NTP
National Toxicology Program

Draft NTP Technical Report TR594
on
p-Chloro-α,α,α-trifluorotoluene
(Inhalation Studies)

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p-Chloro-α,α,α-trifluorotoluene (PCTFT)

- Classification as a non-ozone depleting solvent results in higher end-user applications due to compliance with Clean Air Act standards
- Used in automobile body coatings and parts cleaning and as an intermediate in the synthesis of other chemicals (e.g. herbicides)
- Production and import was reported as 29 million pounds in 2011 and generally estimated to be 10 to 50 million from 1985 to 2015

NTP 2-Week Study

- 2-week gavage (NTP 1992, Toxicity Report 14)
  - F344/N rats and B6C3F1/N mice
  - Increased liver and kidney weight (male and female rats)
  - Hepatocyte hypertrophy (all sex/species) and nephropathy (male rats)
  - α2u-globulin and hyaline droplet: additional kidney slides were evaluated from male rats for hyaline droplet nephropathy
    - α2u-globulin was determined to comprise 11, 17, 34 and 55% of total protein in the 0, 50, 400 and 1,000 mg/kg groups

Nomination

- Nomination
  - 2001: Public comments submitted to the NTP Center for the Evaluation of Risks to Human Reproduction (CERHR)
    - Suggested evaluation of chronic data needs based on use
    - Concerns for male and female reproductive health
  - 2006: Representative from Kowa American Corporation
    - Expanding use and lack of occupational exposure limits
    - Included nomination of other benzotrifluoride compounds
      - Based on production volume and use pattern, PCTFT was selected for further examination

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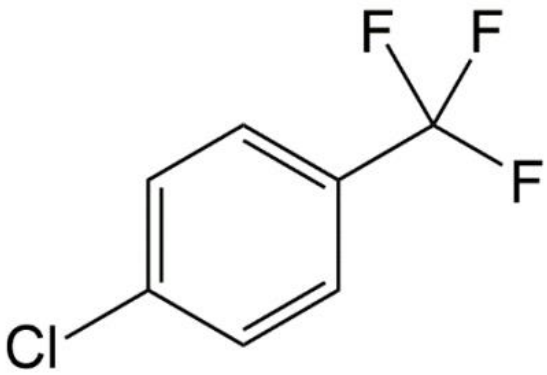
3-Month Studies

Whole Body Inhalation

3-Month Toxicology Studies

- Male and female Hsd:Sprague Dawley SD rats and B6C3F1/N mice
- Whole body inhalation exposure
  - 6 hours per day, 5 days per week
  - 0, 125, 250, 500, 1,000 or 2,000 ppm
  - 10 animals/sex/species/concentration
- Endpoints
  - In-life (survival, body weights, clinical observations)
  - Clinical pathology
  - Micronucleus
  - SCVCE
  - Organ weights and histopathology



3-Month Results

In-Life

	Rats	Mice
Survival	No effect	No effect
Body Weights	↑ ≥ 500 ppm (F)	↑ ≥ 500 ppm (M) ↑ ≥ 250 ppm (F)
Clinical Chemistry	↑ ALT, SDH, ALP, Cholesterol, Triglycerides (M/F)	N/A
Organ Weights	↑ Liver weight (M/F) ↑ Kidney weight (M)	↑ Liver weights (M/F) ↑ Kidney weight (M/F) ↓ Thymus weight (F)

3-Month Results

In-Life

	Rats	Mice
Survival	No effect	No effect
Body Weights	↑ ≥ 500 ppm (F)	↑ ≥ 250 ppm (F) ↑ ≥ 500 ppm (M)
Clinical Chemistry	↑ ALT, SDH, ALP, Cholesterol, Triglycerides (M/F)	N/A
Rel. Liver Weight Percent higher than control at 2,000 ppm	Male rats 88% Female rats 96%	Male mice 155% Female mice 101%

Sperm Counts and Vaginal Cytology Evaluations

	Rats	Mice
Male	↓ sperm motility (≥ 1,000 ppm) ↓ left cauda weight (2,000 ppm) ↓ left epididymis weights (2,000 ppm) ↓ numbers of sperm per cauda (2,000 ppm) **Associated histopathological changes:** Testes, germ cell degeneration (2,000 ppm) Testes, spermatid retention (≥ 1,000 ppm) Epididymis, duct, exfoliated germ cell (2,000 ppm)	↓ sperm motility (≥ 500 ppm)
Female	↑ probability of extended diestrus (2,000 ppm) Also observed ↓ frequency of estrus, ↑ frequency of diestrus, ↓ number of cycles, ↓ number of cycling rats	↑ probability of extended estrus (≥ 500 ppm) ↑ estrous cycle length (≥ 1,000 ppm)

Sperm Counts and Vaginal Cytology Evaluations

	Rats	Mice
Male	↓ sperm motility (≥ 1,000 ppm) ↓ left cauda weight (2,000 ppm) ↓ left epididymis weights (2,000 ppm) ↓ numbers of sperm per cauda (2,000 ppm) **Associated histopathological changes:** Testes, germ cell degeneration (2,000 ppm) Testes, spermatid retention (≥ 1,000 ppm) Epididymis, duct, exfoliated germ cell (2,000 ppm)	↓ sperm motility (≥ 500 ppm)
Female	↑ probability of extended diestrus (2,000 ppm) Also observed ↓ frequency of estrus, ↑ frequency of diestrus, ↓ number of cycles, ↓ number of cycling rats	↑ probability of extended estrus (≥ 500 ppm) ↑ estrous cycle length (≥ 1,000 ppm)

PCTFT exhibits the potential to be a reproductive toxicant in male and female Hsd:Sprague Dawley SD rats and B6C3F1/N mice

Histopathology: Rats

	Male	Female
Adrenal cortex		
Vacuolization cytoplasmic	2,000 ppm	≥ 1,000 ppm
Harderian gland		
Degeneration	≥ 250 ppm	≥ 250 ppm
Kidney		
Accumulation, hyaline droplet (severity)	Mild/moderate	
Nephropathy, chronic (severity)	Mild/moderate	Minimal/mild
Liver		
Centrilobular hepatocyte hypertrophy	≥ 250 ppm	≥ 1,000 ppm
Mammary gland		
Hyperplasia		≥ 1,000 ppm

Histopathology: Mice

	Male	Female
Adrenal Cortex		
Zona fasciculata, hypertrophy	2,000 ppm	2,000 ppm
X-zone, degeneration		2,000 ppm
Forestomach		
Epithelium, hyperplasia	≥ 500 ppm	≥ 500 ppm
Inflammation, granulomatous	2,000 ppm	2,000 ppm
Spleen		
Red pulp, hematopoetic cell proliferation erythrocyte	2,000 ppm	≥ 250 ppm
Red pulp, hematopoetic cell proliferation, megakeratocyte	≥ 1,000 ppm	≥ 250 ppm
Liver		
Centrilobular hepatocyte hypertrophy	≥ 250 ppm	≥ 500 ppm
Centrilobular necrosis	≥ 500 ppm	≥ 1,000 ppm
Multinucleated hepatocytes	≥ 500 ppm	≥ 1,000 ppm

Histopathology: Mice

	Male	Female
Adrenal Cortex		
Zona fasciculata, hypertrophy	2,000 ppm	2,000 ppm
X-zone, degeneration		2,000 ppm
Forestomach		
Epithelium, hyperplasia	≥ 500 ppm	≥ 500 ppm
Inflammation, granulomatous	2,000 ppm	2,000 ppm
Spleen		
Red pulp, hematopoetic cell proliferation erythrocyte	2,000 ppm	≥ 250 ppm
Red pulp, hematopoetic cell proliferation, megakeratocyte	≥ 1,000 ppm	≥ 250 ppm
Liver		
Centrilobular hepatocyte hypertrophy	≥ 250 ppm	≥ 500 ppm
Centrilobular necrosis	≥ 500 ppm	≥ 1,000 ppm
Multinucleated hepatocytes	≥ 500 ppm	≥ 1,000 ppm

3-Month Study Results: Mice

Nonneoplastic Liver Lesions

	Control	125 ppm	250 ppm	500 ppm	1,000 ppm	2,000 ppm
Males	**10**	**10**	**10**	**10**	**10**	**10**
Centrilobular Hepatocyte, Hypertrophy	0	0	10** (1.5)[a]	10** (2.7)	10** (3.9)	10** (4.0)
Centrilobular Hepatocyte, Necrosis	0	0	2 (1.0)	10** (1.2)	10**(1.5)	10** (2.0)
Hepatocyte, Multinucelated	0	0	1 (1.0)	8** (1.6)	10** (3.5)	10** (4.0)
Females	**10**	**10**	**10**	**10**	**10**	**10**
Centrilobular Hepatocyte, Hypertrophy	0	0	2 (1.5)	4* (1.0)	10** (2.2)	10** (3.9)
Centrilobular Hepatocyte, Necrosis	0	0	1 (1.0)	2 (1.0)	8**(1.1)	10** (1.6)
Hepatocyte, Multinucelated	0	0	0	0	6** (1.5)	10** (3.3)

* Significantly different ($p \leq 0.05$) from chamber controls by Poly-3 test

** Significantly different ($p \leq 0.01$) from chamber controls by Poly-3 test; positive trend if associated with a control incidence

[a] Average severity of affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

3-Month Study Results: Mice

Nonneoplastic Liver Lesions

	Control	125 ppm	250 ppm	500 ppm	1,000 ppm	2,000 ppm
Males	**10**	**10**	**10**	**10**	**10**	**10**
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Hepatocyte, Multinucelated	0	0	1 (1.0)	8** (1.6)	10** (3.5)	10** (4.0)
Females	**10**	**10**	**10**	**10**	**10**	**10**
Centrilobular Hepatocyte, Hypertrophy	0	0	2 (1.5)	4* (1.0)	10** (2.2)	10** (3.9)
Centrilobular Hepatocyte, Necrosis	0	0	1 (1.0)	2 (1.0)	8**(1.1)	10** (1.6)
Hepatocyte, Multinucelated	0	0	0	0	6** (1.5)	10** (3.3)

* Significantly different ($p \leq 0.05$) from chamber controls by Poly-3 test

** Significantly different ($p \leq 0.01$) from chamber controls by Poly-3 test; positive trend if associated with a control incidence

[a] Average severity of affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Summary

- Higher body weights
- Higher liver weights and higher incidence of nonneoplastic lesions of the liver in all sex/species
- Increased severity of nonneoplastic lesions of the kidney in male rats
- PCTFT via inhalation exposure exhibits the potential to be a **reproductive toxicant** in male and female Hsd:Sprague Dawley SD rats and B6C3F1/N mice

Exposure Concentration Selection

- **Rats**
  - Highest exposure concentration: 1,000 ppm
    - Based on exposure related increases in relative liver weight; 90% higher than controls (M/F) in 2,000 ppm group

- **Mice**
  - Highest exposure concentration: 400 ppm
    - Based on exposure related increases in liver weight (>100% higher than controls) and increased incidences of liver necrosis and other liver lesions at 500 ppm (males) and 1,000 ppm (females)

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2-Year Studies

Whole Body Inhalation

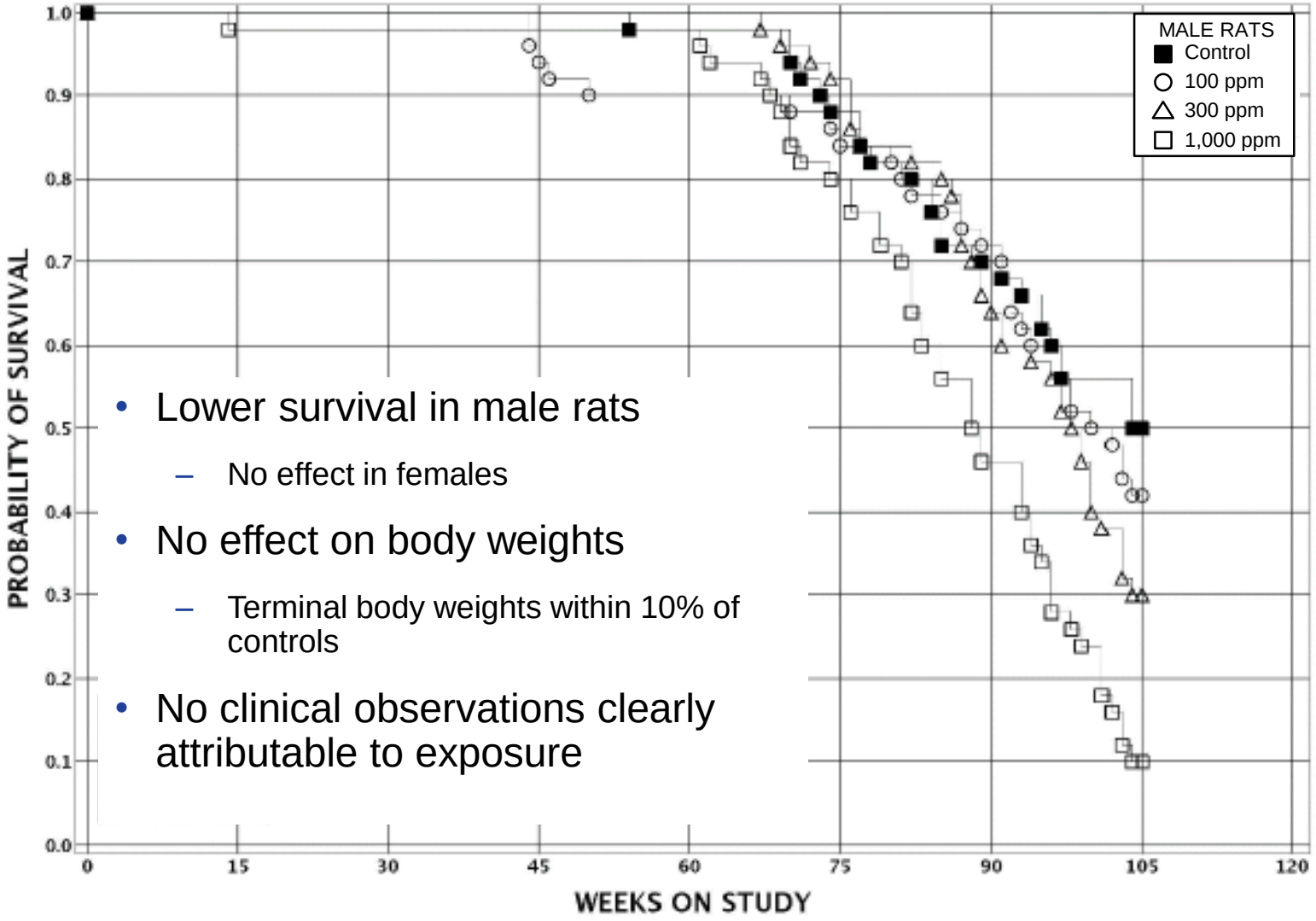
2-Year Study Design

- Male and female Hsd:Sprague Dawley SD rats and B6C3F1/N mice
- Whole body inhalation exposure
  - 6 hours per day, 5 days per week, 104 weeks
  - 0, 100, 300, 1,000 ppm (rats)
  - 0, 100, 200, 400 ppm (mice)
- 50 animals/species/sex/concentration
- Endpoints
  - In-life (survival, body weights, clinical observations)
  - Histopathology
  - Molecular pathology (mouse liver)

2-Year Study Results: Rats

Survival, Body weights and Clinical Observations

Male rat survival



- Lower survival in male rats
  - No effect in females
- No effect on body weights
  - Terminal body weights within 10% of controls
- No clinical observations clearly attributable to exposure

Histopathology

REMINDER:

- One previous study using Hsd:Sprague Dawley SD rats
- Non-inhalation route
- Used to provide context but considered to have limited utility in the interpretation of data in this study

2-Year Study Results: Rats

Incidence of Neoplasms in the Thyroid

	Control	100 ppm	300 ppm	1,000 ppm
Males	**50**	**49**	**49**	**50**
C-cell Adenoma (includes bilateral)	2**	5	3	12**
C-cell Carcinoma	1	0	1	1
C-cell Adenoma or Carcinoma	3**	5	4	13**
Females	**50**	**50**	**50**	**50**
C-cell Adenoma (includes bilateral)	2**	8	8	14**
C-cell Carcinoma	0	2	0	1
C-cell Adenoma or Carcinoma	2**	10*	8	15**

* Significantly different ($p \leq 0.05$) from chamber controls by Poly-3 test

** Significantly different ($p \leq 0.01$) from chamber controls by Poly-3 test; positive trend if associated with a control incidence

2-Year Study Results: Rats

Incidence of Neoplasms in the Thyroid

	Control	100 ppm	300 ppm	1,000 ppm
Males	**50**	**49**	**49**	**50**
C-cell Adenoma (includes bilateral)	2** (13/50)[a]	5	3	12**
C-cell Carcinoma	1	0	1	1
C-cell Adenoma or Carcinoma	3**	5	4	13**
Females	**50**	**50**	**50**	**50**
C-cell Adenoma (includes bilateral)	2** (11/49)	8	8	14**
C-cell Carcinoma	0	2	0	1
C-cell Adenoma or Carcinoma	2**	10*	8	15**

[a] Tumor incidence in controls from other SD rat NTP study

* Significantly different ($p \leq 0.05$) from chamber controls by Poly-3 test

** Significantly different ($p \leq 0.01$) from chamber controls by Poly-3 test; positive trend if associated with a control incidence

Considered **some evidence** of carcinogenic activity

2-Year Study Results: Rats

Incidence of Neoplasms in the Lung

	Control	100 ppm	300 ppm	1,000 ppm
Males	**50**	**50**	**50**	**50**
Alveolar/bronchiolar Adenoma	0	2	0	1
Alveolar/bronchiolar Carcinoma	0*	0	0	2
Alveolar/bronchiolar Adenoma or Carcinoma	0	2	0	3

* Significantly different ($p \leq 0.05$) from chamber controls by Poly-3 test; positive trend if associated with a control incidence

2-Year Study Results: Rats

Incidence of Neoplasms in the Lung

	Control	100 ppm	300 ppm	1,000 ppm
Males	**50**	**50**	**50**	**50**
Alveolar/bronchiolar Adenoma	0	2	0	1
Alveolar/bronchiolar Carcinoma	0*	0	0	2
Alveolar/bronchiolar Adenoma or Carcinoma	0 (0/49)[a]	2	0	3

[a] Tumor incidence in controls from other SD rat NTP study

* Significantly different ($p \leq 0.05$) from chamber controls by Poly-3 test; positive trend if associated with a control incidence

May have been related to exposure (equivocal evidence)

2-Year Study Results: Rats

Incidence of Neoplasms in the Lung

	Control	100 ppm	300 ppm	1,000 ppm
Males	**50**	**50**	**50**	**50**
Alveolar/bronchiolar Adenoma	0	2	0	1
Alveolar/bronchiolar Carcinoma	0*	0	0	2
Alveolar/bronchiolar Adenoma or Carcinoma	0 (0/49)[a]	2	0	3

[a] Tumor incidence in controls from other SD rat NTP study
* Significantly different ($p \leq 0.05$) from chamber controls by Poly-3 test; positive trend if associated with a control incidence

May have been related to exposure (equivocal evidence)

- Robust response in nonneoplastic lesions in lung
  - Not considered pre-neoplastic

Incidence of Neoplasms in the Adrenal Medulla

	Control	100 ppm	300 ppm	1,000 ppm
Females	**49**	**50**	**50**	**50**
Hyperplasia	17 (1.1)[a]	25 (1.6)	34** (1.1)	36** (1.5)
Benign Pheochromocytoma	0	3	4	6*
Malignant Pheochromocytoma	0	1	0	0

[a] Average severity grade of lesion in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
* Significantly different ($p \leq 0.05$) from chamber controls by Poly-3 test

2-Year Study Results: Rats

Incidence of Neoplasms in the Adrenal Medulla

	Control	100 ppm	300 ppm	1,000 ppm
Females	**49**	**50**	**50**	**50**
Hyperplasia	17 (1.1)[a]	25 (1.6)	34** (1.1)	36** (1.5)
Benign Pheochromocytoma	0 (0/49)[b]	3	4	6*
Malignant Pheochromocytoma	0	1	0	0

[a] Average severity grade of lesion in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[b] Tumor incidence in controls from other SD rat NTP study
* Significantly different (p≤0.05) from chamber controls by Poly-3 test

Considered **some evidence** of carcinogenic activity

Incidence of Neoplasms in the Uterus

	Control	100 ppm	300 ppm	1,000 ppm
Females	**50**	**50**	**50**	**50**
Endometrium, Atypical Hyperplasia	0	0	1 (2.0)[a]	3 (2.3)
Adenoma	0	1	0	0
Adenocarcinoma[b]	1*	1	0	5

* Significantly different ($p \leq 0.05$) from chamber controls by Poly-3 test; positive trend if associated with a control incidence

[a] Average severity of lesion in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

2-Year Study Results: Rats

Incidence of Neoplasms in the Uterus

	Control	100 ppm	300 ppm	1,000 ppm
Females	**50**	**50**	**50**	**50**
Endometrium, Atypical Hyperplasia	0	0	1 (2.0)[a]	3 (2.3)
Adenoma	0	1	0	0
Adenocarcinoma[b]	1*	1	0	5

* Significantly different ($p \leq 0.05$) from chamber controls by Poly-3 test; positive trend if associated with a control incidence

[a] Average severity of lesion in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

[b] No historical control available for this tissue due to differences in sectioning

Considered **some evidence** of carcinogenic activity

Incidence of Neoplasms in the Uterus

	Control	100 ppm	300 ppm	1,000 ppm
Females	**50**	**50**	**50**	**50**
Stromal Polyp, Multiple	0	1	4	2
Stromal Polyp (includes multiple)	7	9	16*	12
Stromal Sarcoma	0	0	1	0
Stromal Polyp or Stromal Sarcoma	7	9	17*	12

*Significantly different ($p \leq 0.05$) from chamber controls by Poly-3 test; positive trend if associated with a control incidence

2-Year Study Results: Rats

Incidence of Neoplasms in the Uterus

	Control	100 ppm	300 ppm	1,000 ppm
Females	**50**	**50**	**50**	**50**
Stromal Polyp, Multiple	0	1	4	2
Stromal Polyp (includes multiple)[a]	7	9	16*	12
Stromal Sarcoma	0	0	1	0
Stromal Polyp or Stromal Sarcoma	7	9	17*	12

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[a] No historical control available for this tissue due to differences in sectioning

Considered **some evidence** of carcinogenic activity

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2-Year Studies

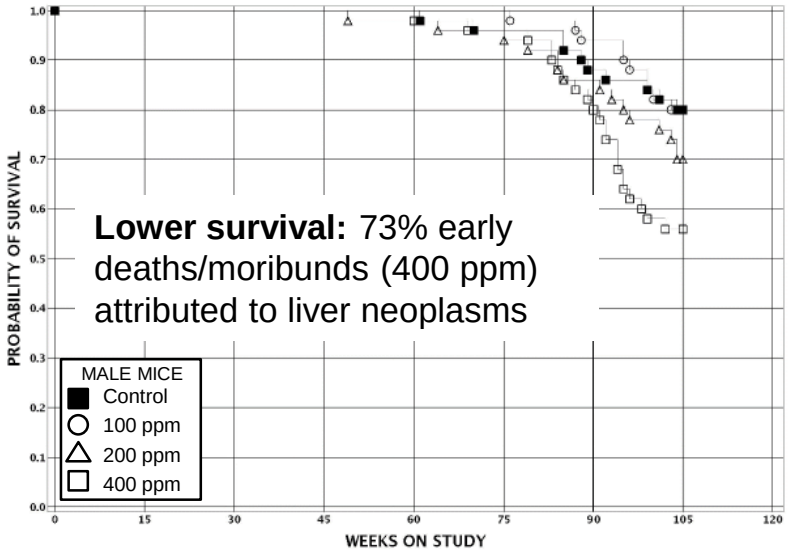
Mice

2-Year Study Results: Mice

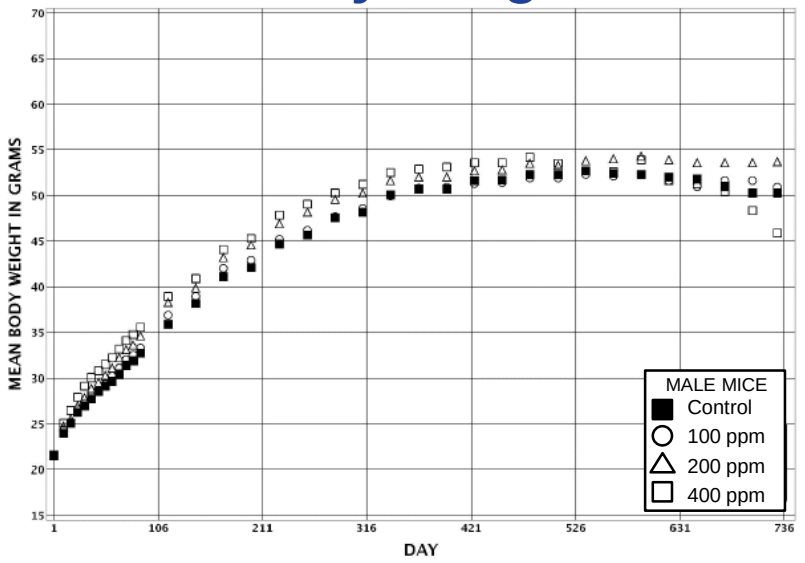
Survival

Body Weights

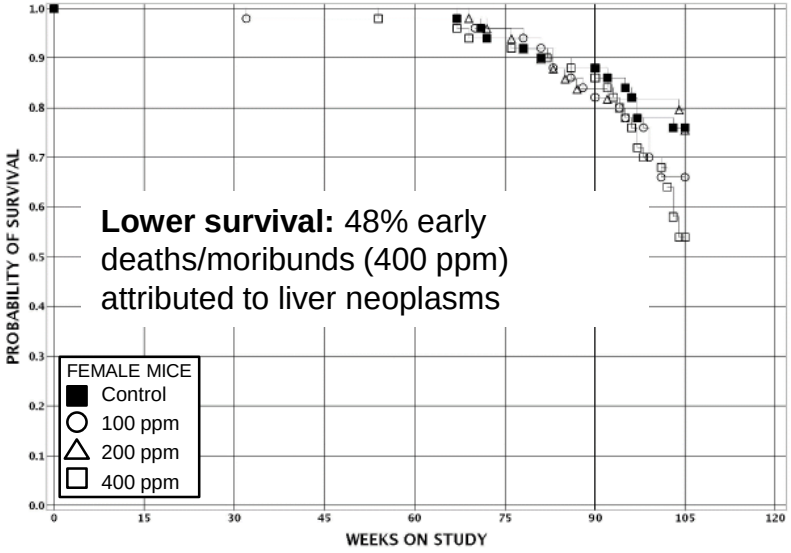
Male



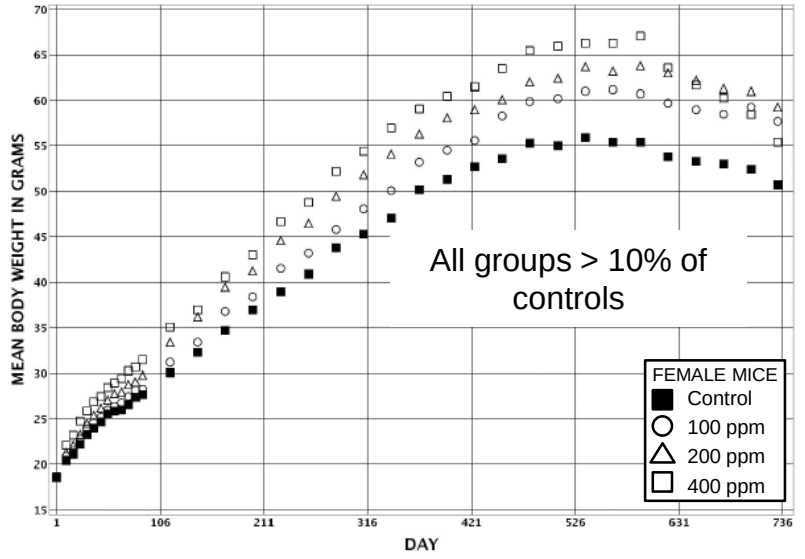
Lower survival: 73% early deaths/moribunds (400 ppm) attributed to liver neoplasms



Female



Lower survival: 48% early deaths/moribunds (400 ppm) attributed to liver neoplasms



All groups > 10% of controls

Clinical observations: Distended abdomen, associated with liver neoplasms

2-Year Study Results: Mice

Incidence of Neoplasms in the Liver

	Control	100 ppm	200 ppm	400 ppm
Males	**50**	**50**	**50**	**50**
Hepatocellular Adenoma, Multiple	9	15	19*	21**
Hepatocellular Adenoma (includes multiple)	25	24	31	29
Hepatocellular Carcinoma, Multiple	2	5	7	30**
Hepatocellular Carcinoma (includes multiple)	8**	19*	16*	35**
Hepatoblastoma, Multiple	0	0	0	5*
Hepatoblastoma (includes multiple)	1**	1	1	15**
Hepatocellular Adenoma, Hepatocellular Carcinoma or Hepatoblastoma	31**	37	40*	48**

*Significantly different ($p \leq 0.05$) from chamber controls by Poly-3 test
**Significantly different ($p \leq 0.01$) from chamber controls by Poly-3 test; positive trend if associated with a control incidence

2-Year Study Results: Mice

Incidence of Neoplasms in the Liver

	Control	100 ppm	200 ppm	400 ppm
Males	**50**	**50**	**50**	**50**
Hepatocellular Adenoma, Multiple	9	15	19*	21**
Hepatocellular Adenoma (includes multiple)	25	24	31	29
Hepatocellular Carcinoma, Multiple	2	5	7	30**
Hepatocellular Carcinoma (includes multiple)[a]	8**	19*	16*	35**
Hepatoblastoma, Multiple	0	0	0	5*
Hepatoblastoma (includes multiple)[b]	1**	1	1	15**
Hepatocellular Adenoma, Hepatocellular Carcinoma or Hepatoblastoma	31**	37	40*	48**

[a] Historical incidence (inhalation) 97/300, 16%-50%, (all routes) 165/550, 16%-50%
[b] Historical incidence (inhalation) 6/300, 0%-4%, (all routes) 18/550, 0%-8%

Considered **clear evidence** of carcinogenic activity

2-Year Study Results: Mice

Incidence of Neoplasms in the Liver

	Control	100 ppm	200 ppm	400 ppm
Females	**50**	**50**	**50**	**50**
Hepatocellular Adenoma, Multiple	3	5	15**	25**
Hepatocellular Adenoma (includes multiple)	12**	14	24**	34**
Hepatocellular Carcinoma, Multiple	2	3	7	28**
Hepatocellular Carcinoma (includes multiple)	7**	8	12	34**
Hepatoblastoma	0**	0	1	8**
Hepatocellular Adenoma, Hepatocellular Carcinoma or Hepatoblastoma	18**	18	29**	46**

**Significantly different ($p \leq 0.01$) from chamber controls by Poly-3 test; positive trend if associated with a control incidence

2-Year Study Results: Mice

Incidence of Neoplasms in the Liver

	Control	100 ppm	200 ppm	400 ppm
Females	**50**	**50**	**50**	**50**
Hepatocellular Adenoma, Multiple	3	5	15**	25**
Hepatocellular Adenoma (includes multiple)[a]	12**	14	24**	34**
Hepatocellular Carcinoma, Multiple	2	3	7	28**
Hepatocellular Carcinoma (includes multiple)[b]	7**	8	12	34**
Hepatoblastoma[c]	0**	0	1	8**
Hepatocellular Adenoma, Hepatocellular Carcinoma or Hepatoblastoma	18**	18	29**	46**

[a] Historical incidence (inhalation) 71/300, 12%-38%, (all routes) 141/549, 10%-67%
[b] Historical incidence (inhalation) 45/300, 10%-20%, (all routes) 71/549, 4%-20%
[c] Historical incidence (inhalation) 3/300, 0%-2%, (all routes) 3/549, 0%-2%

Considered **clear evidence** of carcinogenic activity

2-Year Study Results: Mice

Incidence of Neoplasms in the Harderian Gland

	Control	100 ppm	200 ppm	400 ppm
Females	**50**	**50**	**50**	**50**
Adenoma	2*	6	6	8*
Adenocarcinoma	0	0	3	0
Adenoma or Adenocarcinoma[a]	2*	6	9*	8*

*Significantly different ($p \leq 0.05$) from chamber controls by Poly-3 test; positive trend if associated with a control incidence

[a] Historical incidence (inhalation) 22/300, 4%-14%, (all routes) 47/550, 4%-20%

2-Year Study Results: Mice

Incidence of Neoplasms in the Harderian Gland

	Control	100 ppm	200 ppm	400 ppm
Females	**50**	**50**	**50**	**50**
Adenoma	2*	6	6	8*
Adenocarcinoma	0	0	3	0
Adenoma or Adenocarcinoma[a]	2*	6	9*	8*

*Significantly different ($p \leq 0.05$) from chamber controls by Poly-3 test; positive trend if associated with a control incidence

[a] Historical incidence (inhalation) 22/300, 4%-14%, (all routes) 47/550, 4%-20%

Also considered to be related to treatment (some evidence)

Nonneoplastic Lesions

Nonneoplastic Lesions: Lung

RATS	Control	100 ppm	300 ppm	1,000 ppm
Males	**50**	**50**	**50**	**50**
Fibrosis	8 (1.4)	22** (1.5)	28** (1.4)	24** (1.8)
Hemorrhage	11 (1.2)	23* (1.7)	28** (1.5)	28** (1.7)
Inflammation, Chronic	32 (1.2)	42* (1.5)	47** (1.4)	45** (1.8)
Females	**50**	**50**	**50**	**50**
Fibrosis	11 (1.0)	17 (1.1)	24** (1.4)	28** (1.4)
Hemorrhage	12 (1.1)	11 (1.5)	18 (1.4)	26* (1.4)
Inflammation, Chronic	35 (1.2)	42* (1.2)	48** (1.4)	46* (1.4)

MICE	Control	100 ppm	200 ppm	400 ppm
Males	**50**	**50**	**50**	**50**
Alveolar/bronchiolar epithelium, hyperplasia	0	49** (1.8)	50** (2.4)	48** (2.8)
Peribronchiolar, fibrosis	0	45** (1.0)	47** (1.0)	44** (1.0)
Females	**50**	**50**	**50**	**50**
Alveolar/bronchiolar epithelium, hyperplasia	0	49** (2.1)	49** (2.3)	50** (2.9)
Peribronchiolar, fibrosis	0	44** (1.0)	44** (1.0)	48** (1.0)

Significantly different (*p≤0.05, **p≤0.01) from chamber controls by Poly-3 test
Data in parentheses are severities (1=minimal, 2=mild, 3=moderate, 4=marked)

Nonneoplastic Lesions: Lung

RATS	Control	100 ppm	300 ppm	1,000 ppm
Males	**50**	**50**	**50**	**50**
Fibrosis	8 (1.4)	22** (1.5)	28** (1.4)	24** (1.8)
Hemorrhage	11 (1.2)	23* (1.7)	28** (1.5)	28** (1.7)
Inflammation, Chronic	32 (1.2)	42* (1.5)	47** (1.4)	45** (1.8)
Females	**50**	**50**	**50**	**50**
Fibrosis	11 (1.0)	17 (1.1)	24** (1.4)	28** (1.4)
Hemorrhage	12 (1.1)	11 (1.5)	18 (1.4)	26* (1.4)
Inflammation, Chronic	35 (1.2)	42* (1.2)	48** (1.4)	46* (1.4)

MICE	Control	100 ppm	200 ppm	400 ppm
Males	**50**	**50**	**50**	**50**
Alveolar/bronchiolar epithelium, hyperplasia	0	49** (1.8)	50** (2.4)	48** (2.8)
Peribronchiolar, fibrosis	0	45** (1.0)	47** (1.0)	44** (1.0)
Females	**50**	**50**	**50**	**50**
Alveolar/bronchiolar epithelium, hyperplasia	0	49** (2.1)	49** (2.3)	50** (2.9)
Peribronchiolar, fibrosis	0	44** (1.0)	44** (1.0)	48** (1.0)

Significantly different (*p≤0.05, **p≤0.01) from chamber controls by Poly-3 test
Data in parentheses are severities (1=minimal, 2=mild, 3=moderate, 4=marked)

Nonneoplastic Lesions: Lung

RATS	Control	100 ppm	300 ppm	1,000 ppm
Males	**50**	**50**	**50**	**50**
Fibrosis	8 (1.4)	22** (1.5)	28** (1.4)	24** (1.8)
Hemorrhage	11 (1.2)	23* (1.7)	28** (1.5)	28** (1.7)
Inflammation, Chronic	32 (1.2)	42* (1.5)	47** (1.4)	45** (1.8)
Females	**50**	**50**	**50**	**50**
Fibrosis	11 (1.0)	17 (1.1)	24** (1.4)	28** (1.4)
Hemorrhage	12 (1.1)	11 (1.5)	18 (1.4)	26* (1.4)
Inflammation, Chronic	35 (1.2)	42* (1.2)	48** (1.4)	46* (1.4)

MICE	Control	100 ppm	200 ppm	400 ppm
Males	**50**	**50**	**50**	**50**
Alveolar/bronchiolar epithelium, hyperplasia	0	49** (1.8)	50** (2.4)	48** (2.8)
Peribronchiolar, fibrosis	0	45** (1.0)	47** (1.0)	44** (1.0)
Females	**50**	**50**	**50**	**50**
Alveolar/bronchiolar epithelium, hyperplasia	0	49** (2.1)	49** (2.3)	50** (2.9)
Peribronchiolar, fibrosis	0	44** (1.0)	44** (1.0)	48** (1.0)

Significantly different (*p≤0.05, **p≤0.01) from chamber controls by Poly-3 test
Data in parentheses are severities (1=minimal, 2=mild, 3=moderate, 4=marked)

Nonneoplastic Lesions: Lung

RATS	Control	100 ppm	300 ppm	1,000 ppm
Males	**50**	**50**	**50**	**50**
Fibrosis	8 (1.4)	22** (1.5)	28** (1.4)	24** (1.8)
Hemorrhage	11 (1.2)	23* (1.7)	28** (1.5)	28** (1.7)
Inflammation, Chronic	32 (1.2)	42* (1.5)	47** (1.4)	45** (1.8)
Females	**50**	**50**	**50**	**50**
Fibrosis	11 (1.0)	17 (1.1)	24** (1.4)	28** (1.4)
Hemorrhage	12 (1.1)	11 (1.5)	18 (1.4)	26* (1.4)
Inflammation, Chronic	35 (1.2)	42* (1.2)	48** (1.4)	46* (1.4)

MICE	Control	100 ppm	200 ppm	400 ppm
Males	**50**	**50**	**50**	**50**
Alveolar/bronchiolar epithelium, hyperplasia	0	49** (1.8)	50** (2.4)	48** (2.8)
Peribronchiolar, fibrosis	0	45** (1.0)	47** (1.0)	44** (1.0)
Females	**50**	**50**	**50**	**50**
Alveolar/bronchiolar epithelium, hyperplasia	0	49** (2.1)	49** (2.3)	50** (2.9)
Peribronchiolar, fibrosis	0	44** (1.0)	44** (1.0)	48** (1.0)

Significantly different (*p≤0.05, **p≤0.01) from chamber controls by Poly-3 test
Data in parentheses are severities (1=minimal, 2=mild, 3=moderate, 4=marked)

Nonneoplastic Lesions: Lung

RATS	Control	250 ppm	500 ppm	1,000 ppm
Males	50	50	50	50
Fibrosis	8 (1.4)	22** (1.5)	28** (1.4)	24** (1.8)
Hemo	11 (1.3)	23* (1.7)	28** (1.5)	28** (1.7)
Inflam				1.8)
Females				
Fibros				1.4)
Hemo				.4)
Inflam				.4)
MICE				pm
Males				
Alveol				2.8)
Peribronchiolar, fibrosis	0	45** (1.0)	47** (1.0)	44** (1.0)
Females	50	50	50	50
Alveolar/bronchiolar epithelium, hyperplasia	0	49** (2.1)	49** (2.3)	50** (2.9)
Peribronchiolar, fibrosis	0	44** (1.0)	44** (1.0)	48** (1.0)

Currently no federal occupational exposure limits

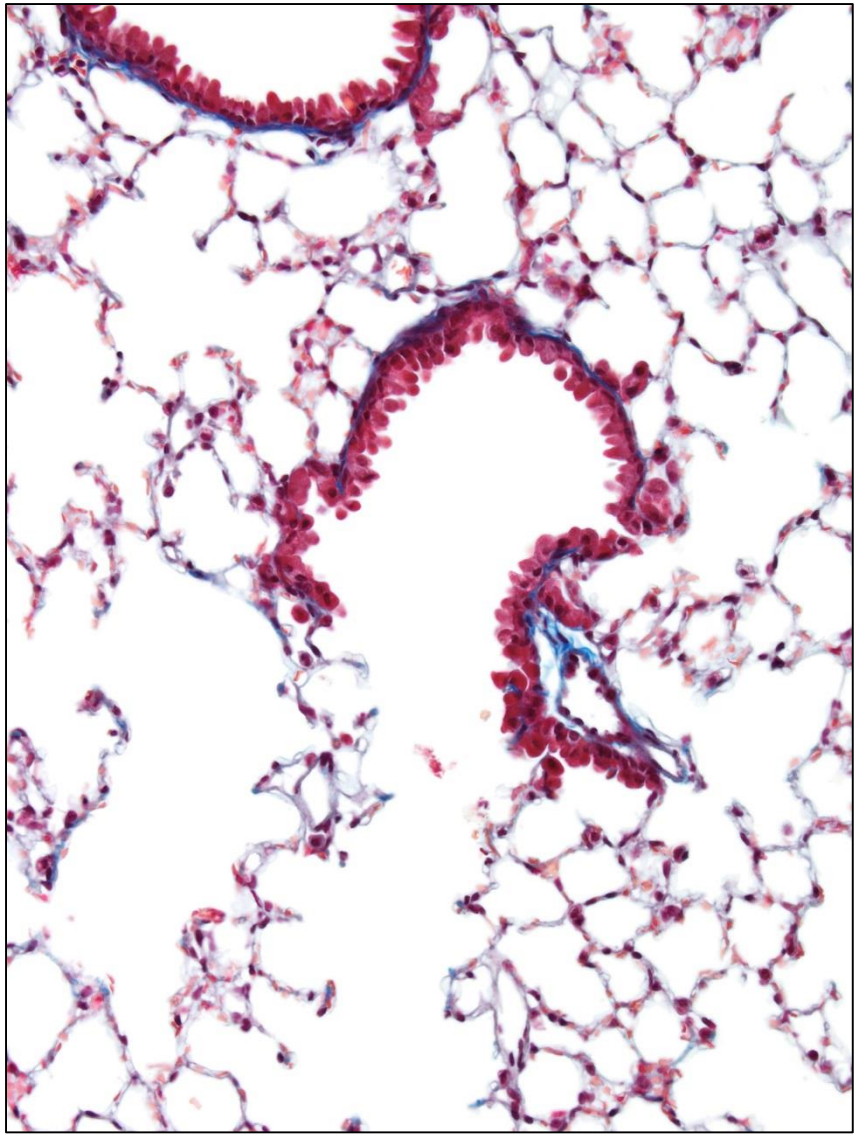
Upon nomination, Kowa was using **20 ppm** as permissible exposure limit (8-hour TWA)

In present study ~100% incidence of fibrosis and alveolar/bronchiolar epithelium, hyperplasia in mice at **100 ppm**

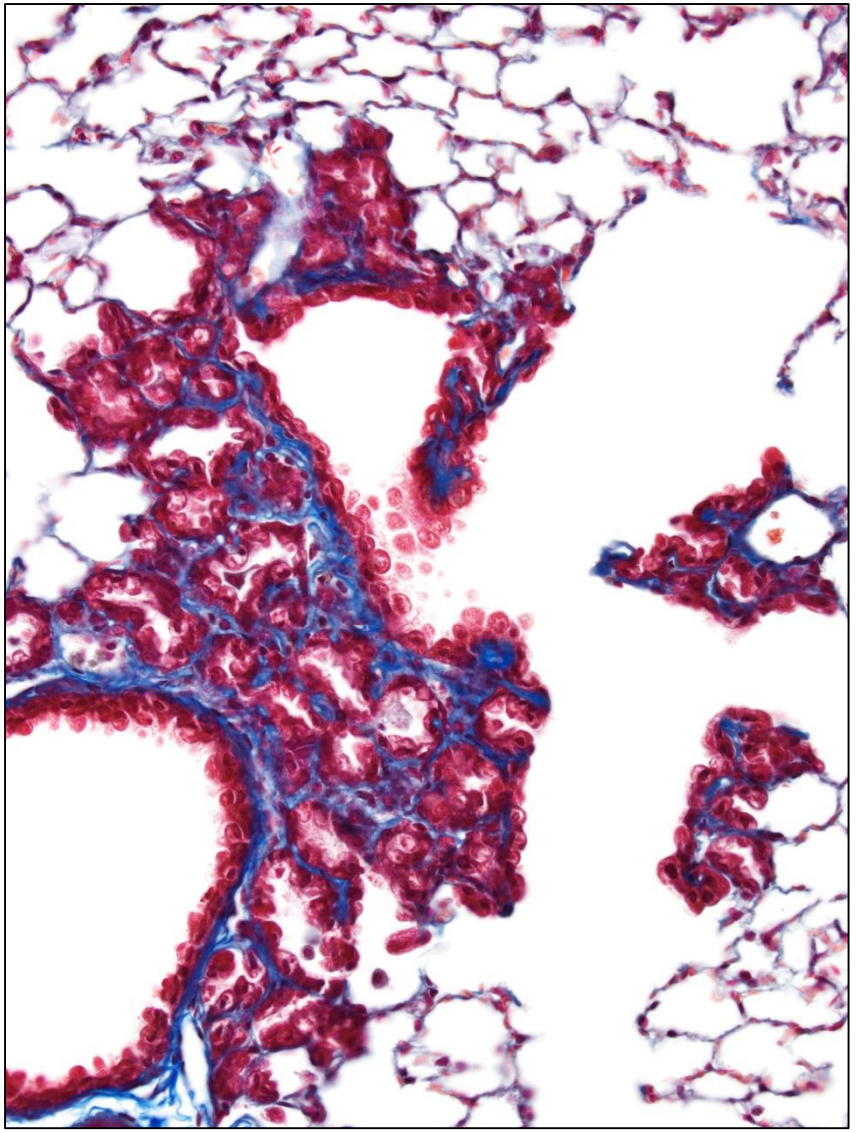
Significantly different (*p≤0.05, **p≤0.01) from chamber controls by Poly-3 test
Data in parentheses are severities (1=minimal, 2=mild, 3=moderate, 4=marked)

Peribronchiolar fibrosis (trichrome stain)

Control



Exposed



Nonneoplastic Lesions: Liver

	Control	Low	Mid	High
	50	**50**	**50**	**50**
Centrilobular, Hepatocyte, Hypertrophy				
Male Rats	2 (1.0)	17** (1.2)	39** (1.5)	47** (1.8)
Female Rats	0	1 (2.0)	10** (1.1)	45** (1.9)
Male Mice	0	8** (1.0)	19** (1.1)	49** (1.5)
Female Mice	0	4 (1.0)	5* (1.2)	40** (1.2)
Eosinophilic Foci				
Male Rats	1	5	6	8**
Female Rats	7	6	7	15
Male Mice	11	14	18	21*
Female Mice	4	8	24**	31**
Fatty Change				
Male Rats	0	3 (1.3)	7** (1.3)	26** (1.2)
Female Rats	2 (1.0)	4 (1.0)	11* (1.2)	10* (1.3)
Hepatocyte, Multinucleated				
Male Mice	2 (1.0)	8 (1.0)	19** (1.1)	49** (1.4)
Female Mice	0	2 (1.0)	2 (1.0)	25** (1.0)
Hepatocyte, Necrosis				
Male Mice	3 (1.0)	4 (2.5)	3 (2.0)	15** (1.5)
Female Mice	2 (2.0)	1 (1.0)	3 (1.7)	10* (1.3)

Significantly different (*p≤0.05, **p≤0.01) from chamber controls by Poly-3 test; Data in parentheses are severities (1=minimal, 2=mild, 3=moderate, 4=marked). Other nonneoplastic lesions of the liver include mixed cell focus (female rats), clear cell focus (female rats), intrahepatocellular erythrocytes (male mice)

Nonneoplastic Lesions: Liver

	Control	Low	Mid	High
	50	**50**	**50**	**50**
Centrilobular, Hepatocyte, Hypertrophy				
Male Rats	2 (1.0)	17** (1.2)	39** (1.5)	47** (1.8)
Female Rats	0	1 (2.0)	10** (1.1)	45** (1.9)
Male Mice	0	8** (1.0)	19** (1.1)	49** (1.5)
Female Mice	0	4 (1.0)	5* (1.2)	40** (1.2)
Eosinophilic Foci				
Male Rats	1	5	6	8**
Female Rats	7	6	7	15
Male Mice	11	14	18	21*
Female Mice	4	8	24**	31**
Fatty Change				
Male Rats	0	3 (1.3)	7** (1.3)	26** (1.2)
Female Rats	2 (1.0)	4 (1.0)	11* (1.2)	10* (1.3)
Hepatocyte, Multinucleated				
Male Mice	2 (1.0)	8 (1.0)	19** (1.1)	49** (1.4)
Female Mice	0	2 (1.0)	2 (1.0)	25** (1.0)
Hepatocyte, Necrosis				
Male Mice	3 (1.0)	4 (2.5)	3 (2.0)	15** (1.5)
Female Mice	2 (2.0)	1 (1.0)	3 (1.7)	10* (1.3)

Significantly different (*p≤0.05, **p≤0.01) from chamber controls by Poly-3 test; Data in parentheses are severities (1=minimal, 2=mild, 3=moderate, 4=marked). Other nonneoplastic lesions of the liver include mixed cell focus (female rats), clear cell focus (female rats), intrahepatocellular erythrocytes (male mice)

Additional Nonneoplastic Lesions

- Kidney: nephropathy severity (male rats)
- Nose: exudate (male rats)
- Larynx: squamous epithelium hyperplasia (male mice)
- Forestomach: inflammation and epithelial hyperplasia (male and female mice)

Genetic Toxicology

- Bacterial mutagenicity
  - Negative
- Micronucleus
  - Rats: negative (all changes in historical range)
  - Mice
    - Positive in males
    - Negative in females (all changes in historical range)

Neoplasms: Rats

- Males
  - ***Some evidence of carcinogenic activity***
    - Increased incidences of C-cell adenoma in the thyroid gland
  - **May have been related to exposure** (equivocal evidence)
    - Combined occurrences of alveolar/bronchiolar adenoma or carcinoma in the lung
- Females
  - ***Some evidence of carcinogenic activity***
    - Increased incidences of C-cell adenoma in the thyroid gland
    - Increased incidences of benign pheochromocytoma in the adrenal medulla
    - Increased incidences of adenocarcinoma in the uterus
    - Increased incidences of stromal polyp in the uterus

Neoplasms: Mice

- Males
  - ***Clear evidence of carcinogenic activity***
    - Increased incidences of hepatocellular carcinoma and hepatoblastoma in the liver
- Females
  - ***Clear evidence of carcinogenic activity***
    - Increased incidences of hepatocellular adenoma, hepatocellular carcinoma, and hepatoblastoma in the liver
  - **Related to treatment** (some evidence)
    - Combined incidences of adenoma or adenocarcinoma in the Harderian gland

Nonneoplastic Lesions

- Increased incidences of nonneoplastic lesions in the:
  - Lung and liver of male and female rats and mice
  - Nose of male rats
  - Adrenal medulla and uterus of female rats
  - Forestomach of male and female mice
  - Larynx in male mice
- Increased severity of nonneoplastic lesions in the kidney of male rats