NTP
National Toxicology Program

Draft NTP Technical Report TR590 on Antimony Trioxide

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National Institute of Environmental Health Sciences

NTP Technical Reports Peer Review Meeting
February 16, 2016

Antimony Trioxide

- Primary and most commercially significant form of antimony
  - Flame retardant synergist – enhances activity of flame retardants
  - Catalyst in the production of polyethylene terephthalate (PET) plastics
- Primary exposure: occupational via inhalation route (metal ore smelting and mining)
- Regulatory status
  - Time weighted average (antimony): 0.5 mg/m^3 (ACGIH/NIOSH/OSHA)
  - Reference Concentration (antimony trioxide): 0.2 $\mu g/m^3$ (US EPA)
- Nominated by Consumer Product Safety Commission and the NIEHS
  - Substantial human exposure in occupational settings
  - Lack of adequate 2-year exposure carcinogenicity studies
    - Three one-year exposure studies in rats with conflicting results on lung carcinogenicity
    - No studies in mice

Study Design

- Wistar Han rats and B6C3F1/N mice
  - 5 animals/sex/species/concentration
  - Additional groups of 5 females/species/concentration held four weeks post-exposure
- Exposure to antimony trioxide particles (MMAD 1.3 – 1.5 µm)
  - Whole body inhalation
  - Concentrations: 0, 3.75, 7.5, 15, 30 and 60 mg/m^3
  - Duration: 6 hr/d, 5 d/wk for 2 wks + 2 days (rats) or 3 days (mice)
- Endpoints
  - In life (survival, body weights, clinical observations)
  - Gross/histopathology, organ weights, lung and blood burdens
  - Post-exposure hold females
    - Lung and blood burdens

MMAD: Mass Median Aerodynamic Diameter

Results

- No deaths or treatment related clinical observations
- Body weights of all groups similar to controls
- Increased lung weights
- No gross observations
- Histopathology findings
  - Lung
    - Chronic active inflammation (rats only at 30 and 60 mg/m^3)
    - Foreign body
  - Larynx
    - Epiglottis, metaplasia, squamous

Design and Exposure Concentration Selection Rationale

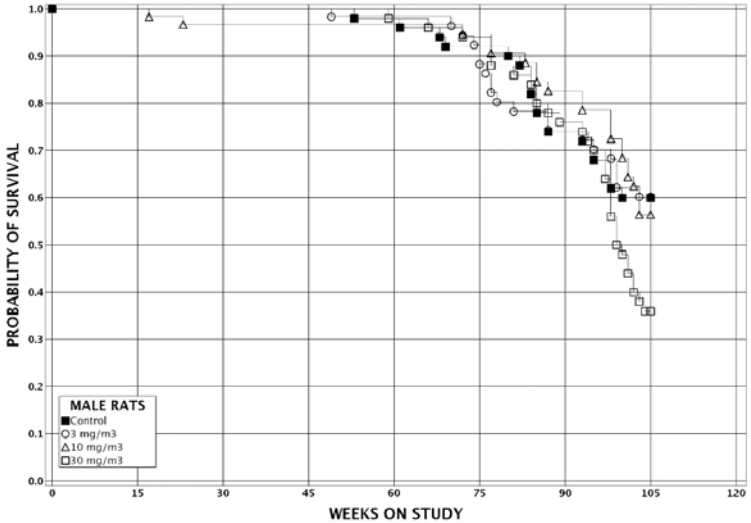
- Two-Year studies designed following
  - Review of the two-week study data
  - Subchronic and chronic studies in the literature
    - Inclusion of one-year interim: comparison to previous studies
- Highest exposure concentration – 30 mg/m^3
  - Similar toxicity in rats at 30 and 60 mg/m^3
  - Lack of findings in previous rat studies that would be anticipated to limit survival
  - Lower concentrations spaced in half log intervals to examine exposure concentration-response
  - Same concentrations used in mice

Study Design

- Wistar Han rats and B6C3F1/N mice
  - Two year study: N=50/sex/species/group
  - One-year interim: N=10/sex/species/group
  - Tissue burden: 25 females/species/group (exposed for 2, 4, 9, 12 or 18 months; N=5)
- Exposure to antimony trioxide particles (MMAD 0.9 – 1.5 µm)
  - Whole body inhalation
  - Concentrations: 0, 3, 10, and 30 mg/m^3
  - Duration: 6 hr/d, 5 d/wk for up to two years
- Endpoints
  - In life (survival, body weights, clinical observations)
  - One-year interim: Organ weights and genotoxicity (micronucleus and comet)
  - One-year interim and two years: Gross/histopathology
  - Lung and blood burden

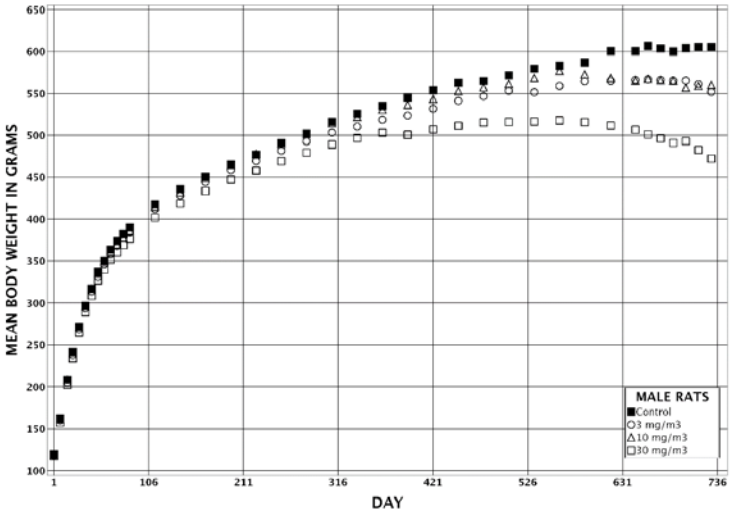
Two-Year Rat Studies

Survival

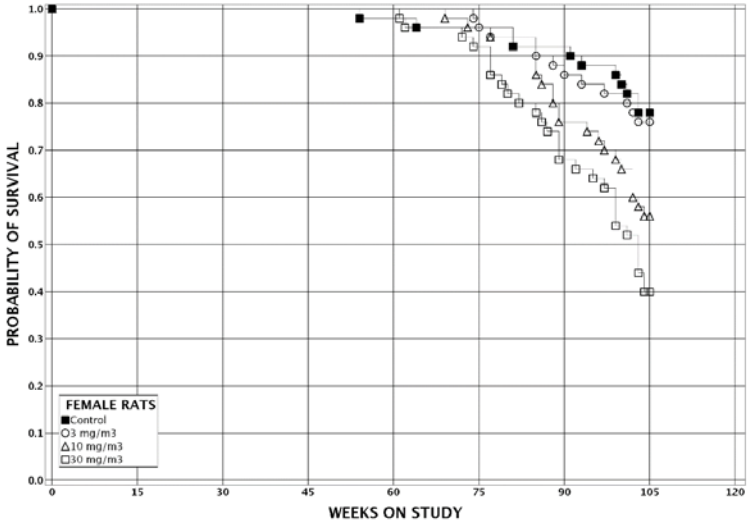


Male rats: Survival similar to controls

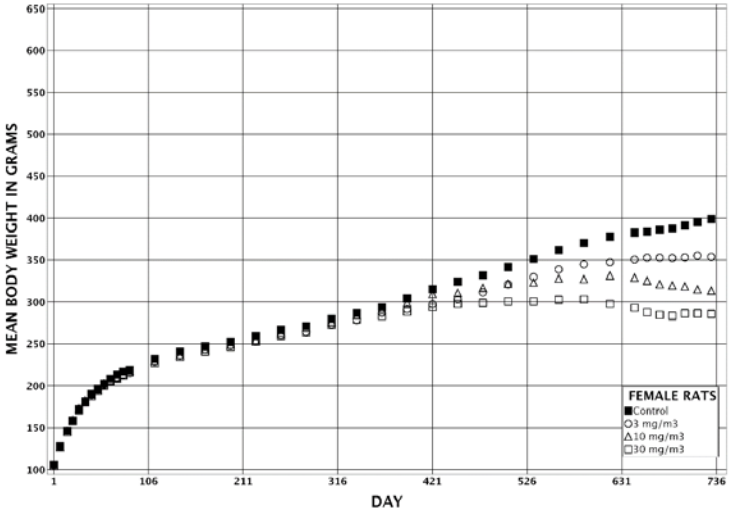
Body Weight



Male rats: 30 mg/m^3 body weights ≥10% less than controls



Female rats: Survival decreased at 10 and 30 mg/m^3



Female rats: Body weight ≥10% less than controls in all groups

Two-Year Rat Studies

- Clinical Observations:
  - Generally occurred during second year of study in males and females
    - Abnormal Breathing
    - Cyanosis
    - Thinness
- Organ Weights (one-year interim)
  - Increased absolute and relative lung weights (males and females)
  - Increased relative liver weights (males)

Lung - Alveolar/Bronchiolar Neoplasms

Some Evidence

	Control	3 mg/m^3	10 mg/m^3	30 mg/m^3
Males	**50**	**50**	**50**	**50**
Adenoma, Includes Multiple (4/150; 0-3/50)	3	4	6	8
Adenoma (Multiple)	0	1	0	3
Carcinoma (0/150)	0	0	2	0
Adenoma or Carcinoma (4/150; 0-3/50)	3	4	8	8
Females	**50**	**50**	**50**	**50**
Adenoma, Includes Multiple (0/150)	0*	2	6*	5*
Adenoma (Multiple)	0	0	1	0
Adenoma (one year interim; N=10)	0	0	0	1

*Significantly different ($p \leq 0.05$) from chamber control by Poly-3 test (positive trend if associated with a control incidence)
Data in parentheses are historical control incidences and incidence ranges, inhalation route in Wistar Han rats

Lung - Squamous Neoplasms

May have been related to exposure (equivocal evidence)

	Control	3 mg/m^3	10 mg/m^3	30 mg/m^3
Females	**50**	**50**	**50**	**50**
Cystic Keratinizing Epithelioma (CKE; 0/150)	0	0	0	2
Squamous Cell Carcinoma (SCC; 0/150)	0	0	0	1
CKE or SCC (0/150)	0*	0	0	3

*Positive trend ($p \leq 0.05$)
Data in parentheses are historical control incidences, inhalation route in Wistar Han rats

Lung – Nonneoplastic Lesions

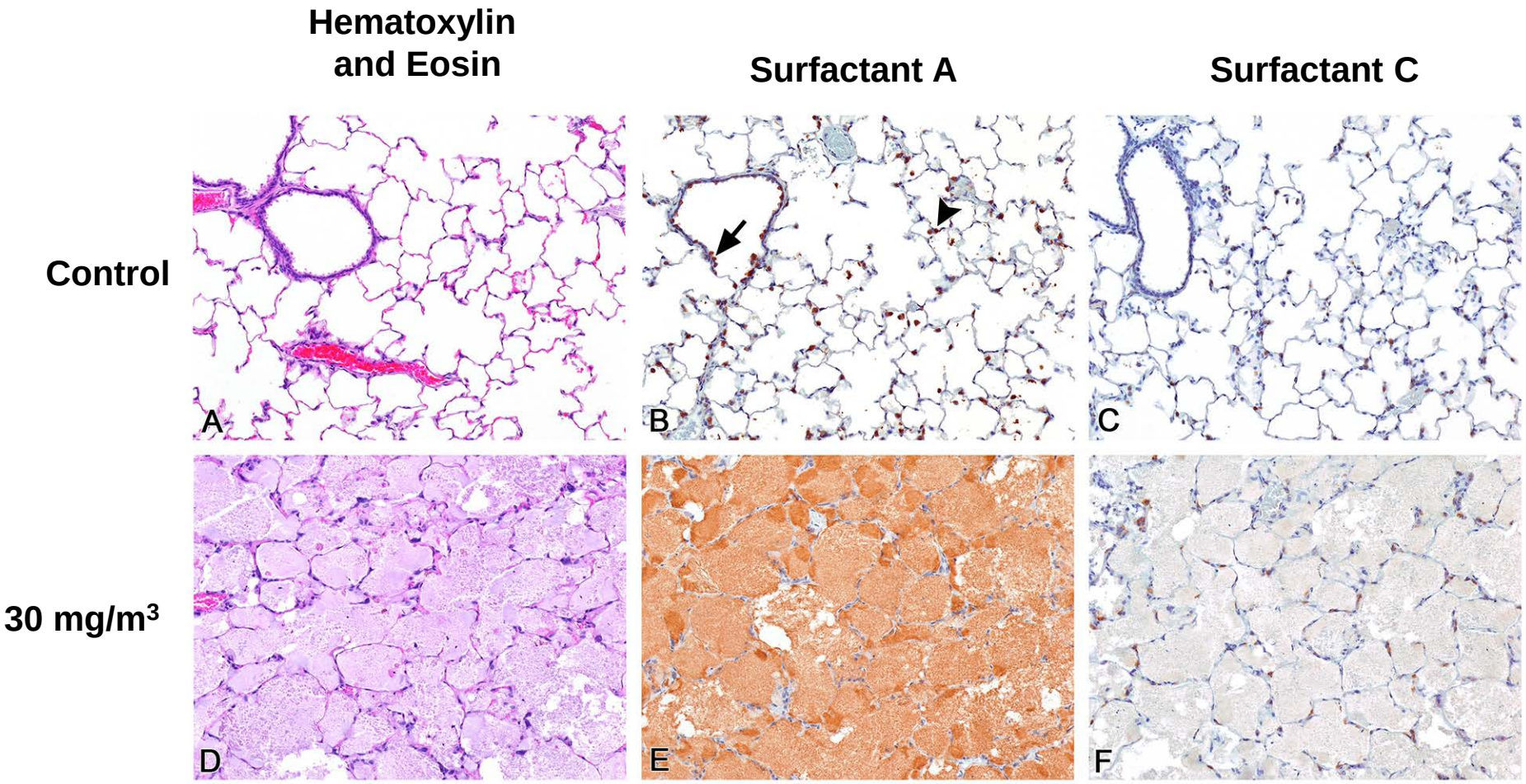
	Control	3 mg/m^3	10 mg/m^3	30 mg/m^3
Males	**50**	**50**	**50**	**50**
Foreign Body§	1	50**	50**	50**
Inflammation, Chronic Active	18 [1.0]	50** [2.5]	50** [2.7]	50** [2.8]
Alveolus, Inflammation, Suppurative	0	12** [1.2]	24** [1.4]	28** [1.7]
Perivascular, Infiltration Cellular, Lymphocyte	3 [1.7]	25** [1.1]	19** [1.2]	9 [1.1]
Proteinosis	0	47** [1.3]	50** [1.9]	50** [2.7]
Alveolar Epithelium, Hyperplasia	*4 [1.3]*	*50** [1.8]*	*48** [2.1]*	*49** [2.2]*
Bronchiole, Epithelium, Hyperplasia	*3 [1.0]*	*34** [1.0]*	*36** [1.1]*	*33** [1.5]*
Fibrosis	2 [1.0]	50** [1.4]	49** [1.6]	49** [1.7]

§ Presumed antimony trioxide particles
**Significantly different ($p \leq 0.01$) from chamber control by Poly-3 test
Data in italics represent nonneoplastic lesions that may progress to neoplasia
Data in brackets are average severities (1=minimal; 2=mild; 3=moderate; 4=marked)

Alveolar Proteinosis

- Proteinosis observed in rats exposed to antimony trioxide
- In humans, pulmonary alveolar proteinosis usually associated with accumulation of surfactant
- Immunohistochemical staining for surfactants A and C performed on antimony trioxide exposed lungs

Alveolar Proteinosis: Accumulation of Surfactant A



Adrenal Medulla – Neoplasms and Nonneoplastic Lesions

Some evidence

	Control	3 mg/m^3	10 mg/m^3	30 mg/m^3
Males	**50**	**50**	**50**	**50**
Benign Pheochromocytoma (5/149; 0-4/50)	1***	0	2	7*
Hyperplasia	*1 [3.0]*	*2 [3.5]*	*4 [1.5]*	*8* [1.5]*
Females	**50**	**50**	**50**	**50**
Benign Pheochromocytoma (1/148; 0-1/50)	0**	2	2	6**
Malignant Pheochromocytoma (1/148; 0-1/50)	0	0	0	1
Benign or Malignant Pheochromocytoma (2/148; 0-2/50)	0***	2	2	7**
Hyperplasia	*0*	*0*	*3 [1.3]*	*5* [2.0]*

*Significantly different ($p \leq 0.05$) from chamber control by Poly-3 test; **$p \leq 0.01$; ***$p < 0.001$ (positive trend if associated with a control incidence)
Data in parenthesis are historical control incidences and incidence ranges, inhalation route in Wistar Han rats
Data in italics represent a nonneoplastic lesion that may progress to neoplasia
Data in brackets are average severities (1=minimal; 2=mild; 3=moderate; 4=marked)

Upper Respiratory Tract – Nonneoplastic Lesions

Males	Control	3 mg/m^3	10 mg/m^3	30 mg/m^3
Nose	**50**	**49**	**50**	**50**
Foreign Body§	0	0	17**	40**
Respiratory Epithelium, Hyperplasia	6 [1.3]	15* [1.1]	13 [1.1]	25** [1.1]
Respiratory Epithelium, Metaplasia, Squamous	0	0	2 [1.0]	6* [1.7]
Larynx	**50**	**50**	**50**	**50**
Foreign Body§	0	50**	50**	50**
Inflammation, Chronic Active	2 [1.5]	5 [1.4]	2 [2.0]	4 [2.0]
Metaplasia, Squamous	0	2 [1.0]	0	1 [1.0]
Trachea	**50**	**50**	**50**	**50**
Foreign Body§	0	28**	43**	48**
Metaplasia, Squamous	1 [1.0]	1 [1.0]	4 [1.0]	3 [1.3]

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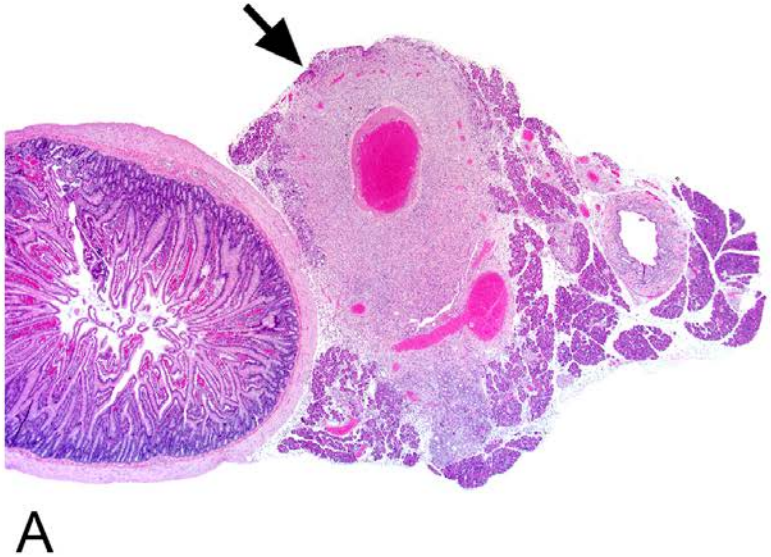
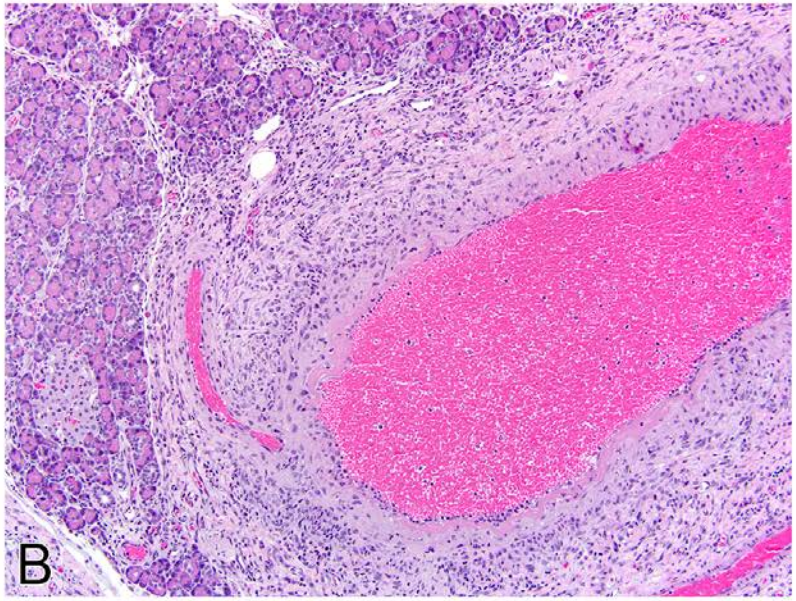
Arterial Chronic Active Inflammation

	Control	3 mg/m^3	10 mg/m^3	30 mg/m^3
Males - Artery (All tissues combined)	**50**	**50**	**50**	**50**
Inflammation, Chronic Active	1	1	5	16**
Females – Artery (All tissues combined)	**50**	**50**	**50**	**50**
Inflammation, Chronic Active	0	0	5*	15**

*Significantly different ($p \leq 0.05$) from chamber control by Poly-3 test; **$p \leq 0.01$
Lesion primarily observed in: mediastinum, pancreas, mesentery, lung, kidney

Arterial Inflammation



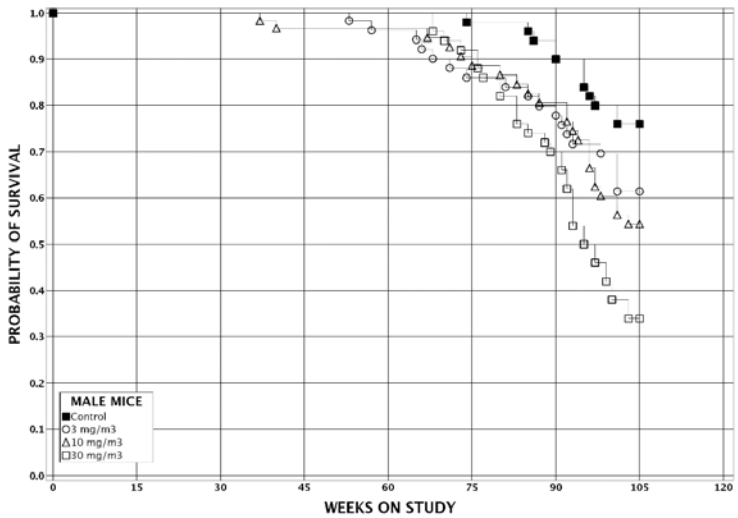


Additional Nonneoplastic lesions

- Bone Marrow: Hyperplasia
- Bronchial and mediastinal lymph nodes: Foreign body, lymphoid hyperplasia, (males only) pigmentation
- Kidney: Renal tubule, hyaline droplet accumulation and (females only) nephropathy
- Eye: Retina, atrophy (females only) and ciliary body, acute inflammation
- Prostate: Epithelium, hyperplasia

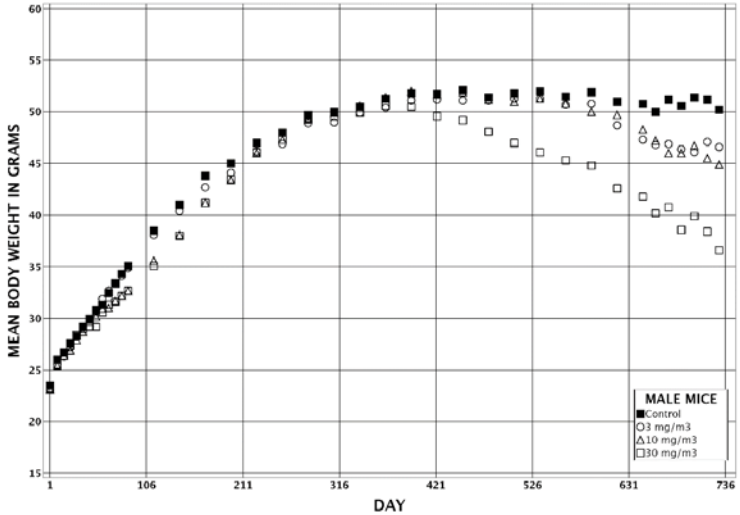
Two-Year Mouse Studies

Survival

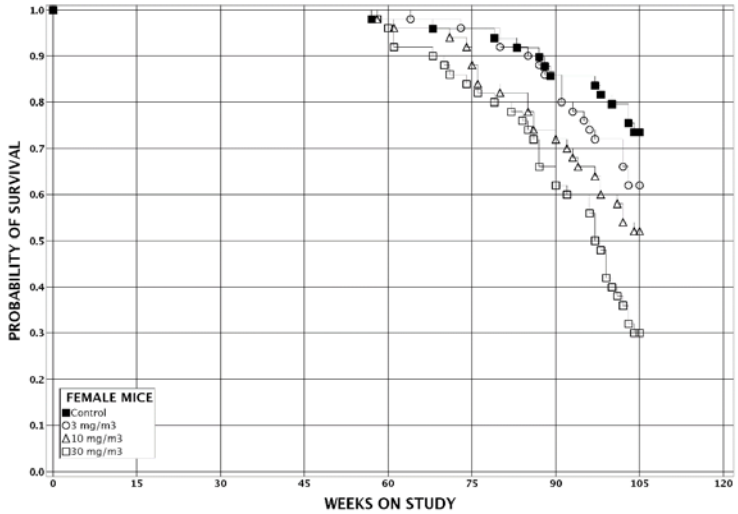


Male mice: Survival decreased at 10 and 30 mg/m^3

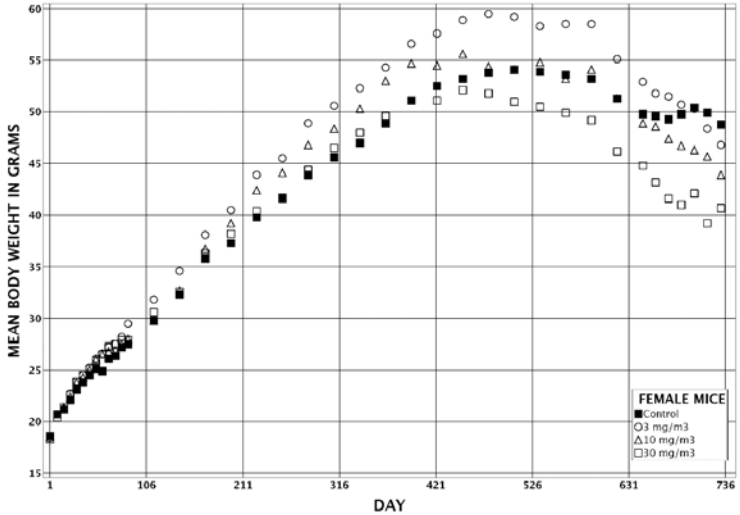
Body Weight



Male mice: 30 mg/m^3 body weights ≥10% less than controls



Female mice: Survival decreased at 10 and 30 mg/m^3



Female mice: 30 mg/m^3 body weights ≥10% less than controls

Two-Year Mouse Studies

- Clinical Observations
  - Generally occurred during second year of study in males and females
    - Abnormal Breathing
    - Thinness
- Organ Weights (one-year interim)
  - Increased absolute and relative lung weights (males and females)
  - Increased absolute and relative thymus weights (males and females)
  - Increased absolute heart and liver weights (females)

Lung - Alveolar/Bronchiolar Neoplasms

Clear Evidence

	Control	3 mg/m^3	10 mg/m^3	30 mg/m^3
Males	**50**	**50**	**50**	**50**
Carcinoma, Includes Multiple (42/250; 4-11/50)	4***	18***	20***	27***
Carcinoma (Multiple)	0	5*	6**	11**
Adenoma or Carcinoma (69/250; 13-16/50)	13***	29***	28***	34***
Females	**50**	**50**	**50**	**50**
Adenoma, Includes Multiple (12/249; 1-4/50)	1	10**	19***	8**
Adenoma (Multiple)	0	3	3	1
Carcinoma, Includes Multiple (17/249; 1-5/50)	2	14***	11**	11**
Carcinoma (multiple)	0	7**	6*	4*
Adenoma or Carcinoma (28/249; 3-9/50)	3*	22***	27***	18***

*Significantly different ($p \leq 0.05$) from chamber control by Poly-3 test; **$p \leq 0.01$; ***$p < 0.001$ (positive trend if associated with a control incidence)
Data in parentheses are historical control incidences and incidence ranges, inhalation route in B6C3F1/N mice

Lung - Alveolar/Bronchiolar Neoplasms (One-Year Interim)

	Control	3 mg/m^3	10 mg/m^3	30 mg/m^3
Males	**10**	**10**	**10**	**10**
Adenoma	0	0	2	0
Carcinoma	0	0	1	2
Females	**10**	**10**	**10**	**10**
Adenoma	0	0	0	1

Lung – Nonneoplastic Lesions

	Control	3 mg/m^3	10 mg/m^3	30 mg/m^3
Males	**50**	**50**	**50**	**50**
Infiltration Cellular, Lymphocyte	13 [1.2]	47** [1.4]	48** [2.1]	45** [2.9]
Foreign Body§	0	50**	50**	50**
Inflammation, Chronic Active	0	48** [2.3]	50** [3.1]	50** [3.7]
Alveolus, Fibrosis	0	12** [1.1]	30** [1.6]	37** [1.9]
Pleura, Fibrosis	0	36** [1.1]	46** [1.4]	50** [2.4]
Pleura, Inflammation	1 [1.0]	40** [1.0]	47** [1.3]	48** [1.8]
Alveolar Epithelium, Hyperplasia	*6 [1.2]*	*39** [1.1]*	*45** [1.8]*	*49** [1.9]*
Bronchiole, Epithelium, Hyperplasia	*0*	*32** [1.2]*	*44** [1.3]*	*44** [1.6]*

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Data in italics represent nonneoplastic lesions that may progress to neoplasia
Data in brackets are average severities (1=minimal; 2=mild; 3=moderate; 4=marked)

Malignant Lymphoma

Clear Evidence

Females	Control	3 mg/m^3	10 mg/m^3	30 mg/m^3
Two-Year	**50**	**50**	**50**	**50**
Malignant Lymphoma (63/250; 7 – 18/50)	7***	17*	20***	27***
One-Year Interim	**10**	**10**	**10**	**10**
Malignant Lymphoma	0	0	0	3

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Data in parentheses are historical control incidences and incidence ranges, inhalation route in B6C3F1/N mice

Skin Neoplasms

Related to exposure in males (some evidence)

May have been related to exposure in females (equivocal evidence)

	Control	3 mg/m^3	10 mg/m^3	30 mg/m^3
Males	**50**	**50**	**50**	**50**
Fibrous Histiocytoma (1/250; 0-1/50)	0*	1	1	4*
Fibrosarcoma (1/250; 0-1/50)	0	0	2	0
Fibrous Histiocytoma or Fibrosarcoma (22/250; 0-1/50)	0*	1	3	4*
Females	**50**	**50**	**50**	**50**
Squamous Cell Carcinoma (0/250)	0	0	0	2

*Significantly different ($p \leq 0.05$) from chamber control by Poly-3 test (positive trend if associated with a control incidence)
Data in parenthesis are historical control incidences and incidence ranges, inhalation route in B6C3F1/N mice

Upper Respiratory Tract – Nonneoplastic Lesions

Males	Control	3 mg/m^3	10 mg/m^3	30 mg/m^3
Nose	**50**	**49**	**49**	**50**
Foreign Body§	0	48**	48**	49**
Respiratory Epithelium, inflammation, chronic active	3 [2.3]	9* [1.2]	9* [1.1]	6 [1.5]
Larynx	**50**	**50**	**50**	**50**
Foreign Body§	0	15**	29**	44**
Respiratory Epithelium, Hyperplasia	1 [1.0]	3 [1.3]	15** [1.1]	30** [1.2]
Respiratory Epithelium, Metaplasia, Squamous	0	0	8** [1.0]	18** [1.1]
Squamous Epithelium, Hyperplasia	2 [1.0]	0	4 [1.3]	13** [1.2]
Trachea	**50**	**50**	**50**	**50**
Foreign Body§	0	3	1	20**
Epithelium, Hyperplasia	0	0	2 [1.5]	5* [1.2]

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**Significantly different ($p \leq 0.01$) from chamber control by Poly-3 test
Data in brackets are average severities (1=minimal; 2=mild; 3=moderate; 4=marked)

Additional Non-Neoplastic Lesions

- Bronchial and mediastinal lymph nodes: Foreign body, Lymphoid hyperplasia, and histiocyte cell infiltration
- Mandibular lymph node: Foreign body
- Bone marrow: Hyperplasia
- Thymus: Cellular depletion
- Spleen (females): Hematopoietic cell proliferation
- Heart: Epicardium, inflammation, chronic active
- Forestomach: Inflammation, chronic active

Genetic Toxicology

Micronucleus

- Mice: (+) in males and females
- Rats: (-) in both males and females

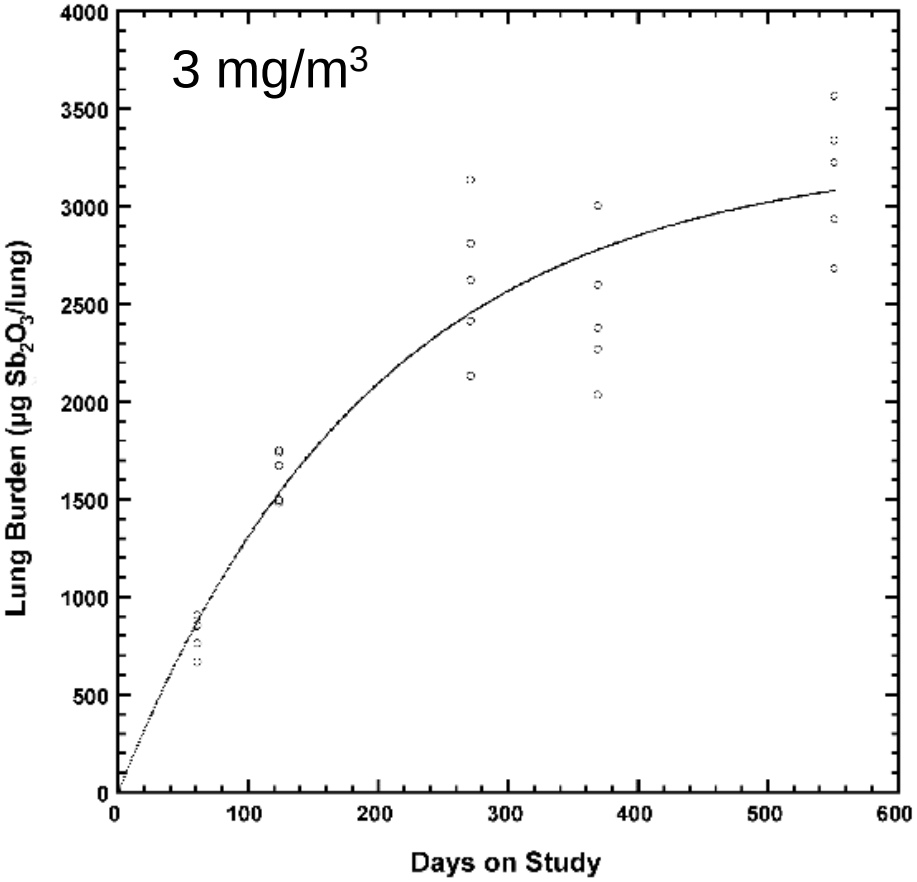
Comet

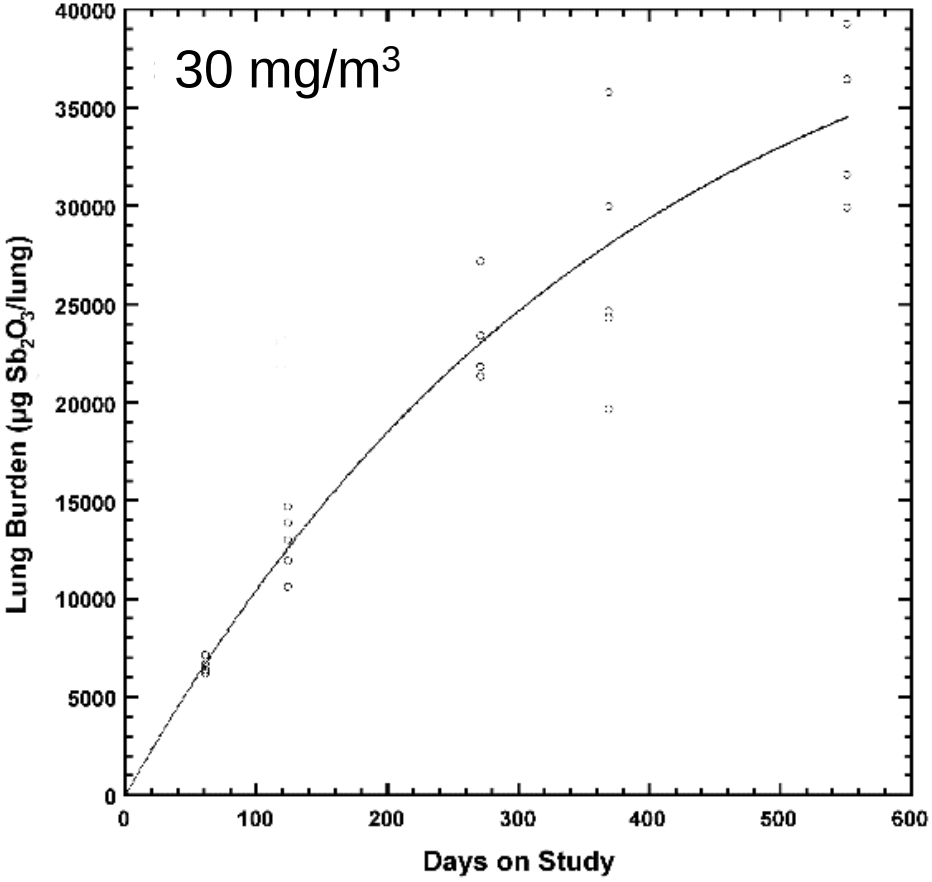
- Mice (males and females): (+) in lung and (-) in leukocytes
- Rats (males and females): (-) in lung and in leukocytes

Tissue Burden Studies

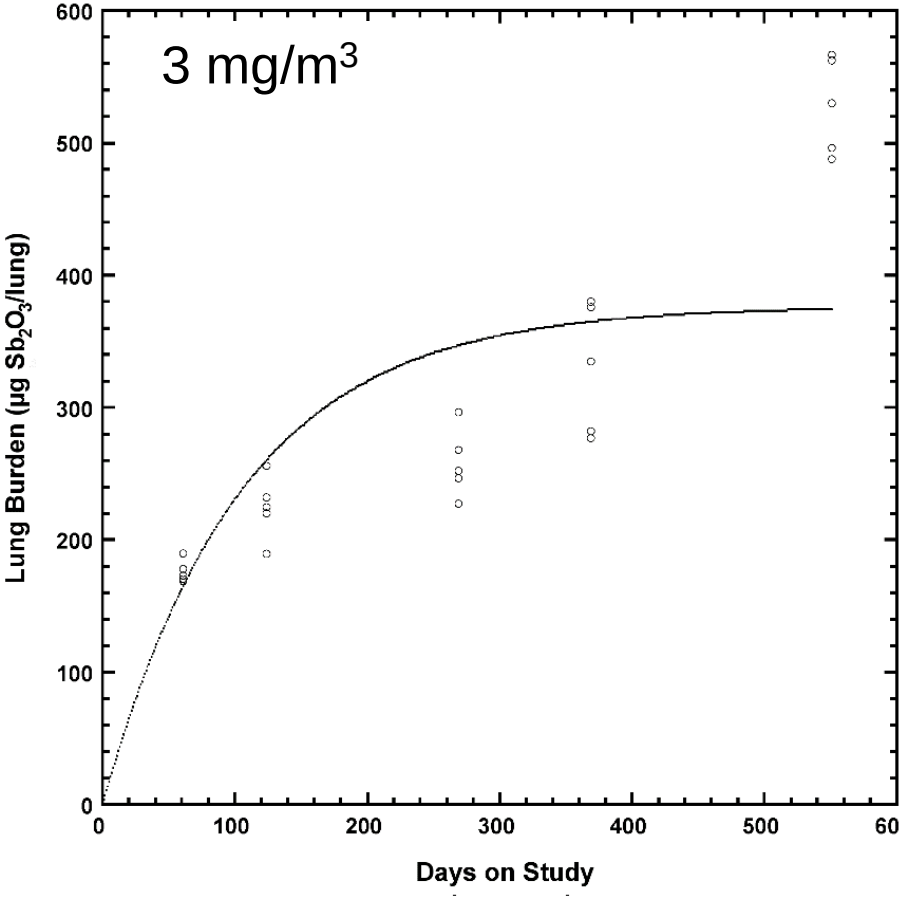
- Lung burdens/concentrations and blood concentrations increased with exposure concentration and duration
  - Lung concentrations > blood concentrations (<1%)
- Two-year studies
  - Lung burdens did not approach steady state in 30 mg/m^3 rats or in mice at any concentration
    - Mouse data did not fit model at any concentration due to unexpectedly high lung burdens at longer exposure durations
  - Half life in 3 mg/m^3 rats similar to those observed in the two-week studies (~4 months)
- Reduced lung clearance due to particle overload in 10 and 30 mg/m^3 in rats and mice
  - Lung neoplastic (mice) and nonneoplastic (rats and mice) observed at 3 mg/m^3

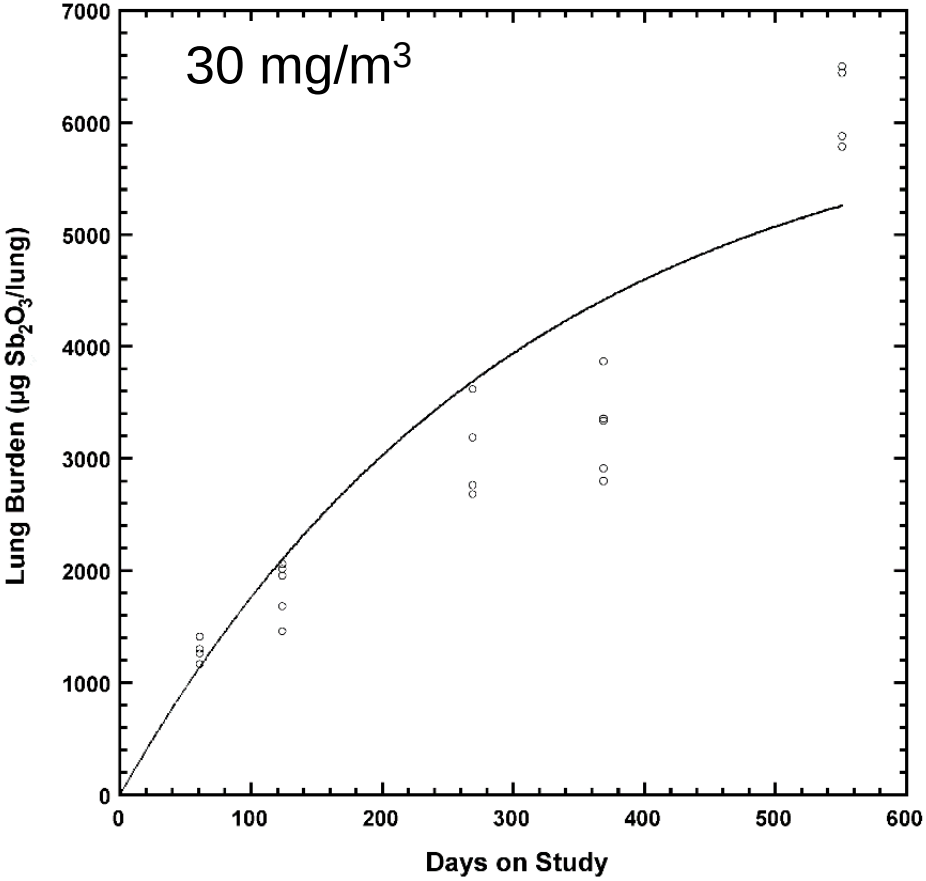
Lung Deposition and Clearance in Rats





Lung Deposition and Clearance in Mice





Lung Neoplasms - Mutations in *Egfr* and *Kras*

- Rats
  - Exposed
    - Point mutations in *Egfr:* 13/26 (50%)
    - Point mutations in *Kras*: 1/26 (4%)
  - Control
    - No *Egfr* mutations
    - No *Kras* mutations
- Mice
  - Exposed
    - Point mutations in *Egfr:* 37/80 (46%)
    - Point mutations in *Kras:* 34/80 (43%)
  - Control
    - No *Egfr* mutations
    - Point mutations in *Kras:* 3/9 (33%)

Egfr and *Kras* are commonly mutated in human lung neoplasms
Egfr mutations only observed in exposed rats and mice
Kras mutations in exposed mice were similar to those in control mice

Neoplasms - Rats

- Males:
  - *Some evidence of carcinogenic activity* based on increased incidences of:
    - Alveolar/bronchiolar adenoma or carcinoma (combined) in the lung
    - Benign pheochromocytoma of the adrenal medulla
- Females:
  - *Some evidence of carcinogenic activity* based on increased incidences of:
    - Alveolar/bronchiolar adenoma in the lung
    - Benign or malignant pheochromocytoma (combined) of the adrenal medulla
  - *May have been related to exposure*:
    - Combined occurrence of cystic keratinizing epithelioma and squamous cell carcinoma in the lung

Neoplasms - Mice

- Males:
  - *Clear evidence of carcinogenic activity* based on increased incidences of:
    - Alveolar/bronchiolar carcinoma of the lung
  - *Considered related to exposure*:
    - Increased incidences of fibrous histiocytoma or fibrosarcoma (combined) in the skin
- Females:
  - *Clear evidence of carcinogenic activity* based on increased incidences of:
    - Alveolar/bronchiolar adenoma and alveolar/bronchiolar carcinoma of the lung
    - Malignant lymphoma
  - *May have been related to exposure*:
    - Occurrence of squamous cell carcinoma of the skin

Nonneoplastic Lesions

- Increased incidences in the:
  - Lung, nose, larynx, trachea, bronchial and mediastinal lymph nodes, and bone marrow of male and female rats and mice
  - Adrenal medulla, arteries of multiple tissues (mesentery, pancreas, mediastinum, kidney and lung) and eye of male and female rats
  - Thymus and heart of male and female mice
  - Forestomach of male mice
  - Spleen of female mice