Draft NTP Technical Report TR 581 on

Cobalt Metal

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Nomination

- Nominated for toxicology and carcinogenesis studies by the United Auto Workers and the Cobalt Development Institute with support from OSHA and NIOSH based on:
  - Widespread occupational exposure and occurrence of hard metal disease associated with exposure to cobalt and its compounds
  - Soluble cobalt compound, cobalt sulfate heptahydrate, previously tested by the NTP is carcinogenic by inhalation exposure
  - Limited data to assess the carcinogenic potential of inhaled insoluble cobalt compounds, particularly cobalt metal

OSHA=Occupational Safety and Health Administration
NIOSH= National Institute of Occupational Safety and Health

Occupational Exposure

- Exposure primarily dermal or through inhalation
  - Skin contact-allergic dermatitis
  - Excess lung-cancer mortality
    - Hard metal pneumoconiosis- progressive interstitial fibrosis from exposure to cobalt-tungsten carbide (hard-metal) industry
- Production of alloys
  - Aircraft engines, grinding and cutting tools, artificial hip and knee joints
- Salts in paint dryers, catalysts and in the production of pigments
- Over a million U.S. workers potentially exposed to cobalt
  - Maximum OSHA permissible level is 0.1 mg/m^3
  - ACGIH TLV-TWA is 0.02 mg/m^3

ACGIH = American Conference of Governmental Industrial Hygienists
TLV=Threshold Limit Value
TWA= Time Weighted Average

Non-Occupational Exposure

- Environmental exposure
  - Present naturally in soil and in groundwater and sediments; mainly derived from erosion of volcanic rocks in the mountains
- Essential trace element
  - Required for production of red blood cells (used clinically to treat anemia in the 1950's)
  - Polycythemia at high doses
- Past use: beer foam stabilizer
  - Beer-drinker's cardiomyopathy (congestive heart failure)

Experimental Design

Genotoxicity:	In vitro and in vivo (mice)
Two-Week Studies: (n=5)	F344/N rats: 0, 2.5, 5, 10, 20, 40 mg/m^3 B6C3F1/N mice: 0, 2.5, 5, 10, 20, 40 mg/m^3
Three-Month Studies: (n=10)	F344/N rats: 0, 0.625, 1.25, 2.5, 5 mg/m^3 B6C3F1/N mice: 0, 0.625, 1.25, 2.5, 5,10 mg/m^3
Two-Year Studies: (n=50)	F344/NTac rats: 0, 1.25, 2.5, 5 mg/m^3 B6C3F1/N mice: 0, 1.25, 2.5, 5 mg/m^3
Tissue- Burden Studies:	Two-week, three-month and two-year studies in additional groups of rats and mice
Molecular Analysis:	Lungs of F344N/Tac rats and B6C3F1/N mice from two-year studies

Genetic Toxicology

- Positive in TA98 strain (without S9) in the Salmonella assay
- Negative in micronucleus assay (male and female mice)

Two-Week Studies in Rats

- Decreased survival in males and females at 40 mg/m^3 and 20 mg/m^3
- Significant decrease in mean body weight in males at 10 mg/m^3 and females at 10 and 20 mg/m^3 relative to controls
- Increased lung weights and nonneoplastic lesions in animals at ≥10 mg/m^3
- Reduced urine volumes with concomitant increase in urine creatinine at ≥ 10 mg/m^3
- Exposure concentration for 3-month studies: 0, 0.625, 1.25, 2.5, 5 mg/m^3

Two-Week Studies in Mice

- Decreased survival in males and females at 40 mg/m^3
- Significant decrease in mean body weight in males (9%) and females (16%) at 20 mg/m^3 relative to controls
- Increased lung weights and nonneoplastic lesions of minimal severity at ≥ 5 mg/m^3
  - Average severity grade of minimal in the 5 and 10 mg/m^3 groups and increases in lung weights in 10 mg/m^3 groups not considered sufficiently severe to preclude use of this concentration
- Exposure concentration for 3-month studies: 0, 0.625, 1.25, 2.5, 5, 10 mg/m^3

Three-Month Studies in Rats

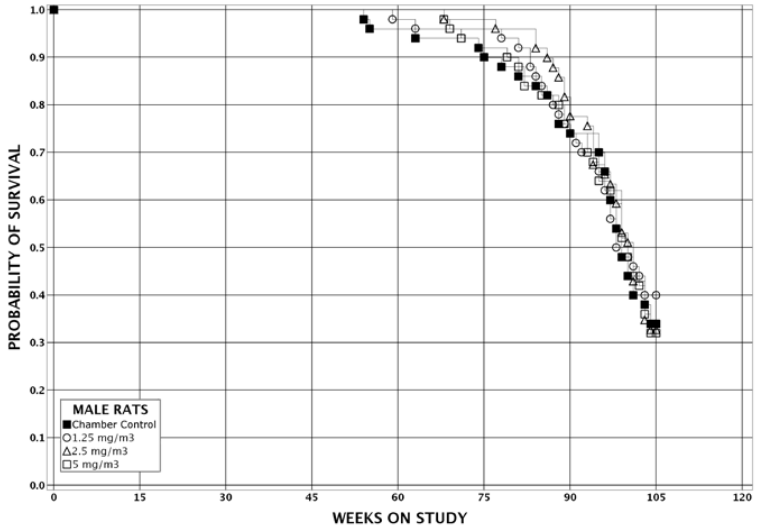
- No exposure-related effect on survival
- Decreased body weight in 5 mg/m^3 males and females, and decreased body weight gain in 5 mg/m^3 males relative to controls
- Increased lung weights and nonneoplastic lesions in nose and lung
- Erythrocytosis in all groups
- Decreased glucose in males exposed to ≥1.25 mg/m^3
- Exposure-concentration related decrease in cholesterol in males and females in the 2.5 and 5 mg/m^3 groups
- Sperm motility significantly decreased in males
- Findings in 5 mg/m^3 not considered sufficiently severe to preclude use of this concentration for 2-year studies

Three-Month Studies in Mice

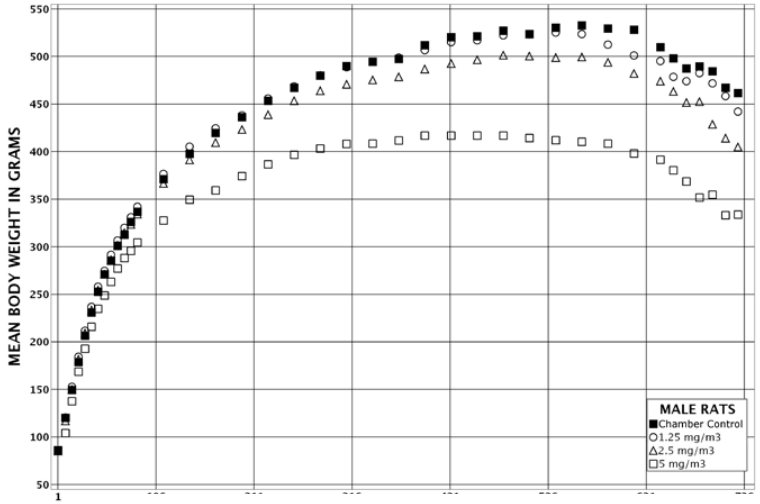
- No exposure-related effect on survival
- Decreased final body weight (~13%) relative to control in 10 mg/m^3
- Increased lung weights and nonneoplastic lesions in the nose and lung
- Exposure concentration-related decreases in testes weights, spermatid and epididymal spermatozoa counts, and sperm motility with histopathologic findings in testis and epididymis
- 5 mg/m^3 used as highest concentration in 2-year studies
  - Based on reductions in body weights and moderate severity of nose and lung lesions in 10 mg/m^3, this was considered too high

Two-Year Studies in Rats: Survival and Body Weight

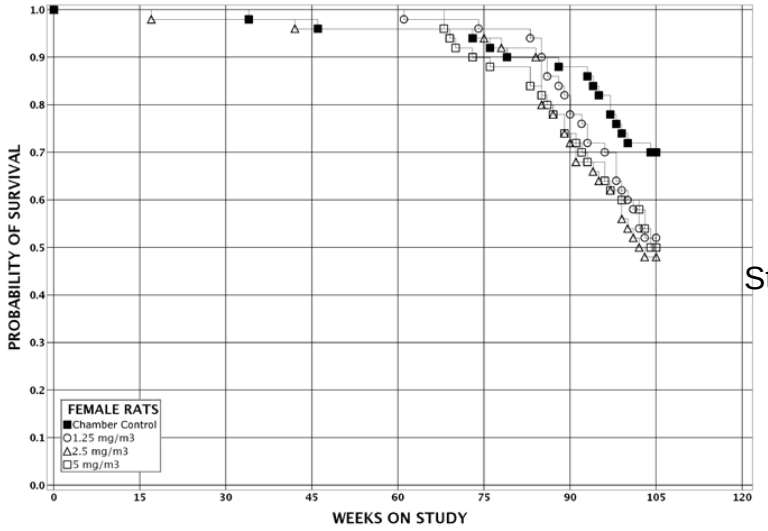
Survival



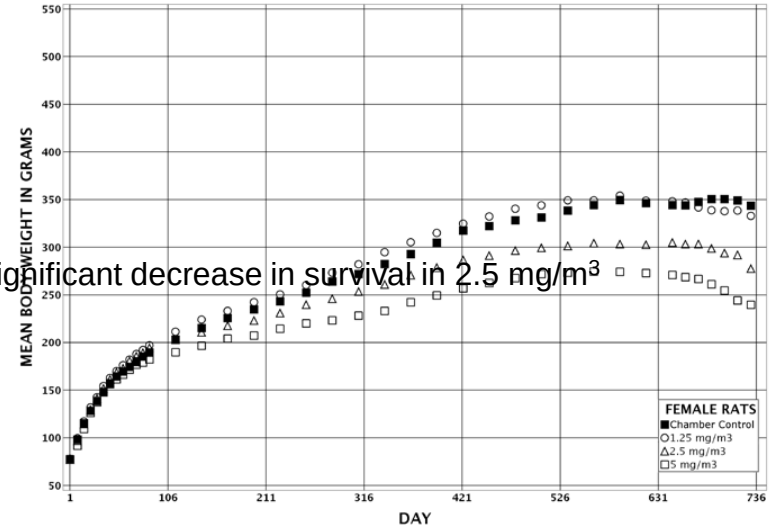
Body Weight



≥ 10% decrease at 2.5 and 5 mg/m^3



Statistically significant decrease in survival in 2.5 mg/m^3



Neoplastic Lesions in Rats: Lung (Clear Evidence)

Male	Control	1.25 mg/m^3	2.5 mg/m^3	5.0 mg/m^3
Alveolar Bronchiolar Neoplasms	50	50	50	50
Adenoma (includes multiple) (3)	2*	10*	10*	14**
Carcinoma, multiple	0	6*	14**	30**
Carcinoma (includes multiple) (0)	0**	16**	34**	36**
Adenoma or Carcinoma (3)	2**	25**	39**	44**
Female	**Control**	**1.25 mg/m^3**	**2.5 mg/m^3**	**5.0 mg/m^3**
Alveolar Bronchiolar Neoplasms	50	50	50	50
Adenoma (includes multiple) (0)	2**	7	9*	13**
Carcinoma, multiple	0	4	3	18**
Carcinoma (includes multiple) (0)	0**	9**	17**	30**
Adenoma or Carcinoma (0)	2**	15**	20**	38**

*Significantly different ($p \leq 0.05$) from chamber control by Poly-3 test; ** $p \leq 0.01$
Parenthesis represent historical controls, all routes in F344/NTac

Neoplastic Lesions in Rats: Lung

Male	Control	1.25 mg/m^3	2.5 mg/m^3	5.0 mg/m^3
Cystic keratinizing epithelioma	0	1	0	1

CKE in males may have been related to exposure

Female	Control	1.25 mg/m^3	2.5 mg/m^3	5.0 mg/m^3
Cystic keratinizing epithelioma	0	4	1	2
Squamous cell carcinoma	0	0	0	1

N = 50

CKE in females was considered related to exposure

Nonneoplastic Lesions in Rats: Lung

Male	Control	1.25 mg/m^3	2.5 mg/m^3	5.0 mg/m^3
Lung	**50**	**50**	**50**	**50**
Alveolar Epithelium, Hyperplasia	3 (1.0)	47 (2.8)**	49 (3.3)**	49 (3.6)**
Alveolus, Proteinosis	0	48 (2.6)**	49 (2.9)**	49 (3.1)**
Inflammation, Chronic Active	22 (1.1)	50 (3.0)**	50 (2.9)**	50 (2.9)**
Bronchiole, Epithelium, Hyperplasia	0	44 (1.5)**	47 (2.7)**	50 (3.7)**

**Significantly different ($p \leq 0.01$) from chamber control by Poly-3 test
In parenthesis: average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Lesions in Rats: Adrenal Medulla (Clear Evidence)

Male	Control	1.25 mg/m^3	2.5 mg/m^3	5.0 mg/m^3
Pheochromocytoma	**50**	**50**	**50**	**50**
Benign (includes bilateral) (10)	**15****	**23**	**37****	**34****
Malignant (includes bilateral) (0)	**2****	**2**	**9***	**16****
Benign or Malignant (10)	**17****	**23**	**38****	**41****
Hyperplasia	*19 (2.3)*	*21 (2.5)*	*9 (3.0)*	*9 (2.4)*
Female	**Control**	**1.25 mg/m^3**	**2.5 mg/m^3**	**5.0 mg/m^3**
Pheochromocytoma	**50**	**50**	**50**	**50**
Benign (includes bilateral) (1)	**6****	**12**	**22****	**36****
Malignant (includes bilateral) (1)	**0****	**2**	**3**	**11****
Benign or malignant (2)	**6****	**13**	**23****	**40****
Hyperplasia	*12 (1.8)*	*27 (2.0)***	*27 (2.3)***	*10 (2.8)*

*Significantly different ($p \leq 0.05$) from chamber control by Poly-3 test; ** $p \leq 0.01$
Text in parenthesis represents historical controls, all routes in F344/NTac
Text in italics represents a pre-neoplastic lesion

Neoplastic Lesions in Rats: Pancreas

Male	Control	1.25 mg/m^3	2.5 mg/m^3	5.0 mg/m^3
Islet Cell	50	50	48	49
Adenoma (0)	0	1	6*	3
Carcinoma (0)	2*	1	5	6
Adenoma or Carcinoma (0)	2**	2	10*	9*

Was considered related to exposure

Female	Control	1.25 mg/m^3	2.5 mg/m^3	5.0 mg/m^3
Islet Cell	50	50	50	50
Adenoma (1)	0	0	0	1
Carcinoma (0)	1	0	0	3
Adenoma or Carcinoma (0)	1	0	0	3

May have been related to exposure

*Significantly different ($p \leq 0.05$) from chamber control by Poly-3 test; ** $p \leq 0.01$
Text in parenthesis represent historical controls, all routes in F344/NTac

Neoplastic Lesions in Rats: Mononuclear Cell Leukemia

Female	Control	1.25 mg/m^3	2.5 mg/m^3	5.0 mg/m^3
Mononuclear cell leukemia (19)	**16**	**29****	**28***	**27***

N= 50
*Significantly different ($p \leq 0.05$) from chamber control by Poly-3 test; ** $p \leq 0.01$
Text in parenthesis represents historical controls, all routes in F344/NTac

Was considered related to exposure

Neoplastic Lesions in Rats: Kidney

Male	Control	1.25 mg/m^3	2.5 mg/m^3	5.0 mg/m^3
Renal Tubule	50	50	50	50
Adenoma (includes multiple) (1)	0	1	0	3
Carcinoma (0,1)	0	0	0	2
Adenoma or Carcinoma (1)	0*	1	0	4
Adenoma (includes multiple) (Original + Step)	3	1	1	6
Carcinoma (Original + Step)	0	0	0	2
Adenoma or Carcinoma (Original + Step)	3*	1	1	7

*Significantly different ($p \leq 0.05$) from chamber control by Poly-3 test; ** $p \leq 0.01$
Text in parenthesis represents historical controls, all routes in F344/NTac

May have been related to exposure

Nonneoplastic Lesions in Rats: Nose

Male	Control	1.25 mg/m^3	2.5 mg/m^3	5.0 mg/m^3
Nose	**48**	**47**	**45**	**50**
Inflammation				
Suppurative	9 (1.0)	12 (1.7)	24 (2.2)**	46 (2.6)**
Chronic Active	28 (1.2)	35 (1.3)*	40 (1.7)**	49 (2.6)**
Olfactory Epithelium				
Atrophy	2 (1.0)	21 (1.0)**	34 (1.0)**	29 (1.2)**
Hyperplasia	0	1 (1.0)	2 (1.5)	7 (1.1)**
Hyperplasia, Basal Cell	0	1 (1.0)	0	13 (1.0)**
Metaplasia, Respiratory	12 (1.1)	26 (1.7)**	37 (1.5)**	50 (2.2)**
Necrosis	0	1 (1.0)	5 (1.6)*	5 (1.8)*
Respiratory Epithelium				
Hyperplasia	20 (1.3)	35 (1.2)**	45 (1.7)**	50 (2.2)**
Metaplasia, Squamous	0	1 (1.0)	11 (1.2)**	35 (1.3)**
Necrosis	1 (1.0)	4 (1.8)	5 (1.4)	13 (1.6)**
Turbinate, Atrophy	1 (1.0)	35 (1.0)**	35 (1.0)**	41 (1.0)**

*Significantly different ($p \leq 0.05$) from chamber control by Poly-3 test; ** $p \leq 0.01$
In parenthesis: average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

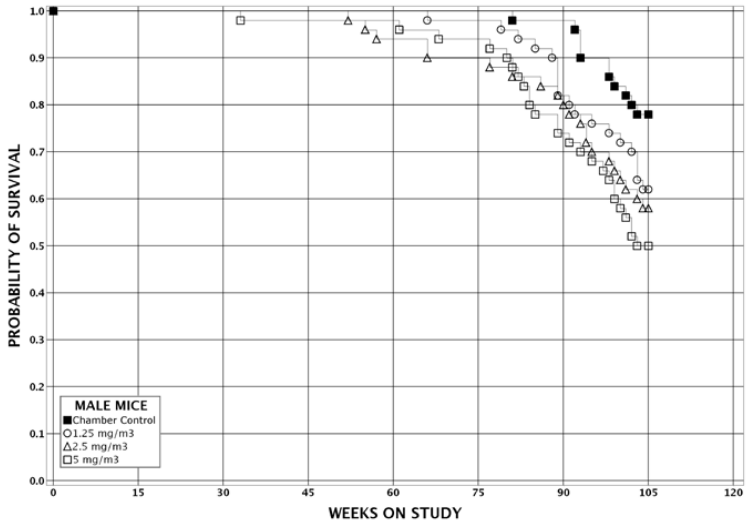
Nonneoplastic Lesions in Rats: Testes

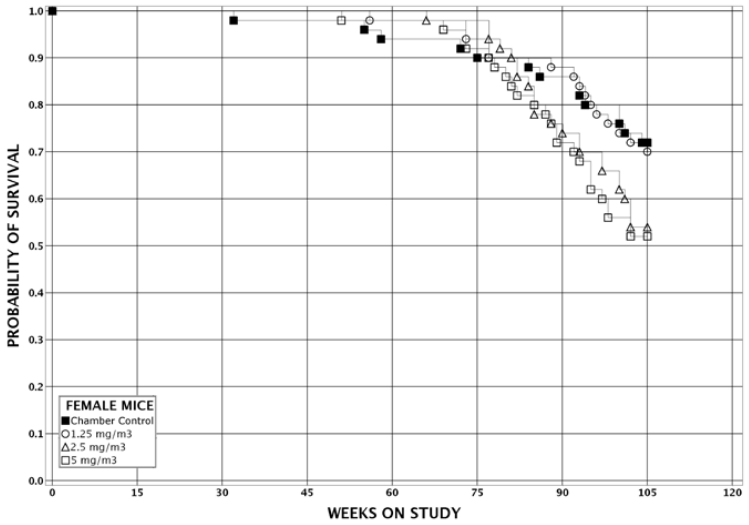
Male	Control	1.25 mg/m^3	2.5 mg/m^3	5.0 mg/m^3
Testes	**50**	**49**	**50**	**50**
Infarct	1 (3.0)	0	2 (3.5)	12 (2.7)**

**Significantly different ($p \leq 0.01$) from chamber control by Poly-3 test
In parenthesis: average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

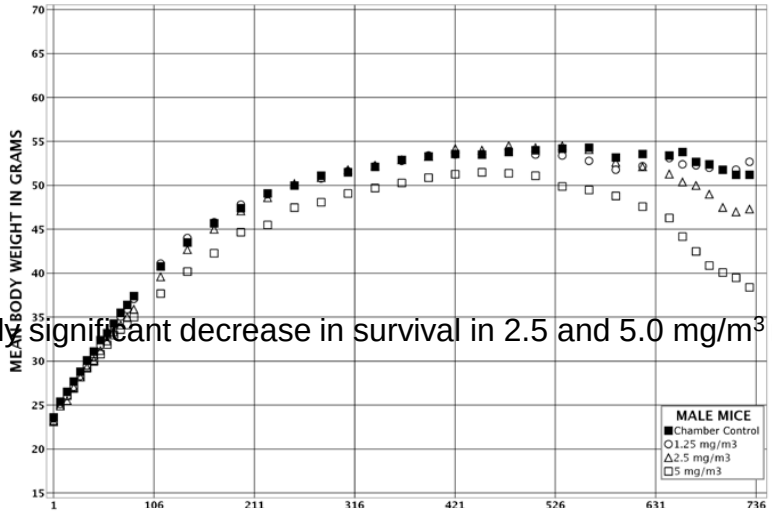
Two-Year Studies in Mice: Survival and Body Weight

Survival



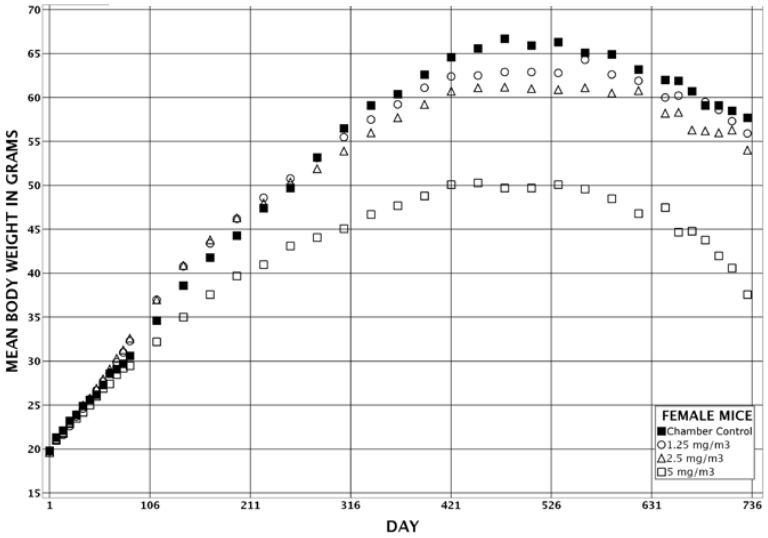


Body Weight



Statistically significant decrease in survival in 2.5 and 5.0 mg/m^3 males

Decrease in ≥ 10% at 5 mg/m^3



Neoplastic Lesions in Mice: Lung (Clear Evidence)

Male	Control	1.25 mg/m^3	2.5 mg/m^3	5.0 mg/m^3
Alveolar Bronchiolar Neoplasms	50	49	50	50
Adenoma (includes multiple) (4-10)	7	11	15*	3
Carcinoma (includes multiple) (8-12)	11**	38**	42**	46**
Adenoma or Carcinoma (13-20)	16**	41**	43**	47**
Female	**Control**	**1.25 mg/m^3**	**2.5 mg/m^3**	**5.0 mg/m^3**
Alveolar Bronchiolar Neoplasms	49	50	50	50
Adenoma (includes multiple) (1-6)	3*	9	8	10*
Carcinoma (includes multiple) (0-5)	5**	25**	38**	43**
Adenoma or Carcinoma (1-8)	8**	30**	41**	45**

*Significantly different ($p \leq 0.05$) from chamber control by Poly-3 test; ** $p \leq 0.01$
Text in parenthesis represents historical incidence for inhalation studies in B6C3F1/N mice

Nonneoplastic Lesions in Mice: Lung

Male	Control	1.25 mg/m^3	2.5 mg/m^3	5.0 mg/m^3
Lung	**50**	**49**	**50**	**50**
Proteinosis	2 (1.0)	46 (1.7)**	49 (3.1)**	50 (3.9)**
Alveolar Bronchiolar Epithelium				
Hyperplasia	0	46 (1.0)**	49 (1.6)**	50 (2.3)**
Vacuolization Cytoplasmic	0	49 (1.1)**	47 (1.9)**	48 (3.1)**
Alveolar Epithelium				
Hyperplasia	4 (2.3)	29 (1.7)**	24 (1.8)**	43 (2.0)**
Bronchiole, Epithelium				
Hyperplasia	4 (2.5)	7 (1.3)	9 (1.3)	11 (1.5)*
Erosion	0	4 (1.0)	10 (1.3)**	2 (1.0)
Alveolus				
Infiltration Cellular Histiocyte	10 (1.8)	49 (1.8)**	48 (2.5)**	48 (3.1)**

*Significantly different ($p \leq 0.05$) from chamber control by Poly-3 test; ** $p \leq 0.01$
In parenthesis: average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Nonneoplastic Lesions in Mice: Larynx and Trachea

Male	Control	1.25 mg/m^3	2.5 mg/m^3	5.0 mg/m^3
Larynx	**48**	**47**	**49**	**50**
Respiratory Epithelium				
Metaplasia, Squamous	7 (1.0)	47 (1.0)**	49 (1.0)**	49 (1.0)**
Vacuolization Cytoplasmic	0	20 (1.0)**	24 (1.0)**	32 (1.1)**
Squamous Epithelium				
Hyperplasia	2 (1.0)	5 (1.0)	5 (1.0)	8 (1.0)*
Trachea (Epithelium)				
Vacuolization, Cytoplasmic	0	14** (1.4)	31** (1.6)	37** (1.4)

*Significantly different ($p \leq 0.05$) from chamber control by Poly-3 test; ** $p \leq 0.01$
In parenthesis: average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Nonneoplastic Lesions in Mice: Nose

Male	Control	1.25 mg/m^3	2.5 mg/m^3	5.0 mg/m^3
Nose	**50**	**49**	**50**	**50**
Inflammation, Suppurative	16 (1.1)	32 (1.9)**	49 (2.7)**	50 (3.1)**
Olfactory Epithelium				
Atrophy	3 (1.0)	46 (1.2)**	42 (1.2)**	31 (1.2)**
Hyperplasia	0	25 (1.2)**	17 (1.0)**	8 (1.1)**
Metaplasia, Respiratory	5 (1.4)	24 (1.3)**	44 (2.3)**	50 (3.1)**
Respiratory Metaplasia, Atypical	0	14 (2.0)**	9 (1.1)**	1 (1.0)
Respiratory Epithelium				
Accumulation, Hyaline Droplet	13 (1.2)	29 (1.1)**	29 (1.1)**	7 (1.0)
Metaplasia, Squamous	3 (1.0)	45 (1.0)**	35 (1.1)**	33 (1.2)**
Vacuolization Cytoplasmic	0	41 (1.2)**	39 (1.2)**	37 (1.4)**
Turbinate, Atrophy	3 (1.3)	25 (1.3)**	49 (2.1)**	50 (3.3)**

**Significantly different ($p \leq 0.01$) from chamber control by Poly-3 test
In parenthesis: average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Nonneoplastic Lesions in Mice: Testes

Male	Control	1.25 mg/m^3	2.5 mg/m^3	5.0 mg/m^3
Testes	**50**	**49**	**50**	**50**
Germinal Epithelium, Degeneration	9 (1.6)	14 (1.6)	8 (1.6)	21** (1.5)

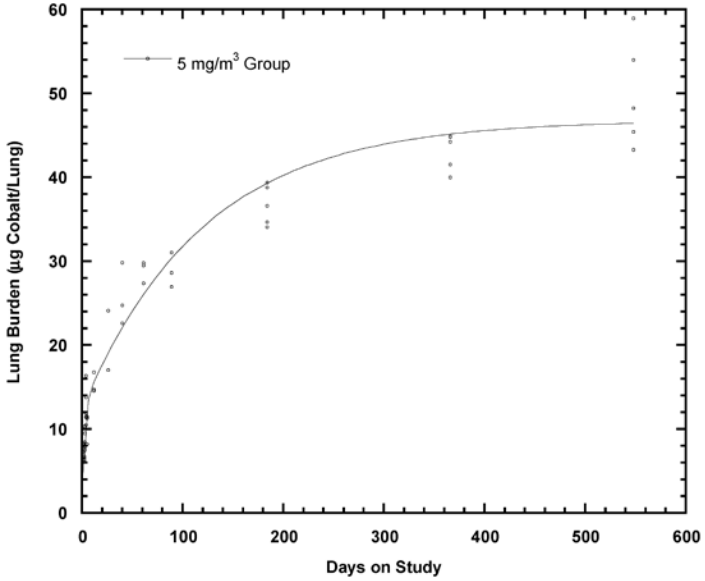
**Significantly different ($p \leq 0.01$) from chamber control by Poly-3 test
In parenthesis: average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

Tissue Burden Studies

- Conducted in rats and mice
  - 2-week studies
    - Males and females at the end of exposure (lung, blood, serum, kidney, liver, heart, femur, testis, urine)
    - Additional groups of females at 3-weeks post-exposure (lung, blood, serum) to estimate clearance
  - 3-month studies
    - Additional groups of females
    - Time-points during exposure, end of exposure (lung, liver, blood), and post-exposure (lung and blood)
  - 2-year studies
    - Additional groups of females
    - Time-points during exposure (lung)
- Endpoints evaluated
  - Tissue weights, concentrations and burdens
  - Lung and blood deposition and clearance parameters

Tissue Burden Studies

- Cobalt concentrations and burdens were increased in all studies, all tissues examined
  - Systemic distribution and toxicity/carcinogenicity of rats and mice to cobalt
- Burden data normalized to exposure concentration similar among exposure groups
  - No disproportionate differences in cobalt deposition/clearance with increasing exposure concentration
- Lung deposition and clearance: Two-compartment model
  - Most of cobalt eliminated rapidly ($t_{1/2}$ of 1-5 days)
  - Lung concentrations reached steady state
    - Low steady state burdens
- No evidence of lung overload



Conclusions: Male F344/NTac Rats

- *Clear evidence of carcinogenic activity* based on increased incidences of:
  - Alveolar/bronchiolar adenoma and carcinoma in the lung including multiples
  - Benign and malignant pheochromocytoma of the adrenal medulla, including bilateral neoplasms

- *Considered related to exposure*
  - Pancreatic islet adenoma or carcinoma (combined)

- *May have been related to exposure*
  - Cystic keratinizing epithelioma of the lung
  - Renal tubule adenoma or carcinoma (combined)

Conclusions: Female F344/NTac Rats

- *Clear evidence of carcinogenic activity* based on increased incidences of:
  - Alveolar/bronchiolar adenoma and carcinoma in the lung including multiples
  - Benign and malignant pheochromocytoma of the adrenal medulla, including bilateral neoplasms

- *Considered related to exposure*
  - Squamous cell neoplasms of lung (cystic keratinizing epithelioma)
  - Mononuclear cell leukemia

- *May have been related to exposure*
  - Pancreatic islet carcinoma

Conclusions: Male and Female B6C3F1/N Mice

- *Clear evidence of carcinogenic activity* based on increased incidences of:
  - Alveolar/bronchiolar neoplasms of the lung (predominantly carcinoma), including multiple carcinoma

Conclusions: Nonneoplastic Lesions

- Increased incidences in:
  - Lung and nose in male and female rats
  - Testes in male rats and mice
  - Adrenal medulla in female rats
  - Lung, nose, larynx, and trachea in male and female mice