



Toxicology – Perfluorocarboxylates

- PFOA**
- PFH_xA**
- PFBA (J. Butenhoff)**

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Perfluorooctanoate (PFOA)



Pharmacokinetics - PFOA

- Readily absorbed after oral intake
- Distributed primarily to blood and liver
- Highly protein bound in serum (albumin)
- Not metabolized
- Enterohepatic recirculation
- Species and sex (within species) differences in urinary elimination



Pharmacokinetics - PFOA

➤ Elimination half-lives:

- Female rat: 2-15 **hours**
- Male rat: 4-6 days
- Male monkey: ~ 21 days
- Female monkey ~ 30 days
- Male dog: 20-23 days
- Female dog: 8-13 days
- Retired workers 3.5 **years**
- Community Study 2.3 **years**



Acute Toxicity - PFOA

- Moderate acute oral toxicity (LD50 ~ 200 - 1800 mg/kg)
- Moderate inhalation toxicity (LD50 980 mg/m³ as dust or condensation aerosol)
- Low dermal toxicity (LD50 4300 - > 7000 mg/kg)



Repeat-Dose Toxicity (Rats) - PFOA

➤ Rat studies

Days	Dietary dose (ppm)	mg/kg
28	30-30,000	1.5-1,500
90	10-1000	0.5-50
90	1-100	0.05-5

Outcomes of 90-day study:

- BW at 5 mg/kg
- Hepatocellular hypertrophy at ≥ 0.5 mg/kg
- Increased PCoA oxidase at ≥ 1.5 mg/kg
- NOAEL = 0.05-0.5 mg/kg

Repeat-Dose Toxicity (Monkey) – PFOA

(Butenhoff et al., 2002)

➤ **Six-Month Monkey (male cynomolgus) Study**

➤ **Doses: oral – capsule**

<u>mg/kg</u>	<u>serum (ppm)</u>
3	77±39
10	86±33
30/20	158±100

➤ **Results:**

- Steady state within 4-6 weeks
- Liver weight increases at all treatment levels
- Body-weight loss at 30 mg/kg and later at 20 mg/kg
- One unscheduled death at 30 mg/kg (Day 29) believed to be attributable to treatment
- One unscheduled death at 3 mg/kg, equivocal as to relationship to treatment



Repeat-Dose Toxicity - PFOA

- Reduced body-weight gain
- Body-weight loss
- Increased liver weight (absolute and relative)
- Hepatocellular hypertrophy and necrosis
- Lipid alterations, decreased cholesterol and triglycerides (primarily in mouse, not in monkey)
- Steep dose-response



Summary of Early Developmental Studies – 1980's

➤ Rat pilot study – lens abnormalities reported;

- Subsequently proven to be an artifact of sectioning technique
(*Staples, 1985*)

➤ Four follow-up studies:

- Rat oral – 150 mg/kg (Gortner, 1981)
- Rabbit oral – 50 mg/kg (Gortner, 1982)
- Rat oral – 100 mg/kg
- Rat inhalation – 21 mg/m³ (*Staples et al., 1984*)

➤ Conclusions:

- Maternal effects: ↓body weight, death
- No embryofetal toxicity or developmental abnormalities in the offspring



Reproduction and Postnatal Development in Rats (Butenhoff et al., 2004)

- Two-Generation Study - dose levels 1, 3, 10, and 30 mg/kg:
 - No effect on reproductive function
 - Significant mortality in F1 pups (30 mg/kg-day dose level)
 - Days 5 – 22 of lactation
 - First four days post-weaning
 - Males and females
 - Delayed sexual maturation (F1)
 - Effects on birth weight (F1 in 30 mg/kg-day group)
- NOAELs: Reproduction > 30 mg/k; Development = 10 mg/kg;
Adult toxicity = < 1 mg/kg



Benchmark Dose Estimates for Developmental Endpoints in Rats

Mean 24-hour serum PFOA concentration associated with the lower 95% confidence limit of the benchmark dose (calculated from *Butenhoff et al., 2004*)

Endpoint	LBMD₁₀ (mg/kg-d)	LBMIC₁₀(ppb)
Days to preputial separation	22	29,000
Post-lactational mortality in female pups	22	29,000
Post-lactational mortality in male pups	24	32,000
Days to vaginal patency	30	40,000
Pre-weaning mortality (both sexes)	34	45,000
Day 22 pup weight (both sexes)	44	59,000



Developmental Toxicity in Mice (Lau et al., 2006)

- Mice – Daily dose throughout gestation
 - Doses (oral gavage): 1, 3, 5, 10, 20, 40 mg/kg-day
 - Maternal NOAEL = <1 mg/kg-day
 - * Maternal effects: liver weights at 1 mg/kg; body weight at 5 mg/kg, resorptions at 10 mg/kg
 - Fetal NOAEL = 3 mg/kg - day
 - * Fetal effects: growth retardation 5 mg/kg; stillbirth neonatal mortality 10 mg/kg; skeletal delayed ossification 10 mg/kg; fetal weight at 20 mg/kg

Notes: 40 mg/kg – total litter resorption

No teratogenic effects

Fetal weights return to normal (wk 6 females, wk 13 males)



Benchmark Dose Estimates for Developmental Endpoints in Mice

Estimated serum PFOA concentration associated with the lower 95% confidence limit of the benchmark dose (calculated from *Lau et al., 2006*)

	BMDL ₅ (mg/kg)	BMDL ₅ (ppb)
Maternal weight gains during pregnancy	3.58	50,000
Maternal liver weight at term	0.170	6,000
Live fetus weight at term	4.3	60,000
Fetal forelimb phalanges ossification at term	0.643	12,000
Fetal hindlimb phalanges ossification at term	0.616	12,000
Neonatal survival at weaning (PD 23)	1.09	19,000
Neonatal body weight at weaning (PD 23)	0.86	16,000
Neonatal eye opening	2.10	32,000



PFOA – Induced Developmental Toxicity in the Mouse is Dependent on Expression of PPAR α (Abbott et al., 2007)

- 129S1/SvImJ wild type (WT) compared to PPAR α knockout (KO) mice
- Oral doses 0, 0.1, 0.3, 0.6, 1, 3, 5, 10 or 20 mg/kg GD 1-17
- No effect on maternal weight, embryonic implantation or number or weight of pups at birth
- Increased litter resorptions both WT and KO at 5 mg/kg
- In WT but not KO:
 - Neonatal survival reduced (≥ 0.6 mg/kg)
 - Eye opening delayed (≥ 1 mg/kg)
- Maternal relative liver weight increase:
 - ≥ 1 mg/kg WT
 - ≥ 3 mg/kg KO
- Conclusions:
 - Delayed eye opening and postnatal weight gain appear to be PPAR α dependent
 - Early pregnancy loss PPAR α independent (only at ≥ 5 mg/kg)



Conclusions – Reproduction and Developmental Effects in Rodents

- **No effects on reproductive function**
- **No developmental malformations observed**
- **Fetal effects observed occur at doses which are toxic to maternal animals and/or appear to be PPAR α dependent**
- **Fetal effects occur at serum concentrations that are thousands of times higher than serum concentrations in the general population (~ 5 ppb)**



No Genotoxicity Observed - PFOA

- *S. typhimurium* strains TA1535, TA1537, TA1538, TA98, and TA100
- Chromosomal aberrations in CHO cells
- Chromosomal aberrations in human lymphocytes
- The *in vivo* mouse micronucleus assay
- C3H 10T1/2 cell transformation



SAB PFOA Final Report

Issued May 31, 2006

- **In general, the SAB endorsed EPA's risk assessment approach, particularly:**
 - the inclusion of non-cancer health endpoints, and
 - the use of PFOA blood levels as a measure of estimated dose.

The SAB strongly urged EPA to consider peer reviewed new information to strengthen the risk assessment.

EPA's Comment:

“The SAB Panel's input will be extremely valuable as EPA continues to develop a full and comprehensive assessment of the risks associated with PFOA. In the year and a half since the draft assessment was submitted to the SAB Panel, a considerable amount of additional research has been initiated, and some has been completed. Some of this new research may impact the Panel's assessment of PFOA. For this reason, **it is premature to draw any conclusions on the potential risks, including cancer, from PFOA until all of this new testing is complete and the data are integrated into the risk assessment.”**

www.epa.gov/oppt/pfoa/pfoarisk.htm



Chronic Toxicity in Sprague-Dawley Rats

3M Study (Sabinski, 1987)

- Doses of 30 & 300 ppm PFOA in diet, males and females
- Interim sacrifice group at one year
- Two-year chronic toxicity and cancer study

DuPont Study (Biegel et al., 2001)

- Two-year mechanistic study
- Single dose of 300 ppm, males only
- Interim sacrifices at multiple times



Tumor Incidence (Rats)

Dose level (ppm in diet):	0	30	300
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Dose level (estimated mg/kg-day):	0	1.3	14
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➤ *3M Study* (Sabinski, 1987)

Leydig cell adenoma	0/44	2/44	7/48*
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➤ *DuPont Study* (Biegel et al., 2001)

Hepatocellular adenoma	1/79		10/79*
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Leydig cell adenoma	2/78		8/76*
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Pancreatic acinar cell adenoma	1/79		7/76*
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*increase from controls statistically significant



Carcinogenicity Studies in Rats

- **All benign, late-life tumors, no increase in carcinoma**
- **All three benign tumors have been observed as class effects of peroxisome proliferators in one 2-yr rat study**
- **Other chemicals in this class include:**
 - **Pharmaceuticals used for many years in humans without evidence of carcinogenicity**
 - **The classic peroxisome proliferator “Wyeth 14,632”**
 - **Industrial chemicals**
- **Human relevance of mode of action:**
 - **Liver tumors – well-defined and not relevant**
 - **Pancreatic acinar-cell tumors – rare in humans**
 - **Leydig-cell tumors – rare in humans**



SAB Panel Recommendations

Mammary Gland Tumors in Rats

- Mammary tumor incidences were an important consideration in the SAB Panel recommendation on Descriptor for Carcinogenic Potential as “Likely”
- However, SAB Panel recommended
 - that EPA “consider new information that has been verified and peer-reviewed prior to use in their revision of the Draft Risk Assessment.”
 - that “an independent, appropriately-designed histopathology review of ... female mammary glands from the Sibinski study be conducted to re-analyze the resulting tumor incidence data”
- Full Pathology Working Group (PWG) review was conducted for mammary tumors in a 2-year study
 - Results not available in time for incorporation into Draft Risk Assessment



Pathology Working Group Review of Mammary Glands – Methods

- **Conducted in general accordance with requirements for a PWG as stated in USEPA PR 94-5**
- **All mammary glands re-examined microscopically by a reviewing pathologist**
- **All primary neoplasms of the mammary gland as diagnosed by the original or review pathologists were evaluated by the PWG pathologists**
 - **Slides examined by PWG without knowledge of treatment group**
 - **Used diagnostic criteria and nomenclature recommended by the Society of Toxicologic Pathologists**

Pathology Working Group Review – Mammary Glands

Concentration (ppm)	0		30		300	
	Original PWG Study		Original PWG Study		Original PWG Study	
Adenocarcinoma (%)	16	18	28	32	10	10
Fibroadenoma (%)	16	32	26	32	38	40
Fibroadenoma, Multiple (%)	4	4	12	12	4	6

50 rats/group



PWG Results and Conclusions: Mammary Tumor Effect

- **No statistically-significant (Fisher's Exact Test, NTP Program Poly-3 procedure) increases in incidence of mammary tumor type, of total benign neoplasms, or total malignant neoplasms**
- **No increase in tumor multiplicity**
- **Morphologic appearance of the neoplasms in treated groups was similar to that of controls**
- **Incidence of mammary gland neoplasms observed in this study was similar to historical control incidences**

(Report on EPA docket; manuscript in prep)



Carcinogenicity Summary

- Non-genotoxic in a battery of *in vitro* and *in vivo* studies
- Carcinogenicity studies in animals
 - Benign tumors in male rats only
 - No effect on incidences of mammary gland tumors
 - Class effect of questionable relevance to humans



Immunotoxicity - Effect of APFO on Rodent Immune Systems Following 28 days of Oral Gavage (Loveless et al., 2008)


Conclusion: No primary effects on the immune system in male rats or mice (0.3, 1, 10 and 30 mg/kg/day)

Rats

- No immune-related changes occurred, even at doses causing significant systemic toxicity

Mice

- No immune-related changes occurred at doses (0.3 and 1 mg/kg) that did not produce a stress response
- Immune-related changes occurred only at doses (10 and 30 mg/kg) causing significant systemic toxicity (e.g., hepatic necrosis) and stress (e.g., increased serum corticosterone)



Immunotoxicity – Review Article

DeWitt J.C. et al. (2009). Immunotoxicity of Perfluorooctanoic Acid and Perfluorooctane Sulfonate and the Role of Peroxisome Proliferator-Activated Receptor Alpha. Critical Reviews in Toxicology 39: 76-94.

Conclusions:

- **PFOA alters immune system in experimental models**
- **Considerable species and strain variability**
- **Less sensitive models – no effects or effects only where there is evidence of other toxicities**
- **Sensitive models – effects at PFOA serum concentrations ~100-fold higher than occupational levels in people**
- **Data indicate both PPAR α -dependent and PPAR α -independent pathways**



Summary

Repeated Dose Toxicity

- primary target is the liver
- lipid alterations primarily in the mouse
- good dose-response characteristics
- species similarity in target and potency
- NOEL/LOEL ~0.5 mg/kg

Reproductive Toxicity

- fertility, reproductive parameters unaffected
- pup weight, survival, markers of sexual maturation altered at high dose only



Summary

Developmental Toxicity

- fetus not a particular target
- no structural abnormalities
- post-natal survival effects in mice;
NOEL 3 mg/kg

Immunotoxicity

- no primary effects on the immune system
- doses in mice that caused systemic toxicity also caused stress and immune-related changes



Summary

Genotoxicity

- inactive in wide range of test systems

Chronic Toxicity

- liver primary target
- at higher concentration produce tumor triad seen with peroxisome proliferators in rodents - liver, testis, pancreas

Point of Departures for Risk Assessment

Endpoint	Species	Effect	Dose Descriptor	Serum Conc. (ppm)
Repro	Rat	None	NOAEL	> 39
Develop	Mouse	↓ postnatal body wt.	BMCL₅	16
Chronic	Monkey	↑ liver/brain wt.	BMCL₁₀	23
Chronic	Monkey	↓ body wt.	BMCL₁₀	60
Cancer	Rat	Leydig cell tumors	BMCL₁₀	125



Perfluorohexanoate (PFH_xA)



PFHx is rapidly absorbed and eliminated when dose orally in rats and mice, has low biopersistence and is not bioaccumulative.

90-Day sub-chronic *repeated-dose*

- PFHx is eliminated almost exclusively in the urine at both dose levels and in both sexes
- *The majority (ie, >90%) of the dose is eliminated within the first 24 hrs* after dosing in both male and female rats (99.83%, 99.95%).
- Half-life in rat plasma : male 5 hrs.; female 2 hrs.

Radiolabelled *single oral dose*

- Male and female rats and mice; 2 and 100 mg/kg
- Greater than 99% of the dose was eliminated in urine within 24 hours in both sexes, rats and mice.
- *By 24 hrs after a single dose, there is almost no detectable PFHx in tissues from rats or mice of either sex.*

NaPFHxA* Toxicity Data

Subchronic toxicity study in rats

- **Crl:CD(SD) Rats (male and female) dosed with either 0, 20, 100, or 500 mg/kg/day via gavage for 90-days**

- **Results @ 500 mg/kg/day:**

Decreased body wt. and wt. gain

Induction of hepatic β -oxidation

Hematological effects (\downarrow RBC, Hg, Hb)

Thyroid follicular cell hypertrophy – likely secondary to enzyme induction

Results @ 100 and 500 mg/kg/day:

Atrophy of olfactory epithelium

- **NOAEL for Subchronic Toxicity was 20 mg/kg/day**

*Perfluorohexanoic Acid, C6 acid sodium salt



NaPFHxA* Toxicity Data

Reproductive toxicity study in rats

- No effects observed on reproductive parameters. Not a selective reproductive toxicant
- **NOAEL for Reproductive Toxicity was 500 mg/kg/day**

Neurobehavioral toxicity study in rats

- No effects seen on neurobehavioral parameters
- **NOAEL for Neurobehavioral Toxicity was 500 mg/kg/day**

*Perfluorohexanoic Acid, C6 acid sodium salt

NaPFHxA Toxicity Data (Cont.)

Developmental Toxicity Study in Rats

- CrI:CD(SD) Rats (time-mated female) Dose groups: 0, 20, 100, or 500 mg/kg/day via gavage during pregnancy
- **NOAEL = 100 mg/kg/day. Not a selective developmental toxicant**

Genotoxicity Studies (*in vitro*)

- No evidence of mutations in the Bacterial Reverse Mutation Assay (OECD 471)
- No evidence of chromosome aberrations in human lymphocytes (OECD 473)

Conclusion: **Not genotoxic**

Hazard Assessment

Benchmark Dose – BMDL10 (mg/kg/day)

PFHxA

PFOA

Non-cancer Systemic Toxicity	13 Nasal lesions*	3.9
Developmental Effects	292	22
Tumorigenic Effects	No Study	5.1

* relevance to humans unknown

Loveless, S.E. et al., 2009. Toxicological evaluation of perfluorohexanoate. Toxicology 264: 32-44.



90-Day Oral Toxicity Study of PFHxA in Rats

(Chengelis et al. Reproductive Toxicology 27(2009) 342-351.)

Doses: 10, 50 and 200 mg/kg/day

Results:

- **Lower body wt. gain at all doses (males only) not dose-responsive and not considered adverse**
- **Minimal hematological /clinical chemical changes @ 200mg/kg/day not considered adverse**
- **Mild elevation in liver enzymes @ 50 and 200 mg/kg/day males (stat. significant at 200 mg/kg/day only)**
- **Minimal liver hypertrophy, ↑ liver wt. and β-oxidation @ 200 mg/kg/day males**
- **No effects on functional observational battery or motor activity evaluations**
- **NOAEL based on liver effects:**
 - **Males – 50 mg/kg/day**
 - **Females – 200 mg/kg/day**



Perfluorobutanoate (PFBA)

Presented by: John Butenhoff



PFBA Effects: Published Studies

Ikeda *et al.*, (1985) J. Biochem. 98(2): 475-482

Just *et al.*, (1989) Hepatology 9(4): 570-581

Kozuka *et al.* (1991) Biochem. Pharmacol. 41(4): 617-623

Takagi *et al.* (1991) Cancer Lett. 57(1): 55-56

Vanden Heuvel *et al.* (1991) Biochem. Pharmacol., 42:295-302

Intrasuksri & Feller (1991) Biochem. Pharmacol. 42(1): 184-888

Permadi *et al.* (1992) Biochem. Pharmacol. 44(6): 1183-1191

Permadi *et al.* (1993) Xenobiotica 23(7): 761-770

Das *et al.* (2008) Toxicol. Sci. 105(1): 173-181

Chang *et al.* (2008) Toxicol. Sci. 104(1): 40-53

Foreman *et al.* (2009) Toxicol. Sci. 110(1): 204-211

Bjork and Wallace (2009) Toxicol. Sci. 111(1): 89-99

Wolf *et al.* (2008) Toxicol. Sci. 106(1): 162-171



PFBA Pharmacokinetics

Comparative Serum Elimination Half Lives

	<u>Males</u>	<u>Females</u>
Mice	~15 hours	~3 hours
Rats	~6-9 hours	~1-2 hours
Monkeys	~1-2 days	~1-2 days
Humans	~2-4 days	~2-4 days



PFBA Developmental Summary

Modeled after EPA studies with PFOA

PFBA doses of 35, 175, and 350 mg/kg-d to pregnant mice

- Doses chosen based on comparative internal dose (AUC) to PFOA

Elevated maternal liver weight at doses of 175 mg/kg and 350 mg/kg

No significant adverse effect the birth outcomes or development

- No increase in malformations
- No reduction in survival or growth after birth
- Slight delay in postnatal development (eye opening)
- Transient increase in neonatal liver weight
- Maintenance of pregnancy affected at 350 mg/kg (5 mg/kg for PFOA)

Relative lack of adverse developmental effects of PFBA vs. PFOA

- In part due to rapid elimination of PFBA vs. PFOA

Das et al. (2008) Toxicol. Sci. 105(1): 173-181



Outcomes of 90-Day Oral Toxicity Study in Rats

Gavage doses of 1.2, 6, and 30 mg/kg/d

Noted effects at the study high dose (30 mg/kg):

- Only males affected
- Increase in liver weight (PPAR α)
- Reduced thyroid hormone
 - Binding displacement
 - TSH normal
 - HPT axis unaffected
- No significant change in thyroid follicle epithelial cell height and colloidal area by histomorphometry

At mid and high dose

- Reduction in bilirubin
 - protective, due to induction of metabolizing enzymes



Important Non-Effects

No effect on body weight or weight gain

No evidence of liver or thyroid injury

No marked progression of effects on longer dosing (28-day vs. 90-day)



How Humans are Different than Rodents

PFBA-induced hepatocellular hypertrophy is mediated by PPAR α .¹

Human liver cells do not express PPAR α -regulated genes when exposed to PFBA in culture.²

Therefore, the liver effects observed in rodents from PFBA-induced PPAR α activation are attenuated or absent in humans.

¹ Foreman et al. (2009) Toxicol. Sci. 110(1): 204-211

² Bjork and Wallace (2009) Toxicol. Sci. 111(1): 89-99



Summary - PFBA

Brief review of prior and new PFBA studies

- Low level of toxicity
- Effects adaptive (non-adverse) and reversible
- Clear no-effect levels established

Rapid elimination in lab animals and humans

- Partly responsible for low toxicity
- Leads to low blood levels in exposed humans

PFBA is very different than PFOA

Elimination Half-Life in plasma (alpha-phase) Single Oral Dose

Elimination $t_{1/2}$ (Days)	PFBA	PFBS	PFHxA	PFHxS	PFOA	PFOS
Rat	0.3	0.2	0.2 – 0.05	7	5	25
Monkey	2	4	1	100	21	45
Human	3 - 4	26	no data	3000	1000	1500

- Data taken from multiple references.
- PFHxA has low biopersistence and is not bioaccumulative



In Summary - PFCAs and PFAS

Not possible to generalize about toxicity of Perfluoroalkyls or to treat as a class

- Similarities, yet significant differences
- Species differences in response
- Mode of action differences
- Qualitative, not just quantitative, differences