Toxicology of Perfluoroalkyl Acids

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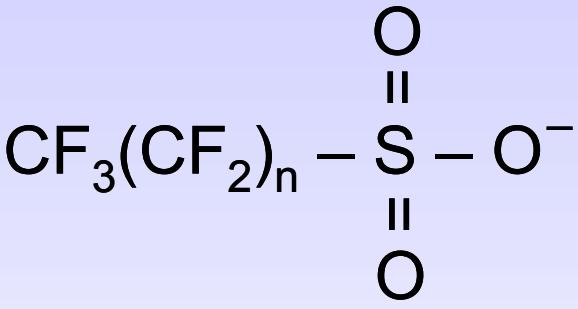
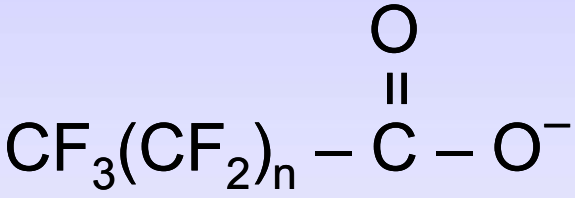
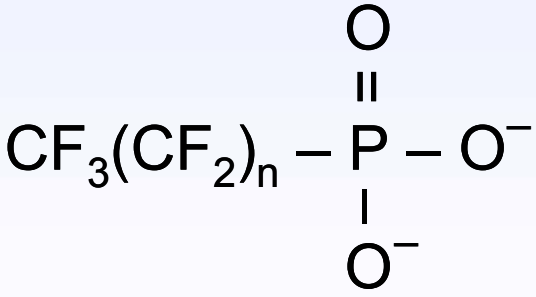
Perfluoroalkyl Acids (PFAAs)



Perfluoroalkyl sulfonic acid
(PFSA)



Perfluoroalkyl carboxylic acid
(PFCA)



Perfluoroalkyl phosphonic acid
(PFPA)

What are PFAAs?

- Stable, synthetic chemicals, produced last ~50-60 years
- Their hydrophobic and oleophobic properties make them ideal surfactants (water and oil resistant).
- The most useful PFAAs are the 8-carbon (C8) chemicals:
  Perfluorooctane Sulfonate (PFOS)
  Perfluorooctanoic Acid (PFOA)
- PFOS, PFOA (Telomer Alcohols) and their derivatives have over 200 industrial and consumer applications:

Fabric coatings
Carpet coatings
Paper coatings
Floor polish/wax
Alkaline cleaners
Denture cleaners
Shampoos
Insecticides (ant/roach)

Fire-fighting foam
Airplane gear lubricant
Mining/oil well surfactants
Acid rust/dust suppressants
Metal electroplating
Electronic etching bath
Polymer additives
Emulsifiers for polymer production

PFAAs Commonly Found in the Environment

- Perfluorooctane Sulfonate (PFOS, C8)
- Perfluorooctanoic Acid (PFOA, C8)
- Perfluorononanoic Acid (PFNA, C9)
- Perfluorohexane Sulfonate (PFHxS, C6)
- Perfluorohexanoic Acid (PFHxA, C6)
- Perfluorobutane Sulfonate (PFBS, C4)
- Perfluorobutyric Acid (PFBA, C4)
- Perfluorodecanoic Acid (PFDA, C10)
- *Perfluorophosphonic Acids (C6, C8, C10)*

Why do we care?

- They are everywhere and environmentally persistent
  - globally distributed, detected in water, air, soil, sediment and sludge
- They are present in humans and wildlife

(ppb)	PFOS	PFOA	PFHxS	PFNA
NHANES 99-00	**30.4**	**5.2**	**2.1**	**0.5**
NHANES 03-04	**20.7**	**3.9**	**1.9**	**1.0**
NHANES 05-06	**15.5**	**3.5**	**1.6**	**1.0**
Lake trout	121	4.4	0.6	2.9
Polar bear	~1,200	~10	--	~100

- They hang around

Serum $t_{½}$	PFBS	PFHxS	PFOS	PFBA	PFOA
Human	**10-20 d**	**8.7 yrs**	**5.4 yrs**	**2-4 d**	**2.3-3.8 yrs**

- They *may* be harmful (based on animal studies)
  - hepatotoxicity, carcinogenicity, immunotoxicity, hormonal imbalance, neurotoxicity, developmental toxicity

General Properties of PFAAs

- Hydrophobic and lipophobic
- Well absorbed orally (> 95% within 24 h)
- Distributed mainly in serum, liver and kidney (lung)
- Highly bound to proteins
- Not metabolized
- Elimination dependent on carbon-chain length (poor with long carbon-chains): urinary and fecal excretion
- Body burden increases linearly with cumulative doses
- Steep dose-response relationship

Hepatotoxicity

- Produce hepatocellular hypertrophy associated with vacuole formation and peroxisome proliferation
- Induce lipid metabolism and alter lipid transport
- Down-regulate cholesterol and bile acid synthesis
- Alter steroid and lipoprotein metabolism
- Actions largely mediated by PPARα molecular signals (PFNA > PFOA > PFOS), but other nuclear receptors such as CAR, PXR, LXR may be involved
- Interfere with cell-cell communication

Gene signatures of PFAAs in mouse liver: PPARα

	PFOA	PFOS
➢Peroxisome biogenesis	+++	+++
➢Xenobiotic metabolism	++	+
➢Acute phase response	++	
➢Proteasome activation	++	
➢Cholesterol biosynthesis	++	
➢Phospholipid metabolism	++	+
➢Bile acid biosynthesis	++	+
➢Glucose metabolism	++	+
➢Lipid metabolism and transport	+++	+++

Rosen et al., 2008 (Tox. Path.); 2009 (Reprod. Tox.)

Comparison of PFAA Activities on PPARα

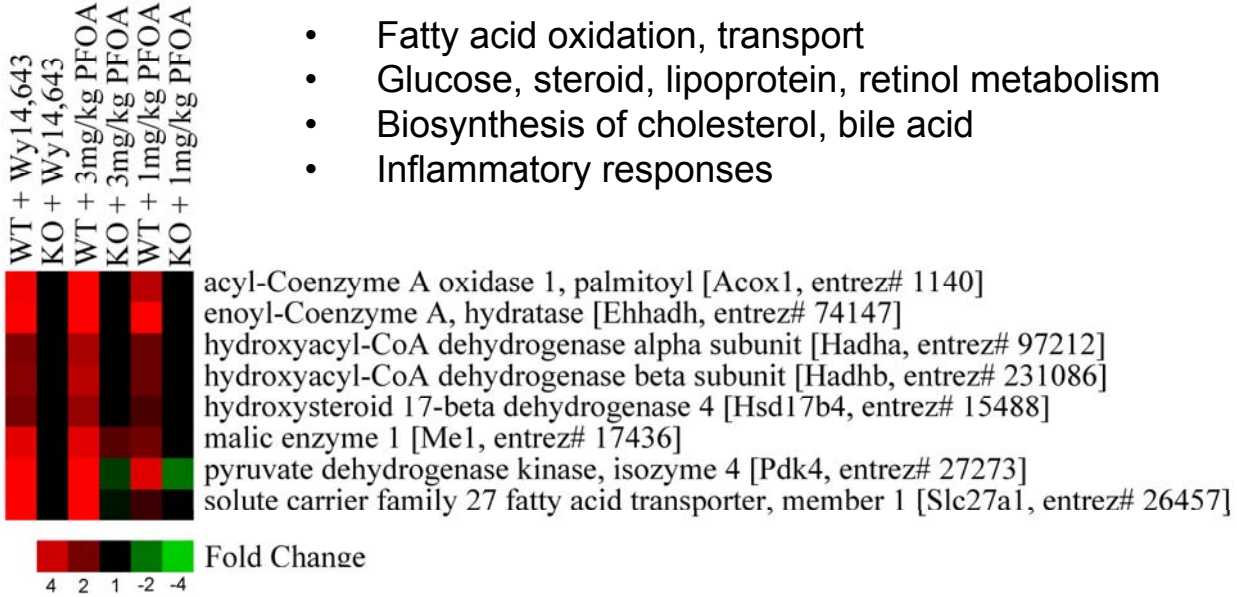
	C_{20max} (μM)	
Compound	Mouse	Human
PFNA (C9)	5	11
PFOA (C8)	6	16
PFDA (C10)	20	no activity
PFHxA (C6)	38	47
PFBA (C4)	51	75
PFHxS (C6)	76	81
PFOS (C8)	94	262
PFBS (C4)	317	206

Wolf et al., 2008

Carcinogenicity

- PFOA
  - Liver adenomas
  - Pancreatic acinar cell tumors
  - Testicular Leydig cell adenomas
  - *Ovarian tubular hyperplasia*
- PFOS
  - Liver adenomas
  - Thyroid adenomas/carcinomas

Study with PPARα-KO Mouse



- Fatty acid oxidation, transport
- Glucose, steroid, lipoprotein, retinol metabolism
- Biosynthesis of cholesterol, bile acid
- Inflammatory responses

	PFOA 1 mg/kg	PFOA 3 mg/kg	WY 14,643
WT	206	879	902
PPAR-KO	35	176	10

Involvement of Constitutive Androstane Receptor (CAR) pathway?

Rosen et al., 2008 (Tox. Path.)

Immunotoxicity

- PFOA reduced thymus and spleen weight: associated with decreases of thymocyte and splenocyte production
- Suppression of adaptive immune responses by PFOA: activation of T and B cells attenuated, IgM synthesis suppressed
- Suppression of NK cell function and decreases of IgM production after *in utero* exposure to PFOS
- Suppression of innate immune (inflammatory) responses by PFOA
- Actions mediated by both PPARα-dependent and independent signals

Yang et al., 2002; Pedan-Adams et al., 2008
Kiel et al., 2008; DeWitt et al., 2008; Qazi et al. 2009

Hormone Imbalance

- Reduction of serum tT4 and T3, but a lack of feedback elevation of TSH (PFOA, PFOS, PFHxS, PFNA)
- Profile of changes does not resemble that of classical hypothyroidism
- PFOS-induced hypothyroxinemia (T4) likely related to displacement of hormones from binding protein – physiological significance remains to be defined
- Decrease in serum testosterone and increase in serum estradiol in male rats (PFOA) -- effects associated with induction of hepatic aromatase
- Estrogenic mechanism in rainbow trout by PFOA – associated with hepatocellular carcinoma

Chang et al., 2007; 2008; Liu et al., 1996; Tilton et al., 2008

Neurotoxicity

- *In vitro* study with PC12 cells: Altered cell replication, differentiation and induced oxidative stress
  - PFOSA > PFOS > PFBS = PFOA
- Behavioral study: Neonatal exposure to PFOS or PFOA in mice led to deranged spontaneous behavior, reduced habituation, and hypoactive response to nicotine challenge at adult age
- Enhanced transport of PFOS into immature rat brain
- However, no significant adverse effects of PFOS were indicated in the developmental neurotoxicity testing with rat
- No overt neurotoxicity after a single dose of PFOS or PFOA at sublethal doses

Slotkin et al., 2008; Johansson et al., 2008; Butenhoff et al., 2009; Seto et al., 2009

Developmental Toxicity

Effects of PFAA exposure by daily oral gavage treatment during pregnancy in the Sprague-Dawley rat and CD-1 mouse

PFOS, PFOA, PFNA, PFBA

Common Features of Maternal Effect

- Exposure to PFAAs during pregnancy did not alter maternal weight gains, except at the very high doses
- PFAAs, particularly the carboxylates produced significant increases in maternal liver weight

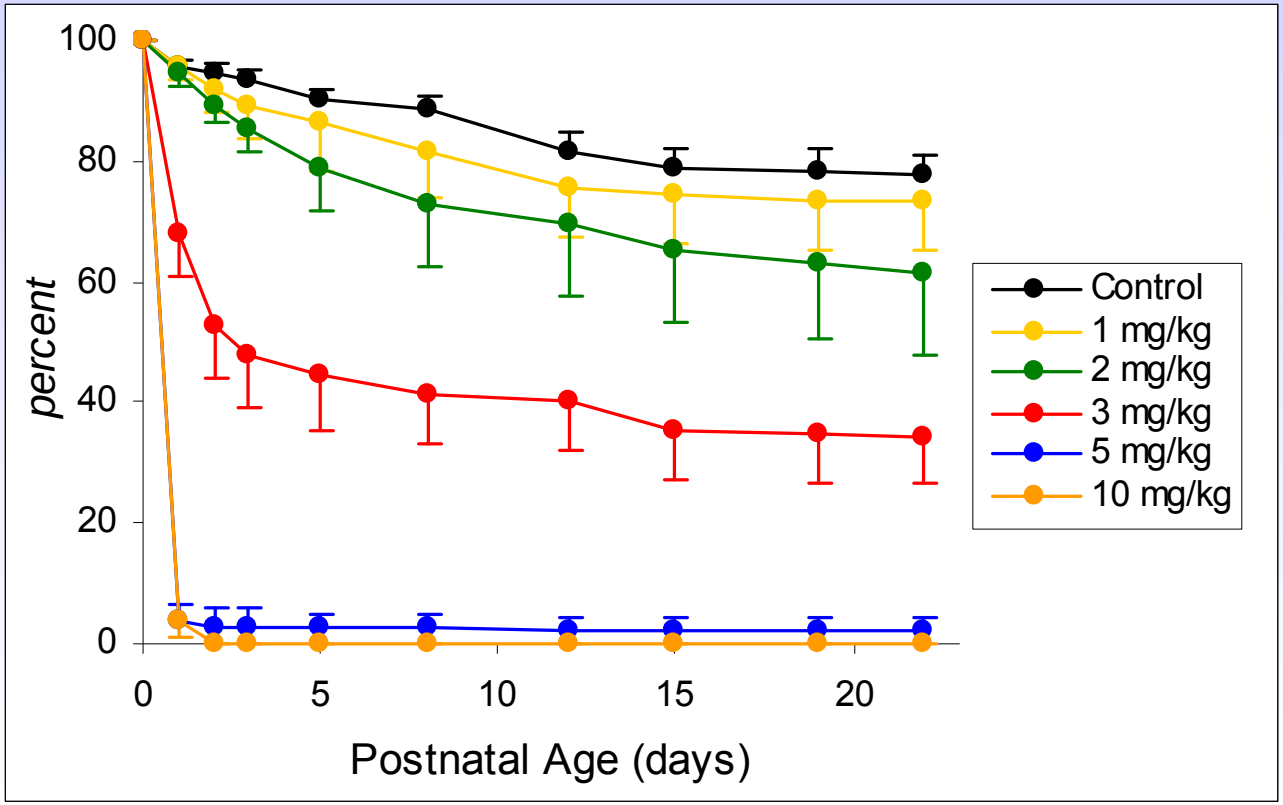
Common Findings of Prenatal Evaluation

- *In utero* exposure to PFAA did not significantly alter implantation, viability or weight of the fetus at term
- A few structural abnormalities and developmental delays were noted, primarily in the highest dose groups of PFOS and PFOA

Thibodeaux et al., (2003)

Postnatal Evaluation

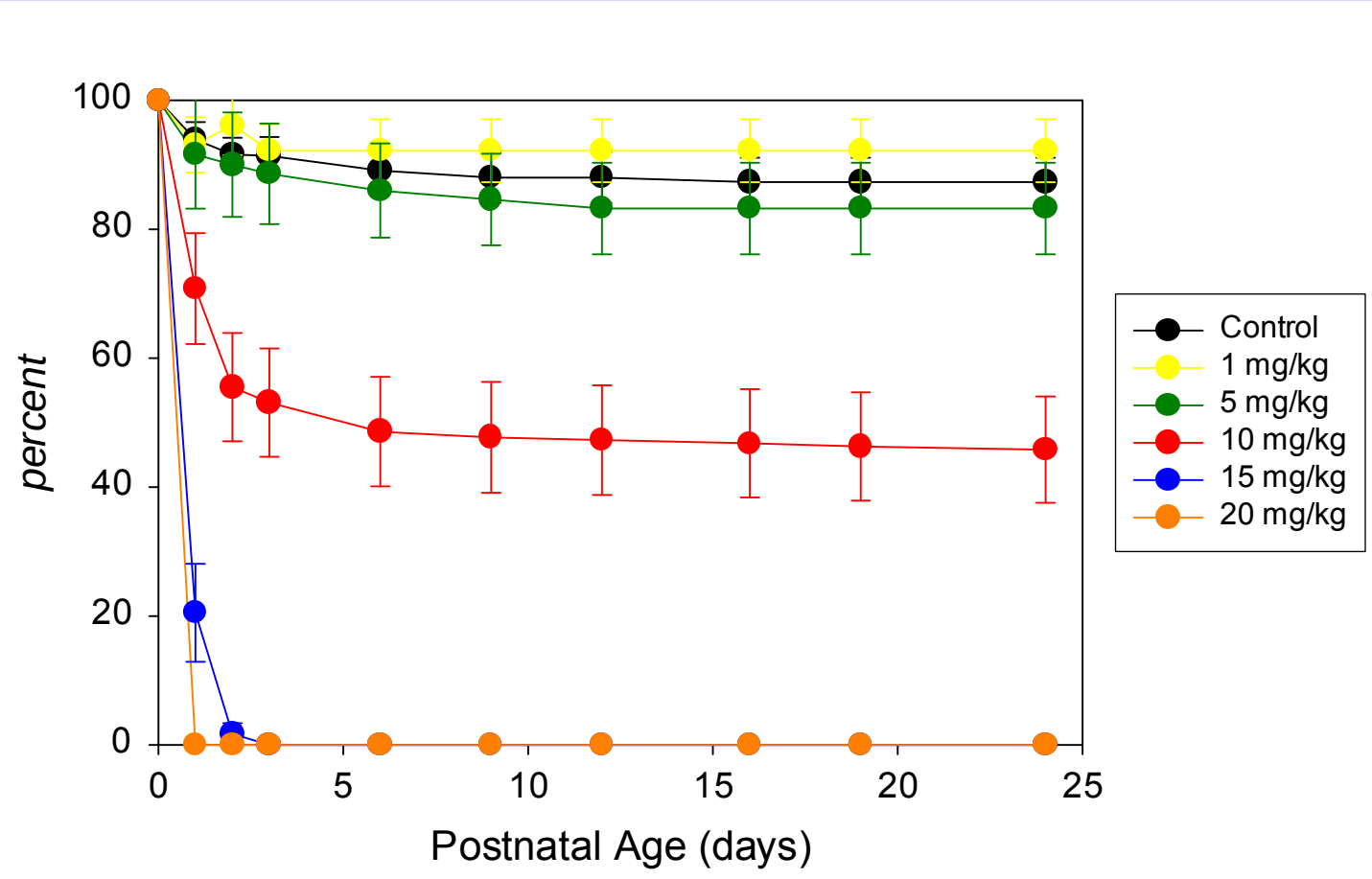
PFOS compromised postnatal survival of neonatal rats



Lau et al., (2003); Luebker et al., (2005)

PFOS

Postnatal Survival: Mouse



Summary of PFOS Postnatal Findings

- While all rats and mice were born alive, postnatal survival was severely compromised
- Neonatal mortality was likely associated with pulmonary insufficiency
- Small growth deficits and developmental delays were noted in the surviving pups
- Persistent liver hypertrophy was seen in the developing mice

Developmental Toxicity of PFOA

- Unremarkable findings in the rat model: *no mortality at birth, slight postnatal growth deficits*
- Likely associated with rapid clearance of the chemical in female rat

Serum $t_{1/2}$	**Male**	**Female**
Rat	6 - 7 days	2 - 4 hours
Monkey	21 days	30 days
Human	2.3 - 3.8 years	

- No gender differences in PFOA elimination in humans or primates

Alternative Model

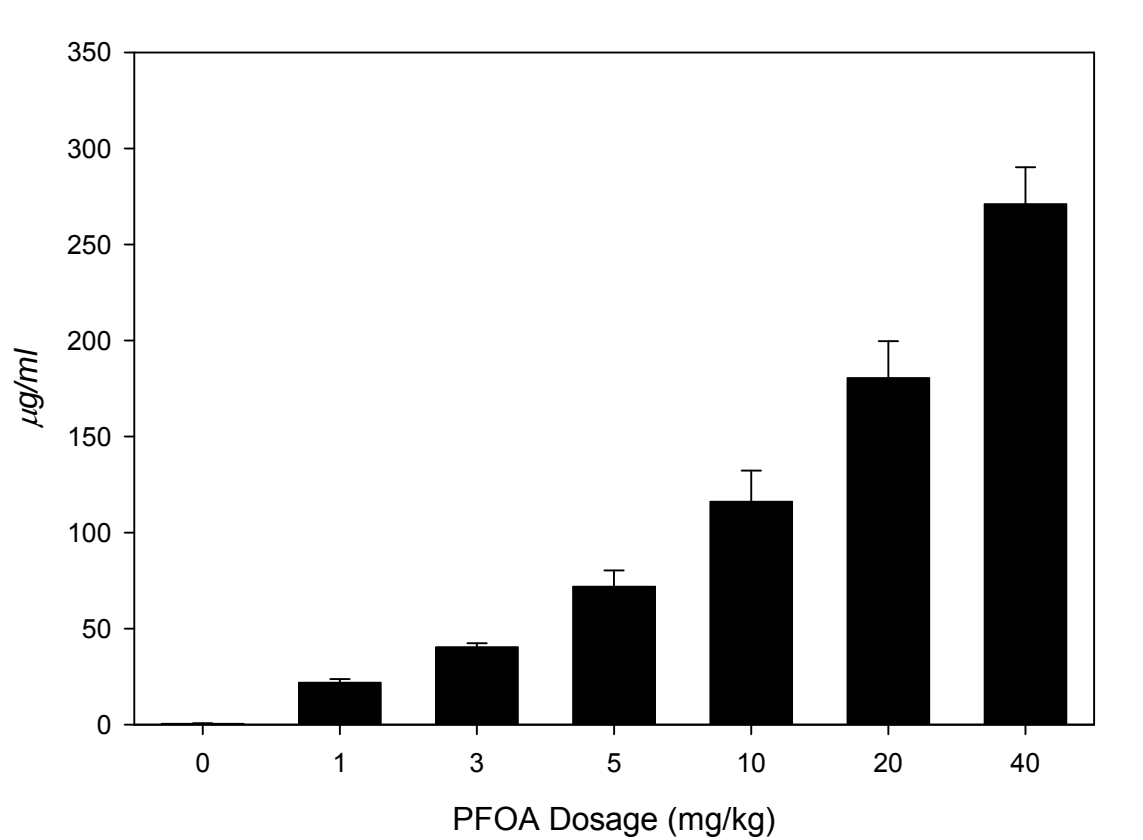
Serum Levels of PFOA

Species	Dose *(mg/kg)*	Males *(µg/mL)*	Females *(µg/mL)*
Rat	10	111 ± 10	0.7 ± 0.2
Mouse	20	199 ± 19	171 ± 15

Serum half-life (days)	**Male**	**Female**
Rat	6 - 7	0.08 – 0.16
Mouse	21.7	15.6

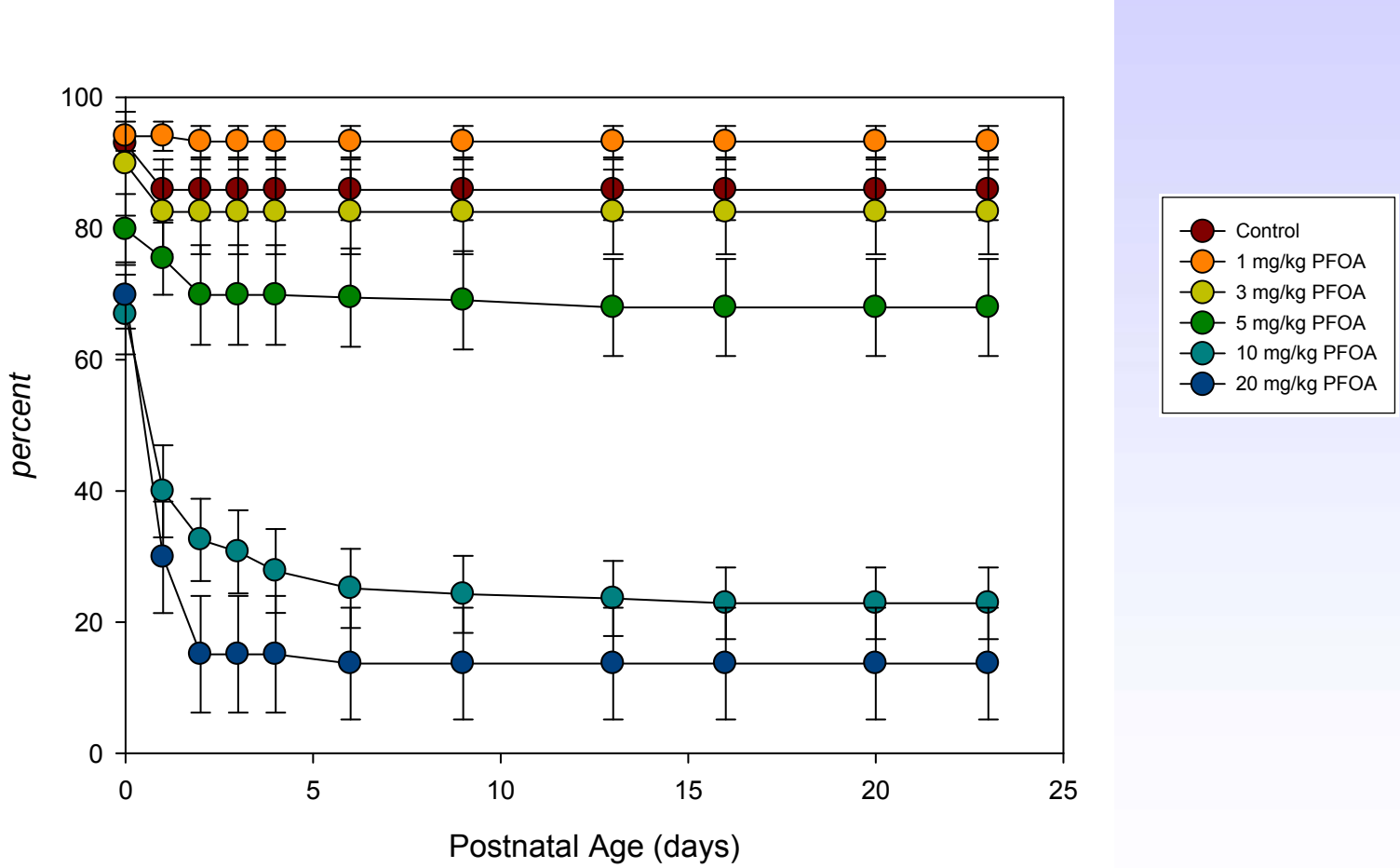
Lau et al. (2006); Lou et al. (2009)

Accumulation of PFOA in pregnant mice at term



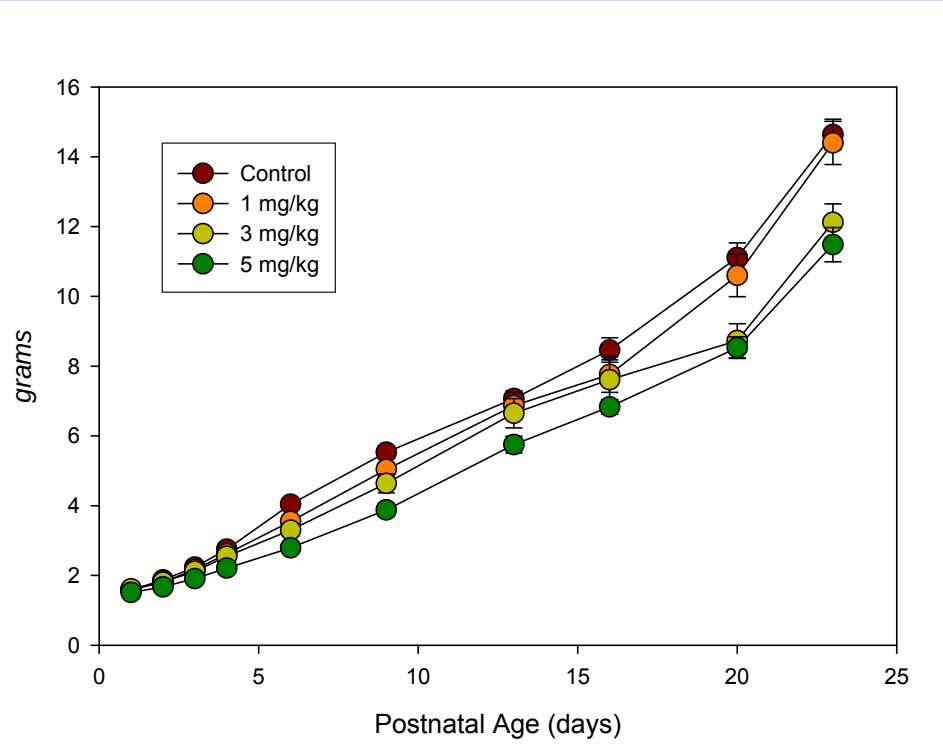
Lau et al., 2006

Postnatal survival of Mice exposed to PFOA

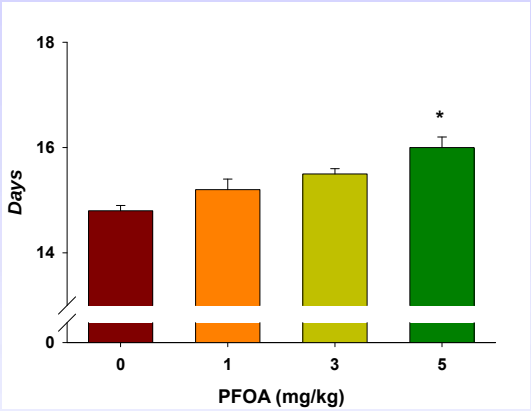


Neonatal Growth and Development

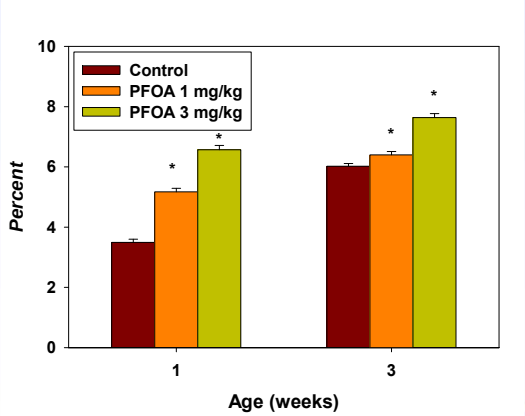
Body Weight



Eye Opening



Relative liver weight



Summary of PFOA Postnatal Findings

- In contrast to the rat, neonatal survival was severely compromised in the mouse, likely reflecting the ability of the females in this species to accumulate PFOA
- The profile of mortality rate was slightly different from that seen with PFOS
- Significant growth deficits and developmental delays were observed among the surviving pups
- Neonatal liver weights were significantly increased

Developmental Effects of PFNA in Rat

- Deficits of maternal weight gain detected at 3 mg/kg or higher doses, severe toxicity seen at 10 mg/kg
- No effect on prenatal parameters
- No effect on neonatal survival
- Small but significant lags in early neonatal growth at 3 mg/kg or higher doses

	Male	Female
Serum $t_{1/2}$	30.7 days	1.8 days

Tatum et al., (submitted); Das et al., (submitted)

Developmental Effects of PFNA in Mouse

- No effect on maternal weight gain during pregnancy at doses up to 5 mg/kg
- No effect on prenatal parameters
- No significant mortality was seen at birth, but pups exposed to 5 mg/kg died in the first two weeks of life
- Significant lags in early neonatal growth were observed at doses as low as 1 mg/kg
- These effects are likely due to the ability of pregnant mice to accumulate PFNA

	Male	Female
Serum $t_{1/2}$	64.4 days	40.7 days

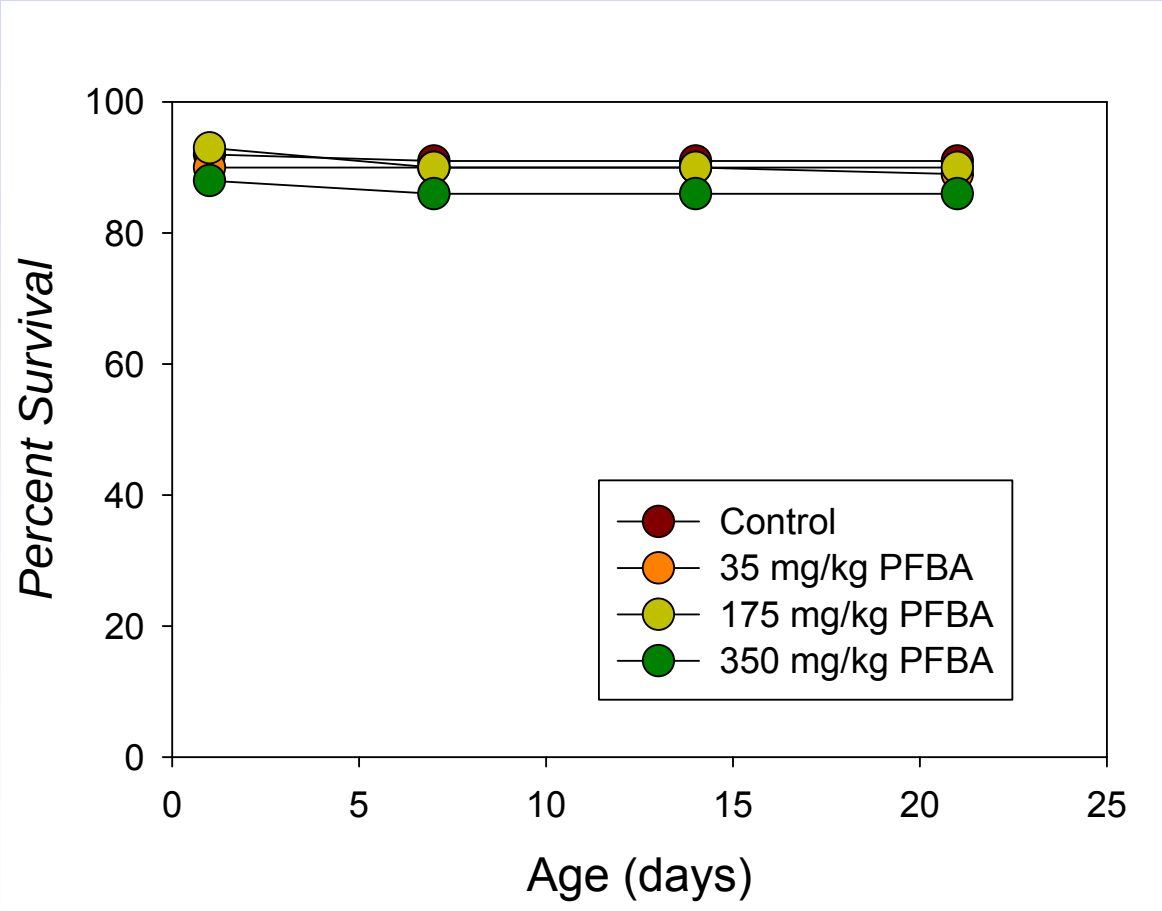
Tatum et al., (submitted); Das et al., (submitted)

Summary of PFNA Postnatal Findings

- Similar to PFOA, exposure to PFNA led to neonatal mortality in mouse, but not in rat, likely due to the ability of female mice to accumulate the chemical
- The profile of mortality rate was slightly different from those seen with PFOS or PFOA
- Significant growth deficits and developmental delays were observed among the surviving pups, and neonatal liver weights were significantly increased
- Actions of PFNA appeared to be more potent than those of PFOA

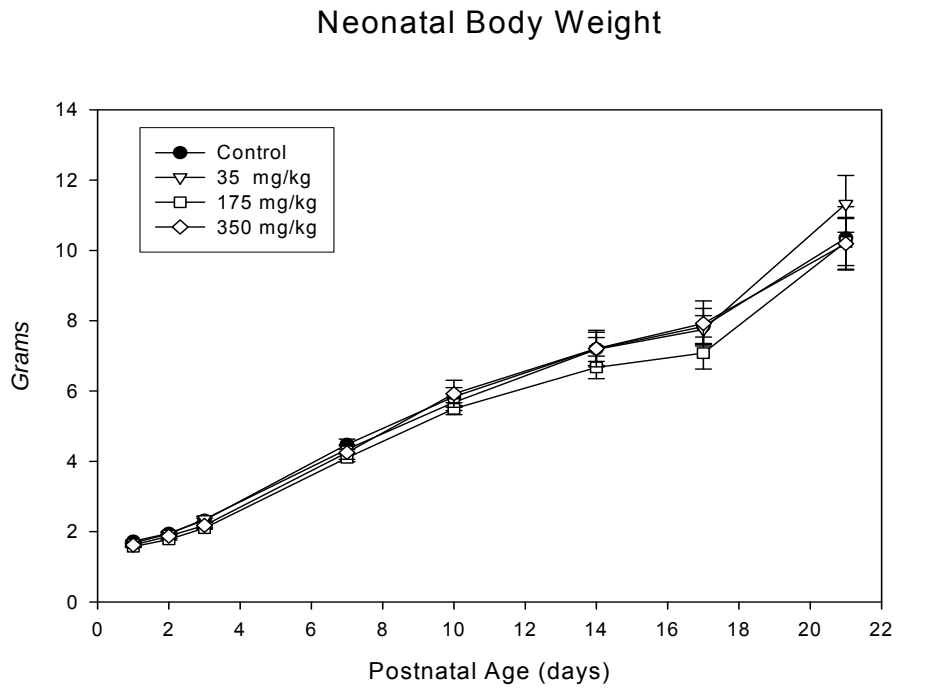
Do all PFAAs produce developmental toxicity?

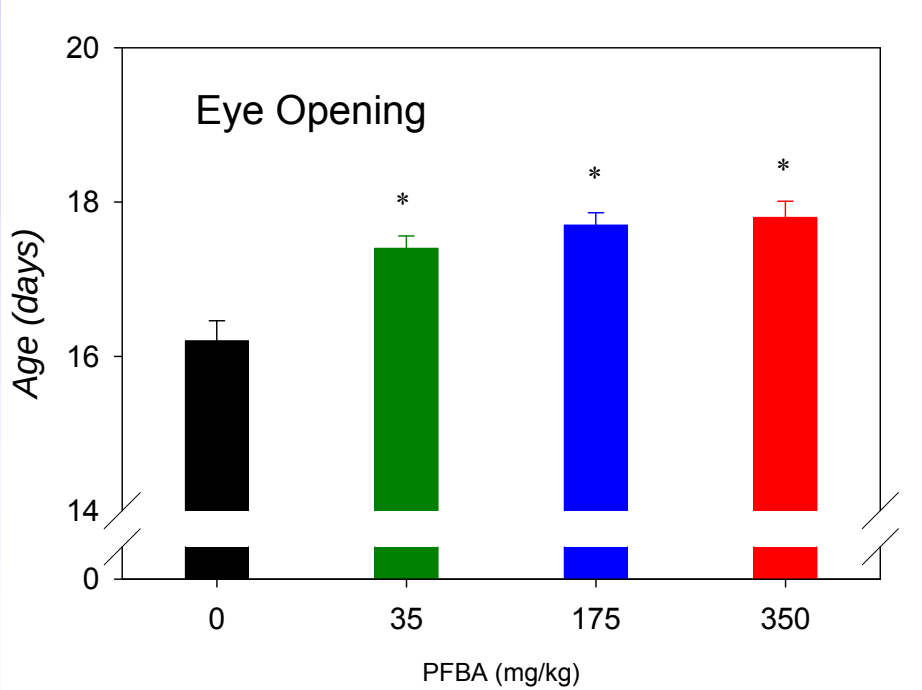
PFBA did not alter neonatal survival



Das et al., (2008)

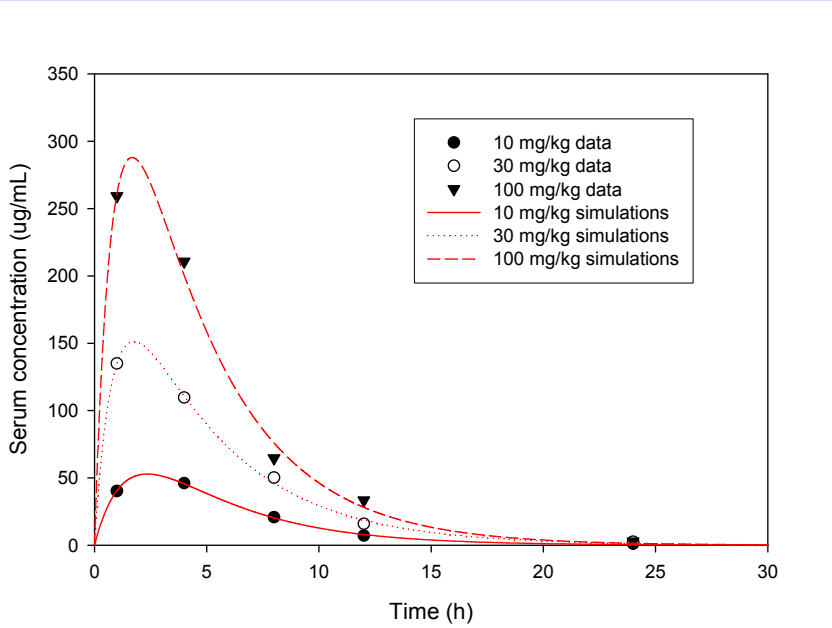
Neonatal Growth and Development



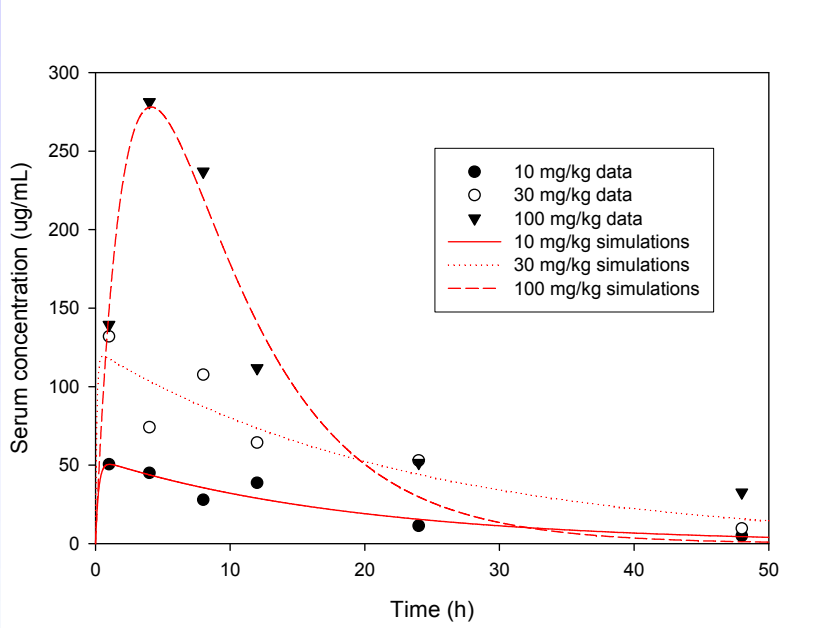


Elimination of PFBA in Mouse

Female



Male



	Male	Female
Serum $t_{1/2}$	11.6 hrs	2.9 hrs

Chang et al., (2008)

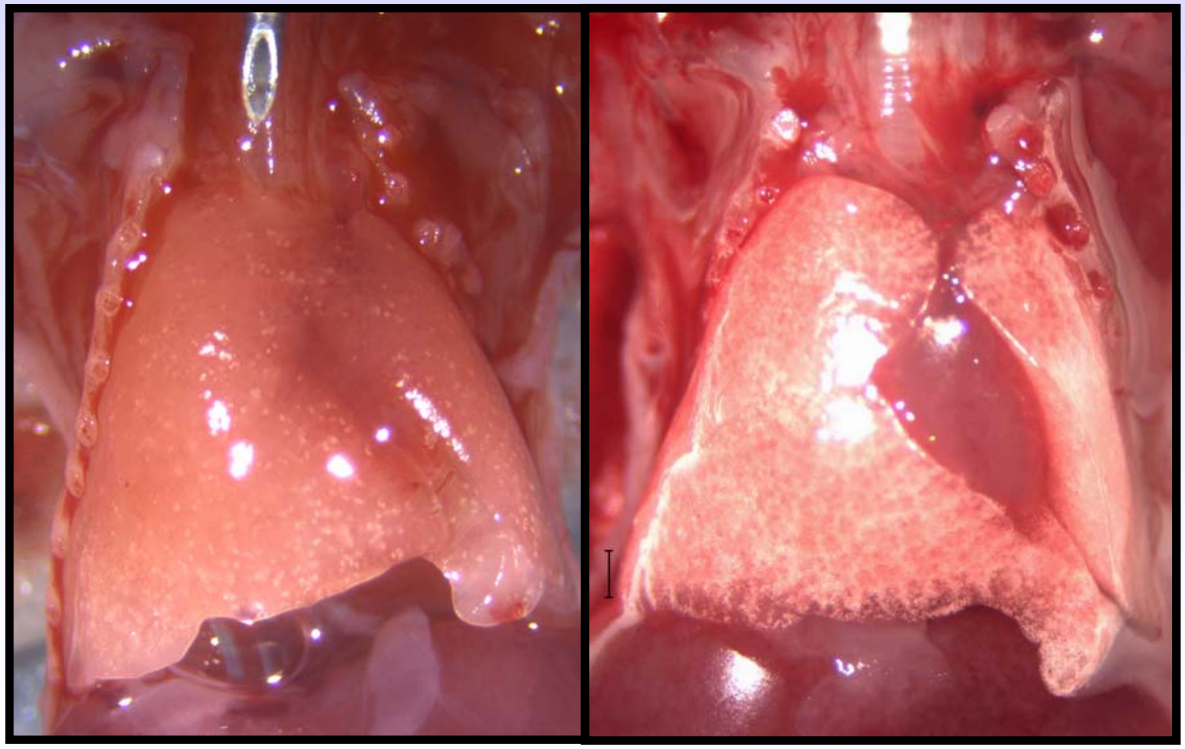
Summary of PFBA Postnatal Findings

- Exposure to high doses of PFBA (up to 350 mg/kg, which matched the effective doses (AUC) of PFOA) did not adversely affect neonatal survival or growth, although some developmental delays were noted
- Transient liver hypertrophy was seen at PD 1, but the liver weight returned to control level by PD 10
- The relative lack of adverse developmental effects of PFBA (compared to PFOA) is in part, due to the rapid elimination of this chemical

Pathophysiological mechanisms of developmental toxicity

Ho: PFOS dev tox = Altered lung function

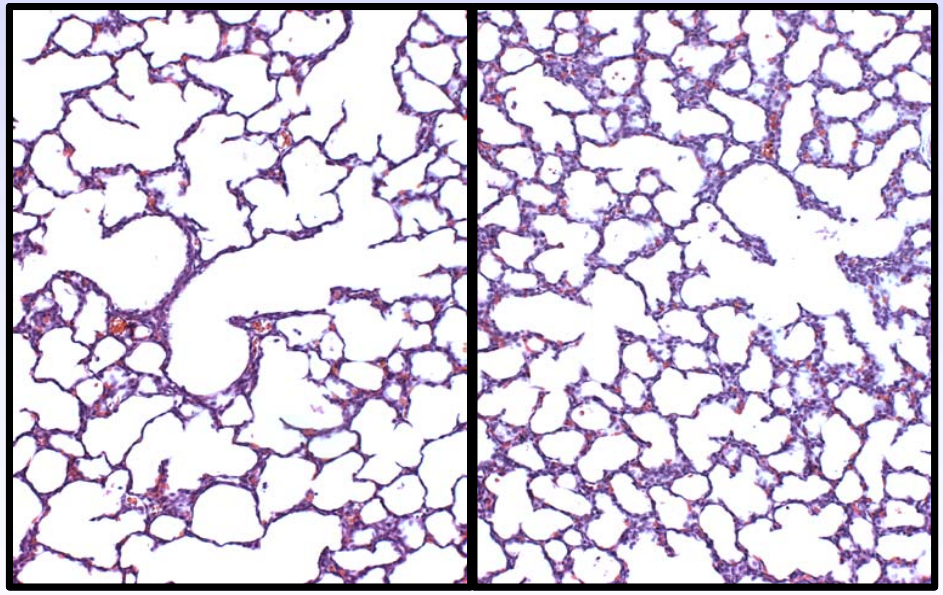
Control **PFOS**



Grasty et al., (2005)

Lung Histology and Morphometry

Control **PFOS**



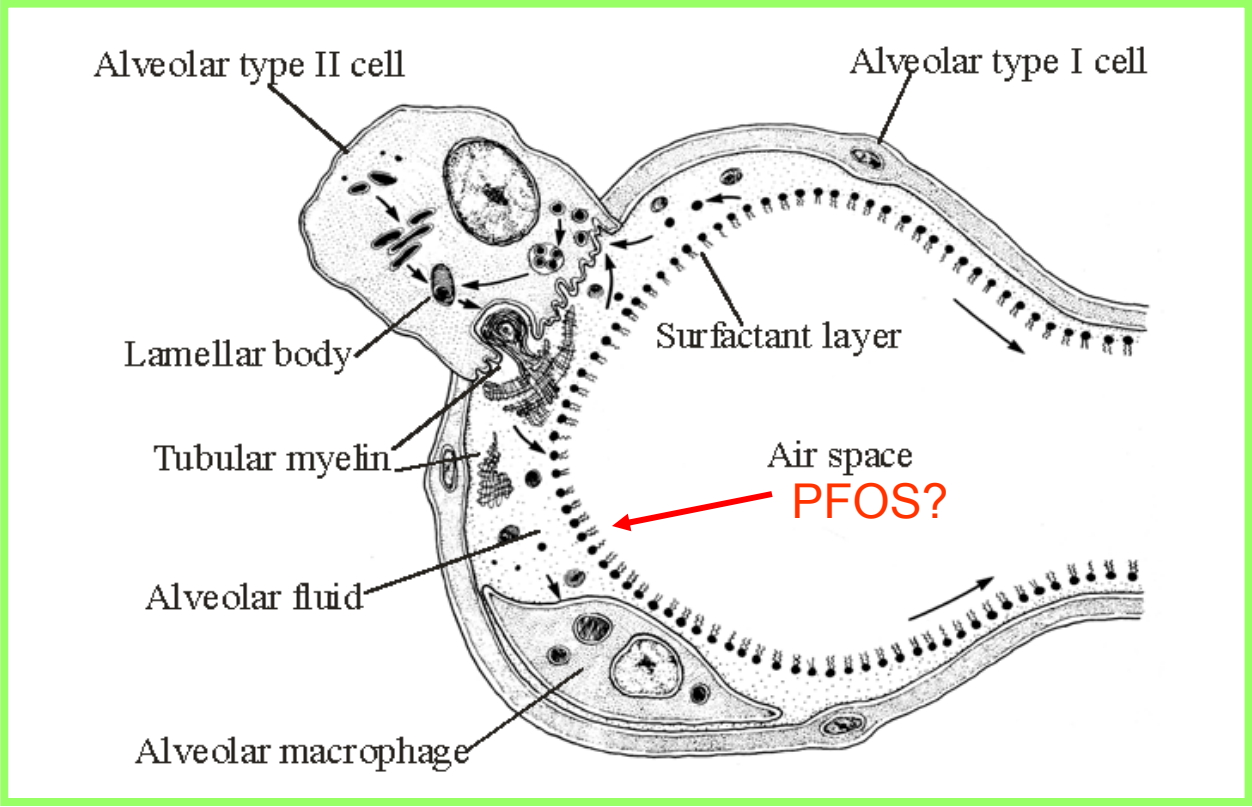
Dose (mg/kg)	Air Space (%)	Septal Space (%)
0	63.9 ± 1.5	31.6 ± 1.3
5	56.7 ± 2.1	41.2 ± 2.0 *
10	55.2 ± 2.2*	43.6 ± 1.9 *

Does PFOS alter lung maturation?

- Surfactant levels and phospholipid composition in newborn rat lungs were not altered.
- Glycogen stores (indicator of lung maturation) was not affected.
- Surfactant transport and secretion were not perturbed significantly.
- Therefore, lung maturation *per se* was not likely hampered by PFOS.
- *Speculation: Rather, PFOS may impede the function of endogenous surfactant to prevent the lung from collapsing.*

Alveolar Structure

Surfactant prevents lungs from collapsing during end-expiration by reducing the surface tension at the air-liquid interface



Modified from Hawgood and Clements (1990)

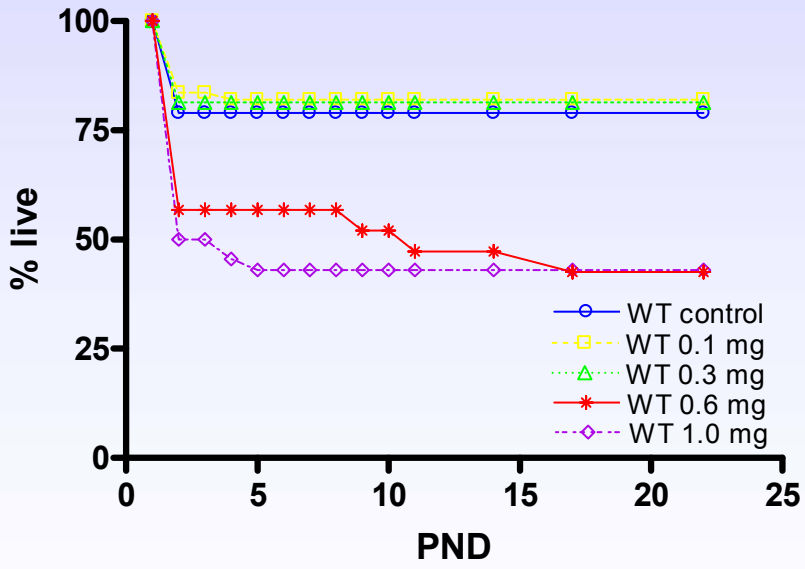
PFOS and Pulmonary Surfactant

- PFOS was detected in amniotic fluid that bathed the fetal lung
- Oral gavage of newborn rats failed to cause mortality – chemical has to reach within the lung
- PFOS interacts with phospholipids
  - Dipalmitoylphosphatidylcholine (DPPC) is a major component of lung surfactant
  - *In vitro* study: PFOS had strong tendency to partition into and disrupt DPPC bilayers
  - PFOS > PFOA >>OS
- Definitive evidence is needed

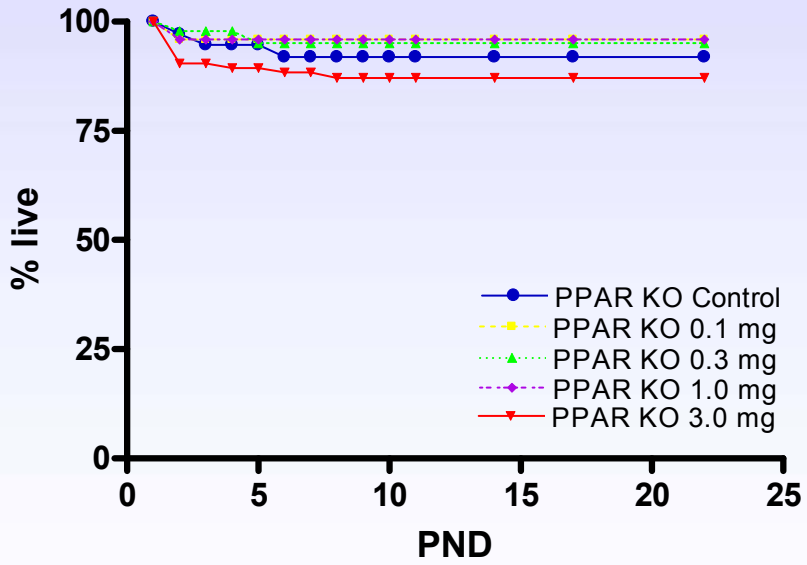
Xie et al., (2007)

PPARα Involvement in PFOA Neonatal Mortality

Wildtype Mice



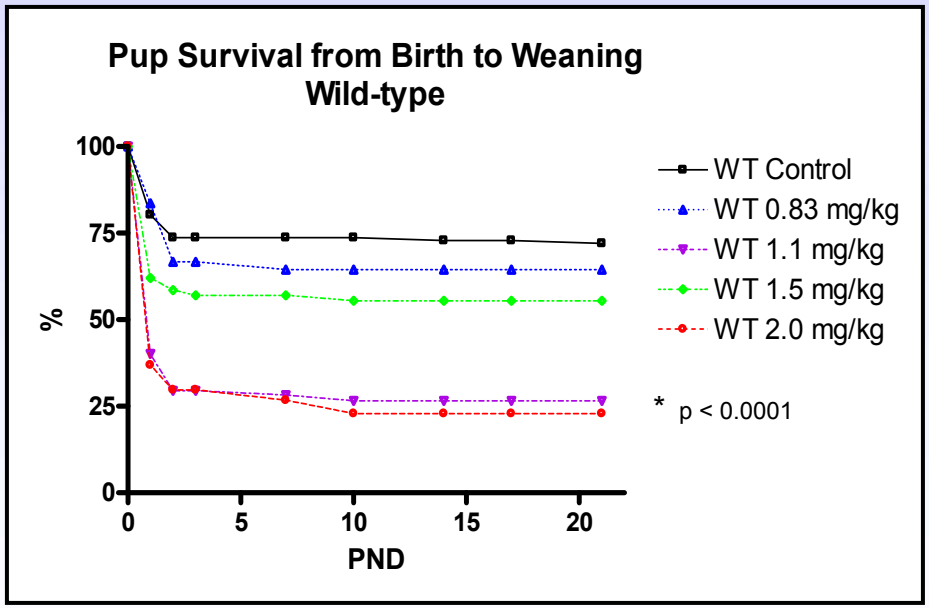
PPARα-null Mice



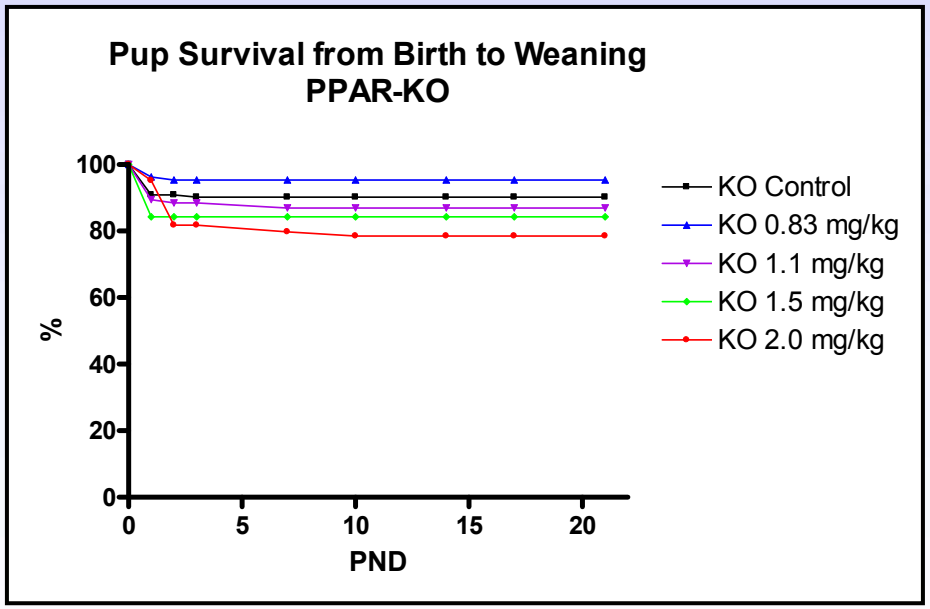
Abbott et al., (2008)

PPARα Involvement in PFNA Neonatal Mortality

WT



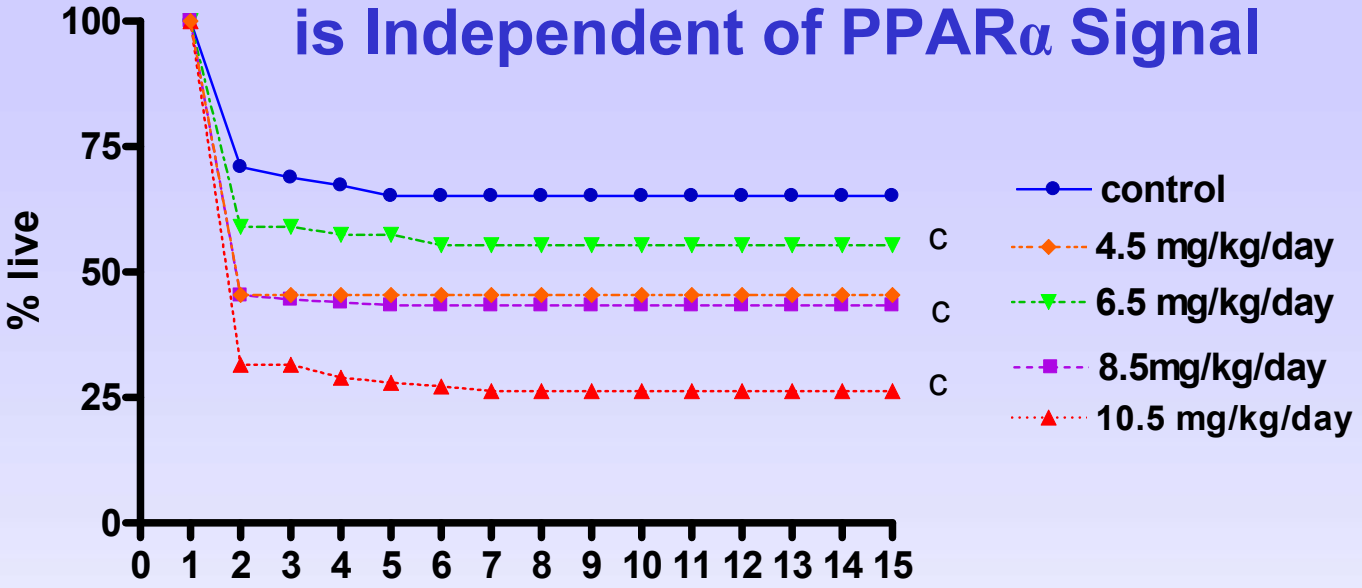
PPARα KO



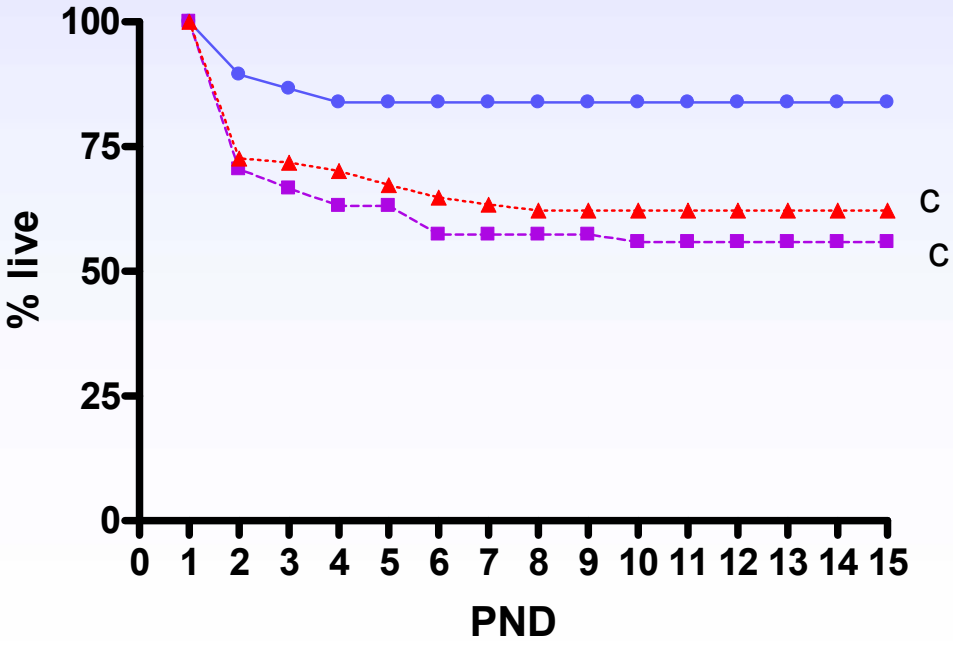
Wolf et al., (submitted)

PFOS-induced Neonatal Mortality is Independent of PPARα Signal

Wild Type



PPAR KO



Abbott et al., (2009)

Summary

- Although *in utero* exposure of both PFOS and PFOA caused neonatal mortality, the adverse effects may be mediated by separate mechanisms
- PFOS likely interacts with phospholipids of lung surfactant and interferes with lung inflation and pulmonary function
- PFOA and PFNA likely acts through the PPARα signaling pathway that *regulates intermediary metabolism*

PFAA toxicity depends on carbon-chain length and functional group

- **Pharmacokinetics**

Serum Half-life	**PFBS** (C4)	**PFHS** (C6)	**PFOS** (C8)	**PFBA** (C4)	**PFHxA** (C6)	**PFOA** (C8)	**PFNA** (C9)	**PFDA** (C10)
Rat			**7 d**	**2 h** **9 h**	**0.42 h** **1 h**	**2-4 h** **6-7 d**	**2 d** **31 d**	**59 d** **40 d**
Mouse				**3 h** **12 h**		**16 d** **22 d**	**41 d** **64 d**	
Monkey	**3-4 d**	**87 d** **141 d**	**150 d**	**41 h** **40 h**	**2.4 h-0.8 d** **5.3 h-1.5 d**	**30 d** **21 d**		
Human	**1 m**	**8.5 y**	**5.4 y**	**1-4 d**		**2.3-3.8 y**		

- **Toxicodynamics**
  - Endpoints dependent on MOA, some share, some do not
  - Rank order of potency among PFAAs with the same MOA
- **PFAAs *in toto***

PFAA Analysis Team

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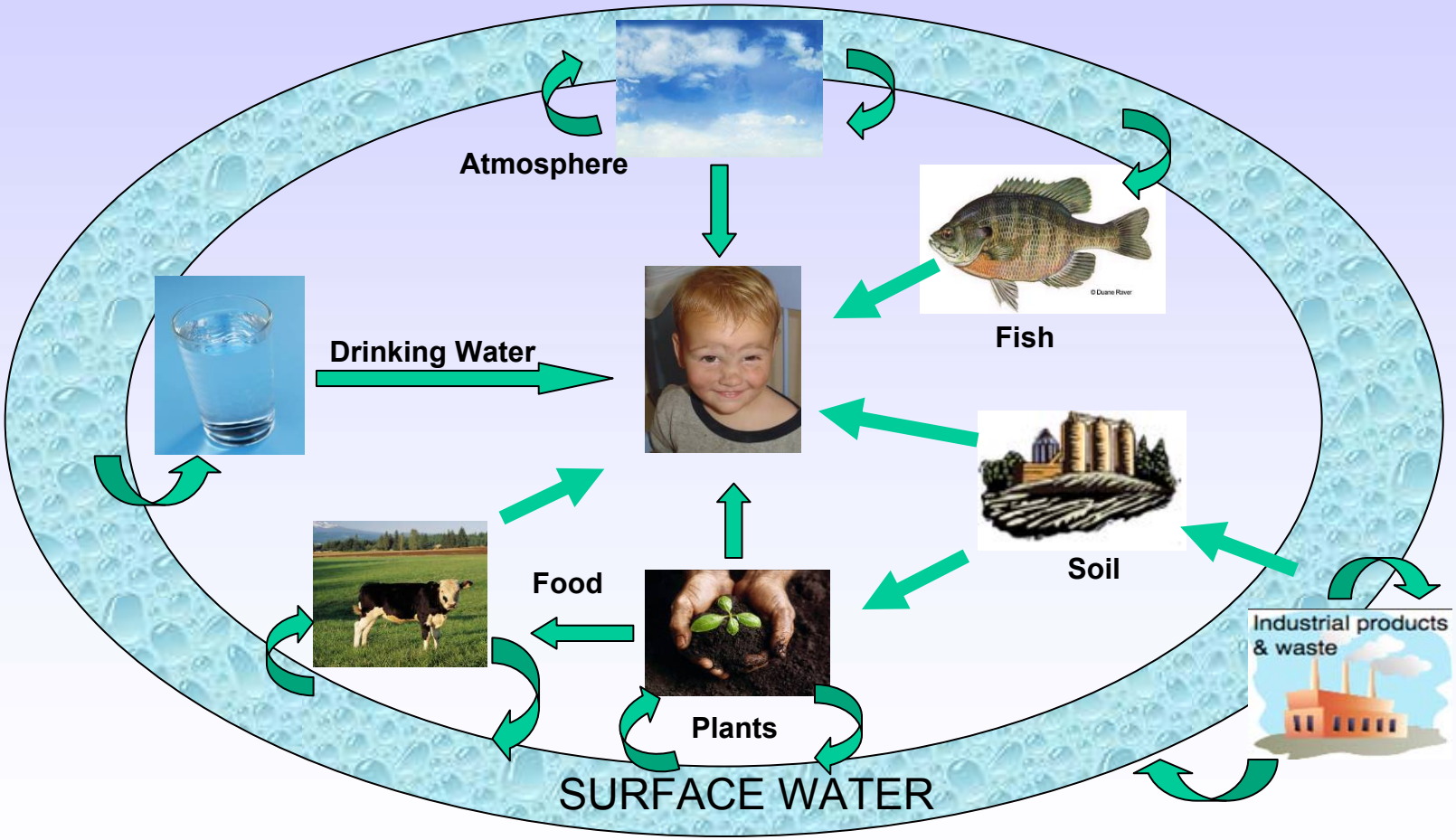
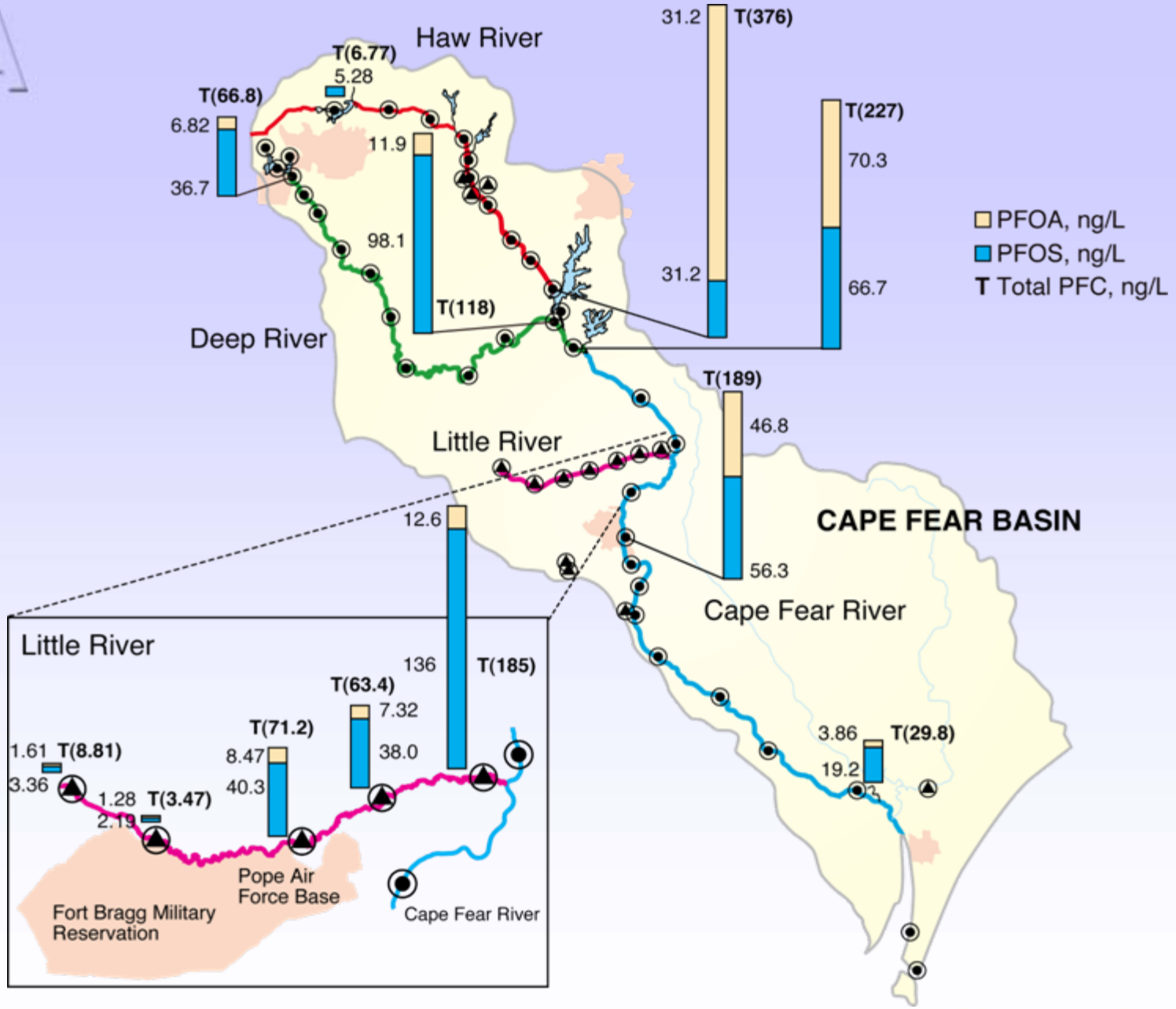
Human Exposure Pathways





Nakayama et al, (2007)

Method Development for Fish Samples

- **Homogenization**
  - water:fish = 3:1; Polytron
- **Alkaline Digestion**
  - 1ml fish homogenate + 9ml 0.1M NaOH in MeOH, for 16 h
- **SPE Clean-up** (Waters 3 cc WAX cartridge)

Condition	Load	Wash	Elute
4 ml NH_4OH/MeOH 4 ml MeOH 4 ml H_2O	1 ml digest 9ml H2O	4 ml Acetate Buffer 4 ml MeOH	4 ml NH_4OH/MeOH

Delinsky et al., (2009)

PFAAs in Bluegill Fillets from MN and NC

(ng/g wet weight) (Delinsky et al., 2009)

Sample Site	PFOS	C10	C11	C12
Miss. River, MN	102 (32.8 – 130)	1.73 (0.56 – 2.78)	1.21 (0.53 – 2.70)	1.07 (0.36 – 3.03)
St. Croix River, MN	2.08 (1.22 – 7.17)	< LOQ	< LOQ	< LOQ
Lake Calhoun, MN	275 (205 – 339)	6.09 (3.40 – 7.05)	4.50 (2.14 – 6.02)	5.91 (2.70 – 6.08)
Haw River, NC	30.3 (15.9 – 47.5)	9.08 (6.07 – 22.8)	23.9 (14.3 – 42.2)	6.60 (4.16 – 16.1)
Deep River, NC	62.2 (21.4 – 136)	2.90 (0.56 – 22.7)	9.15 (1.31 – 50.5)	3.46 (0.36 – 24.3)

MN Fish Consumption Advisory:
PFOS: 40 ng/g (once/week); 200 ng/g (once/month)

C10? C11? C12?

Summary

- PFAA signatures in NC fish fillet generally reflect those of the river water
- Species differences in fillet PFAA concentrations were observed
- Ratios of fillet:whole fish and liver:whole fish will help to better understand the PFAA disposition, and to relate the fish liver PFAA values reported in the literature to human exposure (fillet)

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