NTP
National Toxicology Program

Draft NTP Technical Report TR598 on Perfluorooctanoic Acid

(Feed Studies)

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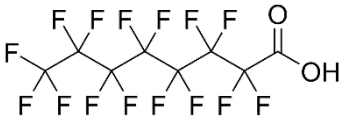
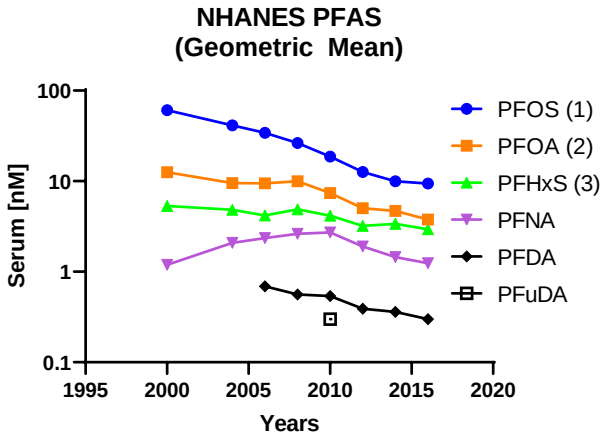
PFOA Background

- Perfluorooctanoic Acid (PFOA) is a perfluoroalkyl substance (PFAS) that was used for decades in creating non-stick properties in a variety of products.
- Manufacturers agreed to discontinue use due to widespread exposure and health concerns.
- Due to a long half-life, measured in years, and resistance to environmental degradation, exposure has continued, but declined.
- It is the second most abundant PFAS measured in the human population, including children and pregnant women.





PFOA Background

- Ammonium perfluorooctanoate was evaluated in two chronic toxicity and carcinogenicity studies in rats, where exposure started in young adult animals.
  - Butenhoff et al., 2012: male and female rats (30 and 300 ppm)
  - Biegel et al., 2001: male rats (300 ppm)

- However, human exposure to PFOA occurs during early development (gestation and lactation = perinatal).

- Does including early perinatal exposure lead to changes in PFOA chronic toxicity and carcinogenicity response in Hsd:Sprague Dawley SD rats?

Study Hypothesis

- NTP tested the hypothesis that including perinatal exposure (gestation and lactation) would quantitively or qualitatively alter the PFOA response.

- Two types of comparisons were made during the analysis and interpretation of the data:

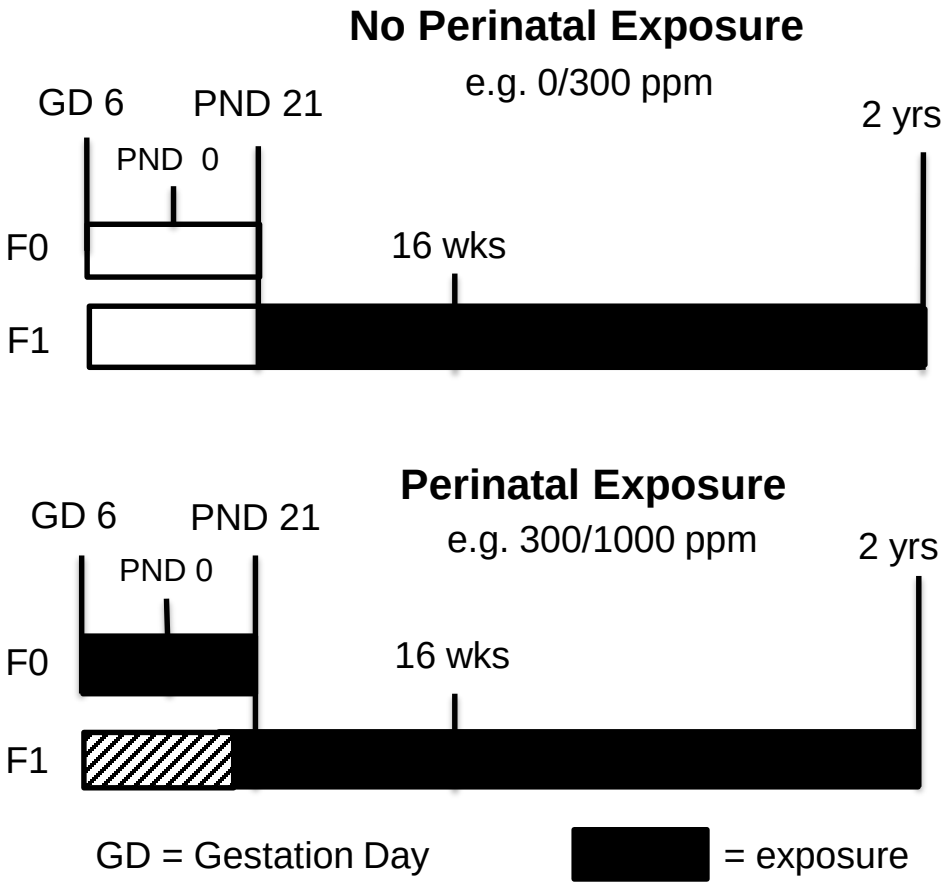
  1) Exposed groups were compared to the control group to determine if exposure increased effects in various endpoints.

  2) Exposed groups were also compared to determine if animals with perinatal exposure had different effects compared to animals without perinatal exposure.

Study Design

- Initial study design (Study #1) was based on previous studies by the NTP to assess early life exposure's contribution to carcinogenicity.

- Due to observed toxicity in males during the interim necropsy, the male portion of the study was stopped and males were restarted (Study #2).

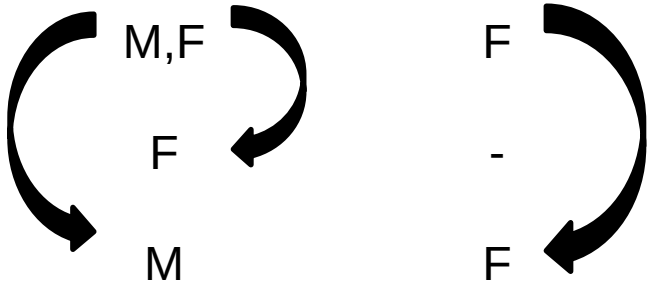


Study #1 Comparisons

Post-weaning exposure

Perinatal exposure

ppm	0	150	300	1000
0	M,F	M	M,F	F
150	-	M	F	-
300	-	-	M	F



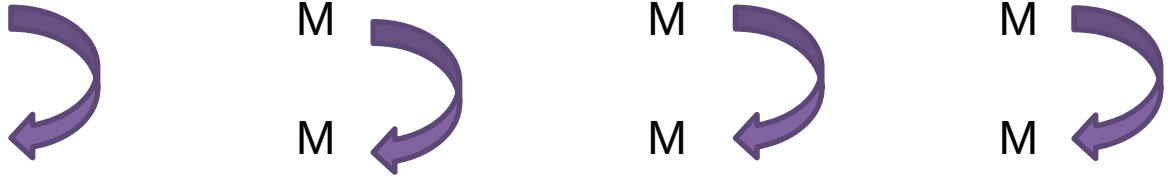
- Higher exposure to females due to faster PFOA elimination
- M = Males; F = Females

Study #2 Comparisons

Post-weaning exposure

Perinatal exposure

ppm	0	20	40	80
0	M	M	M	M
300	M	M	M	M



- A perinatal exposure level of 0 or 300 ppm

Study Design

- **Perinatal evaluation (gestation through lactation)**
  - Dam and pup body weights and littering parameters (e.g. litter size)
  - Dam and fetal (GD 18) and dam and pup (PND 4) PFOA concentrations in Study #2
- **Interim evaluation at 16 weeks (n = 10/group):**
  - Body and organ weights
  - Histopathology and clinical chemistry
  - Plasma concentrations
  - Acyl-CoA oxidase and aromatase enzyme activity to assess potential mechanistic pathways
- **Terminal evaluation at 104 weeks (n = 50/group):**
  - Histopathology

Results:
Exposure and Perinatal Findings

Perinatal PFOA Exposure

300 ppm Average (Study #2)

Timepoint/Age	Endpoint	0 ppm	300 ppm
GD 6 - 18	Chemical Consumption	-	~19 mg/kg/d
GD 18	Dam Plasma	BD	31 ± 1 µg/mL
GD 18	Fetal^	BD	9.4 ± 1.8 µg/g
PND 1 – 4	Chemical Consumption	-	~38 mg/kg/d
PND 4	Dam Plasma	BD	31 ± 2.8 µg/mL
PND 4	Male Pup#	BD	4.5 ± 0.3 µg/g
PND 4	Female Pup#	BD	4.1 ± 0.5 µg/g

^ Pooled by Litter; # Individual whole Pup
BD = Below Detection
GD = Gestation Day; PND = Postnatal Day

- There was exposure to the fetus
- There was exposure to the pup: male and female have similar levels

Perinatal PFOA Findings

- No effects on dam weight (< 3% change), pregnancy, littering (Study #1 & #2).
- No effects on pup litter size or survival (Study #1 & #2).
- Marginal effects (3-8%) on pup body weight (no sex difference) compared to 0 ppm control:

Study #1 (M & F)

Pup Weights	150 ppm	300 ppm
PND 1	-3%	-7%*
PND 4	1%	-2%
PND 7	0%	-8%*
PND 14	-2%	-6%*
PND 21	-2%	-5%*

Study #2 (M Only)

Pup Weights	150 ppm	300 ppm
PND 1	-	-3%*
PND 4	-	-4%*
PND 7	-	-5%*
PND 14	-	-5%*
PND 21	-	-7%*

* $p < 0.05$ from control (0 ppm)

Results:
Male and Female Interim
(Study 1 and 2 combined)

16 Week PFOA Exposure

Gen/Sex	Diet (ppm)	Consumption (mg/kg/day)	Plasma (µg/mL)
F1 Male	0/20; 300/20	~2	~80
	0/40; 300/40	~4	~124
	0/80; 300/80	~8	~152
	0/150; 150/150	~16	~184
	0/300; 300/300	~32	~233
F1 Female	0/300; 150/300	~30	~21
	0/1000; 300/1000	~102	~71

- Values for consumption and plasma are averaged and rounded for presentation
- Minimal differences between perinatal exposure and no perinatal exposure

Body Weight

Percent difference from 0/0 ppm control

MALE

Post-weaning exposure

Perinatal exposure ppm	0	20	40	80	150	300	1000
0	-	-9%*	-18%*	-19%*	-21%**	-45%**	
150					-24%**		
300	-4%	-9%	-14%*	-21%*		-45%**	

Study 1

FEMALE

Perinatal exposure ppm	0	20	40	80	150	300	1000
0	-					-2%	-12%**
150						-5%	
300							-12%**

* p < 0.05; ** p < 0.01 from control (0/0 ppm)
# p < 0.05 from non-perinatal exposure

Liver Weight (absolute)

Percent difference from 0/0 ppm control

MALE

Post-weaning exposure

Perinatal exposure ppm	0	20	40	80	150	300	1000
0		23%*	29%*	35%*	42%**	18%*	
150					30%**		
300	-7%	19%*	26%*	27%*		13%*	

FEMALE

Perinatal exposure ppm	0	20	40	80	150	300	1000
0	-					5%	36%**
150						0%	
300							36%**

* p < 0.05; ** p < 0.01 from control (0/0 ppm)
# p < 0.05 from non-perinatal exposure

Liver Acyl-CoA Oxidase Activity

Fold Increase over control (0/0 ppm)

Post-weaning exposure

Perinatal exposure

MALE

ppm	0	20	40	80	150	300	1000
0		4.3*	7.3*	9.5*	9.6**	10.0**	
150					8.7**		
300	1.0	5.2*	7.0*	9.6*		10.5**	

FEMALE

ppm	0	20	40	80	150	300	1000
0						0.4**	5.5**
150						0.4**	
300							4.5**

* $p < 0.05$; ** $p < 0.01$ from control (0/0 ppm)
# $p < 0.05$ from non-perinatal exposure

Clinical Chemistry Highlights

MALE

Perinatal exposure

Post-weaning exposure

ppm	0	20	40	80	150	300	1000
0		↓Globulin ↑Liver ↓TGs	↓Globulin ↑Liver ↓TGs	↓Globulin ↑Liver	↓Globulin ↑Liver ↓TGs	↓Globulin ↑Liver ↓TGs	
150					↓Globulin ↑Liver ↓TGs		
300		↓ Globulin ↑Liver ↓TGs	↓ Globulin ↑Liver	↓ Globulin ↑Liver ↓TGs		↓ Globulin ↑Liver ↓TGs	

FEMALE

ppm	0	20	40	80	150	300	1000
0						↓Globulin	↓Globulin ↑Liver
150						↓Globulin	
300							↓Globulin ↑Liver

Hepatocyte, Cytoplasmic Alteration

MALE

Perinatal exposure

Post-weaning exposure

ppm	0	20	40	80	150	300	1000
0	0	10** [1.0]^	10** [1.8]	10** [2.0]	10** [2.2]	10** [2.6]	
150					10** [2.1]		
300	0	9** [1.2]	10** [1.7]	10** [1.9]		10** [2.8]	

FEMALE

ppm	0	20	40	80	150	300	1000
0	0					0	10** [1.3]
150						0	
300							10** [2.0]

* p < 0.05; ** p < 0.01 from control (0/0 ppm)
# p < 0.05 from non-perinatal exposure

^Average severity grade [1=minimal, 2=mild, 3=moderate, 4=marked]
n = 10/group

Liver Necrosis

MALE

Post-weaning exposure

Perinatal exposure ppm	0	20	40	80	150	300	1000
0	0/1 [1.0]^	1 [1.0]	6* [1.0]	4 [1.5]	6** [1.2]	2 [1.0]	
150					2 [1.5]		
300	0	2 [1.0]	3 [1.0]	1 [1.0]		4* [1.8]	

FEMALE

Perinatal exposure ppm	0	20	40	80	150	300	1000
0	0				0	0	2 [2.5]
150						0	
300							0

^ Study #1/Study #2
* p < 0.05; ** p < 0.01 from control (0/0 ppm)
# p < 0.05 from non-perinatal exposure

Average severity grade [1=minimal, 2=mild, 3=moderate, 4=marked]
n = 10/group

Hepatocyte Single Cell Death

MALE

Perinatal exposure

Post-weaning exposure

ppm	0	20	40	80	150	300	1000
0	0	7* [1.0]^	9* [1.0]	10* [1.0]	10** [1.3]	10** [1.0]	
150					9** [1.1]		
300	0	5* [1.0]	8* [1.0]	10* [1.0]		10** [1.0]	

FEMALE

ppm	0	20	40	80	150	300	1000
0	0					0	1 [1.0]
150						0	
300							0

* p < 0.05; ** p < 0.01 from control (0/0 ppm)
# p < 0.05 from non-perinatal exposure

^Average severity grade [1=minimal, 2=mild, 3=moderate, 4=marked]
n = 10/group

Other Effects

- Changes in absolute and/or relative spleen (M), thymus (M), right kidney weights (M)

- Marginal increase in liver aromatase activity (study # 2)

- Increased incidence of hepatocyte hypertrophy and pigment (M & F), thyroid gland follicular cell hypertrophy (M & F), kidney renal tubule mineral (M & F) and papilla urothelium hyperplasia (F), and glandular stomach submucosa chronic active inflammation (M)

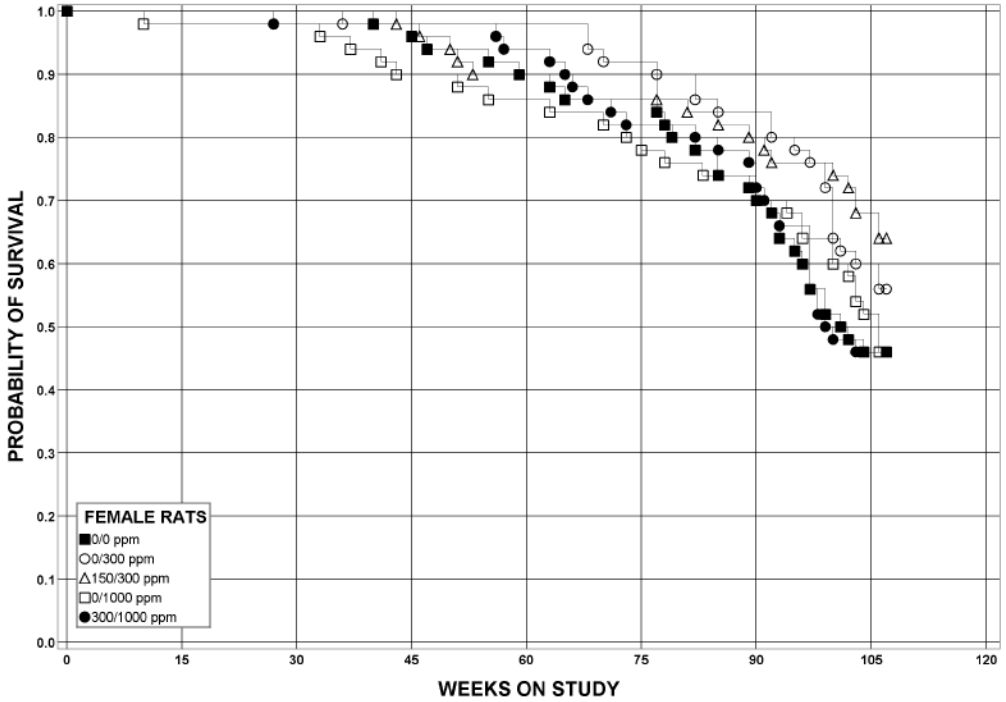
Results: Chronic Toxicity and Carcinogenicity

Carcinogenicity LOE

- A weight of evidence was used based on the several factors described in the preface of this report and on the consistent findings in animals exposed either perinatally and postweaning or postweaning alone.

- A few differences between exposure paradigms are noted, but in general, the additional effect of including perinatal exposure on the chronic toxicity or carcinogenic response of PFOA appeared to be minimal.

Female Survival

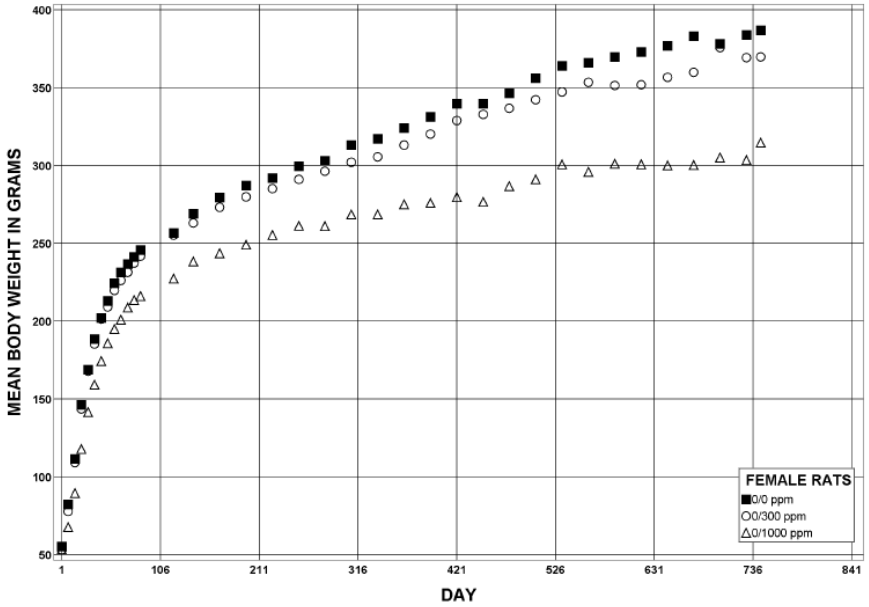


Percent Survival

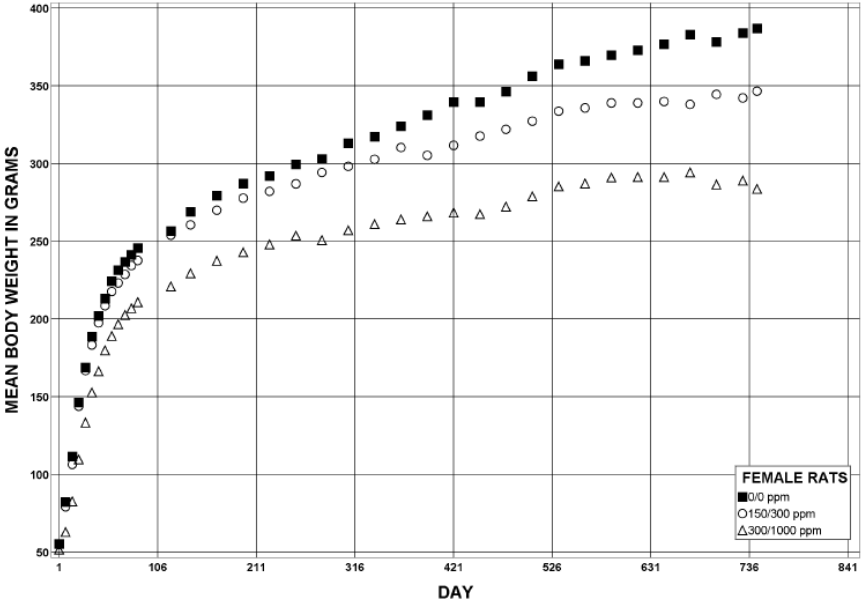
ppm	0	300	1000
0	46%	56%	46%
150		64%	
300			46%

Female Body Weights

Without Perinatal Exposure



With Perinatal Exposure



-Lower weights in high dose (0/1000 ppm = -19%; 300/1000 ppm = -27%) compared to control (0/0 ppm)

Female Pancreas Acinar Cell

Some Evidence

Lesion	ppm	0	300	1000
Acinus Hyperplasia	0	0/50	1/50 [2.0]^	1/50 [2.0]
Acinus Hyperplasia	150		0/50	
Acinus Hyperplasia	300			1/50 [4.0]
Adenoma	0	0/50	0/50	1/49
Adenoma	150		0/50	
Adenoma	300			3/50
Adenocarcinoma	0	0/50	0/50	1/49
Adenocarcinoma	150		0/50	
Adenocarcinoma	300			2/50
Adenoma or Adenocarcinoma	0	0/50	0/50	2/49
Adenoma or Adenocarcinoma	150		0/50	
Adenoma or Adenocarcinoma	300			5/50

Adenoma Historical Control: 0/340
Adenocarcinoma Historical Control: 0/340
Adenoma or Adenocarcinoma Historical Control: 0/340

^Average severity grade [1=minimal, 2=mild, 3=moderate, 4=marked]

Female Liver

Lesion	ppm	0	300	1000
Necrosis	0	0	1 [1.0]^	8* [1.5]
Necrosis	150		4 [1.3]	
Necrosis	300			5 [2.4]
Hepatocyte Single Cell Death	0	0	4 [1.0]	29** [1.3]
Hepatocyte Single Cell Death	150		5* [1.0]	
Hepatocyte Single Cell Death	300			32** [1.2]
Hepatocyte Increased Mitoses	0	2 [1.0]	3 [1.0]	4 [1.5]
Hepatocyte Increased Mitoses	150		5 [1.6]	
Hepatocyte Increased Mitoses	300			10* [1.3]

* $p < 0.05$; ** $p < 0.01$ from control (0/0 ppm)
# $p < 0.05$ from non-perinatal exposure
^Average severity grade [1=minimal, 2=mild, 3=moderate, 4=marked]
n = 50 animal/group

Also observed increased incidence of:
-Hepatocyte Hypertrophy
-Pigment
-Bile Duct Hyperplasia

Equivocal Evidence

Neoplasm	ppm	0	300	1000
Adenoma	0	2/50	0/50	1/49
Adenoma	150		0/50	
Adenoma	300			3/50
Carcinoma	0	1/50	1/50	3/49
Carcinoma	150		0/50	
Carcinoma	300			4/50
Adenoma or Carcinoma	0	3/50	1/50	4/49
Adenoma or Carcinoma	150		0/50	
Adenoma or Carcinoma	300			6/50

Adenoma Historical Control: 14/340; 0/50 – 4/50
Carcinoma Historical Control: 1/340; 0/50 – 1/50
Adenoma or Carcinoma Historical Control: 15/340; 0/50 – 4/50

Equivocal Evidence

Lesion	ppm	0	300	1000
Hyperplasia, Atypical	0	3/50 [2.0]^	4/49 [2.0]	3/48 [2.7]
Hyperplasia, Atypical	150		7/50 [2.1]	
Hyperplasia, Atypical	300			3/48 [4.0]
Adenoma	0	1/50	1/50	0/50
Adenoma	150		0/50	
Adenoma	300			0/50
Adenocarcinoma	0	1/50*	5/50	8/50*
Adenocarcinoma	150		3/50	
Adenocarcinoma	300			5/50
Adenoma or Carcinoma	0	2/50*	5/50	8/50*
Adenoma or Carcinoma	150		3/50	
Adenoma or Carcinoma	300			5/50

Adenoma Historical Control: 1/50, 0/50, 0/50
Carcinoma Historical Control: 1/50, 5/50, 5/50
^Adenoma or Carcinoma Historical Control: 2/50, 5/50, 5/50

* $p < 0.05$; ** $p < 0.01$ from control (0/0 ppm)
# $p < 0.05$ from non-perinatal exposure

Female Kidney

Lesion	ppm	0	300	1000
Papilla, Urothelium Hyperplasia	0	4 [1.0]^	21** [1.0]	40** [1.9]
Papilla, Urothelium Hyperplasia	150		8## [1.0]	
Papilla, Urothelium Hyperplasia	300			45** [1.8]
Papilla Necrosis	0	0	0	12** [2.3]
Papilla Necrosis	150		0	
Papilla Necrosis	300			22** [2.1]
Renal Tubule Mineral	0	5 [1.2]	6 [1.3]	16** [1.0]
Renal Tubule Mineral	150		8 [1.0]	
Renal Tubule Mineral	300			8# [1.5]

* p < 0.05; ** p < 0.01 from control (0/0 ppm)
# p < 0.05, ## p < 0.01 from non-perinatal exposure
^Average severity grade [1=minimal, 2=mild, 3=moderate, 4=marked]
n = 50

Female Forestomach

Lesion	ppm	0	300	1000
Ulcer	0	2 [1.5]^	2 [1.5]	9* [1.6]
Ulcer	150		1 [1.0]	
Ulcer	300			11* [2.1]
Epithelium Hyperplasia	0	4 [2.3]	5 [1.8]	22** [2.8]
Epithelium Hyperplasia	150		3 [2.3]	
Epithelium Hyperplasia	300			21** [2.5]
Submucosa Inflammation Chronic	0	3 [2.3]	2 [2.5]	16** [2.6]
Submucosa Inflammation Chronic	150		2 [2.0]	
Submucosa Inflammation Chronic	300			18** [2.5]

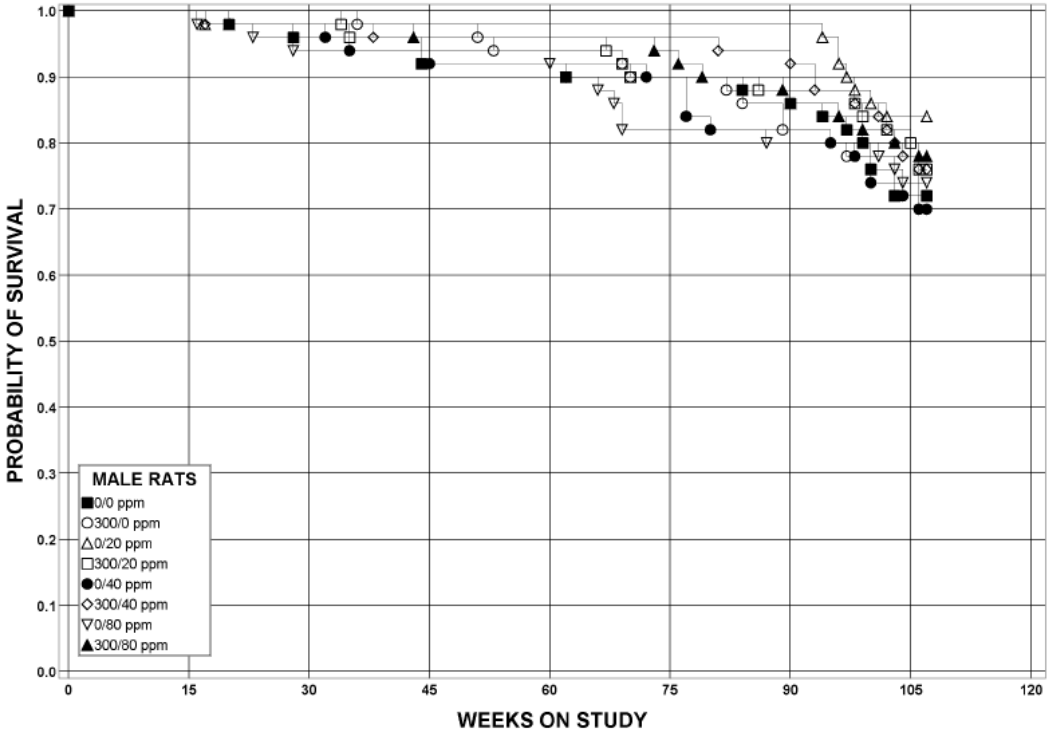
* p < 0.05; ** p < 0.01 from control (0/0 ppm)
# p < 0.05 from non-perinatal exposure
^Average severity grade [1=minimal, 2=mild, 3=moderate, 4=marked]
n = 50

Female Thyroid

Lesion	ppm	0	300	1000
Follicular Cell Hypertrophy	0	4 [2.3]^	8 [2.3]	28** [2.0]
Follicular Cell Hypertrophy	150		9 [1.6]	
Follicular Cell Hypertrophy	300			19** [1.7]

* p < 0.05; ** p < 0.01 from control (0/0 ppm)
# p < 0.05 from non-perinatal exposure
^Average severity grade [1=minimal, 2=mild, 3=moderate, 4=marked]
n = 50

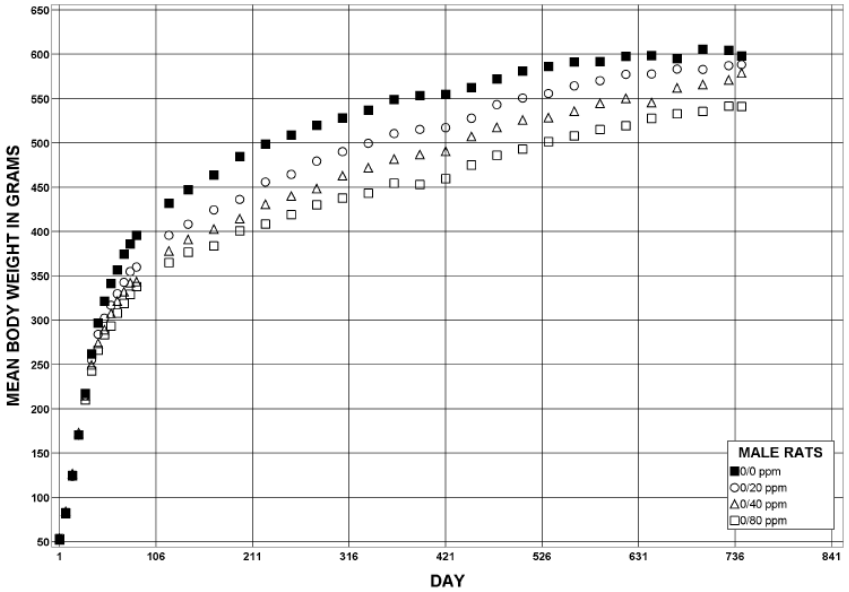
Male Survival

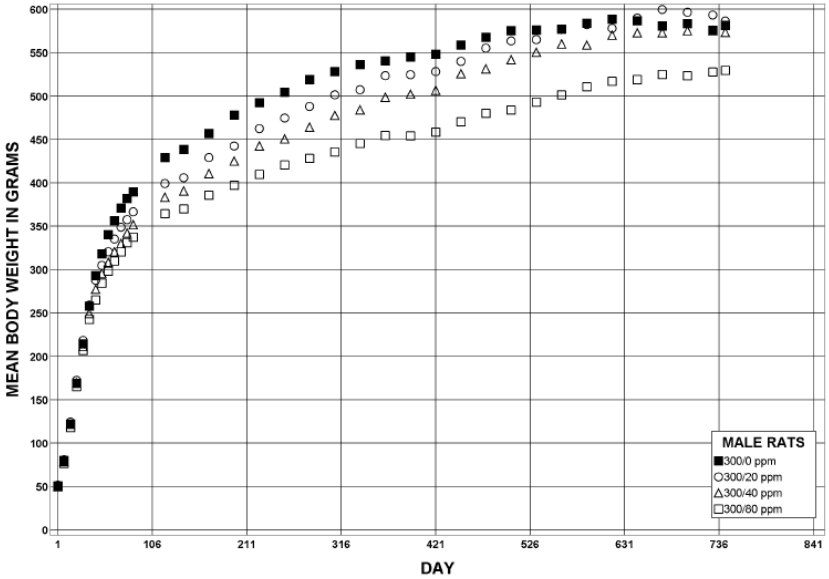


Percent Survival

ppm	0	20	40	80
0	72%	84%	70%	74%
300	70%	76%	76%	78%

Male Body Weights





~10% lower weights in 0/80 ppm and 300/80 ppm compared to 0/0 ppm control at termination

Male Pancreas Acinar Cell

Clear Evidence

Lesion	ppm	0	20	40	80
Acinus Hyperplasia	0	18/50 [2.7]^	32/50* [3.7]	37/50** [3.2]	31/50** [3.2]
Acinus Hyperplasia	300	23/50 [2.7]	27/50 [3.2]	38/50** [3.3]	33/50 [3.4]
Adenoma	0	3/50	28/50**	26/50**	32/50**
Adenoma	300	7/50	18/50**	30/50**	30/50**
Adenocarcinoma	0	0/50	3/50	1/50	3/50
Adenocarcinoma	300	0/50	2/50	1/50	3/50
Adenoma or Adenocarcinoma	0	3/50	29/50**	26/50**	32/50**
Adenoma or Adenocarcinoma	300	7/50	20/50**	30/50**	30/50**

Adenoma Historical Control: 45/340; 0/50 – 14/50
Adenocarcinoma Historical Control: 2/340; 0/50 – 1/50
Adenoma or Adenocarcinoma Historical Control: 145/340; 0/50 – 14/50
^ Average severity grade [1=minimal, 2=mild, 3=moderate, 4=marked]

* $p < 0.05$; ** $p < 0.01$ from control (0/0 ppm)
# $p < 0.05$ from non-perinatal exposure

Male Hepatocellular Neoplasms

Clear Evidence

Lesion	ppm	0	20	40	80
Adenoma	0	0/50	0/50	7/50*	11/50**
Adenoma	300	0/50	1/50	5/50	10/50**
Carcinoma	0	0	0	0	0
Carcinoma	300	0	0	0	4/50
Adenoma or Carcinoma	0	0/50	0/50	7/50*	11/50**
Adenoma or Carcinoma	300	0/50	1/50	5/50	12/50**

Adenoma Historical Control: 2/340; 0/50 – 1/50
Carcinoma Historical Control: 0/340
Adenoma or Carcinoma Historical Control: 2/340; 0/50 – 1/50

* $p < 0.05$; ** $p < 0.01$ from control (0/0 ppm)
# $p < 0.05$ from non-perinatal exposure

Similar lesions in the liver (necrosis, hepatocyte cytoplasmic alteration, hypertrophy, single cell death, pigment) as observed in 16-week males/females and 104-week females in addition to various foci.

Study Comparison

Pancreas Acinar Cell (Male Rat)

Publication	Perinatal Exposure?	Neoplasm	0 ppm	20 ppm	30 ppm	40 ppm	80 ppm	300 ppm
Rae et al.[#]	No	Adenoma	0%		0%			0%
Biegel et al.	No	Adenoma	0%					9%
NTP Draft	No	Adenoma	6%	56%		52%	64%	
NTP Draft	Yes	Adenoma	14%	36%		60%	60%	
Rae et al.[#]	No	Carcinoma	0%		0%			2%
Biegel et al.	No	Carcinoma	0%					1%
NTP Draft	No	Carcinoma	0%	6%		2%	6%	
NTP Draft	Yes	Carcinoma	0%	4%		2%	6%	

[#] Review of lesions found in chronic study reported by Butenhoff et al. 2012

Male Conclusions

- *Clear evidence of carcinogenic activity*
  - Increased incidences of hepatocellular neoplasms (predominately hepatocellular adenomas)
  - Increased incidences of acinar cell neoplasms (predominately acinar cell adenomas) of the pancreas
- Exposure to perfluorooctanoic acid resulted in increased incidences of nonneoplastic lesions in the liver and pancreas.
- The additional effect of combined perinatal and postweaning exposure was limited to a higher incidence of hepatocellular carcinomas compared to postweaning exposure alone.

Female Conclusions

- *Some evidence of carcinogenic activity*
  - Increased incidences of pancreatic acinar cell adenoma or adenocarcinoma (combined) neoplasms
- May have been related to exposure (*equivocal evidence*)
  - Higher incidence of hepatocellular carcinomas
  - Higher incidence of adenocarcinomas of the uterus
- Exposure to perfluorooctanoic acid resulted in increased incidences of nonneoplastic lesions in the liver, kidney, forestomach, and thyroid gland
- The combined perinatal and postweaning exposure was not observed to change the neoplastic or nonneoplastic response compared to postweaning exposure alone

Questions?