Comparison of health effects following oral exposures to PFOA and HFPO-DA (GenX) in pregnant mice

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Research Triangle Environmental Health Collaborative
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Developmental PFOA Exposure Sensitive Targets

- **Fetal development**
  - **Birth weight decrements in humans and mice**

- **Adipose**
  - **Overweight if developmentally exposed**
  - **Insulin and glucose tolerance**
  - **Hines et al, 2009, *Mol. Cell Endocrinol.***

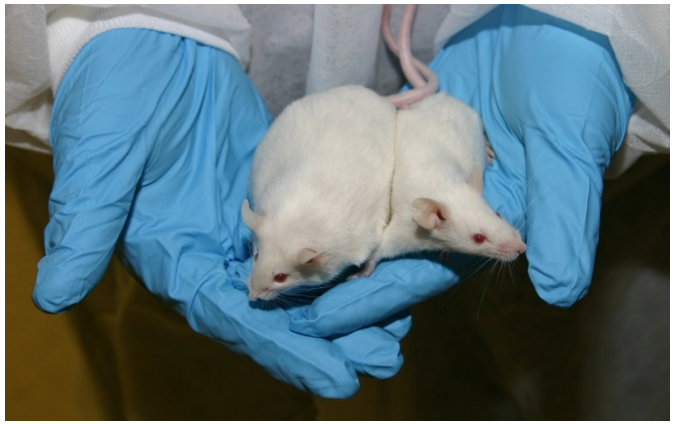


Photo from *Environ Health Perspect* Focus

- **Breast/Mammary gland**
  - **Decreased breastfeeding duration/efficiency/ability in women and mice**
  - **Mammary developmental delays with no change in other pubertal timepoints (in studies that have evaluated this tissue) – permanent change in those studies that have evaluated latent effects**

- **Liver**
  - **Hepatocellular hypertrophy, lipid deposition, enlarged relative liver weight**
  - **Liver disease (altered enzyme levels, cancer, etc)**
  - **Increased mitochondrial number in developmentally exposed mice**

NTP PFAS Program Project

Focused research projects under REACT:
Responsive Evaluation and Assessment of Chemical Toxicity

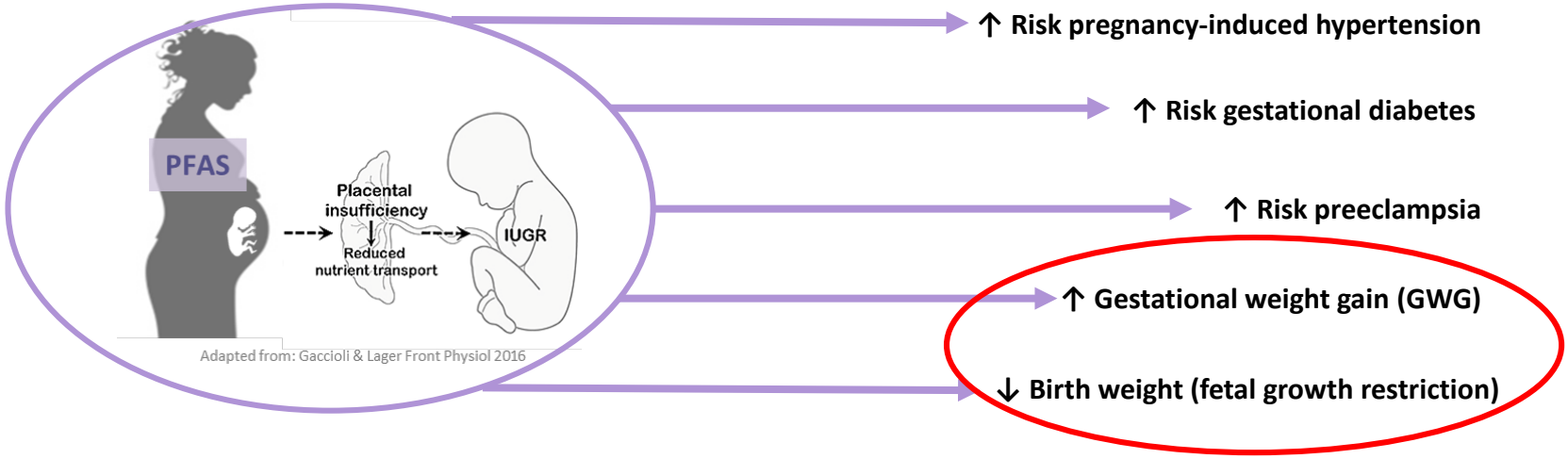
Primary goals:

Using mice, compare GenX* to PFOA on already established sensitive endpoints

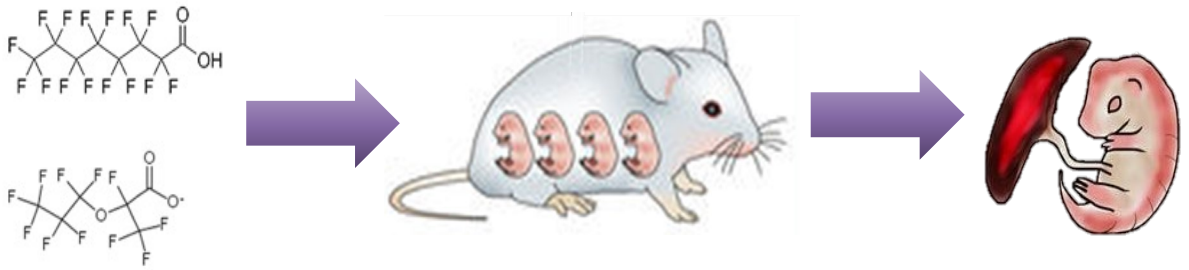
- *Evaluate effects on fetal weight gain (PFOA Navigation Guide)*
- *Determine effects on metabolic end points and weight gain*
- *Examine puberty timing and mammary endpoints (dam and pup)*
- *Examine adult and developing liver for pathology and mechanisms*
- *Establish relationship(s) between histopathology and other end points*
- *Understand internal dose and transfer to offspring*

*PFOA (Perfluorooctanoic acid ammonium salt, CAS# 3825-26-1) and GenX (Hexafluoropropylene Oxide Dimer Acid or [Ammonium 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)propanoate], CAS# 62037-80-3)

Adverse Pregnancy Outcomes



Evaluate PFOA concurrently with its replacement compound, GenX, for adverse effects on the maternal-***placental***-embryo unit in a mouse model

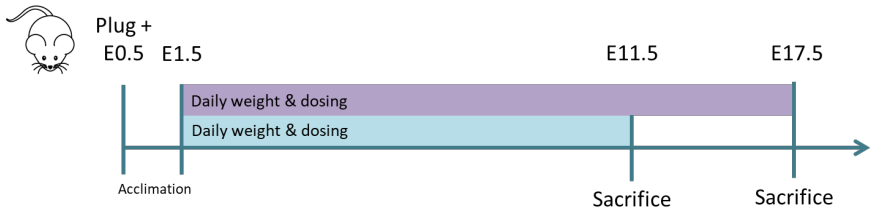


Study design and experimental methods

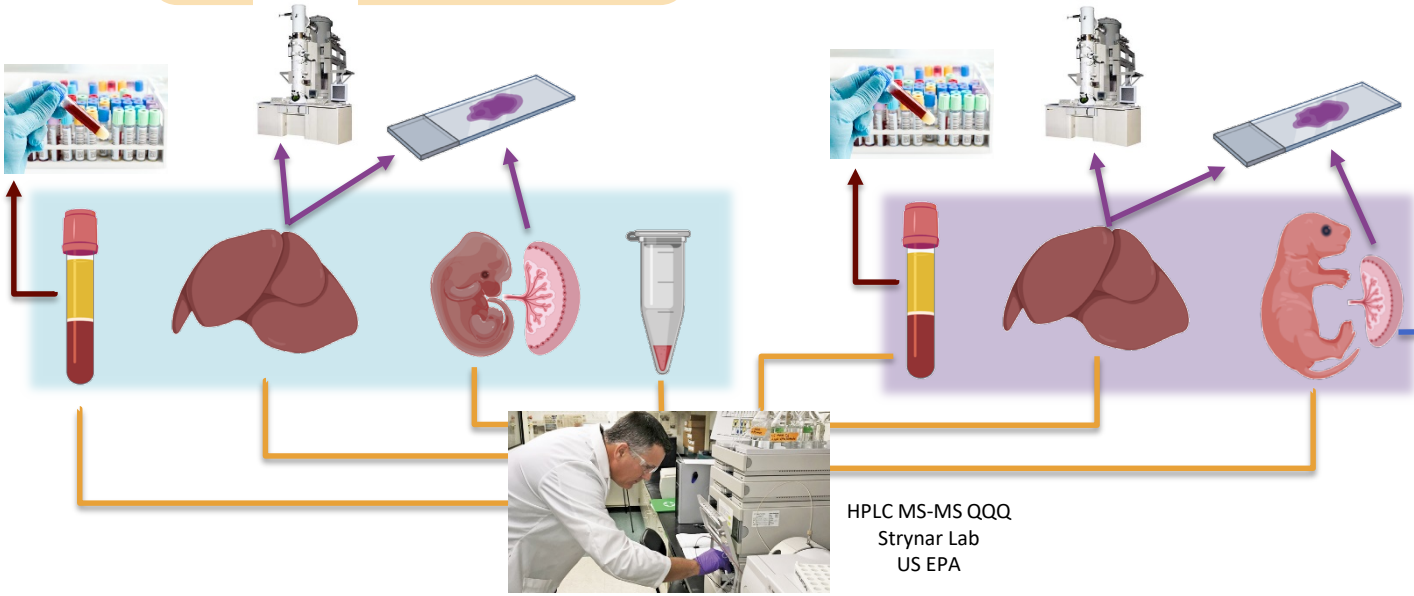
Treatment Groups N = 11-13 dams
Control (water)
1 mg/kg/day PFOA
5 mg/kg/day PFOA
2 mg/kg/day GenX
10 mg/kg/day GenX

Drinking Water Standards	
PFOA	70 ppt (US EPA)
GenX	140 ppt (NC DHHS/DEQ)

- PFOA doses: selected based on previous studies
- GenX doses: selected to serve as "equivalent" doses



Clinical Chemistry,
Histology & Transmission Electron Microscopy (TEM)
Cellular & Molecular Pathology Branch
NIEHS



HPLC MS-MS QQQ
Strynar Lab
US EPA

HPLC-MS/MS-ESI
Stapleton Lab
Duke University

Blake *et al* 2019, under review

GenX and PFOA Disposition

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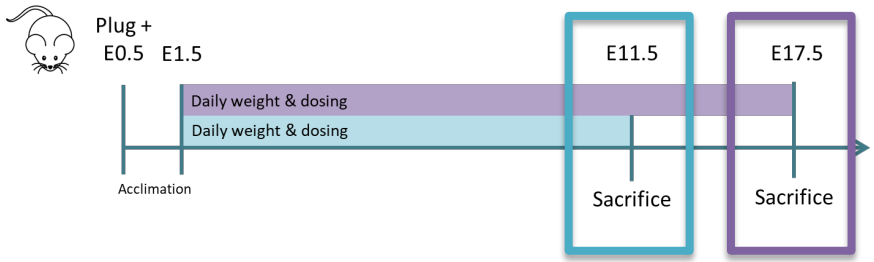

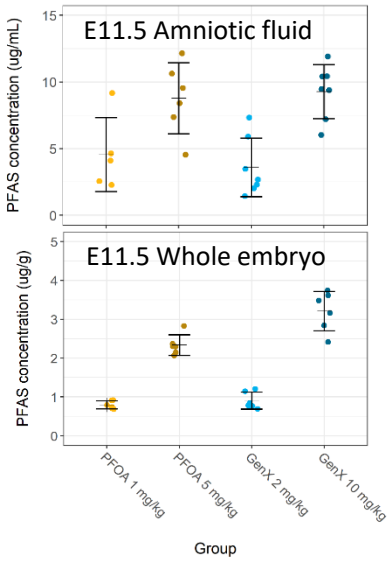

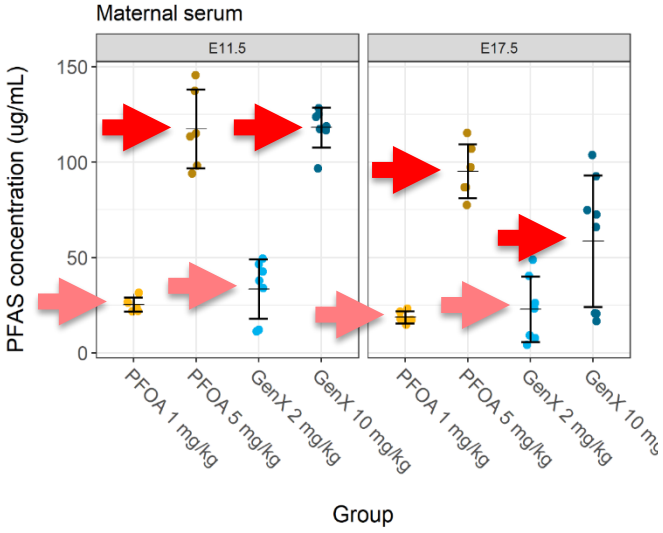

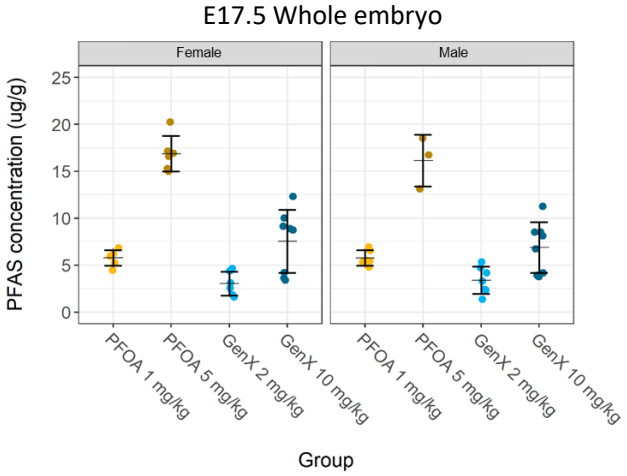

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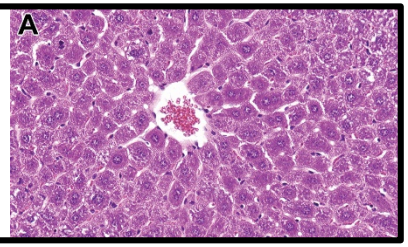
GenX and PFOA affect liver

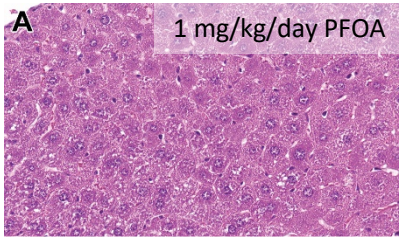
100% of livers from dams exposed to PFOA (1 or 5 mg/kg) or GenX (2 or 10 mg/kg) showed some degree of cytoplasmic alteration

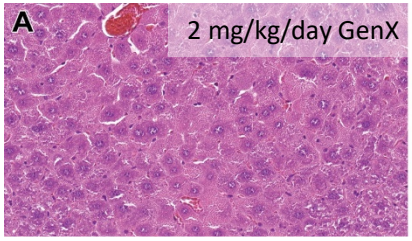
Normal liver histology & TEM at E17.5

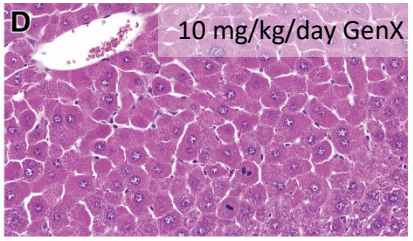
Representative images of pathology induced by PFOA or GenX at E17.5

Centrilobular hepatocellular hypertrophy with karyomegaly, increased basophilic granular cytoplasm and decreased glycogen





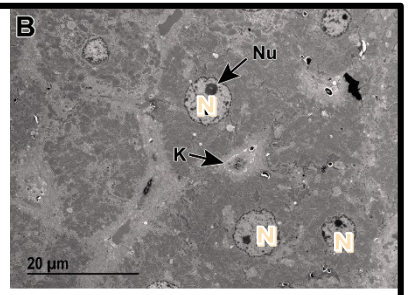


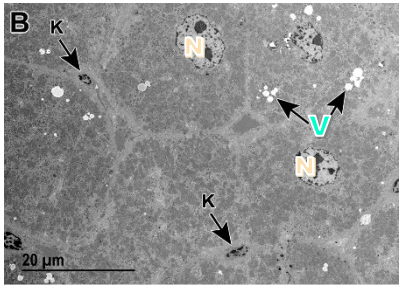


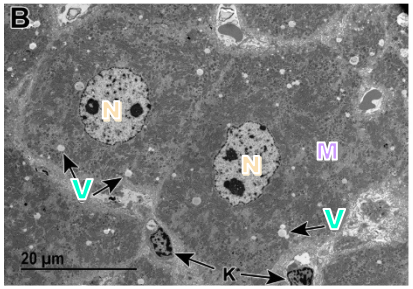
Arrows: prominent rough endoplasmic reticulum with abundant ribosomes

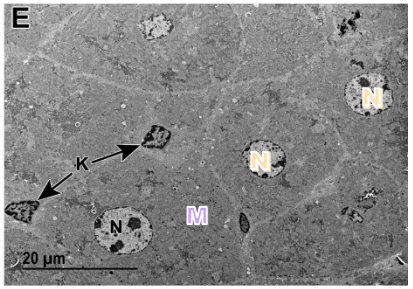
* Asterisks: evenly dispersed, abundant glycogen

Nu = nucleolus
N = nucleus
K = Kupffer cell
* = glycogen
P = peroxisomes
M = mitochondria
V = vacuole









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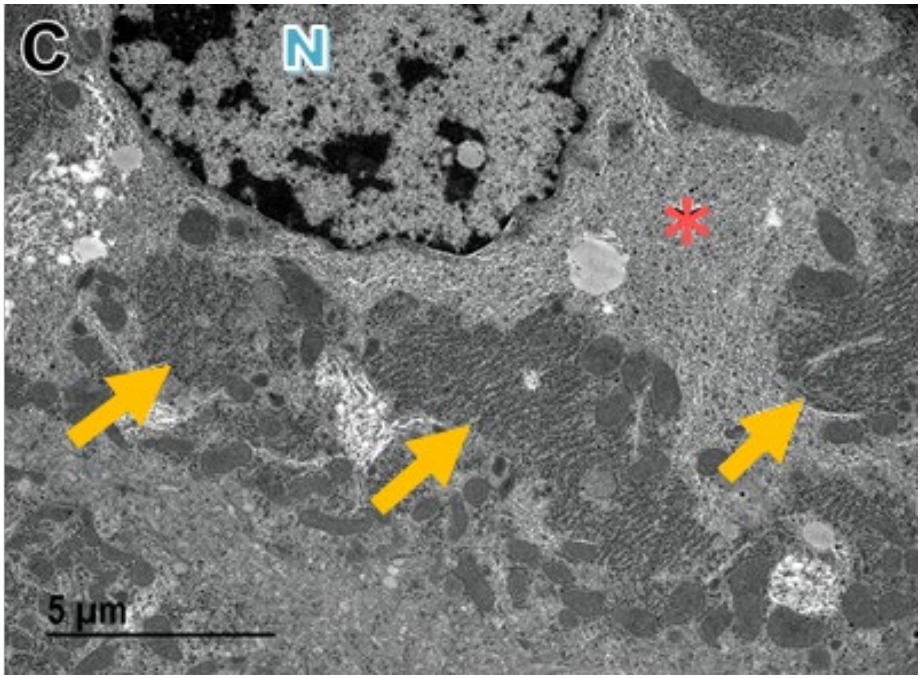
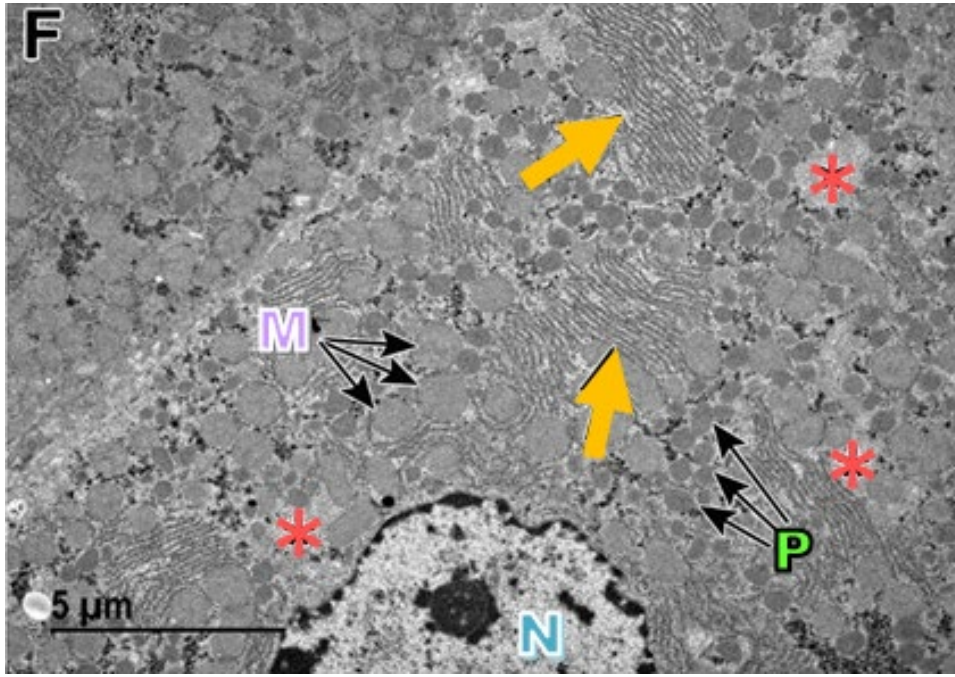
GenX and PFOA affect liver

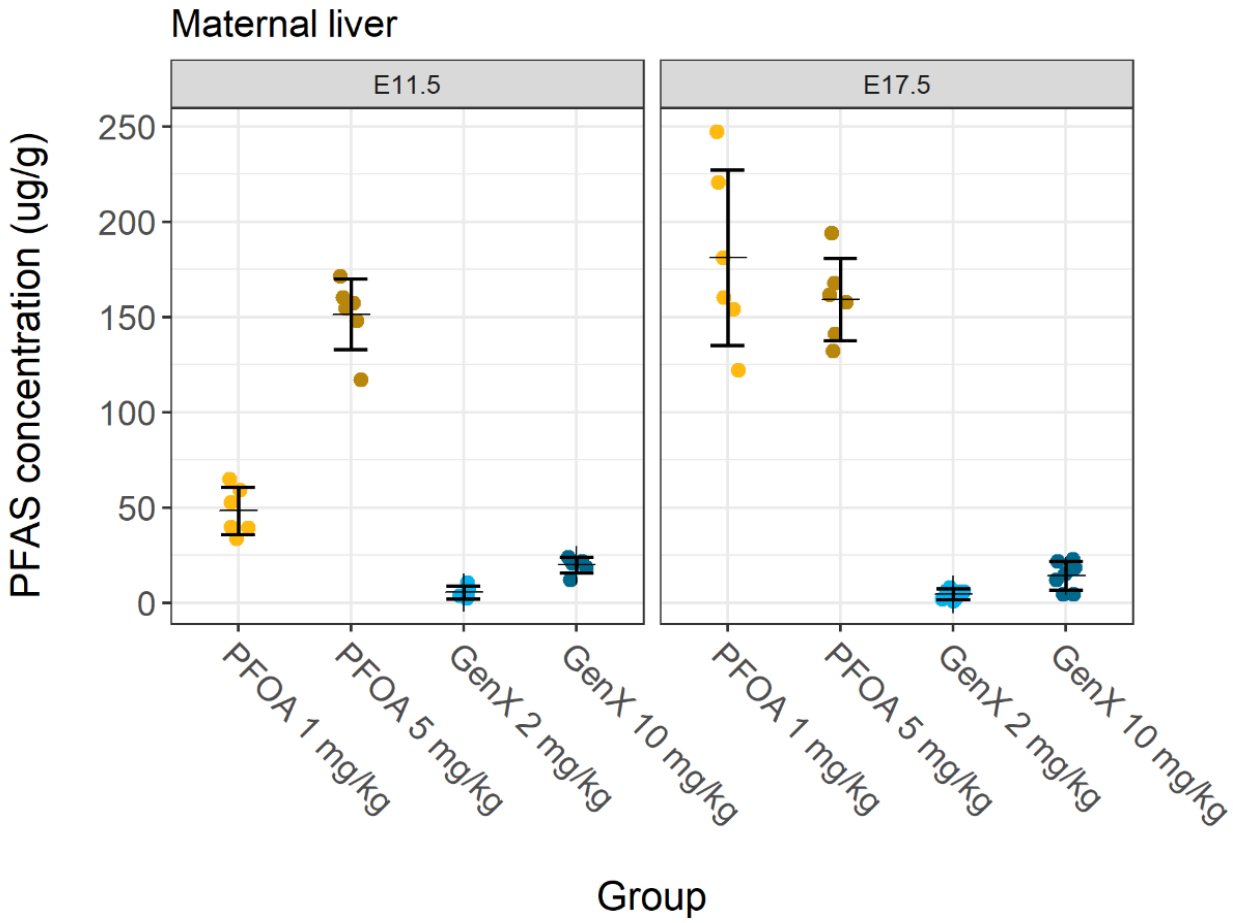




Transmission electron microscopy (TEM) of liver from a control (left) and 10mg/kg/day GenX treated pregnant dam at gestation day 17.5. Note the abundance of mitochondria (M), increased vacuolation, altered rough endoplasmic reticulum (arrows) and depletion of glycogen (asterisks) in treated liver. P = peroxisomes, N = nucleus.

Liver levels of GenX and PFOA

GenX induces similar adverse maternal liver pathology as PFOA at internal liver concentrations ~10x lower



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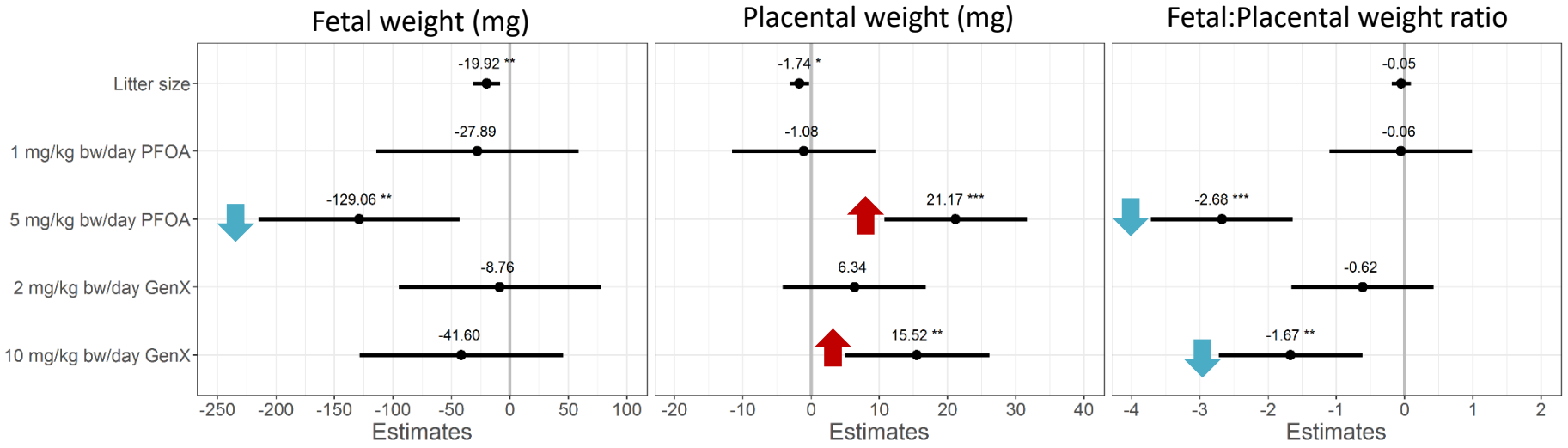
Placenta is a sensitive target of both PFOA and GenX

E17.5 estimates and 95% CI

	Fetal weight (g)	Placental weight (mg)	Fetal:Placental weight ratio
Vehicle Control	1378.6 (1206.3, 1550.8)	130.8 (109.8, 151.8)	11.2 (9.2, 13.3)
1 mg/kg PFOA	1350.7 (1091.9, 1609.4)	129.7 (98.2, 161.2)	11.1 (8.0, 14.3)
5 mg/kg PFOA	**1249.5 (991.0, 1508.0)***	**151.9 (120.5, 183.4)***	**8.5 (5.4, 11.6)***
2 mg/kg GenX	1369.8 (1111.3, 1628.4)	137.1 (105.6, 168.6)	10.6 (7.5, 13.7)
10 mg/kg GenX	1337.0 (1077.5, 1596.4)	**146.3 (114.7, 177.9)***	**9.5 (6.4, 12.7)***

*Beta estimate 95% confidence intervals do not overlap zero (Mixed effect model adjusting *a priori* for litter size as fixed effect and the dam as random effect); N = 11-13 dams with 62-80 observations per group



Mixed effect models fit using likelihood ratio test; model estimates with 95% CI
N = 11-13 litters with 1-3 observations per litter

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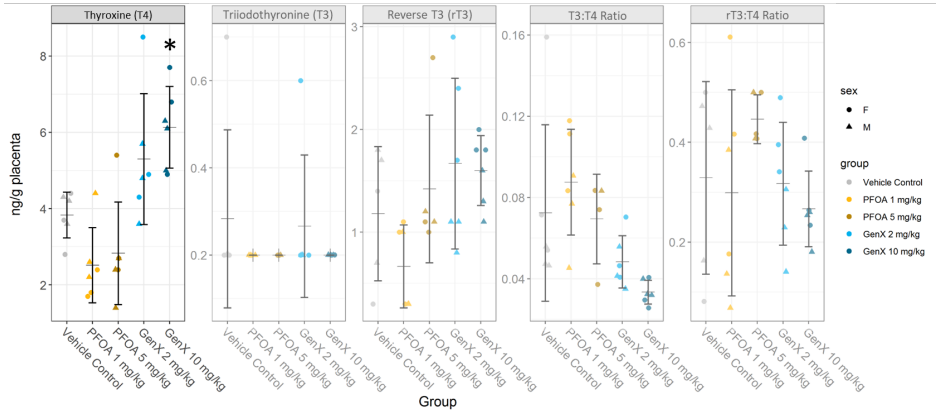
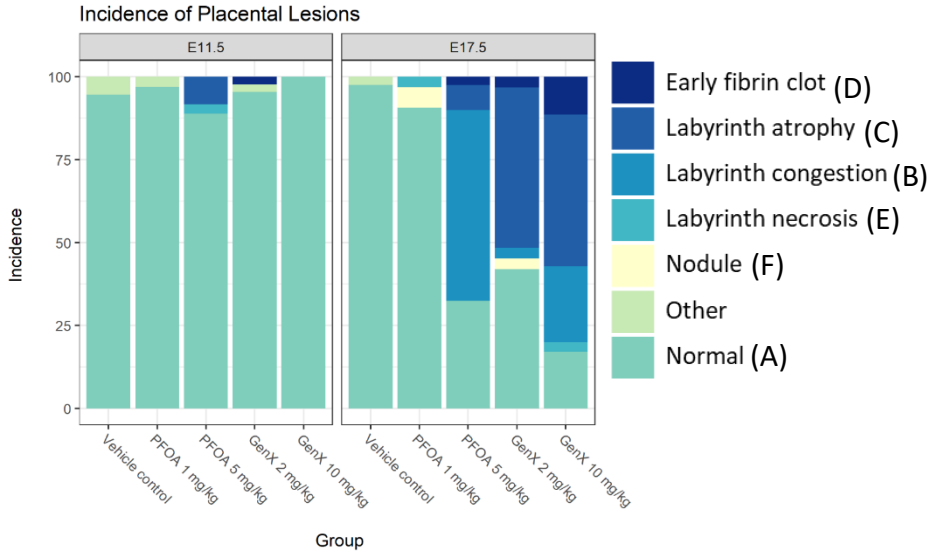
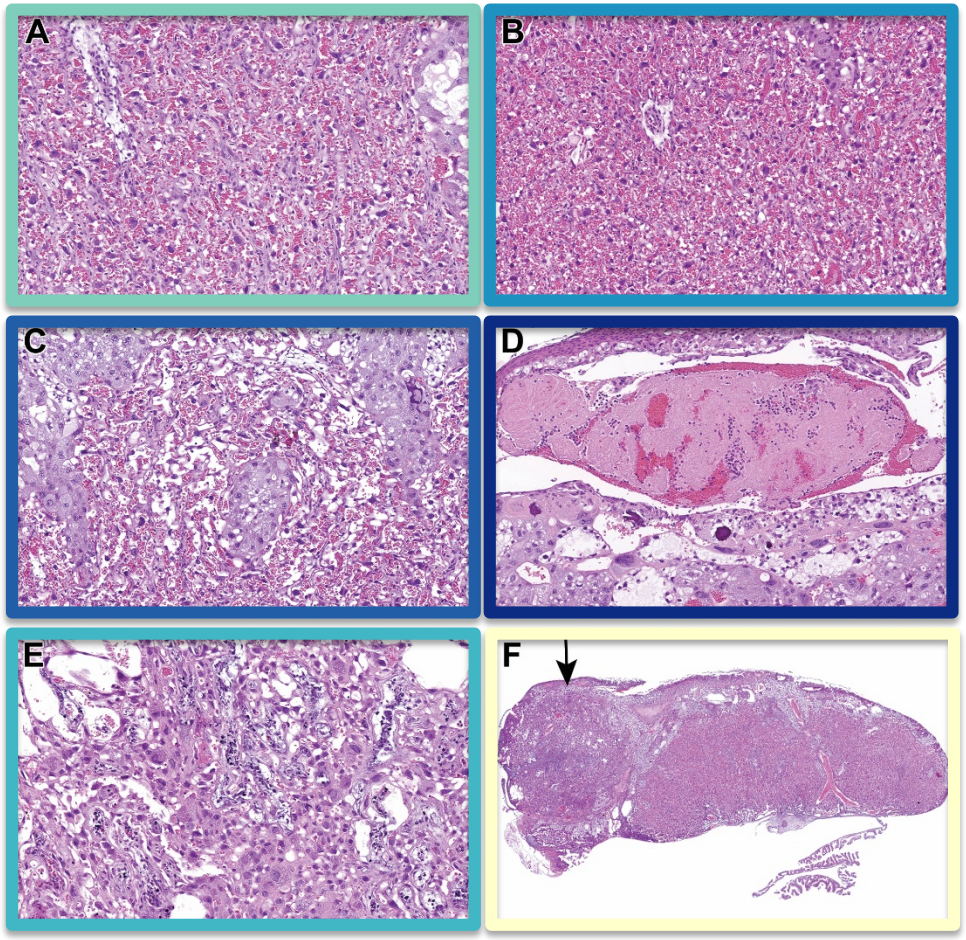
Congener-specific placental lesion profiles







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Summary

- Similar effects of PFOA and GenX in liver, with lower GenX burden in liver
- Unique placental effects, and difference in response for fetal growth
- No sex specific differences in fetal burden of PFOA or GenX
- Mammary gland of offspring – sex specific effects
  - Pup mammary effects at 1 mg/kg GenX and 0.1 mg/kg PFOA
- Ongoing work addressing maternal mammary gland development, metabolic effects in offspring and other reproductive tissues in pups
- PFOA and GenX-induced transcriptomic pathways that are shared and unique in placenta, liver, and mammary tissue are being determined
- Future studies to address lower doses and adverse outcome pathways

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