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

• 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
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 endpoints 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 endpoints
- 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

Gestational weight gain (GWG) ↑
Risk pregnancy-induced hypertension ↑
Risk gestational diabetes ↑
Risk preeclampsia ↑
Gestational weight gain (GWG) ↑
Birth weight (fetal growth restriction) ↓

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**

<table>
<thead>
<tr>
<th>Substance</th>
<th>US EPA</th>
<th>NC DHHS/DEQ</th>
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<td>PFOA</td>
<td>70 ppt</td>
<td>140 ppt</td>
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<tr>
<td>GenX</td>
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- 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

**Study design and experimental methods**

- Plug + E0.5 E1.5
- E11.5 E17.5
- Daily weight & dosing
- Acclimation
- Daily weight & dosing
- Sacrifice
- Sacrifice

**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

Treatment Groups
N = 11-13 dams
- Control (water)
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Blake et al 2019, under review
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.

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

**A** Normal liver histology & TEM at E17.5

**B** Arrows: prominent rough endoplasmic reticulum with abundant ribosomes

**C** Asterisks: evenly dispersed, abundant glycogen

**D** Nu = nucleolus

**E** N = nucleus

**F** K = Kupffer cell

**G** * = glycogen

**H** P = peroxisomes

**I** M = mitochondria

**J** V = vacuole

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

1 mg/kg/day PFOA

2 mg/kg/day GenX

10 mg/kg/day GenX

Blake *et al* 2019, under review
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

Blake et al 2019, under review
Placenta is a sensitive target of both PFOA and GenX

### E17.5 estimates and 95% CI

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

*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.

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**Mixed effect models fit using likelihood ratio test; model estimates with 95% CI**

**N = 11-13 litters with 1-3 observations per litter**
Congener-specific placental lesion profiles

Early fibrin clot (D)
Labyrinth atrophy (C)
Labyrinth congestion (B)
Labyrinth necrosis (E)
Nodule (F)
Other
Normal (A)
• 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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