Toxicity of Perfluoroalkyl Substances

David Klein

PFAS in the Northeast: State of Practice & Regulatory Perspectives

May 8-10th 2017

Outline

- How did the advisory come about?

- What is the evidence for PFAS toxicity?
  - Developmental delay
  - Hepatotoxicity
  - Immunosuppression

How did this advisory come about?

- No observed adverse effect level (animal studies).
- If NOAEL isn't observed, takes LOAEL and divide by 10.
- Assume 10 fold lower than the LOAEL is NOAEL.

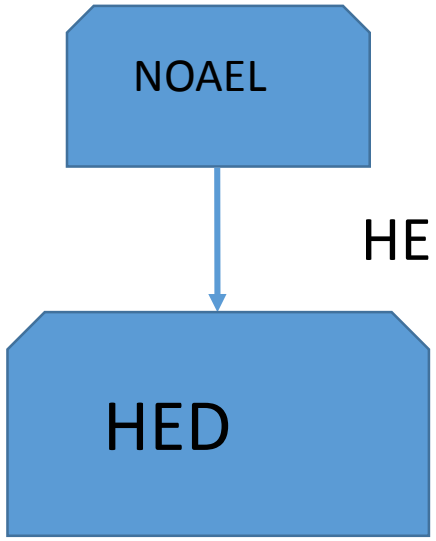
NOAEL

Example:
Lau et al., 2006
1.0 mg/kg LOAEL

How did this advisory come about?

- Human equivalent dose: predicted oral dose for humans to have serum concentrations equal to that of the animal at NOAEL.

- Calculate average serum level at NOAEL (pharmacokinetic model from Wambaugh) and multiply by clearance.



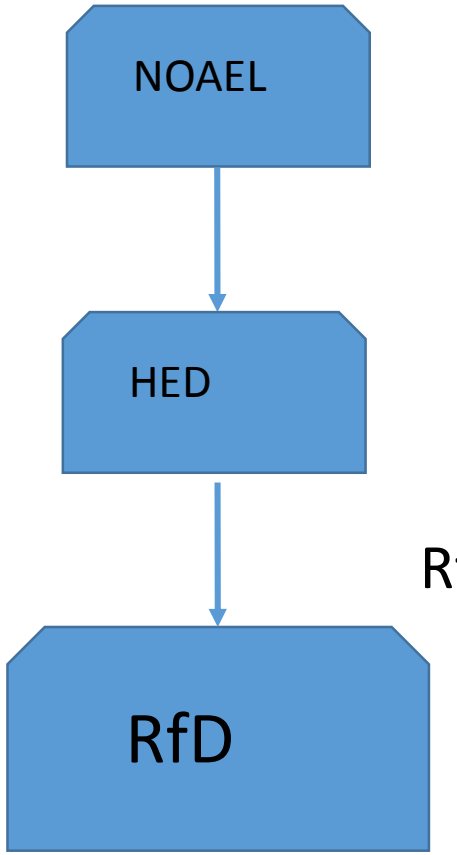
Average serum concentration:
38mg/L
38 * 0.00014 (Cl) = 5.3ng/kg/day

HED = Avg serum level (NOAEL) * Clearance

How did this advisory come about?

- Reference Dose: estimate of a daily exposure to the human population that is likely to be without an appreciable risk of deleterious effects during a lifetime. (mg/kg/day)

- Divide by total uncertainty factors associated with study (extrapolating LOAEL to NOAEL, interspecies uncertainty, and intraindividual uncertainty).



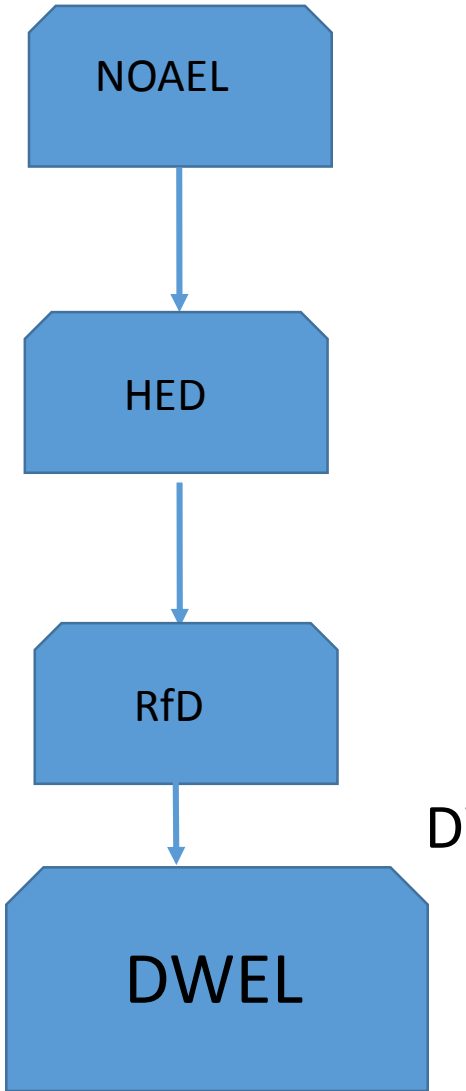
5.3ng/kg/day (HED) / 300 (UF)
~20ng/kg/day RfD

RfD = HED (from NOAEL) / UF

How did this advisory come about?

- Drinking Water Equivalent Level: Estimate maximum safe level a person could intake via drinking water.
  - Assumes 100% exposure from drinking water.

- Drinking water intake / body weight was estimate at 0.054L/kg/day (90th percentile for lactating women)

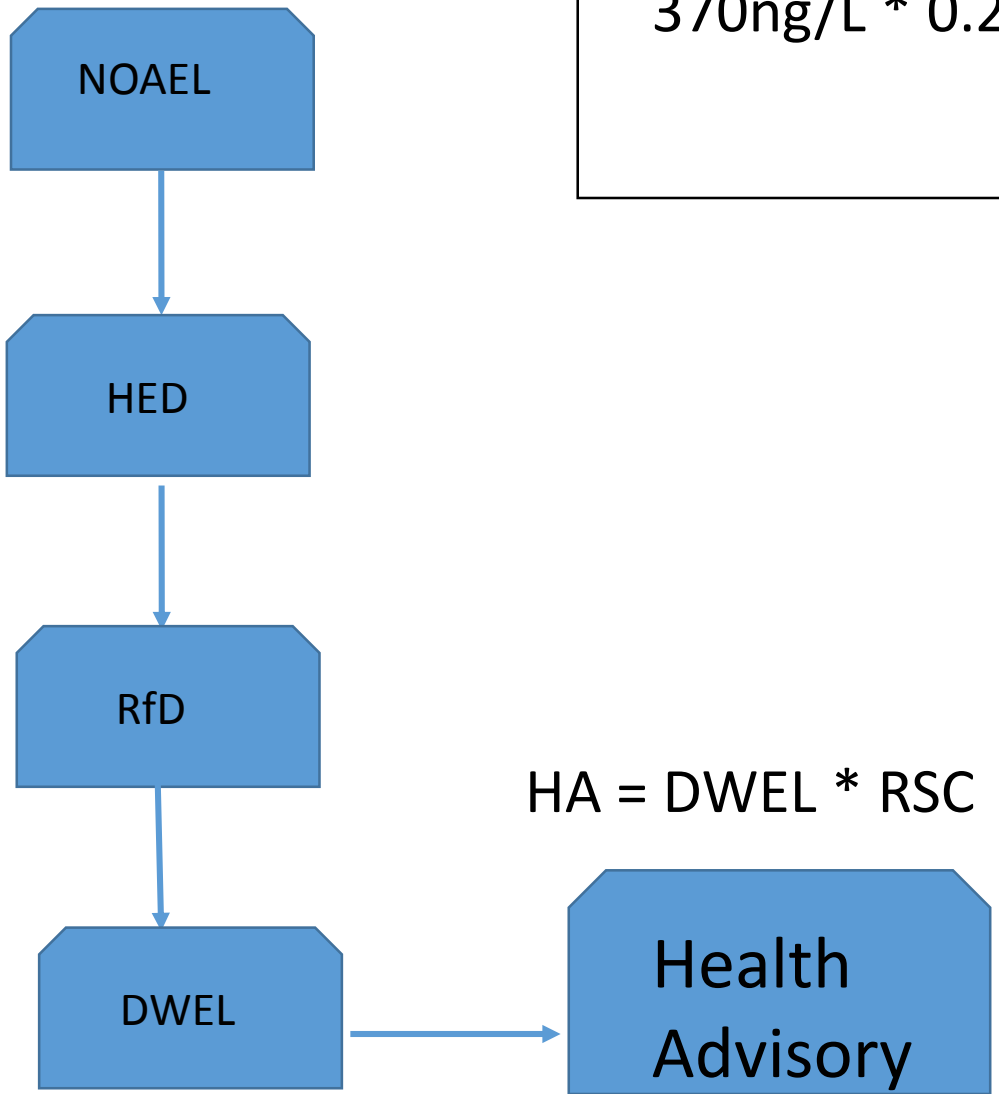


20ng/kg/day / .054L/kg/day) = 370ng/L DWEL

DWEL = RfD / (DWI / BW)

How did this advisory come about?

- Relative Source Contribution: accounts for the contribution of sources of exposure besides drinking water (soil, food, air, etc.)
- Estimated 20% (0.2 for the formula)
- 20% is the standard estimate for when scientific data on what it should be is lacking.



370ng/L * 0.2 = ~70 ng/L

HA = DWEL * RSC

State Health Advisories?

- Vermont (20ppt) used same RfD but had a different drinking water intake (0.175 instead of 0.054) based on 95th percentile of first year of life rather than lactating women.

- New Jersey (14ppt) used a different endpoint of an increase in relative liver weight (Loveless et al., 2006) seen for mice at 0.3mg/kg dose PFOA.

What is the evidence for PFAS toxicity?

- "Studies indicate that PFOA and PFOS can cause reproductive and developmental, liver and kidney, and immunological effects in laboratory animals. Both chemicals have caused tumors in animal studies. The most consistent findings from human epidemiology studies are increased cholesterol levels among exposed populations, with more limited findings related to low infant birth weights, effects on the immune system, cancer for PFOA, and thyroid hormone disruption for PFOS." -EPA

- Mechanism Unknown.

What is the evidence for PFAS toxicity? PPARα

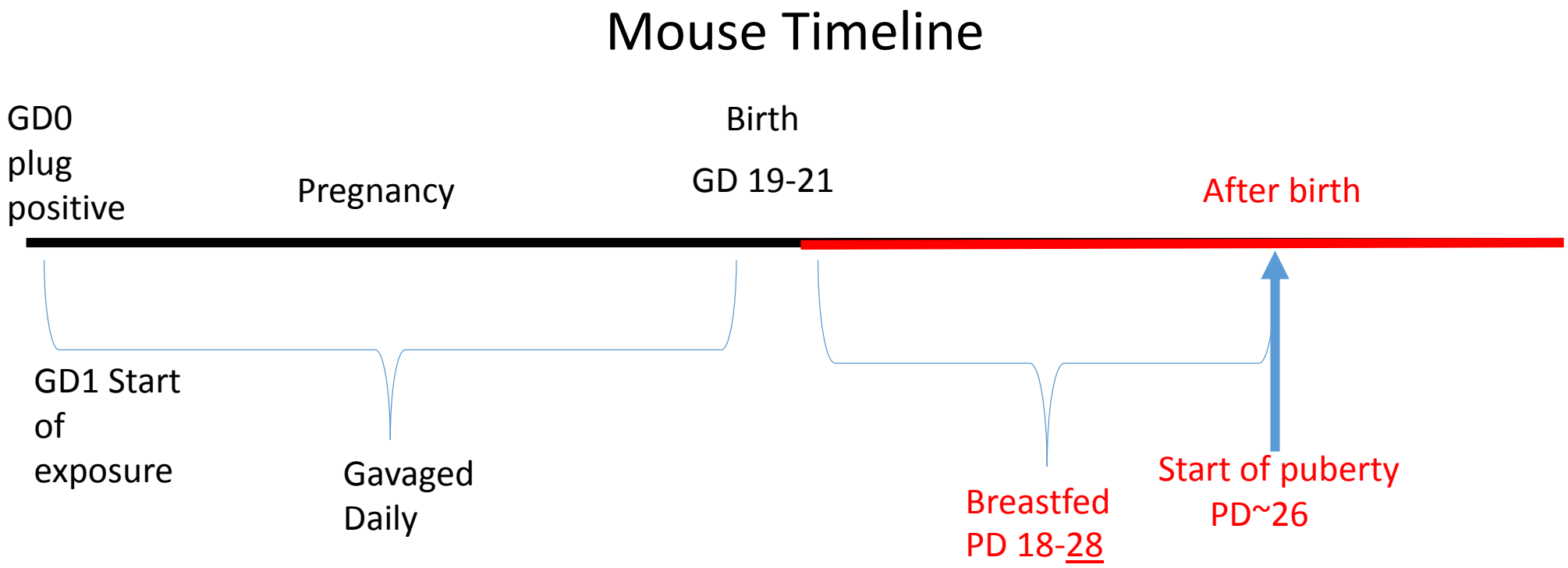
- Peroxisome proliferator-activated receptor alpha (PPARα).
  - PFAS are agonists.

- Nuclear receptor that controls genes for lipid homeostasis, catabolism etc.

- Much more prevalent in rodents than humans.
  - If liver weight increase is related to PPARα activation, then we don't worry about it.

- Activation is linked to hepatocellular, pancreatic acinar cell, and Leydig cell adenomas in rats (Not usually seen in humans).

Developmental Delay



Developmental Delay: Phalanges Ossification

Mouse Reproductive Outcome and Fetal Teratology, Examined at Term

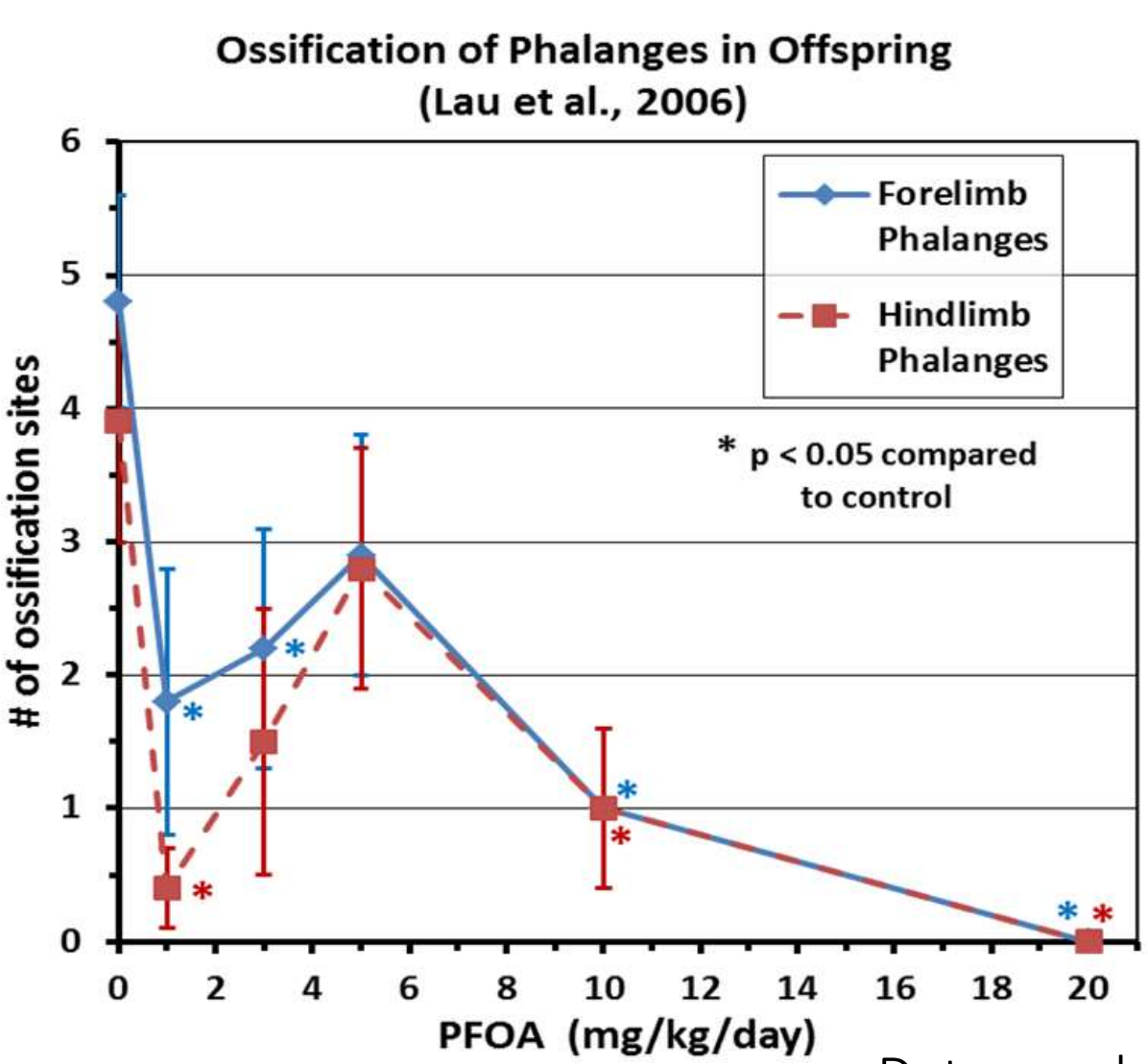
	PFOA dosage (mg/kg)						
	0	1	3	5	10	20	40
Dams examined (#)	45	17	17	27	26	42	9
Dams with FLR (#)	3	2	1	7	12	37	9
Dams with FLR (%)	6.7	11.8	5.9	25.9*	46.1*	88.1*	100*
Implants (# per litter with FLR)	7.0 ± 4.0	10.0 ± 3.0	13.0	11.6 ± 1.2	10.8 ± 1.2	11.5 ± 0.6	11.9 ± 0.5
Implants (# per live litter)	12.9 ± 0.4	13.1 ± 0.4	11.6 ± 0.9	11.5 ± 0.5	12.6 ± 0.6	10.2 ± 2.1	—
Live fetuses (# per live litter)	12.5 ± 0.4	13.0 ± 0.4	10.8 ± 0.9	11.1 ± 0.4	11.7 ± 0.8	7.2 ± 2.0*	—
Prenatal loss (% per live litter)	4.1 ± 1.4	1.0 ± 0.7	7.4 ± 2.5	2.4 ± 0.8	7.7 ± 3.3	25.9 ± 11.7*	—
Fetal body weight (g)	1.05 ± 0.02	0.98 ± 0.03	1.03 ± 0.04	1.03 ± 0.04	0.98 ± 0.05	0.86 ± 0.11*	—
Notable skeletal findings (*n*)	13	6	7	11	5	5	—
Ossification (number of sites):							
Sternebrae	5.9 ± 0.1	6.0 ± 0.1	6.0 ± 0.1	5.5 ± 0.3	5.7 ± 0.2	4.0 ± 1.1*	—
Caudal vertebrae	4.3 ± 0.3	4.1 ± 0.1	4.0 ± 0.2	4.3 ± 0.3	3.7 ± 0.2	2.1 ± 0.7*	—
Metacarpals	7.7 ± 0.2	7.3 ± 0.3	7.6 ± 0.2	6.6 ± 0.5	6.8 ± 0.4	5.2 ± 1.4*	—
Metatarsals	9.3 ± 0.3	8.9 ± 0.4	9.1 ± 0.3	8.2 ± 0.6	8.6 ± 0.4	6.2 ± 1.6*	—
Proximal phalanges (forelimb)	4.8 ± 0.8	1.8 ± 1.0*	2.2 ± 0.9*	2.9 ± 0.9	1.0 ± 0.6*	0.0 ± 0.0*	—
Proximal phalanges (hindlimb)	3.9 ± 0.9	0.4 ± 0.3*	1.5 ± 1.0	2.8 ± 0.9	1.0 ± 0.6*	0.0 ± 0.0*	—
Reduced ossification (%):							
Calvaria	13.5 ± 9.2	62.5 ± 15.5*	66.7 ± 13.0*	22.7 ± 10.4	35.0 ± 12.7	55.0 ± 20.0*	—
Supraoccipital	14.7 ± 4.0	33.3 ± 10.5	28.6 ± 8.5	27.3 ± 9.2	45.0 ± 9.4*	90.0 ± 10.0*	—
Unossified hyoid	0	0	0	0	0	26.7 ± 19.4*	—
Enlarged fontanel	17.3 ± 9.1	66.7 ± 21.1*	53.6 ± 15.8*	18.2 ± 9.6	45.0 ± 20.0	95.0 ± 5.0*	—
Notable visceral findings (*n*)	10	6	6	11	5	5	
Tail defects (curly, bent) (%)	0	0	0	20.5 ± 5.7*	5.0 ± 5.0*	11.7 ± 7.3*	—
Limb defects (club, bent) (%)	0	0	0	5.7 ± 2.8*	0	5.8 ± 3.9*	—
Microcardia (%)	0	0	0	0	5.0 ± 5.0*	30.0 ± 18.3*	—

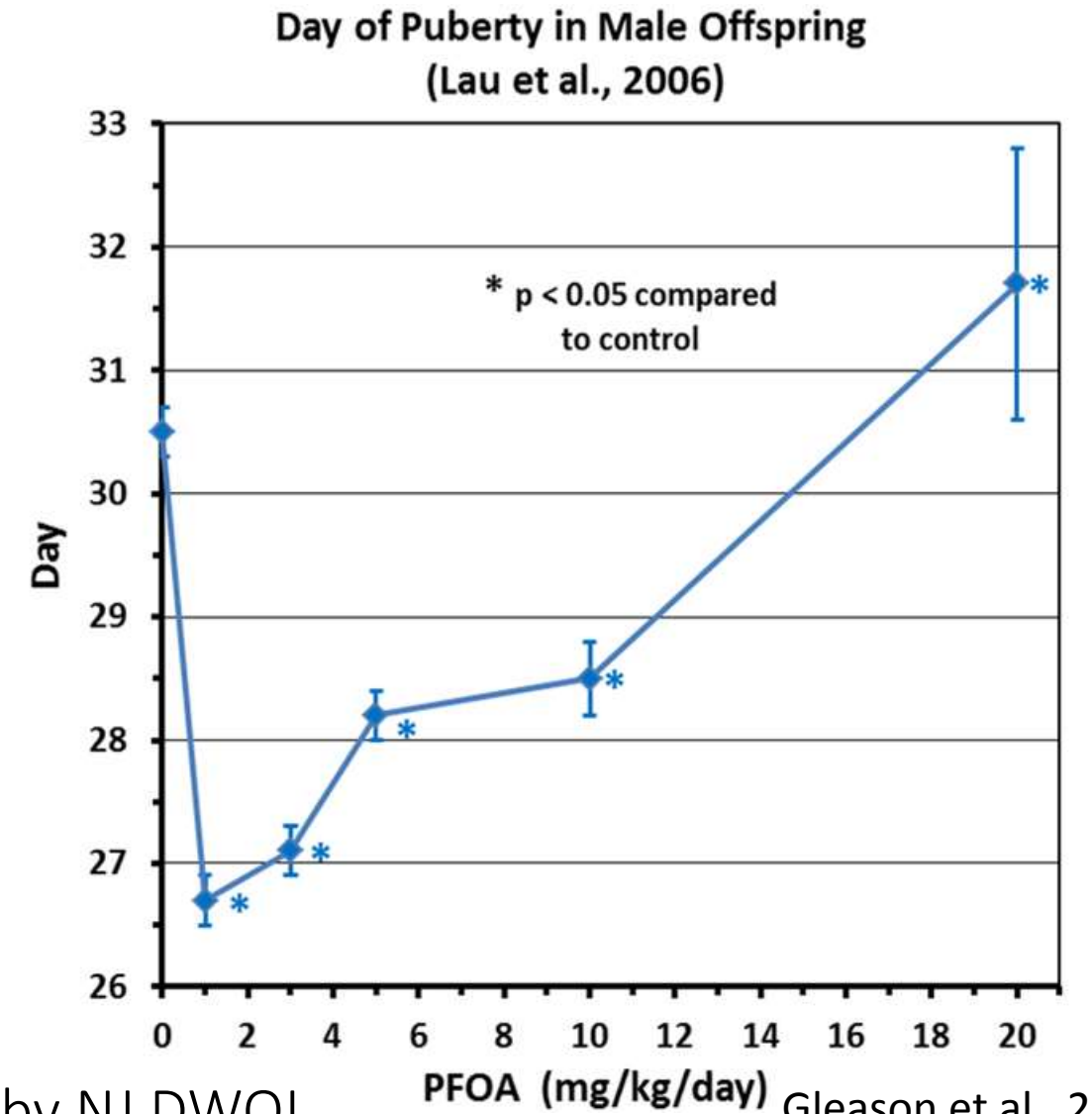
Note. Data represent means ± SE of litters examined as indicated. One-way ANOVA indicates significant differences ($p < 0.05$) in number of live fetuses and prenatal loss. Asterisks denote significant differences from controls ($p < 0.05$) by Fisher's exact test for full litter resorptions (FLR) and by Dunnett's *t*-test for other parameters.

Later on, growth caught up, so no long term functional consequence.

Lau et al., 2006

Developmental Delay: Phalanges Ossification





Data graphed by NJ DWQI

Gleason et al., 2016

Developmental Delay: Phalanges Ossification

- Showed pubertal effects and delay in phalanges ossification at low dose (1mg/kg/day).
  - No functional consequence for this delay in phalanges ossification
  - Dose-response is non-monotonic.
    - Difficult to explain biologically
  - No mechanism of action proposed.
  - Human relevance is unclear.
  - Lack of repetition.

Common theme for low-dose PFOA/PFOS

- It does provide evidence of physiological effects at low levels.
  - Precautionary principle

Hepatotoxicity: Relative Liver Weight

Liver and kidney weights and liver and kidney to body weight ratios in rats and mice treated with various forms of APFO

Dose (mg/kg)	Test material	Rats				Mice			
		Liver weight (g)	Liver/body weight (g/100 g)	Kidney weight (g)	Kidney/body weight (g/100 g)	Liver weight (g)	Liver/body weight (g/100 g)	Kidney weight (g)	Kidney/body weight (g/100
0	Linear/branched	11.18 ± 1.78[a]	3.43 ± 0.40	2.84 ± 0.25	0.88 ± 0.05	1.64 ± 0.11	5.14 ± 0.27	0.50 ± 0.04	1.57 ± 0.16
	Linear	12.09 ± 0.96	3.63 ± 0.35	2.89 ± 0.27	0.87 ± 0.06	1.70 ± 0.12	5.52 ± 0.24	0.56 ± 0.05	1.84 ± 0.16
	Branched	11.05 ± 1.43	3.38 ± 0.29	2.63 ± 0.29	0.80 ± 0.06	1.62 ± 0.14	5.14 ± 0.31	0.52 ± 0.04	1.64 ± 0.16
0.3	Linear/branched	11.57 ± 0.99	3.63 ± 0.23	2.76 ± 0.21	0.86 ± 0.05	1.92 ± 0.14	6.12 ± 0.25	0.54 ± 0.07	1.73 ± 0.18
	Linear	12.54 ± 1.09	3.86 ± 0.14	2.72 ± 0.59	0.83 ± 0.15	2.00 ± 0.11	6.45 ± 0.23*	0.53 ± 0.03	1.71 ± 0.09
	Branched	11.73 ± 1.52	3.58 ± 0.27	2.72 ± 0.34	0.83 ± 0.07	1.95 ± 0.19	6.12 ± 0.34*	0.55 ± 0.04	1.72 ± 0.15
1	Linear/branched	12.72 ± 1.00	3.94 ± 0.20*	2.91 ± 0.31	0.90 ± 0.07	2.55 ± 0.15	7.92 ± 0.49	0.54 ± 0.06	1.66 ± 0.15
	Linear	13.41 ± 2.16	4.13 ± 0.33*	2.83 ± 0.17	0.88 ± 0.08	2.73 ± 0.14*	8.33 ± 0.31*	0.56 ± 0.08	1.72 ± 0.19
	Branched	13.14 ± 1.75*	3.91 ± 0.33*	2.79 ± 0.31	0.83 ± 0.06	2.53 ± 0.26	7.85 ± 0.85*	0.55 ± 0.05	1.69 ± 0.12
3	Linear/branched	17.92 ± 2.33*	3.94 ± 0.20*	3.14 ± 0.23*	0.96 ± 0.06*	3.47 ± 0.35*	10.72 ± 0.63*	0.54 ± 0.07	1.66 ± 0.15
	Linear	15.42 ± 1.20*	3.94 ± 0.20*	2.88 ± 0.29	0.92 ± 0.07	3.70 ± 0.49*	11.37 ± 1.06*	0.57 ± 0.09	1.74 ± 0.18
	Branched	14.02 ± 1.24*	3.94 ± 0.20*	2.70 ± 0.25	0.85 ± 0.06	4.03 ± 0.32*	11.94 ± 0.83*	0.53 ± 0.06	1.58 ± 0.18
10	Linear/branched	16.29 ± 1.72*	5.67 ± 0.52*	2.62 ± 0.27	0.91 ± 0.06	4.61 ± 0.42*	16.27 ± 1.05*	0.45 ± 0.04	1.60 ± 0.08
	Linear	18.81 ± 1.85*	6.03 ± 0.41*	2.94 ± 0.23	0.94 ± 0.06	4.83 ± 0.71*	17.18 ± 1.13*	0.46 ± 0.08*	1.63 ± 0.17*
	Branched	18.55 ± 2.81*	5.77 ± 0.75*	2.84 ± 0.27	0.88 ± 0.07*	5.90 ± 0.63*	17.71 ± 1.81*	0.54 ± 0.04	1.63 ± 0.10
30	Linear/branched	17.67 ± 1.54*	6.82 ± 0.56*	2.49 ± 0.19*	0.96 ± 0.08*	4.56 ± 0.58*	18.28 ± 1.57*	0.40 ± 0.05*	1.60 ± 0.14
	Linear	17.41 ± 1.59*	6.64 ± 0.51*	2.42 ± 0.19*	0.92 ± 0.07	4.64 ± 0.51*	17.96 ± 1.06*	0.44 ± 0.06*	1.70 ± 0.17
	Branched	19.82 ± 2.62*	6.37 ± 0.70*	2.72 ± 0.21	0.88 ± 0.05*	6.51 ± 0.65*	21.01 ± 1.40*	0.48 ± 0.05	1.57 ± 0.11

* Statistically significant difference from control ($p < 0.05$).

[a] Mean ± standard deviation.

Loveless et al., 2006

Hepatotoxicity: Relative Liver Weight

Body and Liver Weights of Female CD-1 Mice

	Control (n)	0.01 mg/kg (n)	0.1 mg/kg (n)	0.3 mg/kg (n)	1.0 mg/kg (n)
Body Weight (g)					
PND 21	11.9 ± 0.2 (20)	12.1 ± 0.2 (22)	12.5 ± 0.3 (22)	11.6 ± 0.3 (22)	10.9 ± 0.2 (21)
PND 35	23.1 ± 0.3 (17)	22.9 ± 0.5 (16)	23.0 ± 0.4 (14)	22.2 ± 0.3 (17)	21.8 ± 0.4 (16)
PND 56	26.6 ± 0.8 (9)	27.7 ± 0.7 (14)	27.6 ± 0.2 (8)	25.69 ± 0.83 (4)	28.5 ± 0.7 (9)
Net Body Weight (g)					
PND 21	11.3 ± 0.2 (19)	11.5 ± 0.2 (22)	11.9 ± 0.3 (22)	11.1 ± 0.3 (22)	10.3 ± 0.2 (21)*
PND 35	22.0 ± 0.3 (17)	21.7 ± 0.4 (16)	21.9 ± 0.4 (14)	21.1 ± 0.3 (17)	20.7 ± 0.3 (16)*
PND 56	25.2 ± 0.8 (9)	26.3 ± 0.7 (13)	26.3 ± 0.2 (8)	25.6 ± 0.5 (10)	27.1 ± 0.6 (9)
Absolute Liver Weight (g)					
PND 21	0.60 ± 0.02 (19)	0.62 ± 0.02 (22)	0.61 ± 0.02 (22)	0.59 ± 0.02 (22)	0.62 ± 0.02 (21)
PND 35	1.16 ± 0.03 (17)	1.14 ± 0.04 (16)	1.13 ± 0.04 (14)	1.13 ± 0.02 (17)	1.13 ± 0.04 (16)
PND 56	1.36 ± 0.05 (9)	1.35 ± 0.04 (13)	1.29 ± 0.03 (8)	1.22 ± 0.02 (10)	1.36 ± 0.07 (9)
Relative Liver					
PND 21	0.051 ± 0.002 (19)	0.051 ± 0.001 (22)	0.049 ± 0.001 (22)	0.051 ± 0.001 (22)	0.057 ± 0.001 (21)*
PND 35	0.050 ± 0.001 (17)	0.050 ± 0.002 (16)	0.049 ± 0.002 (14)	0.051 ± 0.001 (17)	0.051 ± 0.001 (16)
PND 56	0.052 ± 0.002 (9)	0.048 ± 0.003 (13)	0.047 ± 0.001 (8)	0.046 ± 0.001 (10)*	0.048 ± 0.001 (9)

Data are represented as mean ± SEM. Significance observed in comparison to control;

* $p \leq 0.05$

Net Body Weight= Body weight (g) – Liver Weight (g); Relative Liver weight = Body weight (g)/Liver weight (g)

(n) = # of animals per dose group, n= 8-22

Tucker et al., 2015

Hepatotoxicity: Relative Liver Weight

Body and Liver Weights of Female C57Bl/6 Mice

	Control (n)	0.01 mg/kg (n)	0.1 mg/kg (n)	0.3 mg/kg (n)	1.0 mg/kg (n)
Body Weight (g)					
PND 21	8.4 ± 0.4 (6)	8.6 ± 0.1 (4)	9.5 ± 0.9 (2)	8.2 ± 0.7 (5)	7.5 ± 0.3 (5)
PND 61	19.1 ± 0.3 (9)	19.8 ± 0.3 (5)	20.1 ± 0.5 (3)	20.1 ± 0.4 (9)	19.9 ± 0.5 (8)
Net Body Weight (g)					
PND 21	8.0 ± 0.4 (6)	8.3 ± 0.03 (4)	9.1 ± 0.9 (2)	7.9 ± 0.6 (5)	7.1 ± 0.3 (5)
PND 61	18.2 ± 0.2 (9)	18.8 ± 0.3 (5)	19.2 ± 0.5 (3)	19.2 ± 0.3 (9)	19.0 ± 0.4 (8)
Absolute Liver Weight (g)					
PND 21	0.37 ± 0.03 (6)	0.43 ± 0.04 (4)	0.45 ± 0.03 (3)	0.38 ± 0.03 (6)	0.39 ± 0.01 (5)
PND 61	0.93 ± 0.02 (9)	0.97 ± 0.03 (5)	0.90 ± 0.05 (3)	0.95 ± 0.05 (9)	0.89 ± 0.03 (8)
Relative Liver					
PND 21	0.044 ± 0.002 (6)	0.049 ± 0.004 (4)	0.048 ± 0.001 (2)	0.045 ± 0.001 (5)	0.052 ± 0.001 (5)
PND 61	0.048 ± 0.001 (9)	0.049 ± 0.001 (5)	0.045 ± 0.002 (3)	0.047 ± 0.002 (9)	0.045 ± 0.002 (8)

Data are represented as mean ± SEM

Net Body Weight= Body weight (g) – Liver Weight (g); Relative Liver weight = Body weight (g)/Liver weight (g)

(n) = # of animals per dose group; N= 2-9

Tucker et al., 2015

Developmental Delay: Mammary Gland

- Tucker et al. show delay in mammary gland development due to *in utero* exposure to PFOA at doses lower than phalanges ossification (.01mg/kg)
  - Using .01mg/kg as a PoD would mean setting a limit of in the ppq (per quadrillion).
- EPA dismisses due to 1) No functional consequence. 2) Unknown mechanism of action. 3) Lack of repetition.
- EPA used phalanges development as a basis for their health advisory.
  - All of their criticism is equally applicable.

CD-1

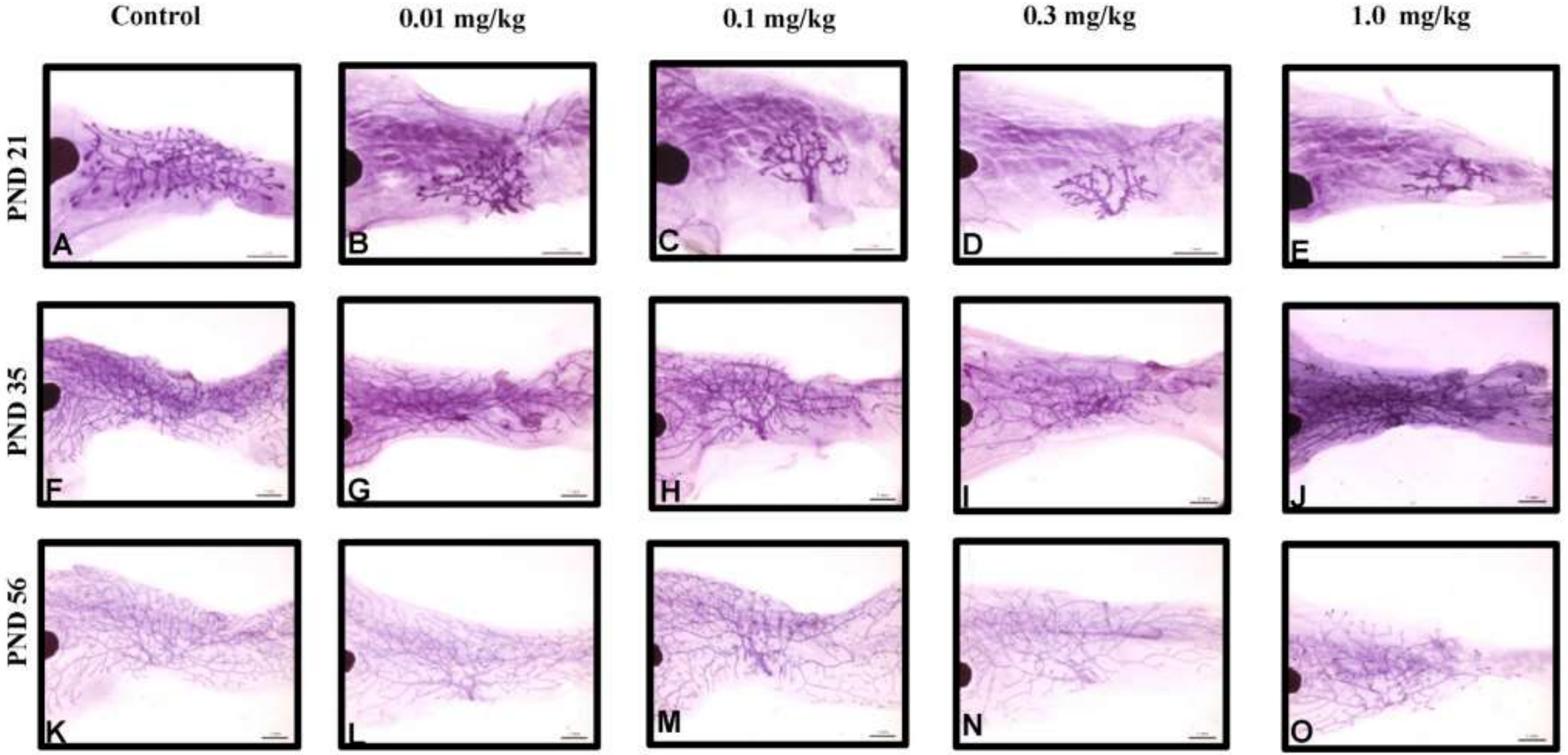


Fig. 2. Mammary whole mount assessment of early and late pubertal glands in CD-1 offspring. Representative image of control (A) PND 21, (F) PND 35, and (K) PND 5
0.01 mg/kg (B) PND 21, (G) PND 35 and (L) PND 56, 0.1 mg/kg (C) PND 21, (H) PND 35 and (M) PND 56; 0.3 mg/kg (D) PND 21, (I) PND 35 and (N) PND 56 and 1.0 mg/kg (
PND 21, (J) PND 35 and (O) PND 56. CD-1 $n = 4–11$ litters/treatment group. Significant inverse trends were noted between developmental scores and PFOA dose, indicati
higher PFOA exposure was related to lower (more severe) developmental scores ($p < 0.05$).

Tucker et al., 2015

Table 5
Mammary gland developmental scores.

	Control (*n*)	0.01 mg/kg (*n*)	0.1 mg/kg (*n*)	0.3 mg/kg (*n*)	1.0 mg/kg (*n*)
CD-1					
PND 21	2.9 ± 0.1 (19)	2.4 ± 0.1 (22)	2.3 ± 0.1 (22)**	2.0 ± 0.1 (21)***	1.7 ± 0.1 (21)****
PND 35	3.1 ± 0.1 (16)	2.3 ± 0.2 (17)**	2.2 ± 0.2 (14)**	2.3 ± 0.1 (16)**	1.9 ± 0.2 (14)****
PND 56	3.3 ± 0.1 (9)	2.3 ± 0.2 (13)**	2.5 ± 0.2 (8)*	2.2 ± 0.1 (10)**	1.9 ± 0.2 (9)****
C57Bl/6					
PND 21	2.9 ± 0.2 (7)	2.5 ± 0.4 (5)	2.1 ± 0.7 (2)	1.8 ± 0.3 (6)*	1.8 ± 0.2 (5)*
PND 61	2.8 ± 0.2 (10)	2.2 ± 0.2 (5)	2.6 ± 0.1 (3)	2.1 ± 0.1 (10)*	1.7 ± 0.1 (8)***

Data are represented as mean ± SEM. Significance observed in comparison to control. Mammary glands scored between 1 (poor development) and 4 (best development). Individual pup scores were averaged and are represented by the mean values for each treatment group. (*n*) = # of animals per dose group; CD-1 *n* = 8–19 and C57Bl/6 *n* = 2–10.

* $p \leq 0.05$.
** $p \leq 0.01$.
*** $p \leq 0.001$
**** $p \leq 0.0001$.

Lactation is just fine.
Offspring are not malnourished.
Human impact?

Tucker et al., 2015

PFAS and Breastfeeding

- Animal studies show that PFAS can influence breast development and lactation hormones
- PFOA/PFOS exposure during pregnancy associated with decreased breastfeeding duration
  - Doubling in PFOA associated with 0.5 month (95% CI: 0.3, 0.7) reduction in exclusive breastfeeding duration

Fei et al., 2010, Romano et al., 2016, Tucker et al. 2015, Yang et al. 2009

Slide courtesy of Joe Braun

Immunosuppression

- Several animal studies report a decrease in antibody production and spleen/thymus weights at high doses.
  - These effects rapidly disappear after cessation of exposure.

- Concern that it could reduce effectiveness of vaccines.

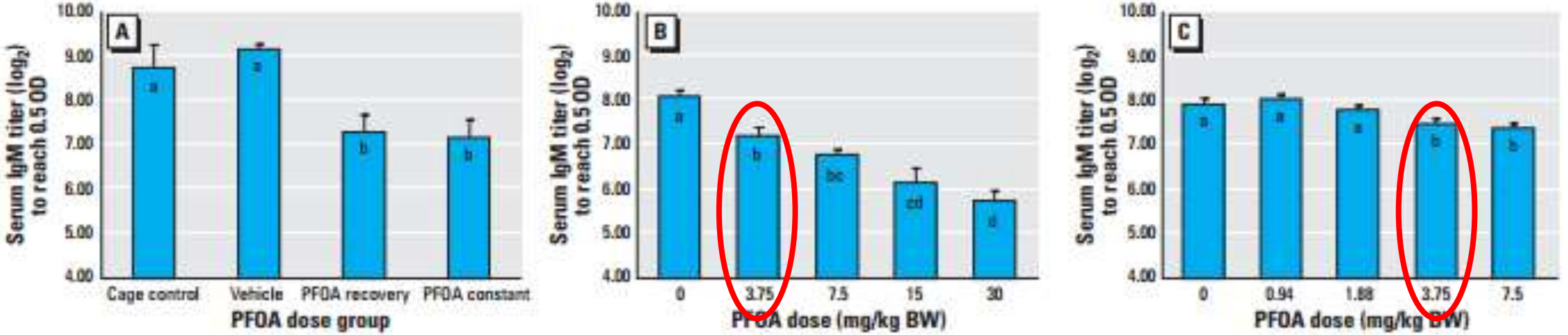


Figure 3. Effects of PFOA exposure on SRBC-specific IgM antibody titers (mean ± SE) in female C57BL/6J (*A*) or C57BL/6N (*B,C*) mice. OD, optical density. (*A*) PFOA was given for 10 days (PFOA recovery) or 15 days (PFOA constant) via gavage; IgM antibody titers were suppressed in both exposed groups compared with controls. (*B,C*) PFOA was given for 15 days via drinking water. (*B*) IgM antibody titers were suppressed relative to controls for all tested doses. (*C*) IgM antibody titers were suppressed relative to controls at the two highest doses. Means with different letters are statistically different ($p < 0.05$).

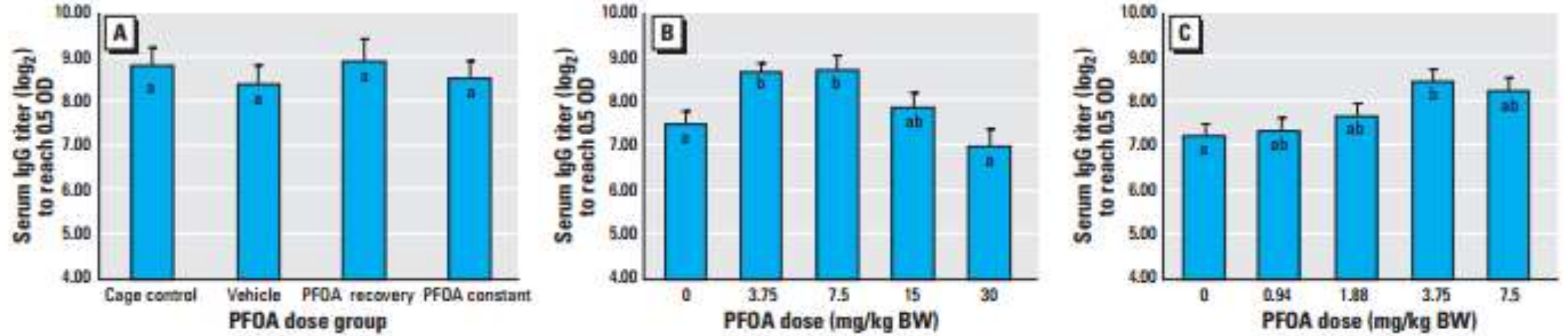


Figure 4. Effects of PFOA exposure on SRBC-specific IgG antibody titers (mean ± SE) in female C57BL/6J (*A*) or C57BL/6N (*B,C*) mice. OD, optical density. (*A*) PFOA (30 mg/kg) was given for 10 days (PFOA recovery) or 15 days (PFOA constant) via gavage. No statistical effect on IgG antibody titers was detected. (*B, C*) PFOA given for 15 days via drinking water. (*B*) IgG antibody titers were elevated compared with controls at 3.75 and 7.5 mg/kg. (*C*) IgG antibody titers were elevated compared with controls at 3.75 mg/kg. Means with different letters are statistically different ($p < 0.05$)

DeWitt et al., 2008

Immunosuppression

- “Taken together, available human studies (Grandjean et al. 2012; Granum et al. 2013; Looker et al. 2014) provide some evidence of a significant association between PFOS exposure and serological vaccine responses in general. Within each study, however, most estimated associations were statistically nonsignificant, and results were inconsistent by vaccine type and by outcome classification… One issue related to use of immune biomarkers and antibody levels in human studies is whether small but statistically significant changes in these endpoints, when analyzed on a continuous scale, are clinically meaningful, particularly when most or all subjects are within the normal range” –EPA Drinking Water Health Advisory for Perfluorooctane Sulfonate (PFOS)

Conclusions

- Drinking water advisory level was driven based on developmental toxicity of PFOA/PFOS following *in utero* exposure.
  - Several studies resulted in RfD 20ng/kg/day

- Phalanges delay in ossification is one of the primary studies determining health advisory levels.

- Mammary gland development may also be altered in even lower levels, although questions around its meaning led EPA to not use the study.

- Immunosuppression also may occur at low doses but has a mild effect. Clinical studies are inconsistent.

Acknowledgements

<u>Boekelheide Lab</u>
Kim Boekelheide
Sue Hall
Sam Madnick
Angela Stermer
Dan Spade
Enrica Bianchi

This speaker is supported by National Institute of Environmental Health Sciences Training Grant [T32 ES007272] and Superfund Research Program [P42 ES013660].

<u>Brown University</u>
Jennifer Guelfo
Joe Braun

References

1. DeWitt JC, Copeland CB, Strynar MJ, and Luebke RW (2008) Perfluorooctanoic Acid–Induced Immunomodulation in Adult C57BL/6J or C57BL/6N Female Mice. *Environ Health Perspect* **116**:644–650.
2. Fei C, and Olsen J (2010) Prenatal Exposure to Perfluorinated Chemicals and Behavioral or Coordination Problems at Age 7 Years. *Environ Health Perspect* **119**:573–578.
3. Gleason, Jessie A, Keith R Cooper, Judith B Klotz, Gloria B Post, and Geroge Van Orden. 2016. "HEALTH-BASED MAXIMUM CONTAMINANT LEVEL SUPPORT DOCUMENT: PERFLUOROOCTANOIC ACID (PFOA); New Jersey Drinking Water Quality Institute Health Effects Subcommittee."
4. Lau C, Thibodeaux JR, Hanson RG, Narotsky MG, Rogers JM, Lindstrom AB, and Strynar MJ (2006) Effects of Perfluorooctanoic Acid Exposure during Pregnancy in the Mouse. *Toxicol Sci* **90**:510–518.
5. Romano ME, Xu Y, Calafat AM, Yolton K, Chen A, Webster GM, Eliot MN, Howard CR, Lanphear BP, and Braun JM (2016) Maternal serum perfluoroalkyl substances during pregnancy and duration of breastfeeding. *Environ Res* **149**:239–246.
6. Tucker DK, Macon MB, Strynar MJ, Dagnino S, Andersen E, and Fenton SE (2015) The mammary gland is a sensitive pubertal target in CD-1 and C57Bl/6 mice following perinatal perfluorooctanoic acid (PFOA) exposure. *Reprod Toxicol* **54**:26–36, NIH Public Access.
7. Yang C, Tan YS, Harkema JR, and Haslam SZ (2009) Differential effects of peripubertal exposure to perfluorooctanoic acid on mammary gland development in C57Bl/6 and Balb/c mouse strains. *Reprod Toxicol* **27**:299–306.