Maternal and neonatal effects of *in utero* exposure to perfluoroalkyl ether acids in the Sprague-Dawley rat

Justin Conley

Reproductive and Developmental Toxicology Branch
Office of Research and Development
U.S. Environmental Protection Agency

Research Triangle Park, NC

Photo credit: NCSU

Research Team

Earl
Gray

Vickie
Wilson

Phillip
Hartig

Mary
Cardon

Christy
Lambright

Nicki
Evans

Elizabeth
Medlock-Kakaley

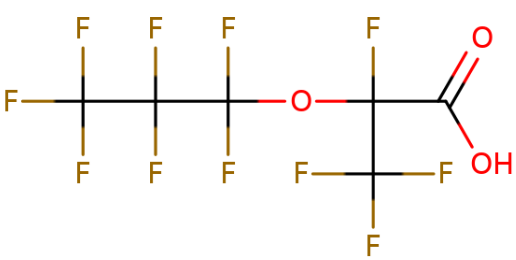
Not pictured: Erin Hines, Aaron Dixon

Collaborators: James McCord, Mark Strynar, Donna Hill

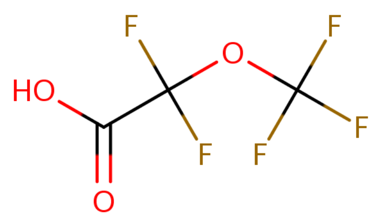
Emerging PFAS

- 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)
- Conley et al. (2019) *Environ. Health Persp*. doi: 10.1289/EHP4372

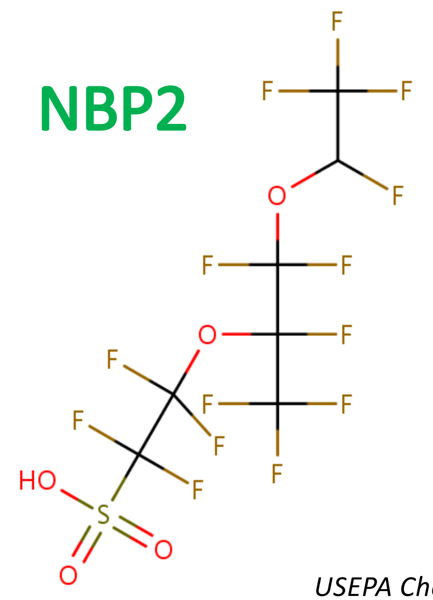
GenX



PFMOAA



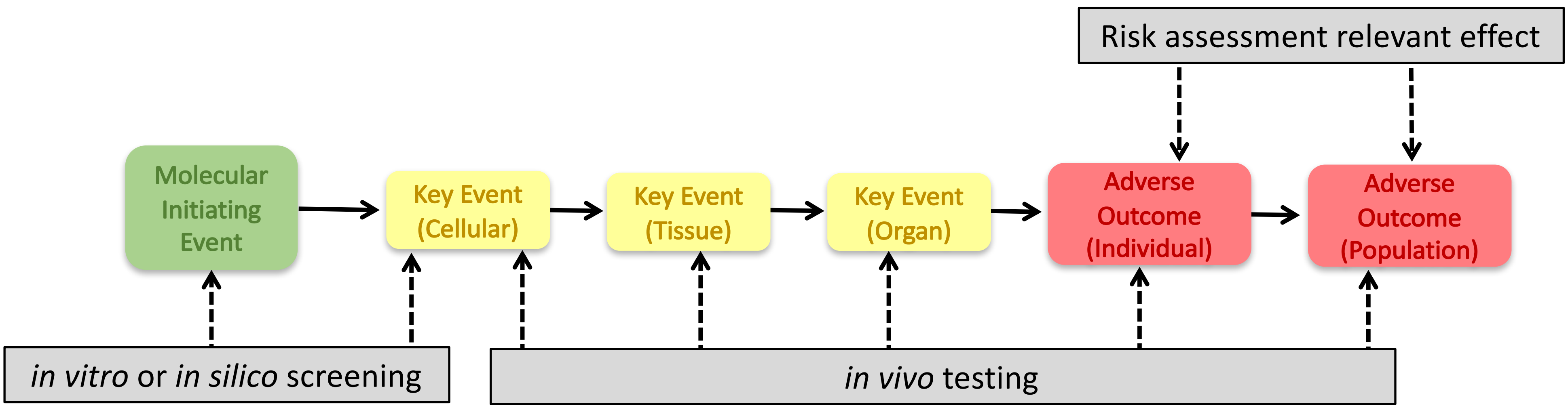
NBP2



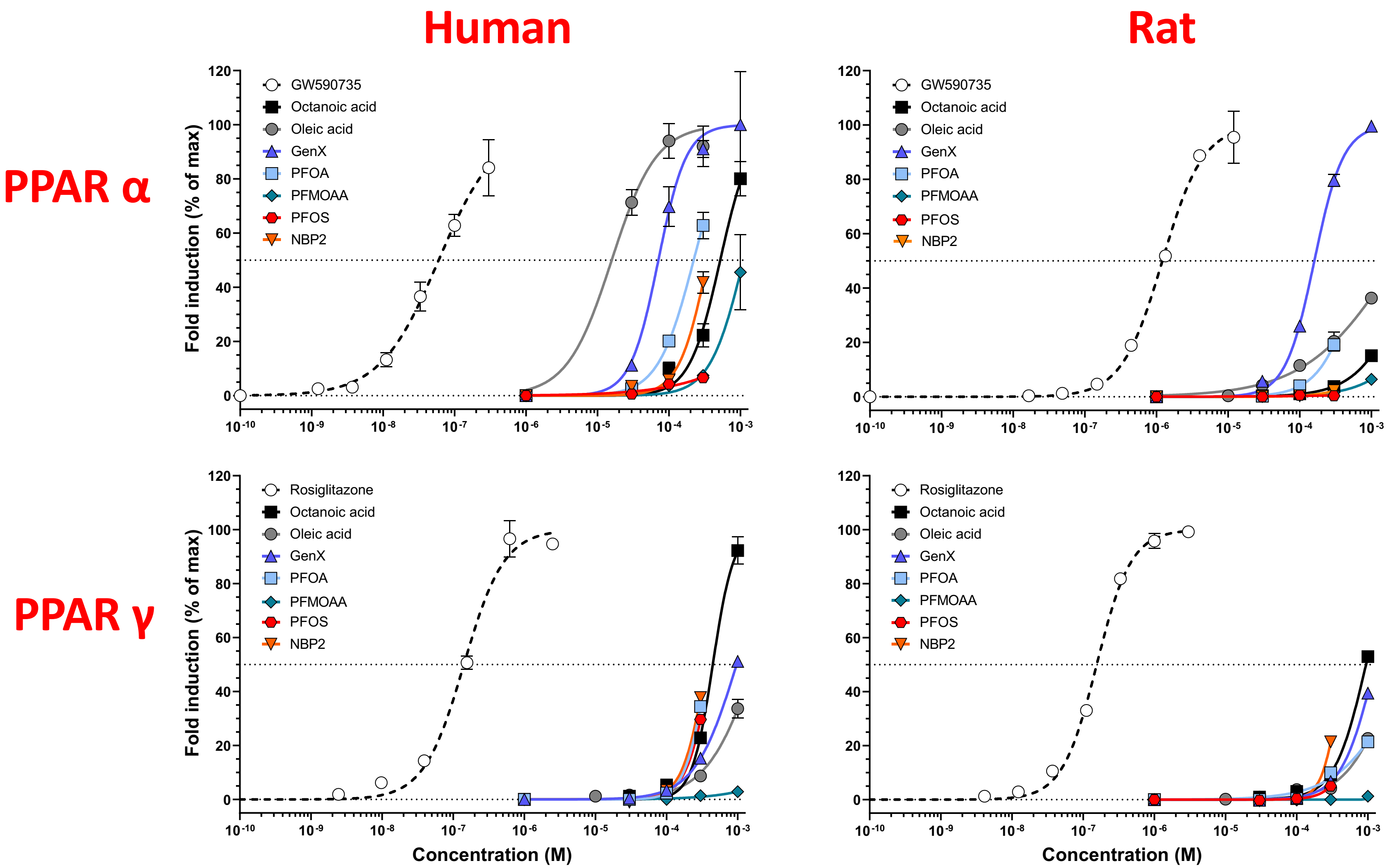
USEPA Chemical Dashboard

Research objectives

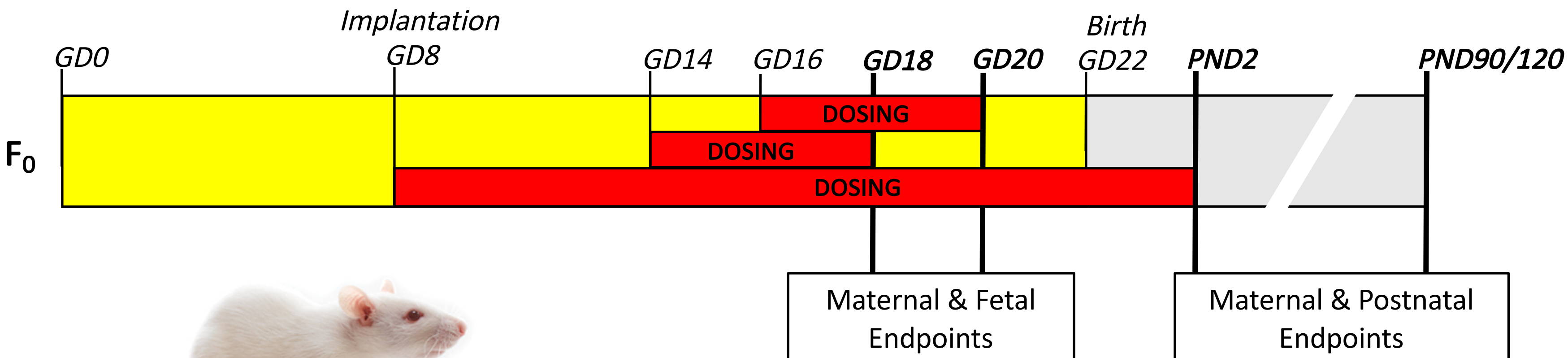
- Assess maternal and perinatal effects of gestational exposure to PFEAs that have documented human exposure but little/no published toxicity data available
- Develop Adverse Outcome Pathways to facilitate the use of *in vitro* or refined *in vivo* assays to predict effects of additional PFAS in future testing



In vitro human and rat PPAR alpha and gamma activity



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

Gene	Ctl	1	3	10	30	62.5	125	250
Ehhadh			4.6	30.5	81.2	144.8	214.8	252.3
Fabp1			3.3	10.9	28.3	56.6	77.8	88.3
Hmgcs2			2.9	4.6	8.0	16.7	20.9	22.0
Cpt1b		1.7	3.0	3.5	8.6	10.3	16.7	16.4
Acox1		1.2	1.5	2.3	3.7	5.7	8.3	8.5
Ech1				1.5	2.1	2.6	4.6	4.3
Cpt2			1.4	2.1	2.9	3.2	4.1	4.0
Scd1					1.7	2.4	2.9	3.6
Rxrg				2.2	4.0	4.4	4.9	3.4
Acaa2				1.8	2.3	2.5	3.2	3.3
Slc22a5			1.6	1.7	2.6	2.4	3.4	3.2
Slc27a2			1.2	1.6	1.8	2.1	2.4	2.5
Acadm				1.4	1.7	1.9	2.4	2.3
Acadl				1.3	1.4	1.5	1.9	2.0
Fads2			1.2	1.1	1.6	1.8	2.0	2.2
Acsl3					1.2	1.3	1.4	1.5
Pck1					4.4	6.6	11.8	8.8
Angptl4		1.6	3.8	6.2	11.3	17.0	19.5	17.3
Cpt1a			2.0	3.4	4.6	6.2	9.7	11.2
Etfdh				1.7	2.1	2.7	3.5	3.6
Acsl1			1.3	1.8	2.4	2.5	2.9	2.7
Mlycd					1.4	2.3	2.6	2.4
Gk					1.4	1.4	1.9	1.7
Fabp5						1.3	1.4	1.5
Aqp7					1.5	1.6	1.7	1.6
Acsl4					1.5	1.6	1.7	1.7
Dgat1				1.4	1.5	1.2	1.5	1.5
Lpl				1.2	1.4	1.3	1.5	1.5

Maternal GenX dose (mg/kg/d)

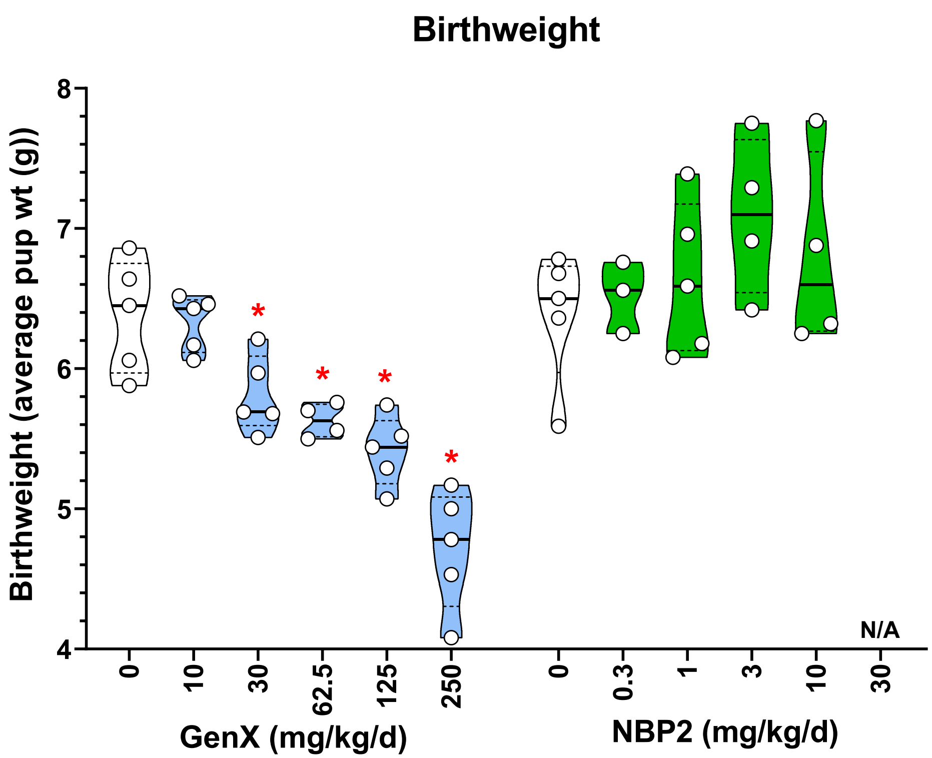
Fetal NBP2 liver

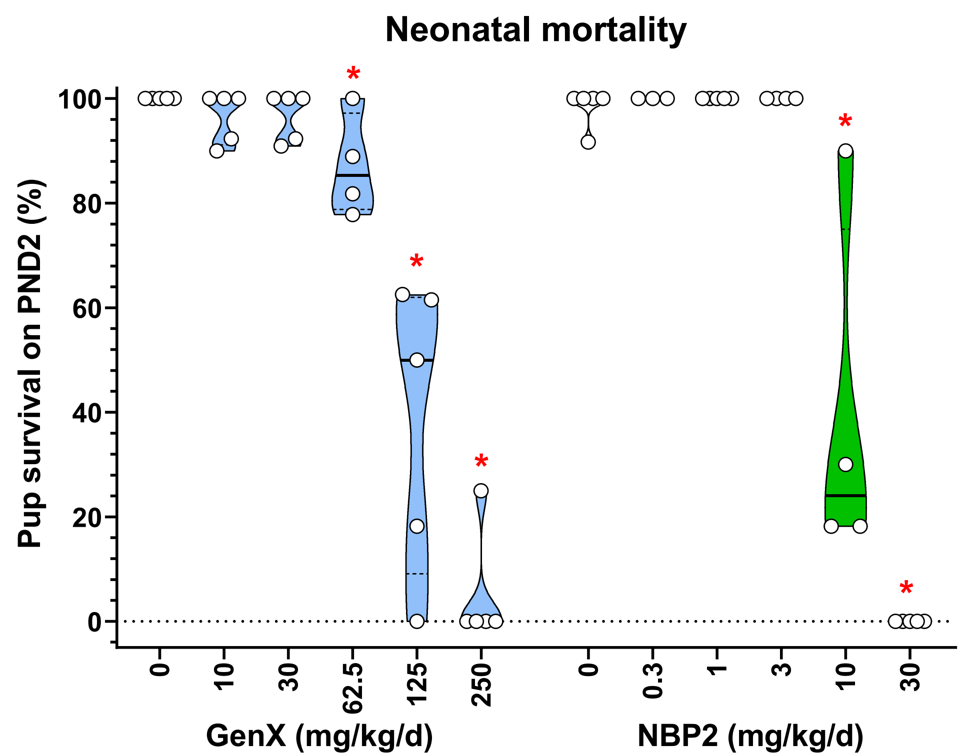
Gene	Ctl	0.1	0.3	1	3	10	30
Fabp1						1.4	1.6

Maternal NBP2 dose (mg/kg/d)

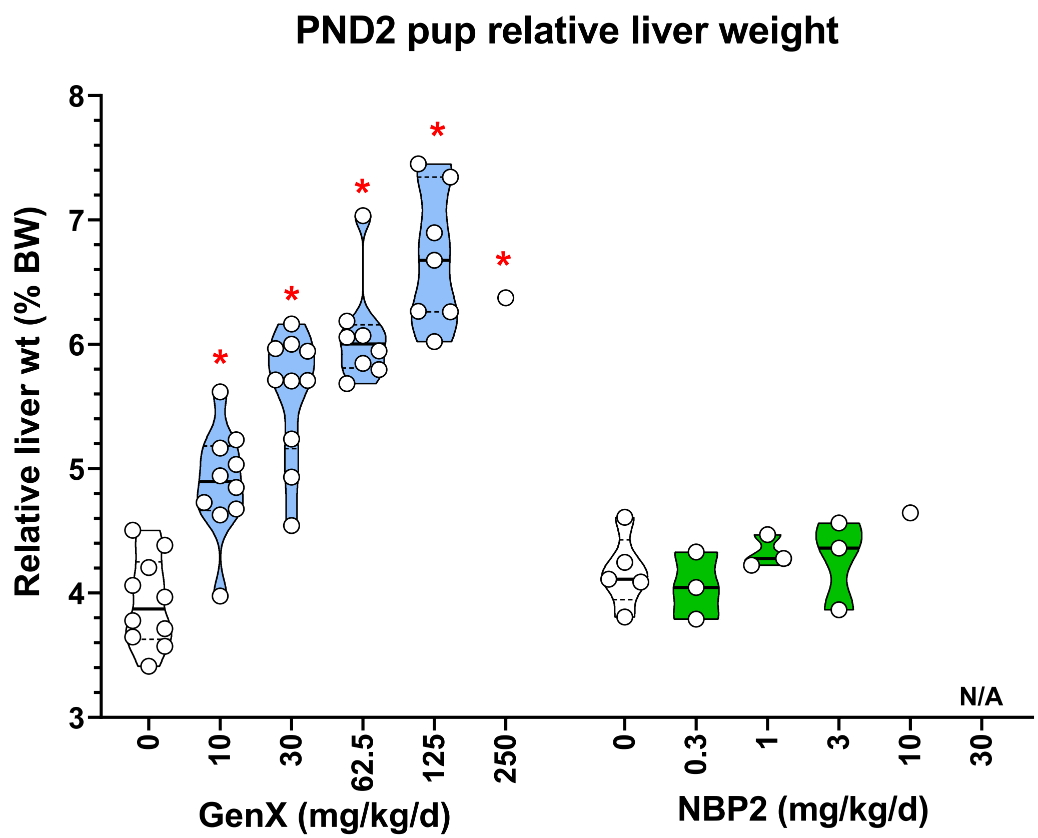
ANOVA p<0.001
Pairwise vs control p<0.01

Adverse neonatal effects
GD 8 – PND 2 exposure



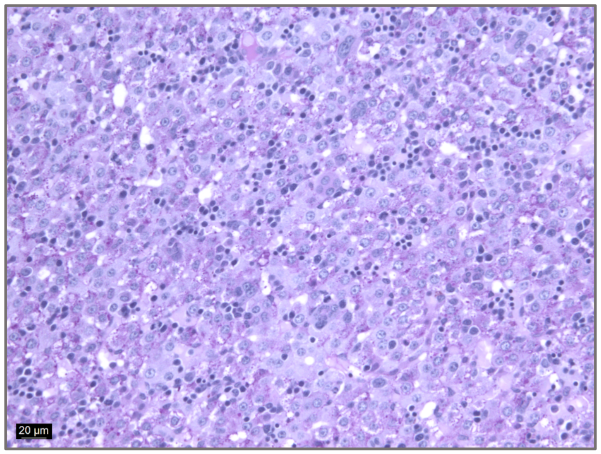


Adverse neonatal effects
GD 8 – PND 2 exposure

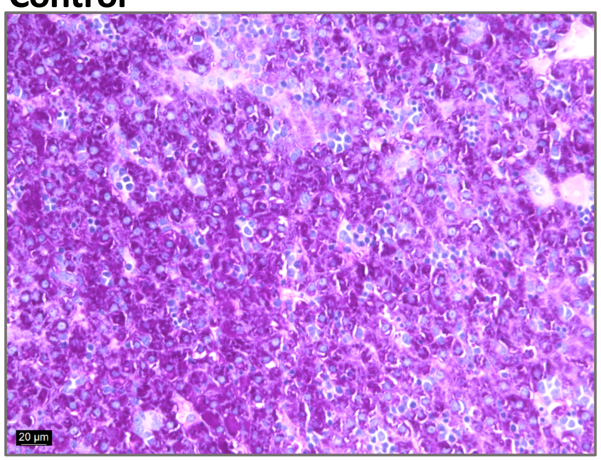


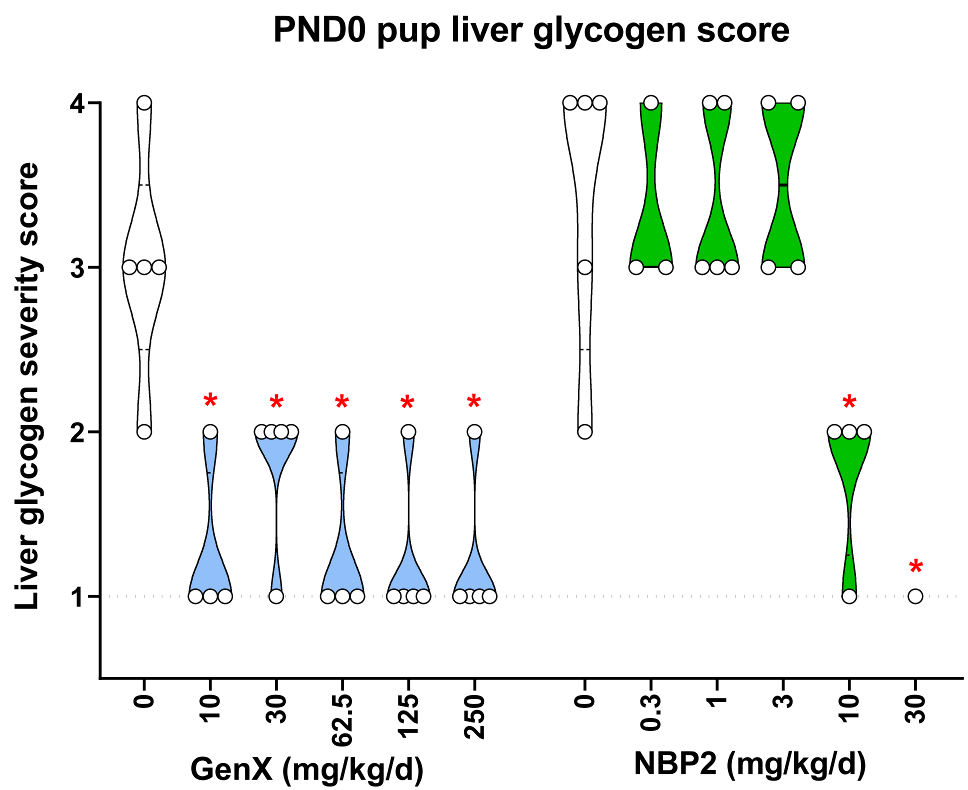
Histopathological evaluation of PND0 pup liver

Treated – GenX 250 mg/kg

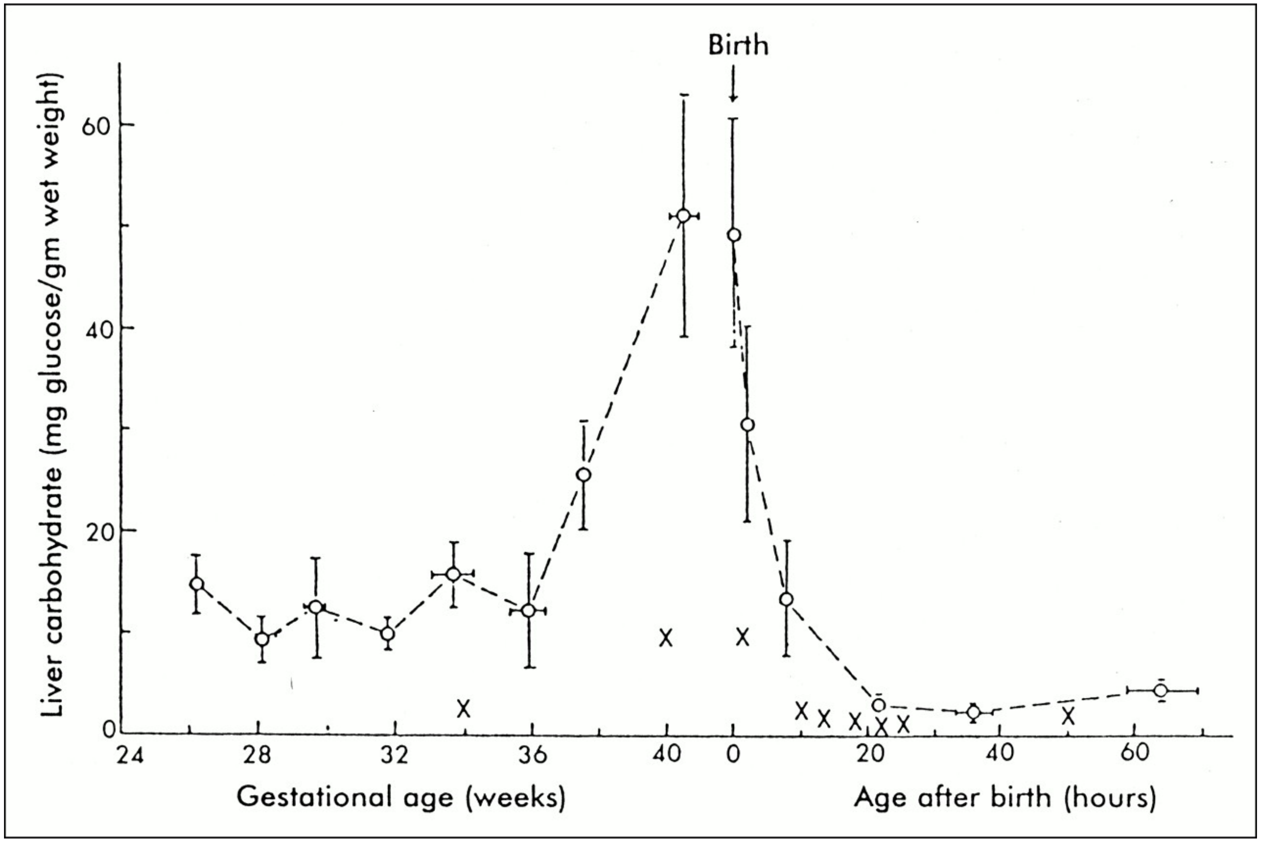


Control





Fetal liver glycogen deposition is critical for neonatal health



Shelley, HJ, Nelligan, GA (1966) Neonatal hypoglycemia. British Medical Bulletin

Neonatal liver glucose metabolism pathway gene expression

Neonatal liver PND(0)

Gene	Control	10	30	62.5	125	250
Ugp2		-5.7	-13.8	-14.6	-23.1	-30.0
Aldob			-7.6	-11.3	-16.7	-24.3
Agl			-4.3	-7.3	-11.5	-13.0
Idh1			-2.0	-2.4	-3.4	-5.4
Fbp1			-2.4	-3.0	-3.7	-5.1
Pygl			-2.1	-3.3	-3.9	-5.1
H6pd				-3.1	-4.0	-5.1
Pdhb			-2.1	-2.3	-3.4	-4.5
Idh2			-2.1	-2.4	-3.1	-4.4
Fh1			-2.8	-3.2	-3.1	-4.3
Pgm2			-2.1	-2.2	-2.5	-4.0
Mdh1			-2.3	-2.3	-3.1	-3.6
Sdhb			-1.7	-1.7	-2.5	-3.2
Mdh2			-2.1	-2.0	-2.3	-3.0
Rbks				-2.2	-2.1	-3.0
Acly						-2.7
Sucla2				-1.5	-2.0	-2.5

Maternal oral GenX dose (mg/kg/d)

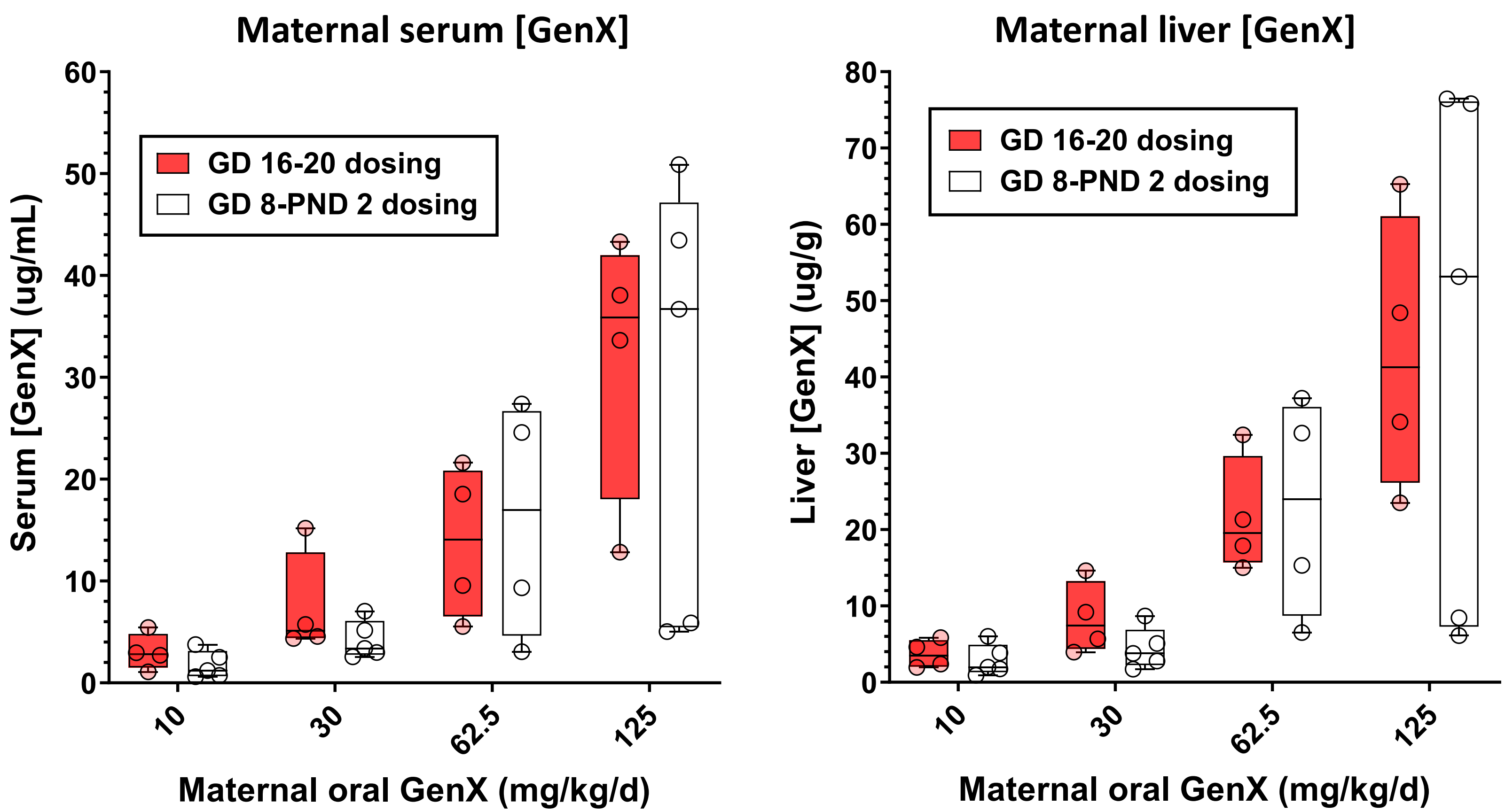
Neonatal liver PND(0)

Gene	Control	0.3	1	3	10	30
Pdhb		-1.4	-1.8	-1.8	-2.2	-1.9

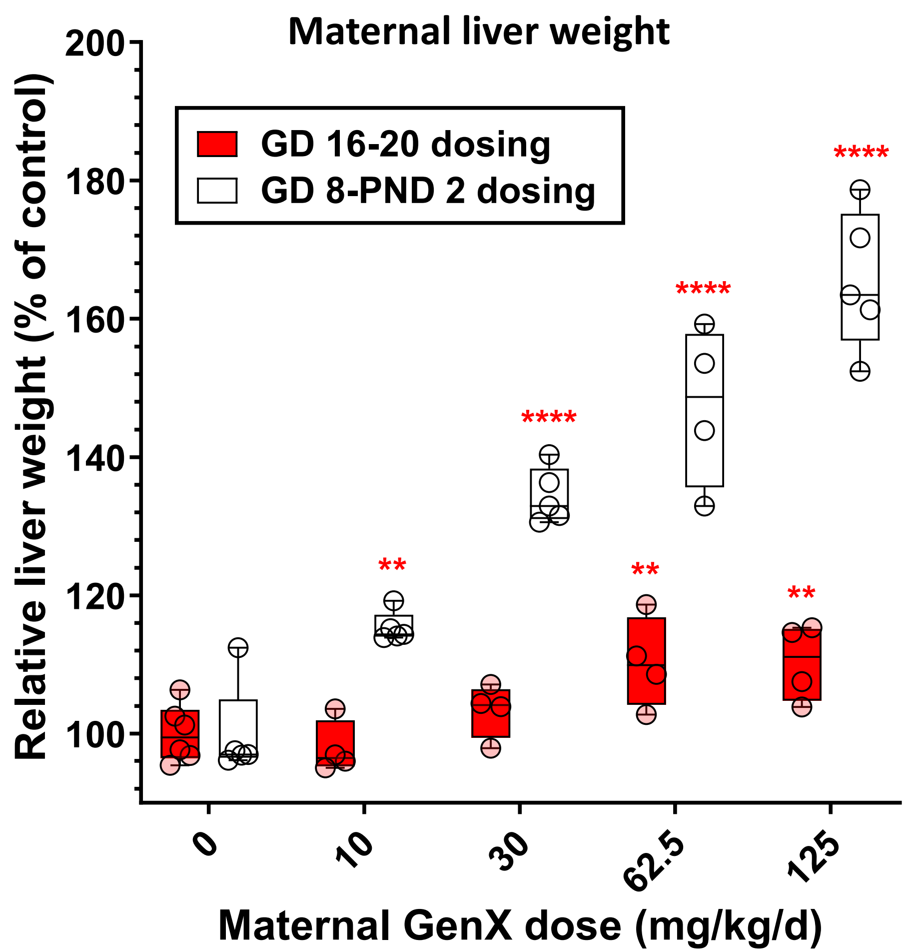
Maternal oral NBP2 dose (mg/kg/d)

ANOVA $p<0.001$
Pairwise vs control $p<0.01$

GenX does not accumulate in maternal serum or liver…

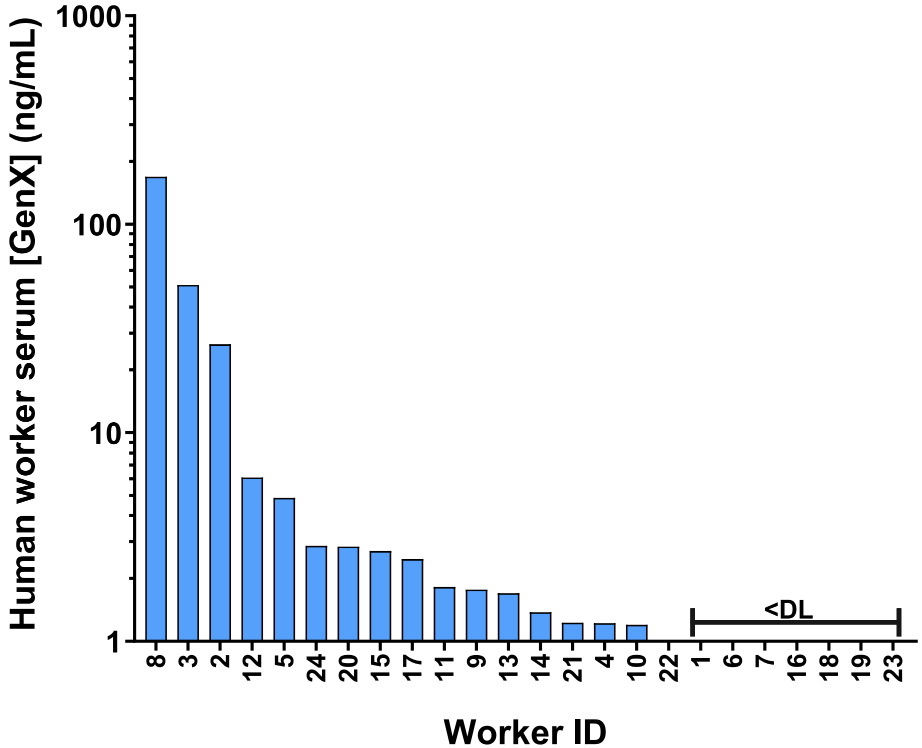


…but exposure duration is important for effects

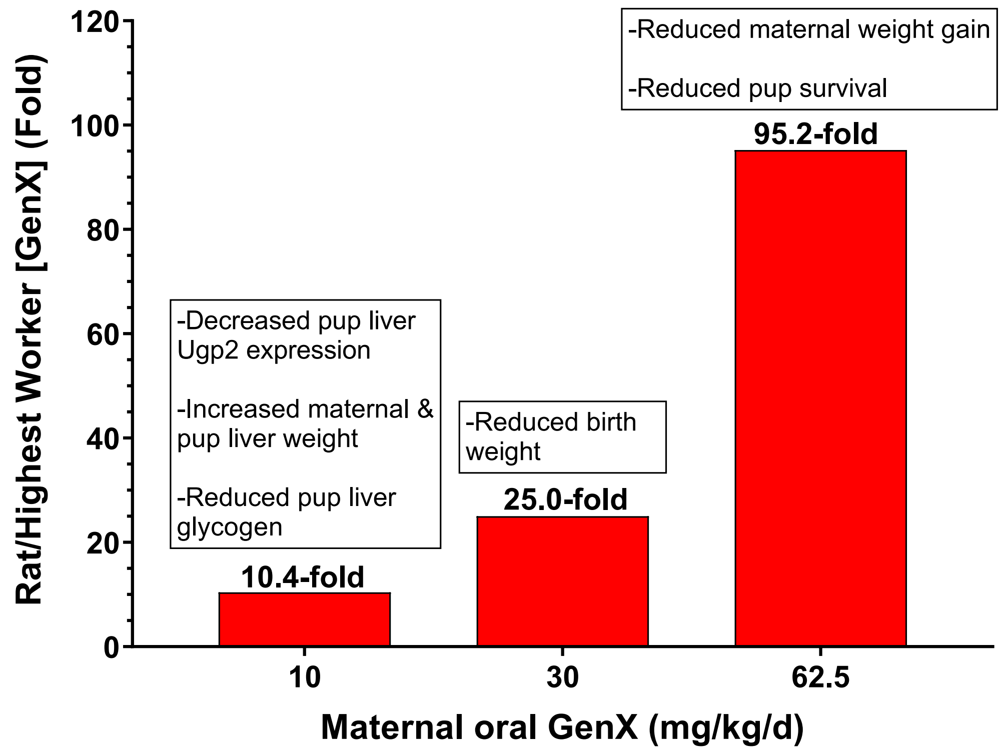


Margin of internal exposure – rat:human

Human factory worker serum [GenX]



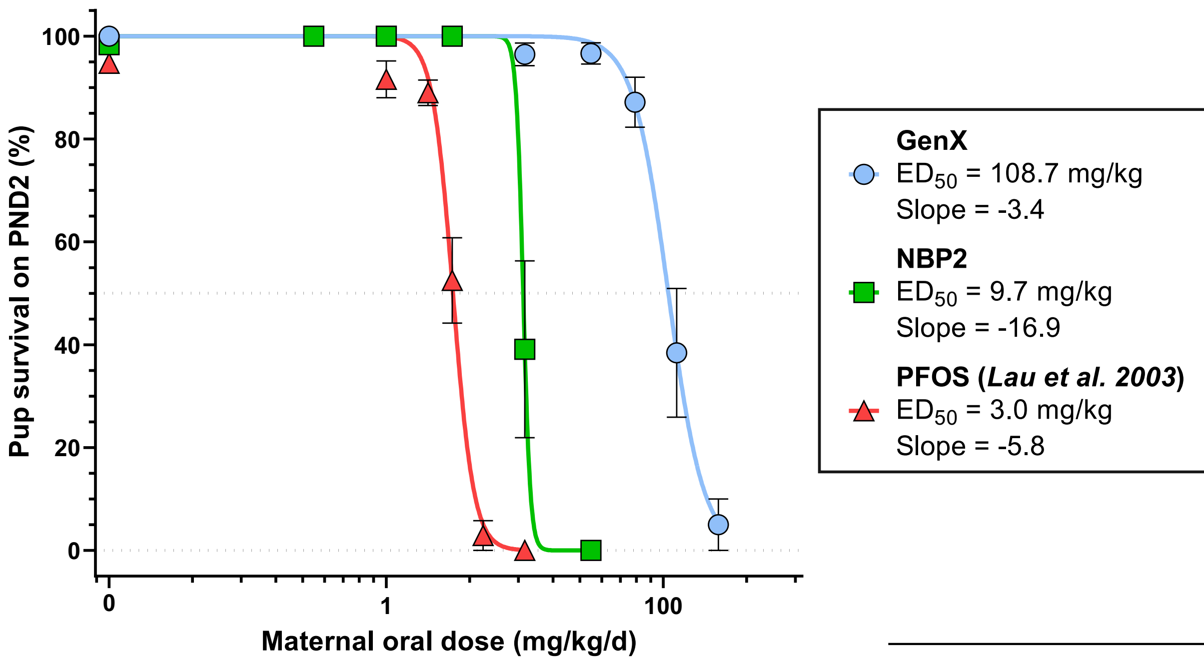
Margin of Internal Exposure: Ratio of Rat/Human serum [GenX]



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

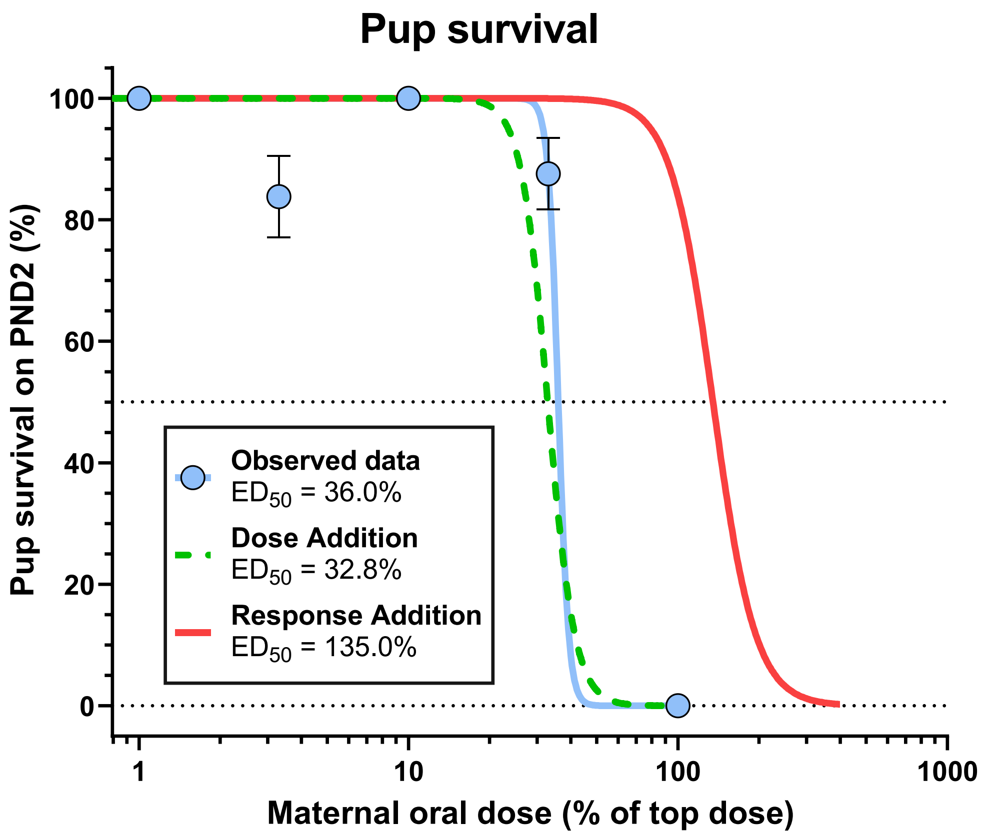
GenX+NBP2+PFOS Mixture study



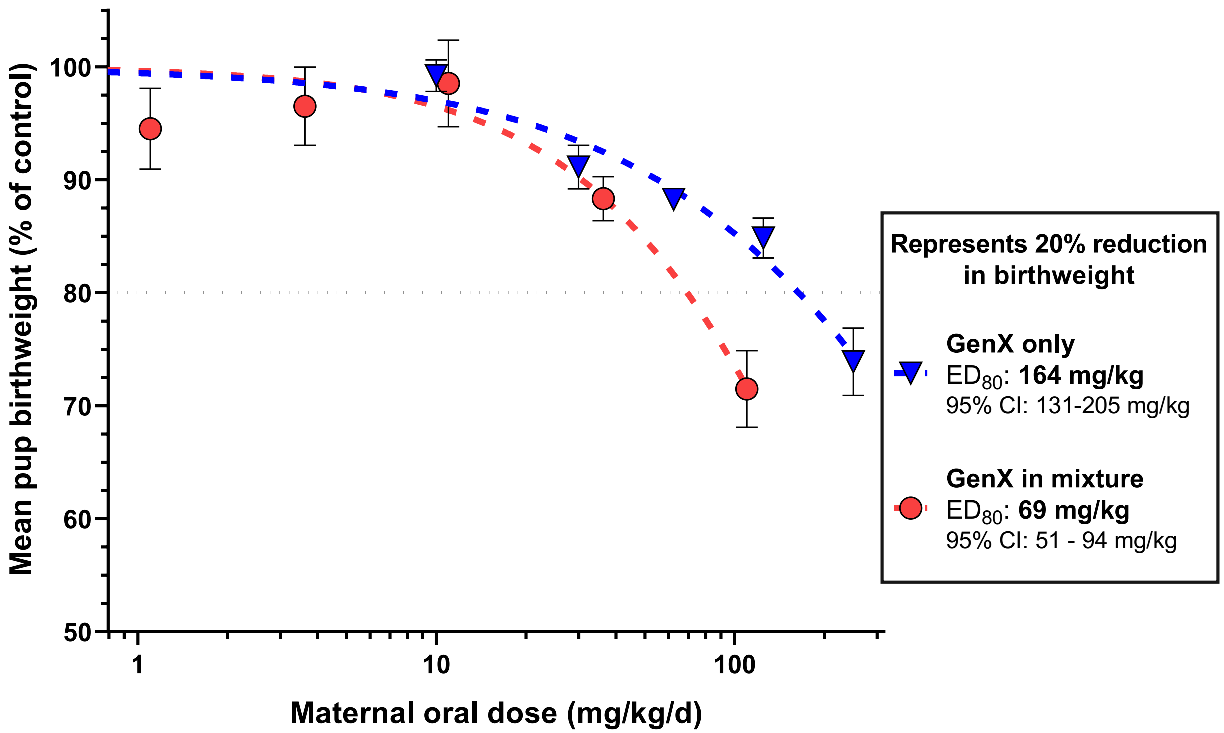
Top dose = each chemical at ED_{50}

	100%	**33%**	**10%**	**3.3%**	**1%**
GenX (mg/kg)	110	36.7	11	3.67	1.1
NBP2 (mg/kg)	10	3.3	1	0.3	0.1
PFOS (mg/kg)	3	1	0.3	0.1	0.03

Mixture effects appear dose additive



Impact of co-exposure on chemical dose-response



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

EPA

conley.justin@epa.gov