Characterization of developmental toxicity and Adverse Outcome Pathways (AOPs) for emerging PFAS – individual compounds and mixtures

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SOT RASS-MixSS Joint Webinar
January 13, 2021

The views expressed in this presentation do not necessary represent the views or policy of the USEPA

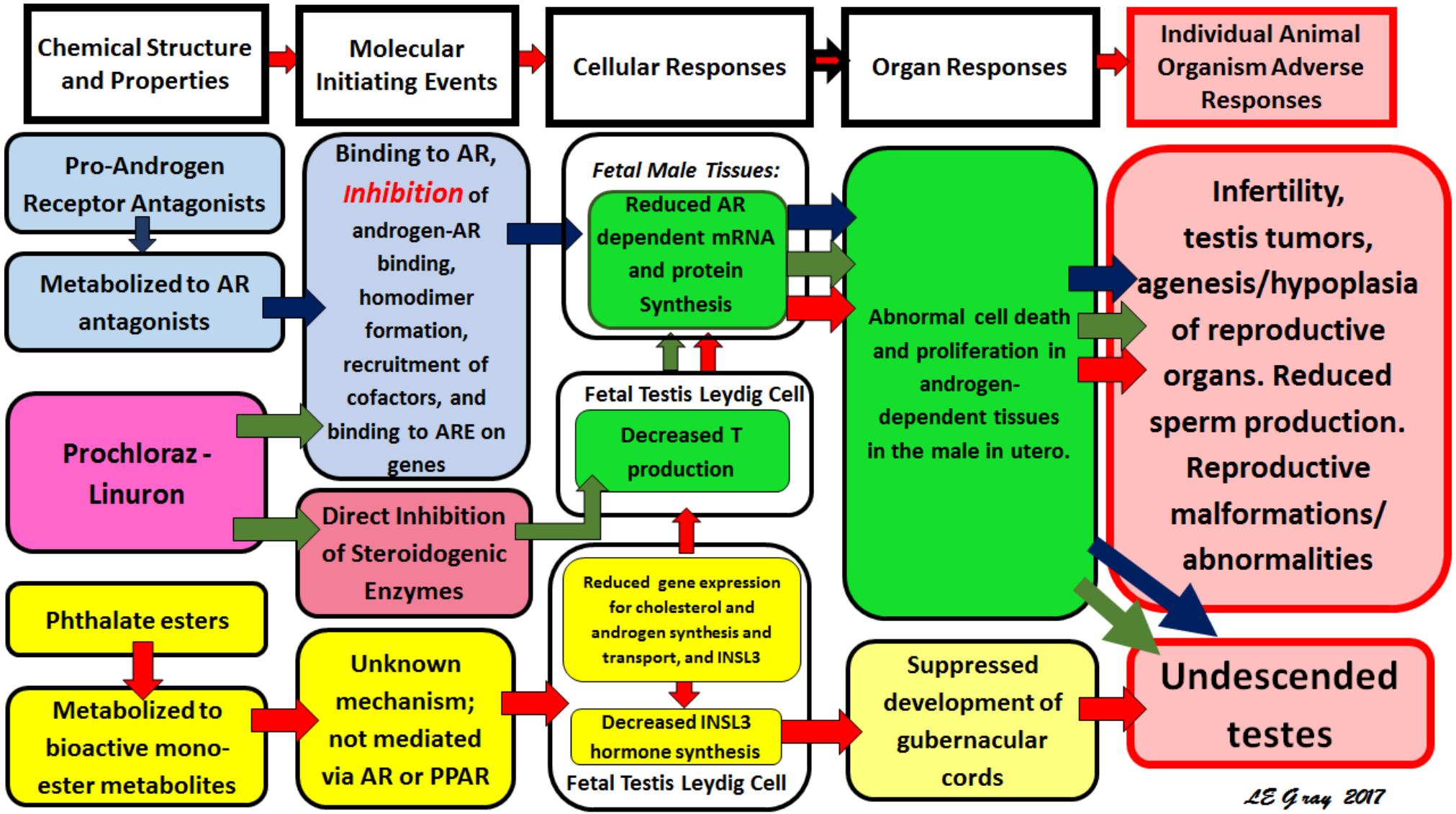
The presenters declare no conflicts of interest

Do not cite or quote as some data are unpublished and should be considered preliminary

Outline

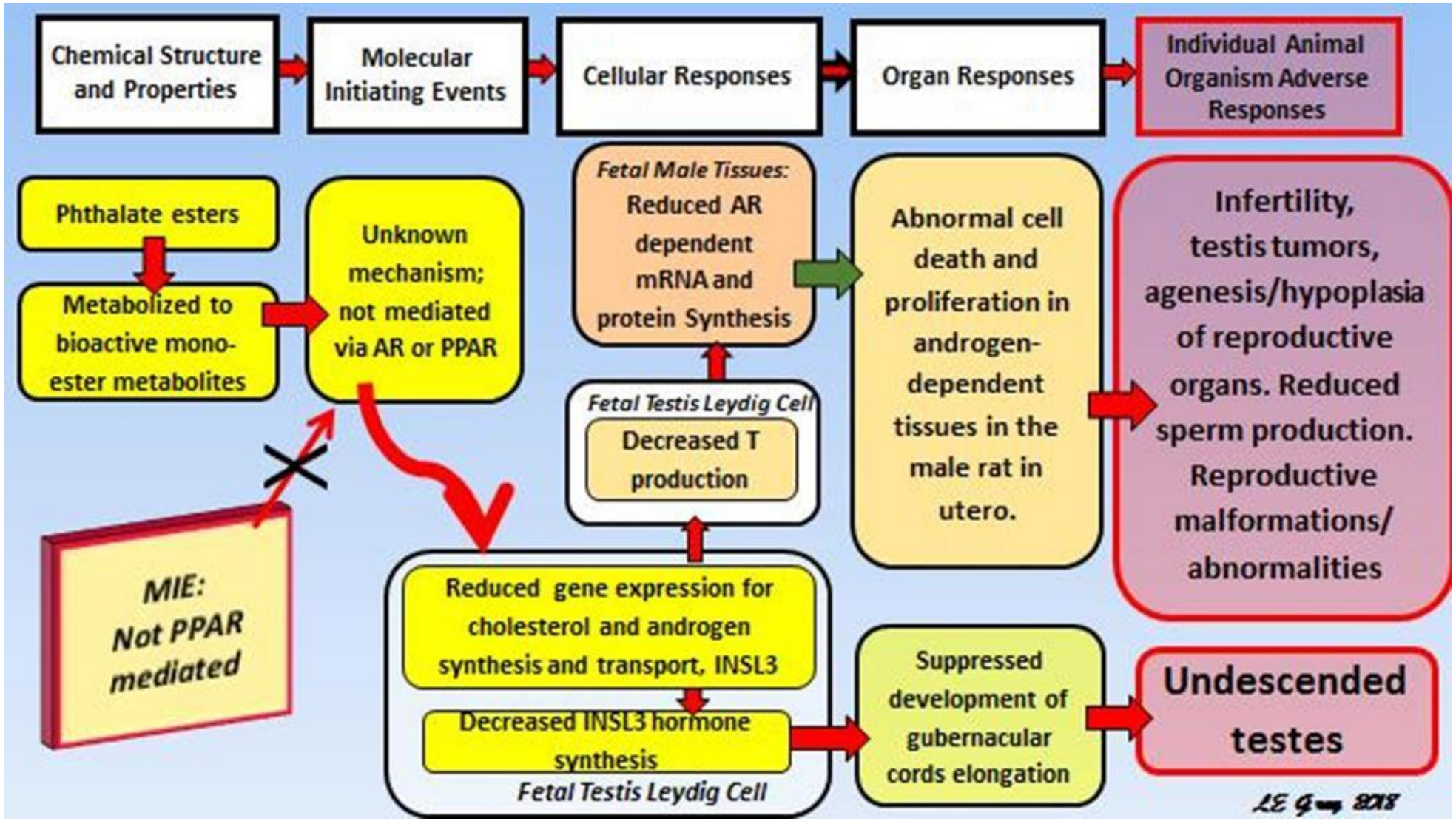
- Context from prior phthalate research
- Emerging PFAS
- Research objectives
- Study data and main points from individual PFAS experiments
- PFAS mixture study
- Publications
- Key findings and impact

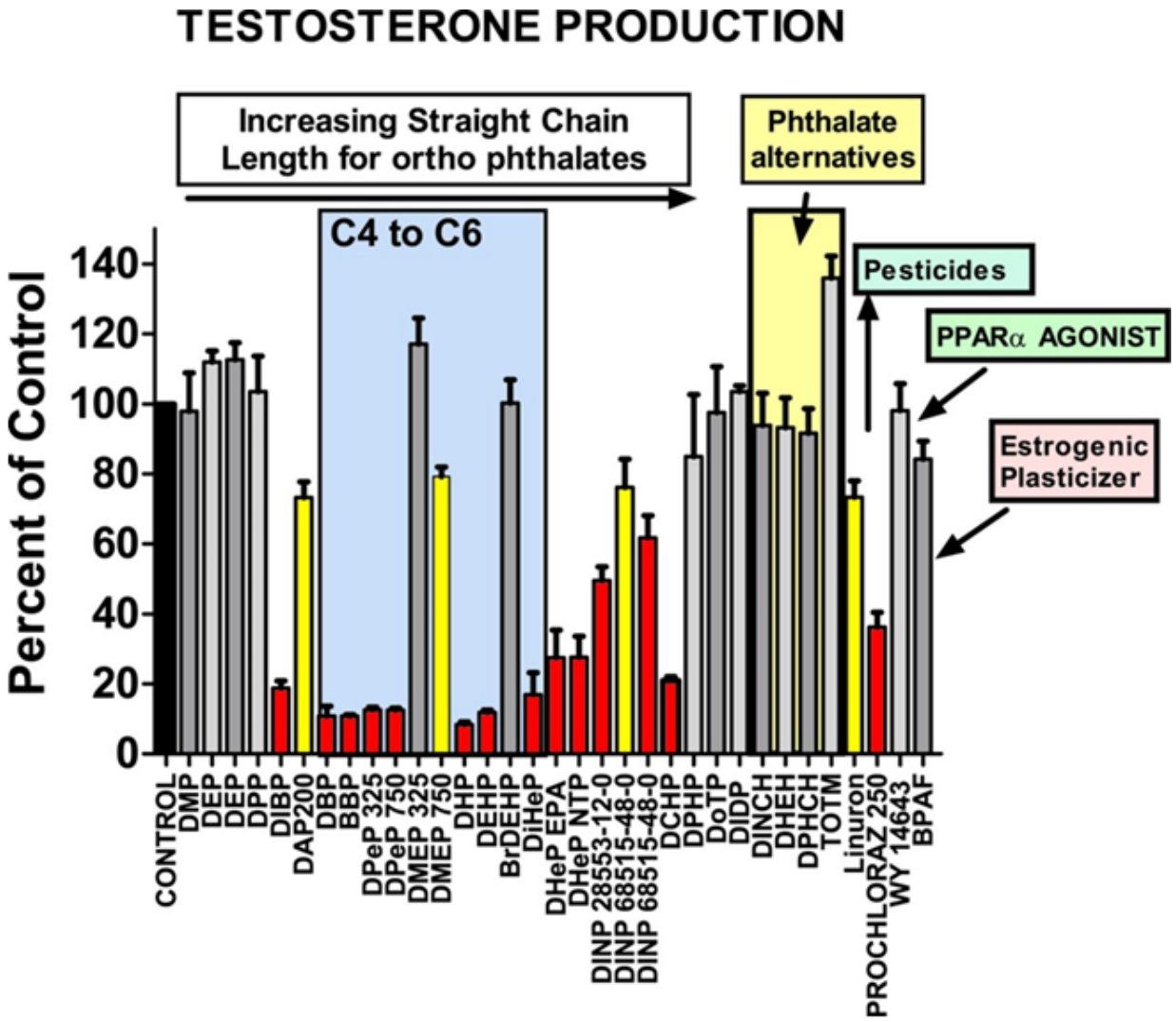
AOP Network linking several MIEs and Key Events to common Adverse Outcomes resulting from disruption of the Androgen Signaling Pathway *in utero.* Other MIEs not shown include inhibition of DHT synthesis, for example.



PHTHALATE (PE) Adverse Outcome Pathway

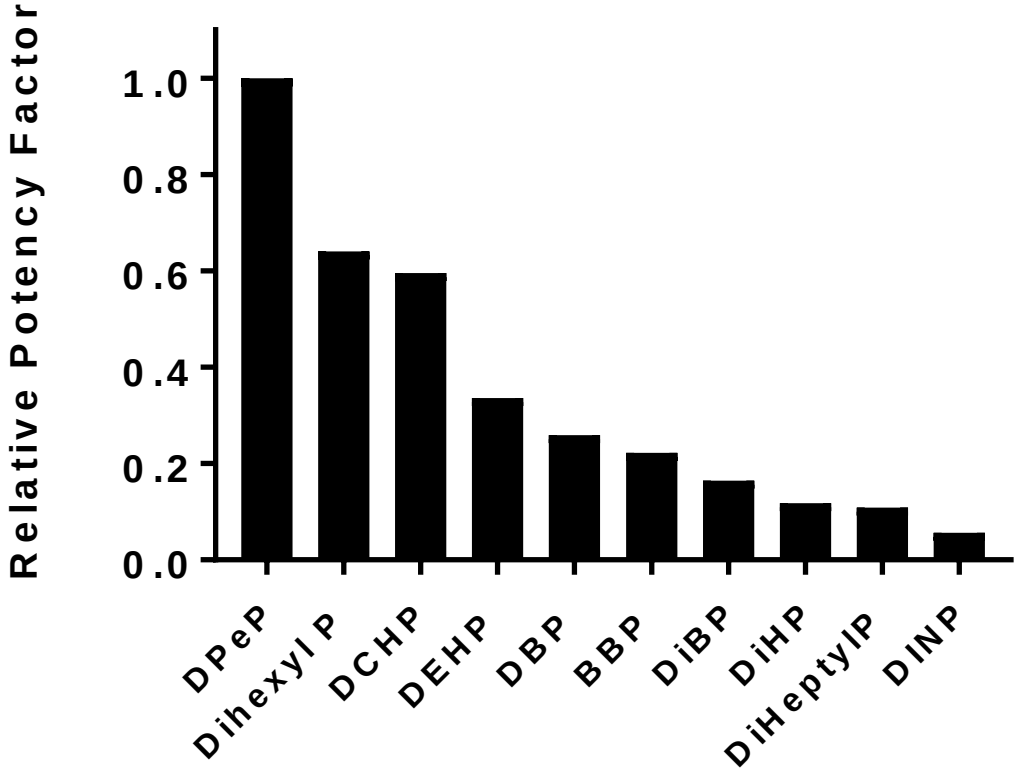
Unknown Molecular Initiating Event(s) that reduces mRNA for about 40 Leydig cell genes involved in steroid hormone transport and synthesis, Insl3 synthesis and cholesterol synthesis. Reduced testicular testosterone and Insl3 synthesis are causally related to reproductive malformations. Reductions in testosterone production in the fetal male rat can be used to predict the in utero doses that induce malformations in F1 male rats after birth.



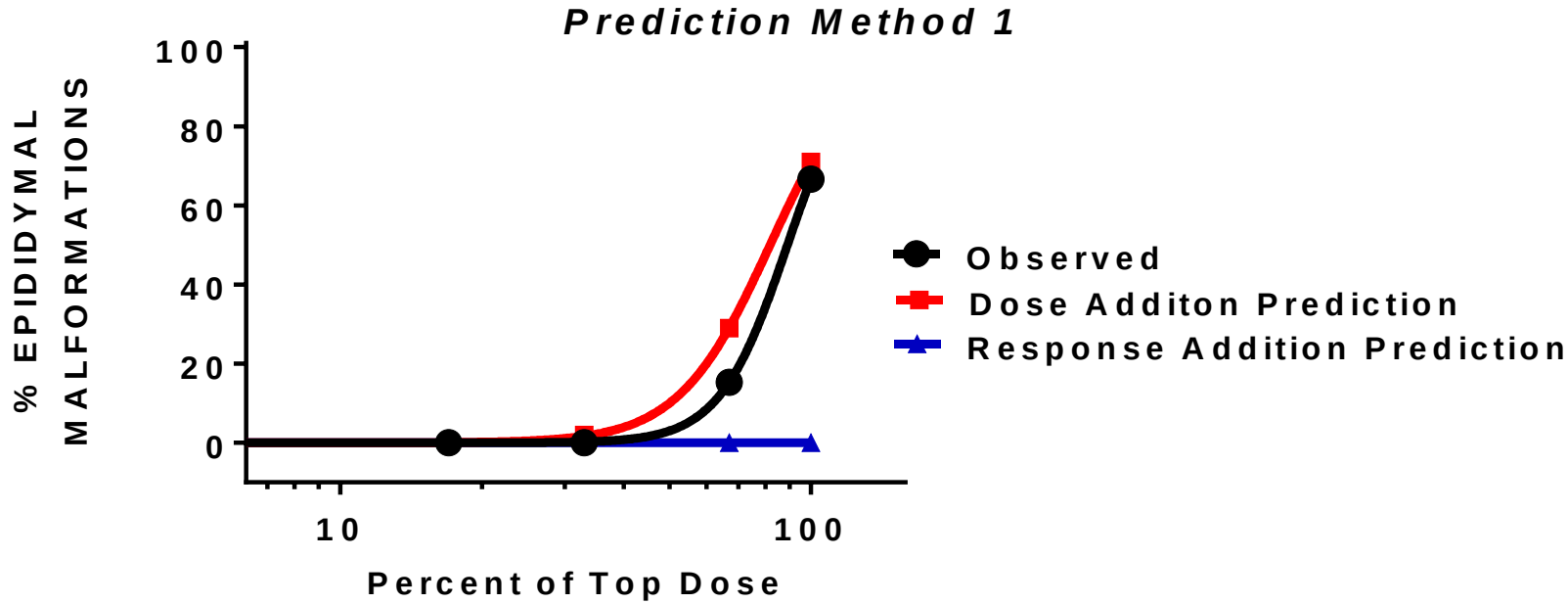


Johnathan R. Furr et al. Toxicol. Sci. 2014;140:403-424

Relative Potency of Phthalates that reduce fetal Testosterone Production using dipentyl phthalate (DPeP) as the reference chemical



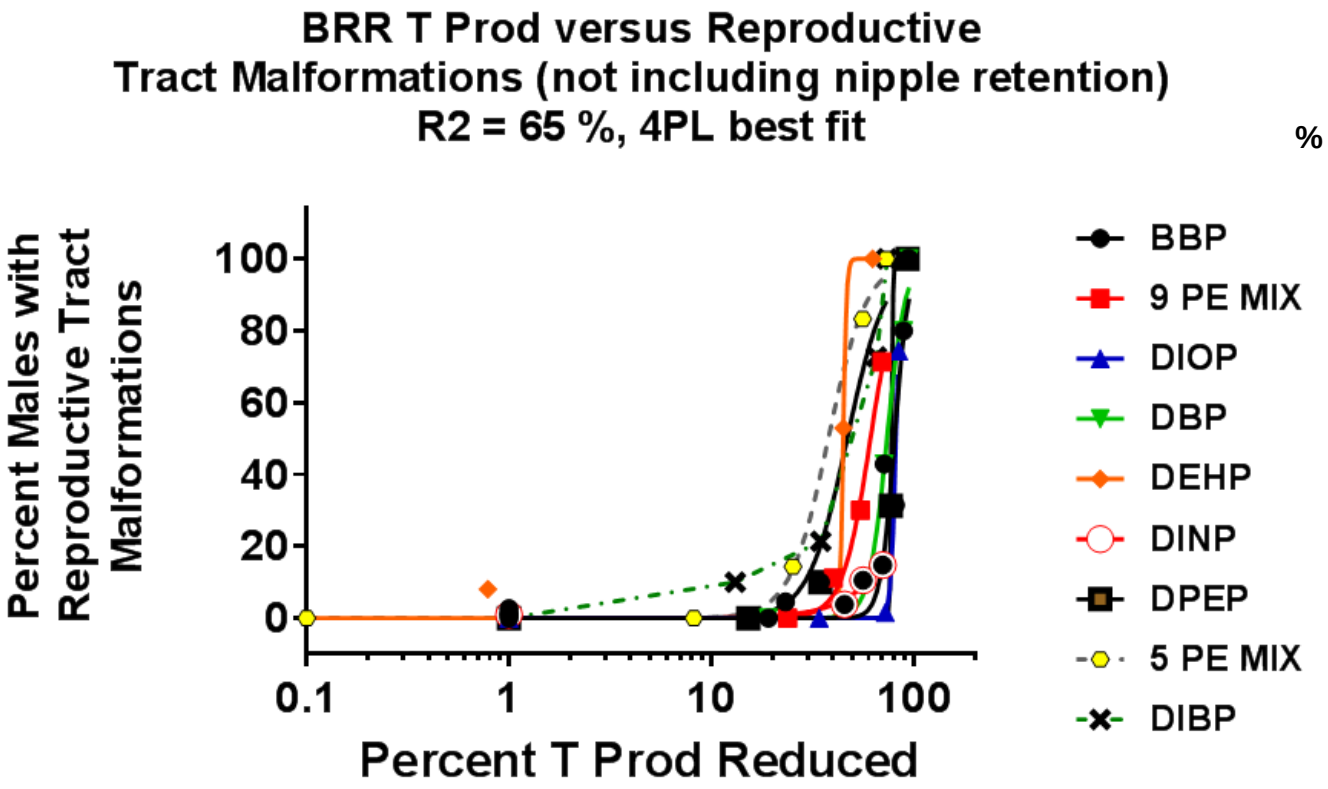
Nine Phthalate *in utero* Mixture Study:
Prediction of the % of males with Epididymal Agenesis Using the Relative Potency Factors for reduced Testosterone Production using Dipentyl Phthalate as the Reference Chemical:

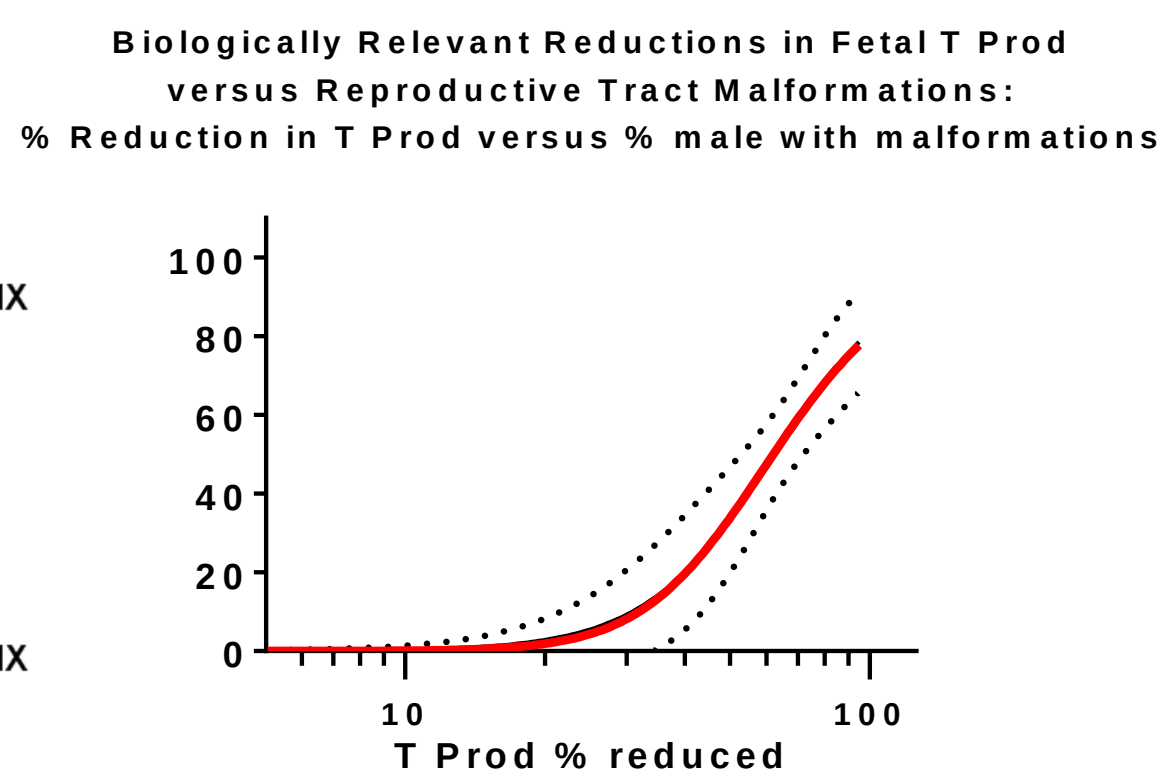


Phthalate ester	DPeP	DHP	DCHP	DEHP	DiHeP	DBP	BBP	DiBP	DHeP
RPF vs DPeP TPROD*	1	0.57	0.69	0.28	0.09	0.25	0.20	0.14	0.04
ED50 multiplier BASED ON	1	1.75	1.46	3.60	10.76	4.00	5.08	7.08	28.20
ED50 EPI AGEN	154.8	271	225	557	1665	620	787	1096	4366

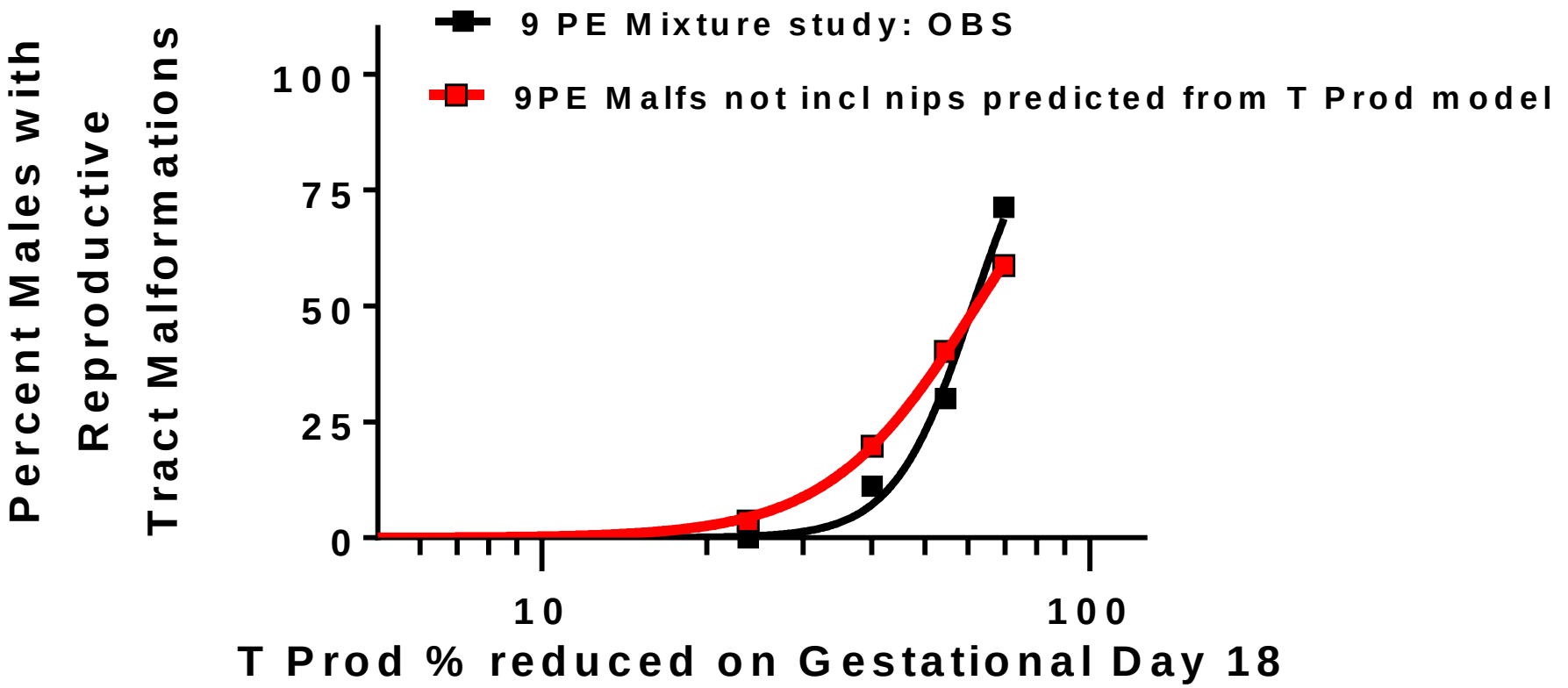
Phthalate ester	DPeP	DHP	DCHP	DEHP	DiHeP	DBP	BBP	DiBP	DHeP	Predicted % Epididymal agenesis
100% DOSE	10	80	80	80	80	80	80	80	80	71
67%	6.7	53.3	53.3	53.3	53.3	53.3	53.3	53.3	53.3	29
33%	3.3	26.7	26.7	26.7	26.7	26.7	26.7	26.7	26.7	2
17%	1.7	13.3	13.3	13.3	13.3	13.3	13.3	13.3	13.3	0
0%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0

Comparison of the relationship between reduced fetal rat Testosterone Production (T Prod) and the % of Male rat Offspring with Reproductive Tract Malformations: Data from Nine Phthalates





Predicting the adverse postnatal effects of a 9 phthalate mixture administered in utero on GD 14-18 from a Predictive Logistic Regression Model of Fetal Testosterone Reductions versus F1 Male Rat Reproductive Abnormalities. *Prediction Method 2*.



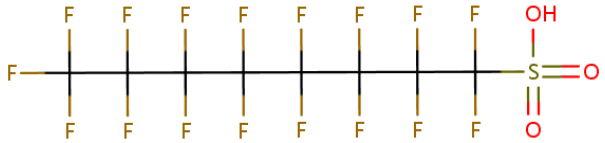
Conclusion: **We can predict the adverse reproductive effects of in utero exposure to a phthalate or a mixture of phthalates solely from the fetal T Production Reductions eliminating the need for a One generation Postnatal Study .**


I bet we could do something similar with PFAS

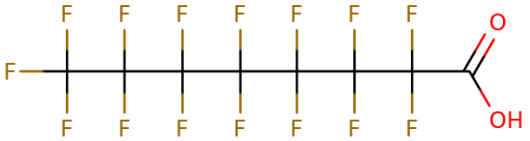
Background

- Legacy PFAS (PFOS, PFOA, PFNA) replaced with perfluoroalkyl ether acids (PFEAs)
- Parent PFEAs and manufacturing byproducts detected in drinking water and/or human serum in multiple locations across the US and globally
- Few or no published toxicity studies on most “emerging” or “next generation” PFAS

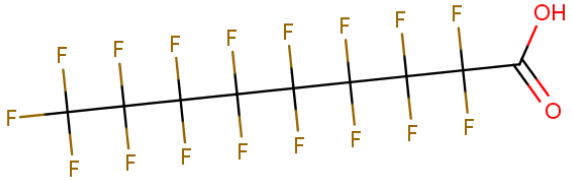
PFOS



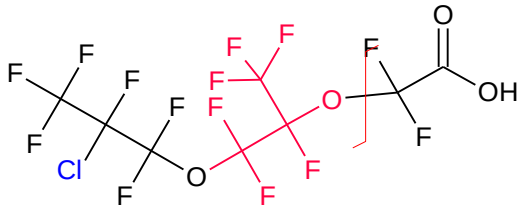
PFOA



PFNA

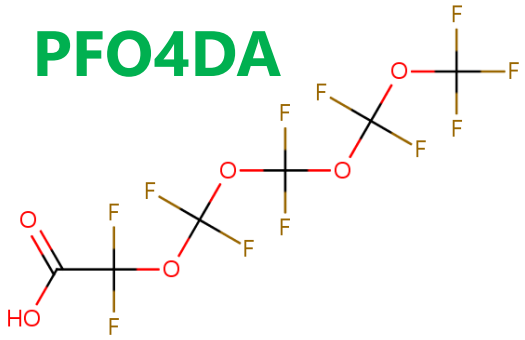


Cl-PFECA

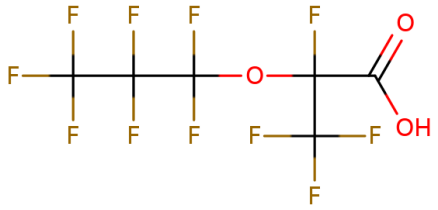


Washington et al. 2020 Science
DOI: 10.1126/science.aba7127

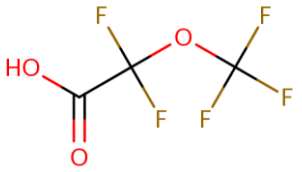
PFO4DA



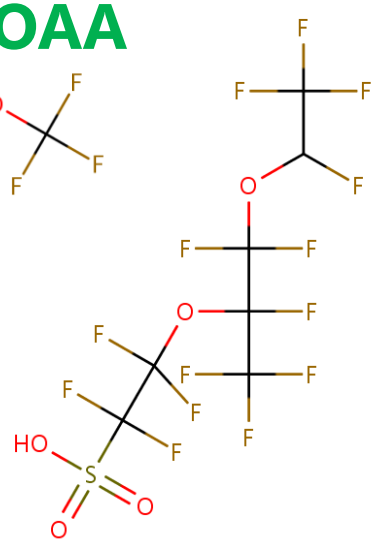
GenX



PFMOAA



NBP2



PFOS=perfluorooctanesulfonate

PFOA=perfluorooctanoic acid

PFNA=perflurornonanoic acid

Cl-PFECA=Chlorinated perfluoroethercarboxylic acid

PFO4DA=perfluoro-3,5,7,9-butaoxadecanoic acid

GenX=hexafluoropropylene oxide dimer acid

PFMOAA=perfluoromethoxyacetic acid

NBP2=Nafion byproduct 2

USEPA Chemical Dashboard

Background

- Legacy PFAS produce adverse developmental, liver, and/or immune toxicity, among other effects, in animal studies (rat and mouse) and are correlated with similar effects in human epidemiological studies

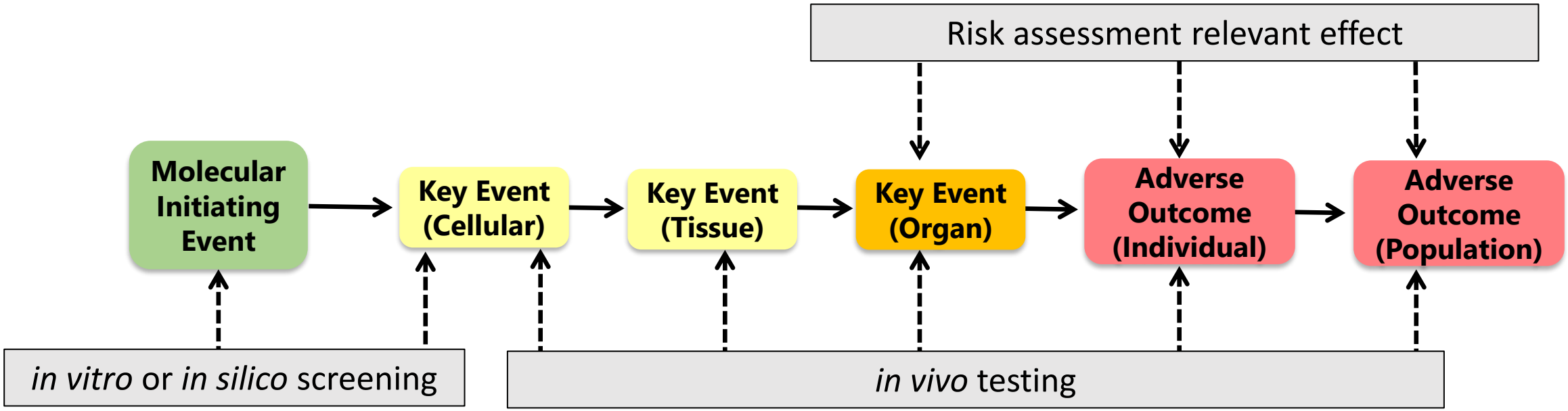
- Adverse developmental, liver, or immune effects are the primary basis for all US state and federal risk assessment reference doses and drinking water advisories or regulations (Post 2020 ET&C DOI: 10.1002/etc.4863)

- Questions:
  - Do emerging PFAS exert similar toxicities and potencies as legacy PFAS in animal studies?
  - Given most humans are known to be exposed to multiple PFAS, how do these compounds interact in mixture-based toxicity studies?
  - Can we design shorter term studies that are quantitatively predictive of developmental effects to produce risk assessment relevant data more quickly than traditional guideline studies?

Research Objectives

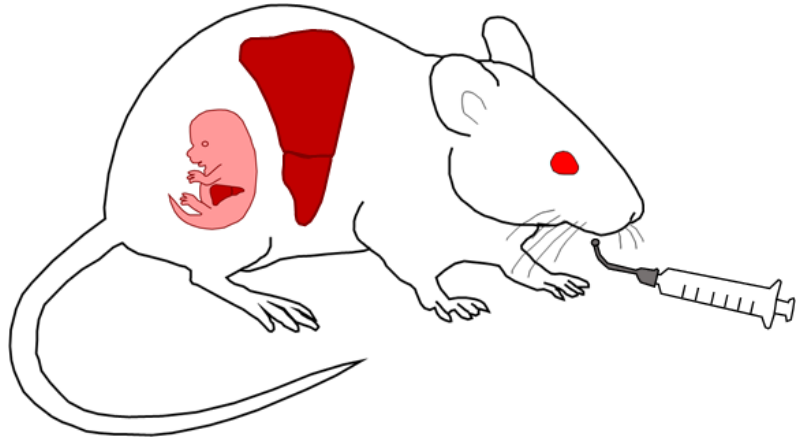
- Assess maternal, fetal, and perinatal effects of gestational exposure to PFEAs that have documented human exposure but little/no published toxicity data available

- 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



PFAS Studies

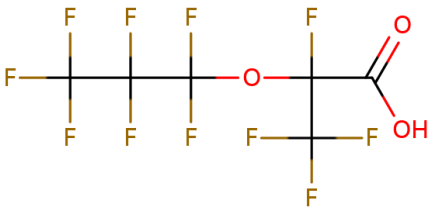
- **Cell-based studies (in vitro)**
  - Peroxisome proliferator activated receptor (PPAR) alpha/beta/gamma
  - Estrogen receptor, Androgen receptor, Glucocorticoid receptor



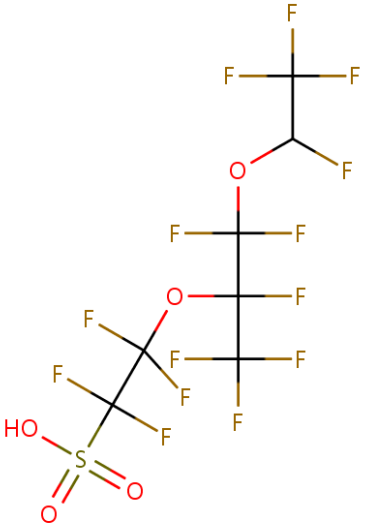
- **Maternal and fetal studies – rat 5-day dosing**
  - Maternal and fetal body weight, liver weight
  - Maternal clinical chemistry and thyroid hormones
  - Maternal and fetal liver gene expression
  - Maternal and fetal serum and liver PFAS concentrations

- **Maternal and postnatal studies – rat 17-day dosing**
  - Maternal and neonatal body weight, liver weight
  - Birthweight
  - Neonatal survival
  - Neonatal liver histopathology
  - Maternal and neonatal clinical chemistry
  - Maternal and neonatal serum and liver PFAS concentrations
  - Maternal and neonatal liver gene expression

GenX



NBP2

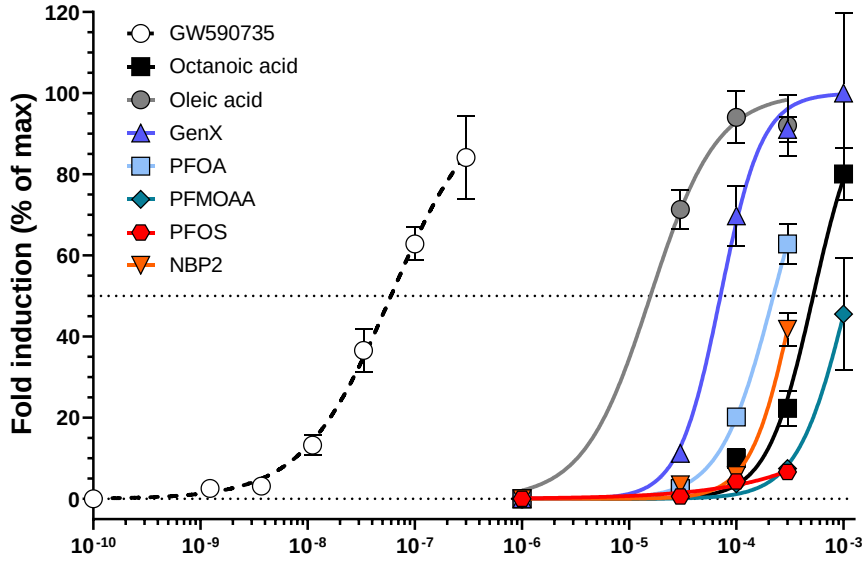


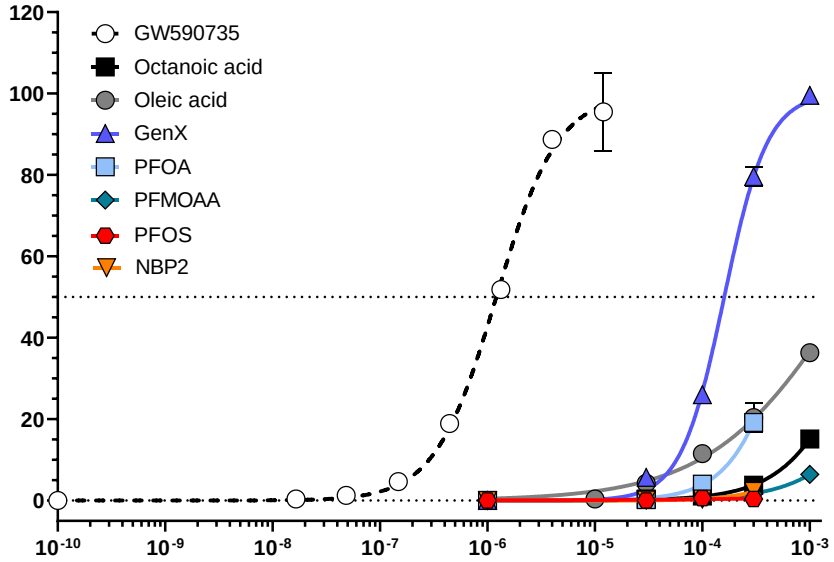
In vitro human and rat PPAR alpha and gamma activity

Human

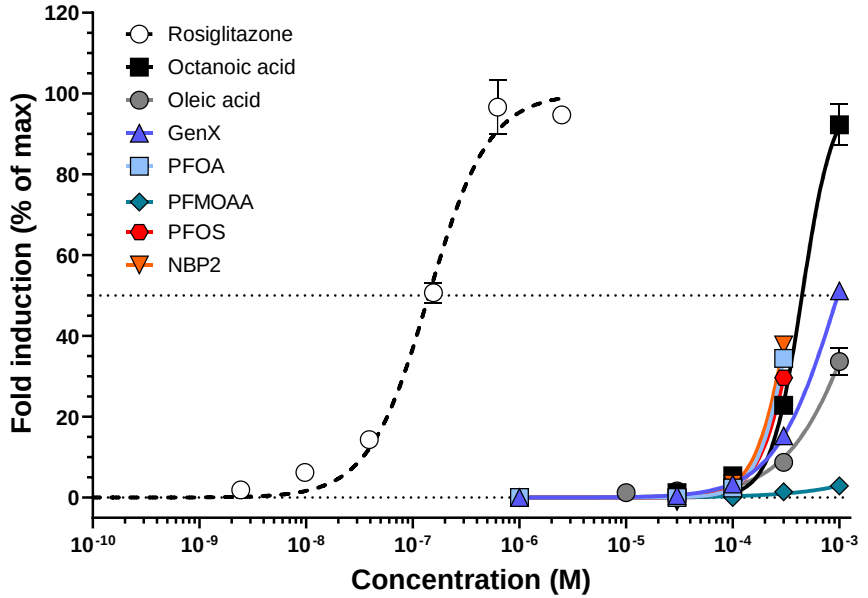
Rat

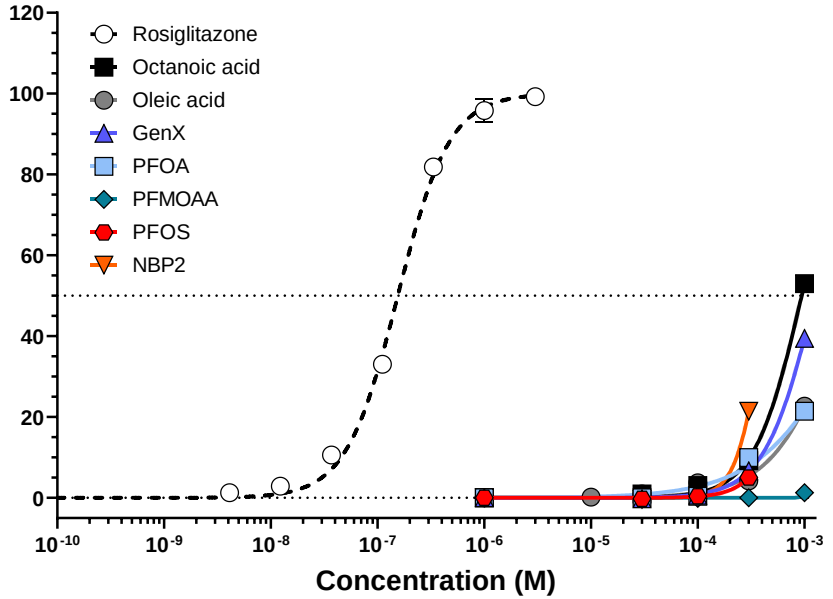
PPAR α



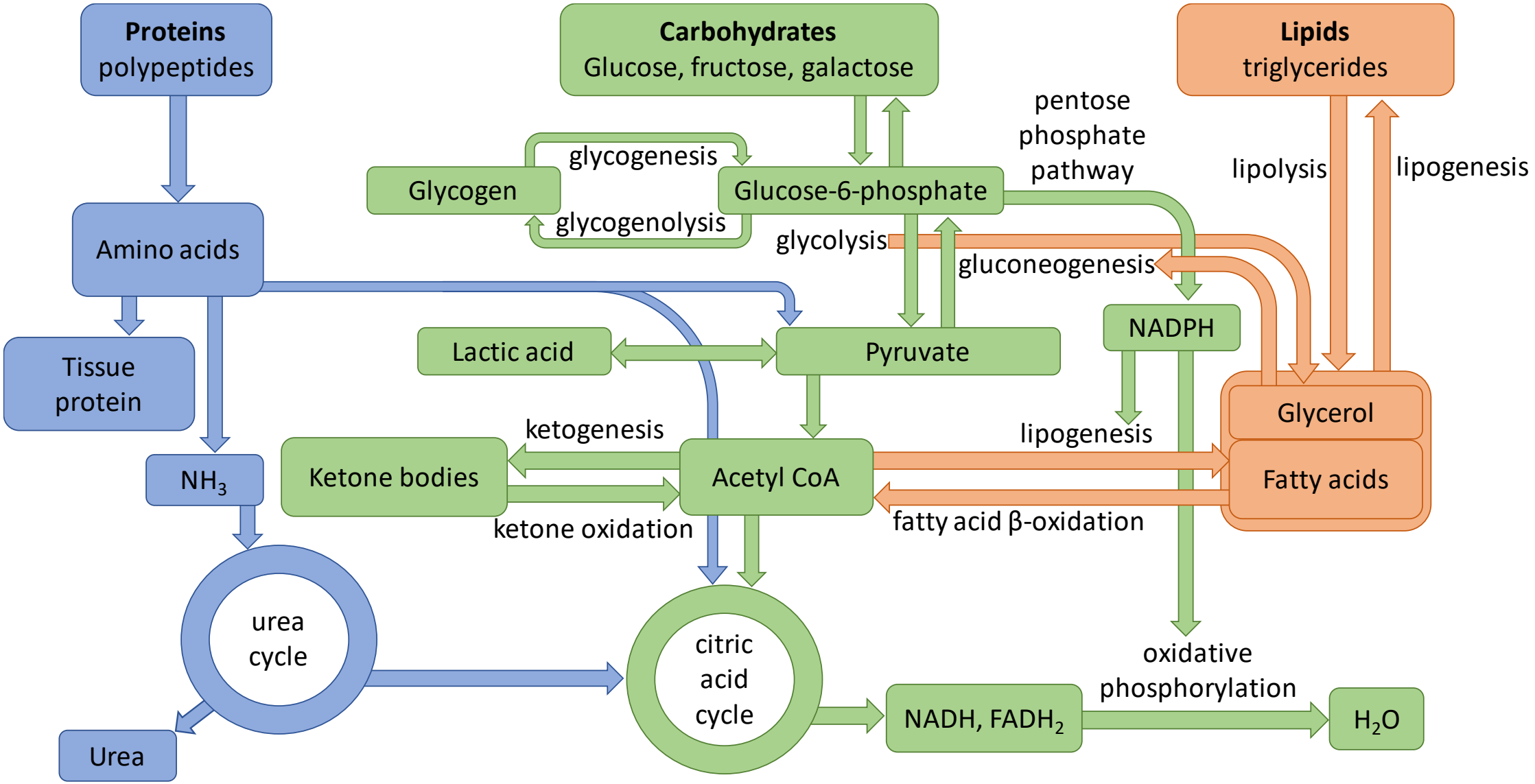


PPAR γ





Protein, carbohydrate, and lipid metabolism



PPARα and PPARγ play major roles in lipid and carbohydrate metabolism pathways

Relevance of PPAR to human health

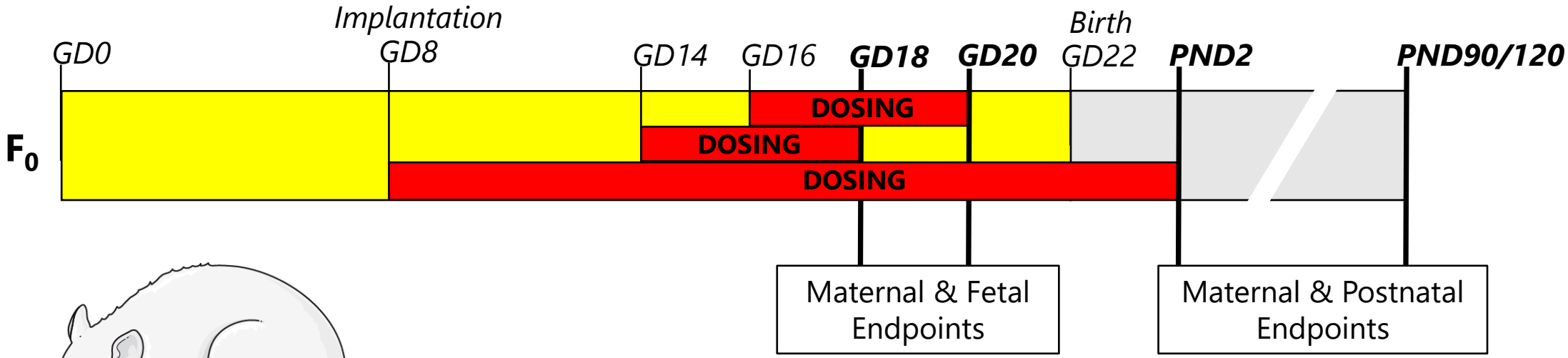
- PPARα-induced rodent hepatocarcinoma described as not relevant to human health (Corton et al. 2018)

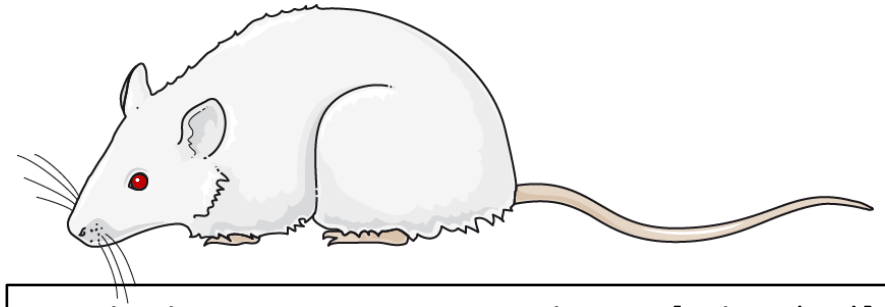
- PPARα is the “master regulator of lipid metabolism” and PPARγ critical for carbohydrate metabolism
  - Multiple pharmaceutical therapeutics target PPARα (fibrates) and PPARγ (thiazolidinediones)
  - Dual receptor therapeutics PPARα+PPARγ (glitazars)
  - Multiple fibrates and thiazolidinediones and all glitazars have failed clinical trials due to toxicity (Hong et al. 2018)

- PPARα well expressed in human liver slices (Janssen et al. 2015) and expression levels similar to mouse (Rakhshandehroo et al. 2009)

- Clearly, PPARα and PPARγ both relevant to human health and alteration of lipid and carbohydrate metabolism conserved across species and critical during pregnancy/gestation

In vivo study designs



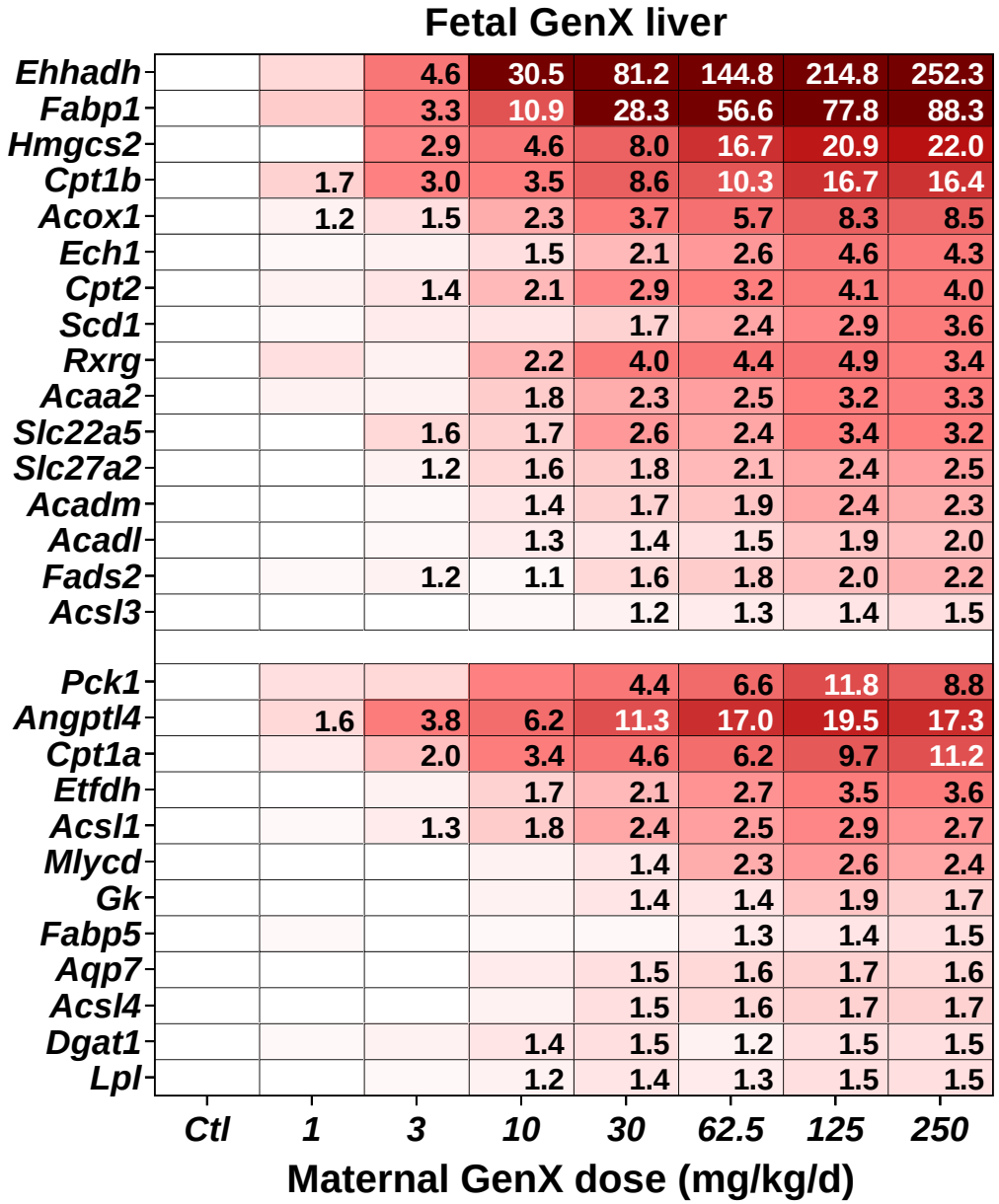


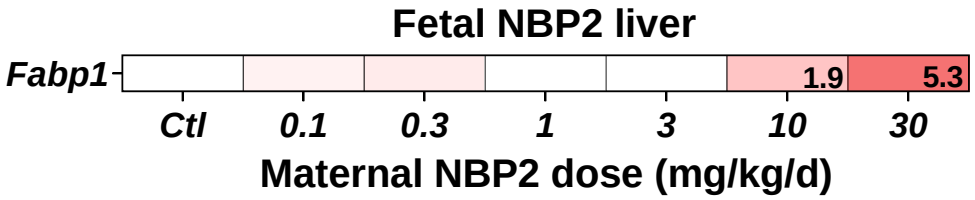
- Charles River Sprague-Dawley rat [Crl:CD(SD)]
- ≥3 dams/litters per dose group
- Oral gavage administration
- Ultra pure water vehicle

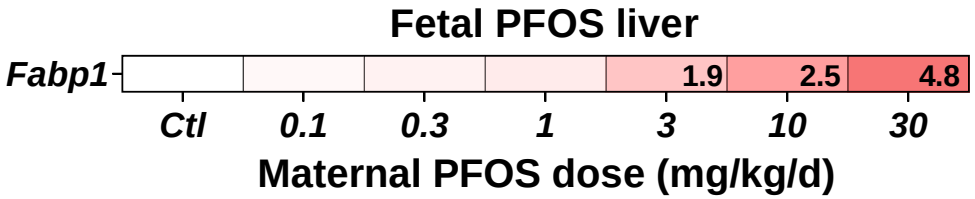
- Body + organ weights
- Serum clinical chemistry
- Tissue-specific gene expression
- Serum & liver PFAS concentration
- Histopathology
- Serum thyroid hormones (T3/T4)

GD=gestation day
PND=postnatal day

Fetal liver PPAR signaling pathway gene expression – GD 14-18

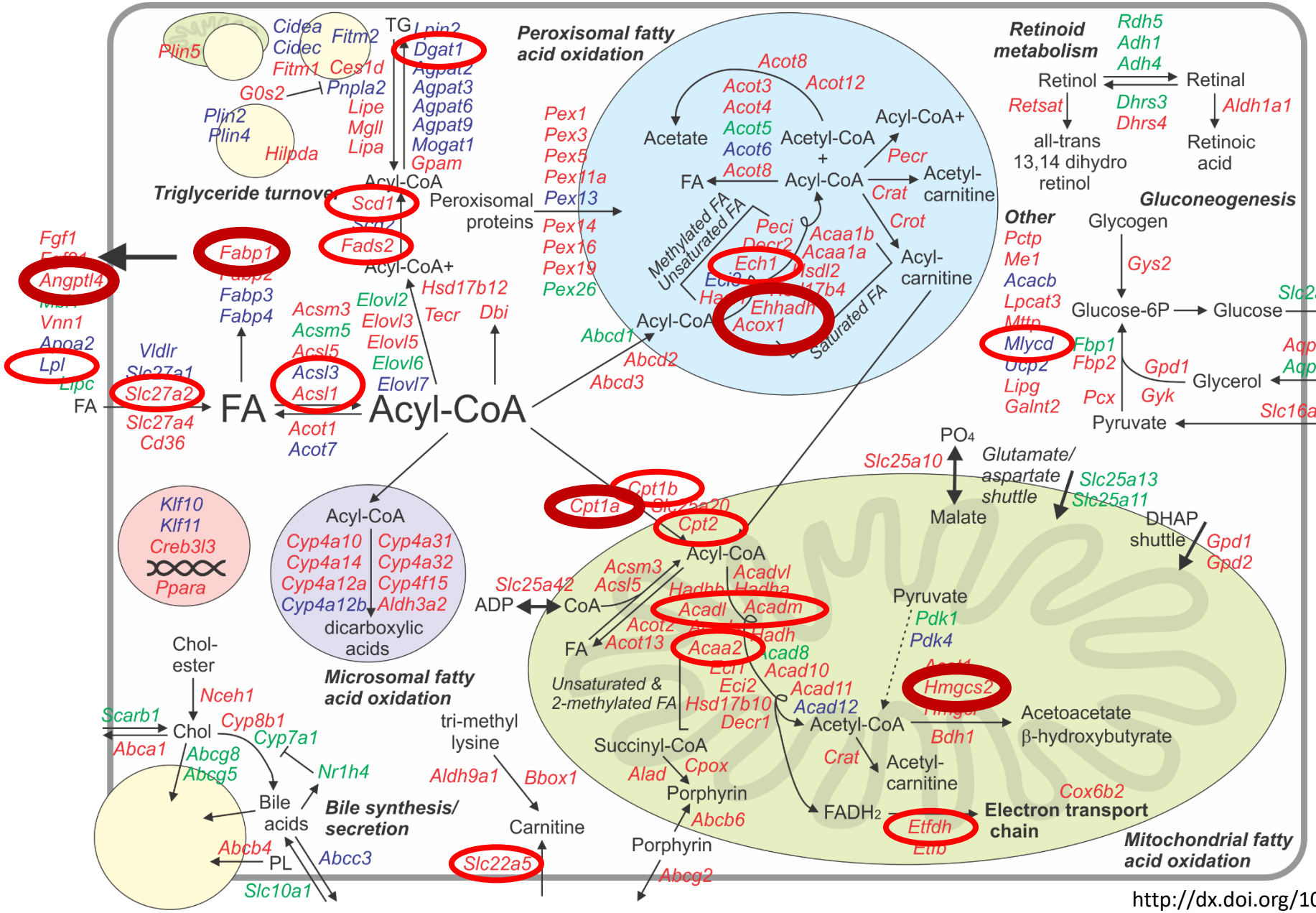






ANOVA p<0.001
Pairwise vs control p<0.01

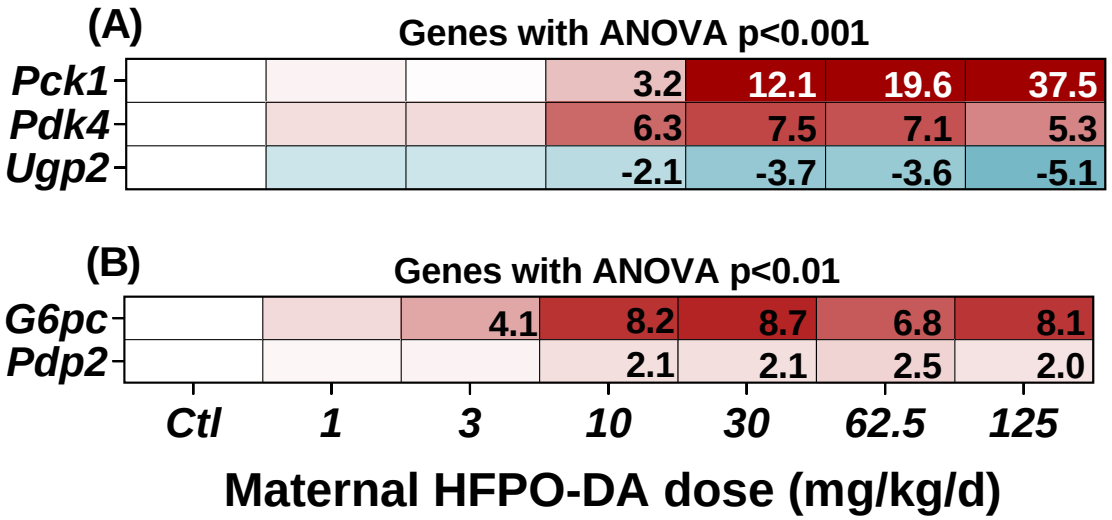
PPAR signaling pathway gene expression – rodent hepatocytes



http://dx.doi.org/10.1016/j.molmet.2014.02.002

Glucose metabolism pathway gene expression – GD16-20 exposure

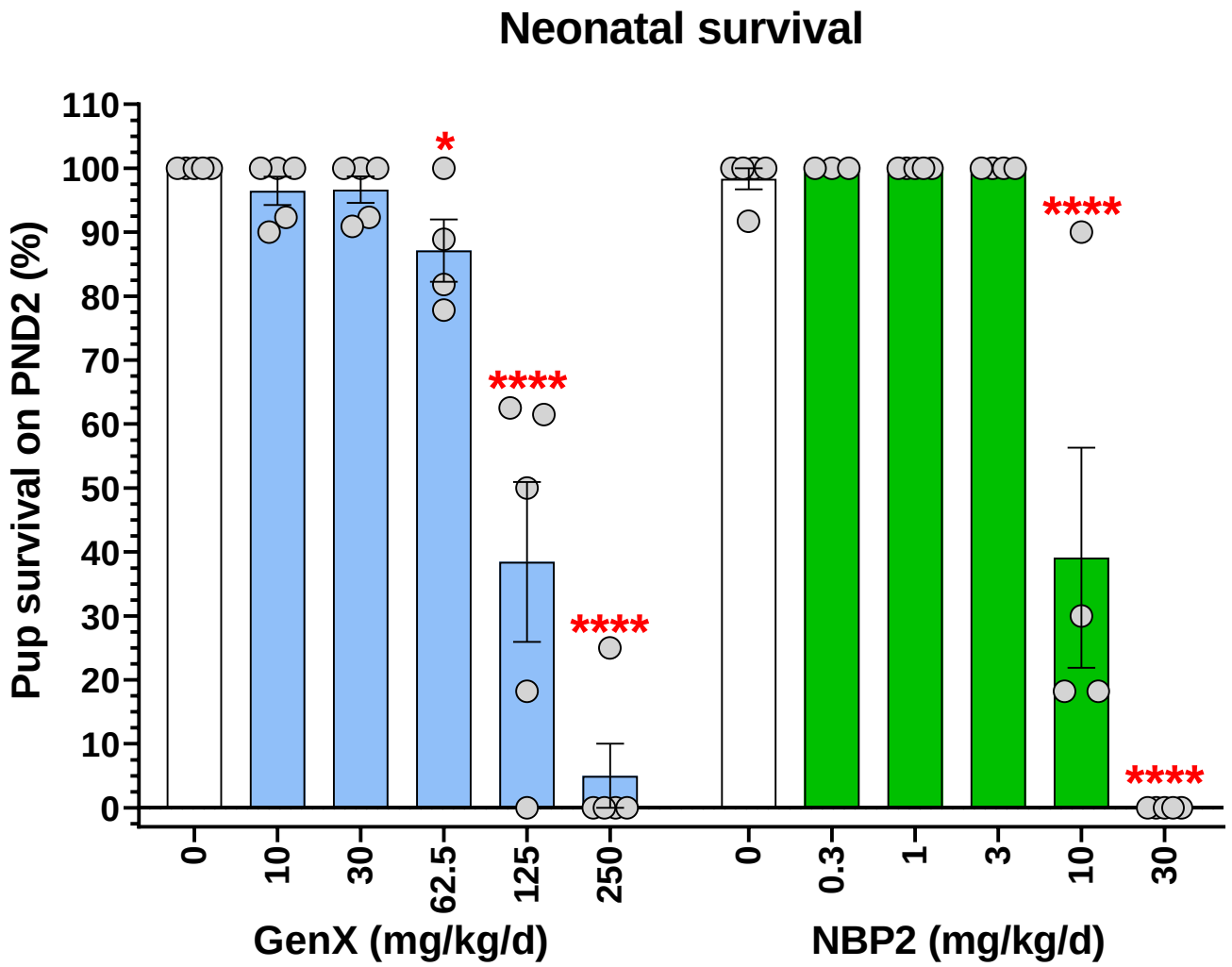
Fetal GenX liver (GD20)



Fetal NBP2 liver (GD20)

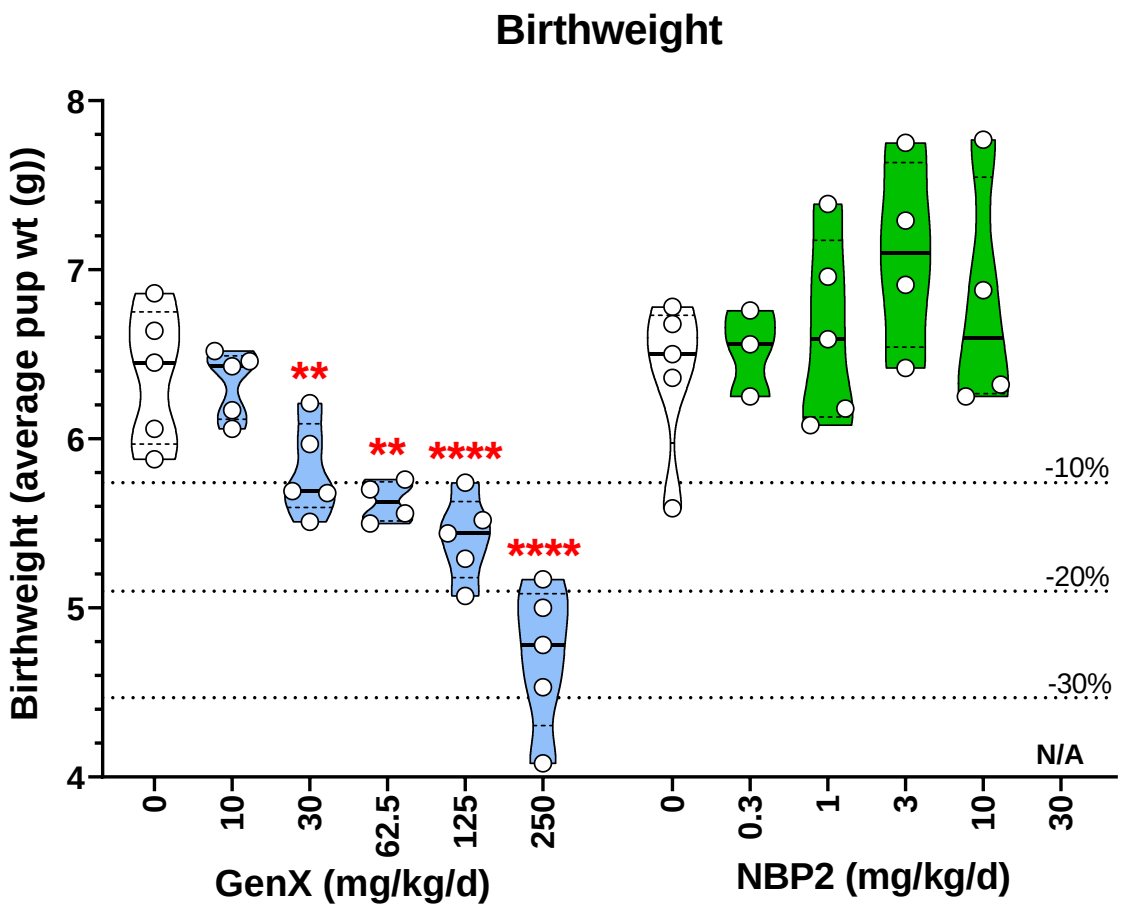
No Glucose Metabolism genes affected

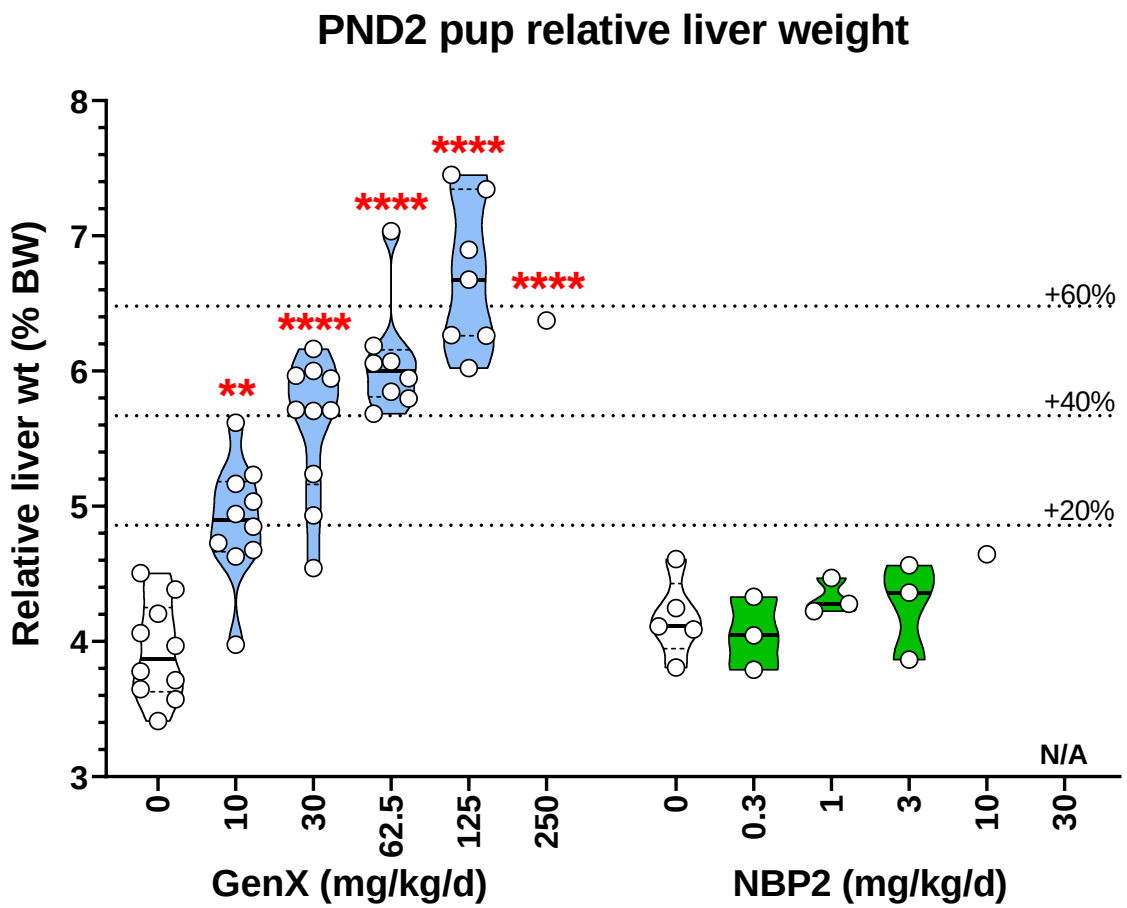
Both GenX and NBP2 produce neonatal mortality, similar to PFOS, PFOA and PFNA



NBP2 ~10-fold more potent than GenX based on oral maternal dose

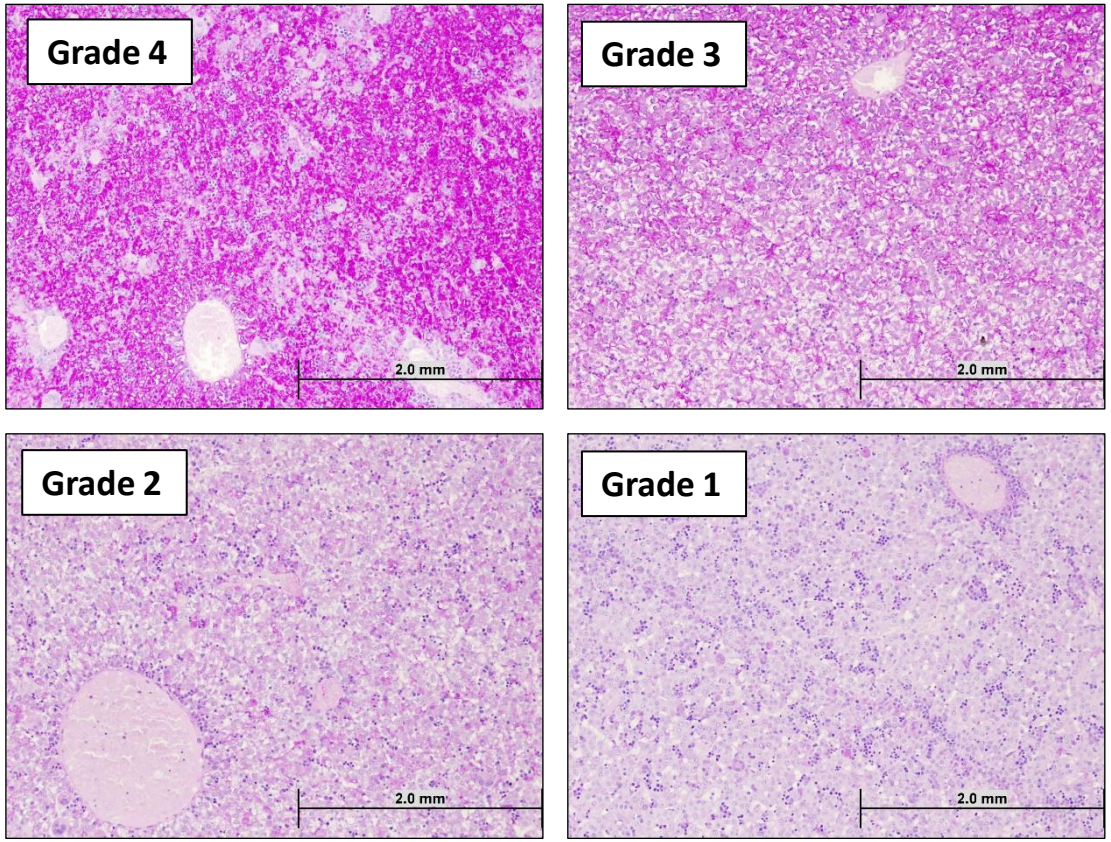
Neonatal effects on birthweight and liver weight differ between GenX and NBP2

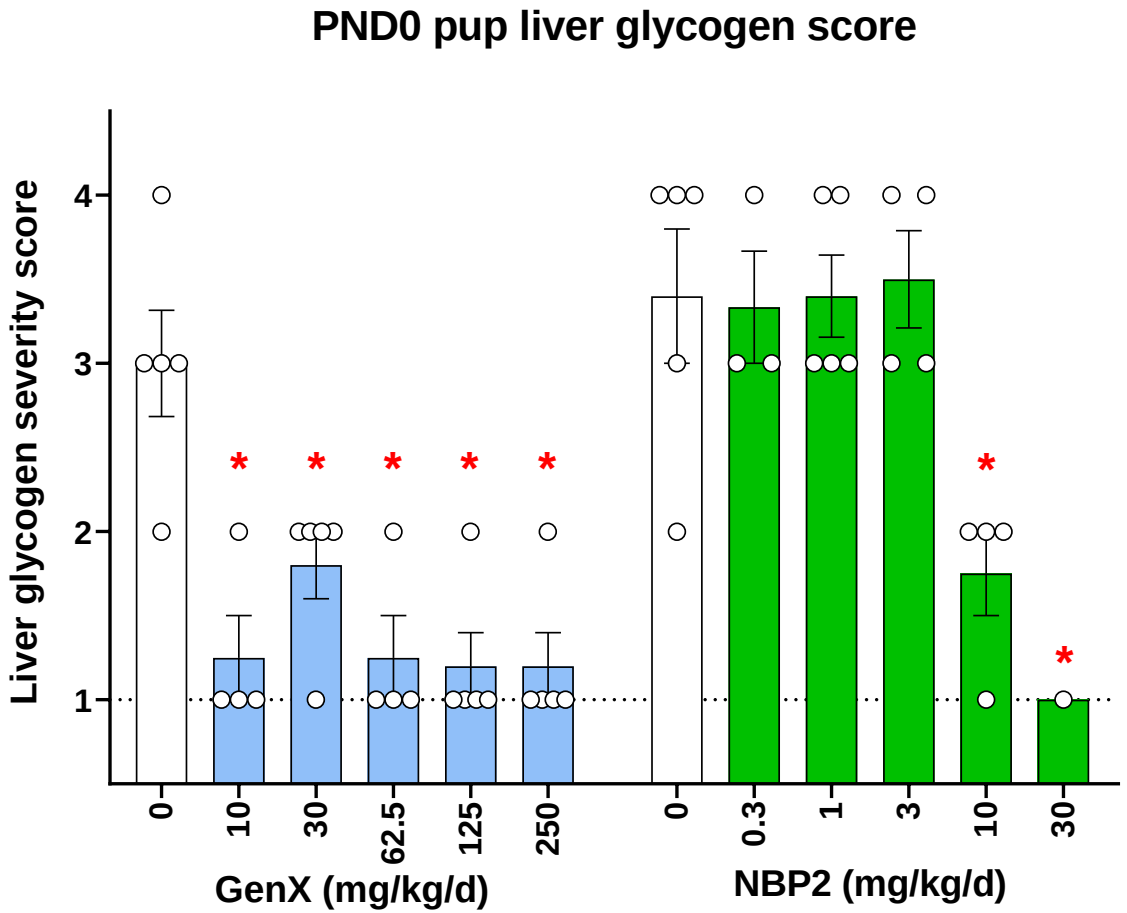




There are notable similarities and differences in GenX vs NBP2 developmental toxicity, likely associated with different chemical functional groups (carboxylate vs sulfonate) and toxicokinetics

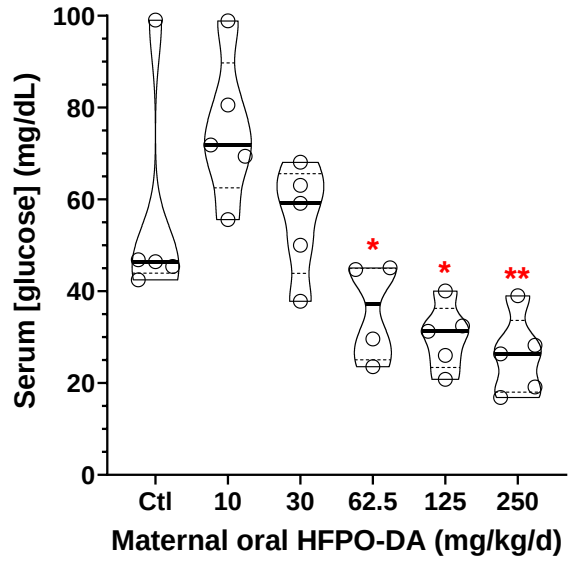
Histopathological neonatal liver glycogen deficit a common key event for GenX and NBP2

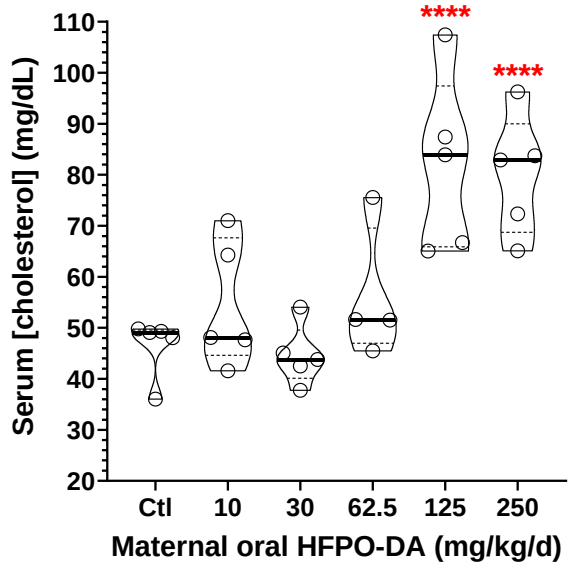


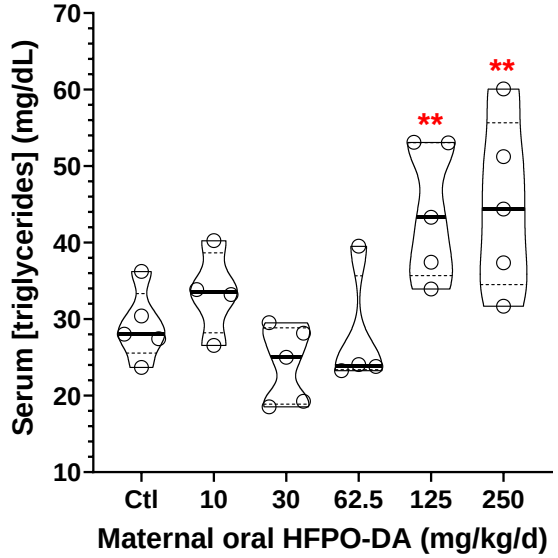


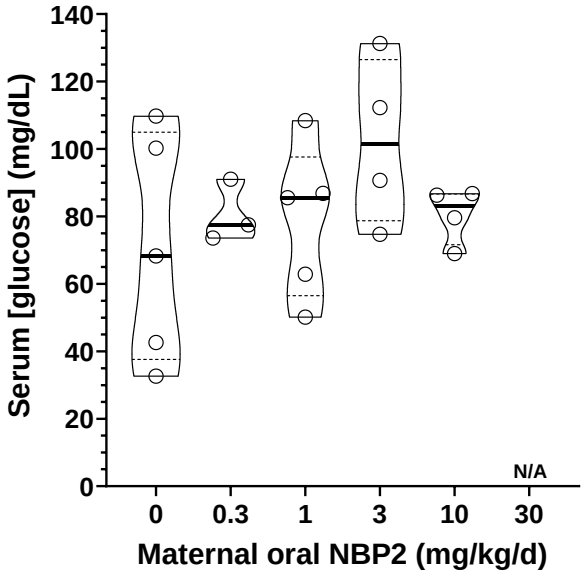
Liver carbohydrate and lipid metabolism are strongly affected by GenX but seemingly less so by NBP2

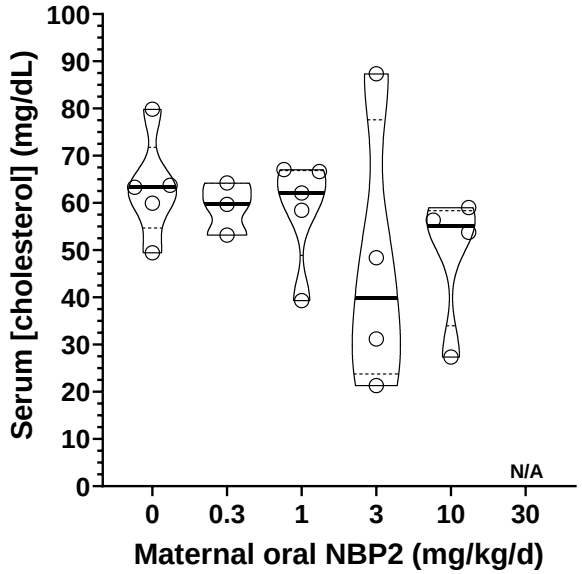
Newborn pup clinical chemistry more affected by GenX than NBP2

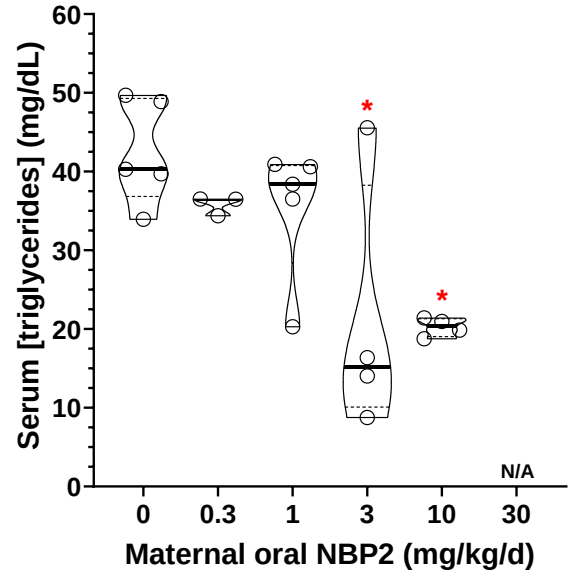








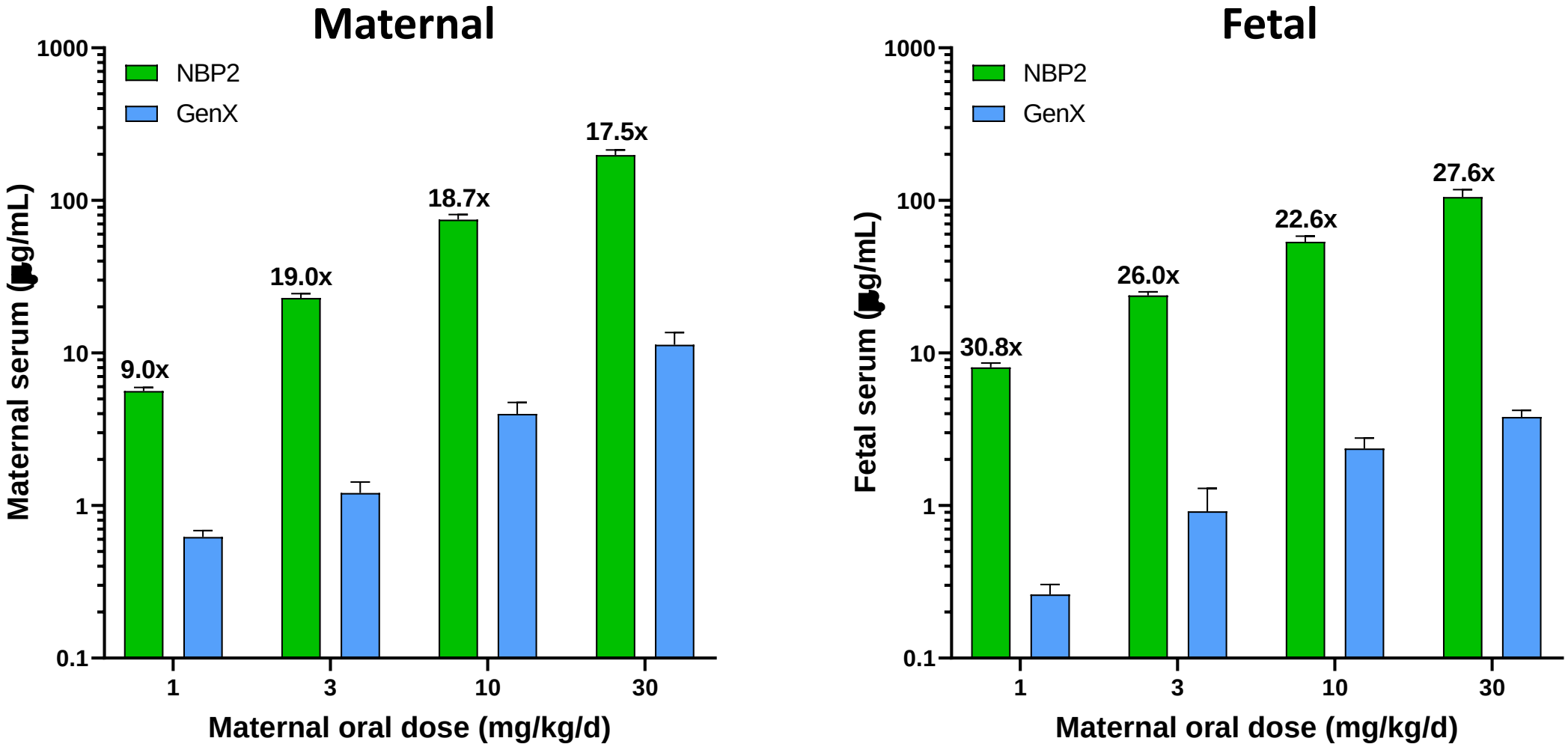




Altered gestational glucose homeostasis

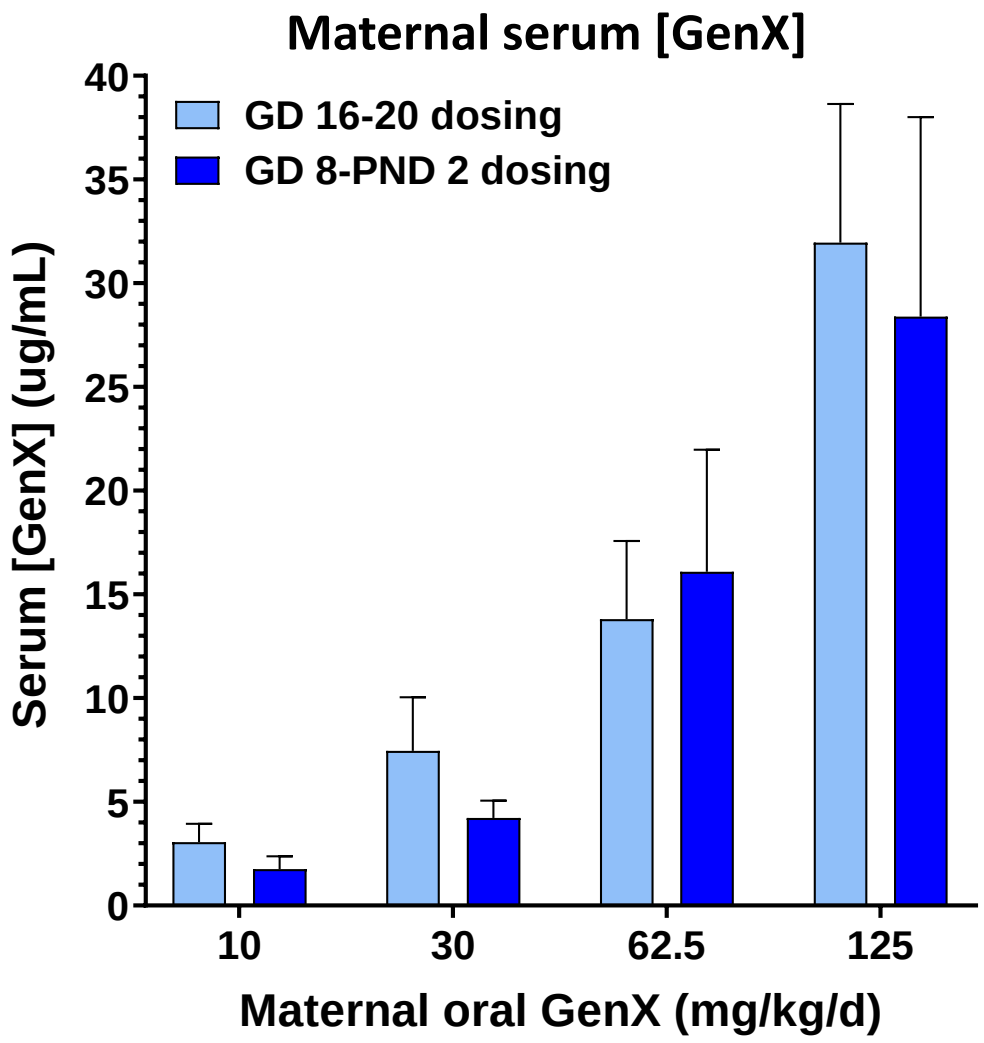
- Fetal nutrition dependent on maternal supply and placental transfer
- Maternal dietary glucose restriction produces low birthweight and neonatal mortality in the rat (*Lanoue et al. 1999*)
- Indicators of Glycogen Storage Disease = ↓ birthweight, ↓ serum glucose, ↑ liver weight, ↑ serum lipids (*Kishnani et al. 2014*)
- Placental Insufficiency and Intrauterine Growth Restriction (*Brown et al. 2015*)
  - ↑ fetal liver *Pck1* expression associated with compensatory gluconeogenesis
  - ↑ fetal liver *Pdk4* expression associated with decreased glucose oxidation
  - ↓ fetal liver *Ugp2* expression associated with decreased glycogen synthesis
- Critical period of maternal dietary glucose restriction similar to critical period for PFOS effects – Gestation Days 19-20 (*Koski et al. 1990; Grasty et al. 2003*)

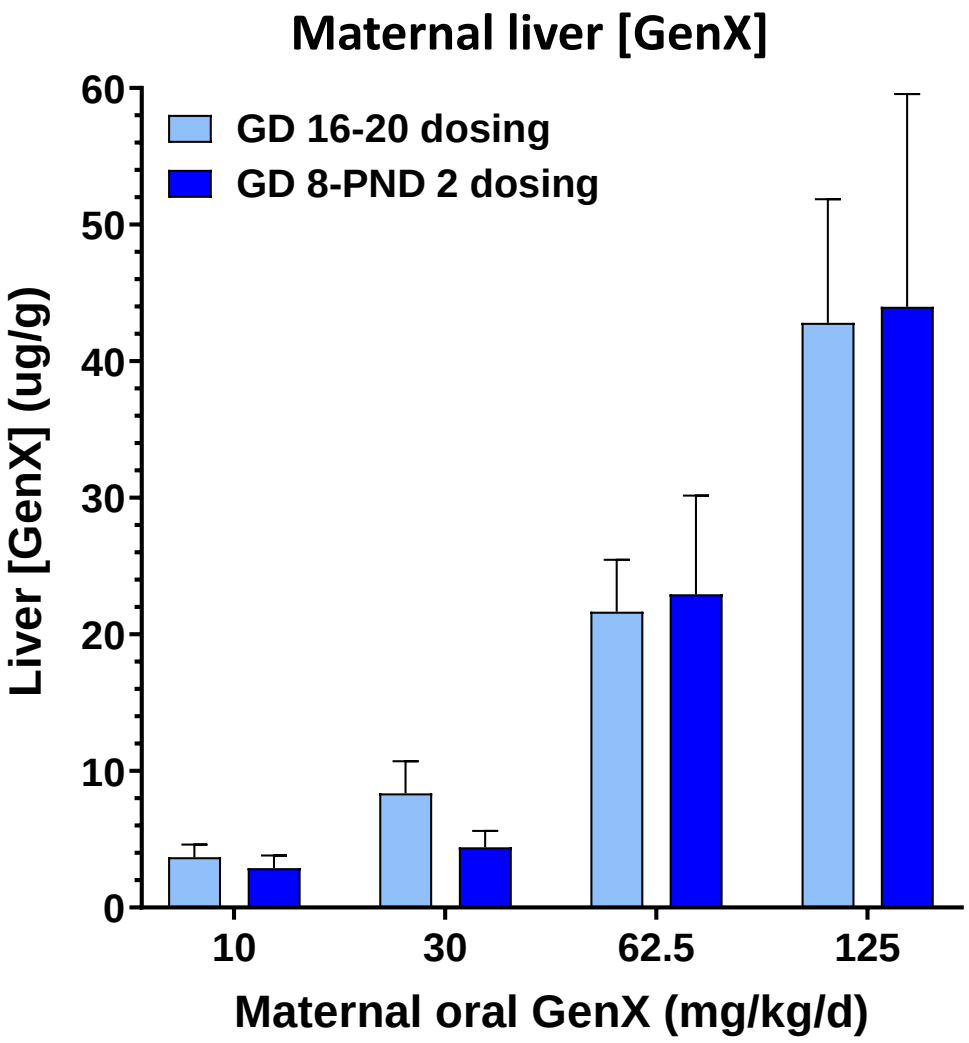
Serum concentrations – GD 16-20 exposure



NBP2 maternal and fetal serum concentrations ~10-30-fold greater than GenX at similar maternal dose

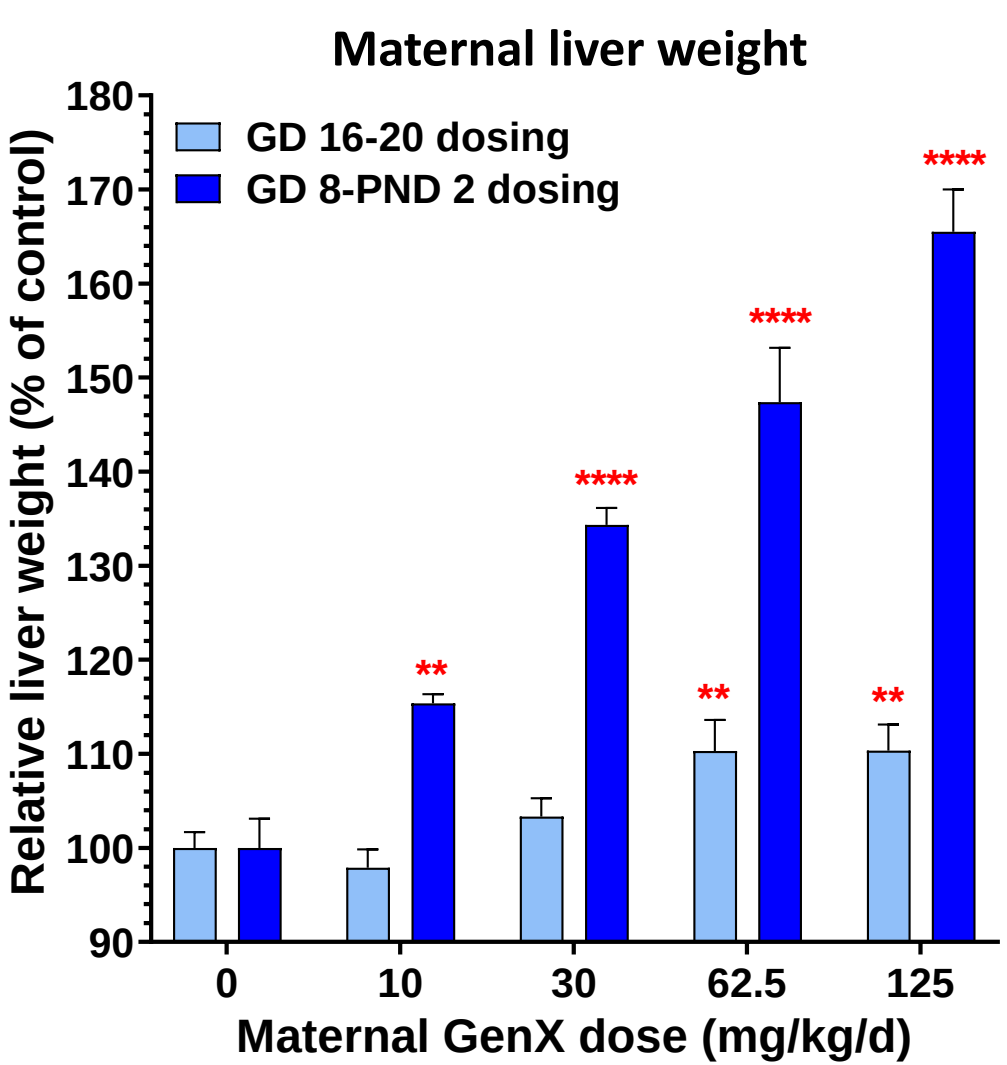
Maternal liver and serum GenX from two dosing intervals

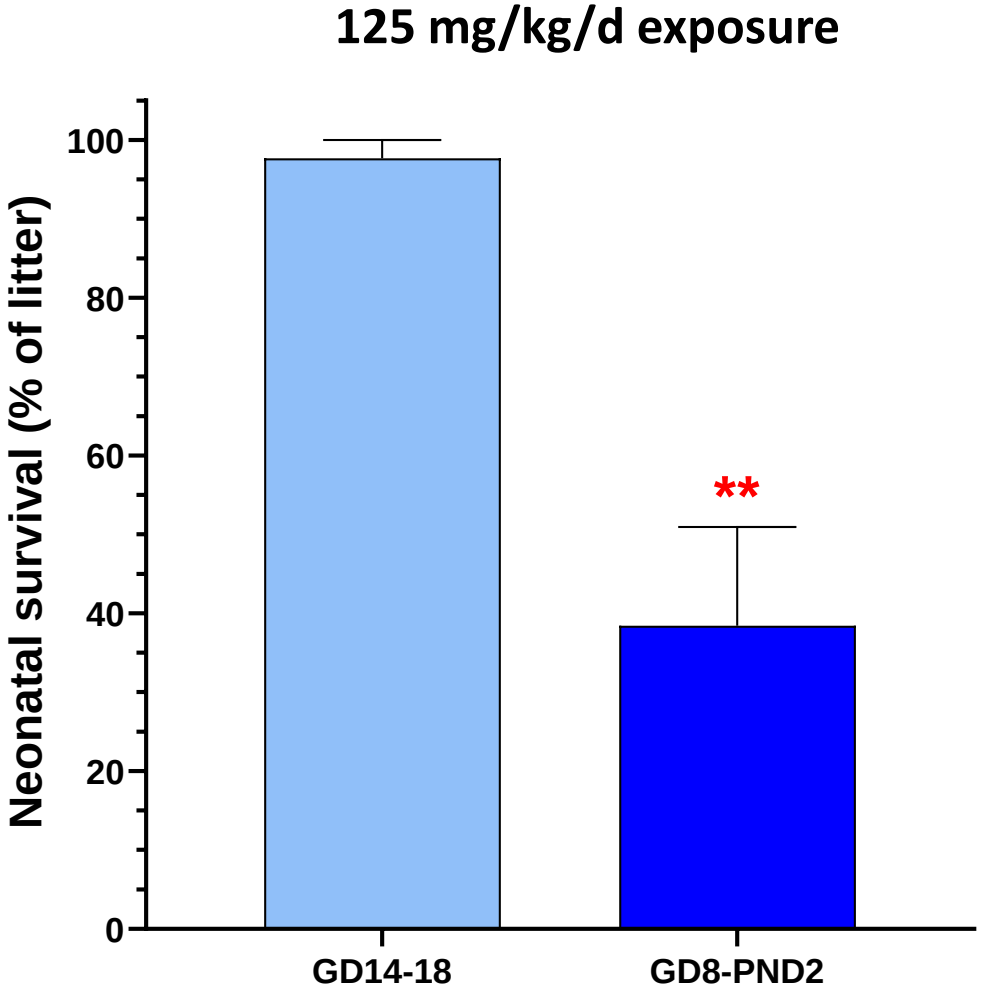




GenX does not accumulate in maternal rat serum or liver from longer exposure

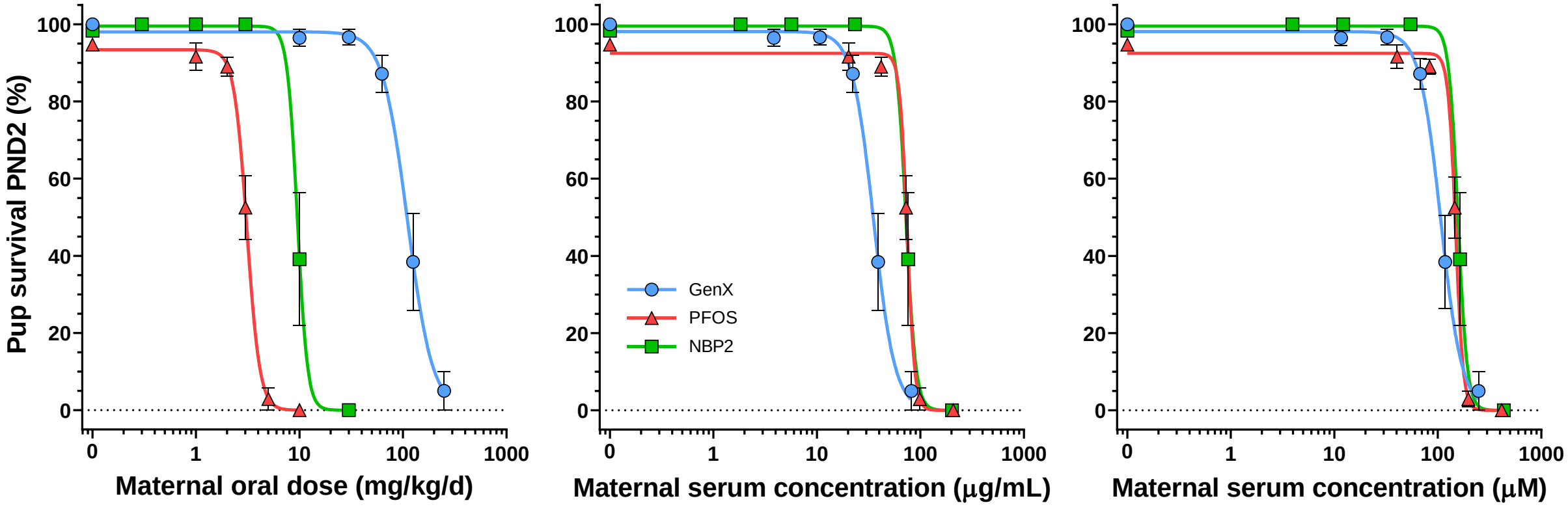
GenX maternal liver weight and neonatal mortality from two dosing intervals





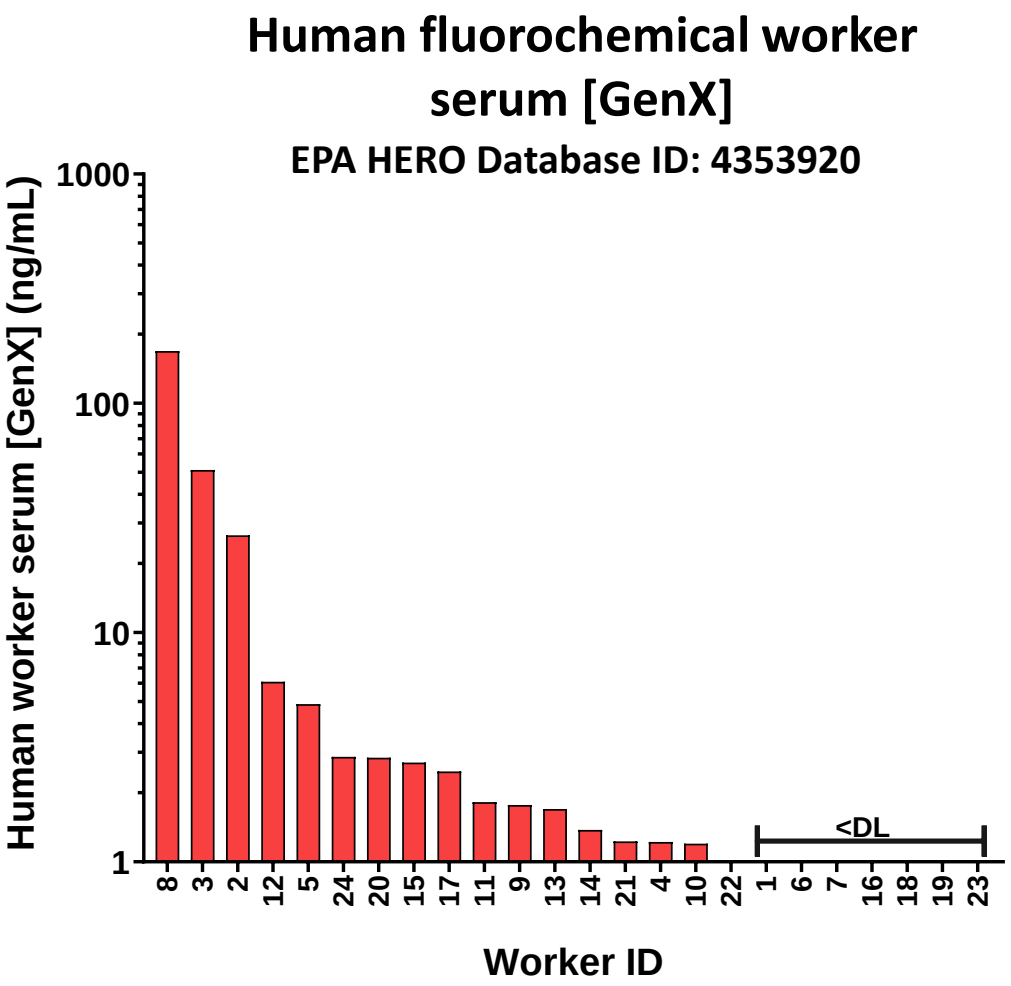
Despite no accumulation, GenX effects more severe from longer exposure interval

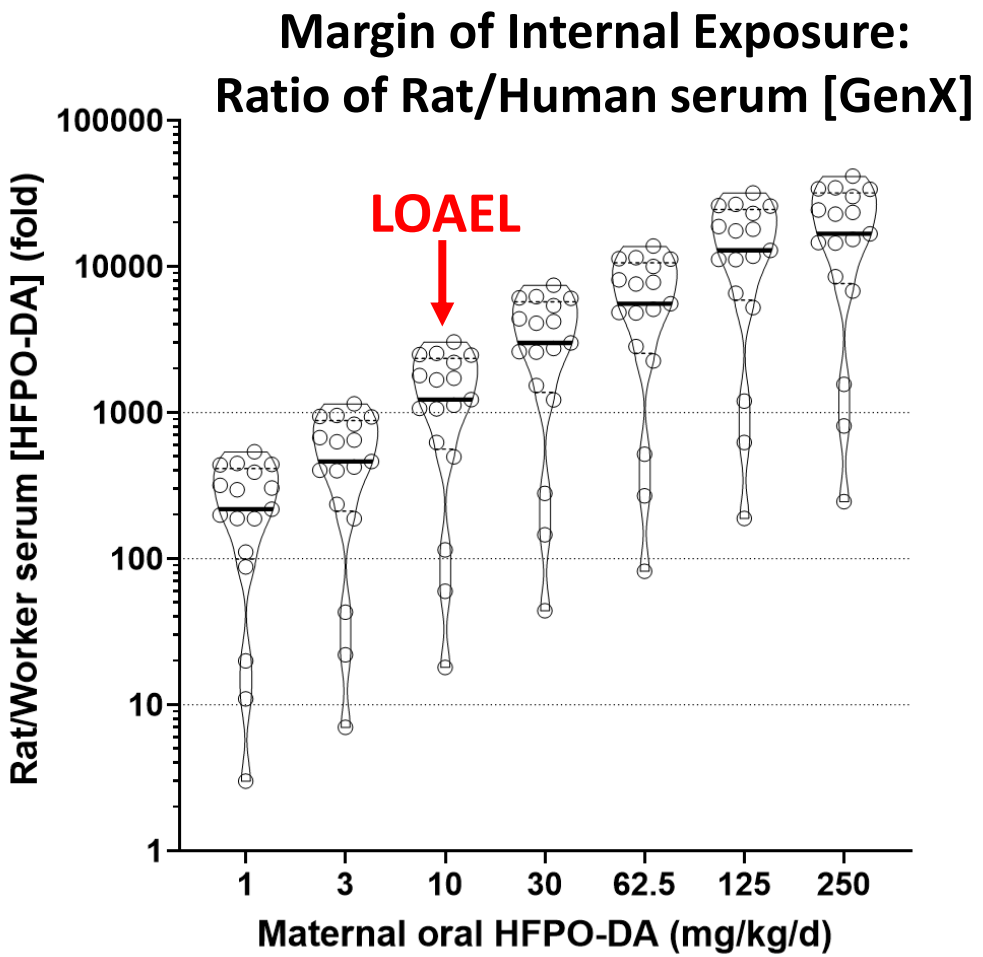
Potency comparisons across GenX, NBP2, and PFOS



	Oral dose ED50 (mg/kg)	Serum ED50 (µg/mL)	Serum ED50 (µM)
GenX	110	35	107
NBP2	9	72	157
PFOS	3	74	149

Maternal rat GenX blood levels compared to human fluorochemical workers in the Netherlands

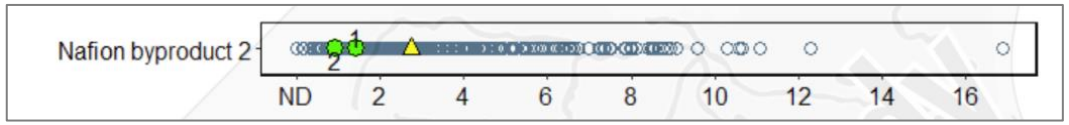




~30% of human fluorochemical worker GenX blood concentrations were within a factor of 1000 of maternal rat blood concentrations at lowest observed adverse effect level (LOAEL)

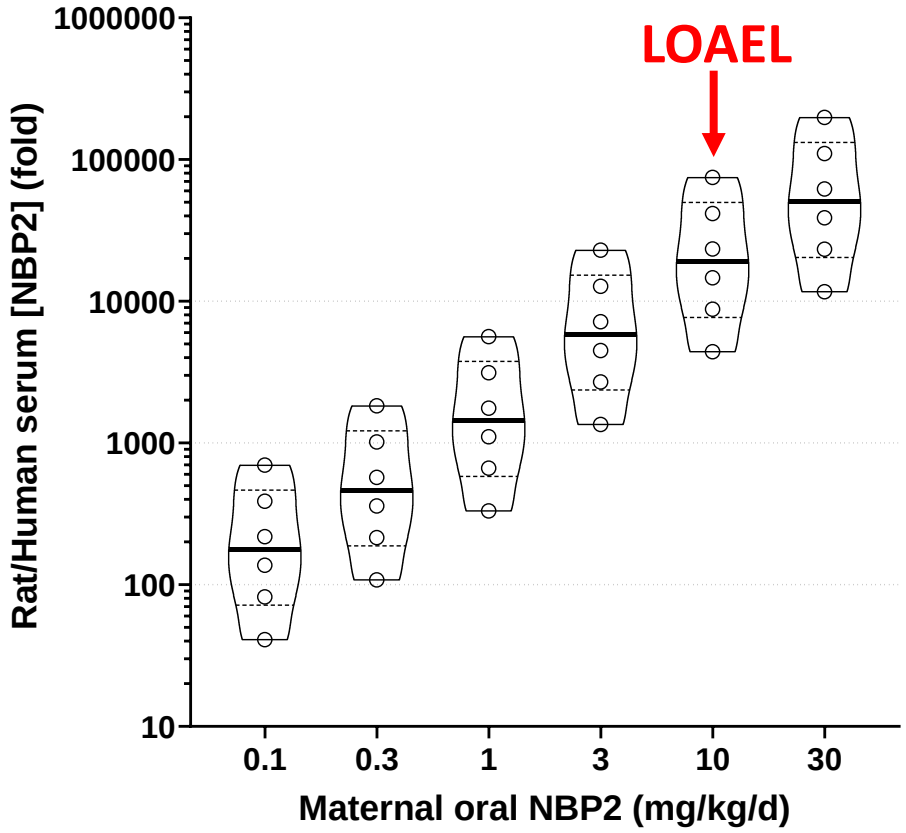
Comparison of NBP2 serum levels – rat to human

Human serum [NBP2]
NC State "GenX Exposure Study"
genxstudy.ncsu.edu
Kotlarz et al. (2020) doi: 10.1289/EHP6837



- Max: 17 ng/mL
- 95th : 8.5 ng/mL
- 75th : 5.1 ng/mL
- 50th : 3.2 ng/mL
- 25th : 1.8 ng/mL
- 10th : 1.0 ng/mL

Margin of Internal Exposure:
Ratio of Rat/Human serum [NBP2]



≥95th centile of human NBP2 blood concentrations were within a factor of 10000 of maternal rat blood concentrations at lowest observed adverse effect level (LOAEL)

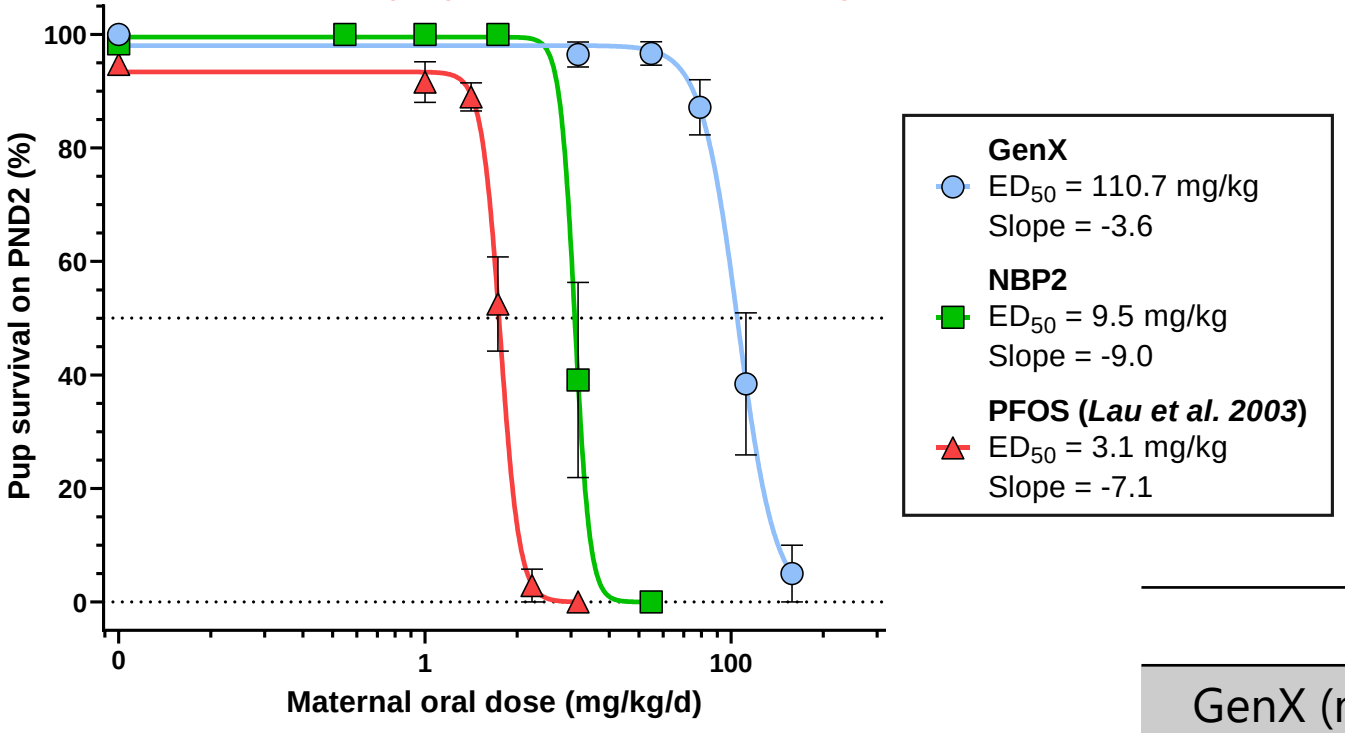
PFAS co-exposures in pregnant women

- ***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
- ***Kashino et al. 2020*** – Hokkaido, Japan prospective birth cohort 2003-2009 (n=1985)
  - >99% with detectable PFOS, PFOA, PFNA, PFDA, PFUnDA

Critical to study mixture-based effects of exposure to multiple PFAS compounds

Mixture study with GenX, NBP2 and PFOS

Individual chemical dose response data used to design equipotent mixture experiment

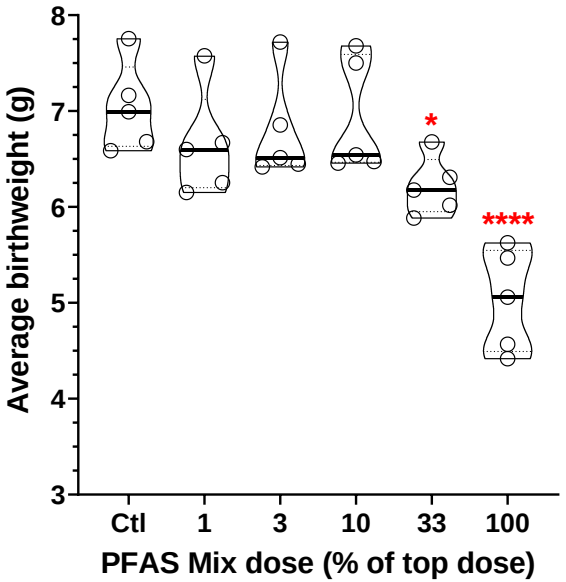


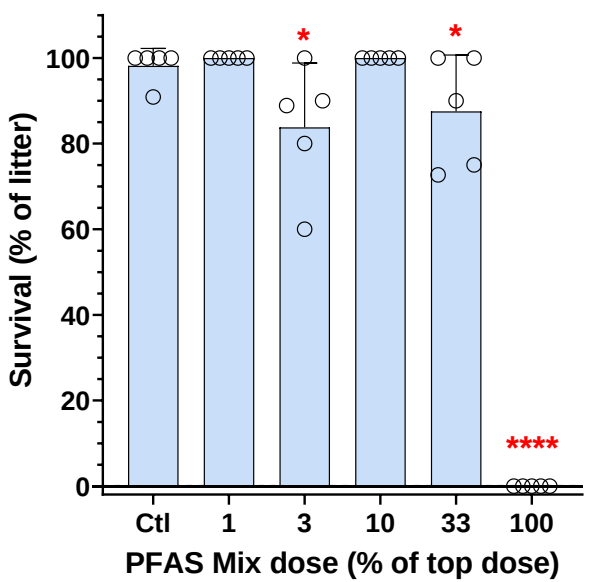
Actual mixture doses for in vivo study

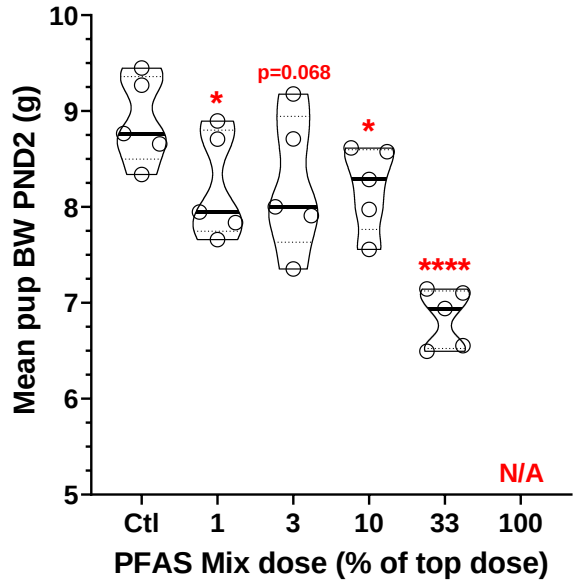
	100%	33%	10%	3.3%	1%
GenX (mg/kg)	110	36.7	11	3.67	1.1
NBP2 (mg/kg)	10	3.3	1	0.3	0.1
PFOS (mg/kg)	3	1	0.3	0.1	0.03

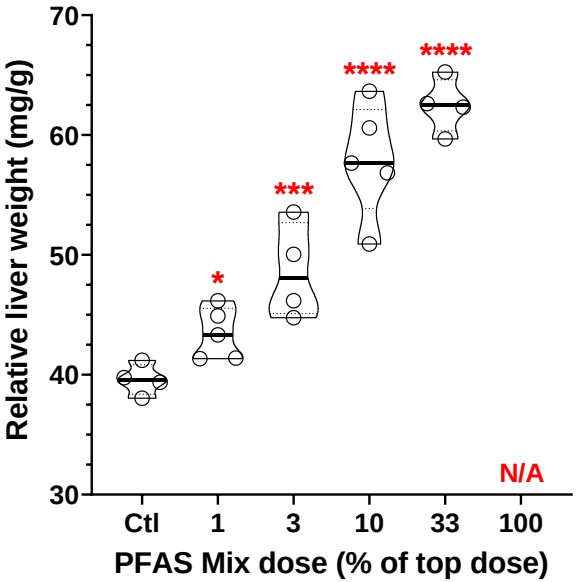
Top dose = each chemical at ED_{50}

Mixture study with GenX + NBP2 + PFOS



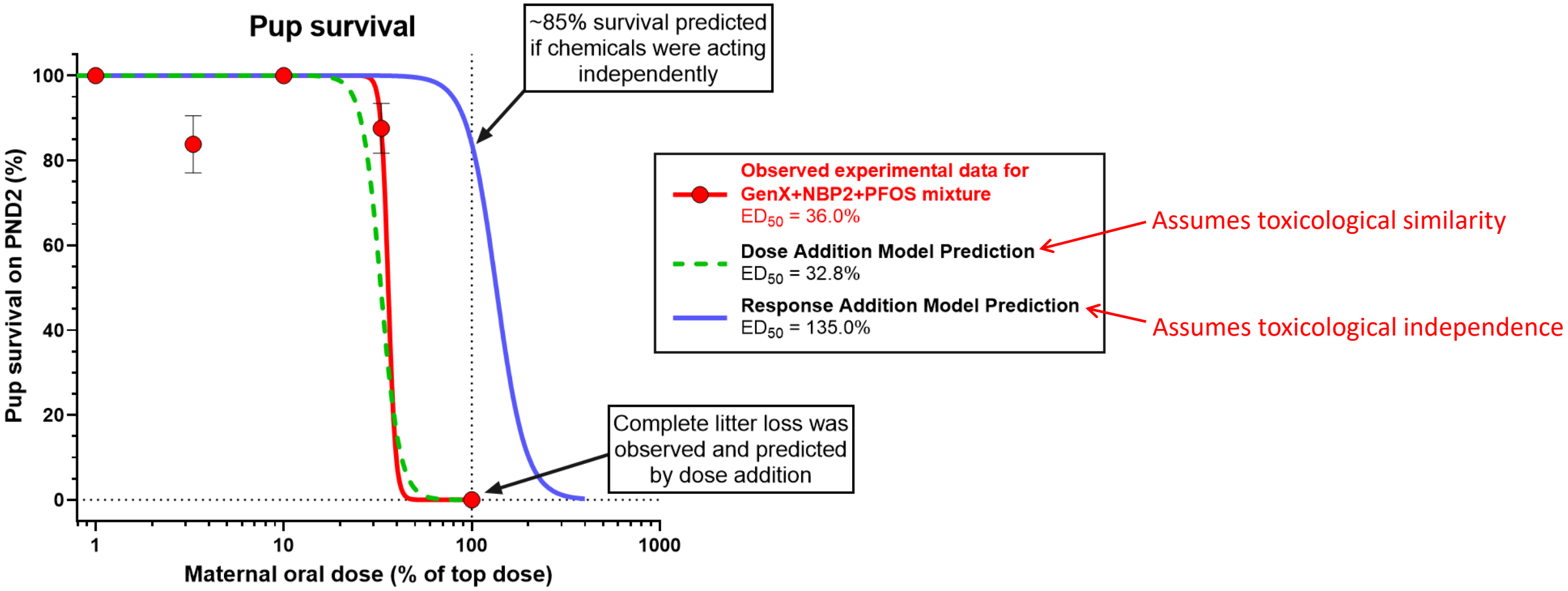






Based on individual study data, body weight and liver weight effects appear driven mostly by GenX

Experimental data compared to mixture model predictions



Based on traditional mixture models, neonatal mortality is dose additive indicating chemicals produced a cumulative effect of co-exposure

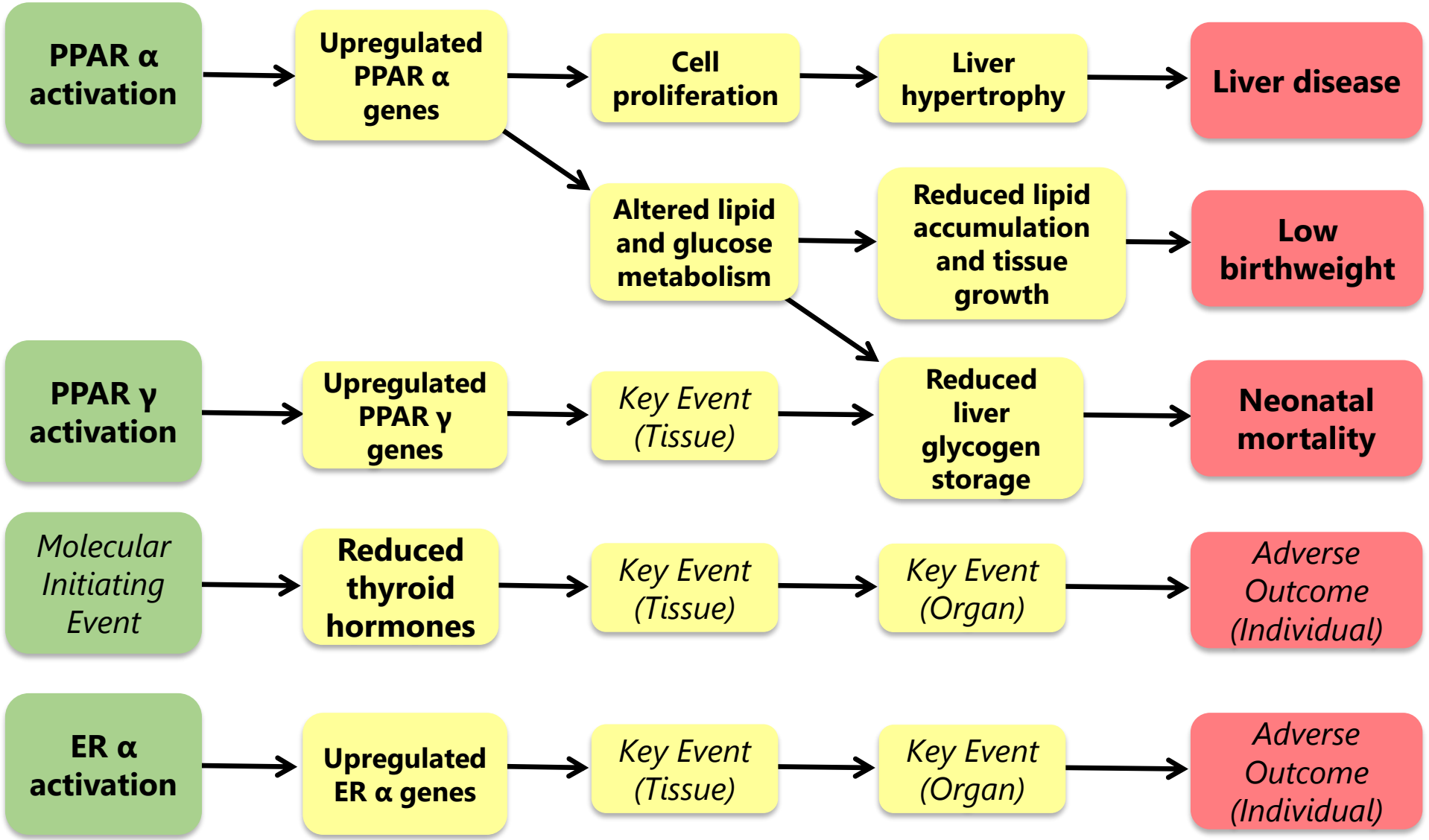
Publications and Ongoing Research

- Two publications on GenX developmental toxicity:
  - Conley et al. (2019) Adverse maternal, fetal, and postnatal effects of hexafluoropropylene oxide dimer acid (GenX) in the Sprague-Dawley rat. *Environmental Health Perspectives* DOI:10.1289/EHP4372.
  - Conley et al. (2021) Hexafluoropropylene oxide-dimer acid (HFPO-DA or GenX) alters maternal and fetal glucose and lipid metabolism and produces neonatal mortality, low birthweight, and hepatomegaly in the Sprague-Dawley rat. *Environment International* DOI: 10.1016/j.envint.2020.106204
- Publications in preparation:
  - Developmental toxicity of Nafion byproduct 2
  - In vitro PPAR alpha/beta/gamma activation by PFAS and fatty acids
- Ongoing research:
  - Developmental toxicity of a mixture of PFOS, GenX and NBP2

Key Findings and Impact

- GenX and NBP2 produced adverse maternal and neonatal effects but with disparate patterns and oral doses generally consistent with those reported for PFOA and PFOS, respectively
- Published GenX data (and future NBP2 data) are useful for state and federal risk assessments and regulatory efforts to characterize hazard and identify points-of-departure
- Mixture effects of GenX+NBP2+PFOS on neonatal mortality are dose additive and support a cumulative risk assessment approach for exposure to multiple PFAS
- Internal dosimetry is important for estimating potency across PFAS and relevance to human exposures
- Development of a short-term (~5-day exposure) *in vivo* developmental toxicity assay that is quantitatively predictive of effects from longer-term studies would allow for rapid generation of risk assessment relevant data on data-poor PFAS

PFAS developmental toxicity AOPs



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