EPA Health Advisory for PFOA and PFOS Drinking Water

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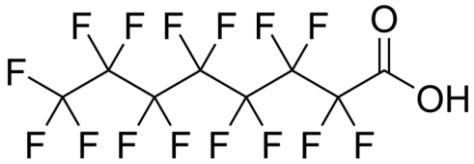
Outline

- PFOA Background
- How did the advisory come about?
- Animal Studies
  - Skeletal Variations
  - Testicular Cancer
  - Persistent Liver Damage
  - Mammary Gland Development

Perfluorinated Compounds

- Entirely manmade products including in non-stick coatings, fire fighting foams and stain resistance materials.
- Composed of an acid group (carboxylic or sulfonic) and a carbon chain tail with F instead of H.
- Resistant to chemical reactions, persists indefinitely, and water soluble.
- Slow elimination (half life of years in humans).



Perfluorooctanoic acid (PFOA, C8)

Perfluorinated Compounds: Reproductive Toxicity

- Pregnant/breastfeeding mothers are the primary sensitive populations.
  - Detected in breastmilk, umbilical cord blood, and amniotic fluid.
- At birth, infants have roughly the same serum levels of PFOA as mother.
  - But will surpass mom during the first few months due to breastmilk exposure (or water from formulae)!
- USEPA set for 70ppt for PFOA + PFOS exposure.

How did this advisory come about?

RfD = NOAEL / (UF x MF)

Reference Dose (RfD): 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)

NOAEL: No observed adverse effect level. If NOAEL isn't established, LOAEL (lowest) is sometimes used. (mg/kg/day).

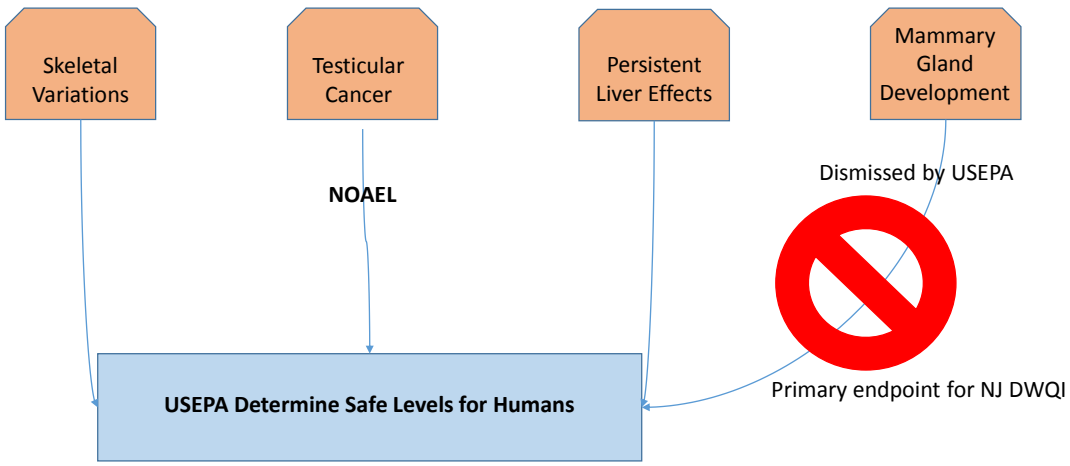
UF: Uncertainty factor to err for safety in relating animal studies to sensitive human populations.

MF: Modifying factor to be determined based on professional judgement

EPA.gov

How did this advisory come about?

Animal Study Endpoints



How did this advisory come about?

RfD (non-cancerous): 20ng/kg/day

LOAEL 1mg/kg/day based on Lau et al 2006 (skeletal variaions and accelerated puberty in males) and then applied additional UF of 10 for NOAEL extrapolation.

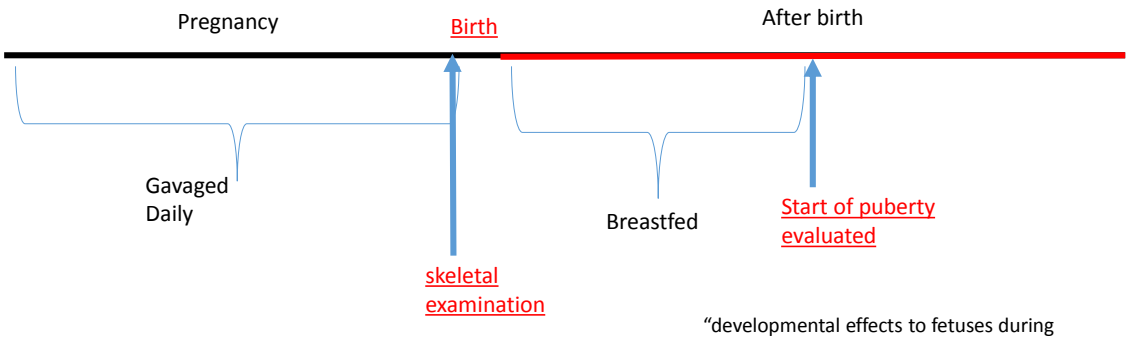
Then pharmacokinetic modeling was used to relate serum level in mice to humans to generate human equivalent doses.

Animal Models for PFAS Toxicity

- Strengths:
  - Ethically carry out controlled studies.
  - Establish mechanism.
  - Provide preliminary data to inform human studies.
- Weaknesses
  - Animals are not people.
    - Especially so in reproduction (puberty timeline, testicular cancer).
  - Half-life varies a bunch by species.
    - Humans 3.5 years
    - Cynomolgus Monkey: ~28.5 weeks Cost prohibitive
    - Mouse: 15-18 days
    - Male rat: 6-10 days
    - Female rat: 3-4 hours Maternal half-life too short for daily dosing.

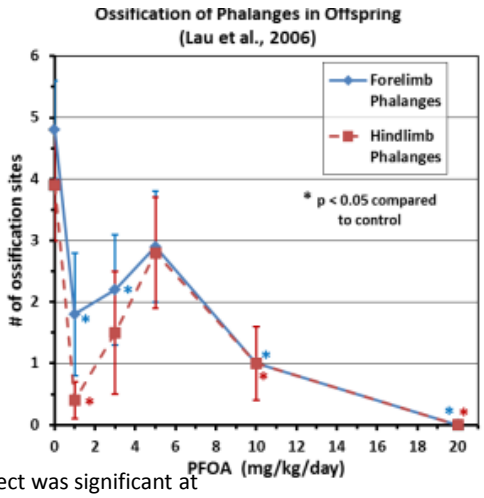
Effects of Perfluorooctanoic Acid Exposure during Pregnancy in the Mouse (Lau et al 2006 Tox Sci)

Timeline



"developmental effects to fetuses during pregnancy or to breastfed infants (e.g., low birth weight, accelerated puberty, skeletal variations)."

Skeletal Variations



Effect was significant at 1mg/kg dose (LOAEL 1)

Mouse reproductive outcome and fetal teratology, examined at term. Data represent means ±S.E 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.

	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*	-

Lau et al 2006

Fetal birth weight

Birth weight was significant at 20mg/kg dose (NOAEL of 10)

Mouse reproductive outcome and fetal teratology, examined at term. Data represent means ± S.E 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.

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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*	-
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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*	-
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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*	-

Lau et al 2006

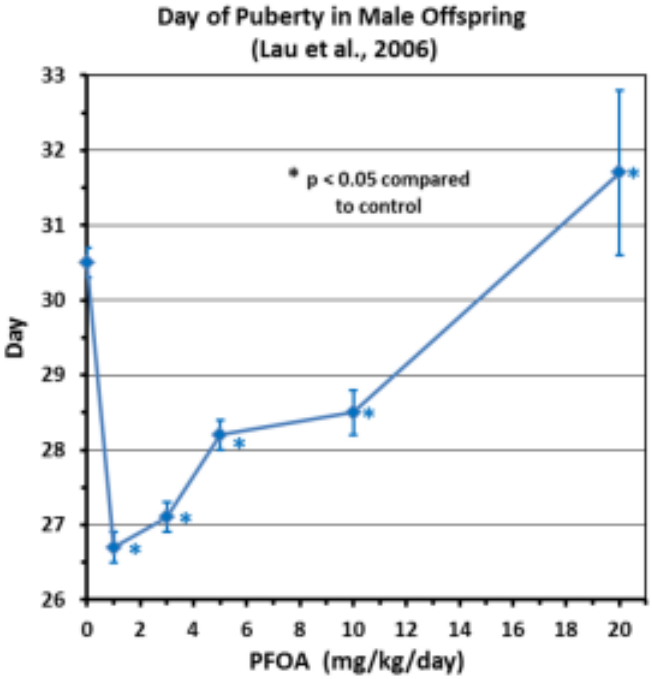
Accelerated Puberty (males)

Developmental landmarks of mouse pups exposed to PFOA *in utero*. Data represent means ± S.E. of numbers of litters (for eye opening) or individual pups (for vaginal opening, first estrus and preputial separation) examined as indicated. For eye opening, N = litter; for other landmarks, N = individual animal, numbers in parenthesis indicate litters represented. ANOVA indicate significant treatment effect in all parameters examined ($p < 0.05$). Significant differences ($p < 0.05$) between each dose group were determined by Duncan's multiple range test and are depicted by different letters (a, b, c and d).

Sign of puberty for females

Sign of puberty for males

	Eye Opening		Vaginal Opening			First Estrus		Preputial Separation		
PFOA (mg/kg)	*N*	*Age (days)*	*N*	*Age (days)*	*Body Weight (g)*	*N*	*Age (days)*	*N*	*Age (days)*	*Body Weight (g)*
0	22	14.8 ± 0.1^{a}	47 (20)	$28.4 \pm 0.3^{a,b}$	18.0 ± 0.2^{a}	47 (20)	$29.9 \pm 0.4^{a,b}$	56 (22)	30.5 ± 0.2^{a}	25.0 ± 0.3^{a}
1	8	15.2 ± 0.2^{a}	21 (8)	27.4 ± 0.5^{b}	18.2 ± 0.5^{a}	21 (8)	28.2 ± 0.6^{b}	22 (8)	26.7 ± 0.2^{b}	$20.3 \pm 0.3^{b,c}$
3	8	$15.5 \pm 0.1^{a,b}$	21 (7)	$28.8 \pm 0.4^{a,b}$	$17.7 \pm 0.4^{a,b}$	21 (7)	$30.2 \pm 0.4^{a,c}$	20 (7)	27.1 ± 0.2^{b}	$19.4 \pm 0.6^{b,c,d}$
5	17	16.0 ± 0.2^{b}	43 (16)	29.9 ± 0.4^{a}	$17.7 \pm 0.4^{a,b}$	43 (16)	31.8 ± 0.5^{c}	46 (16)	28.2 ± 0.2^{c}	$18.3 \pm 0.5^{c,d}$
10	13	17.2 ± 0.3^{c}	27 (12)	29.3 ± 0.3^{a}	16.7 ± 0.3^{b}	27 (12)	$30.2 \pm 0.3^{a,c}$	28 (11)	28.5 ± 0.3^{c}	17.5 ± 0.7^{d}
20	3	17.9 ± 0.8^{c}	8 (2)	31.3 ± 0.5^{c}	19.3 ± 0.4^{a}	8 (2)	31.3 ± 0.5^{c}	4 (2)	31.7 ± 1.1^{d}	20.8 ± 1.2^{b}



Chronic Dietary Toxicity and Carcinogenicity Study with Ammonium Perflurooctanoate in Sprague-Dawley Rats (Butenhoff et al 2012) Toxicology

Cited for testicular cancer effects.

Rats were given 0, 30 and 300ppm PFOA fed via diet for 1 or 2 years.

Table 8
Incidence of neoplastic microscopic findings for male and female rats in either control groups or groups fed 30 ppm or 300 ppm APFO in their diet for 2 years.

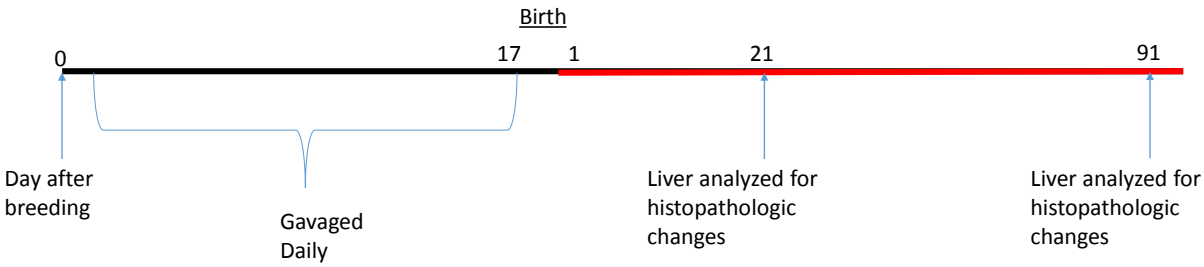
Organ/lesion	Dietary dose group (ppm APFO)					
	Males			Female		
	0	30	300	0	30	300
Adrenal						
Pheochromocytoma, benign	2/49 (4)[a]	4/50 (8)	4/50 (8)	2/50 (4)	0/50 (0)	0/49 (0)
Pheochromocytoma, malignant	0/49 (0)	1/50 (2)	0/50 (2)	0/50 (0)	0/50 (0)	1/49 (2)
Liver						
Hepatocellular adenoma	0/49 (0)	0/50 (0)	0/50 (0)	0/50 (0)	0/50 (0)	0/50 (0)
Hepatocellular carcinoma	3/49 (6)	1/50 (2)	5/50 (10)	0/50 (0)	0/50 (0)	1/50 (2)
Mammary gland						
Adenocarcinoma	–	–	–	7/46 (15)	14/45 (31)	5/44 (11)
Adenoma	–	–	–	3/46 (7)	0/45 (0)	0/44 (0)
Carcinoma	–	–	–	1/46 (2)	0/45 (0)	0/44 (0)
Fibroadenoma	–	–	–	10/46 (22)	19/45 (42)	**21/44 (48)***
Lymphangiosarcoma	–	–	–	0/46 (0)	0/45 (0)	1/44 (2)
Reevaluation by PWG[b]						
Adenocarcinoma				9/50 (18)	16/50 (32)[c]	5/50 (10)[c]
Adenoma				1/50 (2)	0/50 (0)	0/50 (0)
Fibroadenoma				16/50 (32)	16/50 (32)	20/50 (40)
Fibroadenoma (multiple)				2/50 (4)	6/50 (12)	3/50 (6)
Pituitary						
Adenoma	17/48 (35)	17/47 (36)	13/46 (28)	33/46 (72)	39/47 (83)	36/50 (72)
Testes/epididymis						
Leydig cell adenoma	0/49 (0)	2/50 (4)	**7/50 (14)***	–	–	–
Thyroid						
C-cell adenoma	0/43 (0)	2/47 (4)	4/47 (9)	1/50 (2)	0/45 (0)	0/41 (0)
C-cell carcinoma	2/43 (5)	0/47 (0)	0/47 (0)	0/50 (0)	0/45 (0)	0/41 (0)

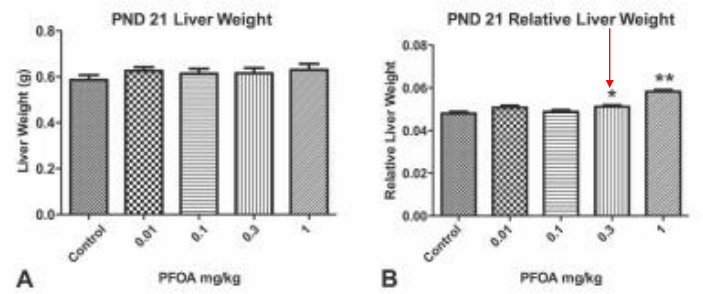
Leydig Cell Tumor

Bolded values are statistically significant.
* Statistically significantly different from controls ($p \leq 0.05$).
[a] Number observed/number examined (%).
[b] Hardisty et al. (2010).
[c] The incidence in the groups sharing this footnote were statistically significantly different from each other ($p < 0.01$, Hardisty et al., 2010).

Hepatic mitochondrial alteration in CD1 mice associated with prenatal exposures to low doses of perfluorooctanoic acid (PFOA) (Quist et al 2015) Toxicol Pathol

- Design: Pregnant CD-1 mice were exposed to 0, 0.01, 0.1, 0.3, and 1mg/kg PFOA by gavage daily from GD1-17.



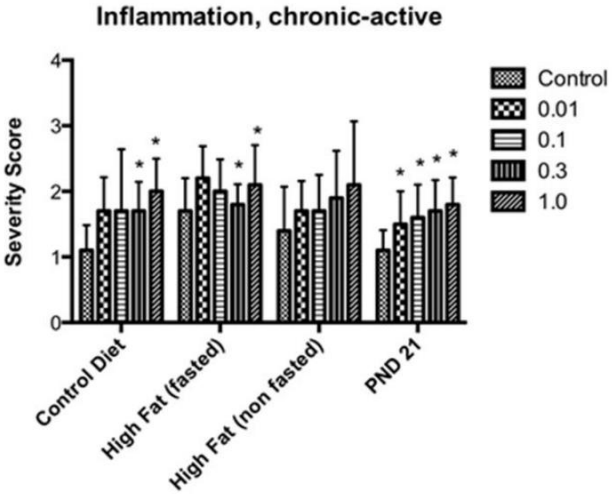


Increase in liver weight to body weight ratio.

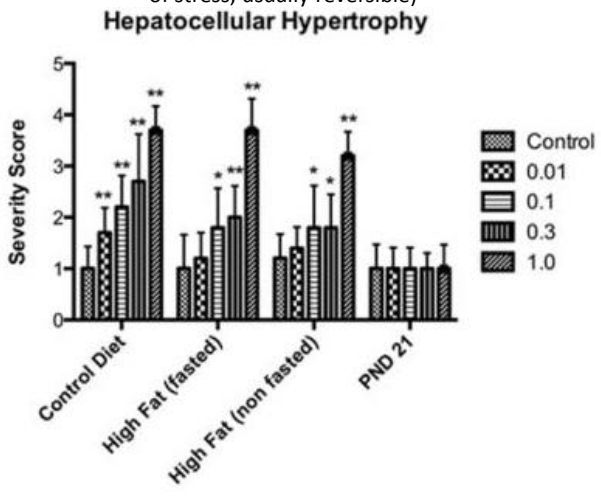
Figure 3.
Liver weight and relative liver weight of PND 21CD-1 mice. Mean liver weights (g) and relative liver weights (liver:body weight ratio) in female offspring following prenatal exposure to PFOA. All pups consumed Purina 5001 diet at this life stage. Data presented as mean ± SEM. n =10/dose group. Significant treatment effect compared with controls (*p≤0.05, **p≤0.01).

Dose-related Chronic Inflammation and Hypertrophy

Chronic inflammation (cancer risk)

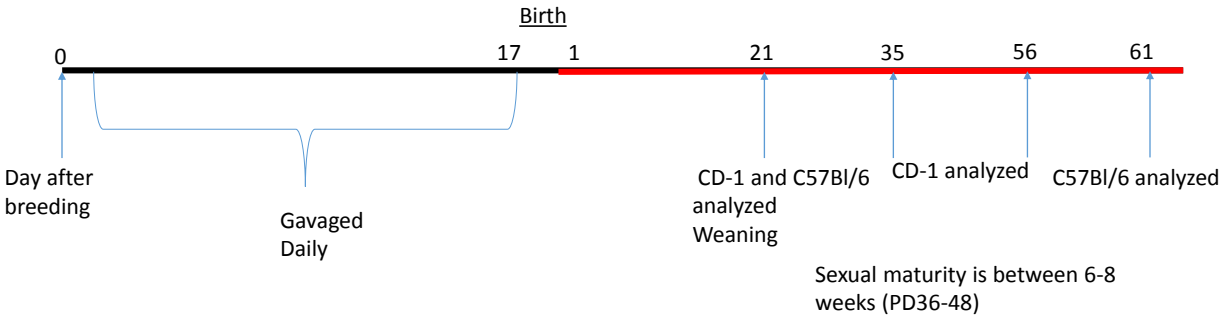


Increase cell size (classic sign of stress, usually reversible)

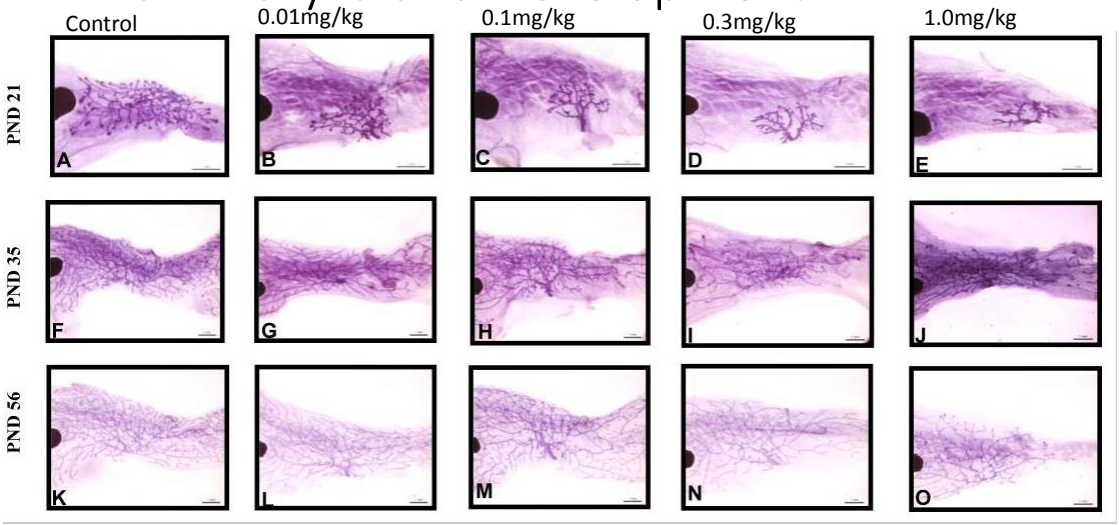


The mammary gland is a sensitive pubertal target in CD-1 and C57Bl/6 mice following perinatal perfluorooctanoic acid (PFOA) exposure (Tucker et al 2015) Repro Tox

Pregnant CD-1 and C57Bl/6 mice were exposed to 0, 0.01, 0.1, 0.3, 1.0mg/kg PFOA for GD1-17. Female offspring were analyzed for mammary gland development.



Mammary Gland Development



CD-1

Mammary Gland Development

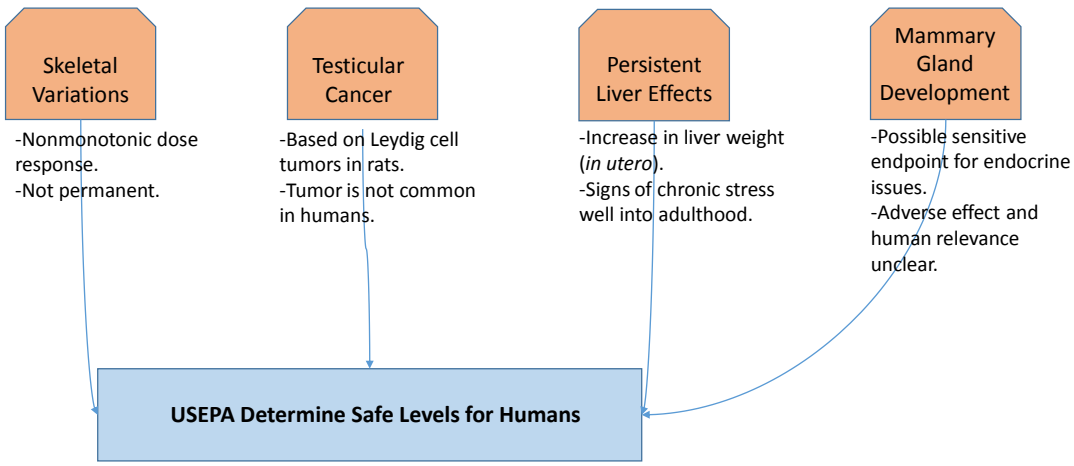
Mammary Gland Developmental Scores

	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. 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.

Relation to humans and toxicity are controversial.

Animal Study Conclusions



Summary

- Mouse represent a decent animal model for PFAS exposure due to longer elimination.
- Animal studies claiming toxicity from low dose PFOA *in utero* exposure is based on several endpoints
  - skeletal variations, low birth weight, accelerated puberty development (Lau et al), testicular cancer (Leydig cell tumor, Butenhoff et al), lasting liver effects (Quist et al) and other effects.
- The impact of *in utero* PFOA exposure on mouse mammary gland development (Tucker et al) remains controversial.
  - Accepted by NJDWQI but not USEPA

National Institute of Environmental Health Sciences
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2. Butenhoff JL, Kennedy GL Jr, Chang SC, Olsen GW. "Chronic dietary toxicity and carcinogenicity study with ammonium perfluorooctanoate in Sprague-Dawley rats."Toxicology. 2012 Aug 16;298(1-3):1-13.

3. Quist EM, Filgo AJ, Cummings CA, Kissling GE, Hoenerhoff MJ, Fenton SE. "Hepatic Mitochondrial Alteration in CD-1 Mice Associated with Prenatal Exposures to Low Doses of Perfluorooctanoic Acid (PFOA)."Toxicol Pathol. 2015 Jun;43(4):546-57.

4. Tucker DK, Macon MB, Strynar MJ, Dagnino S, Andersen E, Fenton SE. "The mammary gland is a sensitive pubertal target in CD-1 and C57Bl/6 mice following perinatal perfluorooctanoic acid (PFOA) exposure " Reprod Toxicol. 2015 Jul;54:26-36