Maternal and neonatal effects of \textit{in utero} exposure to perfluoroalkyl ether acids in the Sprague-Dawley rat

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Photo credit: NCSU
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Emerging PFAS

- PFOS and PFOA phased out and replaced in some instances with perfluoroalkyl ether acids (PFEAs)
- Parent compounds and manufacturing byproducts detected in drinking water and/or human serum in multiple locations globally
- Few or no peer-reviewed toxicity studies on hexafluoropropylene oxide dimer acid (GenX), Nafion byproduct 2 (NBP2), or perfluoro-methoxyacetic acid (PFMOAA)

GenX

PFMOOA

NBP2

*USEPA Chemical Dashboard*
Research objectives

- Assess maternal and perinatal effects of gestational exposure to PFEAs that have documented human exposure but little/no published toxicity data available.

- Develop Adverse Outcome Pathways to facilitate the use of \textit{in vitro} or refined \textit{in vivo} assays to predict effects of additional PFAS in future testing.

\begin{center}
\includegraphics[width=\textwidth]{research_objectives_diagram.png}
\end{center}
In vitro human and rat PPAR alpha and gamma activity

### Human

#### PPAR α

- GWS90735
- Octanoic acid
- Oleic acid
- GenX
- PFOA
- PFMOAA
- PFOS
- NBP2

#### PPAR γ

- Rosiglitazone
- Octanoic acid
- Oleic acid
- GenX
- PFOA
- PFMOAA
- PFOS
- NBP2

### Rat

#### PPAR α

- GWS90735
- Octanoic acid
- Oleic acid
- GenX
- PFOA
- PFMOAA
- PFOS
- NBP2

#### PPAR γ

- Rosiglitazone
- Octanoic acid
- Oleic acid
- GenX
- PFOA
- PFMOAA
- PFOS
- NBP2
In vivo study designs

- Charles River Sprague-Dawley rat
- 3-9 dams/litters per dose group
- Oral gavage administration
- Ultra pure water vehicle

- Body weight
- Liver weight
- Fetal testis testosterone production
- Serum thyroid hormones (T3/T4)
- Clinical chemistry
- Liver gene expression
- Serum & liver chemical concentration

GD=gestation day
PND=postnatal day
### Fetal liver PPAR signaling pathway gene expression

**GD 14-18 exposure**

#### Fetal GenX liver

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<tr>
<th>Gene</th>
<th>Ctl</th>
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#### Fetal NBP2 liver

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#### Maternal NBP2 dose (mg/kg/d)

ANOVA p<0.001
Pairwise vs control p<0.01
Adverse neonatal effects
GD 8 – PND 2 exposure

Birthweight

Neonatal mortality

Birthweight (average pup wt (g))

GenX (mg/kg/d)

NBP2 (mg/kg/d)

Pup survival on PND2 (%)

GenX (mg/kg/d)

NBP2 (mg/kg/d)
Adverse neonatal effects
GD 8 – PND 2 exposure

PND2 pup relative liver weight

Relative liver wt (% BW)

GenX (mg/kg/d)

NBP2 (mg/kg/d)

N/A
Histopathological evaluation of PND0 pup liver

Treated – GenX 250 mg/kg

Control

PND0 pup liver glycogen score

Liver glycogen severity score

GenX (mg/kg/d)

NBP2 (mg/kg/d)
Fetal liver glycogen deposition is critical for neonatal health

**Neonatal liver glucose metabolism pathway gene expression**

### Neonatal liver PND(0)

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**ANOVA p<0.001**

Pairwise vs control p<0.01
GenX does not accumulate in maternal serum or liver...

Maternal serum [GenX]

Maternal liver [GenX]
...but exposure duration is important for effects

Maternal liver weight

GD 16-20 dosing
GD 8-PND 2 dosing

Maternal GenX dose (mg/kg/d)

Relative liver weight (% of control)
Margin of internal exposure – rat:human

Human factory worker serum [GenX]

Margin of Internal Exposure:
Ratio of Rat/Human serum [GenX]

- Reduced maternal weight gain
- Reduced pup survival

- Decreased pup liver Ugp2 expression
- Increased maternal & pup liver weight
- Reduced pup liver glycogen

Worker ID

Maternal oral GenX (mg/kg/d)

Rat/Highest Worker [GenX] (Fold)

<DL
PFAS co-exposures in pregnant women

• **Woodruff et al. 2011** – US pregnant women from NHANES 2003-2004 (n=268)
  • 99% with detectable PFOS and PFOA

• **Dereumeaux et al. 2016** – Elfe Cohort French pregnant women 2011 (n=277)
  • >99% with detectable PFOA, PFOS, PFHxS, PFNA

• **Berg et al. 2014** – Northern Norway Mother-and-Child Contaminant Cohort Study 2007-2009 (n=391)
  • >99% with detectable PFHxS, PFOS, PFOA, PFNA, PFDA, PFUnDa

• **Hopkins et al. 2018** – Drinking water derived from Cape Fear River water
  • Frequent detection of GenX, NBP2, PFMOAA, PFO2HxA

• **NCSU GenX Exposure Study (genxstudy.ncsu.edu)** – 388 participants from Wilmington, NC area
  • Detectable NBP2 (99%), PFO4DA (98%), PFO5DoDA (87%)
  • PFOS, PFOA, PFHxS, PFNA, PFDA also detected

• **Critical to study mixture-based effects of co-exposure to multiple PFAS compounds**
GenX+NBP2+PFOS Mixture study

GenX
- ED$_{50}$ = 108.7 mg/kg
- Slope = -3.4

NBP2
- ED$_{50}$ = 9.7 mg/kg
- Slope = -16.9

PFOS (Lau et al. 2003)
- ED$_{50}$ = 3.0 mg/kg
- Slope = -5.8

Top dose = each chemical at ED$_{50}$

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<th></th>
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<th>33%</th>
<th>10%</th>
<th>3.3%</th>
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<td>PFOS (mg/kg)</td>
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Mixture effects appear dose additive
Impact of co-exposure on chemical dose-response

Represented by 20% reduction in birthweight

GenX only
- ED₈₀: 164 mg/kg
  95% CI: 131-205 mg/kg

GenX in mixture
- ED₈₀: 69 mg/kg
  95% CI: 51 - 94 mg/kg
Summary

- GenX and NBP2 produced adverse maternal and neonatal effects but with disparate patterns and oral dose ranges.

- Effects for GenX and NBP2 generally consistent with those reported for PFOA and PFOS but at slightly higher oral doses.

- Both PPAR α and γ appear to be involved as MIEs.

- Exposure duration is important - despite rapid clearance, longer exposure produced greater adverse effects for GenX.

- Internal dosimetry is important for estimating potency and relevance to human exposures.

- Mixture effects of exposure to GenX+NBP2+PFOS appear dose additive.

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