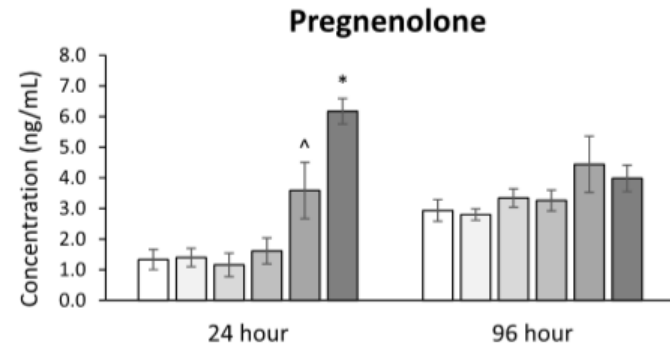
□ Control □ 0.065 µg/mL □ 0.65 µg/mL
 □ 6.5 µg/mL □ 65 µg/mL □ 325 µg/mL

* $p \leq 0.05$

Effects of the Metabolite Mixture on Sex Steroid Hormone Levels



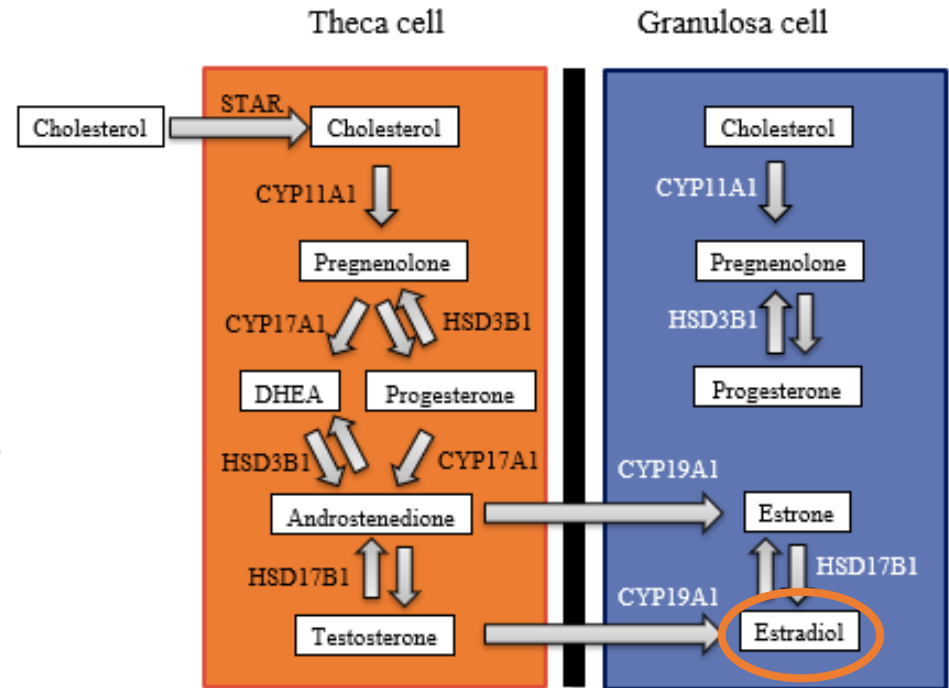
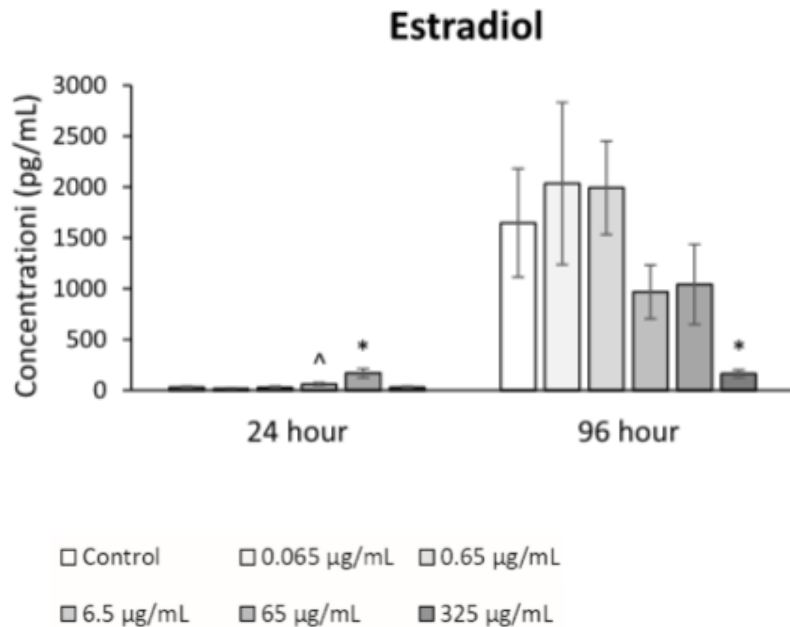
The Metabolite Mixture Alters Hormone Levels



Control
 0.065 µg/mL
 0.65 µg/mL
 6.5 µg/mL
 65 µg/mL
 325 µg/mL

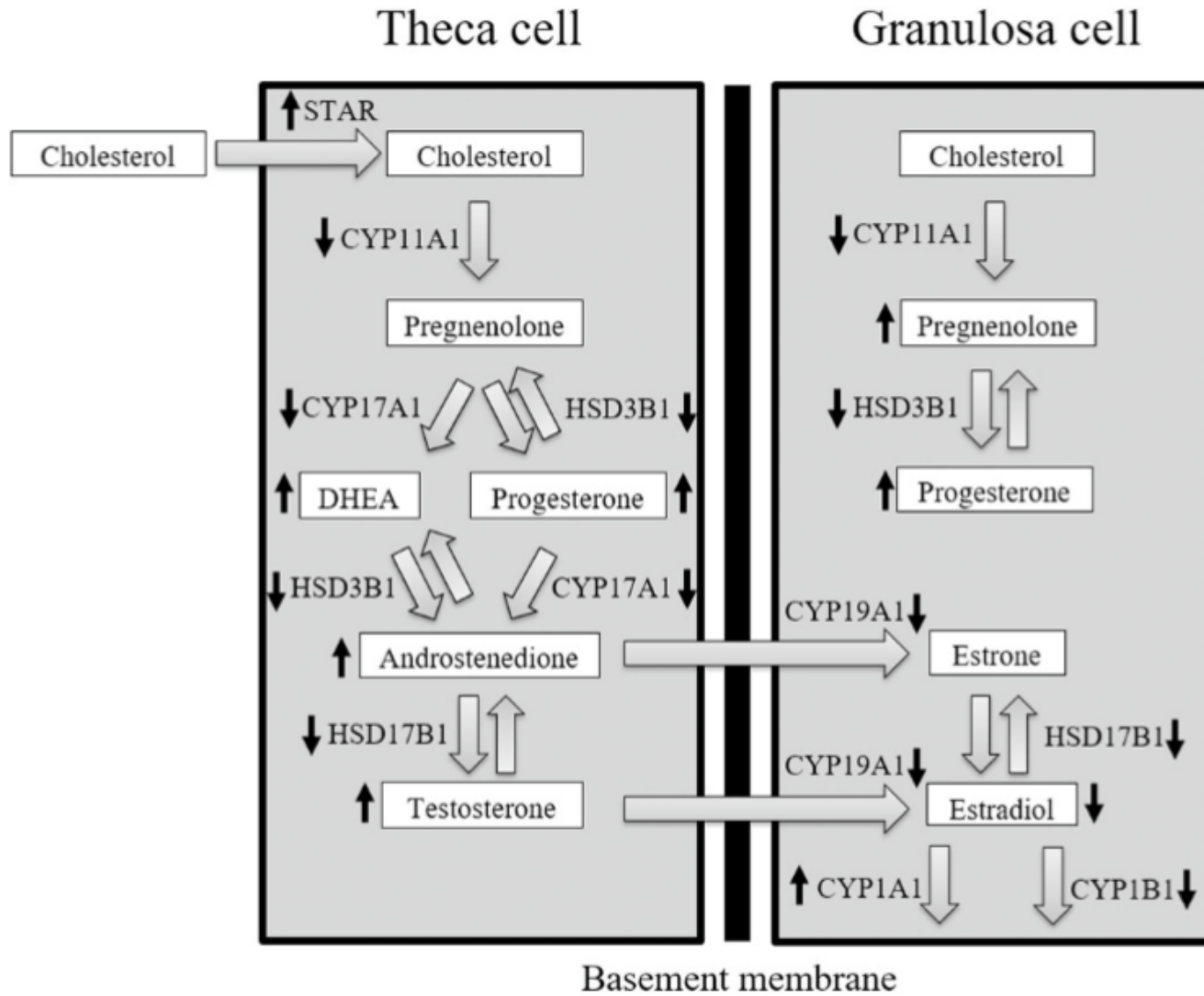
* $p \leq 0.05$, ^ $0.05 < p \leq 0.10$

The Metabolite Mixture Alters Estradiol Levels



* $p \leq 0.05$, ^ $0.05 < p \leq 0.10$

Summary



Conclusion

Exposure to a mixture of phthalate metabolites decreases growth and alters sex steroid synthesis in antral follicles.

Future Directions

- Determine how mixtures of phthalates affect the ovary in vivo
- Develop methods to reduce/eliminate exposure to phthalates
 - Reduce use of products that contain phthalates
 - Develop policy solutions for reducing/eliminating toxic phthalates in products/materials

Acknowledgments



NIH R01 ES 028661
NIH R56 ES 025147
NIH T32 ES 007326