

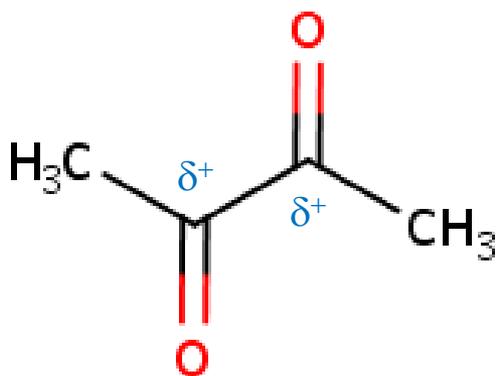
Draft NTP Technical Report TR593
on
2,3-Butanedione
(Inhalation Studies)

Study Scientist: Daniel Morgan, PhD, DABT
Study Pathologist: Gordon Flake, MD
National Institute of Environmental Health Sciences

NTP Technical Reports Peer Review Meeting
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2,3-Butanedione Properties and Uses

- Vicinal diketone
 - Reactive with nucleophiles
 - Amines; guanosine, arg, lys, his
 - Volatile, water soluble
- Naturally present in butter, coffee, honey, fruits
- Fermentation product dairy products, beer, wine
- Component of artificial flavorings used in food, cooking oil, beverages



Non-occupational Exposure

- Primarily ingestion - low concentrations
- No reports of toxicity from ingestion
- GRAS status as a direct food additive
- Vaping – e-cigarettes
e-liquids contain flavorings



Occupational Exposure

- Primarily inhalation – potential for high exposure concentrations
- Microwave popcorn, coffee roasting, bakeries, flavoring industries
- Inhalation exposure not regulated (NIOSH REL = 5 ppb TWA)



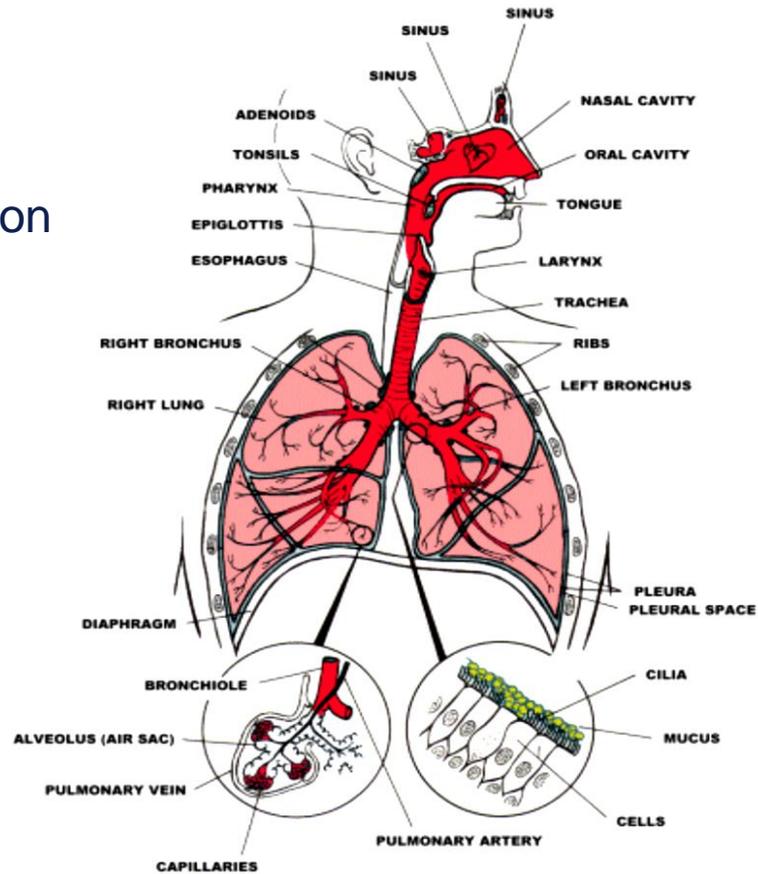
Microwave Popcorn Packaging Plant

- Cluster of cases of bronchiolitis obliterans in workers of a microwave popcorn plant
- NIOSH health hazard evaluation:
 - Strong correlation between exposure to butter flavoring vapors and bronchiolitis obliterans.
 - 2,3-Butanedione and acetoin were the major volatile organics.
- Increasing number of cases diagnosed in industries where butter flavoring is used or made

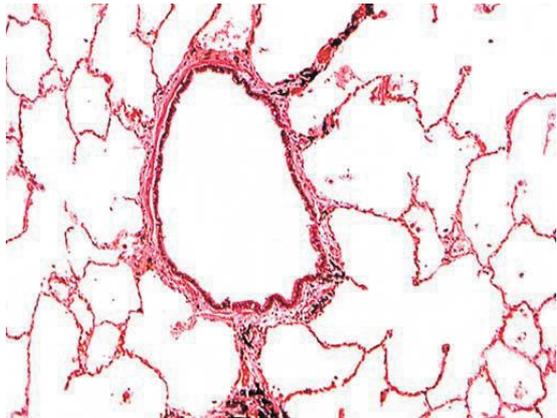
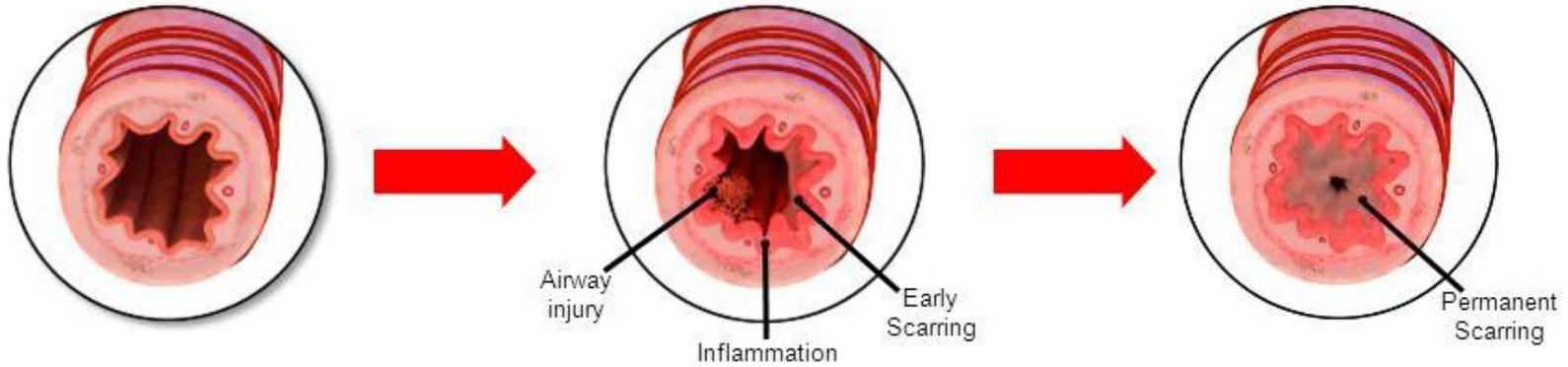


“Popcorn Workers Lung”

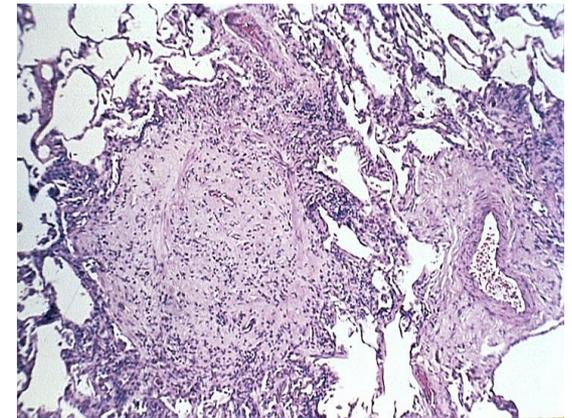
- Nose, eye irritation
- Nonproductive cough
- Shortness of breath on exertion
- X-rays typically normal
- Obstruction fixed
- Decreased FEV1



Bronchiolitis Obliterans



Normal human bronchiole



End stage bronchiolitis obliterans

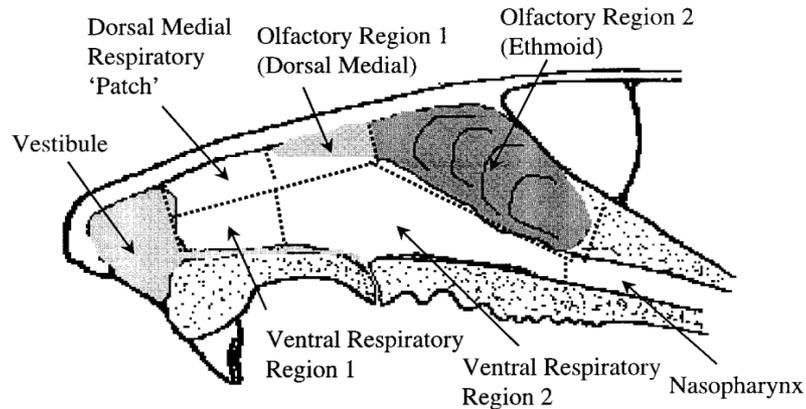
Nomination

- Artificial butter flavoring, 2,3-butanedione and acetoin nominated by UFCW union for long-term testing for respiratory and other toxicity and carcinogenicity by the inhalation route
 - Based upon prevalence of bronchiolitis obliterans in exposed workers, and the lack of chronic inhalation toxicity data
- NIOSH was conducting studies on artificial butter flavoring mixture
- NTP conducted studies on 2,3-butanedione

NTP Short-term Inhalation Studies in Rodents

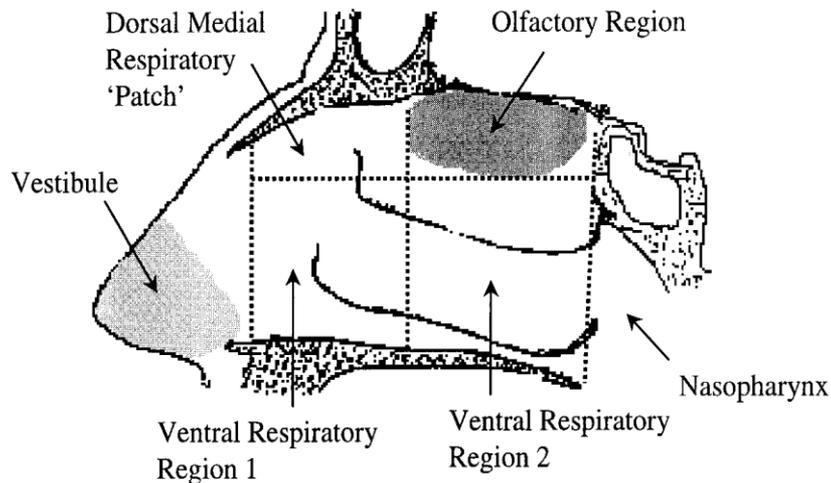
- Provide concentration-response data
 - Multiple exposure scenarios similar to worker exposures
- Does 2,3-butanedione cause bronchiolitis obliterans?
 - ≥ 150 ppm x 10d causes bronchiolitis obliterans in rats
- Characterize the toxicity of inhaled 2,3-butanedione
 - Toxicity limited to respiratory tract
 - Damage to epithelium and disruption of basement membrane
 - Dysregulated repair resulting in progressive fibrosis
 - In rodents the most severe toxicity occurs in the nasal cavity with decreasing toxicity in more distal regions of the respiratory tract

Species Differences in Target Sites



Rodents – Toxicity: Nose

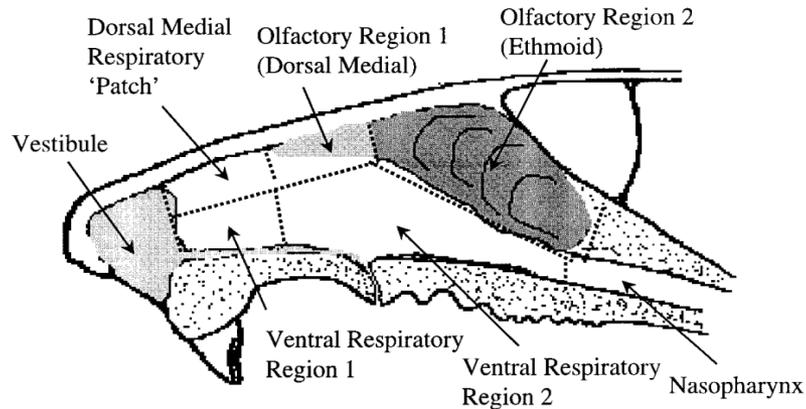
- Obligate nose breathers
- All inhaled air scrubbed by nose
- More complex turbinate scrolls
- Greater surface area
- More chemical absorbed by nose



Humans – Toxicity: Bronchioles

- Nose and mouth breathers
- Less inhaled air scrubbed by nose
- More chemical reaches bronchioles

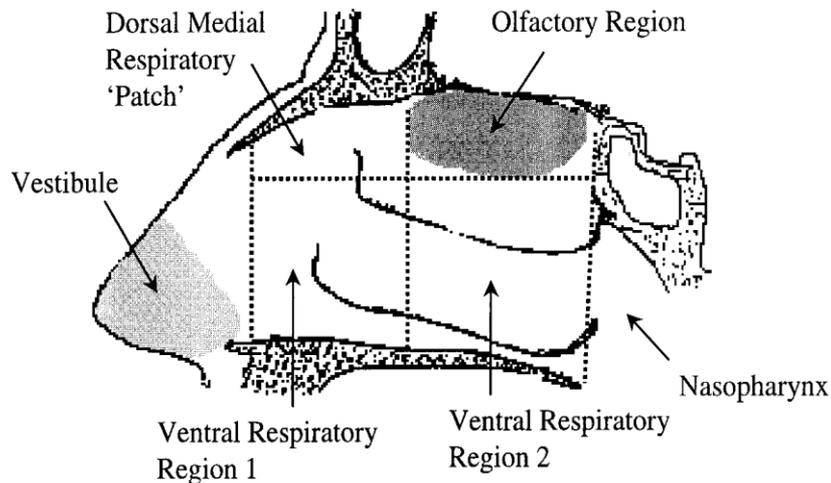
Species Differences in Target Sites



Rodents – Toxicity: Nose

- Obligate nose breathers
- All inhaled air scrubbed by nose
- More complex turbinate scrolls
- Greater surface area
- More chemical absorbed by nose

100 ppm → 62 ppm in bronchioles



Humans – Toxicity: Bronchioles

- Nose and mouth breathers
- Less inhaled air scrubbed by nose
- More chemical reaches bronchioles

100 ppm → 97 ppm in bronchioles

Bronchiolitis Obliterans in Rodents

- Nasal scrubbing
 - Protection of bronchioles
 - Greater toxicity in nose
- Reflex apnea – inhaled irritants
 - Trigeminal nerve endings in nasal mucosa
 - Decrease respiratory rate
 - Decrease tidal volume (or compensatory increase)
 - Greater in mice than rats
- Concentration ≥ 150 ppm required to cause bronchiolitis obliterans in 2-week studies
- Can chronic exposure to lower concentrations cause bronchiolitis obliterans?

3-Month Studies

Whole Body Inhalation

3-Month Study Design

- Wistar Han [Crl:WI (Han)] rats and B6C3F1/N mice
 - 10 animals/species/sex/concentration
- Whole body inhalation exposure
 - 6 hours/day, 5 days/week
 - 0, 6.25, 12.5, 25, 50, or 100 ppm
- Endpoints
 - In-life (survival, body weights, clinical observations)
 - Clinical pathology
 - Organ weights, Micronucleus and Histopathology

3-Month Study Results

	<u>Rats</u>	<u>Mice</u>
Survival:	8/10 ♂ at 100 ppm	no effect
Clin Obs:	abnormal breathing, sneezing, at ≥ 50 ppm	sneezing at ≥ 50 ppm
Body Wt:	♂ ♀: ↓ at 100 ppm	♂: ↓ at ≥ 50 ppm ♀: ↓ at ≥ 12.5 ppm
Clinical Path:	minimal effects secondary to respiratory toxicity	
Micronucleus:	negative	negative
Mutagenicity:	+ <i>S. typhimurium</i> TA97a (+S9) + <i>E. coli</i> WP2 <i>uvrA</i> /pKM101 (+/- S9)	

3-Month Study – Selected Histopathology

Nasal Cavity (site of most severe lesions)

- Suppurative inflammation
 - All rats and mice at 100 ppm (moderate-marked severity)
 - All rats (mild-moderate) and mice (mild) at 50 ppm
- Respiratory epithelium, squamous metaplasia
 - All rats and mice at 50 (mild-moderate) and 100 ppm (moderate-marked)
- Respiratory epithelium, hyperplasia
 - Most rats at 50 and 100 ppm (mild-moderate)

3-Month Study - Selected Histopathology

Larynx

- Respiratory epithelium, squamous metaplasia, atypical
 - Most male mice at 50 ppm (minimal-mild severity)
 - All mice at 100 ppm (moderate-marked)
- Respiratory epithelium, squamous metaplasia
 - Most rats at 50 (mild-moderate) and all rats at 100 ppm (moderate–marked)

Trachea

- Epithelial necrosis
 - All rats at 100 ppm (mild-marked)
- Epithelium, squamous metaplasia, atypical
 - All mice at 100 ppm (mild-marked)

3-Month Study - Selected Histopathology

Lung

- No lung lesions in rats at ≤ 50 ppm, mild lesions at 100 ppm
- No lung lesions in mice at ≤ 25 ppm
- Bronchial epithelium, atypical hyperplasia, and atypical squamous metaplasia
 - Most mice at 100 ppm (mild-moderate severity)
- Bronchus, chronic inflammation
 - Most mice at 100 ppm (mild-moderate severity)
 - Most mice at 50 ppm (minimal-mild severity)
- Bronchiolitis obliterans not present in rats or mice

2-Year Exposure Concentration Selection

- **100 ppm – NOT SELECTED**
 - Mortality – 2 male rats; all mice survived
 - Significantly decreased body weights of rats and mice
 - Severity of lesions in nasal cavity and larynx of rats and mice
- **50 ppm – HI CONCENTRATION**
 - Decreased body weight of female mice (16%)
 - Nasal lesions were minimal to mild severity in mice
 - Some airway lesions in mice (potential for bronchiolitis obliterans)
 - Occupationally relevant exposure concentration
- **25 ppm – MID CONCENTRATION**
 - Minimal nasal cavity lesions in rats and mice
 - No lung lesions in rats or mice
 - Considered unlikely to cause bronchiolitis obliterans in 2-yr study

2-Year Studies

Whole Body Inhalation

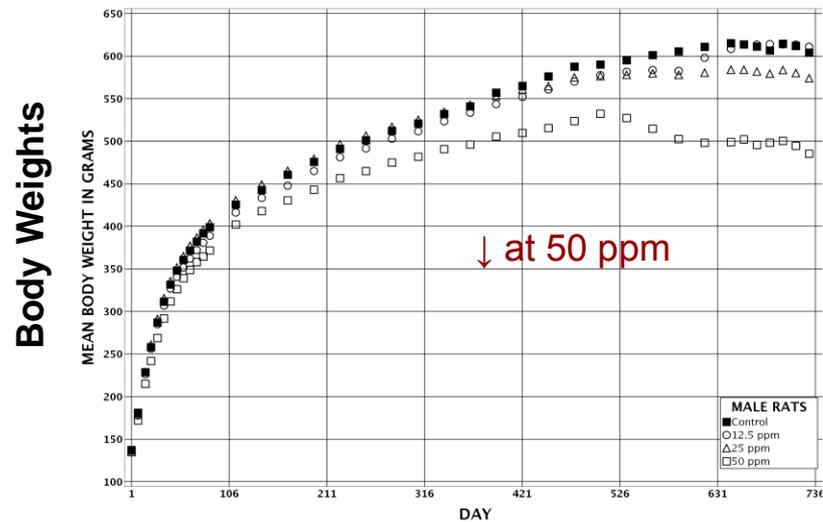
Wistar Han Rats

2-Year Study Design

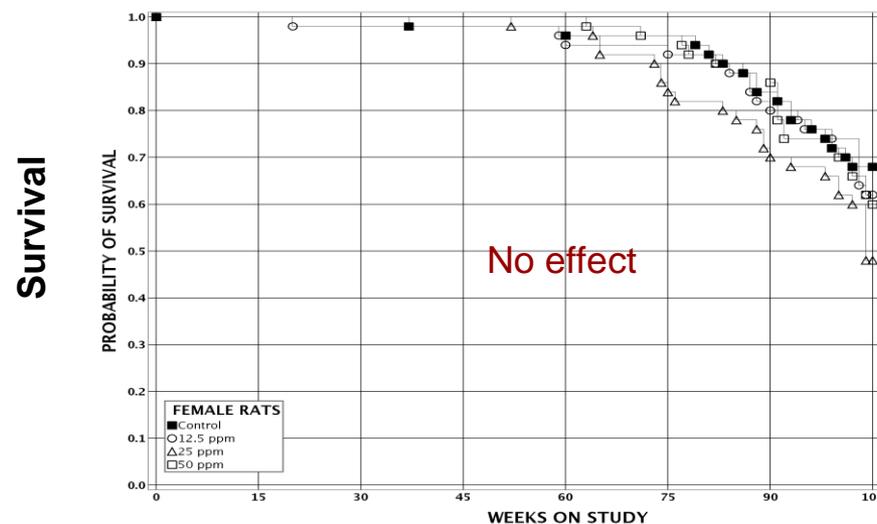
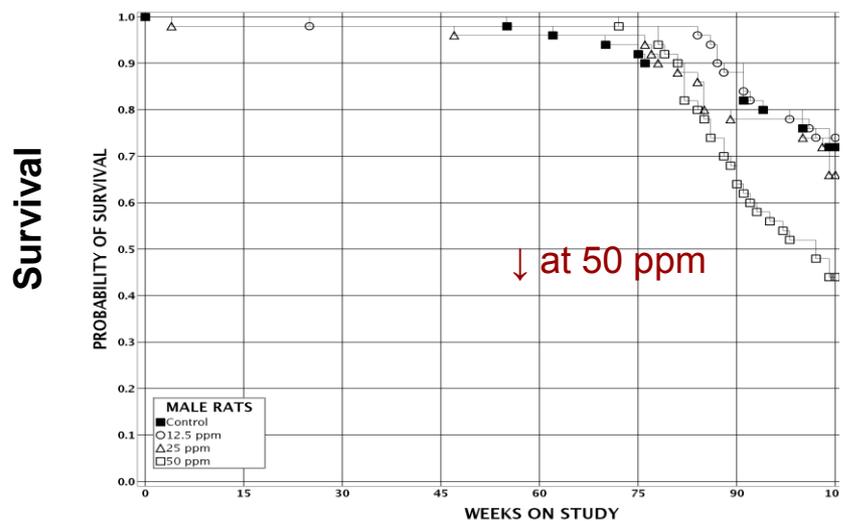
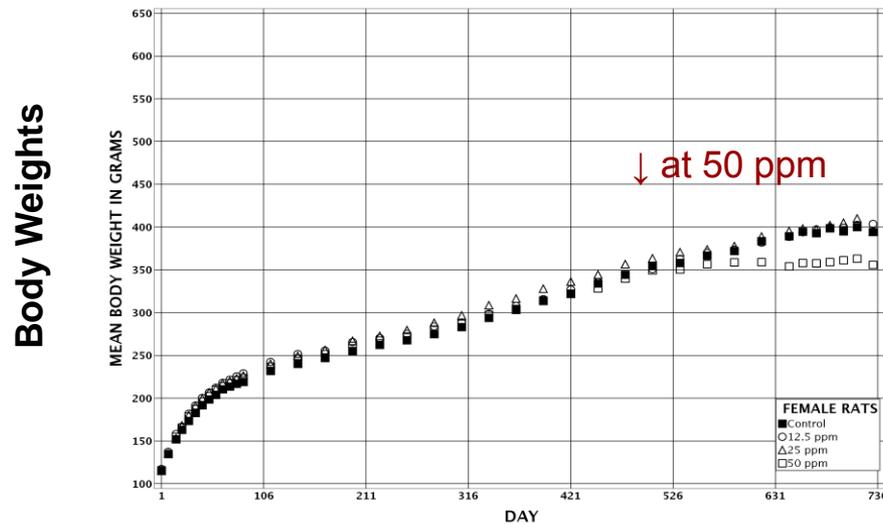
- Wistar Han [Crl:WI (Han)] rats and B6C3F1/N mice
 - 50 animals/species/sex/concentration
- Whole body inhalation exposure
 - 6 hours/day, 5 days/week, 104 weeks
 - 0, 12.5, 25, or 50 ppm
- Endpoints
 - In-life (survival, body weights, clinical observations)
 - Histopathology

Rats: Growth and Survival Curves

Male



Female



Male Rats: Neoplastic Lesions

NOSE	Concentration (ppm):	0	12.5	25	50
Squamous cell papilloma ^a		0/50	0/50	0/50	1/50
Squamous cell carcinoma ^a		0/50**	0/50	0/50	3/50
Squamous cell papilloma or carcinoma ^a		0/50**	0/50	0/50	4/50 *

^a Historical control incidence for all routes in 2-year studies: 0/349

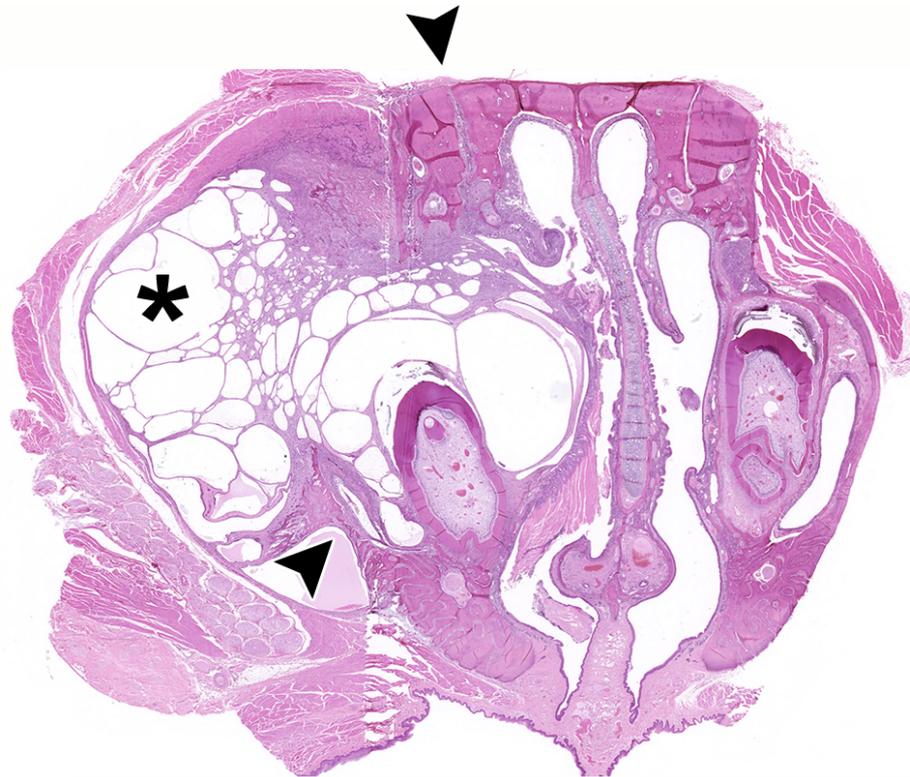
* $p < 0.05$

** Statistically significant trend; ** $p < 0.01$

Considered **Some Evidence** of carcinogenic activity

Squamous Cell Carcinoma of the Nose

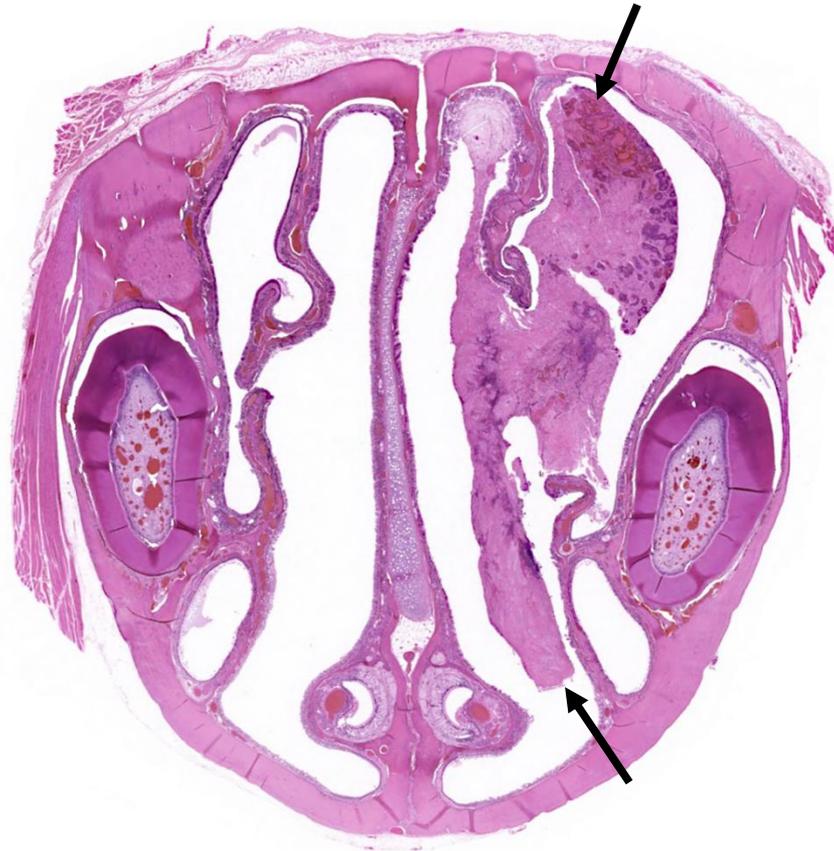
Male Rat Exposed to 50 ppm 2,3-Butanedione



Large solid cystic mass filling one side of nasal cavity

Squamous Cell Papilloma of the Nose

Male Rat Exposed to 50 ppm 2,3-Butanedione



Polypoid mass extending from dorsal end to ventral end of nasal cavity

Male Rats: Select Nonneoplastic Lesions

NOSE	Concentration (ppm):	0	12.5	25	50
Respiratory Epithelium, Hyperplasia		0/50	2/50	5/50*	50/50**
Respiratory Epithelium, Metaplasia, Squamous		0/50	0/50	5/50*	34/50**
Olfactory Epithelium, Metaplasia, Respiratory		1/50	3/50	6/50	50/50**
Olfactory Epithelium, Atrophy		0/50	5/50*	27/50**	22/50**
Lamina Propria, Fibrosis		0/50	0/50	28/50**	38/50**
LARYNX					
Respiratory epithelium, squamous metaplasia		0/50	1/50	0/50	45/50**
TRACHEA					
Epithelium, Hyperplasia		0/50	0/50	1/50	32/50**
Submucosa, Fibrosis		0/50	0/50	0/50	27/50**
LUNG					
Bronchus, Submucosa, Fibrosis		0/50	0/50	0/50	5/50*
Interstitium, Fibrosis		0/50	1/50	1/50	11/50**

*Significantly greater than control, $p \leq 0.05$; ** $p \leq 0.01$

Male Rats: Select Nonneoplastic Lesions

NOSE	Concentration (ppm):	0	12.5	25	50
Respiratory Epithelium, Hyperplasia		0/50	2/50	5/50*	50/50**
Respiratory Epithelium, Metaplasia, Squamous		0/50	0/50	5/50*	34/50**
Olfactory Epithelium, Metaplasia, Respiratory		1/50	3/50	6/50	50/50**
Olfactory Epithelium, Atrophy		0/50	5/50*	27/50**	22/50**
Lamina Propria, Fibrosis		0/50	0/50	28/50**	38/50**
LARYNX					
Respiratory epithelium, squamous metaplasia		0/50	1/50	0/50	45/50**
TRACHEA					
Epithelium, Hyperplasia		0/50	0/50	1/50	32/50**
Submucosa, Fibrosis		0/50	0/50	0/50	27/50**
LUNG					
Bronchus, Submucosa, Fibrosis		0/50	0/50	0/50	5/50*
Interstitium, Fibrosis		0/50	1/50	1/50	11/50**

*Significantly greater than control, $p \leq 0.05$; ** $p \leq 0.01$

Female Rats: Neoplastic Lesions

NOSE	Concentration (ppm):	0	12.5	25	50
Squamous Cell Carcinoma ^a		0/50 ^{**}	0/50	0/50	3/50 ^b

^a Historical control incidence for all routes of 2-year studies: 0/350

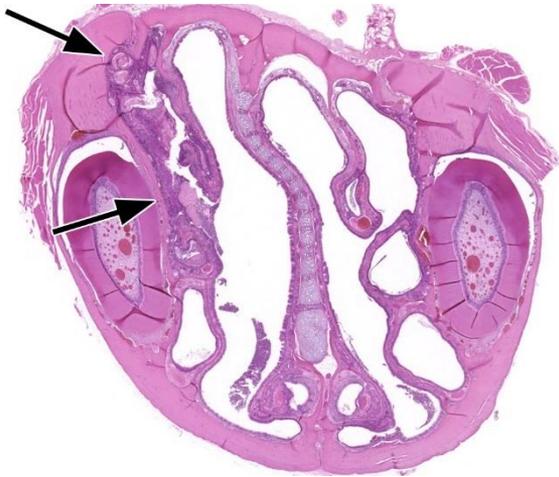
^b $p=0.118$

^{**} Statistically significant trend ($p<0.01$)

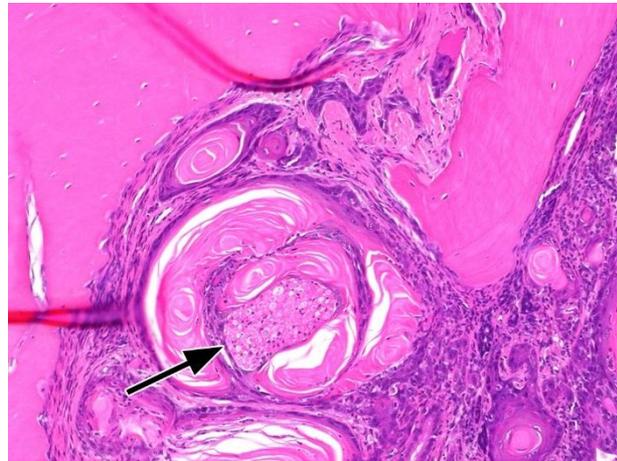
Considered **Some Evidence** of carcinogenic activity

Squamous Cell Carcinoma of the Nose

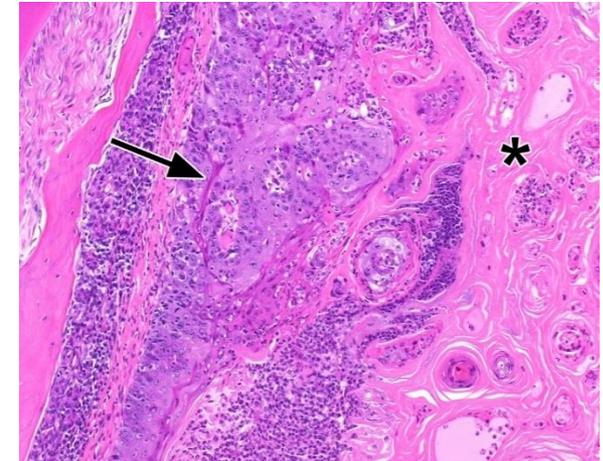
Female Rat Exposed to 50 ppm 2,3-Butanedione



Squamous cell carcinoma involves much of the lateral wall (between arrows) on one side of Level I.



Perineural invasion and invasion of suture between nasal bone and premaxillary bone



Atypical squamous metaplasia (↑) with marked hyperkeratosis (*) is replacing the respiratory epithelium of lateral wall.

Female Rats: Select Nonneoplastic Lesions

NOSE	Concentration (ppm):	0	12.5	25	50
Respiratory Epithelium, Hyperplasia		1/50	0/50	2/50	44/50**
Respiratory Epithelium, Metaplasia, Squamous		1/50	0/50	1/50	44/50**
Olfactory Epithelium, Metaplasia, Respiratory		1/50	0/50	18/50**	46/50**
Olfactory Epithelium, Atrophy		1/50	1/50	14/50**	24/50**
Lamina Propria, Fibrosis		1/50	1/50	17/50**	46/50**
LARYNX					
Respiratory epithelium, squamous metaplasia		0/50	0/50	0/50	35/50**
TRACHEA					
Epithelium, Hyperplasia		0/50	0/50	0/50	30/50**
Submucosa, Fibrosis		0/50	0/50	0/50	19/50**
LUNG					
Interstitialium, Fibrosis		1/50	1/50	1/50	9/50**

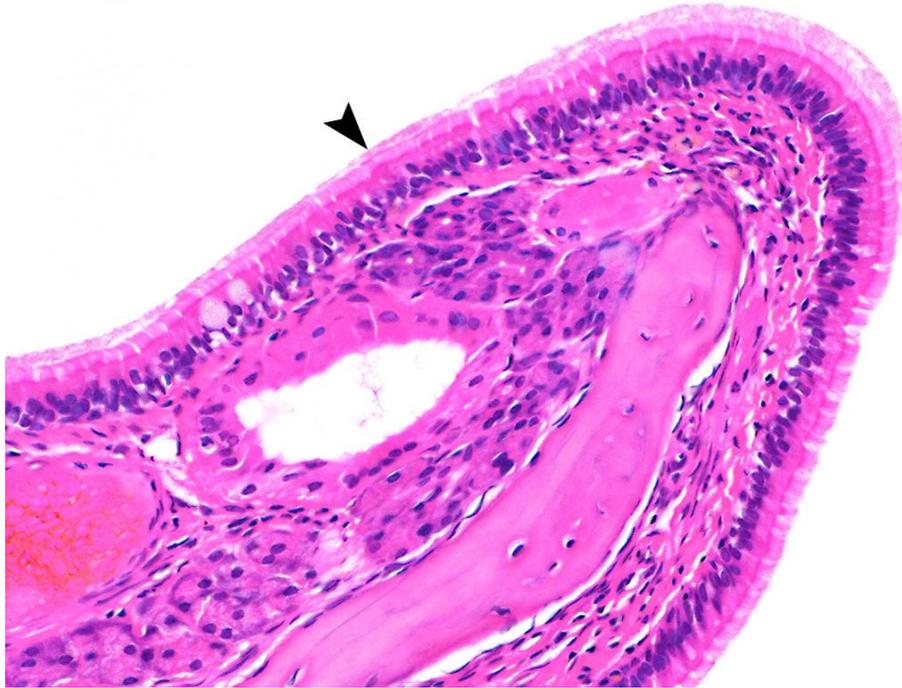
*Significantly greater than control, $p \leq 0.05$; ** $p \leq 0.01$

Female Rats: Select Nonneoplastic Lesions

NOSE	Concentration (ppm):	0	12.5	25	50
Respiratory Epithelium, Hyperplasia		1/50	0/50	2/50	44/50**
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TRACHEA					
Epithelium, Hyperplasia		0/50	0/50	0/50	30/50**
Submucosa, Fibrosis		0/50	0/50	0/50	19/50**
LUNG					
Interstitialium, Fibrosis		1/50	1/50	1/50	9/50**

*Significantly greater than control, $p \leq 0.05$; ** $p \leq 0.01$

Nasal Turbinate Fibrosis in Rats

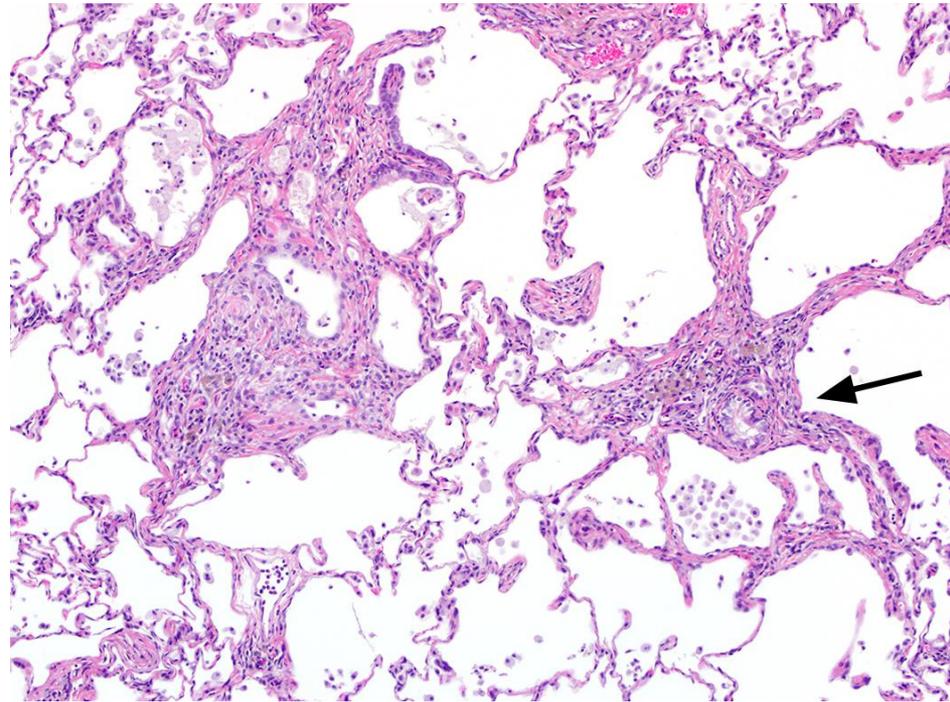
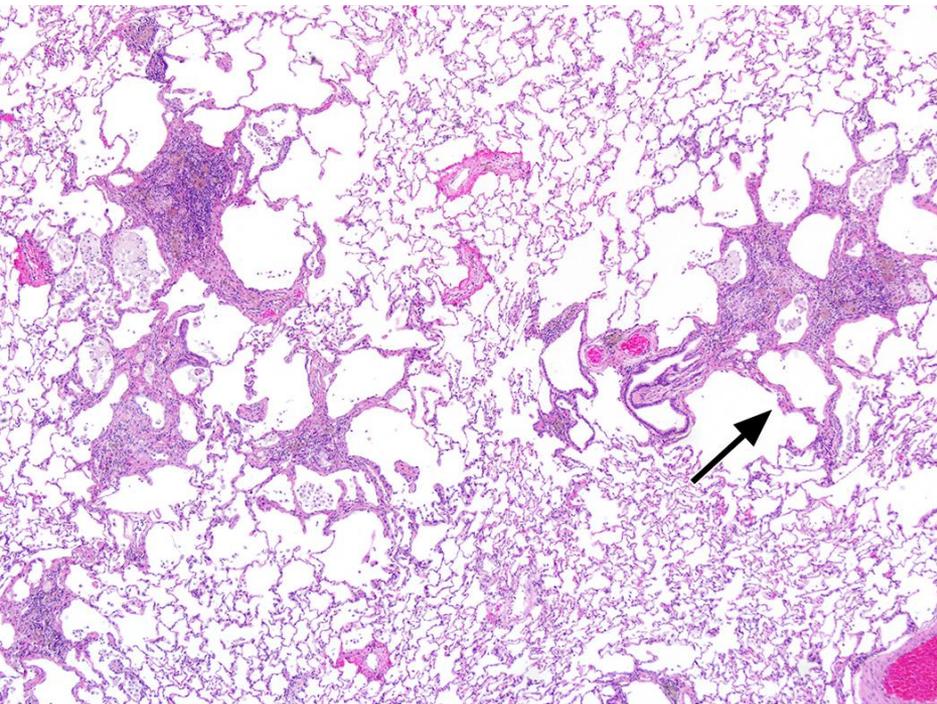


Male rat, control. Normal mucosal respiratory epithelium, Level II (▲).



Male rat, 50 ppm. Fibrosis of the lamina propria Level II, with replacement of glands (long ↑). Hyperplastic epithelium (▲). Hyperostosis of turbinate bone (short ↑).

Interstitial Fibrosis in Lungs of Rats



Male rat, 50 ppm. Nodular foci of interstitial fibrosis of the alveolar walls accompanied by inflammatory infiltrate, extend irregularly into the surrounding alveolar septa.

2-Year Studies

Whole Body Inhalation

B6C3F1/N Mice

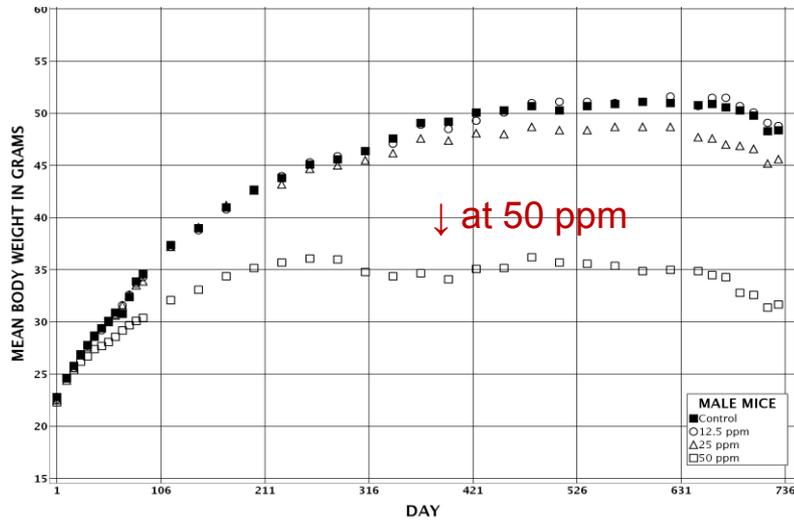
2-Year Study Design

- B6C3F1/N mice
 - 50 animals/species/sex/concentration
- Whole body inhalation exposure
 - 6 hours/day, 5 days/week, 104 weeks
 - 0, 12.5, 25, or 50 ppm
- Endpoints
 - In-life (survival, body weights, clinical observations)
 - Histopathology

Mice: Growth and Survival Curves

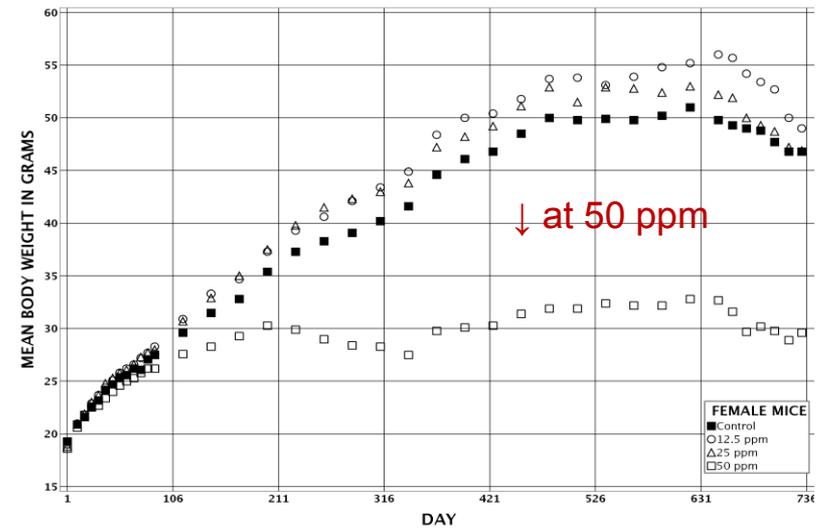
Male

Body Weights

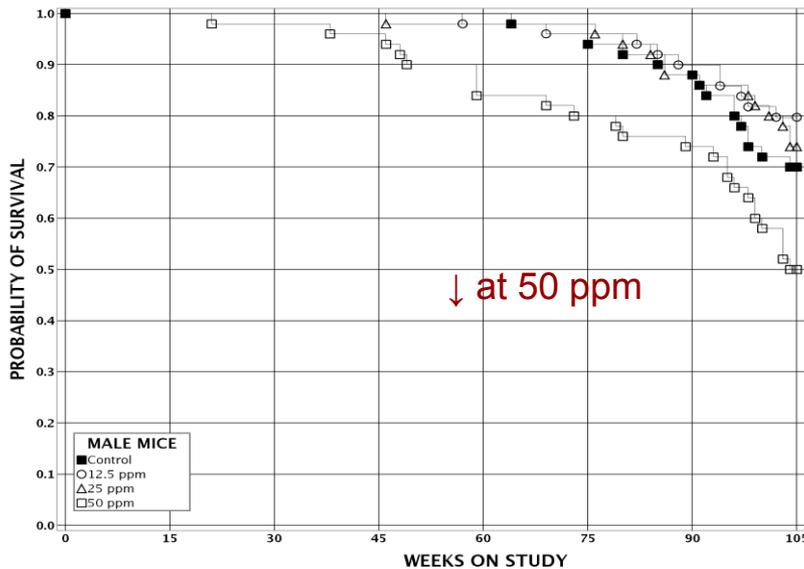


Female

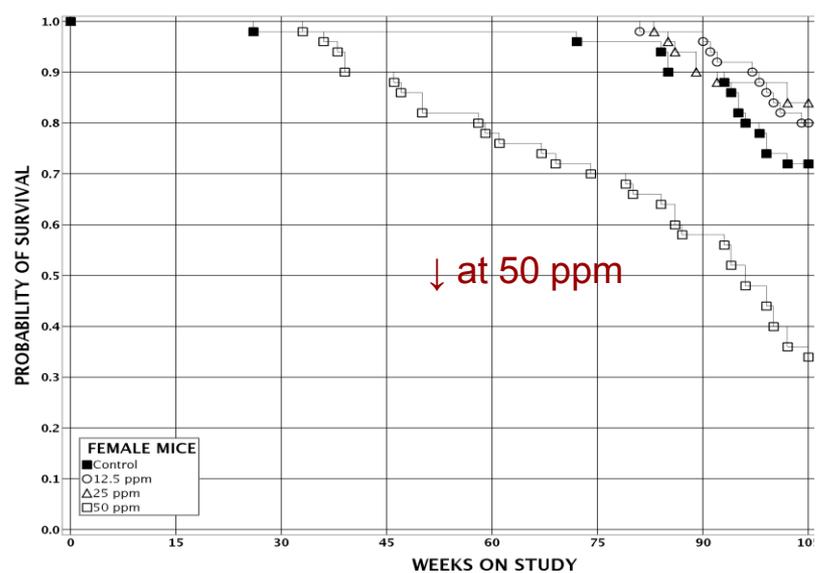
Body Weights



Survival



Survival



Male Mice: Neoplastic Lesions

- There were no treatment-related increases in the incidence of neoplastic lesions in male mice

Considered **No Evidence** of carcinogenic activity

Male Mice: Select Nonneoplastic Lesions

NOSE	Concentration (ppm):	0	12.5	25	50
Respiratory Epithelium, Necrosis		0/50	0/50	34/50**	50/50**
Respiratory Epithelium, Metaplasia, Squamous		0/50	6/50*	47/50**	50/50**
Olfactory Epithelium, Metaplasia, Respiratory		1/50	0/50	39/50**	45/50**
Olfactory Epithelium, Atrophy		0/50	14/50**	48/50**	38/50**
Lamina Propria, Fibrosis		0/50	0/50	44/50**	50/50**
LARYNX					
Respiratory epithelium, squamous metaplasia		3/50	0/50	6/50	50/50**
TRACHEA					
Submucosa, Fibrosis		0/50	0/50	0/50	46/50**
Necrosis		0/50	0/50	0/50	47/50**
LUNG					
Bronchus, Epithelium, Regeneration		0/50	0/50	0/50	34/50**

*Significantly greater than control, $p \leq 0.05$; ** $p \leq 0.01$

Male Mice: Select Nonneoplastic Lesions

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Respiratory Epithelium, Necrosis		0/50	0/50	34/50**	50/50**
Respiratory Epithelium, Metaplasia, Squamous		0/50	6/50*	47/50**	50/50**
Olfactory Epithelium, Metaplasia, Respiratory		1/50	0/50	39/50**	45/50**
Olfactory Epithelium, Atrophy		0/50	14/50**	48/50**	38/50**
Lamina Propria, Fibrosis		0/50	0/50	44/50**	50/50**
LARYNX					
Respiratory epithelium, squamous metaplasia		3/50	0/50	6/50	50/50**
TRACHEA					
Submucosa, Fibrosis		0/50	0/50	0/50	46/50**
Necrosis		0/50	0/50	0/50	47/50**
LUNG					
Bronchus, Epithelium, Regeneration		0/50	0/50	0/50	34/50**

*Significantly greater than control, $p \leq 0.05$; ** $p \leq 0.01$

Female Mice: Neoplastic Lesions

NOSE	Concentration (ppm):	0	12.5	25	50
Adenocarcinoma ^a		0/50 ^{**}	0/50	0/50	2/50 ^b

^a Historical control incidence for all routes of 2-year studies: 0/548

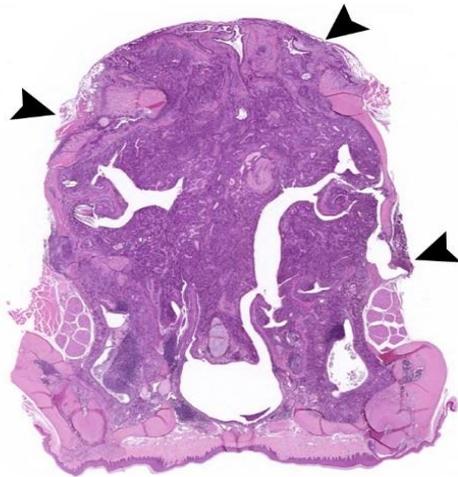
^b $p=0.171$

^{**} Statistically significant trend ($p=0.038$)

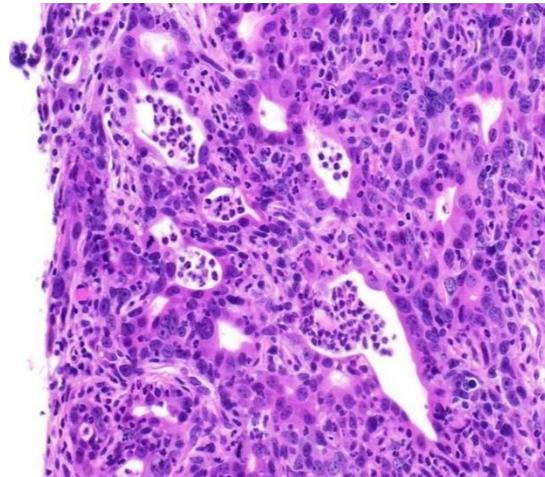
Considered **Equivocal Evidence** of carcinogenic activity

Adenocarcinoma of the Nose

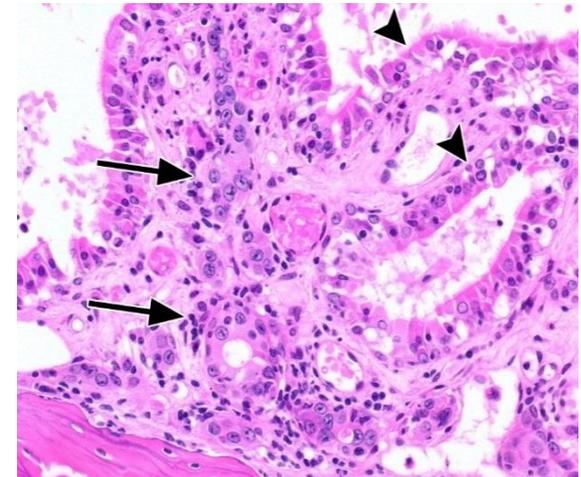
Female Mice Exposed to 50 ppm 2,3-Butanedione



Neoplasm fills the nasal cavity Level III, and invades the bony wall on both sides and dorsally.



Higher magnification of the infiltrating neoplasm within an inflamed fibrous stroma.



Adenocarcinoma (↑) arising in the background of extensive respiratory metaplasia (▲) of the olfactory epithelium, Level III.

Female Mice: Select Nonneoplastic Lesions

NOSE	Concentration (ppm):	0	12.5	25	50
Respiratory Epithelium, Necrosis		1/50	5/50	33/50**	50/50**
Respiratory Epithelium, Metaplasia, Squamous		1/50	9/50*	48/50	50/50**
Olfactory Epithelium, Metaplasia, Respiratory		0/50	22/50**	46/50**	49/50**
Olfactory Epithelium, Atrophy		0/50	41/50**	49/50**	45/50**
Lamina Propria, Fibrosis		0/50	0/50	47/50**	49/50**
LARYNX					
Respiratory epithelium, squamous metaplasia		2/50	0/50	6/50	48/50**
TRACHEA					
Submucosa, Fibrosis		0/50	0/50	0/50	44/50**
Necrosis		0/50	0/50	3/50	48/50**
LUNG					
Bronchus, Epithelium, Regeneration		2/50	0/50	0/50	38/50**
Bronchus, Submucosa, Fibrosis		0/50	0/50	0/50	5/50*

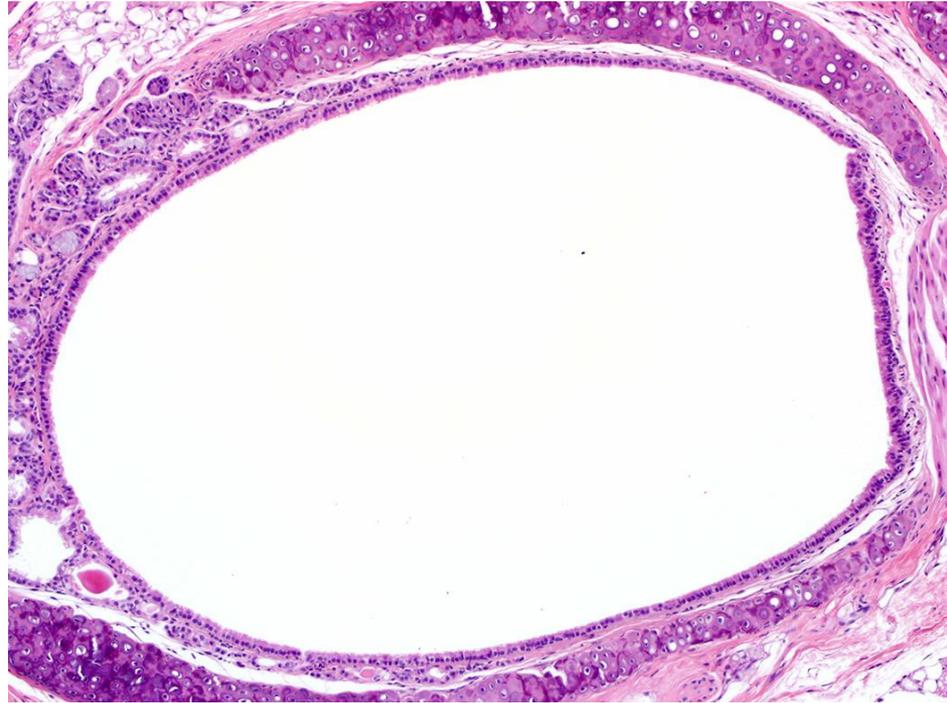
*Significantly greater than control, $p \leq 0.05$; ** $p \leq 0.01$

Female Mice: Select Nonneoplastic Lesions

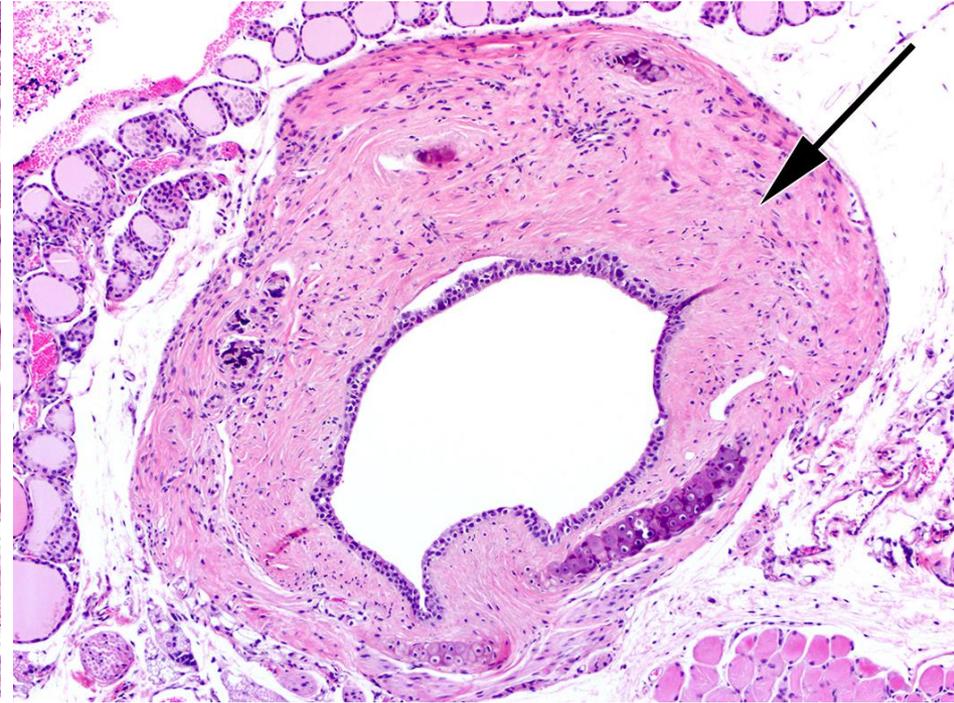
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Lamina Propria, Fibrosis		0/50	0/50	47/50**	49/50**
LARYNX					
Respiratory epithelium, squamous metaplasia		2/50	0/50	6/50	48/50**
TRACHEA					
Submucosa, Fibrosis		0/50	0/50	0/50	44/50**
Necrosis		0/50	0/50	3/50	48/50**
LUNG					
Bronchus, Epithelium, Regeneration		2/50	0/50	0/50	38/50**
Bronchus, Submucosa, Fibrosis		0/50	0/50	0/50	5/50*

*Significantly greater than control, $p \leq 0.05$; ** $p \leq 0.01$

Tracheal Fibrosis in Exposed Mice

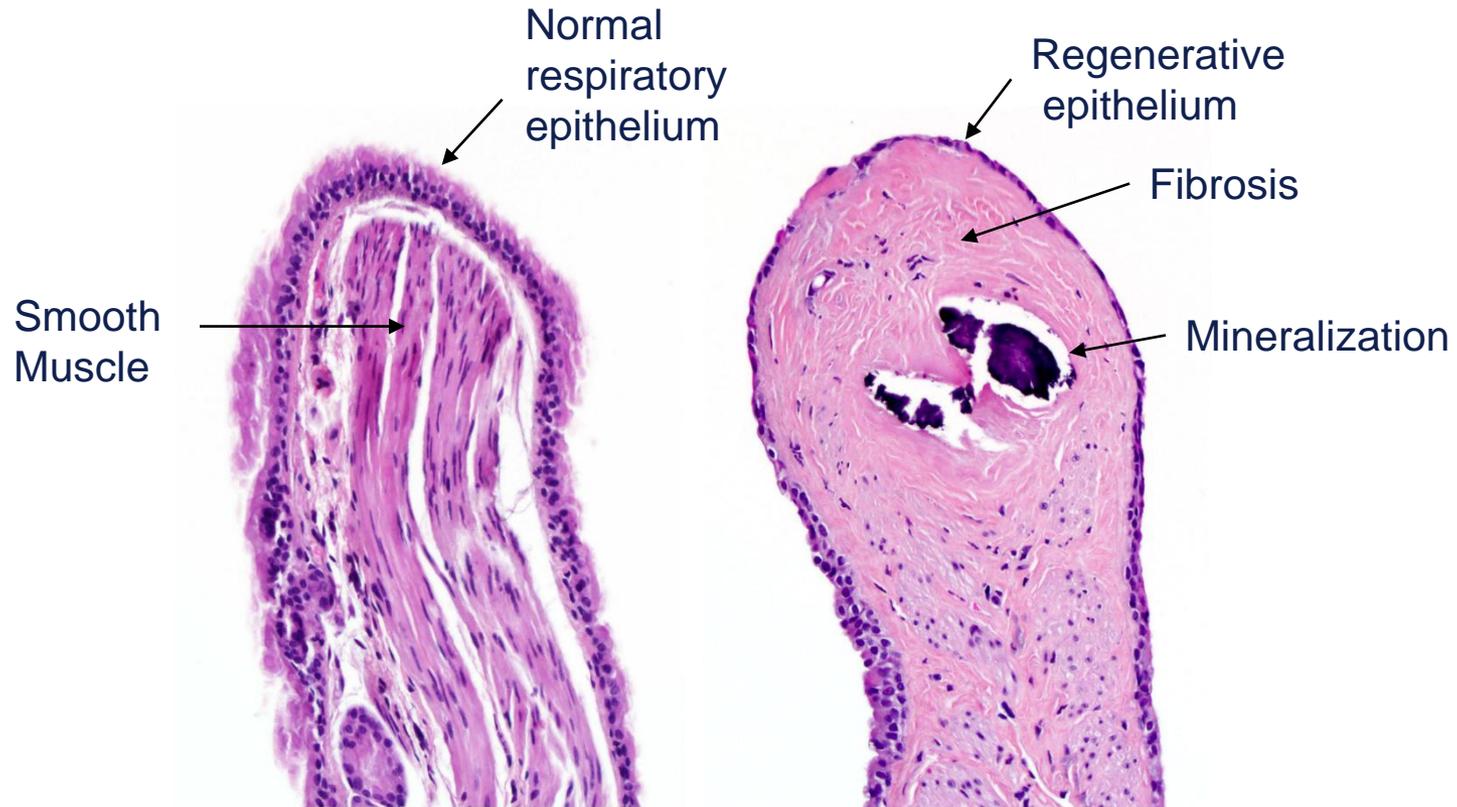


Female mouse, control. Normal trachea.



Male mouse, 50 ppm. Submucosal fibrosis in trachea.

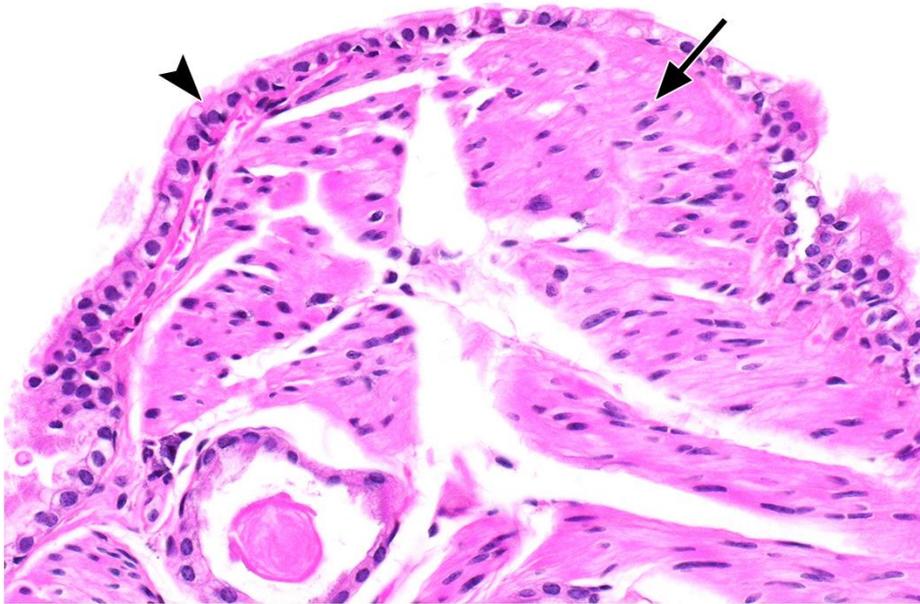
Fibrosis of Tracheal Carina



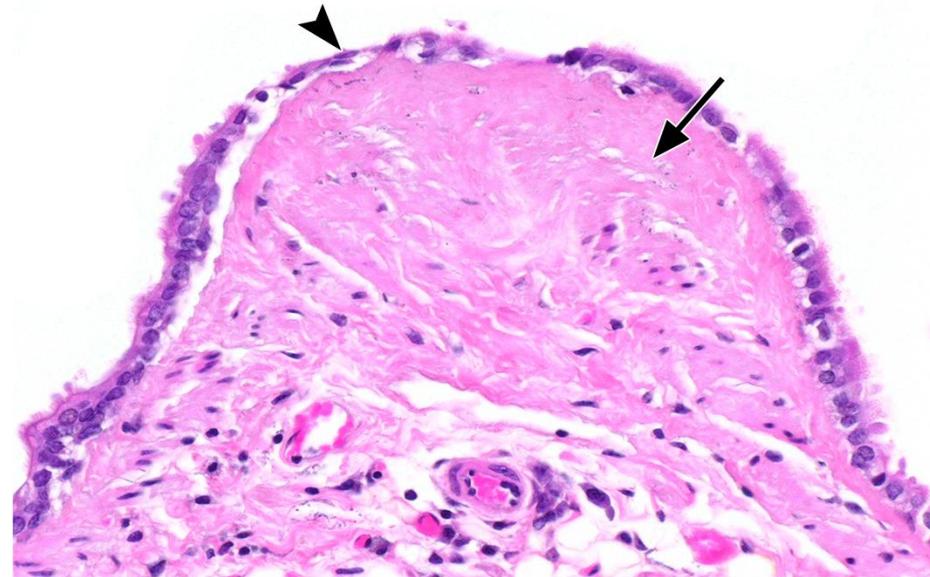
Male mouse, control

Male mouse, 50 ppm

Bronchial Fibrosis in Exposed Mice



Female mouse, control. Normal mainstem bronchus and bifurcation knob. Normal respiratory epithelium (▲) with underlying smooth muscle (↑)



Female mouse, 50 ppm. Submucosa fibrosis in the mainstem bronchus and bifurcation knob. The surface epithelium is slightly attenuated (▲) and the subepithelial muscle has been replaced by dense fibrous tissue (↑)

2,3-Butanedione: Conclusions

Male Wistar Han Rats

- **Some evidence of carcinogenic activity**
 - Combined incidences of squamous cell papilloma and squamous cell carcinoma of the nose.

Female Wistar Han Rats

- **Some evidence of carcinogenic activity**
 - Incidences of squamous cell carcinoma of the nose.

2,3-Butanedione: Conclusions

Male B6C3F1/N Mice

- **No evidence of carcinogenic activity** exposed to 12.5, 25, or 50 ppm

Female B6C3F1/N Mice

- **Equivocal evidence of carcinogenic activity**
 - Occurrences of adenocarcinoma of the nose.

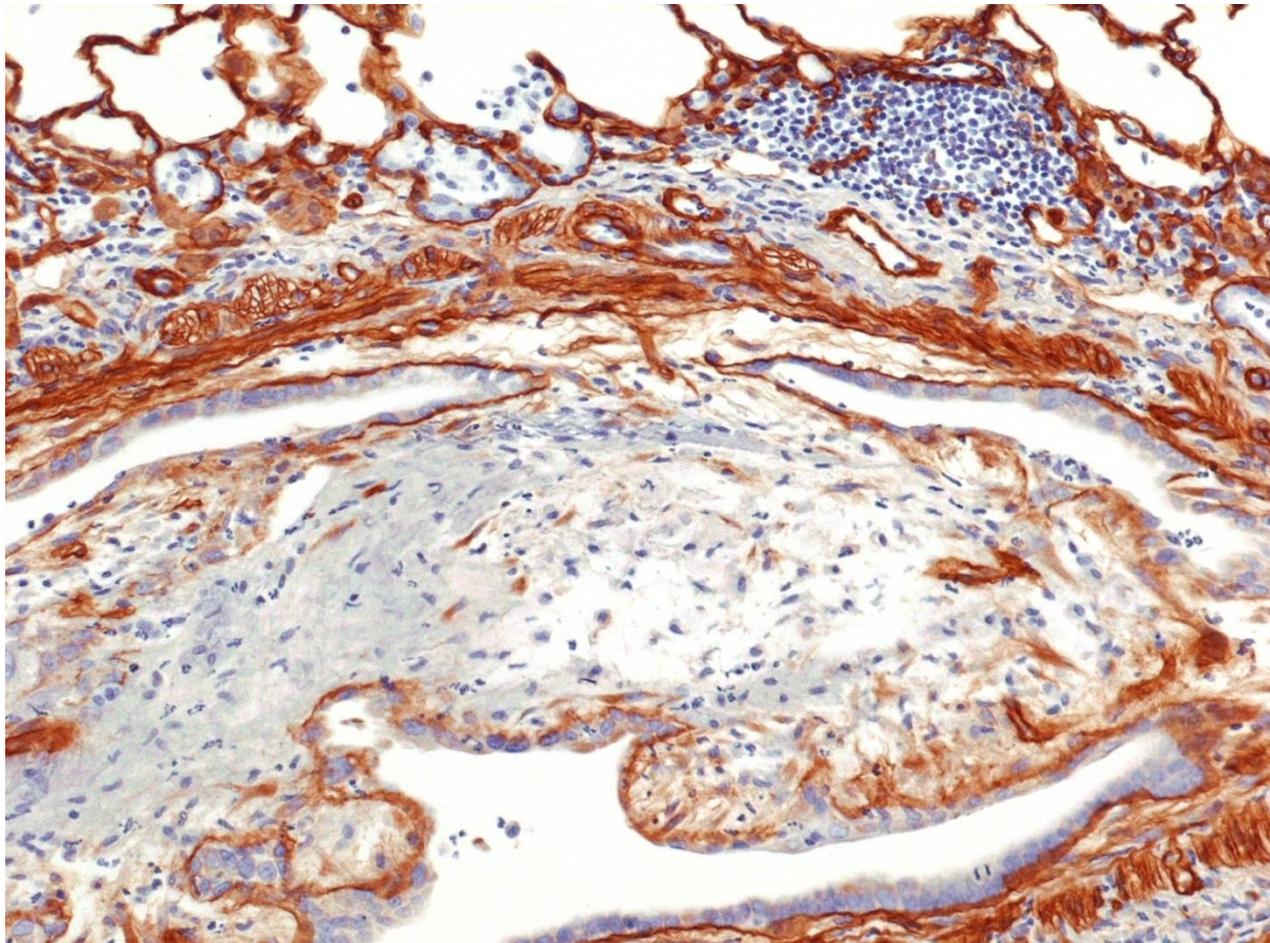
2,3-Butanedione: Conclusions

- **Non-Neoplastic Lesions**

Exposure to 2,3-butanedione resulted in increased incidences of nonneoplastic lesions of the nose, larynx, trachea, lung and eye in male and female rats and mice.



Basement Membrane Disruption (Laminin stain)



Fibrosis caused by 2,3-Butanedione Inhalation

