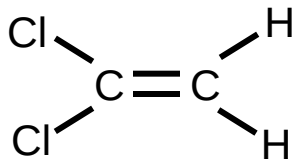
NTP
National Toxicology Program

Draft NTP Technical Report TR582 on Vinylidene Chloride in F344/N Rats and B6C3F1/N Mice

(Inhalation Studies)



Michael Wyde, Ph.D.

National Institute of Environmental Health Sciences

NTP Technical Reports Peer Review Meeting
October 29, 2013

Background

- Nominated by Agency for Toxic Substances and Disease Registry (ATSDR) based on insufficient critical information concerning health effects
- Used as intermediate in organic synthesis reactions and in production of polyvinylidene chloride polymers and copolymers
- Occupational exposure via inhalation or dermal contact; primary source of environmental contamination through air emissions and effluent waters from processing facilities
  - Common in household products, artificial turf, pipes, in lacquer resins and latex, and flame-resistant carpet backing
- US annual production estimated at 79,000 tons (2003)
- ACGIH Threshold Limit Value (TLV) 5 ppm; EPA Category C carcinogen – a possible human carcinogen

NTP Program of Study for VDC

- **2-Week** inhalation toxicity studies in male and female Fisher 344/N rats and B6C3F_1/N mice
- **3-Month** inhalation studies in rats and mice
- **2-year** inhalation studies in rats and in mice
- **Genotoxicity** testing
  - Salmonella, Drosophila reciprocal translocation/sex-linked recessive lethal, mouse lymphoma, micronucleus

Genetic Toxicity Test Results for Vinylidene Chloride

Test	Result
Bacterial Mutagenicity	Negative (+/- S9)
Mouse Lymphoma Cell Mutagenicity	Positive (+ S9) Equivocal (- S9)
Drosophila Sex-Linked Recessive Lethals	Negative
Erythrocyte Micronucleus	Negative (♂/♀ Mice)

Vinylidene Chloride 2-Week Studies

- Male and female F344/N rats (up to 400 ppm)
  - No survival in males or females at 200 or 400 ppm
  - Decreased body weight gain in 100 ppm females
  - Increased kidney weights and centrilobular hepatocyte necrosis and cytoplasmic alteration in males and females
- Male and female B6C3F1/N mice (up to 400 ppm)
  - No survival in females at 200 or 400 ppm, or in males at ≥ 100 ppm
  - Decreased body weight gain in 25 and 50 ppm males
  - Increased lung weight in females; increased liver weights in males and females
  - Liver necrosis in 100 ppm males and females and regeneration in 100 ppm females
  - Tubule necrosis, regeneration, granular casts in male kidney (25-50ppm)

3-Month Studies

- Male and female F344/N rats and B6C3F1/N mice (n=10)
- Exposure concentrations
  - Rats: 0, 6.25, 12.5, 25, 50, and 100 ppm
  - Female mice: 0, 6.25, 12.5, 25, 50, and 100 ppm
  - Male mice: 0, 6.25, 12.5, 25, and 50 ppm
- 6 hours a day, 5 days a week

3-Month Results in Rats

- No effect on mortality, body weight gain, hematological indices in males or females
- Increases in kidney weights in females ≥12.5 ppm
- In the liver, cytoplasmic vacuolization (females ≥50 ppm) and centrilobular cytoplasmic alteration (males ≥12.5 ppm) were observed
  - Transient increase in sorbitol dehydrogenase and alanine aminotransferase
- Increased olfactory epithelium atrophy, mineralization, and necrosis, and turbinate atrophy in male and females
  - Nasal lesions were not considered to be sufficient to preclude 100 ppm as exposure concentration for the chronic rat studies
  - Selected **0, 25, 50, and 100 ppm** for chronic inhalation studies in rats

3-Month Results in Mice

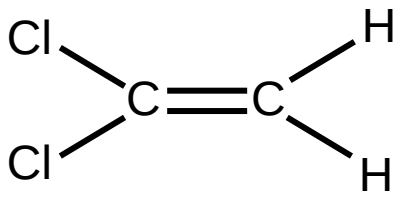
- Decreased survival in 50 ppm males and 100 ppm females
- Lower mean body weight in all exposed females (9-18%) and ≥12.5 ppm males (10-16%)
- Exposure concentration-related decreases in red blood cell indices, males affected at lower exposure concentrations than females
- Increased liver weight in females at ≥12.5 ppm; increased kidney and lung weights in 100 ppm females
- Lesions observed in 100 ppm females:
  - Liver necrosis, centrilobular hypertrophy; lung histiocytic inflammation and necrosis of the bronchus epithelium; respiratory epithelium necrosis and turbinate atrophy in nose
- Increased incidence of squamous metaplasia of the respiratory epithelium of the larynx of ≥ 50 ppm females and 50 ppm males
- Increased incidence and severity of nephropathy in males at ≥12.5 ppm

Exposure Selection for Chronic Studies in Mice

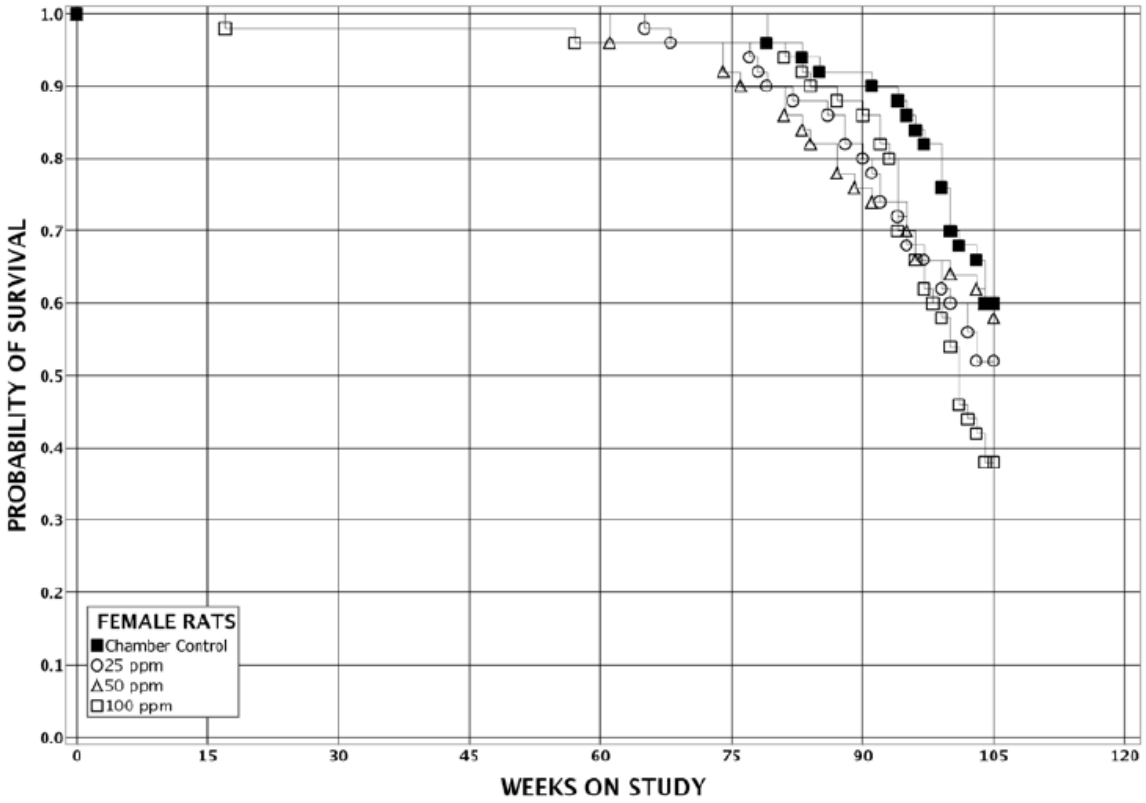
- Chronic exposure concentration selection primarily dependent on survival and body weight reductions
  - Increased mortality in males at 50 ppm; only observed decreased body weight in males at 25 ppm (absence of other overt toxicity)
  - Smaller decrease in body weight in 6.25, 12.5, and 25 ppm females (9-12%) compared to the 50 ppm group (18%)
  - No treatment-related histopathology
- Selected **0, 6.25, 12.5, and 25 ppm** for chronic inhalation studies in mice

Chronic Rat Studies

Vinylidene Chloride



Decreased Survival in 100 ppm Females



DOSE	Control	25 ppm	50 ppm	100 ppm
SURVIVAL AT END OF STUDY (KAPLAN-MEIER)	60.0%	52.0%	58.0%	38.0%
SIGNIFICANCE (B) (LIFE TABLE)	P=0.046	P=0.337	P=0.709	P=0.029

Chronic Study Results – Histopathological Findings

- **Systemic Neoplasms**
  - Malignant mesothelioma (males), Mononuclear cell leukemia (females)
- **Thyroid gland** (females)
  - C-cell adenoma, carcinoma
- **Kidney** (males)
  - Neoplasms: Renal tubular adenoma (step sections), renal tubular carcinoma
  - Non-neoplastic: Renal tubular hyperplasia
- **Nose** (males and females)
  - Neoplasms: Respiratory epithelium adenoma (males)
  - Non-neoplastic lesions: Turbinate atrophy, turbinate hyperostosis, chronic active inflammation, olfactory epithelial metaplasia, respiratory inflammatory polyp (females only)
- **Liver** (males and females)
  - Non-neoplastic: chronic inflammation, diffuse fatty change, necrosis, cystic degeneration

Incidence of Malignant Mesotheliomas in Rats

SYSTEMIC LESIONS	0 ppm	25ppm	50ppm	100ppm
Males				
Mesothelioma, malignant[a]	1**	12**	28**	23**
Females				
Mesothelioma, malignant[b]	0	1	1	0

* p<0.05; ** p < 0.01; (n=50)
[a] Historical control same route 1/200 (range 0-2%), all routes 26/699 (range 0-8%)
[b] Historical control same route 0/200, all routes 0/700

- Grossly observed fluid in the abdomen and multiple nodules on the peritoneum particularly on the testicular tunics and epididymides
  - Lesions clearly associated with exposure to VDC, resulting from the occurrence of mesothelioma

Incidence of Thyroid Gland and Systemic Neoplasms in Female Rats

THYROID	0 ppm	25 ppm	50 ppm	100 ppm
C-cell adenoma[a]	3**	4	6	11*
C-cell carcinoma[b]	0	6*	2	2
C-cell adenoma or carcinoma[c]	3**	10*	8	13**
SYSTEMIC				
Mononuclear Cell Leukemia[d]	10**	11	13	25**

*p < 0.05, **p < 0.01; n=50

[a] Historical control same route 13/200 (range 6-8%), all routes 81/690 (range 6-22%)

[b] Historical control same route 1/200 (range 0-2%), all routes 6/690 (range 0-7%)

[c] Historical control same route 14/200 (range 6-8%), all routes 87/690 (range 6-22%)

[d] Historical control same route 58/200 (range 20-34%), all routes 165/700 (range 10-36%)

Incidence of Kidney Lesions in Males

KIDNEY (Standard Single Sections)	0 ppm	25 ppm	50 ppm	100 ppm
Renal tubule, hyperplasia[a]	0	1 [2.0]	1 [1.0]	1 [4.0]
Renal tubule, carcinoma[b]	0	2	1	1

[a] Data presented as Incidence [Average severity grade]; 1=minimal, 2=mild, 3=moderate, 4=marked
[b] Historical control same route 0/200; all routes 1/697 (range 0-2%)

Vinylidene Chloride-Induced Lesions in the Nose (Males)

NOSE	0 ppm	25 ppm	50 ppm	100 ppm
Respiratory epithelium adenoma[b]	0**	0	1	4
Turbinate atrophy	0	50 [2.2]**	50 [3.2]**	50 [3.8]**
Turbinate hyperostosis	0	49 [2.1]**	50 [2.6]**	50 [2.9]**
Olfactory epithelium, respiratory metaplasia	3 [1.0]	49 [2.5]**	49 [3.2]**	48 [3.5]**
Olfactory epithelium, squamous metaplasia	0	0	1 [2.0]	5 [1.2]*
Respiratory epithelium, hyperplasia	5 [1.6]	8 [1.5]	22 [2.5]**	31 [2.3]**
Inflammation, chronic active	9 [1.2]	36 [2.0]**	45 [2.7]**	48 [3.2]**
Thrombosis	4 [2.3]	4 [3.0]	11 [3.3]*	7 [2.7]

* $p < 0.05$, **$p < 0.01$, n=50
[a] Data presented as Incidence [Average severity grade]; 1=minimal, 2=mild, 3=moderate, 4=marked
[b] Historical control same route 0/198, all routes 0/697

Nonneoplastic Lesions in the Liver

Males	0 ppm	25 ppm	50 ppm	100 ppm
Inflammation, chronic	28 [1.0]	46 [1.2]**	46 [1.3]**	44 [1.9]**
Fatty change, diffuse	4 [2.0]	19 [1.7]**	18 [1.7]**	26 [2.0]**
Necrosis	2 [2.5]	6 [2.8]	8 [2.6]*	6 [2.3]
Degeneration, cystic	2 [2.0]	5 [2.8]	7 [1.9]	12 [2.1]**
Females				
Inflammation, chronic	42 [1.0]	48 [1.4]*	49 [1.4]**	48 [2.1]**
Fatty change, diffuse	19 [1.2]	30 [1.7]*	26 [1.7]	30 [2.0]**
Necrosis	0	3 [1.7]	5 [2.2]*	11 [1.8]**
Degeneration, cystic	0	2 [3.0]	4 [2.3]*	7 [2.7]**

Data presented as Incidence [Average severity grade]; 1=minimal, 2=mild, 3=moderate, 4=marked
*p < 0.05, **p < 0.01, n=50

Evidence for Carcinogenic Activity in Rats

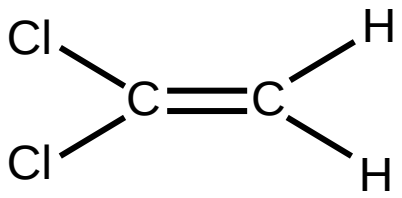
- **Males**
  - *Clear evidence* in male rats based on increased incidences of malignant mesothelioma.
  - Increased incidences of renal tubule carcinomas and respiratory epithelium adenomas in the nose *were also* related to vinylidene chloride exposure
- **Females**
  - *Some evidence* in female rats based on increased incidences of C-cell adenoma or carcinoma in the thyroid gland and systemic mononuclear cell leukemia
  - Occurrences of malignant mesothelioma may have been related to vinylidene chloride exposure

Treatment-Related Nonneoplastic Lesions in Rats

- **Males**
  - *Kidney* - renal tubular hyperplasia
  - *Nose* - turbinate atrophy and hyperostosis, olfactory epithelial hyperplasia, olfactory epithelial respiratory and squamous metaplasia, chronic active inflammation
  - *Liver* - chronic inflammation, diffuse fatty change, necrosis, cystic degeneration
- **Females**
  - *Nose* - turbinate atrophy and hyperostosis, olfactory epithelial respiratory and squamous metaplasia, respiratory epithelial hyperplasia, chronic active inflammation
  - *Liver* - chronic inflammation, diffuse fatty change, necrosis, cystic degeneration

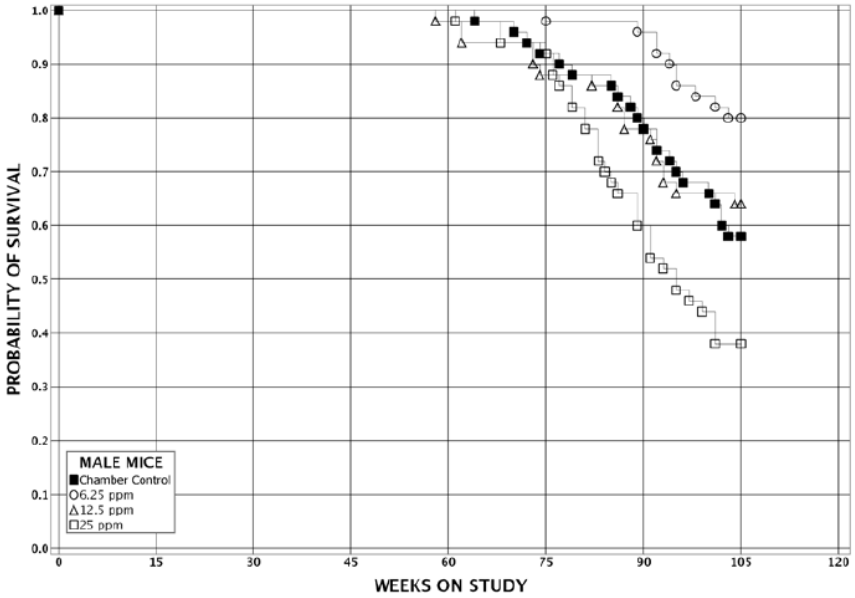
Chronic Mouse Studies

Vinylidene Chloride

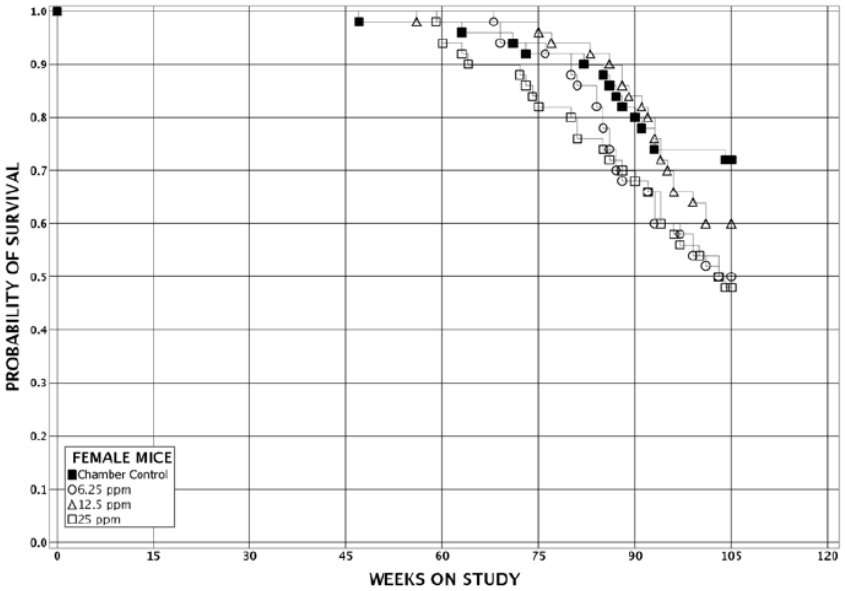


Survival in Mice Exposed to Vinylidene Chloride

Males



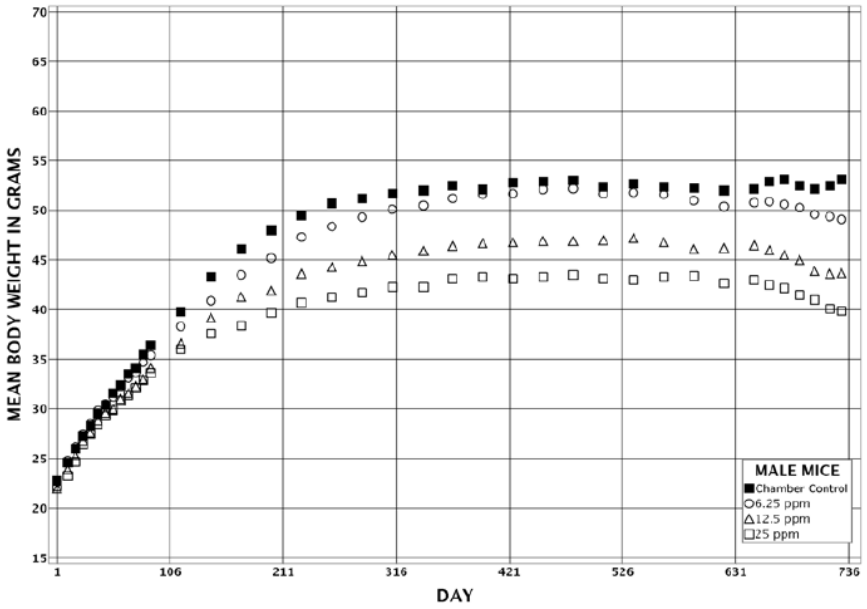
Females



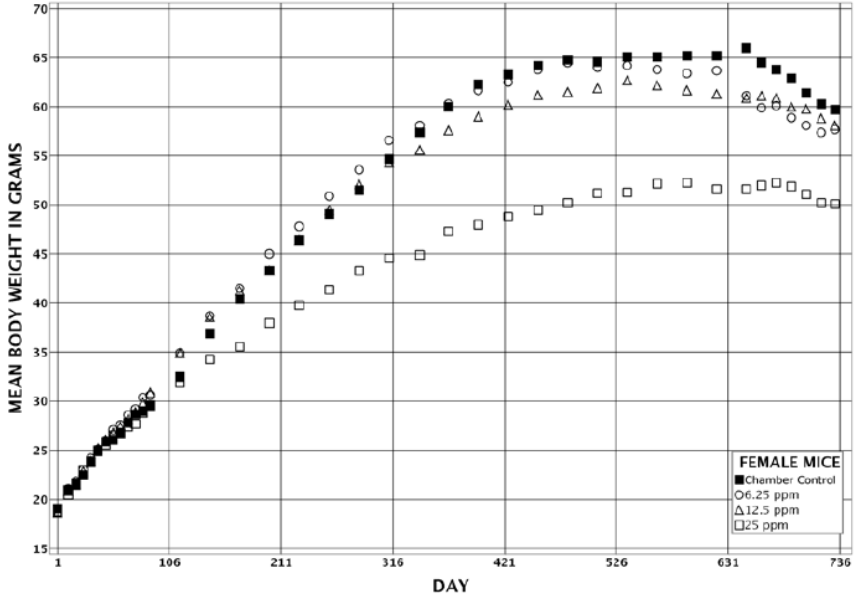
- Decreased survival in males at 25 ppm and females at 6.25 and 25 ppm
- Increased survival in 6.25 ppm males

Body Weights in Mice Exposed to Vinylidene Chloride

Males



Females



- Mean body weights were decreased in 12.5 ppm males (10-17%) and 25 ppm males (10-24%)
- Mean body weights were decreased in 25 ppm females (12-23%)

Chronic Study Results – Histopathological Findings

- **Kidney**
  - Neoplasms: Renal tubular adenoma, carcinoma (males)
  - Non-neoplastic: Renal tubular hyperplasia and cyst (males)
- **Liver**
  - Neoplastic: Hepatocellular adenoma and carcinoma (females), hepatocholangiocarcinoma (males and females)
- **Systemic**
  - Hemangioma or Hemangiosarcoma (females)
- **Lungs**
  - Alveolar/bronchiolar adenoma or carcinoma (females)
- **Small intestine**
  - Carcinoma
- **Nose** (males and females)
  - Non-neoplastic: Hyperostosis, turbinate atrophy; olfactory epithelial metaplasia, respiratory; hyaline droplet accumulation of olfactory epithelium; respiratory epithelial hyperplasia

Incidences of Kidney Lesions in Male Mice

Neoplasms	0 ppm	6.25 ppm	12.5 ppm	25 ppm
Renal tubule adenoma[a]	0**	5*	19**	10**
Renal tubule carcinoma[b]	0**	7*	31**	18**
Renal tubule adenoma or carcinoma[c]	0**	11**	37**	27**
Nonneoplastic lesions				
Renal tubule, hyperplasia	0	8 [1.8]**	22 [1.7]**	16 [1.7]**

* p<0.05; ** p < 0.01; (n=50)
[a] Historical control same route 0/298, all routes 8/944 (range 0-4%)
[b] Historical control same route 0/298, all routes 3/944 (range 0-4%)
[c] Historical control same route 0/298, all routes 11/944 (range 0-6%)

Incidences of Hemangioma and Hemangiosarcoma in Female Mice

LIVER	0ppm	6.25ppm	12.5ppm	25ppm
Hemangiosarcoma	1**	1	1	6*
ALL ORGANS				
Hemangioma[a]	0	2	2	2
Hemangiosarcoma[b]	4	4	4	9
Hemangioma or Hemangiosarcoma[c]	4*	6	6	11*

* $p<0.05$; ** $p < 0.01$; (n=50)
[a]Historical control same route 0/300, all routes 5/950 (range 0-2%)
[b]Historical control same route 21/300 (range 4-10%), all routes 50/950 (range 0-12%)
[c]Historical control same route 21/300 (range 4-10%), all routes 55/950 (range 2-14%)

Incidence of Liver Neoplasms in Mice

Females	0 ppm	6.25 ppm	12.5 ppm	25 ppm
Hepatocellular adenoma[a]	25*	21	36*	29
Hepatocellular carcinoma[b]	8*	14	12	17*
Hepatocellular adenoma or carcinoma[c]	28**	30	37*	38**
Hepatocholangiocarcinoma[d]	0	1	1	2
Males				
Hepatocholangiocarcinoma[e]	1	2	2	3

* $p<0.05$; ** $p < 0.01$; (n=50)
[a]Historical control same route 105/300 (range 28-50%), all routes 378/948 (range 14-78%)
[b]Historical control same route 44/300 (range 10-20%), all routes 152/948 (range 4-46%)
[c]Historical control same route 133/300 (range 32-56%), all routes 448/948 (range 20-82%)
[d]Historical control same route 0/300, all routes 0/948
[e]Historical control same route 2/299 (range 0-2%), all routes 10/949 (range 0-8%)

Incidences of Neoplasms in the Lung and Small Instestine of Female Mice

LUNG	0ppm	6.25ppm	12.5ppm	25ppm
Alveolar/bronchiolar adenoma	3	4	2	2
Alveolar/bronchiolar carcinoma[a]	1*	2	7*	5
Alveolar/bronchiolar adenoma or carcinoma	4	5	9	7
SMALL INTESTINE				
Carcinoma[b]	1	1	1	3
Adenoma or Carcinoma[c]	2	1	2	4

* p<0.05; ** p < 0.01; (n=50)
[a]Historical control same route 13/299 (range 0-10%), all routes 38/949 (range 0-14%)
[b]Historical control same route 2/300 (range 0-2%), all routes 5/950 (range 0-2%)
[c]Historical control same route 4/300 (range 0-4%), all routes 10/950 (range 0-4%)

Nonneoplastic Lesions of the Nose

Males	0 ppm	6.25 ppm	12.5 ppm	25 ppm
Turbinate atrophy	0	46 [1.1]**	46 [2.1]**	47 [2.8]**
Hyperostosis	1 [2.0]	27 [1.3]**	45 [2.1]**	48 [2.2]**
Olfactory epithelium, hyaline droplet accumulation	2 [1.0]	5 [1.0]	13 [1.3]**	11 [1.3]**
Olfactory epithelium, respiratory metaplasia	17 [1.2]	39 [1.2]**	47 [1.6]**	48 [1.8]**
Females				
Turbinate atrophy	0	46 [1.0]**	50 [2.3]**	49 [2.8]**
Hyperostosis	0	13 [1.2]**	45 [2.0]**	48 [2.2]**
Olfactory epithelium, hyaline droplet accumulation	18 [1.6]	18 [1.5]	13 [1.4]	32 [1.8]**
Olfactory epithelium, respiratory metaplasia	3 [1.0]	29 [1.1]**	49 [1.6]**	50 [1.9]**
Respiratory epithelium hyperplasia	33 [1.1]	41 [1.2]	39 [1.5]	43 [1.8]**

Evidence for Carcinogenic Activity in Mice

- **Males**
  - *Clear evidence* in male mice based on increased incidences of renal tubule adenoma and carcinoma
  - Increased incidences of hepatocholangiocarcinoma may have been related to vinylidene chloride exposure.
- **Females**
  - *Clear evidence* in female mice based on increased incidences systemic hemangioma or hemangiosarcoma (combined)
  - Hepatocholangiocarcinoma and hepatocellular adenoma and carcinoma (combined) in the liver of female mice *were also* considered to be related to vinylidene chloride exposure.
  - Increased incidences of alveolar/bronchiolar carcinoma in the lungs and carcinoma of the small intestine may have been related to treatment.

Treatment-Related Nonneoplastic Lesions in Mice

- **Males**
  - *Kidney* - renal tubule hyperplasia, cysts
  - *Nose* - turbinate atrophy and hyperostosis, olfactory epithelium respiratory metaplasia, olfactory epithelium hyaline droplet accumulation
- **Females**
  - *Nose* - turbinate atrophy and hyperostosis, olfactory epithelium respiratory metaplasia, olfactory epithelium hyaline droplet accumulation, respiratory epithelium hyperplasia

END