

# NIPERA<sup>INC.</sup>

Nickel  
Producers  
Environmental  
Research  
Association

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Dear Sir or Madam:

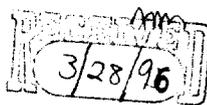
To has come to my attention that the enclosures were not included in NiPERA's letter to you dated December 6, 1994 (enclosed). This letter was in reference to the TSCA Section 8(e) Report/Notification requirement. I apologize for this oversight and am including a copy the draft summary NTP Technical Reports at this time.

If you have any questions regarding this letter, please contact me at (919) 544-7722.

Sincerely,

Connie Lawson  
Technical Information Specialist

Enclosures



# NIPERA INC.

Nickel  
Producers  
Environmental  
Research  
Association

December 6, 1994

Via Certified Mail

Document Processing Center (TS-790)  
Attention: Section 8(e) Coordinator  
Office of Pollution Prevention and Toxics  
U.S. Environmental Protection Agency  
401 M Street, SW  
Washington, DC 20460

Re: *TSCA Section 8(e) Report/Notification*

Dear Sir or Madam:

Pursuant to Section 8(e) of the Toxic Substances Control Act ("TSCA"), the Nickel Producers Environmental Research Association, Inc. ("NiPERA") hereby submits this report/notification regarding the availability of Draft NTP Technical Reports on the Toxicology and Carcinogenesis Studies of Nickel Oxide (CAS No. 1313-99-1), Nickel Subulfide (CAS No. 12035-72-2), and Nickel Sulfate Hexahydrate (CAS No. 10101-97-0). The NIH publication numbers of the three Reports are: 94-3363, 94-3369, and 94-3370, respectively.

The Draft Technical Reports present the results of inhalation studies conducted in F344/N Rats and B6C3F<sub>1</sub> Mice by the National Toxicology Program ("NTP") of the U.S. Department of Health and Human Services. Summaries of the three Technical Reports, including descriptions of the neoplastic and nonneoplastic effects that were found in the studies, are presented in the Abstracts of the Reports, copies of which are submitted herewith. EPA can obtain copies of the complete Reports from:

NTP Central Data Management  
NIEHS  
P.O. Box 12233  
MD AO-01  
Research Triangle Park, NC 27709  
(919) 541-1371

Alston Technical Park, 100 Capitola Drive, Suite 104, Durham, NC 27713  
Telephone 919/544-7722 • Fax 919/544-7507

**NTP TECHNICAL REPORT**  
**ON THE**  
**TOXICOLOGY AND CARCINOGENESIS**  
**STUDIES OF**  
**NICKEL OXIDE**  
**(CAS NO. 1313-99-1)**  
**IN F344/N RATS AND B6C3F<sub>1</sub> MICE**  
**(INHALATION STUDIES)**

**Scheduled Peer Review Date: November 29, 1994**

**NOTICE**

This is a DRAFT Technical Report prepared for public review and comment. Until this DRAFT has been reviewed and approved by the NTP Board of Scientific Counselors' Technical Reports Review Subcommittee in public session, the interpretations described herein do not represent the official scientific position of the National Toxicology Program. Following peer review, readers should contact NTP for the final version of this Technical Report.

**NTP TR 451**

**NIH Publication No. 94-3363**

**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES**  
**Public Health Service**  
**National Institutes of Health**

## NOTE TO THE READER

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge while supplies last from NTP Central Data Management, NIEHS, P.O. Box 12233, MD A0-01, Research Triangle Park, NC 27709 (919-541-1371).

**NTP TECHNICAL REPORT**  
**ON THE**  
**TOXICOLOGY AND CARCINOGENESIS**  
**STUDIES OF**  
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**NTP TR 451**

**NIH Publication No. 94-3363**

**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES**  
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# CONTENTS

<b>ABSTRACT</b>		7
<b>EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY</b>		16
<b>TECHNICAL REPORTS REVIEW SUBCOMMITTEE</b>		17
<b>SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS</b>		18
<b>INTRODUCTION</b>		19
<b>MATERIALS AND METHODS</b>		51
<b>RESULTS</b>		71
<b>DISCUSSION AND CONCLUSIONS</b>		121
<b>REFERENCES</b>		145
<b>APPENDIX A</b>	<b>Summary of Lesions in Male Rats in the 2-Year Inhalation Study of Nickel Oxide</b>	A-1
<b>APPENDIX B</b>	<b>Summary of Lesions in Female Rats in the 2-Year Inhalation Study of Nickel Oxide</b>	B-1
<b>APPENDIX C</b>	<b>Summary of Lesions in Male Mice in the 2-Year Inhalation Study of Nickel Oxide</b>	C-1
<b>APPENDIX D</b>	<b>Summary of Lesions in Female Mice in the 2-Year Inhalation Study of Nickel Oxide</b>	D-1
<b>APPENDIX E</b>	<b>Genetic Toxicology</b>	E-1
<b>APPENDIX F</b>	<b>Organ Weights and Organ-Weight-to-Body-Weight Ratios</b>	F-1
<b>APPENDIX G</b>	<b>Hematology Results</b>	G-1
<b>APPENDIX H</b>	<b>Tissue Burden in Rats</b>	H-1
<b>APPENDIX I</b>	<b>Tissue Burden in Mice</b>	I-1
<b>APPENDIX J</b>	<b>Reproductive Tissue Evaluations and Estrous Cycle Characterization</b>	J-1
<b>APPENDIX K</b>	<b>Chemical Characterization and Generation of Chamber Concentrations</b>	K-1

APPENDIX L	Ingredients, Nutrient Composition, and Contaminant Levels in NIH-07 Rat and Mouse Ration .....	L-1
APPENDIX M	Sentinel Animal Program .....	M-1
APPENDIX N	The Immunotoxicity of Three Nickel Compounds Following 13-Week Inhalation Exposure in the Mouse .....	N-1
APPENDIX O	Biochemical Responses of Rat and Mouse Lung to Inhaled Nickel Compounds ...	O-1
APPENDIX P	Fate of Inhaled Nickel Oxide and Nickel Subsulfide in F344/N Rats .....	P-1



# ABSTRACT

## NiO

### NICKEL OXIDE

CAS No. 1313-99-1

Chemical Formula: NiO      Molecular Weight: 74.71

**Synonyms:** Bunsenite; C.I. 77777; green nickel oxide; mononickel oxide; nickel monoxide; nickelous oxide; nickel protoxide; nickel oxide sinter 75; nickel (II) oxide; nickel (T+) oxide

Nickel oxide (NiO), a major component of nickel ore, is used in the welding and manufacture of stainless steel. It was nominated by the National Cancer Institute to the NTP for testing due to the increased incidences of lung and nasal sinus cancers that have occurred among workers in certain nickel refining facilities and is part of a class study of nickel compounds. Although the agent(s) responsible for the nasal sinus cancers have not been positively identified, nickel oxide is one of several forms of nickel to which workers are exposed in the refineries. Male and female F344/N rats and B6C3F<sub>1</sub> mice were exposed to nickel oxide (high temperature, green nickel oxide; mass median diameter  $2.8 \pm 1.8 \mu\text{m}$ ; at least 99% pure) by inhalation for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in peripheral blood of B6C3F<sub>1</sub> mice exposed to nickel oxide for 13 weeks.

### 16-DAY STUDY IN RATS

Groups of five male and five female F344/N rats were exposed to 0, 1.2, 2.5, 5, 10, or 30 mg nickel oxide/m<sup>3</sup> (equivalent to 0, 0.9, 2.0, 3.9, 7.9, or 23.6 mg nickel/m<sup>3</sup>) by inhalation for 6 hours per day, 5 days per week for a total of 12 exposure days during a 16-day period. Additional groups of five male

and five female rats were exposed to 0, 1.2, 5, or 10 mg/m<sup>3</sup> for tissue burden studies. All core study rats survived until the end of the study, final mean body weights of exposed male and female rats were similar to those of the controls, and there were no clinical findings related to nickel oxide exposure. Absolute and relative lung weights of male and female rats exposed to 10 or 30 mg/m<sup>3</sup> were significantly greater than those of the controls. Pigment particles in alveolar macrophages or within the alveolar spaces were observed in the lungs of exposed groups of males and females. Inflammation and accumulation of macrophages in alveolar spaces of the lungs, and hyperplasia in the respiratory tract lymph nodes were most severe in 10 and 30 mg/m<sup>3</sup> males and females. Hyperplasia of bronchial lymph nodes occurred in 30 mg/m<sup>3</sup> rats. Atrophy of the olfactory epithelium was observed in one male and one female exposed to 30 mg/m<sup>3</sup>. The concentrations of nickel oxide in the lungs of exposed groups of rats were significantly greater than those in the lungs of control groups (males, 42 to 267 µg nickel/g lung; females, 54 to 340 µg/g lung).

### 16-DAY STUDY IN MICE

Groups of five male and five female B6C3F<sub>1</sub> mice were exposed to 0, 1.2, 2.5, 5, 10, or 30 mg nickel oxide/m<sup>3</sup> (equivalent to 0, 0.9, 2.0, 3.9, 7.9, or 23.6 mg nickel/m<sup>3</sup>) by inhalation for 6 hours per day, 5 days per week for a total of 12 exposure days during a 16-day period. Additional groups of five male and five female mice were exposed to 0, 1.2, 2.5, or 5 mg/m<sup>3</sup> for tissue burden studies. No exposure-related deaths occurred among core study mice, and final mean body weights of exposed male and female mice were similar to those of the controls. There were no chemical-related clinical findings. Pigment particles were present in the lungs of mice exposed to 2.5 mg/m<sup>3</sup> or greater. Accumulation of macrophages in alveolar spaces was observed in the lungs of 10 and 30 mg/m<sup>3</sup> males and females. The concentrations of nickel oxide in the lungs of exposed groups of mice were significantly greater than those in the lungs of control animals (males, 32 to 84 µg nickel/g lung; females, 31 to 71 µg/g lung).

### 13-WEEK STUDY IN RATS

Groups of 10 male and 10 female F344/N rats were exposed to 0, 0.6, 1.2, 2.5, 5, or 10 mg nickel oxide/m<sup>3</sup> (equivalent to 0, 0.4, 0.9, 2.0, 3.9, or 7.9 mg nickel/m<sup>3</sup>) by inhalation for 6 hours per day, 5 days per week for 13 weeks. Additional groups of 18 male and 18 female rats were exposed to 0, 0.6, 2.5, or 10 mg/m<sup>3</sup> for tissue burden studies. No exposure-related deaths occurred among core study rats, final mean body weights of exposed male and female rats were similar to those of the controls, and no clinical findings in any group were related to nickel oxide exposure. Lymphocyte, neutrophil, monocyte, and erythrocyte counts; hematocrit values; and hemoglobin and mean cell hemoglobin concentrations in exposed rats were minimally to mildly greater than those of the controls; these differences were most pronounced in females. Mean cell volumes in exposed rats were generally less than those in the controls. Absolute and relative lung weights of exposed groups of males and females were generally significantly greater than those of controls.

Chemical-related nonneoplastic lesions were observed in the lungs of male and female rats exposed to concentrations of 2.5 mg/m<sup>3</sup> or higher, and the severity of these lesions generally increased with exposure concentration. Accumulation of alveolar macrophages, many of which contained black, granular pigment, was generally observed in all exposed groups of males and females, and increased incidences of inflammation occurred in males and females exposed to 2.5 mg/m<sup>3</sup> or higher. In addition, lymphoid hyperplasia and pigment occurred in the bronchial and mediastinal lymph nodes of 2.5, 5, and 10 mg/m<sup>3</sup> males and females.

The concentration of nickel oxide in the lung of 0.6, 2.5, and 10 mg/m<sup>3</sup> males was significantly greater than that in the lung of controls at 4, 9, and 13 weeks, and nickel continued to accumulate in the lung at the end of the 13-week exposures (4 weeks, 33 to 263 µg nickel/g lung; 9 weeks, 53 to 400 µg/g lung; 13 weeks, 80 to 524 µg/g lung).

## 13-WEEK STUDY IN MICE

Groups of 10 male and 10 female B6C3F<sub>1</sub> mice were exposed to 0, 0.6, 1.2, 2.5, 5, or 10 mg nickel oxide/m<sup>3</sup> (equivalent to 0, 0.4, 0.9, 2.0, 3.9, or 7.9 mg nickel/m<sup>3</sup>) by inhalation for 6 hours per day, 5 days per week for 13 weeks. Additional groups of six male and six female mice were exposed to 0, 0.6, 2.5, or 10 mg/m<sup>3</sup> for tissue burden studies. No exposure-related deaths occurred among core study animals, final mean body weights of exposed male and female mice were similar to those of the controls, and no clinical findings in any group were related to nickel oxide exposure. Hematocrit values and erythrocyte counts in 5 and 10 mg/m<sup>3</sup> females were minimally greater than those of the controls, as was the hemoglobin concentration in 5 mg/m<sup>3</sup> females. Absolute and relative lung weights of 10 mg/m<sup>3</sup> males and females were significantly greater than those of controls, and absolute and relative liver weights of 10 mg/m<sup>3</sup> males were significantly less than those of controls.

Accumulation of alveolar macrophages, many of which contained pigment particles, occurred in all groups of mice exposed to nickel oxide. Inflammation (chronic active perivascular infiltrates or granulomatous) occurred in 2.5, 5, and 10 mg/m<sup>3</sup> males and females. In addition, lymphoid hyperplasia and pigment occurred in the bronchial lymph nodes of males and females exposed to 2.5 mg/m<sup>3</sup> or higher.

The concentration of nickel in the lung was significantly increased in 0.6, 2.5, and 10 mg/m<sup>3</sup> males at 13 weeks (42 to 736 µg nickel/g lung).

## 2-YEAR STUDY IN RATS

### *Survival, Body Weights, Clinical Findings, and Hematology*

Groups of 65 male and 65 female F344/N rats were exposed to 0, 0.62, 1.25, or 2.5 mg nickel oxide/m<sup>3</sup> (equivalent to 0, 0.5, 1.0, or 2.0 mg nickel/m<sup>3</sup>) by inhalation for 6 hours per day, 5 days per week for 104 weeks. Survival of exposed male and female rats was similar to that of the controls. Mean body

weights of 0.62 mg/m<sup>3</sup> males and females and 1.25 mg/m<sup>3</sup> males were similar to those of the controls. Mean body weights of 1.25 mg/m<sup>3</sup> females and 2.5 mg/m<sup>3</sup> males and females were slightly lower than those of the controls during the second year of the study. No chemical-related clinical findings were observed in male or female rats during the 2-year study. No chemical-related differences in hematology parameters were observed in male or female rats at the 15-month interim evaluation.

### *Pathology Findings*

Absolute and relative lung weights of 1.25 and 2.5 mg/m<sup>3</sup> males and females were significantly greater than those of the controls at 7 and 15 months. At 2 years, there was an exposure-related increased incidence of alveolar/bronchiolar adenoma in females and exposure-related increased incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in males and females. The incidence of alveolar/bronchiolar carcinoma in 1.25 mg/m<sup>3</sup> females was significantly greater than that of the controls, and the incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in 1.25 and 2.5 mg/m<sup>3</sup> males were significantly greater than that of the controls. Incidences of atypical alveolar epithelial hyperplasia in the lungs generally increased with increasing exposure concentration in male and female rats. Chronic inflammation of the lung was observed in most exposed rats at 7 and 15 months and at 2 years; the incidences in exposed males and females at 2 years were significantly greater than those in the controls, and the severity of the inflammation increased in exposed groups. The incidences of pigmentation in the alveolus of exposed groups of males and females were significantly greater than those of the controls at 7 and 15 months and at 2 years.

Pigmentation in the bronchial lymph nodes similar to that in the lungs was observed in all exposure groups with the exception of 0.62 mg/m<sup>3</sup> males and females at 7 months. Lymphoid hyperplasia was observed in the bronchial lymph nodes of 1.25 and 2.5 mg/m<sup>3</sup> males and females at 7 and 15 months, and the incidence at 2 years generally increased with exposure concentration.

At 2 years, there was an exposure-related increase in the incidence of benign pheochromocytoma in males and females. The incidences of benign pheochromocytoma and adrenal medulla hyperplasia in 2.5 mg/m<sup>3</sup> females and the incidence of benign or malignant pheochromocytoma (combined) in 2.5 mg/m<sup>3</sup> males were significantly greater than those in the controls.

### *Tissue Burden Analyses*

Nickel concentrations in the lung of exposed rats were significantly greater than those in the controls at 7 and 15 months (7 months, 173 to 713 µg nickel/g lung; 15 months, 262 to 1,116 µg/g lung), and nickel concentrations increased with increasing exposure concentration and with time.

## **2-YEAR STUDY IN MICE**

### *Survival, Body Weights, Clinical Findings, and Hematology*

Groups of 74 to 79 B6C3F<sub>1</sub> mice were exposed to 0, 1.25, 2.5, or 5 mg nickel oxide/m<sup>3</sup> (equivalent to 0, 1.0, 2.0, or 3.9 mg nickel/m<sup>3</sup>) by inhalation for 6 hours per day, 5 days per week for 104 weeks.

Survival of exposed male and female mice was similar to that of the controls. Mean body weights of exposed males and of 1.25 and 2.5 mg/m<sup>3</sup> females were similar to those of the controls. Mean body weights of 5 mg/m<sup>3</sup> females were slightly lower than those of the controls during the second year of the study. No chemical-related clinical findings were observed in male or female mice during the 2-year study. No chemical-related differences in hematology parameters were observed in male or female mice at the 15-month interim evaluation.

### *Pathology Findings*

At 2 years, the incidence of alveolar/bronchiolar adenoma in 2.5 mg/m<sup>3</sup> females was significantly greater than that of the controls, as was the incidence of alveolar/bronchiolar adenoma or carcinoma (combined) in 1.25 mg/m<sup>3</sup> females. Generally, incidences of chronic inflammation increased with exposure

concentration in males and females at 7 and 15 months. Bronchialization (alveolar epithelial hyperplasia) of minimal severity in exposed animals and proteinosis were first observed at 15 months. At 2 years, the incidences of chronic inflammation, bronchialization, and proteinosis in exposed groups of males and females were significantly greater than those of the controls. The severity of chronic inflammation increased with exposure concentration in females, and proteinosis was most severe in 5 mg/m<sup>3</sup> males and females. Pigment occurred in the lungs of nearly all exposed mice at 7 and 15 months and at 2 years, and the severity increased with exposure concentration.

Lymphoid hyperplasia occurred in one 2.5 mg/m<sup>3</sup> male and one 1.25 mg/m<sup>3</sup> female at 7 months. At 15 months, lymphoid hyperplasia occurred in males exposed to 2.5 and 5 mg/m<sup>3</sup> and in all exposed groups of females. At 2 years, lymphoid hyperplasia occurred in some control animals, but this lesion was still observed more often in exposed males and females and the incidence increased with exposure concentration. Pigmentation was observed in the bronchial lymph nodes of exposed males and females at 7 and 15 months and in nearly all exposed animals at 2 years.

### *Tissue Burden Analyses*

Nickel concentrations in the lung of exposed mice were significantly greater than those in the controls at 7 and 15 months (7 months, 162 to 1,034 µg nickel/g lung; 15 months, 331 to 2,258 µg/g lung), and nickel concentrations increased with increasing exposure concentration and with time.

## GENETIC TOXICOLOGY

No increase in the frequency of micronucleated normochromatic erythrocytes was observed in peripheral blood samples from male or female mice exposed to nickel oxide.

## CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity\** of nickel oxide in male F344/N rats based on increased incidences of alveolar/bronchiolar adenoma or carcinoma (combined) and increased incidences of benign or malignant pheochromocytoma (combined) of the adrenal medulla. There was *some evidence of carcinogenic activity* of nickel oxide in female F344/N rats based on increased incidences of alveolar/bronchiolar adenoma or carcinoma (combined) and increased incidences of benign pheochromocytoma of the adrenal medulla. There was *no evidence of carcinogenic activity* of nickel oxide in male B6C3F<sub>1</sub> mice exposed to 1.25, 2.5, or 5 mg/m<sup>3</sup>. There was *equivocal evidence of carcinogenic activity* of nickel oxide in female B6C3F<sub>1</sub> mice based on marginally increased incidences of alveolar/bronchiolar adenoma in 2.5 mg/m<sup>3</sup> females and of alveolar/bronchiolar adenoma or carcinoma (combined) in 1.25 mg/m<sup>3</sup> females.

Exposure of rats to nickel oxide by inhalation for 2 years resulted in inflammation and pigmentation in the lung and lymphoid hyperplasia and pigmentation in the bronchial lymph nodes. Exposure of mice to nickel oxide by inhalation for 2 years resulted in inflammation and pigmentation in the lung and lymphoid hyperplasia and pigmentation in the bronchial lymph nodes.

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\* Explanation of Levels of Evidence of Carcinogenic Activity is on page 16.

## Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Nickel Oxide

	Male F344/N Rats	Female F344/N Rats	Male B6C3F <sub>1</sub> Mice	Female B6C3F <sub>1</sub> Mice
<b>Doses.</b>	0, 0.62, 1.25, or 2.5 mg nickel oxide/m <sup>3</sup> (0, 0.5, 1.0, or 2.0 mg nickel/m <sup>3</sup> )	0, 0.62, 1.25, or 2.5 mg nickel oxide/m <sup>3</sup> (0, 0.5, 1.0, or 2.0 mg nickel/m <sup>3</sup> )	0, 1.25, 2.5, or 5 mg nickel oxide/m <sup>3</sup> (0, 1.0, 2.0, or 3.9 mg nickel/m <sup>3</sup> )	0, 1.25, 2.5, or 5 mg nickel oxide/m <sup>3</sup> (0, 1.0, 2.0, or 3.9 mg nickel/m <sup>3</sup> )
<b>Body weights</b>	2.5 mg/m <sup>3</sup> group slightly lower than controls	1.25 and 2.5 mg/m <sup>3</sup> groups slightly lower than controls	5 mg/m <sup>3</sup> group slightly lower than controls	5 mg/m <sup>3</sup> group slightly lower than controls
<b>2-Year survival rates</b>	14/54, 15/53, 15/53, 12/52	21/53, 26/53, 20/53, 26/54	19/57, 23/67, 29/66, 23/69	41/64, 40/66, 42/63, 38/64
<b>Nonneoplastic effects</b>	<u>Lung:</u> chronic inflammation (28/54, 53/53, 53/53, 52/52); pigment (1/54, 53/53, 53/53, 52/52) <u>Bronchial lymph node:</u> lymphoid hyperplasia (0/52, 7/51, 10/53, 18/52); pigment (0/52, 45/51, 51/53, 51/52)	<u>Lung:</u> chronic inflammation (18/53, 52/53, 53/53, 54/54); pigment (0/53, 52/53, 53/53, 54/54) <u>Bronchial lymph node:</u> lymphoid hyperplasia (1/49, 5/50, 20/53, 13/52); pigment (0/49, 43/50, 52/53, 47/52)	<u>Lung:</u> chronic inflammation (0/57, 21/67, 34/66, 55/69); pigment (0/57, 65/67, 66/66, 68/69); <u>Bronchial lymph node:</u> lymphoid hyperplasia (5/45, 18/56, 28/61, 33/62); pigment (0/45, 55/56, 61/61, 60/62)	<u>Lung:</u> chronic inflammation (7/64, 43/66, 53/63, 52/64); pigment (0/64, 64/66, 61/63, 64/64); <u>Bronchial lymph node:</u> lymphoid hyperplasia (14/54, 37/63, 40/59, 44/62); pigment (0/54, 58/63, 56/59, 60/62)
<b>Neoplastic effects</b>	<u>Lung:</u> alveolar/bronchiolar adenoma or carcinoma or squamous cell carcinoma (1/54, 1/53, 6/53, 4/52) <u>Adrenal medulla:</u> benign or malignant pheochromocytoma (27/54, 24/52, 27/53, 35/52)	<u>Lung:</u> alveolar/bronchiolar adenoma or carcinoma (1/53, 0/53, 6/53, 5/54) <u>Adrenal medulla:</u> benign pheochromocytoma (4/51, 7/52, 6/53, 18/53)	None	None
<b>Uncertain findings</b>	None	None	None	<u>Lung:</u> alveolar/bronchiolar adenoma (2/64, 4/66, 10/63, 3/64); alveolar/bronchiolar adenoma or carcinoma (6/64, 15/66, 12/63, 8/64)
<b>Level of evidence of carcinogenic activity</b>	Some evidence	Some evidence	No evidence	Equivocal evidence
<b>Genetic toxicology</b>				
Micronucleated erythrocytes				
Mouse peripheral blood <i>in vivo</i> :		Negative in male and female mice		

## EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (clear evidence and some evidence); one category for uncertain findings (equivocal evidence); one category for no observable effects (no evidence); and one category for experiments that cannot be evaluated because of major flaws (inadequate study). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- Clear evidence of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- Some evidence of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- Equivocal evidence of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- No evidence of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- Inadequate study of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase.
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

**NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS  
TECHNICAL REPORTS REVIEW SUBCOMMITTEE**

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on nickel oxide on November 29, 1994, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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**SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS**

NOTE: A summary of the Technical Reports Review Subcommittee's remarks will appear in a future draft of this report.

# INTRODUCTION

## NiO

### NICKEL OXIDE

CAS No. 1313-99-1

Chemical Formula: NiO    Molecular Weight: 74.71

**Synonyms:** Bunsenite; C.I. 77777; green nickel oxide; mononickel oxide; nickel monoxide; nickelous oxide; nickel protoxide; nickel oxide sinter 75; nickel (II) oxide; nickel (T+) oxide

### CHEMICAL AND PHYSICAL PROPERTIES

Nickel oxide (high temperature green nickel oxide, oxidized at 870°-900° C and heated to 1,350° C; Boldt, 1967) is an olive gray powder with a melting point of 2,090° C and a density of 7.45 g/cm<sup>3</sup>. It is insoluble in water and soluble in acids (USEPA, 1986; *Merck Index*, 1989). The mass median aerodynamic diameter of the nickel oxide particles used in these inhalation studies was 2.8 ± 1.8 μm.

### PRODUCTION, USE, AND HUMAN EXPOSURE

Nickel was first isolated in 1751 and is found primarily as an oxide (laterite) or sulfide ore (pentlandite) (NIOSH, 1977; Warner, 1984; U.S. Bureau of Mines, 1984, 1985a). In 1991, the six largest nickel producing countries were the Soviet Union, Canada, Australia, New Caledonia, Indonesia, and Cuba. Approximately 55% of the nickel currently used is extracted from sulfide ore, and the remainder is extracted from oxide ore. The total annual world production of nickel is estimated at 1,000,000 tons (907,000 metric tons) (U.S. Bureau of Mines, 1991).

The United States consumption of nickel is approximately 200,000 tons (180,000 metric tons) annually (U.S. Bureau of Mines, 1991). The United States consumes unwrought nickel (68%), ferronickel (17.3%), nickel oxide (11.4%), nickel salts (1.2%), and other forms (2.1%) (U.S. Bureau of Mines, 1984, 1985b). The National Occupational Exposure Survey (NIOSH) reported that 56,843 and 18,165 United States workers are potentially exposed to nickel sulfate and nickel oxide, respectively (information on nickel subsulfide exposure not reported) (NIOSH, 1994).

Half of the nickel sold per year is used to make stainless steel (Warner, 1984), which contains up to 8% nickel. The ability of nickel to impart corrosion resistance and strength leads to its wide use in chemicals and allied products and in petroleum refining (24%); in electrical equipment and supplies (11%); in aircraft and parts (10%); in construction (10%); in fabricated metal products (9%); household appliances (8%); machinery (7%); in ship and boat building (4%) and miscellaneous uses (7%) (U.S. Bureau of Mines, 1984).

Nickel constitutes about 0.008% of the earth's crust. Low levels of nickel are found in air, soil, water, food, and household objects. The average concentration of nickel in finished drinking water is less than 10 ppb. Nickel concentration in United States air has been found to range from 1 to 86 ng/m<sup>3</sup>. The most probable nickel species present in the atmosphere include nickel oxide and nickel sulfate, and the most probable species found in water includes nickel sulfate hexahydrate (ATSDR, 1992). The average amount of nickel in mainstream particulate fractions of cigarette smoke is 79 ng/cigarette (Bache *et al.*, 1985). Dietary intake of nickel per person from foods is estimated at 170 µg per day; intake from inhalation is estimated at 0.1 to 1 µg nickel per day (excluding cigarette smoke), and intake from drinking water is estimated at 2 µg per day (ATSDR, 1992). Nickel is listed as one of the most frequently occurring chemicals in waste deposit sites in the United States (*Fed. Regist.*).

The threshold limit values adopted by the American Conference of Government Industrial Hygienists (ACGIH) are 1 mg/m<sup>3</sup> for nickel metal and water-insoluble salts and 0.1 mg/m<sup>3</sup> for water-soluble salts, but the ACGIH published notice of an intended change to 0.05 mg nickel/m<sup>3</sup> for water-soluble and water-insoluble nickel compounds (ACGIH, 1993). NIOSH recommended that the permissible exposure limit for nickel be reduced to 0.015 mg nickel/m<sup>3</sup> averaged over a work shift of up to 10 hours per day, 40 hours per week (NIOSH, 1977).

Atomic absorption spectroscopy is a widely used method for quantifying nickel in the environment and in the workplace. This method of analysis measures total nickel without discerning the forms of nickel present, and most studies of environmental or industrial exposure report total nickel and not the occurrence of individual nickel species (ATSDR, 1992).

## ABSORPTION, DISTRIBUTION, AND EXCRETION

### *Experimental Animals*

Animal model systems have been used to obtain information on the absorption, distribution, and excretion of nickel after inhalation exposure (water-soluble and water-insoluble forms of nickel), oral exposure (water-soluble forms of nickel), and dermal exposure (water-soluble forms of nickel).

Intratracheal administration of nickel compounds was one method used by several investigators to study the fate of nickel in the lung. English *et al.* (1981) reported on a comparative toxicokinetic study after intratracheal administration of [<sup>63</sup>Ni]-labeled nickel chloride or nickel oxide (low temperature nickel oxide calcined at 250° C) in Wistar rats. Nickel, after nickel chloride administration, was excreted primarily in the urine. After nickel oxide administration, nickel was equally excreted in the feces and urine. Nickel oxide persisted in the lung for more than 90 days, while nickel chloride was rapidly excreted from the lung with greater than 50% of the nickel cleared from the lungs within 3 days.

Nickel chloride administered as an intratracheal dose to Sprague-Dawley rats was excreted primarily in the urine. By day 3, 90% of the instilled chemical was eliminated from the lungs. The lungs retained 29% of their initial burden at day 1, and this decreased to 0.1% on day 21; 96% of the chemical was excreted in the urine (Carvalho and Ziemer, 1982).

The pulmonary clearance of intratracheally administered nickel subsulfide ( $\text{Ni}_3\text{S}_2$ ) in mice has two distinct components with initial and final biological half-lives corresponding to 1.2 and 12.4 days, respectively. The excretion of the chemical (measured as  $^{63}\text{Ni}$ ) was 60% in the urine and 40% in the feces; 57% of the administered dose was excreted after 3 days with 33% appearing in the urine (Valentine and Fisher, 1984). In another experiment, the calculated clearance times of nickel subsulfide administered intratracheally to mice was also biphasic with a clearance half-life of 2 hours for the first phase and 119 hours for the second phase (Finch *et al.*, 1987).

In F344/N rats administered [ $^{63}\text{Ni}$ ]-labeled nickel oxide (high temperature, green oxide) or nickel subsulfide by pernasal inhalation, the lung half-life was estimated at 120 days for nickel oxide and 5 days for nickel subsulfide (Benson *et al.*, 1994; Appendix P). Benson *et al.* (1994) found that, following nickel oxide exposure, nickel was not distributed to the extrarespiratory tract tissue, and the material was only excreted in the feces during the first few days after exposure. In contrast, after nickel subsulfide exposure, nickel was detected in extrarespiratory tract tissue including blood and kidney, and nickel was excreted in the urine and the feces. The half-life of [ $^{63}\text{Ni}$ ]-labeled nickel sulfate administered to F344/N rats by pernasal administration was 1 to 3 days; nickel was present in extrarespiratory tract tissues (including blood, kidney, and intestine); and urine was the major route for excretion of nickel (Medinsky *et al.*, 1987).

Other studies also indicated that nickel oxide has a relatively long half-life in the rodent lung. Nickel oxide (formed at 550° C; mass median aerodynamic diameter (MMAD) of 0.15  $\mu\text{m}$ , geometric standard

deviation ( $\sigma_p$  of 1.5) given as an aerosol of  $750 \mu\text{g}/\text{m}^3$  to Wistar rats had a bronchial clearance half-life of 1 day and an alveolar clearance half-life of 36 days (Hochrainer *et al.*, 1980). Hochrainer *et al.* (1980) estimated that with continuous exposure to nickel oxide, a steady state would be reached after 1 year.

In Wistar rats after exposure to 0.6 or 8.0 mg nickel oxide/ $\text{m}^3$  (high temperature, green oxide; MMAD of  $1.2 \mu\text{m}$ ,  $\sigma_g$  of 2.5) for 6 to 7 hours per day for 1 to 2 months, the lung clearance was estimated to be 100  $\mu\text{g}$  per year. There was no apparent deposition of nickel in the liver, kidney, spleen, heart, brain, or blood (Kodama *et al.*, 1985). Lung clearance half-lives for nickel oxide (high temperature, green oxide) in Wistar rats exposed for 1 month were estimated to be 8, 11, and 21 months for nickel oxide with a MMAD of 0.6, 1.2, and  $4.0 \mu\text{m}$ , respectively (Tanaka *et al.*, 1985, 1988).

In summary, in absorption and distribution studies for nickel administered intratracheally or by inhalation exposure, the lung half-life was 1 to 3 days for nickel sulfate, 5 days for nickel subsulfide, and greater than 100 days for nickel oxide. Nickel was detected in extrarespiratory tract tissue after exposure to nickel sulfate or nickel subsulfide, but not after exposure to nickel oxide.

The present studies also report findings on the deposition of nickel sulfate hexahydrate and nickel oxide in the lungs and tissues of rats and mice after 16 days, 13 weeks, and at 7 and 15 months in the 2-year studies. These data show a relatively short half-life in the lung for nickel sulfate hexahydrate, a longer half-life for nickel subsulfide, and the longest half-life for nickel oxide (Benson *et al.*, 1987; Dunnick *et al.*, 1989).

Studies of other routes of nickel exposure in rats, mice, and dogs indicate that 1% to 10% given as nickel sulfate hexahydrate or nickel chloride was absorbed after oral administration, and only a small percentage (<1%) of nickel chloride was absorbed through the skin of guinea pigs within 24 hours (ATSDR, 1992; Nielsen *et al.*, 1993).

## ***Humans***

In the industrial setting, a major route of nickel exposure in humans is by inhalation (Sunderman, 1992); it is estimated that 35% of inhaled nickel is absorbed into the blood from the respiratory tract (Bennet, 1984; Grandjean, 1984; Sunderman and Oskarsson, 1991). There is evidence that, in nickel refinery workers, there are storage depots in the body that retain nickel for long periods of time; nickel was excreted in the urine of workers for periods of up to 6 months after facility closing (Morgan and Rouge, 1983). There were elevated nickel concentrations in specimens of urine, plasma, and nasal mucosa biopsies obtained from retired workers years after cessation of employment, although the specific form of nickel to which these workers were exposed was not identified (Torjussen and Andersen, 1979; Boysen *et al.*, 1984).

Andersen and Svenes (1989) found elevated levels of nickel in the lung of nickel refinery workers, although workers who were diagnosed as having lung cancer had the same concentrations of nickel in the lung at autopsy as those who died of other types of cancer. In the workplace setting, exposure to nickel is monitored by analyzing urine, hair, or fingernails for levels of total nickel.

When nickel sulfate was administered to human volunteers, 27% of the administered dose was absorbed when given in drinking water, while only 0.7% was absorbed when administered in food. The elimination half-life for absorbed nickel averaged 28 hours; 100% of the absorbed nickel was eliminated in either the feces or urine within 4 days (Sunderman, 1989, 1992). In studies in humans, reported absorption of radioactive nickel varied from 55% to 77% of nickel sulfate applied to occluded skin to 3% of nickel chloride applied to occluded skin (ATSDR, 1992).

## **TOXICITY**

Studies of nickel toxicity after experimental or industrial exposure have been summarized in various reviews (NAS, 1975; IARC, 1976, 1984, 1987, 1990; NIOSH, 1977; Brown and Sunderman, 1985;

USEPA, 1986; European Chemical Industry, 1989; WHO, 1991; ATSDR, 1992; Nieboer and Nriagu, 1992). In experimental animals and in humans, the primary toxic response to nickel after inhalation occurred in the respiratory system.

Information on the dissolution half-lives for nickel subsulfide and nickel oxide in water and rat serum have been reported. The calculated dissolution half-lives (based on *in vitro* studies) for nickel subsulfide and nickel oxide in water are greater than 7 or 11 years, respectively. In rat serum, the estimated dissolution half-life is 23 days for nickel subsulfide and greater than 11 years for nickel oxide (Sunderman *et al.*, 1987). While nickel subsulfide and nickel oxide are both relatively insoluble in water, nickel subsulfide is more soluble than nickel oxide in biological fluids. Soluble nickel salts (nickel hydroxide) have been shown to be more soluble in human serum than nickel subsulfide (Kasprzak *et al.*, 1983). The comparative toxicity of nickel sulfate hexahydrate, nickel subsulfide, and nickel oxide parallels the solubility of the compounds in biological fluids.

### *Experimental Animals*

The acute toxicity values for selected nickel compounds are summarized in Table 1. Nickel carbonyl ( $\text{NiC}_4\text{O}_4$ ) is the most acutely toxic form of nickel, but the use or formation of this nickel compound in manufacturing processes is limited (NAS, 1975). Exposure to nickel oxide, nickel sulfate hexahydrate, or nickel subsulfide is more common in the workplace.

In animals, after inhalation exposure to water-soluble and water-insoluble nickel compounds, the primary toxic response is seen in the respiratory system. Changes in a variety of parameters, including dose-related reduction in body weight, reduced leukocyte count, increase in urine alkaline phosphatase and alkaline phosphatase, and increased erythrocyte count, were observed in Wistar rats continuously exposed to nickel oxide at 200, 400, or 800  $\mu\text{g}/\text{m}^3$  for 120 days (except for daily cleaning and feeding periods) (Weischer *et al.*, 1980).

**TABLE 1**  
**Toxicity Values for Nickel Carbonyl, Nickel Oxide, Nickel Sulfate Hexahydrate, Nickel Sulfate,**  
**and Nickel Subsulfide<sup>a</sup>**

Nickel Compound	Species	Route	Toxicity Value <sup>b</sup>
Nickel carbonyl	Rat	Inhalation	35 ppm (LC <sub>50</sub> )
		Subcutaneous	63 mg/kg (LD <sub>50</sub> )
		Intravenous	66 mg/kg (LD <sub>50</sub> )
		Intraperitoneal	39 mg/kg (LD <sub>50</sub> )
	Mouse	Inhalation	67 mg/m <sup>3</sup> (LC <sub>50</sub> )
Nickel oxide	Dog	Inhalation	360 ppm (LCLo)
	Cat	Inhalation	1,890 mg/m <sup>3</sup> (LC <sub>50</sub> )
	Rabbit	Inhalation	73 g/m <sup>3</sup> (LCLo)
	Rat	Subcutaneous	25 mg/kg (LD <sub>50</sub> )
Intramuscular		180 mg/kg (TDLo)	
Nickel sulfate hexahydrate	Mouse	Intratracheal	90 mg/kg (TDLo)
		Subcutaneous	50 mg/kg (LD <sub>50</sub> )
Nickel sulfate	Dog	Intraperitoneal	400 mg/kg (TDLo)
		Subcutaneous	500 mg/kg (LDLo)
	Cat	Intravenous	89 mg/kg (LDLo)
		Subcutaneous	500 mg/kg (LDLo)
	Rabbit	Intravenous	72 mg/kg (LDLo)
		Subcutaneous	500 mg/kg (LLLo)
	Guinea pig	Intravenous	36 mg/kg (LDLo)
		Subcutaneous	62 mg/kg (LDLo)
Nickel sulfate	Rat	Intraperitoneal	500 mg/kg (LD <sub>50</sub> )
	Mouse	Intraperitoneal	21 mg/kg (LD <sub>50</sub> )
		Intravenous	7 mg/kg (LDLo)
	Dog	Subcutaneous	38 mg/kg (LDLo)
		Intravenous	38 mg/kg (LDLo)
Cat	Subcutaneous	24 mg/kg (LDLo)	
Rabbit	Subcutaneous	33 mg/kg (LDLo)	
	Intravenous	33 mg/kg (LDLo)	

(continued)

**TABLE 1**  
**Toxicity Values for Nickel Carbonyl, Nickel Oxide, Nickel Sulfate Hexahydrate, Nickel Sulfate, and Nickel Subsulfide (continued)**

Nickel Compound	Species	Route	Toxicity Value
Nickel subsulfide	Rat	Inhalation	1 mg/kg (TCLo)
		Subcutaneous	125 mg/kg (TDLo)
		Intravenous	10 mg/kg (TDLo)
		Intramuscular	20 mg/kg (TDLo)
	Mouse	Intramuscular	200 mg/kg (TDLo)

<sup>a</sup> From RTECS (1987)

<sup>b</sup> LC<sub>50</sub> = median lethal concentration; LCLo = lowest lethal concentration; LD<sub>50</sub> = median lethal dose; LDLo = lowest lethal dose; TCLo = lowest toxic concentration; TDLo = lowest toxic dose.

Alveolar macrophages from lung lavage fluid from rats exposed to nickel oxide at 120  $\mu\text{g}/\text{m}^3$  for 12 hours per day, 6 days per week for 28 days or by intratracheal injection (10 mg nickel oxide/ml and killed 1 week later) were examined by electron microscopy. Compared to controls, alveolar macrophages from exposed animals were increased in number and enlarged. In the cytoplasm of alveolar macrophages, phagosomes contained osmophilic nickel oxide particles as well as membranous and lamellar structures consistent with accumulation of phospholipid material (Migally *et al.*, 1982; Murthy and Niklowitz, 1983).

Respiratory toxicity to F344/Crl rats administered a single dose of either nickel subsulfide, nickel chloride, nickel sulfate, or nickel oxide by intratracheal instillation was evaluated by examining treatment-related changes in lung lavage fluid (Benson *et al.*, 1986). No significant changes in lung lavage fluid were seen after exposure to nickel oxide. After exposure to nickel subsulfide, nickel sulfate hexahydrate, and nickel chloride, there were increases in the following parameters in lung lavage fluid: lactate dehydrogenase,  $\beta$ -glucuronidase, total protein, glutathione reductase, glutathione peroxidase, and sialic acid. This evaluation was continued by exposing rats or mice to nickel oxide, nickel sulfate hexahydrate, or nickel subsulfide for 13 weeks and looking for treatment-related markers of lung toxicity in lung lavage fluid (Benson *et al.*, 1989; Appendix O). Increases in  $\beta$ -glucuronidase, total protein, neutrophil number,

and macrophage number were observed in the lavage fluid after exposure of rats and mice to all three nickel compounds, although there were quantitative differences in the magnitude of the response. Inflammation was observed histologically in the lung of rats and mice exposed to the three nickel compounds. The severity of lung toxicity as measured by the changes in lung lavage fluid paralleled the severity of histologic changes in the lung. Nickel sulfate hexahydrate was the most toxic, and nickel oxide was the least toxic (Benson *et al.*, 1989).

Treatment of rats and mice with water-soluble and water-insoluble nickel salts may cause an alteration of local and systemic immunity, and this toxicity has been studied under various conditions and experiments (Table 2).

Toxic responses to the immune system were measured in B6C3F<sub>1</sub> mice after inhalation exposure to nickel subsulfide, nickel oxide, or nickel sulfate hexahydrate for 6 hours per day and 5 days per week for 13 weeks. Exposure concentrations were 0.11, 0.45, and 1.8 mg nickel/m<sup>3</sup> for nickel subsulfide; 0.47, 2.0, and 7.9 mg nickel/m<sup>3</sup> for nickel oxide; and 0.027, 0.11, and 0.45 mg nickel/m<sup>3</sup> for nickel sulfate hexahydrate. Thymic weights in mice exposed to 1.8 mg nickel/m<sup>3</sup> of nickel subsulfide were lower than those of the controls. Lung-associated lymph nodes were increased in size after exposure to all compounds. The number of alveolar macrophages in lavage samples was increased in mice exposed to the highest concentrations of nickel sulfate hexahydrate and nickel oxide and to 0.45 and 1.8 mg nickel/m<sup>3</sup> nickel subsulfide. Numbers of antibody-forming cells in lung-associated lymph nodes of mice exposed to 2.0 and 7.9 mg nickel/m<sup>3</sup> nickel oxide and 1.8 mg nickel/m<sup>3</sup> nickel subsulfide were greater than those in the controls. Low numbers of antibody-forming cells were observed in the spleens of mice exposed to nickel oxide and in mice exposed to 1.8 ng nickel/m<sup>3</sup> nickel subsulfide. Only mice exposed to 1.8 mg nickel/m<sup>3</sup> nickel subsulfide had a low mixed lymphocyte response. All concentrations of nickel oxide resulted in low levels of alveolar macrophage phagocytic activity, as did 0.45 and 1.8 mg nickel/m<sup>3</sup> nickel subsulfide. None of the nickel compounds affected the phagocytic activity of peritoneal macrophages.

**TABLE 2**  
**Studies on the Immunologic Effects of Nickel Compounds**

Nickel Compound	Species/Route	Treatment	Response	Reference
<b>Cell-Mediated Immunity</b>				
Nickel chloride	CBA/J mice/ intramuscular	Single injection, 18 mg/kg	Reduced T-lymphocyte proliferation	Smialowicz <i>et al.</i> (1984)
	Guinea pig	<i>In vitro</i> study on spleen cells	Inhibited macrophage migration	Hennighausen and Lange (1980)
Nickel sulfate	B6C3F <sub>1</sub> mice (female)/oral	Up to 4,000 mg/kg/day for 23 weeks	Depressed spleen lymphoproliferative response to LPS (no effect on NK activity; PFC assay; mitogen response in spleen cells; resistance to <i>Listeria</i> challenge)	Dieter <i>et al.</i> (1988)
<b>Humoral Immunity</b>				
Nickel chloride	CBA/J mice/ intramuscular	Single injection, 18 mg/kg	Reduced antibody response to T-cell dependent sheep red blood cells	Smialowicz <i>et al.</i> (1984)
	C57BL/6J mouse spleen cells	<i>In vitro</i> exposure to nickel chloride	Decreased response	Lawrence (1981)
	Swiss albino mice/ intramuscular	3-12 $\mu$ g Ni/kg body weight followed by immunization with sheep red blood cells	Depressed antibody formation	Graham <i>et al.</i> (1975a)
	Swiss mice/ inhalation	2-hour inhalation exposure at 250 $\mu$ g/m <sup>3</sup>	Depressed antibody response to sheep red blood cells	Graham <i>et al.</i> (1978)
Nickel acetate	Sprague-Dawley rats/intraperitoneal	11 mg/kg body weight immunized with <i>E. coli</i> bacteriophage	Depressed circulating antibody response	Figoni and Treagan (1975)
Nickel oxide	Wistar rats/ inhalation	25-800 $\mu$ g/m <sup>3</sup> for 4 weeks to 4 months	Decreased ability to form spleen antibodies to sheep red blood cells	Spiegelberg <i>et al.</i> (1984)
<b>Macrophage Function</b>				
Nickel chloride	CBA/J mice/ intramuscular	Single injection, 18 mg/kg	No effect on phagocytic capacity of peritoneal macrophages	Smialowicz <i>et al.</i> (1984)
	Rabbits	Alveolar macrophage <i>in vitro</i> exposure	Reduced viability of macrophages	Graham <i>et al.</i> (1975b)
Nickel oxide and nickel chloride	Wistar rats/ inhalation	12 hours/day, 6 days/week for 2 weeks at 0.1 mg/m <sup>3</sup>	Increased number of alveolar macrophages after nickel oxide; no change after nickel chloride	Bingham <i>et al.</i> (1972)
Nickel oxide	Wistar rats/ inhalation	800 $\mu$ g/m <sup>3</sup> for 2 weeks	Decrease in alveolar macrophage phagocytic ability	Spiegelberg <i>et al.</i> (1984)

(continued)

**TABLE 2**  
**Studies on the Immunologic Effects of Nickel Compounds (continued)**

Nickel Compound	Species/Route	Treatment	Response	Reference
<b>Natural Killer Cell Activity</b>				
Nickel chloride	CBA/J and C57BL/6J mice/ intramuscular	Single injection, 18 mg/kg	Depressed NK activity (against Yac-1 murine lymphoma cells)	Smialowicz <i>et al.</i> (1984, 1985, 1986)
<b>Host Resistance</b>				
Nickel chloride and nickel oxide	CD mice and Sprague-Dawley rats/ inhalation	0.5 mg/m <sup>3</sup> for 2 hours	Enhanced respiratory infection to <i>Streptococcus</i>	Adkins <i>et al.</i> (1979)

Only 1.8 mg nickel/m<sup>3</sup> nickel subsulfide caused a depressed spleen natural killer cell activity. Results indicate that inhalation exposure of mice to nickel can have varying effects on the immune system, depending on dose and physicochemical form of the nickel compound, and these effects were observed at occupationally relevant exposure concentrations (Haley *et al.*, 1990; Appendix N).

Administration of nickel sulfate in the drinking water for 180 days (1 to 10 g/L drinking water, estimated to deliver 116 to 396 mg/kg body weight) resulted in a depressed proliferating response in the bone marrow and spleen of B6C3F<sub>1</sub> mice (Dieter *et al.*, 1988).

While experimental studies in animals show the potential of nickel to affect the immune system, the clinical significance of these studies in humans has not been determined (Nicklin and Nielsen, 1992). Further, there are no studies to examine if there is a relationship between effects on the immune system and the carcinogenic effects of nickel.

### *Humans*

Most of the toxicity information on nickel and nickel compounds came from studies of workers in nickel refineries where the primary toxicity is to the respiratory system. In the industrial setting, nickel exposures were associated with rhinitis, sinusitis, and nasal-septal perforations. Hypersensitive allergic asthmatic reactions to nickel are rare (Nemery, 1990). There were also reports of pulmonary fibrosis in workers inhaling nickel dust (WHO, 1991). While respiratory toxicity has been observed in workers exposed to nickel in the industrial setting, these workers are often exposed to other toxic metals and/or cigarette smoke, and it has not always been possible to conclude that nickel is the sole causative agent of toxicity (ATSDR, 1992).

Nickel contact hypersensitivity has been seen in the general population and in exposed workers. In the general population contact sensitivity to nickel-containing jewelry and/or prosthesis is another form of nickel toxicity (ATSDR, 1992). Other toxic reactions to nickel were reported in humans in isolated cases including: cardiovascular effects in a child ingesting nickel sulfate; and gastrointestinal effects, transient increases in blood reticulocytes, or muscular pain in workers exposed to nickel-contaminated water (ATSDR, 1992).

## CARCINOGENICITY

### *Experimental Animals*

The International Agency for Cancer Research (IARC, 1990) summarized the results of experimental studies which studied the carcinogenic potential of nickel compounds after local injection (e.g., subcutaneous or intramuscular injection). Nickel oxide, nickel subsulfide, nickel carbonyl, and nickel powder cause neoplasms at the injection site, while the soluble nickel salts such as nickel sulfate have generally not been associated with a carcinogenic response at the injection site. A portion of the IARC

(1990) listing and tabulation of over 100 experiments on the carcinogenic potential of nickel compounds is presented in Table 3.

Information for the carcinogenic potential of nickel oxide, nickel subsulfide, and nickel sulfate hexahydrate by inhalation exposure is limited. Ottolenghi *et al.* (1975) reported that nickel subsulfide (70% of particles were smaller than 1  $\mu\text{m}$  in diameter; 25% of particles were between 1 and 1.5  $\mu\text{m}$ ) caused an increased incidence in lung tumors in F344/N rats exposed to 1  $\text{mg}/\text{m}^3$  by inhalation (6 hours/day and 5 days/week for 108 weeks). In the exposed groups, 12% to 14% of the animals (208 animals examined histologically) had lung tumors compared to less than 0.5% of control animals (215 animals examined histologically). At the end of the 108-week exposure period, fewer than 5% of the animals in exposed groups were alive compared with a survival of 31% in control groups.

Other experimental studies indicated carcinogenic potential of nickel subsulfide for the respiratory tract mucosa. Yarita and Nettesheim (1978) reported that a single nickel subsulfide intratracheal dose of 1 or 3  $\text{mg}/\text{kg}$  caused tumors in heterotrophic tracheal transplants in female F344 rats. These authors noted that toxicity might decrease a carcinogenic response resulting in a misleadingly low carcinoma incidence, based on the finding that the more toxic dose (3  $\text{mg}/\text{kg}$ ) caused only a 1.5% incidence of carcinomas (there was a high incidence of tracheal hyperplastic change) versus a 10% carcinoma incidence in the 1  $\text{mg}/\text{kg}$  group (generally with only a low incidence of toxic lesions).

Hamsters exposed to 53  $\text{mg}$  nickel oxide/ $\text{m}^3$  (median diameter of 0.3  $\mu\text{m}$ ; geometric standard deviation of 2.2) for 2 years did not have an increase in the incidence of lung tumors (Wehner *et al.*, 1975). The hamster may be less sensitive than the rat to the carcinogenic effects of nickel (Furst and Schlauder, 1971).

**TABLE 3**  
**Summary of Studies Used to Evaluate the Carcinogenicity of Nickel Compounds**  
**in Experimental Animals<sup>a</sup>**

Nickel Compound	Species/Route	Lesion Incidence <sup>b</sup>	Reference
<b>Nickel oxides and hydroxides</b>			
Nickel monoxide (green)	Rat/inhalation	0.6 mg/m <sup>3</sup> : 0/6 lung lesion 8 mg/m <sup>3</sup> : 1/8 lung lesion	Horie <i>et al.</i> (1985)
Nickel monoxide	Rat/inhalation	0.06 mg/m <sup>3</sup> : 0/40 lesion 0.2 mg/m <sup>3</sup> : 0/20 lesion	Glaser <i>et al.</i> (1986)
	Rat/intrapeural	Controls: 0/32 local lesions 31/32 local lesions	Skaug <i>et al.</i> (1985)
	Rat/intratracheal	Controls: 0/40 lesions 10 × 5 mg: 10/37 lung lesions 10 × 15 mg: 12/38 lung lesions	Pott <i>et al.</i> (1987)
	Rat/intramuscular	21/32 local lesions	Gilman (1962)
	Rat/intramuscular	2/20 local lesions	Gilman (1966)
	Rat/intramuscular	0/20 local lesions	Sosiński (1975)
	Rat/intramuscular	14/15 local lesions	Sunderman and McCully (1983)
	Rat/intramuscular	0/20 local lesions	Berry <i>et al.</i> (1984)
	Rat/subperiosteal	0/20 local lesions	Berry <i>et al.</i> (1984)
	Rat/intraperitoneal	46/47 local lesions	Pott <i>et al.</i> (1987)
Nickel monoxide (green)	Rat/intraperitoneal	25 mg: 12/34 local lesions 100 mg: 15/36 local lesions	Pott <i>et al.</i> (1989, 1992)
	Rat/intrarenal	0/12 local lesions	Sunderman <i>et al.</i> (1984)
Nickel monoxide	Mouse/intramuscular	33/50 and 23/52 local lesions	Gilman (1962)
	Hamster/inhalation	1/51 osteosarcoma	Wehner <i>et al.</i> (1975, 1979)
	Hamster/intratracheal	Controls: 4/50 lung lesions 1/49 lung lesions	Farrell and Davis (1974)
Nickel hydroxide	Rat/intramuscular	15/20 local lesions	Gilman (1966)
	Rat/intramuscular	Dried gel: 5/19 local lesions Crystalline: 3/20 local lesions Colloidal: 0/13 local lesions	Kasprzak <i>et al.</i> (1983)
	Rat/intramuscular	0/10 local lesions	Judde <i>et al.</i> (1987)
Nickel trioxide	Rat/intramuscular	0/10 local lesions	Judde <i>et al.</i> (1987)
	Rat/intracerebral	3/20 local lesions	Sosiński (1975)

(continued)

**TABLE 3**  
**Summary of Studies Used to Evaluate the Carcinogenicity of Nickel Compounds**  
**in Experimental Animals (continued)**

Nickel Compound	Species/Route	Lesion Incidence	Reference
<b>Nickel sulfides</b>			
Nickel disulfide	Rat/intramuscular	12/14 local lesions	Sunderman (1984)
	Rat/intrarenal	2/10 local lesions	Sunderman <i>et al.</i> (1984)
Nickel sulfide (amorphous)	Rat/intramuscular	5.6 mg: 0/10 local lesions	Sunderman and Maenza (1976)
		22.4 mg: 0/10 local lesions	
$\beta$ -Nickel sulfide	Rat/intramuscular	14/14 local lesions	Sunderman (1984)
Nickel sulfide (amorphous)	Rat/intramuscular	3/25 local lesions	Sunderman (1984)
Nickel sulfide	Rat/intrarenal	0/18 local lesions	Jasmin and Riopelle (1976)
$\beta$ -Nickel sulfide	Rat/intrarenal	8/14 local lesions	Sunderman <i>et al.</i> (1984)
Nickel sulfide (amorphous)	Rat/intrarenal	0/15 local lesions	Sunderman <i>et al.</i> (1984)
Nickel subsulfide	Rat/inhalation	14/208 malignant lung lesions 15/208 benign lung lesions	Ottolenghi <i>et al.</i> (1975)
	Rat/intratracheal	0.94 mg: 7/47 lung lesions	Pott <i>et al.</i> (1987)
		1.88 mg: 13/45 lung lesions	
		3.75 mg: 12/40 lung lesions	
	Rat/intrapleural	28/32 local lesions	Skaug <i>et al.</i> (1985)
	Rat/subcutaneous	3.3 mg: 37/39 local lesions	Mason (1972)
		10 mg: 37/40 local lesions	
	Rat/subcutaneous	18/19 local lesions	Shibata <i>et al.</i> (1989)
Rat/intramuscular	25/28 local lesions	Gilman (1962)	
Rat/intramuscular	Controls: 1/19 local lesion 10 mg powder: 19/20 local lesions 10 mg diffusion chamber: 14/17 local lesions 500 mg fragments: 5/7 local lesions 500 mg discs: 14/17 local lesions	Gilman and Herchen (1963)	

(continued)

**TABLE 3**  
**Summary of Studies Used to Evaluate the Carcinogenicity of Nickel Compounds**  
**in Experimental Animals (continued)**

Nickel Compound	Species/Route	Lesion Incidence	Reference
Nickel sulfides (continued)			
Nickel subsulfide (disc)	Rat/intramuscular	Removal of disc after 64 days: 4/10 local lesions Removal of disc after 128 days: 7/10 local lesions Removal of disc after 206 days: 10/10 local lesions	Herchen and Gilman (1964)
Nickel subsulfide	Rat/intramuscular	NIH black: 28/28 local lesions Hooded: 14/23 local lesions	Daniel (1966)
	Rat/intramuscular	3.3 mg: 38/39 local lesions 10 mg: 34/40 local lesions	Mason (1972)
	Rat/intramuscular	5 mg: 8/20 local lesions 20 mg: 9/9 local lesions	Sunderman and Maenza (1976)
	Rat/intramuscular	Fischer: 59/63 local lesions Hooded: 11/20 local lesions	Yamashiro <i>et al.</i> (1980)
	Rat/intramuscular	0.6 mg: 7/30 local lesions 1.2 mg: 23/30 local lesions 2.5 mg: 28/30 local lesions 5 mg: 29/30 local lesions	Sunderman <i>et al.</i> (1976)
	Rat/intramuscular	0.63 mg: 7/29 local lesions 20 mg: 9/9 local lesions	Sunderman (1981)
$\alpha$ -Nickel subsulfide	Rat/intramuscular	9/9 local lesions	Sunderman (1984)
Nickel subsulfide	Rat/intramuscular	10/20 local lesions	Berry <i>et al.</i> (1984)
	Rat/intramuscular	2/100 local lesions	Judde <i>et al.</i> (1987)
	Rat/intramuscular	19/20 local lesions	Shibata <i>et al.</i> (1989)
	Rat/intraperitoneal	9/37 local lesions	Gilman (1966)
	Rat/intraperitoneal	27/42 local lesions	Pott <i>et al.</i> (1987)
	Rat/intraperitoneal	6 mg: 20/36 local lesions 12 mg: 25/35 local lesions 25 mg: 25/34 local lesions	Pott <i>et al.</i> (1989, 1992)
	Rat/subperiosteal	0/20 local lesions	Berry <i>et al.</i> (1984)
	Rat/intrafemoral	10/20 local lesions	Berry <i>et al.</i> (1984)
	Rat/intrarenal	In glycerin: 7/16 local lesions In saline: 11/24 local lesions	Jasmin and Raopelle (1976)

(continued)

**TABLE 3**  
**Summary of Studies Used to Evaluate the Carcinogenicity of Nickel Compounds**  
**in Experimental Animals (continued)**

Nickel Compound	Species/Route	Lesion Incidence	Reference
Nickel sulfides (continued)			
$\alpha$ -Nickel subsulfide	Rat/intrarenal	Wistar Lewis: 7/11 local lesions NIH black: 6/12 local lesions Fischer 344: 9/32 local lesions Long-Evans: 0/12 local lesions	Sunderman <i>et al.</i> (1979)
Nickel subsulfide	Rat/intratesticular	16/19 local lesions	Damjanov <i>et al.</i> (1978)
	Rat/intraocular	14/15 local lesions	Albert <i>et al.</i> (1980); Sunderman (1983a)
	Rat/transplacental	No difference in lesion incidence	Sunderman <i>et al.</i> (1981)
	Rat/pellet implantation into subcutaneous implanted tracheal grafts	5 mg: 9/60 local lesions 15 mg: 45/64 local lesions	Yarita and Nettesheim (1978)
	Rat/intra-articular	16/19 local lesions	Shibata <i>et al.</i> (1989)
	Rat/intra-fat	9/20 local lesions	Shibata <i>et al.</i> (1989)
	Mouse/intratracheal	No increase in lung lesion incidence	Fisher <i>et al.</i> (1986)
	Mouse/subcutaneous	5 mg: 4/3 local lesions 10 mg: 7/8 local lesions	Oskarsson <i>et al.</i> (1979)
	Mouse/intramuscular	Swiss: 27/45 local lesions C3H: 9/18 local lesions	Gilman (1962)
	Mouse/intramuscular	5 mg: 4/8 local lesions 10 mg: 4/8 local lesions	Oskarsson <i>et al.</i> (1979)
$\alpha$ -Nickel subsulfide	Hamster/intratracheal	0/62 lung lesions	Muhle <i>et al.</i> (1992)
Nickel subsulfide	Hamster/intramuscular	Controls: 0/14 local lesions 5 mg: 4/15 local lesions 10 mg: 12/17 local lesions	Sunderman (1983b)
$\alpha$ -Nickel subsulfide	Hamster/topical	54 mg total: 0/6 local lesions 108 mg total: 0/7 local lesions 540 mg total: 0/15 local lesions 1080 mg total: 0/13 local lesions	Sunderman (1983a)
Nickel subsulfide	Rabbit/intramuscular	16 local lesions	Hildebrand and Bisette (1979a,b)
(continued)			

**TABLE 3**  
**Summary of Studies Used to Evaluate the Carcinogenicity of Nickel Compounds**  
**in Experimental Animals (continued)**

Nickel Compound	Species/Route	Lesion Incidence	Reference
Nickel sulfides (continued)			
$\alpha$ -Nickel subsulfide	Rabbit/intramuscular	0/4 local lesions	Sunderman (1983b)
Nickel subsulfide	Salamander/intraocular	7/8 local lesions	Okamoto (1987)
Nickel ferrosulfide	Rat/intramuscular	15/15 local lesions	Sunderman (1984)
	Rat/intrarenal	1/12 local lesions	Sunderman <i>et al.</i> (1984)
Nickel salts			
Basic nickel carbonate tetrahydrate	Rat/intraperitoneal	Controls: 1/33 lung lesions	Pott <i>et al.</i> (1989, 1992)
		25 mg: 1/35 lung lesions 50 mg: 3/33 lung lesions	
Nickel acetate	Mouse/intraperitoneal	72 mg: 8/18 lung lesions 180 mg: 7/14 lung lesions 360 mg: 12/19 lung lesions	Stoner <i>et al.</i> (1976)
	Rat/intramuscular	1/35 local lesions	Payne (1964)
Nickel acetate tetrahydrate	Mouse/intraperitoneal	Controls: 0.32 lung lesions/animal 1.5 lung lesions/animal	Poirier <i>et al.</i> (1984)
	Rat/intraperitoneal	Controls: 1/33 lung lesions 25 mg: 3/35 lung lesions 50 mg: 5/31 lung lesions	Pott <i>et al.</i> (1989, 1992)
Nickel ammonium sulfate	Rat/intramuscular	0/35 local lesions	Payne (1964)
Nickel carbonate	Rat/intramuscular	6/35 local lesions	Payne (1964)
Nickel chloride	Rat/intramuscular	0/35 local lesions	Payne (1964)
Nickel chloride hexahydrate	Rat/intraperitoneal	Controls: 1/33 lung lesions 4/32 lung lesions	Pott <i>et al.</i> (1989, 1992)
Nickel chromate	Rat/intramuscular	1/16 local lesions	Sunderman (1984)
Nickel fluoride	Rat/intramuscular	3/18 local lesions	Gilman (1966)
Nickel sulfate	Rat/intramuscular	1/35 local lesions	Payne (1964)
	Rat/intramuscular	0/20 local lesions	Gilman (1966)
	Rat/intramuscular	0/20 local lesions	Kasprzak <i>et al.</i> (1983)

(continued)

**TABLE 3**  
**Summary of Studies Used to Evaluate the Carcinogenicity of Nickel Compounds**  
**in Experimental Animals (continued)**

Nickel Compound	Species/Route	Lesion Incidence	Reference
<b>Nickel salts (continued)</b>			
Nickel sulfate hexahydrate	Rat/intramuscular	0/32 local lesions	Gilman (1962)
Nickel sulfate heptahydrate	Rat/intraperitoneal	Controls: 1/33 lung lesions 6/30 lung lesions	Pott <i>et al.</i> (1989, 1992)
<b>Other</b>			
Nickel carbonyl	Rat/inhalation	30 mg/m <sup>3</sup> for 32 weeks: 1/64 pulmonary lesions 60 mg/m <sup>3</sup> for 32 weeks: 1/32 pulmonary lesions 250 mg/m <sup>3</sup> once: 1/80 pulmonary lesion	Sunderman <i>et al.</i> (1957, 1959)
	Rat/inhalation	Controls: 0/32 lung lesions 1/71 lung lesions	Sunderman and Donnelly (1965)
	Rat/intravenous	19/120 lung lesions	Lau <i>et al.</i> (1972)

<sup>a</sup> From IARC (1990)

<sup>b</sup> Number of animals with lesion per effective number

Sunderman *et al.* (1959) found a low incidence of lung tumors in groups of Wistar rats exposed to nickel carbonyl (0.03 to 0.25 mg/m<sup>3</sup> for 30 minutes 3 times/week for 1 year). Follow-up studies also showed a low incidence of lung tumors in rats exposed to nickel carbonyl (Sunderman and Donnelly, 1965).

Information on the carcinogenic potential of nickel after oral administration is limited (IARC, 1990).

Life-long exposure to nickel acetate at low concentrations (5 ppm) induced no lung lesions in Swiss mice (Schroeder *et al.*, 1964; Schroeder and Mitchener, 1975); the maximum tolerated dose was not reached.

Ambrose *et al.* (1976) administered nickel sulfate hexahydrate in the diet of Wistar rats or dogs (0, 100, 1,000, 2,500 ppm) for 2 years, and no treatment-related lesions were observed.

### *Humans*

Exposure to nickel in the workplace has been associated with an increase in lung and nasal sinus tumors (IARC, 1976, 1987; Doll, 1990). Based on the finding of lung and/or nasal sinus tumors in nickel refinery workers, IARC classified nickel and nickel compounds as human carcinogens (Group 1), although there was insufficient information available to evaluate the carcinogenic risk for individual nickel compounds or the risk for cancer based on exposure to different concentrations of nickel compound(s) (IARC, 1987).

Information on the hazards associated with exposure to nickel came from studies on occupational exposure in nickel refineries and mines in Clydach, South Wales; Kristiansand, Norway; the International Nickel Company (INCO) refineries in Ontario, Canada; or from other studies of nickel refineries or mining operations throughout the world (Doll, 1984).

The United States Environmental Protection Agency (USEPA, 1986) and the International Committee on Nickel Carcinogenesis in Man (Doll, 1990) reviewed the epidemiological evidence for cancer after exposure to nickel in mining or refinery operations. A complete analysis on the type of ore mined and the calcining, smelting, and refining operations in 10 different mines or refineries throughout the world can be found in Doll (1990) and in other more recent summaries (Courtin, 1994; McIlveen and Negusante, 1994; Nieboer and Templeton, 1994; Norseth, 1994). Doll (1990) also estimates the type of nickel exposures encountered based on knowledge of the nickel process procedures used and a few relatively recent measurements of total airborne nickel.

The first indication that some form of nickel can give rise to lung and nasal sinus cancers was obtained from refinery workers at Clydach, South Wales (Bridge, 1933; Doll, 1958; Morgan, 1958). The Clydach Nickel Refinery (Mond Nickel Works) opened in 1902 and received nickel sulfide matte primarily from INCO (Port Colborne refinery, Canada). In 1933, nasal sinus and lung tumors were first noted in workers

who were employed prior to 1925. After 1925, the copper and sulfate content of the matte was reduced, the arsenic contamination in sulfuric acid used to extract copper was reduced, the use of respirators and masks was introduced, and improvements were made in factory design that reduced exposure to nickel (USEPA, 1986). An increased risk for lung and nasal sinus tumors was particularly noted in refinery work involving roasting, sintering, and calcining processes that converted impure nickel-copper matte to an oxide (Doll, 1990).

Peto *et al.* (1984) analyzed the incidence of lung and nasal sinus cancers found in workers in the Clydach plant and found the highest incidence of cancer in those workers employed in the copper sulfate and furnace areas. There was no increased risk to workers in the reduction area where nickel carbonyl concentrations were highest.

Other evidence for nasal sinus and lung cancer come from studies of workers in the INCO (Ontario, Canada) mines and refineries (Roberts *et al.*, 1989a,b; Muir *et al.*, 1994). Facilities operated include the Sudbury area mines (Copper Cliff Smelter and the Port Colborne refinery) that use an ore that is primarily pentlandite ( $\text{NiFeS}_2$ ). Men working in mining operations in Ontario had a two-fold increase in lung cancer risk, but no nasal sinus cancers (Doll, 1990).

The Falconbridge refinery in Kristiansand, Norway, receives nickel ore (a nickel copper sulfide matte) from Canada and uses an electrolysis process to refine the ore. Workers in roasting and smelting operations are exposed to dry dust containing nickel subsulfide and nickel oxide. Electrolysis workers are also exposed to nickel sulfate and nickel chloride. In this cohort, nasal sinus and lung cancer risks were increased in men working in the electrolysis department, thus implicating the soluble forms of nickel as the cause for the cancer (USEPA, 1986; Doll, 1990). The electrolysis workers had the highest average plasma and urine nickel concentrations (Høgetveit *et al.*, 1978).

Enterline and Marsh (1982) and Goldberg *et al.* (1994) studied cancer rates in a refinery in Huntington, West Virginia, which received nickel sulfide matte from Canada. The Doll Committee reported no clear evidence for an increased incidence in lung cancer in this population, although the data from this cohort provided weak evidence for an increased incidence in lung cancer in men exposed to sulfidic nickel at 4 mg nickel/m<sup>3</sup> for more than a year (Doll, 1990).

Results of epidemiology studies of workers in the nickel mining, smelting, and refinery operations in New Caledonia (French territory in the South Pacific) showed no increased incidence of lung or upper respiratory tract cancers. Nickel at this site is mined from nickel oxide or the silicate form of the ore. The Doll Committee also reported little evidence for an increased incidence in lung or upper respiratory tract cancer in this group of nickel workers (Doll, 1990).

The ten cohorts of nickel workers studied by the Doll Committee include the six cohorts mentioned above (nickel refinery operations, Clydach, South Wales; Falconbridge Nickel Mines, Ontario, Canada; INCO mines and refineries [Copper Cliff and Port Colborne], Ontario, Canada; Falconbridge refinery, Kristiansand, Norway; Huntington Alloys, West Virginia; and New Caledonia mines) as well as the Hanna Nickel Smelting Co., Oregon; Oak Ridge Gaseous Diffusion Plant, Tennessee; Outokumpu Oy nickel refinery, Finland; and Henry Wiggin Alloy Co., England (Doll, 1990).

The results within the individual cohorts varied, but the overall conclusion by the Doll Committee suggested that more than one form of nickel gives rise to lung and nasal sinus cancer. Much of the respiratory cancer risk was attributed to exposure to a mixture of oxidic and sulfidic nickel. Exposure to oxidic nickel in the absence of sulfidic nickel was also associated with increased lung and nasal sinus cancer risks. There was evidence that exposure to soluble nickel salts increased the risk of lung and nasal sinus cancer and that it may enhance risks associated with exposure to less soluble forms of nickel. There was no evidence that metallic nickel was associated with increased lung and nasal sinus cancer risks.

There was no evidence to suggest that exposure to metallic nickel or any of its compounds was likely to produce cancers elsewhere than in the lung or nose. These investigators were not able to provide dose-specific estimates of risks for individual nickel species. However, the evidence from these studies suggests that respiratory cancer risks are primarily related to exposure to water-soluble nickel compounds at concentrations in excess of 1 mg nickel/m<sup>3</sup> and to exposure to less soluble forms at concentrations greater than 10 mg nickel/m<sup>3</sup>.

There are no studies evaluating the potential carcinogenic effect in humans specifically after oral exposure to nickel (ATSDR, 1992).

While nickel and nickel compounds are classified by the IARC as Group 1 (human) carcinogens, the mechanism for this carcinogenic activity is not fully understood (Sunderman, 1989; Costa, 1991; Snow, 1992). The mechanisms involved in the induction of cancer by nickel compounds may be related to the ability of nickel ions to interact with chromatin proteins and/or the ability of nickel to generate intracellular oxidants (Costa *et al.*, 1994). Recent studies suggest that nickel generates free radicals, and the subsequent oxidative reactions lead to DNA damage and cancer. Studies show that: 1) incubation of nickel ions with cysteine under aerobic conditions generated hydroxyl radicals and carbon-centered alkyl radicals, suggesting free radicals are generated by nickel (II)-thiol complexes and molecular oxygen (Shi *et al.*, 1993); 2) in forward mutation assays with bacterial DNA, nickel ions produce tandem double CC → TT mutations consistent with damage to DNA by either ultraviolet irradiation or oxygen-free radicals (Tkeshelashvili *et al.*, 1993); 3) and, in *in vitro* studies, nickel ions induce increases in 8-hydroxy-2'-deoxyguanosine (8-OH-dG), a biomarker of oxidatively damaged DNA (Littlefield *et al.*, 1991).

After subcutaneous or intramuscular injection of nickel compounds, the water-insoluble nickel compounds are the most potent carcinogens. These findings may be related to the fact that water-insoluble nickel compounds are more readily phagocytized than are the water-soluble nickel salts, which passively diffuse

through the cell membrane. Phagocytized nickel particles are internalized in vacuoles whose acidity accelerates the dissolution of nickel ions and results in a higher concentration of nickel than would be achieved by the cellular uptake of water-soluble nickel salts (Costa *et al.*, 1994).

## REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

### *Experimental Animals*

Leonard and Jacquet (1984) reviewed studies which show that water-soluble nickel compounds administered orally or by peritoneal routes have the potential to cause embryotoxicity in rodents. In these studies, the nickel compounds were generally administered at higher doses than humans would be exposed to in drinking water or in the diet.

Studies in rodents have indicated that water-soluble nickel compounds can cross the placenta or be excreted in the milk of lactating animals. When [<sup>63</sup>Ni]-labeled nickel chloride was administered as an oral bolus dose (10  $\mu$ mol or 0.58 mg/kg body weight) to pregnant mice, the label was detected in various fetal tissues including liver, kidney, lung, brain, and heart. In another experiment, when [<sup>63</sup>Ni]-labeled nickel chloride was injected into pregnant mice, nickel was found to cross the placenta, and a marked uptake of nickel was seen in the embryo as measured by whole-body autoradiography (Olsen and Jonsen, 1979). When nickel chloride hexahydrate was given as a single subcutaneous dose (10 to 100  $\mu$ mol NiCl<sub>2</sub> · 6H<sub>2</sub>O/kg body weight or 23 mg/kg) to lactating rats, nickel was excreted in the milk and was found in the plasma of the pups (Dostal *et al.*, 1989). The doses used in these studies are higher than the average concentration of nickel found in drinking water in the United States (48  $\mu$ g/L water) (NAS, 1975).

Nickel chloride administered in the drinking water (50 and 250 ppm, estimated to deliver 7 or 31 mg/kg of nickel compound) to female rats for 11 weeks prior to mating and then during two successive gestation and lactation periods, caused an increase in the proportion of dead pups per litter (Smith *et al.*, 1993).

Other studies in rodents administered nickel chloride by intramuscular or intraperitoneal injection during gestation also showed developmental toxicity or fetal death. Nickel chloride injected intraperitoneally (1, 2, or 4 mg/kg body weight) to pregnant Wistar Porton rats on day 8, 12, or 16 of pregnancy caused skeletal retardation (poor ossification), hydrocephalus, hydronephrosis, heart defects, and hemorrhage. At these doses, there was an increase in maternal plasma glucose concentration (Mas *et al.*, 1985).

Nickel chloride injected intramuscularly (16 mg/kg) on day 8 of gestation to Fischer rats reduced the mean number of live pups per dam and diminished fetal body weights on day 20 (Sunderman *et al.*, 1978).

Nickel chloride injected into chicken eggs at doses of 0.02 to 0.8 mg per egg on days 0, 1, 2, 3, and 4 after fertilization caused malformations in the embryo examined at day 8 including exencephaly, everted viscera, abnormalities in the limb development, microphthalmia, and reduced body size (Gilani and Marano, 1980).

Groups of pregnant hamsters were exposed to nickel carbonyl by inhalation (0.06 mg/L/15 minutes) on days 4, 5, 6, 7, or 8 of gestation; dams were evaluated on day 15 of gestation. Teratogenic effects observed included cystic lung, exencephaly, cleft palate, and fused ribs. In another series of experiments, dams were allowed to deliver the pups; neonatal mortality was increased in the exposed groups (Sunderman *et al.*, 1980). Nickel carbonyl administered to pregnant dams by intravenous injection (11 mg/kg) on day 7 of gestation caused an increase in fetal mortality, diminished body weight of live pups, and increased incidences of fetal abnormalities including anophthalmia, microphthalmia, cystic lungs, and hydronephrosis (Sunderman *et al.*, 1983).

In a study of nickel oxide, Wistar rats were exposed to 1.6 mg nickel/m<sup>3</sup> by inhalation on gestation days 1 through 20. There was no evidence for embryotoxicity (Weischer *et al.*, 1980).

These and other studies show that water-soluble nickel salts have the potential to cause embryotoxicity in rodents. The metal can cross the fetomaternal barrier and enter the fetus. The embryotoxicity of nickel may be related to several factors including the mutagenic properties of nickel, direct effects on the mammalian embryo, or indirect effects through maternal toxicity. Further work is needed to understand the mechanisms for these effects (Leonard and Jacquet, 1984).

### *Humans*

Until recently, there have been few studies of reproductive effects in humans after exposure to nickel (ATSDR, 1992). A study of nickel refinery workers in Norway who were exposed to water-soluble nickel salts in electrolysis departments notes a suggested increased risk of pregnancy complications in female workers. The authors point out that the results of their studies should be considered preliminary data, and further investigations are needed (Chashschin *et al.*, 1994).

## GENETIC TOXICITY

Recent detailed reviews of the mutagenicity of nickel compounds and the possible mechanisms involved in the production of these effects were presented by Coogan *et al.* (1989), Christie and Katisifis (1990), Costa (1991), Snow (1992), and Costa *et al.* (1994). Nickel compounds are not typically detected as bacterial mutagens, but they often give positive results in *in vitro* assays designed to identify compounds that induce chromosomal damage in mammalian cells in the form of sister chromatid exchanges, chromosomal aberrations, and DNA strand breaks. Nickel salts have been shown to inhibit DNA replication and to increase replication errors in mammalian cells *in vitro*, possibly by competing with magnesium for essential binding sites on DNA polymerases (Christie *et al.*, 1991). In addition, positive results were demonstrated in mammalian cell forward mutation assays (TK locus in mouse lymphoma cells and hypoxanthine phosphoribosyl transferase locus in hamster V79 cells), although these responses are usually weak (Nishimura and Umeda, 1979; Amacher and Paillet, 1980; Morita *et al.*, 1991; Lee *et al.*,

1993). Insoluble crystalline nickel compounds are more active in genetic toxicity assays than the soluble or amorphous forms of nickel. Presumably, this differential activity derives from the more efficient entry of insoluble nicks into the cell through phagocytosis (Costa, 1991), longer retention of these compounds within the cell, and the consequent higher intracellular concentration of nickel (II) ions. Soluble nickel salts cannot be efficiently phagocytized, and do not accumulate in high concentration within the cell.

Based on the results of cell transformation studies in cultured rodent cells, Costa (1983) concluded that the nickel sulfide compounds must be in the crystalline, rather than in the amorphous state to be efficiently phagocytized into the cell and cause genetic damage. Particle size (Costa and Mollenhauer, 1980) and surface charge (Costa *et al.*, 1982) are also important factors in the phagocytosis of nickel compounds. Insoluble nickel compounds, once inside the cell, aggregate near the nucleus (Bryan, 1981; Evans *et al.*, 1982) where they are dissolved by lysosomes, releasing nickel (II) ions that proceed to effect DNA damage (Costa *et al.*, 1994).

The induced DNA damage resulting from nickel exposure has been attributed to one or more of the following mechanisms. It may follow the generation of short-lived reactive oxygen species inside the nucleus, produced by the oxidation of  $\text{Ni}^{+2}$  to  $\text{Ni}^{+3}$  by hydrogen peroxide or other oxidants subsequent to the binding of nickel ions to ligands such as amino acids, glutathione, and amino acid side chains of nuclear proteins (Biggart and Costa, 1986; Inoue and Kawanishi, 1989; Nieboer *et al.*, 1989; Cotelle *et al.*, 1992; Tkeshelashvili *et al.*, 1993; Sugiyama, 1994). The formation of persistent DNA-protein crosslinks is implicated in the generation of nickel (II)-induced DNA damage (Ciccarelli and Weterhahn, 1982; Lee *et al.*, 1982; Patierno and Costa, 1985; Sen and Costa, 1986a). Factors involved in the binding of nickel ions to DNA, nuclear proteins, and other nuclear structures are reviewed by Coogan *et al.* (1989). The binding affinity of nickel to protein is far greater than its binding affinity to purified DNA (Eichorn and Shin, 1968) and therefore the mutagenic activity of nickel (II) ions probably derives in greater part from the binding of nickel to chromosomal protein rather than directly to DNA (Costa, 1991). Nickel binds preferentially to heterochromatic regions of the chromosomes such as the long arm of the

X chromosome in cultured Chinese hamster cells (Sen and Costa, 1986a,b; Sen *et al.*, 1987; Costa, 1991); binding of nickel ions to the long arm of the X chromosome and subsequent deletions in this region were postulated to cause the loss of a gene controlling senescence in cultured Chinese hamster cells and to promote immortality in transformed cultured Chinese hamster cell lines (Klein *et al.*, 1991). A schematic representation of some of the proposed mechanisms of nickel-induced genotoxicity, based upon the current understanding of the activities of nickel ions within mammalian cells, is presented in Figure 1. The genetic toxicity data for each of the three nickel compounds under study by the NTP are described below.

The mutagenicity data for nickel oxide are limited; however, there are clear indications of genotoxicity in some *in vitro* test systems. Although exposure to nickel oxide did not result in growth inhibition due to DNA damage in repair-deficient strains of *Bacillus subtilis* (Kanematsu *et al.*, 1980), an S-phase block (determined by flow cytometric analysis) was induced in cycling Chinese hamster ovary cells incubated with 5 µg/mL nickel oxide (Costa *et al.*, 1982). No increase in gene mutations was detected at the ouabain resistance locus in C3H/10T<sub>1/2</sub> mouse embryo cells (Miura *et al.*, 1989) or at the HPRT locus in hamster V79 cells after exposure to nickel oxide (Kargacin *et al.*, 1993). However, positive effects were reported in mutation assays using a different site, the *gpt* gene, in V79 cells as the target for nickel oxide activity (Kargacin *et al.*, 1993). No induction of chromosomal aberrations was detected in human fibroblast or leukocyte cultures exposed to nickel oxide for 24, 48, or 72 hours (Paton and Allison, 1972); however, the experimental protocol used in this test was designed for water-soluble compounds and may not have been suitable for testing insoluble nickel oxide. Data from human epidemiology studies indicate that exposure to nickel oxide-containing fumes or smelter dusts may induce chromosomal aberrations (Waksvik *et al.*, 1984) and DNA-crosslinks (Costa *et al.*, 1993) in peripheral blood lymphocytes of workers, but the evidence is weak. The link between nickel oxide and these genetic endpoints is confounded because smelter dusts and welding fumes contain other nickel compounds as well as other metals such as chromium and magnesium. Also, the genetic effects noted were not correlated with nickel concentrations in urine or blood, whereas increased DNA-crosslink frequencies noted after exposure to

chromium-containing fumes, for example, were correlated with urine concentrations of the metal (Popp *et al.*, 1992).

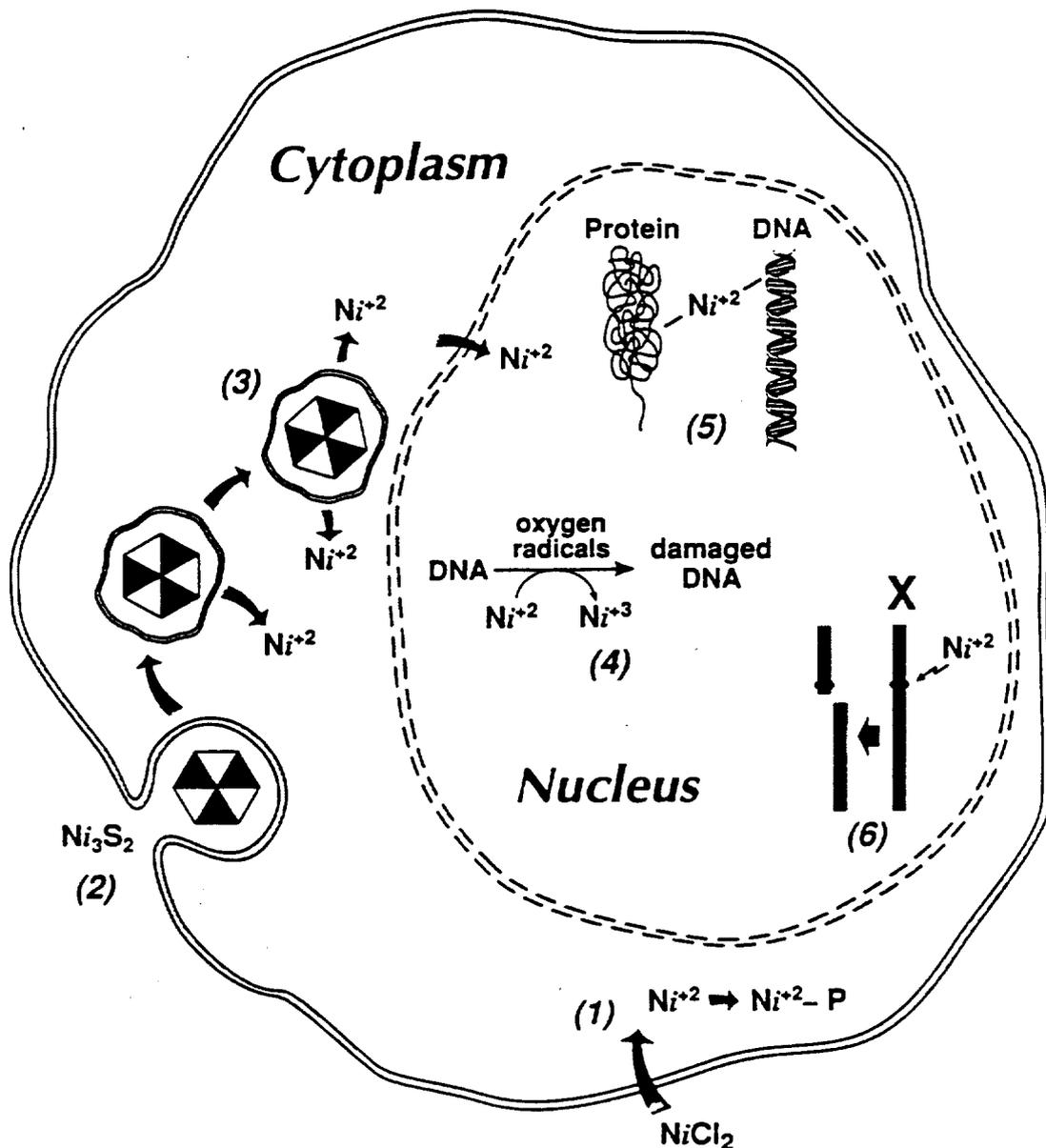
Nickel sulfate hexahydrate did not induce gene mutations in *Escherichia coli* or *Salmonella typhimurium* (Arlauskas *et al.*, 1985), and (in contrast to results reported for nickel oxide) no increases in *gpt* mutants were observed in hamster V79 cells treated with nickel sulfate hexahydrate (Christie, 1989; Lee *et al.*, 1993). However, nickel sulfate hexahydrate did induce mutations in L5178Y mouse lymphoma TK<sup>+</sup> cells, treated with 500 to 1,000  $\mu\text{g}/\text{mL}$  in the absence of S9 metabolic activation enzymes (McGregor *et al.*, 1988). In addition, nickel sulfate hexahydrate, administered by injection at doses of 200, 300, and 400 ppm, induced sex-linked recessive lethal mutations in germ cells of male *Drosophila* (Rodriguez-Arnaiz and Ramos, 1986). The pre- and post-meiotic cell stages were affected; the broods obtained from sperm cells undergoing meiosis at the time of treatment showed no evidence of increased lethal mutations. In another test for germ cell effects in male *Drosophila*, the test for sex chromosome loss, only the highest dose of nickel sulfate hexahydrate (400 ppm) resulted in the production of XO males (Rodriguez-Arnaiz and Ramos, 1986). Induction of sister chromatid exchanges and chromosomal aberrations was observed in hamster cells (Larramendy *et al.*, 1981; Ohno *et al.*, 1982), as well as human peripheral lymphocytes (Larramendy *et al.*, 1981) treated with nickel sulfate hexahydrate *in vitro*. However, no induction of DNA single strand breaks was detected in human xeroderma pigmentosum fibroblasts treated with 250  $\mu\text{g}/\text{mL}$  nickel sulfate hexahydrate (Fornace, 1982). *In vivo*, no induction of chromosomal aberrations was observed in rat bone marrow or spermatogonial cells after injection of nickel sulfate hexahydrate at doses that provided 3 or 6 mg nickel/kg body weight. Also, no change in the mitotic index of bone marrow cells was noted in treated animals (Mathur *et al.*, 1978).

As with the two nickel compounds discussed above, there are limited published mutagenicity data for the third nickel compound in the present studies, nickel subsulfide. However, results of *in vitro* tests performed with this insoluble nickel compound were mainly positive. In the *Salmonella typhimurium* gene

mutation assay, crystalline nickel subsulfide gave equivocal results in one study that used a preincubation protocol (Zeiger *et al.*, 1992) and negative results in a standard plate incorporation assay (Arrouijal *et al.*, 1990). It induced lethal mutations in *Paramecium tetraurelia*, without S9 (Smith-Sonneborn *et al.*, 1986) and unscheduled DNA repair in cultured Syrian hamster embryo cells (Robison *et al.*, 1983). Treatment of cultured Chinese hamster ovary cells for 24 hours with 10  $\mu\text{g}/\text{mL}$  nickel subsulfide resulted in an increase in the number of DNA strand breaks detected by alkaline sucrose gradient techniques (Robison *et al.*, 1982). Nickel subsulfide, in the absence of S9, was a weak inducer of hypoxanthine phosphoribosyl transferase mutations in cultured Chinese hamster ovary cells (Rossetto *et al.*, 1994) and sister chromatid exchanges in cultured human lymphocytes (Saxholm *et al.*, 1981). Nickel subsulfide induced significant dose-related increases in chromosomal aberrations (Arrouijal *et al.*, 1990) and micronuclei (Arrouijal *et al.*, 1992) in human lymphocytes *in vitro*. One reported *in vivo* test with nickel subsulfide, a measure of DNA synthesis inhibition in rats administered 10  $\mu\text{g}/\text{rat}$  (6 mg/100 g body weight) by intrarenal injection, was negative (Hui and Sunderman, 1980). A second *in vivo* study, a mouse bone marrow micronucleus test, reportedly produced positive results (Arrouijal *et al.*, 1990). This second study, however, employed only a single dose (250 mg/kg nickel subsulfide administered by intraperitoneal injection), and no confirmatory study was conducted.

## STUDY RATIONALE

The National Cancer Institute nominated nickel compounds for study because there was little information on the toxic and carcinogenic properties of specific nickel compounds after inhalation exposure. Nickel oxide and nickel sulfate hexahydrate (NTP, 1994a) were selected as compounds that are commonly found in the workplace in the United States. Nickel subsulfide (NTP, 1994b) was selected for study based on a previous study in which lung tumors were observed in rats (Ottolenghi *et al.*, 1975). The NTP toxicity and carcinogenicity studies of nickel oxide, nickel subsulfide, and nickel sulfate hexahydrate were performed to provide comparative toxicology and carcinogenicity information on these nickel compounds. The results of the nickel oxide studies are presented in this technical report.



**FIGURE 1**  
Possible Mechanisms of Nickel-Induced Genotoxicity

1. Soluble nickel compounds such as nickel chloride diffuse into the cell;  $\text{Ni}^{+2}$  ions are rapidly bound to cytoplasmic proteins (P) (Lee *et al.*, 1993). 2. Insoluble nickel compounds such as nickel subsulfide are phagocytosed into the cell and move toward the nucleus (Costa *et al.*, 1982). 3. Lysosomal breakdown of insoluble nickel compounds releases large quantities of  $\text{Ni}^{+2}$  ions which concentrate adjacent to the nuclear membrane (Costa and Heck, 1983). 4. Oxidative damage is induced in DNA by nickel ions bound to nuclear proteins ( $\text{Ni}^{+2} \rightarrow \text{Ni}^{+3}$ ), releasing active oxygen species (Tkeshelashvili *et al.*, 1993; Sugiyama, 1994). 5. DNA-protein crosslinks are produced by  $\text{Ni}^{+2}$  ions binding to heterochromatin (Lee *et al.*, 1982; Patierno and Costa, 1985; Sen and Costa, 1986a). 6. Binding of nickel ions to the heterochromatic regions of the long arm of the X chromosome, which may contain a senescence gene and a tumor suppressor gene, can cause deletion of all or part of this region, leading to an immortalization of the cell and clonal expansion (Conway and Costa, 1989; Klein *et al.*, 1991).

## DISCUSSION AND CONCLUSIONS

Occupational exposure to nickel oxide may occur during refining and processing operations, in high temperature operations such as production of stainless steel, and in mining of laterite ore (Doll, 1990). No previous 2-year studies have been reported that establish toxic dose-response relationships or toxic effects in organs other than the lung after inhalation exposure to nickel oxide in rats or mice.

In the 16-day (1.2 to 30 mg nickel oxide/m<sup>3</sup>, equivalent to 0.9 to 23.6 mg nickel/m<sup>3</sup>) and 13-week studies (0.6 to 10 mg nickel oxide/m<sup>3</sup>, equivalent to 0.4 to 7.9 mg nickel/m<sup>3</sup>), there were no chemical-related deaths in rats or mice. There were no biologically significant chemical-related effects on sperm morphology or vaginal cytology in rats or mice. The major toxic effects were in the lung, as measured by an exposure-related increase in lung weights and the occurrence of pigment and inflammation. In both the 16-day and 13-week studies, pigment and lymphoid hyperplasia occurred in the respiratory tract lymph nodes.

Analysis of bronchoalveolar lavage fluid has been used in human medicine for diagnosing the type or stage of various forms of interstitial lung disease and as a rapid *in vivo* method of evaluation of lung injury in toxicologic studies (Henderson *et al.*, 1985). In these 13-week studies of nickel oxide, evaluation of bronchoalveolar lavage fluid indicated the presence of an inflammatory response in the lung of rats exposed to 2.5 and 10 mg/m<sup>3</sup> and mice exposed to 10 mg/m<sup>3</sup> (Benson *et al.*, 1989; Appendix O). This increase was mostly due to an increase in the number of alveolar macrophages, although numbers of polymorphonuclear leukocytes were also significantly increased. Nickel oxide exposure resulted in decreased alveolar macrophage phagocytic activity and increased numbers of nucleated cells in lung lavage samples (Haley *et al.*, 1990; Appendix N). No nasal lesions were observed in either rats or mice in the

13-week studies, which contrasts with the findings for the more water-soluble nickel compounds (nickel sulfate hexahydrate and nickel subsulfide). Toxic effects were more severe in rats than in mice.

In this series of 16-day and 13-week nickel compound studies, nickel oxide was the least toxic, followed by nickel subsulfide and nickel sulfate hexahydrate, which was the most toxic (Tables 30 and 31). This is reflected by the increased mortality and reduced body weight gain or body weight loss seen with nickel sulfate hexahydrate and nickel subsulfide but not nickel oxide. The lung and nasal toxicity reflects the relative solubility of the nickel compounds in water and biological fluids, with the most soluble nickel compound (nickel sulfate hexahydrate) being the most toxic. The soluble nickel compounds are thought to be more toxic than the insoluble nickel compounds because the nickel ions are in a form that can diffuse across the cell membrane and interact with cytoplasmic proteins, thereby causing toxicity. In contrast, it may be that the water-insoluble nickel compounds are phagocytized and do not cause extensive damage to cytoplasmic components of the alveolar/bronchiolar epithelium (Lee *et al.*, 1993; Costa *et al.*, 1994).

The spectrum of inflammatory lesions in the lungs of rats and mice after 13 weeks of exposure to nickel oxide was similar to that observed with other particulates including nickel sulfate hexahydrate, nickel subsulfide, gallium arsenide (NTP unpublished data), gallium oxide (NTP unpublished data), and cadmium oxide (NTP 1994c). Lymphoid hyperplasia with or without inflammation was present in the respiratory tract lymph nodes of rats and mice from all of these studies. Nickel oxide pigment granules were present in the lung and respiratory lymph nodes; although pigment granules were not present in the lymph nodes of animals from the nickel sulfate hexahydrate, nickel subsulfide, or cadmium oxide studies, the morphologic appearance of the hyperplasia in the paracortical region of the lymph nodes was otherwise generally similar in each study.

In contrast to the findings with nickel sulfate hexahydrate and nickel subsulfide where the amount of nickel present in the lungs reached a steady state after a 13-week exposure period, the amount of nickel present

in the lungs continued to increase during the 13-week exposure to nickel oxide; a steady state was not reached. Even though the lungs of rats and mice exposed to nickel oxide contained more nickel than the lungs of animals exposed to nickel sulfate hexahydrate or nickel subsulfide (Table 32), the toxic effects were less severe at 13 weeks.

The threshold limit value for water-insoluble nickel compounds is  $1 \text{ mg/m}^3$ . In the 13-week studies of nickel oxide, a no-observed-adverse-effect level was not reached in either rats or mice since lung toxicity was observed at the lowest exposure evaluated ( $0.6 \text{ mg nickel oxide/m}^3$ , equivalent to  $0.4 \text{ mg nickel/m}^3$ ).

The highest exposure concentrations for these 2-year studies were limited to  $2.5 \text{ mg/m}^3$  for rats and  $5 \text{ mg/m}^3$  for mice, because of the increased severity and spectrum of inflammatory lesions in the lung and increased lung weights that occurred at the higher exposure concentrations in the 13-week studies. The nickel compound exposure concentrations for the 16-day, 13-week, and 2-year nickel studies and their nickel equivalents are presented in Table 33.

Two-year exposure of rats and mice to nickel oxide by inhalation had no effect on survival. Mean body weights of  $2.5 \text{ mg/m}^3$  male and female rats and  $1.25 \text{ mg/m}^3$  female rats were 2% to 10% lower than those of controls during the last year of the study. Mean body weights of  $5 \text{ mg/m}^3$  male and female mice were also reduced during the last year of the study. Final mean body weights of  $2.5 \text{ mg/m}^3$  male and female rats and  $5 \text{ mg/m}^3$  male and female mice were 93%, 90%, 93%, and 90% that of controls, respectively.

At 7 months and at 15 months, the amount of nickel in lungs was similar in males and females. The lung nickel burden represents the difference between the amount of nickel deposited in the lung and the amount removed by the clearance mechanisms. Inhaled particles deposited on the mucosal surface of the trachea, bronchi, or bronchioles are transported up the airways and from the lung through the ciliary activity of the respiratory epithelium, while particles reaching the alveolar region are phagocytized by alveolar macrophages and, to a lesser extent, other phagocytic inflammatory cells. Some alveolar macrophages

migrate to the ciliated epithelium of the airways while others cross the alveolar epithelium to enter the interstitium and, finally, the lymphatics. Phagocytic cells reaching the lymphatics are transported in the lymph to the bronchial and mediastinal lymph nodes. In the current studies, the pigment in the lymph nodes probably represented the clearance of some of nickel oxide from the lungs. Depending on the physiochemical properties of the inhaled particles, they may be partially or completely degraded within phagolysosomes of the macrophages and soluble components released from the cell. Nickel oxide is relatively insoluble in biologic fluids, and a relatively large amount of nickel oxide remains in the lung.

The carcinogenic response in the lung of rats exposed to nickel oxide was considered to be chemical related because the incidence of lung neoplasms in the 1.25 and 2.5 mg/m<sup>3</sup> groups exceeded the historical control rate at this laboratory, and the effect was observed in both males and females. The incidences of lung neoplasms in 1.25 males (6/53, 11%) and females (6/53, 11%) and 2.5 mg/m<sup>3</sup> males (4/52, 8%) and females (5/54, 9%) were significantly greater ( $P < 0.05$ ) than the historical control incidences at Lovelace Inhalation Toxicology Research Institute, which are 1.4% (3/210) for males and 1.9% (4/208) for females. Some of these alveolar/bronchiolar neoplasms also had morphologic features (prominent scirrhous reaction and squamous differentiation) that were different from the spontaneous alveolar/bronchiolar neoplasms in control rats.

The incidence of alveolar/bronchiolar adenoma in the 2.5 mg/m<sup>3</sup> groups of female mice was significantly greater than that of the controls and exceeded the historical control range from inhalation studies. The incidence of alveolar/bronchiolar adenoma or carcinoma (combined) in 1.25 mg/m<sup>3</sup> females was significantly greater than that of the controls, and the incidences in 1.25 and 2.5 mg/m<sup>3</sup> females exceeded the historical control range. However, the incidences of these neoplasms were not increased at the highest exposure concentration (5 mg/m<sup>3</sup>) in females.

While both nickel oxide and nickel subsulfide caused lung neoplasms in male and female rats (Table 34), the response was not proportional to the amount of nickel deposited in the lungs (Table 32). In the 2-year

nickel oxide study, there was approximately 300 to 1,100  $\mu\text{g}$  nickel/g lung at 15 months; in the 2-year nickel subsulfide study, there was 3 to 7  $\mu\text{g}$  nickel/g lung at 15 months. However, the incidence of chemical-related lung neoplasms was greater in the nickel subsulfide rats than in the nickel oxide rats. The type of nickel compound is probably important in the eventual carcinogenic response, and under the conditions of these studies, the nickel compound that was more rapidly cleared from the lungs (nickel subsulfide) gave a stronger carcinogenic response than the nickel compound retained in the lungs (nickel oxide).

A number of studies have reported sarcomas at the site of nickel oxide injection into the muscle or the pleural or peritoneal cavity of rodents (Table 3). In one inhalation study in hamsters exposed to a nickel oxide concentration of 50  $\text{mg}/\text{m}^3$ , there were extensive nonneoplastic pulmonary lesions but no increase in lung neoplasms (Wehner *et al.*, 1975, 1979). Intratracheal administration of nickel oxide to female rats also caused a significant increase in adenocarcinoma and squamous cell carcinoma of the lung (Pott *et al.*, 1987).

Some generalities can be made about the comparative lung pathology in rats and mice after 2 years of exposure to nickel oxide, nickel subsulfide, or nickel sulfate hexahydrate. Exposure-related pigment occurred in lungs and bronchial lymph nodes of rats and mice exposed to nickel oxide, but was not observed in the lungs of animals in the nickel subsulfide or nickel sulfate hexahydrate studies. All three studies were similar in that mice were less susceptible to proliferative and fibrotic lung lesions than rats exposed to the same compound. Morphologic features of the proliferative lesions in rats exposed to nickel oxide or nickel subsulfide were clearly different from spontaneous lesions in control rats. Five of the alveolar/bronchiolar carcinomas in rats exposed to nickel oxide and four of the alveolar/bronchiolar carcinomas and two of the alveolar/bronchiolar adenomas in rats exposed to nickel subsulfide had marked squamous differentiation. Alveolar/bronchiolar neoplasms in exposed mice from all the studies and in rats

exposed to nickel sulfate hexahydrate had morphologic features similar to those observed in spontaneously occurring tumors.

The nonneoplastic lung lesions in nickel oxide exposed rats had evidence of recurrent parenchymal damage secondary to inflammation. The resulting fibrosis and consolidation was multifocally extensive in many nickel oxide exposed rats, differing greatly from the minute fibrotic lesions that were occasionally observed in control rats. Similar exposure-related fibrotic lesions were also observed in nickel subsulfide exposed rats but not in nickel sulfate hexahydrate exposed rats.

With the exception of pigment observed in the nickel oxide study, nonneoplastic lesions in the lungs of exposed mice were similar in all three nickel compound studies. The components of the inflammatory reaction (intra-alveolar protein and macrophages; mononuclear inflammatory cells around vessels; and multifocal intra-alveolar aggregates of inflammatory cells) were similar in exposed mice in all three studies. Inflammatory foci with neutrophils and necrotic cell debris were relatively common in mice exposed to nickel sulfate hexahydrate, while inflammatory foci in mice exposed to nickel oxide and nickel subsulfide were predominantly mononuclear cells with little evidence of necrotic cell debris.

The inflammatory lesions in the lung were similar to those reported in rodents exposed to talc (NTP, 1993a), cadmium compounds (Aufderheide *et al.*, 1989), titanium dioxide (Lee *et al.*, 1985), chromium dioxide (Lee *et al.*, 1988), antimony trioxide and antimony ore concentrate (Groth *et al.*, 1986), or volcanic ash (Wehner *et al.*, 1986). Aerosols of each of these particulate substances were reported to elicit pulmonary inflammation (characterized primarily by the accumulation of alveolar macrophages), hyperplasia, and, in some cases, squamous metaplasia of the alveolar epithelium and fibrosis.

In the NTP studies, more chemical-related lung neoplasms were observed in nickel oxide and nickel subsulfide exposed rats than in mice. In cadmium carcinogenicity inhalation studies performed at the

Fraunhofer Institute, cadmium induced alveolar/bronchiolar neoplasms in rats but not in mice

(Aufderheide *et al.*, 1989; Heinrich *et al.*, 1989; Thiedemann *et al.*, 1989; Glaser *et al.*, 1990; Takenaka *et al.*, 1990). In the series of approximately 450 chemical studies by NTP, the rat is more susceptible to the formation of lung neoplasms after exposure to metals (e.g., nickel oxide, nickel subsulfide), while the mouse is more susceptible to the formation of lung neoplasms after exposure to epoxide-forming chemicals (e.g., coumarin, NTP, 1993b; benzene, NTP, 1986; glycidol, NTP, 1990a). The mouse is also more susceptible than the rat to formation of lung neoplasms after exposure to halogenated chemicals (2,2-bis(bromomethyl)-1,3-propanediol, NTP, 1994d; 1,2-dibromo-3-chloropropane, NTP, 1982a; 1,2-dibromomethane, NCI, 1978a, NTP 1982b; 2,3-dibromo-1-propanol, NTP, 1994e; 1,2-dichloroethane, NCI, 1978b; and tris(2,3-dibromopropyl)phosphate, NCI, 1978c).

The morphological types of lung neoplasms induced by various particulates in rodents vary. In the talc studies, a significant increase in alveolar/bronchiolar neoplasms was observed only in female rats (NTP, 1993a), and the type of neoplasm was similar to that observed in the present nickel oxide studies. Other chemicals producing alveolar/bronchiolar neoplasms in rats after inhalation exposure include antimony trioxide (Groth *et al.*, 1986) and cadmium (Aufderheide *et al.*, 1989). Alternatively, most of the pulmonary neoplasms induced by quartz (Dagel *et al.*, 1986), volcanic ash (Wehner *et al.*, 1986), or chromium dioxide (Lee *et al.*, 1988) were squamous cell (epidermoid) carcinomas. In refinery workers, pulmonary neoplasms attributed to nickel exposure have been classified primarily as squamous cell carcinomas with fewer anaplastic carcinomas and adenocarcinomas (Sunderman *et al.*, 1989).

Lung tissue specimens from 39 nickel refinery workers showed increased lung nickel levels (Andersen and Svenes, 1989). The average nickel concentration for workers in roasting and smelting operations was  $330 \pm 380$   $\mu\text{g}$  nickel/g dry lung weight; for workers in electrolysis departments,  $34 \pm 48$   $\mu\text{g}$ .g. and for lung tissue from unexposed people,  $0.76 \pm 0.39$   $\mu\text{g}$ /g. Dry lung represents approximately 20% "wet" lung weight, the lung weight used in the NTP nickel studies (Henderson and Escobedo, 1976). Workers

who were diagnosed with lung cancer (14 cases) had the same lung nickel concentrations at autopsy as nickel workers (25 cases) who died of other causes. The lung nickel concentration was independent of smoking habits. Anderson and Svenes (1989) felt that the retained nickel in the lung was probably nickel oxide because an earlier study using energy dispersive X-ray analysis did not detect sulfides (e.g.,  $\text{Ni}_3\text{S}_2$ ) in the lung. This study also found that lung cancer occurred in workers from the electrolysis department (8/24) as well as those from the roasting and smelting operations (6/15) even though those from the electrolysis department had lower lung nickel levels.

Nasopharyngeal carcinoma in humans has been attributed to nickel exposure. The preponderance of these sinonasal neoplasms in humans have been classified as anaplastic, undifferentiated, or squamous cell carcinoma (Sunderman *et al.*, 1989). In rodent studies, the olfactory epithelium, rather than the respiratory or squamous mucosa, has been the target site for chemical-related toxicity. Although the atrophic changes were present in the olfactory epithelium of rats and mice in the 13-week and 2-year studies of nickel subsulfide and nickel sulfate hexahydrate, the nasal mucosa was not affected in the nickel oxide study. Furthermore, after 2 years, there was no evidence of a chemical-related increase in the incidences of proliferative lesions in the nasal cavity of rats or mice exposed to any of the three nickel compounds tested.

The increase in proliferative lesions of the adrenal medulla in male and female rats was considered to be chemical related. In male rats, the overall increase in benign and malignant pheochromocytomas was related primarily to an increase in malignant pheochromocytomas. In female rats, only the incidence of benign pheochromocytoma was increased, but the number of rats with bilateral pheochromocytoma and the number with hyperplasia were also increased in the 5 mg/m<sup>3</sup> group. A chemical-related increase in pheochromocytomas was also observed in male and female rats exposed to nickel subsulfide and in male and female rats exposed to talc (NTP, 1993a). However, similar increased incidences were not observed in rats exposed to nickel sulfate hexahydrate or to other particulates including antimony trioxide or

trisulfide (Groth *et al.*, 1986) and titanium dioxide (Lee *et al.*, 1988). Chemical-related increases in the incidences of pheochromocytoma have also been reported in rats exposed by inhalation to bromoethane (NTP, 1990b) and orally to 2-mercaptobenzothiazole (NTP, 1988) and reserpine (NCI 1982). The mechanism for this increased incidence of adrenal medulla neoplasms is not known. There was no morphologic evidence that the pigment granules of phagocytized nickel oxide reached the adrenal gland. This suggests that the mechanism for the carcinogenic response in the adrenal gland may be related to factors other than direct interaction of the chemical with adrenal cells. No histopathologic changes were observed in the adrenal medulla in the short-term studies of either nickel oxide or talc.

## CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *some evidence of carcinogenic activity\** of nickel oxide in male F344/N rats based on increased incidences of alveolar/bronchiolar adenoma or carcinoma (combined) and increased incidences of benign or malignant pheochromocytoma (combined) of the adrenal medulla. There was *some evidence of carcinogenic activity* of nickel oxide in female F344/N rats based on increased incidences of alveolar/bronchiolar adenoma or carcinoma (combined) and increased incidences of benign pheochromocytoma of the adrenal medulla. There was *no evidence of carcinogenic activity* of nickel oxide in male B6C3F<sub>1</sub> mice exposed to 1.25, 2.5, or 5 mg/m<sup>3</sup>. There was *equivocal evidence of carcinogenic activity* of nickel oxide in female B6C3F<sub>1</sub> mice based on the marginally increased incidences of alveolar/bronchiolar adenoma in 2.5 mg/m<sup>3</sup> females and of alveolar/bronchiolar adenoma or carcinoma (combined) in 1.25 mg/m<sup>3</sup> females.

Exposure of rats to nickel oxide by inhalation for 2 years resulted in inflammation and pigmentation in the lung and lymphoid hyperplasia and pigmentation in the bronchial lymph nodes. Exposure of mice to nickel oxide by inhalation for 2 years resulted in inflammation and pigmentation in the lung and lymphoid hyperplasia and pigmentation in the bronchial lymph nodes.

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\* Explanation of Levels of Evidence of Carcinogenic Activity is on page 16.

TABLE 30  
Selected Results in the 16-Day Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide<sup>a</sup>

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate					Nickel Subsulfide					Nickel Oxide							
	0	3.5 (0.7)	7 (1.4)	15 (3.1)	30 (6.1)	60 (12.2)	0	0.6 (0.44)	1.2 (0.88)	2.5 (1.83)	5 (3.65)	10 (7.33)	0	1.2 (0.9)	2.5 (2.0)	5 (3.9)	10 (7.9)	30 (23.6)
<b>Male Rats</b>																		
Survival	5	5	5	5	5	3	5	5	5	5	5	5	5	5	5	5	5	5
Final Mean Body Weights (Relative to Controls)	—	72%	60%	56%	55%	45%	—	109%	105%	92%	72%	52%	—	99%	101%	99%	99%	96%
Absolute Lung Weights <sup>b</sup>	0.98	1.44**	1.45**	1.40*	1.40*	1.62**	1.13	1.41	1.60*	1.59*	1.82**	1.54**	1.06	1.00	1.06	0.96	1.20*	1.36**
<b>Female Rats</b>																		
Survival	5	5	5	5	4	0	5	5	5	5	5	5	5	5	5	5	5	5
Final Mean Body Weights (Relative to Controls)	—	82%	71%	68%	63%	—	—	99%	97%	91%	78%	57%	—	103%	103%	104%	101%	99%
Absolute Lung Weights	0.76	1.28*	1.28*	1.32*	1.40**	1.52**	0.82	1.12**	1.12**	1.36**	1.42**	1.25**	0.78	0.86	0.90	0.82	1.04**	1.12**

(continued)

TABLE 30  
Selected Results in the 16-Day Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide (continued)

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate				Nickel Subsulfide				Nickel Oxide										
	0	3.5 (0.7)	7 (1.4)	15 (3.1)	30 (6.1)	60 (12.2)	0	0.6 (0.44)	1.2 (0.88)	2.5 (1.83)	5 (3.65)	10 (7.33)	30	0	1.2 (0.9)	2.5 (2.0)	5 (3.9)	10 (7.9)	30 (23.6)
<b>Male Mice</b>																			
Survival	5	5	0	0	0	0	4	5	4	5	5	0	5	5	5	4	5	5	5
Final Mean Body Weights (Relative to Controls)	—	95%	—	—	—	—	—	99%	90%	92%	86%	—	—	100%	100%	98%	102%	94%	94%
Absolute Lung Weights	0.20	0.24	0.40**	0.36**	0.36**	0.38**	0.22	0.20	0.22	0.28	0.31**	0.38**	0.20	0.16	0.20	0.13**	0.20	0.20	0.20
<b>Female Mice</b>																			
Survival	5	5	0	0	0	0	4	5	5	5	5	0	5	5	5	5	5	5	5
Final Mean Body Weights (Relative to Controls)	—	96%	—	—	—	—	—	106%	104%	101%	99%	—	—	100%	96%	100%	95%	95%	95%
Absolute Lung Weights	0.16	0.22	0.36**	0.36**	0.38**	0.40**	0.20	0.21	0.22	0.27	0.36*	0.25	0.16	0.16	0.14	0.18	0.12	0.20	0.20

\* Significantly different ( $P \leq 0.05$ ) from the control by Williams' or Dunnett's test

\*\*  $P \leq 0.01$

<sup>a</sup> Survival data indicate number of animals surviving. Five animals initially in group. Final mean body weights are not presented for groups with 100% mortality.

<sup>b</sup> Organ weights are given in grams.



TABLE 31  
Selected Results in the 13-Week Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide (continued)

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate			Nickel Subsulfide			Nickel Oxide											
	0	0.12 (0.03)	0.25 (0.06)	0.5 (0.11)	1 (0.22)	2 (0.44)	0	0.15 (0.11)	0.3 (0.22)	0.6 (0.44)	1.2 (0.88)	2.5 (1.83)	0	0.6 (0.4)	1.2 (0.9)	2.5 (2.0)	5 (3.9)	10 (7.9)
<b>Female Rats</b>																		
Survival	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Final Mean Body Weights (Relative to Controls)	—	96%	98%	98%	101%	95%	—	101%	104%	101%	100%	99%	—	101%	101%	98%	98%	100%
Absolute Lung Weights	1.02	1.02	1.16**	1.34**	1.72**	1.72**	1.01	1.29**	1.39**	1.82**	1.85**	1.81**	0.98	1.03	1.13*	1.55**	1.61**	2.11**
<b>Nonneoplastic Lung Lesions</b>																		
Alveolar Macrophage Hyperplasia (Severity)	0	8 (1.0)	10 (1.0)	10 (1.1)	10 (2.2)	10 (3.6)	0	10 (1.0)	10 (1.7)	10 (1.8)	10 (2.9)	10 (3.8)	0	10 (1.0)	8 (1.0)	10 (1.0)	10 (1.4)	10 (2.2)
Inflammation, Chronic Active (Severity)	0	0	0	4 (1.0)	10 (1.3)	10 (1.0)	0	3 (1.0)	9 (1.0)	10 (1.9)	10 (2.6)	10 (3.8)	0	0	0	1 (1.0)	7 (1.3)	7 (2.7)
Inflammation, Granulomatous (Severity)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4 (2.1)	4 (2.0)
Interstitial Infiltrate (Severity)	0	0	0	6 (1.0)	10 (1.0)	10 (1.0)	0	0	2 (1.0)	9 (1.7)	10 (2.4)	5 (1.6)	0	0	0	2 (1.0)	10 (1.2)	10 (1.8)
Pigment (Severity)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4 (1.0)	8 (1.0)	8 (1.0)	10 (1.2)
<b>Nonneoplastic Nasal Lesions</b>																		
Atrophy, Olfactory Epithelium	0	0	1	2	10	10	0	0	0	8	9	10	0	0	0	0	0	0

(continued)

**TABLE 31**  
Selected Results in the 13-Week Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide (continued)

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate				Nickel Subsulfide				Nickel Oxide									
	0	0.12 (0.03)	0.25 (0.06)	0.5 1 2 (0.11) (0.22) (0.44)	0	0.15 (0.11)	0.3 (0.22)	0.6 1.2 2.5 (0.44) (0.88) (1.83)	0	0.6 (0.4)	1.2 (0.9)	2.5 (2.0)	5 (3.9)	10 (7.9)				
<b>Male Mice</b>																		
Survival	6	8 <sup>c</sup>	10	10	10	10	10	8	9	10	10	10	10	9				
Final Mean Body Weights (Relative to Controls)	—	105%	100%	104%	104%	102%	—	102%	106%	103%	101%	97%	97%	97%				
Absolute Lung Weights	0.20	0.20	0.20	0.21	0.25**	0.31**	0.19	0.20	0.22	0.21	0.23*	0.28**	0.21	0.22	0.21	0.21	0.24	0.29**
<b>Nonneoplastic Lung Lesions</b>																		
Alveolar Macrophage Hyperplasia (Severity)	0	0	0	10	10	10	0	0	8	8	9	10	0	10	10	10	10	9
Fibrosis, Local (Severity)	0	0	0	0	2	10	0	0	0	0	5	10	0	0	0	0	0	0
Inflammation, Chronic Active (Severity)	0	0	0	0	2	2	0	0	0	0	5	7	0	0	0	0	0	3
Inflammation, Granulomatous (Severity)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Interstitial Infiltrate (Severity)	0	0	0	0	2	8	0	1	0	2	3	2	0	0	0	1	3	8
Pigment (Severity)	0	0	0	0	0	0	0	0	0	0	0	0	0	10	10	10	10	9
<b>Nonneoplastic Nasal Lesions</b>																		
Atrophy, Olfactory Epithelium	0	0	0	0	0	10	0	0	0	5	5	10	0	0	0	0	0	0

(continued)

TABLE 31  
Selected Results in the 13-Week Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide (continued)

	Nickel Sulfate Hexahydrate				Nickel Subsulfide				Nickel Oxide									
	0	0.12 (0.03)	0.25 (0.06)	0.5 (0.11)	1 (0.22)	2 (0.44)	0	0.15 (0.11)	0.3 (0.22)	0.6 (0.44)	1.2 (0.88)	2.5 (1.83)	0	0.6 (0.4)	1.2 (0.9)	2.5 (2.0)	5 (3.9)	10 (7.9)
<b>Female Mice</b>																		
Survival	7	10	10	10	10	10	10	8	10	9	10	8	9	10	7	10	10	9
Final Mean Body Weights (Relative to Controls)	--	105%	104%	105%	103%	97%	--	101%	100%	101%	101%	99%	--	97%	100%	96%	94%	97%
Absolute Lung Weights	0.20	0.20	0.20	0.20	0.22	0.27**	0.19	0.18	0.20	0.21	0.26**	0.29**	0.20	0.20	0.19	0.21	0.22	0.27**
<b>Nonneoplastic Lung Lesions</b>																		
Alveolar Macrophage Hyperplasia (Severity)	0	0	0	10	10	10	0	0	4	9	10	10	0	10	7	10	10	9
Fibrosis Focal (Severity)	0	0	0	0	1	8	0	0	0	0	1	9	0	0	0	0	0	0
Inflammation, Chronic Active (Severity)	0	0	0	0	1	9	0	0	0	0	10	7	0	0	0	0	1	3
Inflammation, Granulomatous (Severity)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Interstitial Infiltrate (Severity)	1 (1.0)	0	0	1	1	8	0	2	3	4	9	8	0	1	0	4	6	8
Pigment (Severity)	0	0	0	0	0	0	0	0	0	0	0	0	0	10	7	10	10	9
														(1.0)	(1.0)	(1.0)	(1.0)	(1.0)
<b>Nonneoplastic Nasal Lesions</b>																		
Atrophy, Olfactory Epithelium	0	0	0	0	0	5	0	0	0	1	6	10	0	0	0	0	0	0

\* Significantly different (P ≤ 0.05) from the control by Williams' or Dunnett's test

\*\* P ≤ 0.01

<sup>a</sup> Survival data indicate number of animals surviving. Ten animals initially in group. Final mean body weights are not presented for groups with 100% mortality.

<sup>b</sup> Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

<sup>c</sup> Nine animals initially in group

TABLE 32  
Lung Burden Analyses in the 16-Day, 13-Week, and 2-Year Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide<sup>a</sup>

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate (22.3% Ni)			Nickel Subsulfide (73.3% Ni)			Nickel Oxide (78.6% Ni)											
	0	0.12	0.5	2	3.5	15	30	0	0.15	0.6	2.5	10	0	0.6	1.2	2.5	5	10
	(0.03)	(0.06)	(0.44)	(0.7)	(3.1)	(6.1)		(0.11)	(0.44)	(1.83)	(7.33)		(0.4)	(0.9)	(2.0)	(3.9)	(7.9)	
<b>16-Day Studies</b>																		
Male Rats	- <sup>b</sup>		5	9	8			7	18	67				42	108	267		
Female Rats	-		8	11	9			9	19	77				54	122	340		
Male Mice	-		3					10	20	13				32	46	84		
Female Mice	-		4					8	20	8				31	43	71		
<b>13-Week Studies</b>																		
Male Rats	-		1	6				5	7	18				80	181	524		
Female Rats	-		2	7				5	7	17								
Male Mice	-		1					3	11	17				42	202	736		
Female Mice	-		4					6	13	23								

(continued)

TABLE 32  
Lung Burden Analyses in the 16-Day, 13-Week, and 2-Year Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide (continued)

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate (22.3% Ni)		Nickel Subsulfide (73.3% Ni)		Nickel Oxide (78.6% Ni)									
	0	0.12	0.25	0.5	1	2	0	0.62	1.25	2.5	5	10		
	(0.03)	(0.06)	(0.11)	(0.22)	(0.44)	(0.88)	(0.11)	(0.44)	(0.73)	(0.88)	(0.5)	(1.0)	(3.9)	(7.9)
<b>7-Month Interim Evaluation</b>														
Male Rats	-	-	-	1	-	-	6	9	-	-	175	388	701	
Female Rats	-	-	-	1	-	-	6	9	-	-	173	477	713	
Male Mice	-	1	1	2	-	-	10	11	-	-	162	442	1,034	
Female Mice	-	1	2	2	-	-	10	14	-	-	169	533	861	
<b>15-Month Interim Evaluation</b>														
Male Rats	-	-	-	1	-	-	4	3	-	-	328	746	1,116	
Female Rats	-	-	-	2	-	-	4	7	-	-	262	706	949	
Male Mice	-	1	1	2	-	-	15	26	-	-	331	959	1,798	
Female Mice	-	1	2	2	-	-	12	20	-	-	451	1,237	2,258	

<sup>a</sup> Values represent mean amounts of nickel ( $\mu\text{g Ni/g lung}$ ). Lung burden groups included five to seven animals.

<sup>b</sup> Results were below the limit of detection.

**TABLE 33**  
**Comparison of Exposure Concentrations in the 16-Day, 13-Week, and 2-Year Studies**  
**of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide<sup>a</sup>**

	<u>Amount of Compound</u>	<u>Amount of Nickel</u>
<b>16-Day Studies</b>		
Nickel Sulfate Hexahydrate (22.3% Ni)	0, 3.5, 7, 15, 30, 60	0, 0.7, 1.4, 3.1, 6.1, 12.2
Nickel Subsulfide (73.3% Ni)	0, 0.6, 1.2, 2.5, 5, 10	0, 0.44, 0.88, 1.83, 3.65, 7.33
Nickel Oxide (78.6% Ni)	0, 1.2, 2.5, 5, 10, 30	0, 0.9, 2.0, 3.9, 7.9, 23.6
<b>13-Week Studies</b>		
Nickel Sulfate Hexahydrate (22.3% Ni)	0, 0.12, 0.25, 0.5, 1, 2	0, 0.03, 0.06, 0.11, 0.22, 0.44
Nickel Subsulfide (73.3% Ni)	0, 0.15, 0.3, 0.6, 1.2, 2.5	0, 0.11, 0.22, 0.44, 0.88, 1.83
Nickel Oxide (78.6% Ni)	0, 0.6, 1.2, 2.5, 5, 10	0, 0.4, 0.9, 2.0, 3.9, 7.9
<b>2-Year Studies</b>		
<b>Nickel Sulfate Hexahydrate (22.3% Ni)</b>		
Rats	0, 0.12, 0.25, 0.5	0, 0.03, 0.06, 0.11
Mice	0, 0.25, 0.5, 1	0, 0.06, 0.11, 0.22
<b>Nickel Subsulfide (73.3% Ni)</b>		
Rats	0, 0.15, 1	0, 0.11, 0.73
Mice	0, 0.6, 1.2	0, 0.44, 0.88
<b>Nickel Oxide (78.6% Ni)</b>		
Rats	0, 0.62, 1.25, 2.5	0, 0.5, 1.0, 2.0
Mice	0, 1.25, 2.5, 5	0, 1.0, 2.0, 3.9

<sup>a</sup> Amounts of nickel and nickel compounds are expressed in mg/m<sup>3</sup>. Occupational exposure limits in the United States: 1 mg Ni/m<sup>3</sup> for nickel metals, 0.1 mg Ni/m<sup>3</sup> for soluble nickel compounds.

TABLE 34  
Selected Results in the 2-Year Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide<sup>a</sup>

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate (22.3% Ni)			Nickel Subsulfide (73.3% Ni)			Nickel Oxide (78.6% Ni)				
	0	0.12 (0.03)	0.25 (0.06)	0.5 (0.11)	0	0.15 (0.11)	1 (0.73)	0	0.62 (0.5)	1.25 (1.0)	2.5 (2.0)
<b>Male Rats</b>											
Survival	16/54	16/55	18/55	21/55	13/53	21/53	18/53	14/54	15/53	15/53	12/52
Final Mean Body Weights (Relative to Controls)	—	99%	101%	98%	—	98%	85%	—	100%	95%	93%
Absolute Lung Weights											
7-Month Interim Evaluation	1.67	1.62	1.65	1.89	1.87	2.38**	3.48**	1.72	1.85	2.43**	2.59**
15-Month Interim Evaluation	2.12	2.48	2.50	3.00**	2.27	3.31**	6.84**	2.20	2.15	3.30**	4.09**
Alveolar/broncholar Proliferative Lesions and Neoplasms											
Alveolar Epithelial											
Hyperplasia, Focal or Atypical	3	2	3	2	2	6	11**	0	2	5*	3
Adenoma	0	0	0	2	0	3	6*	0	1	3	2
Carcinoma	2 <sup>b</sup>	0	1	1	0	3	6*	1 <sup>b</sup>	0	3	2
Adenoma or Carcinoma (Combined)	2 <sup>b</sup>	0	1	3	0	6*	11**	1 <sup>b</sup>	1	6 <sup>c</sup>	4 <sup>c</sup>
Adrenal Medulla Proliferative Lesions and Neoplasms											
Hyperplasia	28	20	18	26	26	22	10	25	27	26	24
Benign Pheochromocytoma	16	16	12	11	13	30**	38**	27	24	26	32
Malignant Pheochromocytoma	0	3	2	1	0	2	10**	0	0	1	6*
Benign or Malignant Pheochromocytoma	16	19	13	12	14	30**	42**	27	24	27	35**
Carcinogenic Activity		No evidence				Clear evidence				Some evidence	

(continued)

TABLE 34  
Selected Results in the 2-Year Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide (continued)

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate (22.3% Ni)		Nickel Subsulfide (73.3% Ni)		Nickel Oxide (78.6% Ni)							
	22/52	17/53	28/53	29/54	26/53	25/53	28/52	21/53	26/53	20/53	26/54	
	0	0.12 (0.03)	0.25 (0.06)	0.5 (0.11)	0	0.15 (0.11)	1 (0.73)	0	0.62 (0.5)	1.25 (1.0)	2.5 (2.0)	
Final Mean Body Weights (Relative to Controls)	—	97%	97%	94%	—	96%	78%	—	96%	92%	90%	
Absolute Lung Weights	1.25	1.22	1.22	1.45*	1.31	1.75**	2.59**	1.14	1.31*	1.65**	1.78**	
7-Month Interim Evaluation	1.37	1.57	1.49	1.82**	1.52	2.52**	4.14**	1.56	1.79	2.41**	3.02**	
15-Month Interim Evaluation												
Alveolar/bronchiolar Proliferative Lesions and Neoplasms												
Alveolar Epithelial Hyperplasia, Focal or Atypical	5	3	7	9	2	10*	11**	2	1	6	6	
Adenoma	0	0	0	1	2	5	5	1	0	1	4	
Carcinoma	0	0	0	0	0	1 <sup>b</sup>	4	0	0	5*	1	
Adenoma or Carcinoma (Combined)	0	0	0	1	2	6 <sup>b,d</sup>	9*	1	0	6 <sup>d</sup>	5 <sup>d</sup>	
Adrenal Medulla Proliferative Lesions and Neoplasms												
Hyperplasia	6	4	8	8	5	11	16**	8	12	14	22**	
Benign Pheochromocytoma	2	4	2	3	2	7	36**	4	7	6	18**	
Malignant Pheochromocytoma	0	0	0	0	1	0	1	0	0	0	0	
Benign or Malignant Pheochromocytoma	2	4	2	3	3	7	36**	4	7	6	18**	
Carcinogenic Activity	No evidence			Clear evidence			Some evidence					

(continued)

TABLE 34  
Selected Results in the 2-Year Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide (continued)

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate (22.3% Ni)		Nickel Subsulfide (73.3% Ni)		Nickel Oxide (78.6% Ni)						
	0	0.25 (0.06)	0.5 (0.11)	1 (0.22)	0	0.6 (0.44)	1.2 (0.88)	0	1.25 (1.0)	2.5 (2.0)	5 (3.9)
<b>Male Mice</b>											
Survival	26/61	23/61	24/62	25/61	26/61	25/59	26/58	19/57	23/67	29/66	23/69
Final Mean Body Weights (Relative to Controls)	—	94%	97%	91%	—	92%	92%	—	93%	93%	93%
Absolute Lung Weights											
7-Month Interim Evaluation	0.21	0.20	0.22	0.23	0.24	0.27	0.34**	0.19	0.21	0.24**	0.24**
15-Month Interim Evaluation	0.24	0.25	0.26	0.31**	0.23	0.40**	0.41**	0.23	0.25	0.31*	0.38**
<b>Alveolar/broncholar Proliferative Lesions and Neoplasms</b>											
Alveolar Epithelial Hyperplasia Focal	0	0	0	0	0	0	0	1	1	2	0
Adenoma	5	5	3	5	6	3	2	7	5	6	11
Carcinoma	9	13	4	3	7	2	4	4	10	9	6
Adenoma or Carcinoma (Combined)	13	18	7	8	13	5	6	9	14	15	14
Carcinogenic Activity		No evidence				No evidence			No evidence		

(continued)





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**NTP TECHNICAL REPORT**  
**ON THE**  
**TOXICOLOGY AND CARCINOGENESIS**  
**STUDIES OF**  
**NICKEL SUBSULFIDE**  
**(CAS NO. 12035-72-2)**  
**IN F344/N RATS AND B6C3F<sub>1</sub> MICE**  
**(INHALATION STUDIES)**

**Scheduled Peer Review Date: November 29, 1994**

**NOTICE**

This is a DRAFT Technical Report prepared for public review and comment. Until this DRAFT has been reviewed and approved by the NTP Board of Scientific Counselors' Technical Reports Review Subcommittee in public session, the interpretations described herein do not represent the official scientific position of the National Toxicology Program. Following peer review, readers should contact NTP for the final version of this Technical Report.

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**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES**  
**Public Health Service**  
**National Institutes of Health**

## NOTE TO THE READER

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

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# CONTENTS

<b>ABSTRACT</b>		6
<b>EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY</b>		16
<b>TECHNICAL REPORTS REVIEW SUBCOMMITTEE</b>		17
<b>SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS</b>		18
<b>INTRODUCTION</b>		19
<b>MATERIALS AND METHODS</b>		51
<b>RESULTS</b>		73
<b>DISCUSSION AND CONCLUSIONS</b>		125
<b>REFERENCES</b>		147
<b>APPENDIX A</b>	<b>Summary of Lesions in Male Rats in the 2-Year Inhalation Study of Nickel Subulfide</b>	A-1
<b>APPENDIX B</b>	<b>Summary of Lesions in Female Rats in the 2-Year Inhalation Study of Nickel Subulfide</b>	B-1
<b>APPENDIX C</b>	<b>Summary of Lesions in Male Mice in the 2-Year Inhalation Study of Nickel Subulfide</b>	C-1
<b>APPENDIX D</b>	<b>Summary of Lesions in Female Mice in the 2-Year Inhalation Study of Nickel Subulfide</b>	D-1
<b>APPENDIX E</b>	<b>Genetic Toxicology</b>	E-1
<b>APPENDIX F</b>	<b>Organ Weights and Organ-Weight-to-Body-Weight Ratios</b>	F-1
<b>APPENDIX G</b>	<b>Hematology Results</b>	G-1
<b>APPENDIX H</b>	<b>Tissue Burden in Rats</b>	H-1
<b>APPENDIX I</b>	<b>Tissue Burden in Mice</b>	I-1
<b>APPENDIX J</b>	<b>Reproductive Tissue Evaluations and Estrous Cycle Characterization</b>	J-1
<b>APPENDIX K</b>	<b>Chemical Characterization and Generation of Chamber Concentrations</b>	K-1
<b>APPENDIX L</b>	<b>Ingredients, Nutrient Composition, and Contaminant Levels in NIH-07 Rat and Mouse Ration</b>	L-1
<b>APPENDIX M</b>	<b>Sentinel Animal Program</b>	M-1

APPENDIX N	The Immunotoxicity of Three Nickel Compounds Following 13-Week Inhalation Exposure in the Mouse . . . . .	N-1
APPENDIX O	Biochemical Responses of Rat and Mouse Lung to Inhaled Nickel Compounds . . .	O-1
APPENDIX P	Fate of Inhaled Nickel Oxide and Nickel Subulfide in F344/N Rats . . . . .	P-1

## ABSTRACT



### NICKEL SUBSULFIDE

CAS No. 12035-72-2

Chemical Formula:  $\text{Ni}_3\text{S}_2$       Molecular Weight: 240.25

**Synonyms:** Heazlewoodite, nickel subsulphide, nickel sulfide (3:2),  $\alpha$ -nickel sulfide (3:2) crystalline, nickel sulphide, nickel trisulphide, trinickel disulfide

Nickel subsulfide is used in the manufacture of lithium batteries and is a major component in the refining of certain nickel ores. Nickel subsulfide was nominated by the National Cancer Institute to the NTP as part of a class study of nickel compounds, for which there was little information on the toxic and carcinogenic effects of inhalation exposure. Male and female F334/N rats and B6C3F<sub>1</sub> mice were exposed to nickel subsulfide (at least 97% pure; mass median aerodynamic diameter  $2.4 \pm 2.2 \mu\text{m}$ ) by inhalation 6 hours per day, 5 days per week, for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, and mouse peripheral blood samples were analyzed for frequency of micronucleated normochromatic erythrocytes.

### 16-DAY STUDY IN RATS

Groups of five male and five female F344/N rats were exposed to atmospheres containing 0, 0.6, 1.2, 2.5, 5, or 10 mg nickel subsulfide/m<sup>3</sup> (equivalent to 0, 0.44, 0.88, 1.83, 3.65, and 7.33 mg nickel/m<sup>3</sup>) 6 hours per day, 5 days per week for a total of 12 exposure days during a 16-day period. Additional groups of three male and three female rats were exposed to 0, 0.6, 2.5, or 10 mg/m<sup>3</sup> for tissue burden studies. One

male exposed to 10 mg nickel subsulfide/m<sup>3</sup> in the core study died on day 14; all other rats survived until the end of the study. Final mean body weights and mean body weight gains of males exposed to 5 or 10 mg nickel subsulfide/m<sup>3</sup> and females exposed to 2.5, 5, or 10 mg/m<sup>3</sup> were significantly lower than those of the controls. Clinical findings of toxicity on day 5 of the study included labored respiration in 10 mg/m<sup>3</sup> males and 5 and 10 mg/m<sup>3</sup> females and dehydration in 5 and 10 mg/m<sup>3</sup> females. Absolute and relative lung weights of 2.5, 5, and 10 mg/m<sup>3</sup> males and all exposed groups of females were significantly greater than those of the controls, as was the absolute lung weight of 1.2 mg/m<sup>3</sup> males. Inflammation of the lung and atrophy of the nasal olfactory epithelium occurred in all exposed groups. The concentrations of nickel subsulfide in the lungs of exposed groups of rats increased with exposure concentration (males, 7 to 67 µg nickel/g lung; females, 9 to 77 µg/g lung).

## 16-DAY STUDY IN MICE

Groups of five male and five female B6C3F<sub>1</sub> mice were exposed to atmospheres containing 0, 0.6, 1.2, 2.5, 5, or 10 mg nickel subsulfide/m<sup>3</sup> (equivalent to 0, 0.44, 0.88, 1.83, 3.65, and 7.33 mg nickel/m<sup>3</sup>) 6 hours per day, 5 days per week for a total of 12 exposure days during a 16-day period. Additional groups of three male and three female mice were exposed to 0, 0.6, 2.5, or 10 mg/m<sup>3</sup> for tissue burden studies. All male and female mice exposed to 10 mg nickel subsulfide/m<sup>3</sup> in the core study died before the end of the study; the death of one female was accidental. One control male, one control female, and one 1.2 mg/m<sup>3</sup> male also died before the end of the study. Final mean body weights and mean body weight gains of 5 mg/m<sup>3</sup> males were significantly lower than those of the controls. Clinical findings at day 5 included labored respiration in 10 mg/m<sup>3</sup> males and females. The absolute lung weight of 5 mg/m<sup>3</sup> males, the absolute and relative lung weights of 10 mg/m<sup>3</sup> males and 5 mg/m<sup>3</sup> females, and the relative lung weight of 10 mg/m<sup>3</sup> females were significantly greater than those of the controls. Inflammation of the lung occurred in 2.5, 5, and 10 mg/m<sup>3</sup> male and female mice, fibrosis of the lung occurred 5 mg/m<sup>3</sup> males and females, and lymphoid hyperplasia of the bronchial lymph nodes and atrophy of the nasal

olfactory epithelium occurred in 1.2, 2.5, 5, and 10 mg/m<sup>3</sup> males and females. Nickel concentrations in the lung of exposed male and female mice were generally greater than those of the controls, but the increase was not exposure related (males, 10 to 13 µg nickel/g lung; females, 8 to 8 µg/g lung).

### 13-WEEK STUDY IN RATS

Groups of 10 male and 10 female F344/N rats were exposed to atmospheres containing 0, 0.15, 0.3, 0.6, 1.2, or 2.5 mg nickel subsulfide/m<sup>3</sup> (equivalent to 0, 0.11, 0.22, 0.44, 0.88, and 1.83 mg nickel/m<sup>3</sup>) 6 hours per day, 5 days per week for 13 weeks. Additional groups of 18 male and 18 female rats were exposed to 0, 0.15, 0.6, or 2.5 mg/m<sup>3</sup> for tissue burden studies. All core study rats survived until the end of the study. Final mean body weights and mean body weight gains of 2.5 mg/m<sup>3</sup> males were significantly lower than those of the controls; final mean body weights of all other exposure groups were similar to those of the controls. Chemical-related clinical findings included labored respiration in 2.5 mg/m<sup>3</sup> males and females during weeks 2 through 7. In general, neutrophil and erythrocyte counts, hematocrit values, and hemoglobin concentrations were minimally increased in exposed rats. Absolute and relative lung weights of all exposed groups were significantly greater than those of the controls.

Increases in the number of alveolar macrophages, interstitial infiltrates, or incidences of chronic inflammation of the lung occurred in all groups exposed to nickel subsulfide concentrations of 0.3 mg/m<sup>3</sup> or greater; the severity of these lesions generally increased with increasing exposure concentration. Increases in the number of alveolar macrophages were observed in 0.15 mg/m<sup>3</sup> males and females. Lymphoid hyperplasia of the bronchial and mediastinal lymph nodes was observed in rats exposed to 0.3 mg/m<sup>3</sup> or greater. Most 0.6, 1.2, and 2.5 mg/m<sup>3</sup> males and females had atrophy of the nasal epithelium, and the severity generally increased with increasing exposure concentration.

Nickel concentrations in the lung increased with exposure concentration and were significantly greater than those in the controls in rats exposed for 13 weeks (males, 5 to 18  $\mu\text{g}$  nickel/g lung; females, 5 to 17  $\mu\text{g}/\text{g}$  lung).

### 13-WEEK STUDY IN MICE

Groups of 10 male and 10 female B6C3F<sub>1</sub> mice were exposed to atmospheres containing 0, 0.15, 0.3, 0.6, 1.2, or 2.5 mg nickel subsulfide/m<sup>3</sup> (equivalent to 0, 0.11, 0.22, 0.44, 0.88, and 1.83 mg nickel/m<sup>3</sup>) 6 hours per day, 5 days per week for 13 weeks. Additional groups of six male and six female mice were exposed to 0, 0.15, 0.6, or 2.5 mg/m<sup>3</sup> for tissue burden studies. Final mean body weights of all exposure groups were similar to those of the controls. No chemical-related clinical findings were observed. Lymphocyte counts in 1.2 and 2.5 mg/m<sup>3</sup> males were minimally greater than that of the controls. Hemoglobin concentrations and erythrocyte counts in 0.3, 0.6, 1.2, and 2.5 mg/m<sup>3</sup> females were minimally greater than those of the controls. Absolute and relative lung weights of 1.2 and 2.5 mg/m<sup>3</sup> males and females were significantly greater than those of the controls. Inflammation and fibrosis were observed in the lung of 1.2 mg/m<sup>3</sup> males and 2.5 mg/m<sup>3</sup> males and females, as was inflammation in 1.2 mg/m<sup>3</sup> females, and the severity of these lesions generally increased with increasing exposure concentration. Interstitial infiltrates of lymphocytes were observed in mice exposed 0.6, 1.2, or 2.5 mg/m<sup>3</sup>. Lymphoid hyperplasia of the bronchial lymph nodes was observed in groups exposed to 1.2 or 2.5 mg/m<sup>3</sup>. Atrophy of the nasal olfactory epithelium occurred in 0.6, 1.2, and 2.5 mg/m<sup>3</sup> males and females, and incidences and severity generally increased with increasing exposure concentration.

At 13 weeks, nickel concentrations in the lung of exposed mice were significantly greater than those of the controls (males, 3 to 17  $\mu\text{g}$  nickel/g lung; females, 6 to 23  $\mu\text{g}/\text{g}$  lung), and these concentrations increased with increasing exposure concentration.

## 2-YEAR STUDY IN RATS

### *Survival, Body Weights, Clinical Findings, and Hematology*

Groups of 70 male and 70 female F344/N rats were exposed to 0, 0.15, or 1 mg nickel subsulfide/m<sup>3</sup> (equivalent to 0, 0.11, or 0.73 mg nickel/m<sup>3</sup>) by inhalation for 6 hours per day, 5 days per week for 101 weeks. Survival of exposed males and female rats was similar to that of the controls. Mean body weights of males and females exposed to 0.15 mg/m<sup>3</sup> were similar to those of the controls. Mean body weights of rats exposed to 1 mg/m<sup>3</sup> were less than those of the controls throughout the second year of the study. Chemical-related clinical findings included rapid and shallow breathing following exposure periods. Hematocrit values and hemoglobin concentrations in 1 mg/m<sup>3</sup> males and females and the erythrocyte count in 1 mg/m<sup>3</sup> males were mildly greater than those in the controls.

### *Pathology Findings*

In general, the absolute and relative lung weights of exposed males females were significantly greater than those of the controls at 7 and 15 months. There were exposure-related increases in the incidences of alveolar/bronchiolar adenoma in males, alveolar/bronchiolar carcinoma in males and females, and alveolar/bronchiolar adenoma or carcinoma (combined) in males and females at 2 years. Nonneoplastic lung lesions generally observed in exposed males and females included fibrosis; chronic active inflammation; focal alveolar epithelial hyperplasia, macrophage hyperplasia, and proteinosis; bronchial lymphoid hyperplasia; and interstitial inflammation.

At 2 years, the incidences of chronic active inflammation of the nose in 1 mg/m<sup>3</sup> females and of olfactory epithelial atrophy in 1 mg/m<sup>3</sup> males and females were significantly greater than those of the controls.

At 2 years, there were significant exposure-related increases in the incidences of benign pheochromocytoma, malignant pheochromocytoma, and benign or malignant pheochromocytoma

(combined) in males and of benign pheochromocytoma and benign or malignant pheochromocytoma (combined) in females.

The incidences of lymphoid hyperplasia of the bronchial lymph node in exposed males at 7 and 15 months and in exposed males and females at 2 years were significantly greater than those of the controls.

Incidences of macrophage hyperplasia in the bronchial lymph node of exposed males at 15 months and exposed males and females at 2 years were greater than those of the controls.

### *Tissue Burden Analyses*

Nickel concentrations in the lung of exposed rats were greater than those of the controls and generally increased with increasing exposure concentration at 7 (males, 6 to 9  $\mu\text{g}$  nickel/g lung; females, 6 to 9  $\mu\text{g}/\text{g}$  lung) and 15 months (males, 4 to 3  $\mu\text{g}$  nickel/g lung; females, 4 to 7  $\mu\text{g}/\text{g}$  lung). By 15 months, nickel concentrations in the lung had reached a steady state.

## 2-YEAR STUDY IN MICE

### *Survival, Body Weights, Clinical Findings, and Hematology*

Groups of 80 male and 80 female B6C3F<sub>1</sub> mice were exposed to 0, 0.6, or 1.2 mg nickel subsulfide/m<sup>3</sup> (equivalent to 0, 0.44, or 0.88 mg nickel/m<sup>3</sup>) by inhalation for 6 hours per day, 5 days per week for 105 weeks. Survival of exposed male and female mice was similar to that of the controls. Mean body weights of 0.6 and 1.2 mg/m<sup>3</sup> males and females were less than those of the controls throughout the second year of the study. Chemical-related clinical findings in male and female mice included labored respiration following exposure periods. The hematocrit value and the segmented neutrophil, monocyte, lymphocyte, and total leukocyte counts in 1.2 mg/m<sup>3</sup> females were greater than those in the controls.

### ***Pathology Findings***

Absolute and relative lung weights of exposed males and females were generally significantly greater than those of the controls at 7 and 15 months. The incidence of alveolar/bronchiolar carcinoma in 0.6 mg/m<sup>3</sup> females and the incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in 0.6 mg/m<sup>3</sup> males and 0.6 and 1.2 mg/m<sup>3</sup> females were significantly less than those of the controls. In general, the incidences of chronic active inflammation; bronchialization (alveolar epithelial hyperplasia), macrophage hyperplasia and proteinosis; interstitial infiltration; and fibrosis in exposed groups of males and females were greater than those of the controls at 7 and 15 months and at 2 years.

The incidences of atrophy of the nasal olfactory epithelium and inflammation of the nose in exposed mice were also generally greater than those of the controls. At 2 years, the incidences of degeneration of olfactory epithelium in exposed females were significantly less than that of the controls.

The incidences of lymphoid hyperplasia of the bronchial lymph node in 1.2 mg/m<sup>3</sup> males at 15 months, in 0.6 and 1.2 mg/m<sup>3</sup> females at 15 months, and in 0.6 and 1.2 mg/m<sup>3</sup> males and females at 2 years were significantly greater than those of the controls. The incidences of macrophage hyperplasia in 1.2 mg/m<sup>3</sup> males at 7 and 15 months, in 0.6 and 1.2 mg/m<sup>3</sup> females at 15 months, and in 0.6 and 1.2 mg/m<sup>3</sup> males and females at 2 years were significantly greater than those of the controls.

### ***Tissue Burden Analyses***

Nickel concentrations in the lung of exposed groups of mice were significantly greater than those of the controls at 7 (males, 10 to 11 µg nickel/g lung; females, 10 to 14 µg/g lung) and 15 months (males, 15 to 26 µg nickel/g lung; females, 12 to 20 µg/g lung), and these concentrations increased with increasing exposure concentration and with time.

## GENETIC TOXICOLOGY

Nickel subsulfide was considered to be equivocal in the *Salmonella* gene mutation assay overall. Sporadic weakly positive and equivocal responses were obtained in strain TA100 with and without S9 metabolic activation enzymes; all other strain/activation combinations gave negative results. No increase in the frequency of micronucleated erythrocytes was observed in peripheral blood samples from male or female mice exposed to nickel subsulfide by inhalation for 13 weeks.

## CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity\** of nickel subsulfide in male F344/N rats based on increased incidences of alveolar/bronchiolar adenoma, carcinoma, and adenoma or carcinoma (combined) and on increased incidences of benign, malignant, and benign or malignant (combined) pheochromocytoma of the adrenal medulla. There was *clear evidence of carcinogenic activity* of nickel subsulfide in female F344/N rats based on increased incidences of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) and an increased incidence of benign pheochromocytoma of the adrenal medulla. There was *no evidence of carcinogenic activity* of nickel subsulfide in male or female B6C3F<sub>1</sub> mice exposed to 0.6 or 1.2 mg/m<sup>3</sup>.

Exposure of rats to nickel subsulfide by inhalation for 2 years resulted in inflammation, hyperplasia, and fibrosis in the lung; inflammation and atrophy of the olfactory epithelium in the nose; and hyperplasia in the adrenal medulla (females). Exposure of mice to nickel subsulfide by inhalation for 2 years resulted in inflammation, bronchialization, hyperplasia, and fibrosis in the lung and inflammation and atrophy of the olfactory epithelium in the nose.

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\* Explanation of Levels of Evidence of Carcinogenic Activity is on page 16.

## Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Nickel Sub sulfide

Variable	Male F344/N Rats	Female F344/N Rats	Male B6C3F <sub>1</sub> Mice	Female B6C3F <sub>1</sub> Mice
Doses	0, 0.15, or 1 mg nickel subsulfide/m <sup>3</sup> (0, 0.11, or 0.73 mg nickel/m <sup>3</sup> )	0, 0.15, or 1 mg nickel subsulfide/m <sup>3</sup> (0, 0.11, or 0.73 mg nickel/m <sup>3</sup> )	0, 0.6, or 1.2 mg nickel subsulfide/m <sup>3</sup> (0, 0.44, or 0.88 mg nickel/m <sup>3</sup> )	0, 0.6, or 1.2 mg nickel subsulfide/m <sup>3</sup> (0, 0.44, or 0.88 mg nickel/m <sup>3</sup> )
Body weights	1 mg/m <sup>3</sup> group lower than controls	1 mg/m <sup>3</sup> group lower than controls	0.6 and 1.2 mg/m <sup>3</sup> groups lower than controls	0.6 and 1.2 mg/m <sup>3</sup> groups lower than controls
2-Year survival rates	13/53, 21/53, 18/53	25/53, 25/53, 28/52	26/61, 25/60, 26/60	36/58, 34/60, 38/60
Nonneoplastic effects	<u>Lung</u> : chronic active inflammation (9/53, 53/53, 51/53); focal alveolar epithelial hyperplasia (2/53, 6/53, 11/53); macrophage hyperplasia (9/53, 48/53, 52/53); fibrosis (2/53, 48/53, 40/53) <u>Nose</u> : chronic active inflammation (12/53, 10/53, 18/52); olfactory epithelial atrophy (2/53, 1/53, 9/52)	<u>Lung</u> : chronic active inflammation (7/53, 51/53, 51/53); focal alveolar epithelial hyperplasia (2/53, 10/53, 11/53); macrophage hyperplasia (8/53, 51/53, 52/53); fibrosis (0/53, 50/53, 44/53) <u>Nose</u> : chronic active inflammation (6/53, 9/53, 20/52); olfactory epithelial atrophy (0/53, 0/53, 16/52) <u>Adrenal medulla</u> : hyperplasia (5/53, 11/53, 16/53)	<u>Lung</u> : chronic active inflammation (1/61, 52/59, 53/58); bronchialization (3/61, 53/59, 54/58); macrophage hyperplasia (6/61, 57/59, 58/58); fibrosis (0/61, 3/59, 16/58) <u>Nose</u> : acute inflammation (0/61, 0/59, 3/59); olfactory epithelial atrophy (1/61, 27/59, 55/59)	<u>Lung</u> : chronic active inflammation (1/58, 46/59, 58/60); bronchialization (3/58, 53/59, 58/60); macrophage hyperplasia (5/58, 57/59, 60/60); fibrosis (0/58, 7/59, 17/60) <u>Nose</u> : acute inflammation (0/58, 11/59, 14/60); olfactory epithelial atrophy (1/58, 11/59, 41/60)
Neoplastic effects	<u>Lung</u> : alveolar/ bronchiolar adenoma (0/53, 3/53, 6/53); alveolar/ bronchiolar carcinoma (0/53, 3/53, 6/53); alveolar/bronchiolar adenoma or carcinoma (0/53, 6/53, 11/53) <u>Adrenal medulla</u> : benign pheochromocytoma (13/53, 30/52, 38/53); malignant pheochromocytoma (0/53, 2/52, 10/53); benign or malignant pheochromocytoma (14/53, 30/52, 42/53)	<u>Lung</u> : alveolar/ bronchiolar carcinoma (0/53, 0/53, 4/53); alveolar/bronchiolar adenoma or carcinoma or squamous cell carcinoma (2/53, 6/53, 9/53); <u>Adrenal medulla</u> : benign pheochromocytoma (2/53, 7/53, 36/53)	None	None

**Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Nickel Subulfide (continued)**

Variable	Male F344/N Rats	Female F344/N Rats	Male B6C3F, Mice	Female B6C3F, Mice
Level of evidence of carcinogenic activity	Clear evidence	Clear evidence	No evidence	No evidence
Genetic toxicology				
<i>Salmonella typhimurium</i> gene mutations:			Equivocal with and without S9 in strain TA100, negative with and without S9 in strains TA97, TA98, TA102, and TA1535	
Micronucleated erythrocytes Mouse peripheral blood <i>in vivo</i> :			Negative in male and female mice	

## EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (clear evidence and some evidence); one category for uncertain findings (equivocal evidence); one category for no observable effects (no evidence); and one category for experiments that cannot be evaluated because of major flaws (inadequate study). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- Clear evidence of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- Some evidence of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- Equivocal evidence of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- No evidence of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- Inadequate study of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

## NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on nickel subsulfide on November 29, 1994, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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**SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS**

**NOTE:** A summary of the Technical Reports Review Subcommittee's remarks will appear in a future draft of this report.

# INTRODUCTION



## NICKEL SUBSULFIDE

CAS No. 12035-72-2

Chemical Formula:  $\text{Ni}_3\text{S}_2$       Molecular Weight: 240.25

**Synonyms:** Heazlewoodite, nickel subsulphide, nickel sulfide (3:2),  $\alpha$ -nickel sulfide (3:2) crystalline, nickel sulphide, nickel trisulphide, trinickel disulfide

## CHEMICAL AND PHYSICAL PROPERTIES

Nickel subsulfide is a black powder with a melting point of 790° C and a density of 5.82 g/cm<sup>3</sup>. It is insoluble in water but soluble in acid (USEPA, 1986). The mass median aerodynamic diameter of nickel subsulfide used in these studies was  $2.4 \pm 2.2 \mu\text{m}$ .

## PRODUCTION, USE, AND HUMAN EXPOSURE

Nickel was first isolated in 1751 and is found primarily as an oxide (laterite) or sulfide ore (pentlandite) (NIOSH, 1977; Warner, 1984; U.S. Bureau of Mines, 1984, 1985a). In 1991, the six largest nickel producing countries were the Soviet Union, Canada, Australia, New Caledonia, Indonesia, and Cuba. Approximately 55% of the nickel currently used is extracted from sulfide ore, and the remainder is extracted from oxide ore. The total annual world production of nickel is estimated at 1,000,000 tons (907,000 metric tons) (U.S. Bureau of Mines, 1991).

The United States consumption of nickel is approximately 200,000 tons (180,000 metric tons) annually (U.S. Bureau of Mines, 1991). The United States consumes unwrought nickel (68%), ferronickel (17.3%), nickel oxide (11.4%), nickel salts (1.2%), and other forms (2.1%) (U.S. Bureau of Mines, 1984, 1985b). The National Occupational Exposure Survey (NIOSH) reported that 56,843 and 18,165 United States workers are potentially exposed to nickel sulfate and nickel oxide, respectively (information on nickel subsulfide exposure not reported) (NIOSH, 1994).

Half of the nickel sold per year is used to make stainless steel (Warner, 1984), which contains up to 8% nickel. The ability of nickel to impart corrosion resistance and strength leads to its wide use in chemicals and allied products and in petroleum refining (24%); in electrical equipment and supplies (11%); in aircraft and parts (10%); in construction (10%); in fabricated metal products (9%); household appliances (8%); machinery (7%); in ship and boat building (4%) and miscellaneous uses (7%) (U.S. Bureau of Mines, 1984).

Nickel constitutes about 0.008% of the earth's crust. Low levels of nickel are found in air, soil, water, food, and household objects. The average concentration of nickel in finished drinking water is less than 10 ppb. Nickel concentration in United States air has been found to range from 1 to 86 ng/m<sup>3</sup>. The most probable nickel species present in the atmosphere include nickel oxide and nickel sulfate, and the most probable species found in water includes nickel sulfate hexahydrate (ATSDR, 1992). The average amount of nickel in mainstream particulate fractions of cigarette smoke is 79 ng/cigarette (Bache *et al.*, 1985). Dietary intake of nickel per person from foods is estimated at 170 µg per day; intake from inhalation is estimated at 0.1 to 1 µg nickel per day (excluding cigarette smoke), and intake from drinking water is estimated at 2 µg per day (ATSDR, 1992). Nickel is listed as one of the most frequently occurring chemicals in waste deposit sites in the United States (*Fed. Regist.*).

The threshold limit values adopted by the American Conference of Government Industrial Hygienists (ACGIH) are 1 mg/m<sup>3</sup> for nickel metal and water-insoluble salts and 0.1 mg/m<sup>3</sup> for water-soluble salts, but the ACGIH published notice of an intended change to 0.05 mg nickel/m<sup>3</sup> for water-soluble and water-insoluble nickel compounds (ACGIH, 1993). NIOSH recommended that the permissible exposure limit for nickel be reduced to 0.015 mg nickel/m<sup>3</sup> averaged over a work shift of up to 10 hours per day, 40 hours per week (NIOSH, 1977).

Atomic absorption spectroscopy is a widely used method for quantifying nickel in the environment and in the workplace. This method of analysis measures total nickel without discerning the forms of nickel present, and most studies of environmental or industrial exposure report total nickel and not the occurrence of individual nickel species (ATSDR, 1992).

## ABSORPTION, DISTRIBUTION, AND EXCRETION

### *Experimental Animals*

Animal model systems have been used to obtain information on the absorption, distribution, and excretion of nickel after inhalation exposure (water-soluble and water-insoluble forms of nickel), oral exposure (water-soluble forms of nickel), and dermal exposure (water-soluble forms of nickel).

Intratracheal administration of nickel compounds was one method used by several investigators to study the fate of nickel in the lung. English *et al.* (1981) reported on a comparative toxicokinetic study after intratracheal administration of [<sup>63</sup>Ni]-labeled nickel chloride or nickel oxide (low temperature nickel oxide calcined at 250° C) in Wistar rats. Nickel, after nickel chloride administration, was excreted primarily in the urine. After nickel oxide administration, nickel was equally excreted in the feces and urine. Nickel oxide persisted in the lung for more than 90 days, while nickel chloride was rapidly excreted from the lung with greater than 50% of the nickel cleared from the lungs within 3 days.

Nickel chloride administered as an intratracheal dose to Sprague-Dawley rats was excreted primarily in the urine. By day 3, 90% of the instilled chemical was eliminated from the lungs. The lungs retained 29% of their initial burden at day 1, and this decreased to 0.1% on day 21; 96% of the chemical was excreted in the urine (Carvalho and Ziemer, 1982).

The pulmonary clearance of intratracheally administered nickel subsulfide ( $\text{Ni}_3\text{S}_2$ ) in mice has two distinct components with initial and final biological half-lives corresponding to 1.2 and 12.4 days, respectively. The excretion of the chemical (measured as  $^{63}\text{Ni}$ ) was 60% in the urine and 40% in the feces; 57% of the administered dose was excreted after 3 days with 33% appearing in the urine (Valentine and Fisher, 1984). In another experiment, the calculated clearance times of nickel subsulfide administered intratracheally to mice was also biphasic with a clearance half-life of 2 hours for the first phase and 119 hours for the second phase (Finch *et al.*, 1987).

In F344/N rats administered [ $^{63}\text{Ni}$ ]-labeled nickel oxide (high temperature, green oxide) or nickel subsulfide by pernasal inhalation, the lung half-life was estimated at 120 days for nickel oxide and 5 days for nickel subsulfide (Benson *et al.*, 1994; Appendix P). Benson *et al.* (1994) found that, following nickel oxide exposure, nickel was not distributed to the extrarepiratory tract tissue, and the material was only excreted in the feces during the first few days after exposure. In contrast, after nickel subsulfide exposure, nickel was detected in extrarepiratory tract tissue including blood and kidney, and nickel was excreted in the urine and the feces. The half-life of [ $^{63}\text{Ni}$ ]-labeled nickel sulfate administered to F344/N rats by pernasal administration was 1 to 3 days; nickel was present in extrarepiratory tract tissues (including blood, kidney, and intestine); and urine was the major route for excretion of nickel (Medinsky *et al.*, 1987).

Other studies also indicated that nickel oxide has a relatively long half-life in the rodent lung. Nickel oxide (formed at 550° C; mass median aerodynamic diameter (MMAD) of 0.15  $\mu\text{m}$ , geometric standard

deviation ( $\sigma_g$ ) of 1.5) given as an aerosol of  $750 \mu\text{g}/\text{m}^3$  to Wistar rats had a bronchial clearance half-life of 1 day and an alveolar clearance half-life of 36 days (Hochrainer *et al.*, 1980). Hochrainer *et al.* (1980) estimated that with continuous exposure to nickel oxide, a steady state would be reached after 1 year.

In Wistar rats after exposure to 0.6 or 8.0 mg nickel oxide/ $\text{m}^3$  (high temperature, green oxide; MMAD of  $1.2 \mu\text{m}$ ,  $\sigma_g$  of 2.5) for 6 to 7 hours per day for 1 to 2 months, the lung clearance was estimated to be 100  $\mu\text{g}$  per year. There was no apparent deposition of nickel in the liver, kidney, spleen, heart, brain, or blood (Kodama *et al.*, 1985). Lung clearance half-lives for nickel oxide (high temperature, green oxide) in Wistar rats exposed for 1 month were estimated to be 8, 11, and 21 months for nickel oxide with a MMAD of 0.6, 1.2, and 4.0  $\mu\text{m}$ , respectively (Tanaka *et al.*, 1985, 1988).

In summary, in absorption and distribution studies for nickel administered intratracheally or by inhalation exposure, the lung half-life was 1 to 3 days for nickel sulfate, 5 days for nickel subsulfide, and greater than 100 days for nickel oxide. Nickel was detected in extrapulmonary tract tissue after exposure to nickel sulfate or nickel subsulfide, but not after exposure to nickel oxide.

The present studies also report findings on the deposition of nickel sulfate hexahydrate and nickel oxide in the lungs and tissues of rats and mice after 16 days, 13 weeks, and at 7 and 15 months in the 2-year studies. These data show a relatively short half-life in the lung for nickel sulfate hexahydrate, a longer half-life for nickel subsulfide, and the longest half-life for nickel oxide (Benson *et al.*, 1987; Dunnick *et al.*, 1989).

Studies of other routes of nickel exposure in rats, mice, and dogs indicate that 1% to 10% given as nickel sulfate hexahydrate or nickel chloride was absorbed after oral administration, and only a small percentage (<1%) of nickel chloride was absorbed through the skin of guinea pigs within 24 hours (ATSDR, 1992; Nielsen *et al.*, 1993).

### *Humans*

In the industrial setting, a major route of nickel exposure in humans is by inhalation (Sunderman, 1992); it is estimated that 35% of inhaled nickel is absorbed into the blood from the respiratory tract (Bennet, 1984; Grandjean, 1984; Sunderman and Oskarsson, 1991). There is evidence that, in nickel refinery workers, there are storage depots in the body that retain nickel for long periods of time; nickel was excreted in the urine of workers for periods of up to 6 months after facility closing (Morgan and Rouge, 1983). There were elevated nickel concentrations in specimens of urine, plasma, and nasal mucosa biopsies obtained from retired workers years after cessation of employment, although the specific form of nickel to which these workers were exposed was not identified (Torjussen and Andersen, 1979; Boysen *et al.*, 1984).

Andersen and Svenes (1989) found elevated levels of nickel in the lung of nickel refinery workers, although workers who were diagnosed as having lung cancer had the same concentrations of nickel in the lung at autopsy as those who died of other types of cancer. In the workplace setting, exposure to nickel is monitored by analyzing urine, hair, or fingernails for levels of total nickel.

When nickel sulfate was administered to human volunteers, 27% of the administered dose was absorbed when given in drinking water, while only 0.7% was absorbed when administered in food. The elimination half-life for absorbed nickel averaged 28 hours; 100% of the absorbed nickel was eliminated in either the feces or urine within 4 days (Sunderman, 1989, 1992). In studies in humans, reported absorption of radioactive nickel varied from 55% to 77% of nickel sulfate applied to occluded skin to 3% of nickel chloride applied to occluded skin (ATSDR, 1992).

### **TOXICITY**

Studies of nickel toxicity after experimental or industrial exposure have been summarized in various reviews (NAS, 1975; IARC, 1976, 1984, 1987, 1990; NIOSH, 1977; Brown and Sunderman, 1985;

USEPA, 1986; European Chemical Industry, 1989; WHO, 1991; ATSDR, 1992; Nieboer and Nriagu, 1992). In experimental animals and in humans, the primary toxic response to nickel after inhalation occurred in the respiratory system.

Information on the dissolution half-lives for nickel subsulfide and nickel oxide in water and rat serum have been reported. The calculated dissolution half-lives (based on *in vitro* studies) for nickel subsulfide and nickel oxide in water are greater than 7 or 11 years, respectively. In rat serum, the estimated dissolution half-life is 23 days for nickel subsulfide and greater than 11 years for nickel oxide (Sunderman *et al.*, 1987). While nickel subsulfide and nickel oxide are both relatively insoluble in water, nickel subsulfide is more soluble than nickel oxide in biological fluids. Soluble nickel salts (nickel hydroxide) have been shown to be more soluble in human serum than nickel subsulfide (Kasprzak *et al.*, 1983). The comparative toxicity of nickel sulfate hexahydrate, nickel subsulfide, and nickel oxide parallels the solubility of the compounds in biological fluids.

### *Experimental Animals*

The acute toxicity values for selected nickel compounds are summarized in Table 1. Nickel carbonyl ( $\text{NiC}_4\text{O}_4$ ) is the most acutely toxic form of nickel, but the use or formation of this nickel compound in manufacturing processes is limited (NAS, 1975). Exposure to nickel oxide, nickel sulfate hexahydrate, or nickel subsulfide is more common in the workplace.

In animals, after inhalation exposure to water-soluble and water-insoluble nickel compounds, the primary toxic response is seen in the respiratory system. Changes in a variety of parameters, including dose-related reduction in body weight, reduced leukocyte count, increase in urine alkaline phosphatase and alkaline phosphatase, and increased erythrocyte count, were observed in Wistar rats continuously exposed to nickel oxide at 200, 400, or 800  $\mu\text{g}/\text{m}^3$  for 120 days (except for daily cleaning and feeding periods) (Weischer *et al.*, 1980).

**TABLE 1**  
**Toxicity Values for Nickel Carbonyl, Nickel Oxide, Nickel Sulfate Hexahydrate, Nickel Sulfate,**  
**and Nickel Subsulfide<sup>a</sup>**

Nickel Compound	Species	Route	Toxicity Value <sup>b</sup>	
Nickel carbonyl	Rat	Inhalation	35 ppm (LC <sub>50</sub> )	
		Subcutaneous	63 mg/kg (LD <sub>50</sub> )	
		Intravenous	66 mg/kg (LD <sub>50</sub> )	
		Intraperitoneal	39 mg/kg (LD <sub>50</sub> )	
	Mouse	Inhalation	67 mg/m <sup>3</sup> (LC <sub>50</sub> )	
	Dog	Inhalation	360 ppm (LCLo)	
Nickel oxide	Rat	Inhalation	1,890 mg/m <sup>3</sup> (LC <sub>50</sub> )	
		Rabbit	Inhalation	73 g/m <sup>3</sup> (LCLo)
		Subcutaneous	25 mg/kg (LD <sub>50</sub> )	
	Mouse	Intramuscular	180 mg/kg (TDLo)	
Intratracheal		90 mg/kg (TDLo)		
Nickel sulfate hexahydrate	Dog	Subcutaneous	500 mg/kg (LDLo)	
		Intravenous	89 mg/kg (LDLo)	
	Cat	Subcutaneous	500 mg/kg (LDLo)	
		Intravenous	72 mg/kg (LDLo)	
	Rabbit	Subcutaneous	500 mg/kg (LDLo)	
		Intravenous	36 mg/kg (LDLo)	
	Guinea pig	Subcutaneous	62 mg/kg (LDLo)	
	Nickel sulfate	Rat	Intraperitoneal	500 mg/kg (LD <sub>50</sub> )
		Mouse	Intraperitoneal	21 mg/kg (LD <sub>50</sub> )
			Intravenous	7 mg/kg (LDLo)
		Dog	Subcutaneous	38 mg/kg (LDLo)
			Intravenous	38 mg/kg (LDLo)
Cat		Subcutaneous	24 mg/kg (LDLo)	
Rabbit		Subcutaneous	33 mg/kg (LDLo)	
		Intravenous	33 mg/kg (LDLo)	

(continued)

**TABLE 1**  
**Toxicity Values for Nickel Carbonyl, Nickel Oxide, Nickel Sulfate Hexahydrate, Nickel Sulfate,**  
**and Nickel Sub sulfide (continued)**

Nickel Compound	Species	Route	Toxicity Value
Nickel subsulfide	Rat	Inhalation	1 mg/kg (TCLo)
		Subcutaneous	125 mg/kg (TDLo)
		Intravenous	10 mg/kg (TDLo)
		Intramuscular	20 mg/kg (TDLo)
	Mouse	Intramuscular	200 mg/kg (TDLo)

<sup>a</sup> From RTECS (1987)

<sup>b</sup> LC<sub>50</sub> = median lethal concentration; LCLo = lowest lethal concentration; LD<sub>50</sub> = median lethal dose; LDLo = lowest lethal dose; TCLo = lowest toxic concentration; TDLo = lowest toxic dose.

Alveolar macrophages from lung lavage fluid from rats exposed to nickel oxide at 120  $\mu\text{g}/\text{m}^3$  for 12 hours per day, 6 days per week for 28 days or by intratracheal injection (10 mg nickel oxide/ml and killed 1 week later) were examined by electron microscopy. Compared to controls, alveolar macrophages from exposed animals were increased in number and enlarged. In the cytoplasm of alveolar macrophages, phagosomes contained osmophilic nickel oxide particles as well as membranous and lamellar structures consistent with accumulation of phospholipid material (Migally *et al.*, 1982; Murthy and Niklowitz, 1983).

Respiratory toxicity to F344/Crl rats administered a single dose of either nickel subsulfide, nickel chloride, nickel sulfate, or nickel oxide by intratracheal instillation was evaluated by examining treatment-related changes in lung lavage fluid (Benson *et al.*, 1986). No significant changes in lung lavage fluid were seen after exposure to nickel oxide. After exposure to nickel subsulfide, nickel sulfate hexahydrate, and nickel chloride, there were increases in the following parameters in lung lavage fluid: lactate dehydrogenase,  $\beta$ -glucuronidase, total protein, glutathione reductase, glutathione peroxidase, and sialic acid. This evaluation was continued by exposing rats or mice to nickel oxide, nickel sulfate hexahydrate, or nickel subsulfide for 13 weeks and looking for treatment-related markers of lung toxicity in lung lavage fluid (Benson *et al.*, 1989; Appendix O). Increases in  $\beta$ -glucuronidase, total protein, neutrophil number,

and macrophage number were observed in the lavage fluid after exposure of rats and mice to all three nickel compounds, although there were quantitative differences in the magnitude of the response. Inflammation was observed histologically in the lung of rats and mice exposed to the three nickel compounds. The severity of lung toxicity as measured by the changes in lung lavage fluid paralleled the severity of histologic changes in the lung. Nickel sulfate hexahydrate was the most toxic, and nickel oxide was the least toxic (Benson *et al.*, 1989).

Treatment of rats and mice with water-soluble and water-insoluble nickel salts may cause an alteration of local and systemic immunity, and this toxicity has been studied under various conditions and experiments (Table 2).

Toxic responses to the immune system were measured in B6C3F<sub>1</sub> mice after inhalation exposure to nickel subsulfide, nickel oxide, or nickel sulfate hexahydrate for 6 hours per day and 5 days per week for 13 weeks. Exposure concentrations were 0.11, 0.45, and 1.8 mg nickel/m<sup>3</sup> for nickel subsulfide; 0.47, 2.0, and 7.9 mg nickel/m<sup>3</sup> for nickel oxide; and 0.027, 0.11, and 0.45 mg nickel/m<sup>3</sup> for nickel sulfate hexahydrate. Thymic weights in mice exposed to 1.8 mg nickel/m<sup>3</sup> of nickel subsulfide were lower than those of the controls. Lung-associated lymph nodes were increased in size after exposure to all compounds. The number of alveolar macrophages in lavage samples was increased in mice exposed to the highest concentrations of nickel sulfate hexahydrate and nickel oxide and to 0.45 and 1.8 mg nickel/m<sup>3</sup> nickel subsulfide. Numbers of antibody-forming cells in lung-associated lymph nodes of mice exposed to 2.0 and 7.9 mg nickel/m<sup>3</sup> nickel oxide and 1.8 mg nickel/m<sup>3</sup> nickel subsulfide were greater than those in the controls. Low numbers of antibody-forming cells were observed in the spleens of mice exposed to nickel oxide and in mice exposed to 1.8 ng nickel/m<sup>3</sup> nickel subsulfide. Only mice exposed to 1.8 mg nickel/m<sup>3</sup> nickel subsulfide had a low mixed lymphocyte response. All concentrations of nickel oxide resulted in low levels of alveolar macrophage phagocytic activity, as did 0.45 and 1.8 mg nickel/m<sup>3</sup> nickel subsulfide. None of the nickel compounds affected the phagocytic activity of peritoneal macrophages.

**TABLE 2**  
**Studies on the Immunologic Effects of Nickel Compounds**

Nickel Compound	Species/Route	Treatment	Response	Reference
<b>Cell-Mediated Immunity</b>				
Nickel chloride	CBA/J mice/ intramuscular	Single injection, 18 mg/kg	Reduced T-lymphocyte proliferation	Smialowicz <i>et al.</i> (1984)
	Guinea pig	<i>In vitro</i> study on spleen cells	Inhibited macrophage migration	Hennighausen and Lange (1980)
Nickel sulfate	B6C3F <sub>1</sub> mice (female)/oral	Up to 4,000 mg/kg/day for 23 weeks	Depressed spleen lymphoproliferative response to LPS (no effect on NK activity; PFC assay; mitogen response in spleen cells; resistance to <i>Listeria</i> challenge)	Dieter <i>et al.</i> (1988)
<b>Humoral Immunity</b>				
Nickel chloride	CBA/J mice/ intramuscular	Single injection, 18 mg/kg	Reduced antibody response to T-cell dependent sheep red blood cells	Smialowicz <i>et al.</i> (1984)
	C57BL/6J mouse spleen cells	<i>In vitro</i> exposure to nickel chloride	Decreased response	Lawrence (1981)
	Swiss albino mice/ intramuscular	3-12 $\mu$ g Ni/kg body weight followed by immunization with sheep red blood cells	Depressed antibody formation	Graham <i>et al.</i> (1975a)
	Swiss mice/ inhalation	2-hour inhalation exposure at 250 $\mu$ g/m <sup>3</sup>	Depressed antibody response to sheep red blood cells	Graham <i>et al.</i> (1978)
Nickel acetate	Sprague-Dawley rats/intraperitoneal	11 mg/kg body weight immunized with <i>E. coli</i> bacteriophage	Depressed circulating antibody response	Figoni and Treagan (1975)
Nickel oxide	Wistar rats/ inhalation	25-800 $\mu$ g/m <sup>3</sup> for 4 weeks to 4 months	Decreased ability to form spleen antibodies to sheep red blood cells	Speigelberg <i>et al.</i> (1984)
<b>Macrophage Function</b>				
Nickel chloride	CBA/J mice/ intramuscular	Single injection, 18 mg/kg	No effect on phagocytic capacity of peritoneal macrophages	Smialowicz <i>et al.</i> (1984)
	Rabbits	Alveolar macrophage <i>in vitro</i> exposure	Reduced viability of macrophages	Graham <i>et al.</i> (1975b)
Nickel oxide and nickel chloride	Wistar rats/ inhalation	12 hours/day, 6 days/week for 2 weeks at 0.1 mg/m <sup>3</sup>	Increased number of alveolar macrophages after nickel oxide; no change after nickel chloride	Bingham <i>et al.</i> (1972)
Nickel oxide	Wistar rats/ inhalation	800 $\mu$ g/m <sup>3</sup> for 2 weeks	Decrease in alveolar macrophage phagocytic ability	Speigelberg <i>et al.</i> (1984)

(continued)

**TABLE 2**  
**Studies on the Immunologic Effects of Nickel Compounds (continued)**

Nickel Compound	Species/Route	Treatment	Response	Reference
<b>Natural Killer Cell Activity</b>				
Nickel chloride	CBA/J and C57BL/6J mice/ intramuscular	Single injection, 18 mg/kg	Depressed NK activity (against Yac-1 murine lymphoma cells)	Smialowicz <i>et al.</i> (1984, 1985, 1986)
<b>Host Resistance</b>				
Nickel chloride and nickel oxide	CD mice and Sprague-Dawley rats/ inhalation	0.5 mg/m <sup>3</sup> for 2 hours	Enhanced respiratory infection to <i>Streptococcus</i>	Adkins <i>et al.</i> (1979)

Only 1.8 mg nickel/m<sup>3</sup> nickel subsulfide caused a depressed spleen natural killer cell activity. Results indicate that inhalation exposure of mice to nickel can have varying effects on the immune system, depending on dose and physicochemical form of the nickel compound, and these effects were observed at occupationally relevant exposure concentrations (Haley *et al.*, 1990; Appendix N).

Administration of nickel sulfate in the drinking water for 180 days (1 to 10 g/L drinking water, estimated to deliver 116 to 396 mg/kg body weight) resulted in a depressed proliferating response in the bone marrow and spleen of B6C3F<sub>1</sub> mice (Dieter *et al.*, 1988).

While experimental studies in animals show the potential of nickel to affect the immune system, the clinical significance of these studies in humans has not been determined (Nicklin and Nielsen, 1992). Further, there are no studies to examine if there is a relationship between effects on the immune system and the carcinogenic effects of nickel.

### *Humans*

Most of the toxicity information on nickel and nickel compounds came from studies of workers in nickel refineries where the primary toxicity is to the respiratory system. In the industrial setting, nickel exposures were associated with rhinitis, sinusitis, and nasal-septal perforations. Hypersensitive allergic asthmatic reactions to nickel are rare (Nemery, 1990). There were also reports of pulmonary fibrosis in workers inhaling nickel dust (WHO, 1991). While respiratory toxicity has been observed in workers exposed to nickel in the industrial setting, these workers are often exposed to other toxic metals and/or cigarette smoke, and it has not always been possible to conclude that nickel is the sole causative agent of toxicity (ATSDR, 1992).

Nickel contact hypersensitivity has been seen in the general population and in exposed workers. In the general population contact sensitivity to nickel-containing jewelry and/or prosthesis is another form of nickel toxicity (ATSDR, 1992). Other toxic reactions to nickel were reported in humans in isolated cases including: cardiovascular effects in a child ingesting nickel sulfate; and gastrointestinal effects, transient increases in blood reticulocytes, or muscular pain in workers exposed to nickel-contaminated water (ATSDR, 1992).

## CARCINOGENICITY

### *Experimental Animals*

The International Agency for Cancer Research (IARC, 1990) summarized the results of experimental studies which studied the carcinogenic potential of nickel compounds after local injection (e.g., subcutaneous or intramuscular injection). Nickel oxide, nickel subsulfide, nickel carbonyl, and nickel powder cause neoplasms at the injection site, while the soluble nickel salts such as nickel sulfate have generally not been associated with a carcinogenic response at the injection site. A portion of the IARC

(1990) listing and tabulation of over 100 experiments on the carcinogenic potential of nickel compounds is presented in Table 3.

Information for the carcinogenic potential of nickel oxide, nickel subsulfide, and nickel sulfate hexahydrate by inhalation exposure is limited. Ottolenghi *et al.* (1975) reported that nickel subsulfide (70% of particles were smaller than 1  $\mu\text{m}$  in diameter; 25% of particles were between 1 and 1.5  $\mu\text{m}$ ) caused an increased incidence in lung tumors in F344/N rats exposed to 1  $\text{mg}/\text{m}^3$  by inhalation (6 hours/day and 5 days/week for 108 weeks). In the exposed groups, 12% to 14% of the animals (208 animals examined histologically) had lung tumors compared to less than 0.5% of control animals (215 animals examined histologically). At the end of the 108-week exposure period, fewer than 5% of the animals in exposed groups were alive compared with a survival of 31% in control groups.

Other experimental studies indicated carcinogenic potential of nickel subsulfide for the respiratory tract mucosa. Yarita and Nettesheim (1978) reported that a single nickel subsulfide intratracheal dose of 1 or 3  $\text{mg}/\text{kg}$  caused tumors in heterotrophic tracheal transplants in female F344 rats. These authors noted that toxicity might decrease a carcinogenic response resulting in a misleadingly low carcinoma incidence, based on the finding that the more toxic dose (3  $\text{mg}/\text{kg}$ ) caused only a 1.5% incidence of carcinomas (there was a high incidence of tracheal hyperplastic change) versus a 10% carcinoma incidence in the 1  $\text{mg}/\text{kg}$  group (generally with only a low incidence of toxic lesions).

Hamsters exposed to 53  $\text{mg}$  nickel oxide/ $\text{m}^3$  (median diameter of 0.3  $\mu\text{m}$ ; geometric standard deviation of 2.2) for 2 years did not have an increase in the incidence of lung tumors (Wehner *et al.*, 1975). The hamster may be less sensitive than the rat to the carcinogenic effects of nickel (Furst and Schlauder, 1971).

**TABLE 3**  
**Summary of Studies Used to Evaluate the Carcinogenicity of Nickel Compounds**  
**in Experimental Animals<sup>a</sup>**

Nickel Compound	Species/Route	Lesion Incidence <sup>b</sup>	Reference
<b>Nickel oxides and hydroxides</b>			
Nickel monoxide (green)	Rat/inhalation	0.6 mg/m <sup>3</sup> : 0/6 lung lesion 8 mg/m <sup>3</sup> : 1/8 lung lesion	Horie <i>et al.</i> (1985)
Nickel monoxide	Rat/inhalation	0.06 mg/m <sup>3</sup> : 0/40 lesion 0.2 mg/m <sup>3</sup> : 0/20 lesion	Glaser <i>et al.</i> (1986)
	Rat/intrapleural	Controls: 0/32 local lesions 31/32 local lesions	Skaug <i>et al.</i> (1985)
	Rat/intratracheal	Controls: 0/40 lesions 10 × 5 mg: 10/37 lung lesions 10 × 15 mg: 12/38 lung lesions	Pott <i>et al.</i> (1987)
	Rat/intramuscular	21/32 local lesions	Gilman (1962)
	Rat/intramuscular	2/20 local lesions	Gilman (1966)
	Rat/intramuscular	0/20 local lesions	Sosiński (1975)
	Rat/intramuscular	14/15 local lesions	Sunderman and McCully (1983)
	Rat/intramuscular	0/20 local lesions	Berry <i>et al.</i> (1984)
	Rat/subperiosteal	0/20 local lesions	Berry <i>et al.</i> (1984)
	Rat/intraperitoneal	46/47 local lesions	Pott <i>et al.</i> (1987)
	Rat/intraperitoneal	25 mg: 12/34 local lesions 100 mg: 15/36 local lesions	Pott <i>et al.</i> (1989, 1992)
Nickel monoxide (green)	Rat/intrarenal	0/12 local lesions	Sunderman <i>et al.</i> (1984)
Nickel monoxide	Mouse/intramuscular	33/50 and 23/52 local lesions	Gilman (1962)
	Hamster/inhalation	1/51 osteosarcoma	Wehner <i>et al.</i> (1975, 1979)
	Hamster/intratracheal	Controls: 4/50 lung lesions 1/49 lung lesions	Farrell and Davis (1974)
Nickel hydroxide	Rat/intramuscular	15/20 local lesions	Gilman (1966)
	Rat/intramuscular	Dried gel: 5/19 local lesions Crystalline: 3/20 local lesions Colloidal: 0/13 local lesions	Kasprzak <i>et al.</i> (1983)
Nickel trioxide	Rat/intramuscular	0/10 local lesions	Judde <i>et al.</i> (1987)
	Rat/intracerebral	3/20 local lesions	Sosiński (1975)

(continued)

**TABLE 3**  
**Summary of Studies Used to Evaluate the Carcinogenicity of Nickel Compounds**  
**in Experimental Animals (continued)**

Nickel Compound	Species/Route	Lesion Incidence	Reference
<b>Nickel sulfides</b>			
Nickel disulfide	Rat/intramuscular	12/14 local lesions	Sunderman (1984)
	Rat/intrarenal	2/10 local lesions	Sunderman <i>et al.</i> (1984)
Nickel sulfide (amorphous)	Rat/intramuscular	5.6 mg: 0/10 local lesions 22.4 mg: 0/10 local lesions	Sunderman and Maenza (1976)
$\beta$ -Nickel sulfide	Rat/intramuscular	14/14 local lesions	Sunderman (1984)
Nickel sulfide (amorphous)	Rat/intramuscular	3/25 local lesions	Sunderman (1984)
Nickel sulfide	Rat/intrarenal	0/18 local lesions	Jasmin and Riopelle (1976)
$\beta$ -Nickel sulfide	Rat/intrarenal	8/14 local lesions	Sunderman <i>et al.</i> (1984)
Nickel sulfide (amorphous)	Rat/intrarenal	0/15 local lesions	Sunderman <i>et al.</i> (1984)
Nickel subsulfide	Rat/inhalation	14/208 malignant lung lesions 15/208 benign lung lesions	Ottolenghi <i>et al.</i> (1975)
	Rat/intratracheal	0.94 mg: 7/47 lung lesions 1.88 mg: 13/45 lung lesions 3.75 mg: 12/40 lung lesions	Pott <i>et al.</i> (1987)
	Rat/intrapleural	28/32 local lesions	Skaug <i>et al.</i> (1985)
	Rat/subcutaneous	3.3 mg: 37/39 local lesions 10 mg: 37/40 local lesions	Mason (1972)
	Rat/subcutaneous	18/19 local lesions	Shibata <i>et al.</i> (1989)
	Rat/intramuscular	25/28 local lesions	Gilman (1962)
	Rat/intramuscular	Controls: 1/19 local lesion 10 mg powder: 19/20 local lesions 10 mg diffusion chamber: 14/17 local lesions 500 mg fragments: 5/7 local lesions 500 mg discs: 14/17 local lesions	Gilman and Herchen (1963)

(continued)

**TABLE 3**  
**Summary of Studies Used to Evaluate the Carcinogenicity of Nickel Compounds**  
**in Experimental Animals (continued)**

Nickel Compound	Species/Route	Lesion Incidence	Reference
Nickel sulfides (continued)			
Nickel subsulfide (disc)	Rat/intramuscular	Removal of disc after 64 days: 4/10 local lesions Removal of disc after 128 days: 7/10 local lesions Removal of disc after 206 days: 10/10 local lesions	Herchen and Gilman (1964)
Nickel subsulfide	Rat/intramuscular	NIH black: 28/28 local lesions Hooded: 14/23 local lesions	Daniel (1966)
	Rat/intramuscular	3.3 mg: 38/39 local lesions 10 mg: 34/40 local lesions	Mason (1972)
	Rat/intramuscular	5 mg: 8/20 local lesions 20 mg: 9/9 local lesions	Sunderman and Maenza (1976)
	Rat/intramuscular	Fischer: 59/63 local lesions Hooded: 11/20 local lesions	Yamashiro <i>et al.</i> (1980)
	Rat/intramuscular	0.6 mg: 7/30 local lesions 1.2 mg: 23/30 local lesions 2.5 mg: 28/30 local lesions 5 mg: 29/30 local lesions	Sunderman <i>et al.</i> (1976)
	Rat/intramuscular	0.63 mg: 7/29 local lesions 20 mg: 9/9 local lesions	Sunderman (1981)
	$\alpha$ -Nickel subsulfide	Rat/intramuscular	9/9 local lesions
Nickel subsulfide	Rat/intramuscular	10/20 local lesions	Berry <i>et al.</i> (1984)
	Rat/intramuscular	2/100 local lesions	Judde <i>et al.</i> (1987)
	Rat/intramuscular	19/20 local lesions	Shibata <i>et al.</i> (1989)
	Rat/intraperitoneal	9/37 local lesions	Gilman (1966)
	Rat/intraperitoneal	27/42 local lesions	Pott <i>et al.</i> (1987)
	Rat/intraperitoneal	6 mg: 20/36 local lesions 12 mg: 25/35 local lesions 25 mg: 25/34 local lesions	Pott <i>et al.</i> (1989, 1992)
	Rat/subperiosteal	0/20 local lesions	Berry <i>et al.</i> (1984)
	Rat/intrafemoral	10/20 local lesions	Berry <i>et al.</i> (1984)
	Rat/intrarenal	In glycerin: 7/16 local lesions In saline: 11/24 local lesions	Jasmin and Ruopelle (1976)
(continued)			

**TABLE 3**  
**Summary of Studies Used to Evaluate the Carcinogenicity of Nickel Compounds**  
**in Experimental Animals (continued)**

Nickel Compound	Species/Route	Lesion Incidence	Reference
Nickel sulfides (continued)			
$\alpha$ -Nickel subsulfide	Rat/intrarenal	Wistar Lewis: 7/11 local lesions NIH black: 6/12 local lesions Fischer 344: 9/32 local lesions Long-Evans: 0/12 local lesions	Sunderman <i>et al.</i> (1979)
Nickel subsulfide	Rat/intratesticular	16/19 local lesions	Damjanov <i>et al.</i> (1978)
	Rat/intraocular	14/15 local lesions	Albert <i>et al.</i> (1980); Sunderman (1983a)
	Rat/transplacental	No difference in lesion incidence	Sunderman <i>et al.</i> (1981)
	Rat/pellet implantation into subcutaneous implanted tracheal grafts	5 mg: 9/60 local lesions 15 mg: 45/64 local lesions	Yarita and Neufshem (1978)
	Rat/intra-articular	16/19 local lesions	Shibata <i>et al.</i> (1989)
	Rat/intra-fat	9/20 local lesions	Shibata <i>et al.</i> (1989)
	Mouse/intratracheal	No increase in lung lesion incidence	Fisher <i>et al.</i> (1986)
	Mouse/subcutaneous	5 mg: 4/8 local lesions 10 mg: 7/8 local lesions	Oskarsson <i>et al.</i> (1979)
	Mouse/intramuscular	Swiss: 27/45 local lesions C3H: 9/18 local lesions	Gilman (1962)
	Mouse/intramuscular	5 mg: 4/8 local lesions 10 mg: 4/8 local lesions	Oskarsson <i>et al.</i> (1979)
$\alpha$ -Nickel subsulfide	Hamster/intratracheal	0/62 lung lesions	Muhle <i>et al.</i> (1992)
Nickel subsulfide	Hamster/intramuscular	Controls: 0/14 local lesions 5 mg: 4/15 local lesions 10 mg: 12/17 local lesions	Sunderman (1983b)
$\alpha$ -Nickel subsulfide	Hamster/topical	54 mg total: 0/6 local lesions 108 mg total: 0/7 local lesions 540 mg total: 0/15 local lesions 1080 mg total: 0/13 local lesions	Sunderman (1983a)
Nickel subsulfide	Rabbit/intramuscular	16 local lesions	Hildebrand and Besere (1979a,b)
(continued)			

**TABLE 3**  
**Summary of Studies Used to Evaluate the Carcinogenicity of Nickel Compounds**  
**in Experimental Animals (continued)**

Nickel Compound	Species/Route	Lesion Incidence	Reference
Nickel sulfides (continued)			
$\alpha$ -Nickel subsulfide	Rabbit/intramuscular	0/4 local lesions	Sunderman (1983b)
Nickel subsulfide	Salamander/intraocular	7/8 local lesions	Okamoto (1987)
Nickel ferrosulfide	Rat/intramuscular	15/15 local lesions	Sunderman (1984)
	Rat/intrarenal	1/12 local lesions	Sunderman <i>et al.</i> (1984)
Nickel salts			
Basic nickel carbonate tetrahydrate	Rat/intraperitoneal	Controls: 1/33 lung lesions	Pott <i>et al.</i> (1989, 1992)
		25 mg: 1/35 lung lesions	
		50 mg: 3/33 lung lesions	
Nickel acetate	Mouse/intraperitoneal	72 mg: 8/18 lung lesions	Stoner <i>et al.</i> (1976)
		180 mg: 7/14 lung lesions	
		360 mg: 12/19 lung lesions	
Nickel acetate tetrahydrate	Rat/intramuscular	1/35 local lesions	Payne (1964)
	Mouse/intraperitoneal	Controls: 0.32 lung lesions/animal 1.5 lung lesions/animal	Poirier <i>et al.</i> (1984)
Nickel acetate tetrahydrate	Rat/intraperitoneal	Controls: 1/33 lung lesions	Pott <i>et al.</i> (1989, 1992)
		25 mg: 3/35 lung lesions	
		50 mg: 5/31 lung lesions	
Nickel ammonium sulfate	Rat/intramuscular	0/35 local lesions	Payne (1964)
Nickel carbonate	Rat/intramuscular	6/35 local lesions	Payne (1964)
Nickel chloride	Rat/intramuscular	0/35 local lesions	Payne (1964)
Nickel chloride hexahydrate	Rat/intraperitoneal	Controls: 1/33 lung lesions 4/32 lung lesions	Pott <i>et al.</i> (1989, 1992)
Nickel chromate	Rat/intramuscular	1/16 local lesions	Sunderman (1984)
Nickel fluoride	Rat/intramuscular	3/18 local lesions	Gilman (1966)
Nickel sulfate	Rat/intramuscular	1/35 local lesions	Payne (1964)
	Rat/intramuscular	0/20 local lesions	Gilman (1966)
	Rat/intramuscular	0/20 local lesions	Kasprzak <i>et al.</i> (1983)
(continued)			

**TABLE 3**  
**Summary of Studies Used to Evaluate the Carcinogenicity of Nickel Compounds**  
**in Experimental Animals (continued)**

Nickel Compound	Species/Route	Lesion Incidence	Reference
<b>Nickel salts (continued)</b>			
Nickel sulfate hexahydrate	Rat/intramuscular	0/32 local lesions	Gilman (1962)
Nickel sulfate heptahydrate	Rat/intraperitoneal	Controls: 1/33 lung lesions 6/30 lung lesions	Pott <i>et al.</i> (1989, 1992)
<b>Other</b>			
Nickel carbonyl	Rat/inhalation	30 mg/m <sup>3</sup> for 32 weeks: 1/64 pulmonary lesions 60 mg/m <sup>3</sup> for 32 weeks: 1/32 pulmonary lesions 250 mg/m <sup>3</sup> once: 1/80 pulmonary lesion	Sunderman <i>et al.</i> (1957, 1959)
	Rat/inhalation	Controls: 0/32 lung lesions 1/71 lung lesions	Sunderman and Donnelly (1965)
	Rat/intravenous	19/120 lung lesions	Lau <i>et al.</i> (1972)

<sup>a</sup> From IARC (1990)

<sup>b</sup> Number of animals with lesion per effective number

Sunderman *et al.* (1959) found a low incidence of lung tumors in groups of Wistar rats exposed to nickel carbonyl (0.03 to 0.25 mg/m<sup>3</sup> for 30 minutes 3 times/week for 1 year). Follow-up studies also showed a low incidence of lung tumors in rats exposed to nickel carbonyl (Sunderman and Donnelly, 1965).

Information on the carcinogenic potential of nickel after oral administration is limited (IARC, 1990).

Life-long exposure to nickel acetate at low concentrations (5 ppm) induced no lung lesions in Swiss mice (Schroeder *et al.*, 1964; Schroeder and Mitchener, 1975); the maximum tolerated dose was not reached.

Ambrose *et al.* (1976) administered nickel sulfate hexahydrate in the diet of Wistar rats or dogs (0, 100, 1,000, 2,500 ppm) for 2 years, and no treatment-related lesions were observed.

### *Humans*

Exposure to nickel in the workplace has been associated with an increase in lung and nasal sinus tumors (IARC, 1976, 1987; Doll, 1990). Based on the finding of lung and/or nasal sinus tumors in nickel refinery workers, IARC classified nickel and nickel compounds as human carcinogens (Group 1), although there was insufficient information available to evaluate the carcinogenic risk for individual nickel compounds or the risk for cancer based on exposure to different concentrations of nickel compound(s) (IARC, 1987).

Information on the hazards associated with exposure to nickel came from studies on occupational exposure in nickel refineries and mines in Clydach, South Wales; Kristiansand, Norway; the International Nickel Company (INCO) refineries in Ontario, Canada; or from other studies of nickel refineries or mining operations throughout the world (Doll, 1984).

The United States Environmental Protection Agency (USEPA, 1986) and the International Committee on Nickel Carcinogenesis in Man (Doll, 1990) reviewed the epidemiological evidence for cancer after exposure to nickel in mining or refinery operations. A complete analysis on the type of ore mined and the calcining, smelting, and refining operations in 10 different mines or refineries throughout the world can be found in Doll (1990) and in other more recent summaries (Courtin, 1994; McIlveen and Negusante, 1994; Nieboer and Templeton, 1994; Norseth, 1994). Doll (1990) also estimates the type of nickel exposures encountered based on knowledge of the nickel process procedures used and a few relatively recent measurements of total airborne nickel.

The first indication that some form of nickel can give rise to lung and nasal sinus cancers was obtained from refinery workers at Clydach, South Wales (Bridge, 1933; Doll, 1958; Morgan, 1958). The Clydach Nickel Refinery (Mond Nickel Works) opened in 1902 and received nickel sulfide matte primarily from INCO (Port Colborne refinery, Canada). In 1933, nasal sinus and lung tumors were first noted in workers

who were employed prior to 1925. After 1925, the copper and sulfate content of the matte was reduced, the arsenic contamination in sulfuric acid used to extract copper was reduced, the use of respirators and masks was introduced, and improvements were made in factory design that reduced exposure to nickel (USEPA, 1986). An increased risk for lung and nasal sinus tumors was particularly noted in refinery work involving roasting, sintering, and calcining processes that converted impure nickel-copper matte to an oxide (Doll, 1990).

Peto *et al.* (1984) analyzed the incidence of lung and nasal sinus cancers found in workers in the Clydach plant and found the highest incidence of cancer in those workers employed in the copper sulfate and furnace areas. There was no increased risk to workers in the reduction area where nickel carbonyl concentrations were highest.

Other evidence for nasal sinus and lung cancer come from studies of workers in the INCO (Ontario, Canada) mines and refineries (Roberts *et al.*, 1989a,b; Muir *et al.*, 1994). Facilities operated include the Sudbury area mines (Copper Cliff Smelter and the Port Colborne refinery) that use an ore that is primarily pentlandite ( $\text{NiFeS}_2$ ). Men working in mining operations in Ontario had a two-fold increase in lung cancer risk, but no nasal sinus cancers (Doll, 1990).

The Falconbridge refinery in Kristiansand, Norway, receives nickel ore (a nickel copper sulfide matte) from Canada and uses an electrolysis process to refine the ore. Workers in roasting and smelting operations are exposed to dry dust containing nickel subsulfide and nickel oxide. Electrolysis workers are also exposed to nickel sulfate and nickel chloride. In this cohort, nasal sinus and lung cancer risks were increased in men working in the electrolysis department, thus implicating the soluble forms of nickel as the cause for the cancer (USEPA, 1986; Doll, 1990). The electrolysis workers had the highest average plasma and urine nickel concentrations (Høgerveit *et al.*, 1978).

Enterline and Marsh (1982) and Goldberg *et al.* (1994) studied cancer rates in a refinery in Huntington, West Virginia, which received nickel sulfide matte from Canada. The Doll Committee reported no clear evidence for an increased incidence in lung cancer in this population, although the data from this cohort provided weak evidence for an increased incidence in lung cancer in men exposed to sulfidic nickel at 4 mg nickel/m<sup>3</sup> for more than a year (Doll, 1990).

Results of epidemiology studies of workers in the nickel mining, smelting, and refinery operations in New Caledonia (French territory in the South Pacific) showed no increased incidence of lung or upper respiratory tract cancers. Nickel at this site is mined from nickel oxide or the silicate form of the ore. The Doll Committee also reported little evidence for an increased incidence in lung or upper respiratory tract cancer in this group of nickel workers (Doll, 1990).

The ten cohorts of nickel workers studied by the Doll Committee include the six cohorts mentioned above (nickel refinery operations, Clydach, South Wales; Falconbridge Nickel Mines, Ontario, Canada; INCO mines and refineries [Copper Cliff and Port Colborne], Ontario, Canada; Falconbridge refinery, Kristiansand, Norway; Huntington Alloys, West Virginia; and New Caledonia mines) as well as the Hanna Nickel Smelting Co., Oregon; Oak Ridge Gaseous Diffusion Plant, Tennessee; Outokumpu Oy nickel refinery, Finland; and Henry Wiggin Alloy Co., England (Doll, 1990).

The results within the individual cohorts varied, but the overall conclusion by the Doll Committee suggested that more than one form of nickel gives rise to lung and nasal sinus cancer. Much of the respiratory cancer risk was attributed to exposure to a mixture of oxidic and sulfidic nickel. Exposure to oxidic nickel in the absence of sulfidic nickel was also associated with increased lung and nasal sinus cancer risks. There was evidence that exposure to soluble nickel salts increased the risk of lung and nasal sinus cancer and that it may enhance risks associated with exposure to less soluble forms of nickel. There was no evidence that metallic nickel was associated with increased lung and nasal sinus cancer risks.

There was no evidence to suggest that exposure to metallic nickel or any of its compounds was likely to produce cancers elsewhere than in the lung or nose. These investigators were not able to provide dose-specific estimates of risks for individual nickel species. However, the evidence from these studies suggests that respiratory cancer risks are primarily related to exposure to water-soluble nickel compounds at concentrations in excess of 1 mg nickel/m<sup>3</sup> and to exposure to less soluble forms at concentrations greater than 10 mg nickel/m<sup>3</sup>.

There are no studies evaluating the potential carcinogenic effect in humans specifically after oral exposure to nickel (ATSDR, 1992).

While nickel and nickel compounds are classified by the IARC as Group 1 (human) carcinogens, the mechanism for this carcinogenic activity is not fully understood (Sunderman, 1989; Costa, 1991; Snow, 1992). The mechanisms involved in the induction of cancer by nickel compounds may be related to the ability of nickel ions to interact with chromatin proteins and/or the ability of nickel to generate intracellular oxidants (Costa *et al.*, 1994). Recent studies suggest that nickel generates free radicals, and the subsequent oxidative reactions lead to DNA damage and cancer. Studies show that: 1) incubation of nickel ions with cysteine under aerobic conditions generated hydroxyl radicals and carbon-centered alkyl radicals, suggesting free radicals are generated by nickel (II)-thiol complexes and molecular oxygen (Shi *et al.*, 1993); 2) in forward mutation assays with bacterial DNA, nickel ions produce tandem double CC → TT mutations consistent with damage to DNA by either ultraviolet irradiation or oxygen-free radicals (Tkeshelashvili *et al.*, 1993); 3) and, in *in vitro* studies, nickel ions induce increases in 8-hydroxy-2'-deoxyguanosine (8-OH-dG), a biomarker of oxidatively damaged DNA (Littlefield *et al.*, 1991).

After subcutaneous or intramuscular injection of nickel compounds, the water-insoluble nickel compounds are the most potent carcinogens. These findings may be related to the fact that water-insoluble nickel compounds are more readily phagocytized than are the water-soluble nickel salts, which passively diffuse

through the cell membrane. Phagocytized nickel particles are internalized in vacuoles whose acidity accelerates the dissolution of nickel ions and results in a higher concentration of nickel than would be achieved by the cellular uptake of water-soluble nickel salts (Costa *et al.*, 1994).

## REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

### *Experimental Animals*

Leonard and Jacquet (1984) reviewed studies which show that water-soluble nickel compounds administered orally or by peritoneal routes have the potential to cause embryotoxicity in rodents. In these studies, the nickel compounds were generally administered at higher doses than humans would be exposed to in drinking water or in the diet.

Studies in rodents have indicated that water-soluble nickel compounds can cross the placenta or be excreted in the milk of lactating animals. When [<sup>63</sup>Ni]-labeled nickel chloride was administered as an oral bolus dose (10  $\mu$ mol or 0.58 mg/kg body weight) to pregnant mice, the label was detected in various fetal tissues including liver, kidney, lung, brain, and heart. In another experiment, when [<sup>63</sup>Ni]-labeled nickel chloride was injected into pregnant mice, nickel was found to cross the placenta, and a marked uptake of nickel was seen in the embryo as measured by whole-body autoradiography (Olsen and Jonsen, 1979).

When nickel chloride hexahydrate was given as a single subcutaneous dose (10 to 100  $\mu$ mol NiCl<sub>2</sub>·6H<sub>2</sub>O/kg body weight or 23 mg/kg) to lactating rats, nickel was excreted in the milk and was found in the plasma of the pups (Dostal *et al.*, 1989). The doses used in these studies are higher than the average concentration of nickel found in drinking water in the United States (48  $\mu$ g/L water) (NAS, 1975).

Nickel chloride administered in the drinking water (50 and 250 ppm, estimated to deliver 7 or 31 mg/kg of nickel compound) to female rats for 11 weeks prior to mating and then during two successive gestation and lactation periods, caused an increase in the proportion of dead pups per litter (Smith *et al.*, 1993).

Other studies in rodents administered nickel chloride by intramuscular or intraperitoneal injection during gestation also showed developmental toxicity or fetal death. Nickel chloride injected intraperitoneally (1, 2, or 4 mg/kg body weight) to pregnant Wistar Porton rats on day 8, 12, or 16 of pregnancy caused skeletal retardation (poor ossification), hydrocephalus, hydronephrosis, heart defects, and hemorrhage. At these doses, there was an increase in maternal plasma glucose concentration (Mas *et al.*, 1985).

Nickel chloride injected intramuscularly (16 mg/kg) on day 8 of gestation to Fischer rats reduced the mean number of live pups per dam and diminished fetal body weights on day 20 (Sunderman *et al.*, 1978).

Nickel chloride injected into chicken eggs at doses of 0.02 to 0.8 mg per egg on days 0, 1, 2, 3, and 4 after fertilization caused malformations in the embryo examined at day 8 including exencephaly, everted viscera, abnormalities in the limb development, microphthalmia, and reduced body size (Gilani and Marano, 1980).

Groups of pregnant hamsters were exposed to nickel carbonyl by inhalation (0.06 mg/L/15 minutes) on days 4, 5, 6, 7, or 8 of gestation; dams were evaluated on day 15 of gestation. Teratogenic effects observed included cystic lung, exencephaly, cleft palate, and fused ribs. In another series of experiments, dams were allowed to deliver the pups; neonatal mortality was increased in the exposed groups (Sunderman *et al.*, 1980). Nickel carbonyl administered to pregnant dams by intravenous injection (11 mg/kg) on day 7 of gestation caused an increase in fetal mortality, diminished body weight of live pups, and increased incidences of fetal abnormalities including anophthalmia, microphthalmia, cystic lungs, and hydronephrosis (Sunderman *et al.*, 1983).

In a study of nickel oxide, Wistar rats were exposed to 1.6 mg nickel/m<sup>3</sup> by inhalation on gestation days 1 through 20. There was no evidence for embryotoxicity (Weischer *et al.*, 1980).

These and other studies show that water-soluble nickel salts have the potential to cause embryotoxicity in rodents. The metal can cross the fetomaternal barrier and enter the fetus. The embryotoxicity of nickel may be related to several factors including the mutagenic properties of nickel, direct effects on the mammalian embryo, or indirect effects through maternal toxicity. Further work is needed to understand the mechanisms for these effects (Leonard and Jacquet, 1984).

### *Humans*

Until recently, there have been few studies of reproductive effects in humans after exposure to nickel (ATSDR, 1992). A study of nickel refinery workers in Norway who were exposed to water-soluble nickel salts in electrolysis departments notes a suggested increased risk of pregnancy complications in female workers. The authors point out that the results of their studies should be considered preliminary data, and further investigations are needed (Chashschin *et al.*, 1994).

## GENETIC TOXICITY

Recent detailed reviews of the mutagenicity of nickel compounds and the possible mechanisms involved in the production of these effects were presented by Coogan *et al.* (1989), Christie and Katisifis (1990), Costa (1991), Snow (1992), and Costa *et al.* (1994). Nickel compounds are not typically detected as bacterial mutagens, but they often give positive results in *in vitro* assays designed to identify compounds that induce chromosomal damage in mammalian cells in the form of sister chromatid exchanges, chromosomal aberrations, and DNA strand breaks. Nickel salts have been shown to inhibit DNA replication and to increase replication errors in mammalian cells *in vitro*, possibly by competing with magnesium for essential binding sites on DNA polymerases (Christie *et al.*, 1991). In addition, positive results were demonstrated in mammalian cell forward mutation assays (TK locus in mouse lymphoma cells and hypoxanthine phosphoribosyl transferase locus in hamster V79 cells), although these responses are usually weak (Nishimura and Umeda, 1979; Amacher and Paillet, 1980; Morita *et al.*, 1991; Lee *et al.*,

1993). Insoluble crystalline nickel compounds are more active in genetic toxicity assays than the soluble or amorphous forms of nickel. Presumably, this differential activity derives from the more efficient entry of insoluble nickels into the cell through phagocytosis (Costa, 1991), longer retention of these compounds within the cell, and the consequent higher intracellular concentration of nickel (II) ions. Soluble nickel salts cannot be efficiently phagocytized, and do not accumulate in high concentration within the cell.

Based on the results of cell transformation studies in cultured rodent cells, Costa (1983) concluded that the nickel sulfide compounds must be in the crystalline, rather than in the amorphous state to be efficiently phagocytized into the cell and cause genetic damage. Particle size (Costa and Mollenhauer, 1980) and surface charge (Costa *et al.*, 1982) are also important factors in the phagocytosis of nickel compounds. Insoluble nickel compounds, once inside the cell, aggregate near the nucleus (Bryan, 1981; Evans *et al.*, 1982) where they are dissolved by lysosomes, releasing nickel (II) ions that proceed to effect DNA damage (Costa *et al.*, 1994).

The induced DNA damage resulting from nickel exposure has been attributed to one or more of the following mechanisms. It may follow the generation of short-lived reactive oxygen species inside the nucleus, produced by the oxidation of  $Ni^{+2}$  to  $Ni^{+3}$  by hydrogen peroxide or other oxidants subsequent to the binding of nickel ions to ligands such as amino acids, glutathione, and amino acid side chains of nuclear proteins (Biggart and Costa, 1986; Inoue and Kawanishi, 1989; Nieboer *et al.*, 1989; Cotellet *et al.*, 1992; Tkeshelashvili *et al.*, 1993; Sugiyama, 1994). The formation of persistent DNA-protein crosslinks is implicated in the generation of nickel (II)-induced DNA damage (Ciccarelli and Wetterhahn, 1982; Lee *et al.*, 1982; Patierno and Costa, 1985; Sen and Costa, 1986a). Factors involved in the binding of nickel ions to DNA, nuclear proteins, and other nuclear structures are reviewed by Coogan *et al.* (1989). The binding affinity of nickel to protein is far greater than its binding affinity to purified DNA (Eichorn and Shin, 1968) and therefore the mutagenic activity of nickel (II) ions probably derives in greater part from the binding of nickel to chromosomal protein rather than directly to DNA (Costa, 1991). Nickel binds preferentially to heterochromatic regions of the chromosomes such as the long arm of the

X chromosome in cultured Chinese hamster cells (Sen and Costa, 1986a,b; Sen *et al.*, 1987; Costa, 1991); binding of nickel ions to the long arm of the X chromosome and subsequent deletions in this region were postulated to cause the loss of a gene controlling senescence in cultured Chinese hamster cells and to promote immortality in transformed cultured Chinese hamster cell lines (Klein *et al.*, 1991). A schematic representation of some of the proposed mechanisms of nickel-induced genotoxicity, based upon the current understanding of the activities of nickel ions within mammalian cells, is presented in Figure 1. The genetic toxicity data for each of the three nickel compounds under study by the NTP are described below.

The mutagenicity data for nickel oxide are limited; however, there are clear indications of genotoxicity in some *in vitro* test systems. Although exposure to nickel oxide did not result in growth inhibition due to DNA damage in repair-deficient strains of *Bacillus subtilis* (Kanematsu *et al.*, 1980), an S-phase block (determined by flow cytometric analysis) was induced in cycling Chinese hamster ovary cells incubated with 5  $\mu\text{g}/\text{mL}$  nickel oxide (Costa *et al.*, 1982). No increase in gene mutations was detected at the ouabain resistance locus in C3H/10T<sub>1/2</sub> mouse embryo cells (Miura *et al.*, 1989) or at the HPRT locus in hamster V79 cells after exposure to nickel oxide (Kargacin *et al.*, 1993). However, positive effects were reported in mutation assays using a different site, the *gpt* gene, in V79 cells as the target for nickel oxide activity (Kargacin *et al.*, 1993). No induction of chromosomal aberrations was detected in human fibroblast or leukocyte cultures exposed to nickel oxide for 24, 48, or 72 hours (Paton and Allison, 1972); however, the experimental protocol used in this test was designed for water-soluble compounds and may not have been suitable for testing insoluble nickel oxide. Data from human epidemiology studies indicate that exposure to nickel oxide-containing fumes or smelter dusts may induce chromosomal aberrations (Waksvik *et al.*, 1984) and DNA-crosslinks (Costa *et al.*, 1993) in peripheral blood lymphocytes of workers, but the evidence is weak. The link between nickel oxide and these genetic endpoints is confounded because smelter dusts and welding fumes contain other nickel compounds as well as other metals such as chromium and magnesium. Also, the genetic effects noted were not correlated with nickel concentrations in urine or blood, whereas increased DNA-crosslink frequencies noted after exposure to

chromium-containing fumes, for example, were correlated with urine concentrations of the metal (Popp *et al.*, 1992).

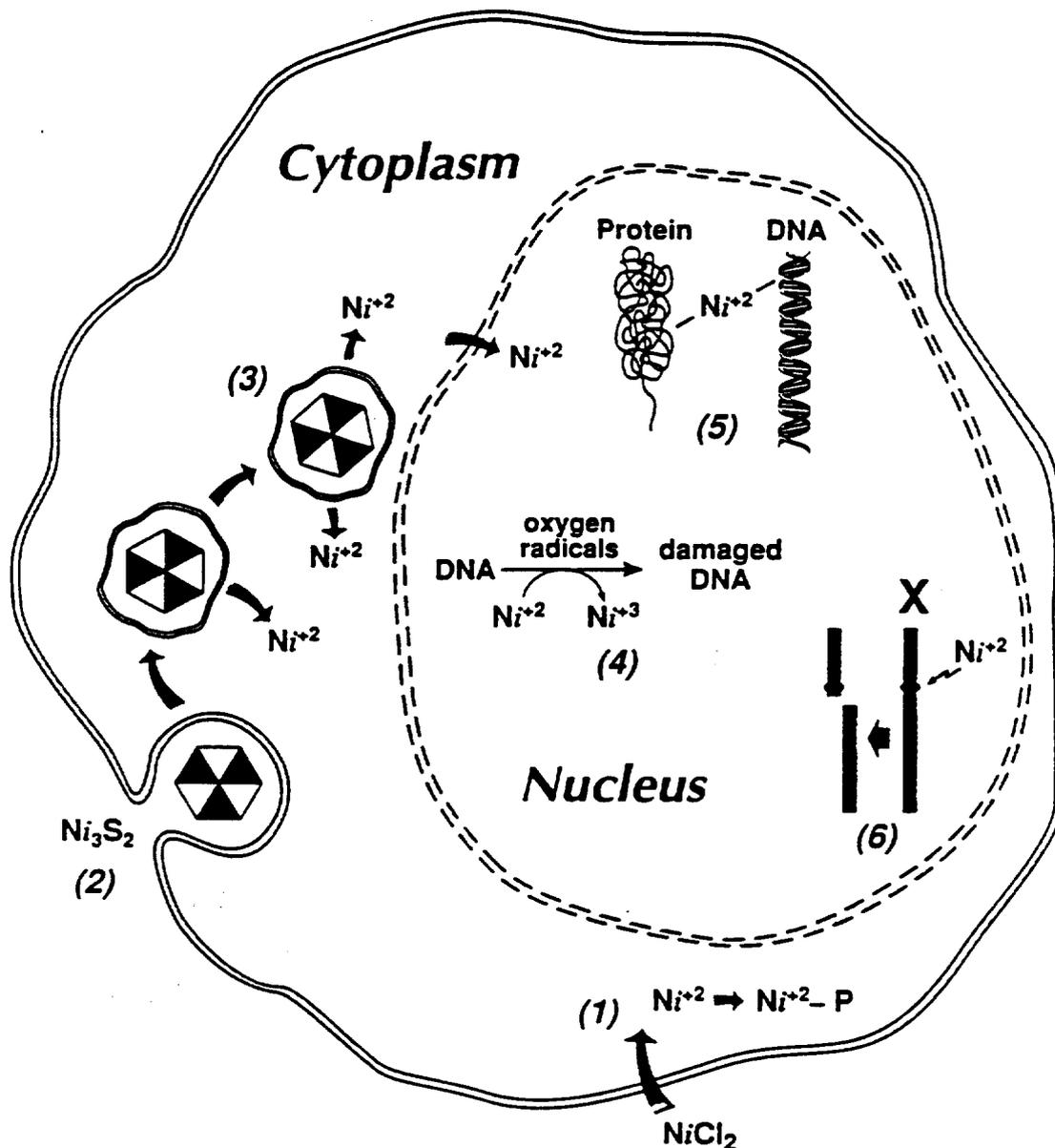
Nickel sulfate hexahydrate did not induce gene mutations in *Escherichia coli* or *Salmonella typhimurium* (Arlauskas *et al.*, 1985), and (in contrast to results reported for nickel oxide) no increases in *gpt* mutants were observed in hamster V79 cells treated with nickel sulfate hexahydrate (Christie, 1989; Lee *et al.*, 1993). However, nickel sulfate hexahydrate did induce mutations in L5178Y mouse lymphoma TK<sup>+</sup> cells, treated with 500 to 1,000  $\mu\text{g}/\text{mL}$  in the absence of S9 metabolic activation enzymes (McGregor *et al.*, 1988). In addition, nickel sulfate hexahydrate, administered by injection at doses of 200, 300, and 400 ppm, induced sex-linked recessive lethal mutations in germ cells of male *Drosophila* (Rodriguez-Arnaiz and Ramos, 1986). The pre- and post-meiotic cell stages were affected; the broods obtained from sperm cells undergoing meiosis at the time of treatment showed no evidence of increased lethal mutations. In another test for germ cell effects in male *Drosophila*, the test for sex chromosome loss, only the highest dose of nickel sulfate hexahydrate (400 ppm) resulted in the production of XO males (Rodriguez-Arnaiz and Ramos, 1986). Induction of sister chromatid exchanges and chromosomal aberrations was observed in hamster cells (Larramendy *et al.*, 1981; Ohno *et al.*, 1982), as well as human peripheral lymphocytes (Larramendy *et al.*, 1981) treated with nickel sulfate hexahydrate *in vitro*. However, no induction of DNA single strand breaks was detected in human xeroderma pigmentosum fibroblasts treated with 250  $\mu\text{g}/\text{mL}$  nickel sulfate hexahydrate (Fornace, 1982). *In vivo*, no induction of chromosomal aberrations was observed in rat bone marrow or spermatogonial cells after injection of nickel sulfate hexahydrate at doses that provided 3 or 6 mg nickel/kg body weight. Also, no change in the mitotic index of bone marrow cells was noted in treated animals (Mathur *et al.*, 1978).

As with the two nickel compounds discussed above, there are limited published mutagenicity data for the third nickel compound in the present studies, nickel subsulfide. However, results of *in vitro* tests performed with this insoluble nickel compound were mainly positive. In the *Salmonella typhimurium* gene

mutation assay, crystalline nickel subsulfide gave equivocal results in one study that used a preincubation protocol (Zeiger *et al.*, 1992) and negative results in a standard plate incorporation assay (Arrouijal *et al.*, 1990). It induced lethal mutations in *Paramecium tetraurelia*, without S9 (Smith-Sonneborn *et al.*, 1986) and unscheduled DNA repair in cultured Syrian hamster embryo cells (Robison *et al.*, 1983). Treatment of cultured Chinese hamster ovary cells for 24 hours with 10  $\mu\text{g}/\text{mL}$  nickel subsulfide resulted in an increase in the number of DNA strand breaks detected by alkaline sucrose gradient techniques (Robison *et al.*, 1982). Nickel subsulfide, in the absence of S9, was a weak inducer of hypoxanthine phosphoribosyl transferase mutations in cultured Chinese hamster ovary cells (Rossetto *et al.*, 1994) and sister chromatid exchanges in cultured human lymphocytes (Saxholm *et al.*, 1981). Nickel subsulfide induced significant dose-related increases in chromosomal aberrations (Arrouijal *et al.*, 1990) and micronuclei (Arrouijal *et al.*, 1992) in human lymphocytes *in vitro*. One reported *in vivo* test with nickel subsulfide, a measure of DNA synthesis inhibition in rats administered 10  $\mu\text{g}/\text{rat}$  (6 mg/100 g body weight) by intrarenal injection, was negative (Hui and Sunderman, 1980). A second *in vivo* study, a mouse bone marrow micronucleus test, reportedly produced positive results (Arrouijal *et al.*, 1990). This second study, however, employed only a single dose (250 mg/kg nickel subsulfide administered by intraperitoneal injection), and no confirmatory study was conducted.

## STUDY RATIONALE

The National Cancer Institute nominated nickel compounds for study because there was little information on the toxic and carcinogenic properties of specific nickel compounds after inhalation exposure. Nickel oxide (NTP, 1994a) and nickel sulfate hexahydrate (NTP, 1994b) were selected as compounds that are commonly found in the workplace in the United States. Nickel subsulfide was selected for study based on a previous study in which lung tumors were observed in rats (Ottolenghi *et al.*, 1975). The NTP toxicity and carcinogenicity studies of nickel oxide, nickel subsulfide, and nickel sulfate hexahydrate were performed to provide comparative toxicology and carcinogenicity information on these nickel compounds. The results of the nickel subsulfide studies are presented in this technical report.



**FIGURE 1**  
Possible Mechanisms of Nickel-Induced Genotoxicity

1. Soluble nickel compounds such as nickel chloride diffuse into the cell;  $\text{Ni}^{2+}$  ions are rapidly bound to cytoplasmic proteins (P) (Lee *et al.*, 1993). 2. Insoluble nickel compounds such as nickel subsulfide are phagocytosed into the cell and move toward the nucleus (Costa *et al.*, 1982). 3. Lysosomal breakdown of insoluble nickel compounds releases large quantities of  $\text{Ni}^{2+}$  ions which concentrate adjacent to the nuclear membrane (Costa and Heck, 1983). 4. Oxidative damage is induced in DNA by nickel ions bound to nuclear proteins ( $\text{Ni}^{2+} \rightarrow \text{Ni}^{3+}$ ), releasing active oxygen species (Tkeshelashvili *et al.*, 1993; Sugiyama, 1994). 5. DNA-protein crosslinks are produced by  $\text{Ni}^{2+}$  ions binding to heterochromatin (Lee *et al.*, 1982; Paterno and Costa, 1985; Sen and Costa, 1986a). 6. Binding of nickel ions to the heterochromatic regions of the long arm of the X chromosome, which may contain a senescence gene and a tumor suppressor gene, can cause deletion of all or part of this region, leading to an immortalization of the cell and clonal expansion (Conway and Costa, 1989; Klein *et al.*, 1991).

## DISCUSSION AND CONCLUSIONS

Workplace exposure to nickel subsulfide can occur during the unloading and crushing of nickel matte, and during roasting, sintering, calcining, and smelting operations (Doll, 1990). These NTP studies were performed to determine the toxicologic and carcinogenic properties of nickel subsulfide in rats and mice exposed by inhalation.

In the 16-day studies [0.6 to 10 mg/m<sup>3</sup> (equivalent to 0.44 to 7.33 mg nickel/m<sup>3</sup>)], one male rat and all male and female mice exposed to 10 mg nickel subsulfide/m<sup>3</sup> died before the end of the studies; these deaths were attributed to respiratory toxicity. In the 13-week studies, where the highest exposure concentration was 2.5 mg nickel subsulfide/m<sup>3</sup> (1.8 mg nickel/m<sup>3</sup>), there were no chemical-related deaths. In the 16-day and 13-week studies, general indications of respiratory toxicity included lung weights greater than those of the controls and incidences of inflammation, hyperplasia, and/or fibrosis in the lung, atrophy of the olfactory epithelium, and lymphoid hyperplasia in the respiratory tract lymph nodes that were greater than those in the controls. The biochemical indices for lung toxicity (as measured in lung lavage fluid) paralleled the toxicity identified by histopathologic analysis of the lung tissue (Benson *et al.*, 1989; Appendix O), and increased numbers of macrophages and neutrophils within lavage samples were consistent with the pulmonary chronic active inflammation.

In the companion 16-day and 13-week nickel compound studies, nickel sulfate hexahydrate was more toxic and nickel oxide was less toxic than nickel subsulfide (Tables 32 and 33). The lung and nasal toxicity reflects the relative solubility of the nickel compounds in water and biological fluids, with the most soluble nickel compound (nickel sulfate hexahydrate) being the most toxic. The soluble nickel compounds may be more toxic than the insoluble nickel compounds because the nickel ions can diffuse across the cell membrane and interact with cytoplasmic proteins, thereby causing toxicity. Alternatively, it may be that

the water-insoluble nickel compounds are phagocytized and do not cause extensive damage to cytoplasmic components of the alveolar/bronchiolar epithelium (Lee *et al.*, 1993; Costa *et al.*, 1994).

The spectrum of inflammatory lesions in the lungs of rats and mice after 13 weeks of exposure to nickel subsulfide was similar to that observed with other particulates including nickel sulfate hexahydrate, nickel oxide, gallium arsenide (NTP, unpublished data), gallium oxide (NTP, unpublished data), and cadmium oxide (NTP, 1994c). Lymphoid hyperplasia with or without inflammation was present in the respiratory tract lymph nodes of rats and mice from all of these studies. Nickel oxide pigment granules were also present in the lung and respiratory tract lymph nodes; although pigment granules were not present in the lymph nodes of animals from the nickel sulfate hexahydrate, nickel subsulfide, or cadmium oxide studies, the morphologic appearance of the hyperplasia in the paracortical region of the lymph nodes was otherwise generally similar in each study.

In nickel subsulfide immunotoxicity studies, a decrease in cell-mediated immunity occurred in mice exposed to 2.5 mg/m<sup>3</sup> for 13 weeks, although there was no evidence for suppression of the humoral immune response (Haley *et al.*, 1990; Appendix N). No significant effects of nickel subsulfide on sperm morphology or vaginal cytology occurred in rats or mice.

The highest exposure concentrations for these 2-year studies were limited to 1 mg/m<sup>3</sup> for rats and 1.2 mg/m<sup>3</sup> for mice because of increased lung weights and incidences and severity of inflammatory lesions in the lung at the higher exposure concentrations in the 13-week studies. The nickel compound exposure concentrations for the 16-day, 13-week, and 2-year nickel studies and their nickel equivalents are presented in Table 34.

In the 2-year nickel subsulfide studies, there were no exposure-related effects on survival. Body weights were reduced in 1 mg/m<sup>3</sup> male and female rats and in 0.6 and 1.2 mg/m<sup>3</sup> male and female mice.

Respiratory toxicity was manifested by clinical findings of respiratory stress (labored breathing patterns compared to controls), lung weights greater than those of the controls, and increased incidences of lesions in the lungs of rats and mice including inflammation, hyperplasia, and/or fibrosis, and atrophy of the olfactory epithelium.

Nickel subsulfide exposure caused lung neoplasms in male and female rats. This was considered clear evidence of a carcinogenic response in rats because there were exposure-related increases in the incidences of alveolar/bronchiolar adenoma, carcinoma, and adenoma or carcinoma (combined) in males and of alveolar/bronchiolar carcinoma and adenoma or carcinoma (combined) in females. The incidence of alveolar/bronchiolar adenoma or carcinoma (combined) in females exceeded the historical control range for this neoplasm in NTP inhalation studies.

While both nickel subsulfide and nickel oxide caused lung neoplasms in male and female rats (Table 35), the response was not proportional to the amount of nickel deposited in the lung (Table 36). In the 2-year nickel oxide study, there was approximately 300 to 1,100  $\mu\text{g}$  nickel/g lung at 15 months; in the 2-year nickel subsulfide study, there was 3 to 7  $\mu\text{g}$  nickel/g lung at 15 months. However, the incidence of chemical-related lung neoplasms was greater in the nickel subsulfide rats than in the nickel oxide rats. The type of nickel compound is probably important in the eventual carcinogenic response, and under the conditions of these studies, the nickel compound that was more rapidly cleared from the lungs (nickel subsulfide) gave a stronger carcinogenic response than the nickel compound retained in the lungs (nickel oxide).

Some generalities can be made about the comparative lung pathology in rats and mice after 2 years of exposure to nickel subsulfide, nickel oxide, or nickel sulfate hexahydrate. The alveolar/bronchiolar neoplasms in rats exposed to nickel sulfate hexahydrate and of mice exposed to each of the three nickel compounds occurred with no greater incidence than the controls and were typical of spontaneously occurring neoplasms. Although the difference in the proliferative responses (focal alveolar epithelial

hyperplasia and alveolar/bronchiolar neoplasms) between rats and mice were not marked, all three studies were similar in that mice were less susceptible to proliferative and fibrotic lung lesions than rats exposed to the same compound. Five of 11 alveolar/bronchiolar carcinomas in rats exposed to nickel oxide had a marked proliferative component with squamous differentiation. Similar squamous differentiation was observed in 2 of 21 alveolar/bronchiolar adenomas and in 4 of 14 alveolar/bronchiolar carcinomas in rats exposed to nickel subsulfide. Such proliferative squamous differentiation is not characteristic of spontaneous alveolar/bronchiolar neoplasms in rats.

The increases in the incidences of proliferative lesions and severity of inflammation in the lungs of rats exposed to nickel subsulfide or nickel oxide were more severe than those in rats exposed to nickel sulfate hexahydrate. The lungs of rats exposed to nickel subsulfide and nickel oxide had significant parenchymal damage secondary to inflammation. The lungs of rats exposed to 1 mg nickel subsulfide/m<sup>3</sup> and 2.5 mg nickel oxide/m<sup>3</sup> had abundant protein accumulations and variable numbers of foamy macrophages in alveoli and multifocally extensive fibrosis. Foci of necrotic cellular debris, regenerative alveolar epithelial proliferation, and collapse or filling of air spaces were somewhat more prominent in rats exposed to nickel subsulfide than in rats exposed to nickel oxide. Exposure-related pigment in the lungs and bronchial lymph nodes was observed only in rats and mice exposed to nickel oxide.

With the exception of the exposure-related pigment observed only in the nickel oxide study, the nonneoplastic lesions in the lungs of exposed mice were similar in all three nickel compound studies. The components of the inflammatory reaction (intra-alveolar protein and macrophages, mononuclear inflammatory cells around vessels, and multifocal intra-alveolar aggregates of inflammatory cells) were similar in exposed mice in all three studies. Inflammatory foci with neutrophils and necrotic cell debris were relatively common in mice exposed to nickel sulfate hexahydrate, while inflammatory foci in mice exposed to nickel oxide and nickel subsulfide were predominantly mononuclear cells with little evidence of necrotic cell debris.

The inflammatory alterations of proteinosis, macrophage hyperplasia, and fibrosis in rats exposed to nickel subsulfide and nickel oxide were similar to those reported after chronic inhalation exposure to talc (NTP, 1993). In the talc study, it was concluded that these lesions resulted in impaired pulmonary function. Rats in the lifetime talc study, like rats in the current 2-year studies of nickel subsulfide and nickel oxide, had significantly increased incidences of adrenal medullary hyperplasia and pheochromocytoma. Whether the catecholamine-synthesizing cells of the adrenal medulla were stimulated either by impaired pulmonary function or by cytokines released by pulmonary macrophages is unknown.

Nasopharyngeal carcinoma in humans has been attributed to nickel exposure. The preponderance of these sinonasal neoplasms in humans have been classified as anaplastic, undifferentiated, or squamous cell carcinoma (Sunderman *et al.*, 1989). In rodent studies, the olfactory epithelium, rather than the respiratory or squamous mucosa, has been the target site for chemical-related toxicity. Although the atrophic changes were present in the olfactory epithelium of rats and mice in the 13-week and 2-year studies of nickel subsulfide and nickel sulfate hexahydrate, the nasal mucosa was not affected in the nickel oxide study. In the nickel subsulfide studies, there was no evidence for nasal sinus neoplasms in either rats or mice. Furthermore, after 2 years, there was no evidence of a chemical-related increase in the incidences of proliferative lesions in the nasal cavity of rats or mice exposed to any of the three nickel compounds tested.

In this 2-year rat study, there were exposure-related increased incidences of benign and malignant pheochromocytomas in male rats and benign pheochromocytoma in female rats. There were also increased incidences of benign or malignant pheochromocytoma (combined) of the adrenal medulla in male and female rats in the talc study (NTP, 1993) and of benign or malignant pheochromocytoma in male and female rats exposed to nickel oxide. However, similar increased incidences were not observed in rats exposed to nickel sulfate hexahydrate or to other particulates including antimony trioxide or trisulfide (Groth *et al.*, 1986) and titanium dioxide (Lee *et al.*, 1988).

Chemical-related increases in the incidences of pheochromocytoma have also been reported in rats exposed by inhalation to bromoethane (NTP, 1990) and orally to 2-mercaptobenzothiazole (NTP, 1988) and reserpine (NCI, 1982).

Other studies have shown that nickel subsulfide causes tumors after local injection (Table 3) and demonstrated the carcinogenic potential of this chemical. A chemical-related increase in lung tumors in 1 mg/m<sup>3</sup> rats was observed in the one previous nickel subsulfide inhalation study (Ottolenghi *et al.*, 1975). Multiple intratracheal instillations of nickel subsulfide induced malignant lung tumors in female Wistar rats (Pott *et al.*, 1987) and in female Fisher 344 rats (Yarita and Nettesheim, 1978), while intratracheally instilled nickel subsulfide did not induce lung tumors in Syrian hamsters (Muhle *et al.*, 1992).

Previous studies of nickel pulmonary carcinogenesis have not shown evidence of carcinogenicity in mice. Hueper (1958) observed no abnormalities of the bronchial mucosa in female C57B1 mice exposed to 15 mg nickel/m<sup>3</sup>, although only three of 12 mice lived longer than 12 months. In earlier studies, Hueper (1955) did not observe chemical-related neoplasms in mice after a single intrapleural injection of nickel metal powder. A study of the pulmonary carcinogenesis of nickel subsulfide, administered intratracheally or intraperitoneally, provided no evidence of an exposure-related increase in lung tumor response in strain A/J mice (McNeill *et al.*, 1990). Results from these nickel subsulfide studies, and from carcinogenicity studies of other metal compounds such as cadmium, suggest that the rat is more susceptible than the mouse to a carcinogenic response in the lung after exposure to certain metals by inhalation.

Recent studies have shown that nickel subsulfide produces a high level of oxidants in the nuclei of cells, which could cause oxidative damage to proteins and DNA (Huang *et al.*, 1994). This damage may lead to specific alterations in oncogene or suppressor gene patterns in *in vitro* studies (Haugen, 1989; Higinbotham *et al.*, 1992; Mæhle *et al.*, 1992; Lin *et al.*, 1994). Nickel subsulfide transforms rat tracheal

epithelial cells more readily than nickel oxide or nickel sulfate (Patierno *et al.*, 1993). Recent studies have also suggested that the carcinogenic properties of nickel subsulfide may be due to the ability of the metal and sulfide portions of the molecule to enhance the generation of genotoxic free radicals (Shi *et al.*, 1994; Tajmir-Riahi *et al.*, 1994). These studies suggest that the mechanism involved in nickel subsulfide carcinogenesis includes specific genetic alterations, and further studies should help elucidate the molecular changes that occur in the rat lung after nickel subsulfide exposures.

The findings of the carcinogenic response in the rat lung after nickel subsulfide exposure agree with epidemiology findings that show that exposure of workers in nickel industries may lead to an increased risk of lung neoplasms (Doll, 1990). Studies from the Sudbury sinter plant (Copper Cliff) show a strong association between exposure to sulfidic nickel and lung cancer at estimated exposure concentrations of 2 to 8 mg nickel/m<sup>3</sup>. Oxidic nickel levels and soluble nickel levels were also at their highest in the areas of sulfidic nickel exposure, and it was not possible to separate the types of exposure and cancer risk.

Lung tissue specimens from 39 nickel refinery workers showed increased lung nickel levels (Andersen and Svenes, 1989). The average nickel concentration for workers in roasting and smelting operations was  $330 \pm 380$   $\mu\text{g}$  nickel/g dry lung weight; for workers in electrolysis departments,  $34 \pm 48$   $\mu\text{g}/\text{g}$ ; and for lung tissue from unexposed people,  $0.76 \pm 0.39$   $\mu\text{g}/\text{g}$ . Dry lung represents approximately 20% "wet" lung weight, the lung weight used in the NTP nickel studies (Henderson and Escobedo, 1976). Workers who were diagnosed with lung cancer (14 cases) had the same lung nickel concentrations at autopsy as nickel workers (25 cases) who died of other causes. The lung nickel concentration was independent of smoking habits. Anderson and Svenes (1989) felt that the retained nickel in the lung was probably nickel oxide because an earlier study using energy dispersive X-ray analysis did not detect sulfides (e.g., Ni<sub>3</sub>S<sub>2</sub>) in the lung. This study also found that lung cancer occurred in workers from the electrolysis department (8/24) as well as those from the roasting and smelting operations (6/15) even though those from the electrolysis department had lower lung nickel levels.

The current threshold limit value (TLV) for water-insoluble nickel compounds is 1 mg/m<sup>3</sup>, and in these 13-week studies, chemical-related toxic lesions were found in the respiratory tract of rodents at exposure concentrations below the TLV. A no-observed-adverse-effect level (NOAEL) for lung toxicity was not reached for rats; exposure-related lung lesions occurred even at the lowest exposure concentration of 0.15 mg/m<sup>3</sup> (0.11 mg nickel/m<sup>3</sup>). The NOAEL for lung toxicity in mice was 0.15 mg/m<sup>3</sup>. The NOAEL for nasal toxicity was approximately 0.3 mg/m<sup>3</sup> for rats and mice. Lung neoplasms occurred below the TLV in rats in the current 2-year study of nickel subsulfide.

## CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *clear evidence of carcinogenic activity\** of nickel subsulfide in male F344/N rats based on increased incidences of alveolar/bronchiolar adenoma, carcinoma, and adenoma or carcinoma (combined) and on increased incidences of benign, malignant, and benign or malignant (combined) pheochromocytoma of the adrenal medulla. There was *clear evidence of carcinogenic activity* of nickel subsulfide in female F344/N rats based on increased incidences of alveolar/bronchiolar carcinoma and alveolar/bronchiolar adenoma or carcinoma (combined) and an increased incidence of benign pheochromocytoma of the adrenal medulla. There was *no evidence of carcinogenic activity* of nickel subsulfide in male or female B6C3F<sub>1</sub> mice exposed to 0.6 or 1.2 mg/m<sup>3</sup>.

Exposure of rats to nickel subsulfide by inhalation for 2 years resulted in inflammation, hyperplasia, and fibrosis in the lung; inflammation and atrophy of the olfactory epithelium in the nose; and hyperplasia in the adrenal medulla (females). Exposure of mice to nickel subsulfide by inhalation for 2 years resulted in inflammation, bronchiolization, hyperplasia, and fibrosis in the lung and inflammation and atrophy of the olfactory epithelium in the nose.

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\* Explanation of Levels of Evidence of Carcinogenic Activity is on page 16.

TABLE 32  
Selected Results in the 16-Day Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide<sup>a</sup>

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate					Nickel Subsulfide					Nickel Oxide							
	0	3.5 (0.7)	7 (1.4)	15 (3.1)	30 (6.1)	60 (12.2)	0	0.6 (0.44)	1.2 (0.88)	2.5 (1.83)	5 (3.65)	10 (7.33)	0	1.2 (0.9)	2.5 (2.0)	5 (3.9)	10 (7.9)	30 (23.6)
<b>Male Rats</b>																		
Survival	5	5	5	5	5	3	5	5	5	5	5	4	5	5	5	5	5	5
Final Mean Body Weights (Relative to Controls)	—	72%	60%	56%	55%	45%	—	109%	105%	92%	72%	52%	—	99%	101%	99%	99%	96%
Absolute Lung Weights <sup>b</sup>	0.98	1.44**	1.45**	1.40*	1.40*	1.62**	1.13	1.41	1.60*	1.59*	1.82**	1.54**	1.06	1.00	1.06	0.96	1.20*	1.36**
<b>Female Rats</b>																		
Survival	5	5	5	5	4	0	5	5	5	5	5	5	5	5	5	5	5	5
Final Mean Body Weights (Relative to Controls)	—	82%	71%	68%	63%	—	—	99%	97%	91%	78%	57%	—	103%	103%	104%	101%	99%
Absolute Lung Weights	0.76	1.28*	1.28*	1.32*	1.40**	1.52**	0.82	1.12**	1.12**	1.36**	1.42**	1.25**	0.78	0.86	0.90	0.82	1.04**	1.12**

(continued)

TABLE 32  
Selected Results in the 16-Day Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide (continued)

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate			Nickel Subsulfide			Nickel Oxide											
	0	3.5 (0.7)	7 (1.4)	15 (3.1)	30 (6.1)	60 (12.2)	0	0.6 (0.44)	1.2 (0.88)	2.5 (1.83)	5 (3.65)	10 (7.33)	0	1.2 (0.9)	2.5 (2.0)	5 (3.9)	10 (7.9)	30 (23.6)
<b>Male Mice</b>																		
Survival	5	5	0	0	0	0	4	5	4	5	5	0	5	5	5	4	5	5
Final Mean Body Weights (Relative to Controls)	—	95%	—	—	—	—	—	99%	90%	92%	86%	—	—	100%	100%	98%	102%	94%
Absolute Lung Weights	0.20	0.24	0.40**	0.36**	0.36**	0.38**	0.22	0.20	0.22	0.28	0.31**	0.38**	0.20	0.16	0.20	0.13**	0.20	0.20
<b>Female Mice</b>																		
Survival	5	5	0	0	0	0	4	5	5	5	5	0	5	5	5	5	5	5
Final Mean Body Weights (Relative to Controls)	—	96%	—	—	—	—	—	106%	104%	101%	99%	—	—	100%	96%	100%	95%	95%
Absolute Lung Weights	0.16	0.22	0.36**	0.38**	0.38**	0.40**	0.20	0.21	0.22	0.27	0.36*	0.25	0.16	0.16	0.14	0.18	0.12	0.20

\* Significantly different (P ≤ 0.05) from the control by Williams' or Dunnett's test

\*\* P ≤ 0.01

a Survival data indicate number of animals surviving. Five animals initially in group. Final mean body weights are not presented for groups with 100% mortality.

b Organ weights are given in grams.

TABLE 33  
Selected Results in the 13-Week Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide<sup>a</sup>

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate					Nickel Subsulfide					Nickel Oxide									
	0	0.12	0.25	0.5	1	2	0	0.15	0.3	0.6	1.2	2.5	5	10	0	0.6	1.2	2.5	5	10
	(0.03)	(0.06)	(0.11)	(0.22)	(0.44)		(0.11)	(0.22)	(0.44)	(0.88)	(1.83)		(0.4)	(0.9)	(2.0)	(3.9)	(7.9)			
Male Rats																				
Survival	10	10	10	10	10	9	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Final Mean Body Weights (Relative to Controls)	—	99%	103%	96%	102%	95%	—	100%	95%	96%	99%	93%	—	103%	104%	99%	102%	100%	100%	100%
Absolute Lung Weights	1.35	1.25	1.51*	1.64**	1.4**	2.22**	1.33	1.74**	1.83**	2.30**	2.63**	2.42**	1.18	1.35**	1.47**	1.70**	1.91**	2.47**	2.47**	2.47**
Nonneoplastic Lung Lesions																				
Alveolar Macrophage	0	10	10	10	10	9	0	10	10	10	10	10	0	10	10	10	10	10	10	10
Hyperplasia (Severity) <sup>b</sup>	(1.0)	(1.0)	(1.0)	(2.4)	(3.6)		(1.1)	(1.5)	(1.6)	(3.4)	(3.8)		(1.0)	(1.0)	(1.0)	(1.5)	(2.5)			
Inflammation, Chronic Active (Severity)	0	0	0	2	10	8	0	2	9	10	10	10	0	0	0	2	10	10	10	10
Inflammation, Granulomatous (Severity)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2	2
Interstitial Infiltrate (Severity)	1	0	1	5	10	9	0	0	1	10	9	8	0	0	1	2	10	10	10	10
Pigment (Severity)	(1.0)	(1.0)	(1.0)	(1.0)	(1.1)		(1.0)	(1.9)	(2.1)	(1.2)			(1.0)	(1.0)	(1.4)	(2.1)				
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	7	9	9	10	10
															(1.0)	(1.0)	(1.0)	(1.0)	(1.8)	(1.8)
Nonneoplastic Nasal Lesions																				
Atrophy, Olfactory Epithelium	0	0	0	1	10	9	0	0	1	5	10	10	0	0	0	0	0	0	0	0

(continued)



TABLE 33  
Selected Results in the 13-Week Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide (continued)

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate					Nickel Subsulfide					Nickel Oxide							
	0	0.12 (0.03)	0.25 (0.06)	0.5 (0.11)	1 (0.22)	2 (0.44)	0	0.15 (0.11)	0.3 (0.22)	0.6 (0.44)	1.2 (0.88)	2.5 (1.83)	0	0.6 (0.4)	1.2 (0.9)	2.5 (2.0)	5 (3.9)	10 (7.9)
Male Mice																		
Survival	6	8 <sup>c</sup>	10	10	10	10	8	10	10	8	9	10	10	10	10	10	10	9
Final Mean Body Weights (Relative to Controls)	—	105%	100%	104%	104%	102%	—	102%	106%	103%	101%	97%	—	101%	99%	97%	98%	97%
Absolute Lung Weights	0.20	0.20	0.20	0.21	0.25**	0.31**	0.19	0.20	0.22	0.21	0.23*	0.28**	0.21	0.22	0.21	0.21	0.24	0.29**
Nonneoplastic Lung Lesions																		
Alveolar Macrophage Hyperplasia (Severity)	0	0	0	10	10	10	0	0	8	8	9	10	0	10	10	10	10	9
Fibrosis, Focal (Severity)	0	0	0	0	2	10	0	0	0	0	5	10	0	0	0	0	0	0
Inflammation, Chronic Active (Severity)	0	0	0	0	2	2	0	0	0	0	5	7	0	0	0	0	0	3
Inflammation, Granulomatous (Severity)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Interstitial Infiltrate (Severity)	0	0	0	0	2	8	0	1	0	2	3	2	0	0	0	1	3	8
Pigment (Severity)	0	0	0	0	0	0	0	0	0	0	0	0	0	10	10	10	10	9
Nonneoplastic Nasal Lesions																		
Atrophy, Olfactory Epithelium	0	0	0	0	0	10	0	0	0	5	5	10	0	0	0	0	0	0

(continued)

TABLE 33 Selected Results in the 13-Week Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide (continued)

	Nickel Sulfate Hexahydrate					Nickel Subsulfide					Nickel Oxide										
	0	0.12	0.25	0.5	1	2	0	0.15	0.3	0.6	1.2	2.5	5	10	0	0.6	1.2	2.5	5	10	
Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	0	0.12	0.25	0.5	1	2	0	0.15	0.3	0.6	1.2	2.5	5	10	0	0.6	1.2	2.5	5	10	
	(0.03)	(0.06)	(0.11)	(0.22)	(0.44)		(0.11)	(0.22)	(0.44)	(0.88)	(1.83)				(0.4)	(0.9)	(2.0)	(3.9)	(7.9)		
<b>Female Mice</b>																					
Survival	7	10	10	10	10	10	10	8	10	9	10	8	10	10	9	10	7	10	10	10	9
Final Mean Body Weights (Relative to Controls)	—	105%	104%	105%	103%	97%	—	101%	100%	101%	101%	99%	—	97%	100%	96%	94%	94%	97%	97%	97%
Absolute Lung Weights	0.20	0.20	0.20	0.20	0.22	0.27**	0.19	0.18	0.20	0.21	0.26**	0.29**	0.20	0.20	0.19	0.21	0.22	0.22	0.27**	0.27**	0.27**
<b>Nonneoplastic Lung Lesions</b>																					
Alveolar Macrophage Hyperplasia (Severity)	0	0	0	10	10	10	0	0	4	9	10	10	10	10	0	10	7	10	10	10	9
Fibrosis, Focal (Severity)	0	0	0	0	1	8	0	0	0	0	1	9	9	9	0	0	0	0	0	0	0
Inflammation, Chronic Active (Severity)	0	0	0	0	1	9	0	0	0	0	10	7	7	7	0	0	0	0	1	3	3
Inflammation, Granulomatous (Severity)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Interstitial Infiltrate (Severity)	1	0	0	1	1	8	0	2	3	4	9	8	8	8	0	1	0	4	6	8	8
Pigment (Severity)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
<b>Nonneoplastic Nasal Lesions</b>																					
Atrophy, Olfactory Epithelium	0	0	0	0	0	5	0	0	0	1	6	10	10	10	0	0	0	0	0	0	0

\* Significantly different (P ≤ 0.05) from the control by Williams' or Dunnett's test

\*\* P ≤ 0.01

a Survival data indicate number of animals surviving. Ten animals initially in group. Final mean body weights are not presented for groups with 100% mortality.

b Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

c Nine animals initially in group.

**TABLE 34**  
**Comparison of Exposure Concentrations in the 16-Day, 13-Week, and 2-Year Studies**  
**of Nickel Sulfate Hexahydrate, Nickel Sub sulfide, and Nickel Oxide<sup>a</sup>**

	<u>Amount of Compound</u>	<u>Amount of Nickel</u>
<b>16-Day Studies</b>		
Nickel Sulfate Hexahydrate (22.3% Ni)	0, 3.5, 7, 15, 30, 60	0, 0.7, 1.4, 3.1, 6.1, 12.2
Nickel Sub sulfide (73.3% Ni)	0, 0.6, 1.2, 2.5, 5, 10	0, 0.44, 0.88, 1.83, 3.65, 7.33
Nickel Oxide (78.6% Ni)	0, 1.2, 2.5, 5, 10, 30	0, 0.9, 2.0, 3.9, 7.9, 23.6
<b>13-Week Studies</b>		
Nickel Sulfate Hexahydrate (22.3% Ni)	0, 0.12, 0.25, 0.5, 1, 2	0, 0.03, 0.06, 0.11, 0.22, 0.44
Nickel Sub sulfide (73.3% Ni)	0, 0.15, 0.3, 0.6, 1.2, 2.5	0, 0.11, 0.22, 0.44, 0.88, 1.83
Nickel Oxide (78.6% Ni)	0, 0.6, 1.2, 2.5, 5, 10	0, 0.4, 0.9, 2.0, 3.9, 7.9
<b>2-Year Studies</b>		
<b>Nickel Sulfate Hexahydrate (22.3% Ni)</b>		
Rats	0, 0.12, 0.25, 0.5	0, 0.03, 0.06, 0.11
Mice	0, 0.25, 0.5, 1	0, 0.06, 0.11, 0.22
<b>Nickel Sub sulfide (73.3% Ni)</b>		
Rats	0, 0.15, 1	0, 0.11, 0.73
Mice	0, 0.6, 1.2	0, 0.44, 0.88
<b>Nickel Oxide (78.6% Ni)</b>		
Rats	0, 0.62, 1.25, 2.5	0, 0.5, 1.0, 2.0
Mice	0, 1.25, 2.5, 5	0, 1.0, 2.0, 3.9

<sup>a</sup> Amounts of nickel and nickel compounds are expressed in mg/m<sup>3</sup>. Occupational exposure limits in the United States: 1 mg Ni/m<sup>3</sup> for nickel metals, 0.1 mg Ni/m<sup>3</sup> for soluble nickel compounds.

TABLE 35  
Selected Results in the 2-Year Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide<sup>a</sup>

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate (22.3% Ni)		Nickel Subsulfide (73.3% Ni)		Nickel Oxide (78.6% Ni)					
	0	0.12 (0.03)	0.25 (0.06)	0.5 (0.11)	0	0.15 (0.11)	0	0.62 (0.5)	1.25 (1.0)	2.5 (2.0)
<b>Male Rats</b>										
Survival	16/54	16/55	18/55	21/55	13/53	21/53	18/53	14/54	15/53	12/52
Final Mean Body Weights (Relative to Controls)	—	99%	101%	98%	—	98%	85%	—	100%	93%
Absolute Lung Weights										
7-Month Interim Evaluation	1.67	1.62	1.65	1.89	1.87	2.38**	3.48**	1.72	1.85	2.43**
15-Month Interim Evaluation	2.12	2.48	2.50	3.00**	2.27	3.31**	6.84**	2.20	2.15	3.30**
<b>Alveolar/bronchial Proliferative Lesions and Neoplasms</b>										
Alveolar Epithelial	3	2	3	2	2	6	11**	0	2	5*
Hyperplasia, Focal or Atypical	0	0	0	2	0	3	6*	0	1	3
Adenoma	2 <sup>b</sup>	0	1	1	0	3	6*	1 <sup>b</sup>	0	3
Carcinoma	2 <sup>b</sup>	0	1	3	0	6*	11**	1 <sup>b</sup>	1	6 <sup>c</sup>
Adenoma or Carcinoma (Combined)	28	20	18	26	26	22	10	25	27	24
<b>Adrenal Medulla Proliferative Lesions and Neoplasms</b>										
Hyperplasia	16	16	12	11	13	30**	38**	27	24	26
Benign Pheochromocytoma	0	3	2	1	0	2	10**	0	0	1
Malignant Pheochromocytoma	16	19	13	12	14	30**	42**	27	24	27
Benign or Malignant Pheochromocytoma	16	19	13	12	14	30**	42**	27	24	27
Carcinogenic Activity		No evidence				Clear evidence			Some evidence	

(continued)

TABLE 35  
Selected Results in the 2-Year Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide (continued)

	Nickel Sulfate Hexahydrate (22.3% Ni)		Nickel Subsulfide (73.3% Ni)		Nickel Oxide (78.6% Ni)	
	Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Survival	Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Survival	Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Survival
Final Mean Body Weights (Relative to Controls)	0 0.12 (0.03) 0.25 (0.06) 0.5 (0.11)	22/52 17/53 28/53 29/54	0 0.15 (0.11) (0.73)	26/53 25/53 28/52	0 0.62 (0.5) (1.0) (2.0)	21/53 26/53 20/53 26/54
Absolute Lung Weights	1.25 1.22 1.22 1.45*		1.31 1.75** 2.59**		1.14 1.31* 1.65** 1.78**	
7-Month Interim Evaluation	1.37 1.57 1.49 1.82**		1.52 2.52** 4.14**		1.56 1.79 2.41** 3.02**	
15-Month Interim Evaluation						
Alveolar/broncholar Proliferative Lesions and Neoplasms						
Alveolar Epithelial	5 3 7 9		2 10* 11**		2 1 6 6	
Hyperplasia, Focal or Atypical	0 0 0 1		2 5 5		1 0 1 4	
Adenoma	0 0 0 0		0 1 <sup>b</sup> 4		0 0 5* 1	
Carcinoma	0 0 0 0		2 6 <sup>b,d</sup> 9*		1 0 6 <sup>d</sup> 5 <sup>d</sup>	
Adenoma or Carcinoma (Combined)	0 0 0 0					
Adrenal Medulla Proliferative Lesions and Neoplasms						
Hyperplasia	6 4 8 8		5 11 16**		8 12 14 22**	
Benign Pheochromocytoma	2 4 2 3		2 7 36**		4 7 6 18**	
Malignant Pheochromocytoma	0 0 0 0		1 0 1		0 0 0 0	
Benign or Malignant Pheochromocytoma	2 4 2 3		3 7 36**		4 7 6 18**	
Carcinogenic Activity	No evidence		Clear evidence		Some evidence	

(continued)

TABLE 35  
Selected Results in the 2-Year Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide (continued)

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate (22.5% Ni)		Nickel Subsulfide (73.3% Ni)		Nickel Oxide (78.6% Ni)		
	0	0.25 (0.06)	0.5 (0.11)	1 (0.22)	0	0.6 (0.44)	1.2 (0.88)
Survival	26/61	23/61	24/62	25/61	26/61	25/59	26/58
Final Mean Body Weights (Relative to Controls)	—	94%	97%	91%	—	92%	92%
Absolute Lung Weights	—	—	—	—	—	—	—
7-Month Interim Evaluation	0.21	0.20	0.22	0.23	0.24	0.27	0.34**
15-Month Interim Evaluation	0.24	0.25	0.26	0.31**	0.23	0.40**	0.41**
Alveolar/broncholar Proliferative Lesions and Neoplasms							
Alveolar Epithelial	0	0	0	0	0	0	0
Hyperplasia, Focal	5	5	3	5	6	3	2
Adenoma	9	13	4	3	7	2	4
Carcinoma	13	18	7	8	13	5	6
Adenoma or Carcinoma (Combined)	13	18	7	8	13	5	6
Carcinogenic Activity	No evidence		No evidence		No evidence		No evidence

(continued)



**TABLE 36**  
**Lung Burden Analyses in the 16-Day, 13-Week, and 2-Year Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide<sup>a</sup>**

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate (22.3% Ni)			Nickel Subsulfide (73.3% Ni)			Nickel Oxide (78.6% Ni)											
	0	0.12 (0.03)	0.5 (0.06)	2 (0.44)	3.5 (0.7)	15 (3.1)	30 (6.1)	0	0.15 (0.11)	0.6 (0.44)	2.5 (1.83)	10 (7.33)	0	0.6 (0.4)	1.2 (0.9)	2.5 (2.0)	5 (3.9)	10 (7.9)
<b>16-Day Studies</b>																		
Male Rats	- <sup>b</sup>		5	9	8				7	18	67				42	108	267	
Female Rats	-		8	11	9				9	19	77				54	122	340	
Male Mice	-		3						10	20	13				32	46	84	
Female Mice	-		4						8	20	8				31	43	71	
<b>13-Week Studies</b>																		
Male Rats	-		1	6					5	7	18				80	181	524	
Female Rats	-		2	7					5	7	17							
Male Mice	-			1					3	11	17				42	202	736	
Female Mice	-			4					6	13	23							

(continued)

**TABLE 36**  
**Lung Burden Analyses in the 16-Day, 13-Week, and 2-Year Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide (continued)**

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate (22.3% Ni)			Nickel Subsulfide (73.3% Ni)			Nickel Oxide (78.6% Ni)										
	0	0.12 (0.03)	0.25 (0.06)	0.5 (0.11)	1 (0.22)	2 (0.44)	0	0.15 (0.11)	0.6 (0.44)	1 (0.73)	1.2 (0.88)	0	0.62 (0.5)	1.25 (1.0)	2.5 (2.0)	5 (3.9)	10 (7.9)
<b>7-Month Interim Evaluation</b>																	
Male Rats	-	-	-	1	-	-	-	6	9	-	-	-	175	388	701	-	-
Female Rats	-	-	-	1	-	-	-	6	9	-	-	-	173	477	713	-	-
Male Mice	-	-	1	1	2	-	-	-	10	11	-	-	162	442	1,034	-	-
Female Mice	-	-	1	2	2	-	-	-	10	14	-	-	169	533	861	-	-
<b>15-Month Interim Evaluation</b>																	
Male Rats	-	-	-	1	-	-	-	4	3	-	-	-	328	746	1,116	-	-
Female Rats	-	-	-	2	-	-	-	4	7	-	-	-	262	706	949	-	-
Male Mice	-	-	1	1	2	-	-	-	15	26	-	-	331	959	1,798	-	-
Female Mice	-	-	1	2	2	-	-	-	12	20	-	-	451	1,237	2,258	-	-

<sup>a</sup> Values represent mean amounts of nickel (µg Ni/g lung). Lung burden groups included five to seven animals.  
<sup>b</sup> Results were below the limit of detection.



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**NTP TECHNICAL REPORT**  
**ON THE**  
**TOXICOLOGY AND CARCINOGENESIS**  
**STUDIES OF**  
**NICKEL SULFATE HEXAHYDRATE**  
**(CAS NO. 10101-97-0)**  
**IN F344/N RATS AND B6C3F<sub>1</sub> MICE**  
**(INHALATION STUDIES)**

**Scheduled Peer Review Date: November 29, 1994**

**NOTICE**

This is a DRAFT Technical Report prepared for public review and comment. Until this DRAFT has been reviewed and approved by the NTP Board of Scientific Counselors' Technical Reports Review Subcommittee in public session, the interpretations described herein do not represent the official scientific position of the National Toxicology Program. Following peer review, readers should contact NTP for the final version of this Technical Report.

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**U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES**  
**Public Health Service**  
**National Institutes of Health**

## NOTE TO THE READER

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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# CONTENTS

<b>ABSTRACT</b>		<b>6</b>
<b>EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY</b>		<b>14</b>
<b>TECHNICAL REPORTS REVIEW SUBCOMMITTEE</b>		<b>15</b>
<b>SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS</b>		<b>16</b>
<b>INTRODUCTION</b>		<b>17</b>
<b>MATERIALS AND METHODS</b>		<b>49</b>
<b>RESULTS</b>		<b>69</b>
<b>DISCUSSION AND CONCLUSIONS</b>		<b>115</b>
<b>REFERENCES</b>		<b>139</b>
<b>APPENDIX A</b>	<b>Summary of Lesions in Male Rats in the 2-Year Inhalation Study of Nickel Sulfate Hexahydrate</b>	<b>A-1</b>
<b>APPENDIX B</b>	<b>Summary of Lesions in Female Rats in the 2-Year Inhalation Study of Nickel Sulfate Hexahydrate</b>	<b>B-1</b>
<b>APPENDIX C</b>	<b>Summary of Lesions in Male Mice in the 2-Year Inhalation Study of Nickel Sulfate Hexahydrate</b>	<b>C-1</b>
<b>APPENDIX D</b>	<b>Summary of Lesions in Female Mice in the 2-Year Inhalation Study of Nickel Sulfate Hexahydrate</b>	<b>D-1</b>
<b>APPENDIX E</b>	<b>Genetic Toxicology</b>	<b>E-1</b>
<b>APPENDIX F</b>	<b>Organ Weights and Organ-Weight-to-Body-Weight Ratios</b>	<b>F-1</b>
<b>APPENDIX G</b>	<b>Hematology Results</b>	<b>G-1</b>
<b>APPENDIX H</b>	<b>Tissue Burden in Rats</b>	<b>H-1</b>
<b>APPENDIX I</b>	<b>Tissue Burden in Mice</b>	<b>I-1</b>
<b>APPENDIX J</b>	<b>Reproductive Tissue Evaluations and Estrous Cycle Characterization</b>	<b>J-1</b>
<b>APPENDIX K</b>	<b>Chemical Characterization and Generation of Chamber Concentrations</b>	<b>K-1</b>

<b>APPENDIX L</b>	<b>Ingredients, Nutrient Composition, and Contaminant Levels in NIH-07 Rat and Mouse Ration</b> .....	<b>L-1</b>
<b>APPENDIX M</b>	<b>Sentinel Animal Program</b> .....	<b>M-1</b>
<b>APPENDIX N</b>	<b>The Immunotoxicity of Three Nickel Compounds Following 13-Week Inhalation Exposure in the Mouse</b> .....	<b>N-1</b>
<b>APPENDIX O</b>	<b>Biochemical Responses of Rat and Mouse Lung to Inhaled Nickel Compounds</b> ...	<b>O-1</b>
<b>APPENDIX P</b>	<b>Fate of Inhaled Nickel Oxide and Nickel Subulfide in F344/N Rats</b> .....	<b>P-1</b>

## ABSTRACT



### NICKEL SULFATE HEXAHYDRATE

CAS No. 10101-97-0

Chemical Formula:  $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$       Molecular Weight: 262.86

**Synonyms:** Blue salt; hexahydrate, nickel (2+) salt; nickel (2+) sulfate hexahydrate; nickel (II) sulfate hexahydrate; nickel monosulfate hexahydrate; nickel sulphate hexahydrate; nickelous sulfate hexahydrate; nickelous sulphate hexahydrate; single nickel salt, sulfuric acid

Nickel sulfate hexahydrate is used in nickel plating, as a mordant in dyeing and printing textiles, as a blackening agent for zinc and brass, and in the manufacture of organic nickel salts. Nickel sulfate hexahydrate was nominated by the National Cancer Institute to the NTP as part of a class study of nickel compounds for which there was little information on the toxic and carcinogenic effects of inhalation exposure. Male and female F344/N rats and B6C3F<sub>1</sub> mice were exposed to nickel sulfate hexahydrate (greater than 98% pure) by inhalation for 16 days, 13 weeks, or 2 years. Genetic toxicology studies were conducted in L5178Y mouse lymphoma cells.

### 16-DAY STUDY IN RATS

Groups of five male and five female F344/N rats were exposed to 0, 3.5, 7, 15, 30, or 60 mg nickel sulfate hexahydrate/m<sup>3</sup> (equivalent to 0, 0.7, 1.4, 3.1, 6.1, or 12.2 mg nickel/m<sup>3</sup>). Rats were exposed on weekdays only, for a total of 12 exposure days during a 16-day period. Additional groups of four or five male and female F344/N rats were exposed to 0, 3.5, 15, or 30 mg nickel sulfate hexahydrate/m<sup>3</sup> for tissue burden studies. In the core study, two 60 mg/m<sup>3</sup> males, one 30 mg/m<sup>3</sup> female, and all 60 mg/m<sup>3</sup>

females died before the end of the study. Final mean body weights of all exposed groups of males and females were significantly less than those of the controls, as were mean body weight gains of male rats. Clinical findings included increased rates of respiration and reduced activity levels in rats in all exposure groups, except those exposed to 3.5 mg/m<sup>3</sup>. Absolute lung weights of 60 mg/m<sup>3</sup> males and of all exposed groups of females were significantly greater than those of the controls, as were the relative lung weights of all exposed groups of males and females. Inflammation (including degeneration and necrosis of the bronchiolar epithelium) occurred in the lungs of exposed groups of males and females. Atrophy of the olfactory epithelium occurred in the nasal passages of all exposed groups of males (except 60 mg/m<sup>3</sup>) and in 15, 30, and 60 mg/m<sup>3</sup> females. Lymphoid hyperplasia in the bronchial or mediastinal lymph nodes was observed in 30 mg/m<sup>3</sup> males and in 60 mg/m<sup>3</sup> males and females. The concentration of nickel in the lungs of all exposed groups of males and females was significantly greater than in control animals.

## 16-DAY STUDY IN MICE

Groups of five male and five female B6C3F<sub>1</sub> mice were exposed to 0, 3.5, 7, 15, 30, or 60 mg nickel sulfate hexahydrate/m<sup>3</sup> (equivalent to 0, 0.7, 1.4, 3.1, 6.1, or 12.2 mg nickel/m<sup>3</sup>). Mice were exposed on weekdays only, for a total of 12 exposure days during a 16-day period. Additional groups of five male and five female B6C3F<sub>1</sub> mice were exposed to 0 or 3.5 mg nickel sulfate hexahydrate/m<sup>3</sup> for tissue burden studies. All core study mice exposed to 7 mg/m<sup>3</sup> or greater died before the end of the study; all control and 3.5 mg/m<sup>3</sup> mice survived to the end of the study. Final mean body weights and weight gains of 7, 15, 30, and 60 mg/m<sup>3</sup> males and females were significantly less than those of the controls, and clinical findings in these groups included emaciation, lethargy, and rapid respiration rates. Absolute and relative lung weights of male and female mice exposed to 7 mg/m<sup>3</sup> or greater were significantly greater than those of the controls. Only tissues from mice exposed to 0, 3.5, or 7 mg/m<sup>3</sup> were examined histopathologically. Inflammation occurred in the lungs of 3.5 and 7 mg/m<sup>3</sup> males and females; necrosis of the alveolar and bronchiolar epithelium was a component of the inflammation in 7 mg/m<sup>3</sup> males and females. In addition,

atrophy of the olfactory epithelium of the nasal passages was observed in 3.5 mg/m<sup>3</sup> males and females. Nickel concentrations in the lungs of mice exposed to 3.5 mg/m<sup>3</sup> were significantly greater than those in the controls.

### 13-WEEK STUDY IN RATS

Groups of ten male and ten female F344/N rats were exposed to 0, 0.12, 0.25, 0.5, 1, or 2 mg nickel sulfate hexahydrate (equivalent to 0, 0.03, 0.06, 0.11, 0.22, or 0.44 mg nickel/m<sup>3</sup>), 5 days per week for 13 weeks. Additional groups of six male and six female F344/N rats were exposed to 0, 0.12, 0.5, or 2 mg nickel sulfate hexahydrate/m<sup>3</sup> for tissue burden studies. In the core study, one 2 mg/m<sup>3</sup> male rat died before the end of the study; all other males and all females survived until the end of the study. Final mean body weights and body weight gains of all exposed groups were similar to those of the controls. There were no significant clinical findings noted during the study. Exposure-related increases in neutrophil and lymphocyte numbers occurred and were most pronounced in female rats. With the exception of 0.12 mg/m<sup>3</sup> rats, absolute and relative lung weights of all exposed groups were generally significantly greater than those of the controls. Treatment- and exposure level-related increases in the incidence and severity of inflammatory lesions (alveolar macrophages, chronic inflammation, and interstitial infiltration) occurred in the lungs of all exposed groups of males and females. Lymphoid hyperplasia of the bronchial and/or mediastinal lymph nodes occurred in males exposed to 0.5 mg/m<sup>3</sup> or greater. Atrophy of the olfactory epithelium occurred in males and females exposed to 0.5, 1, and 2 mg/m<sup>3</sup> and in 0.25 mg/m<sup>3</sup> females. The concentration of nickel in the lungs of 0.5 and 2 mg/m<sup>3</sup> rats was significantly greater than that in the lungs of control animals at 4, 9, and 13 weeks for males and at 13 weeks for females.

## 13-WEEK STUDY IN MICE

Groups of ten male and ten female B6C3F<sub>1</sub> mice were exposed to 0, 0.12, 0.25, 0.5, 1, or 2 mg nickel sulfate hexahydrate (equivalent to 0, 0.03, 0.06, 0.11, 0.22, or 0.44 mg nickel/m<sup>3</sup>), 5 days per week for 13 weeks. Additional groups of five or six male and five or six female B6C3F<sub>1</sub> mice were exposed to 0, 0.12, 0.5, or 2 mg nickel sulfate hexahydrate/m<sup>3</sup> for tissue burden studies. In the core study, four control males, three control females, and one 0.12 mg/m<sup>3</sup> male died before the end of the study; the deaths were not considered to be chemical related, and all other mice survived to the end of the study. The final mean body weights and body weight gains of all exposed groups were similar to those of the controls. There were no chemical-related clinical findings. Hematology changes similar to those reported in female rats occurred in female mice, but the mice were minimally affected. The absolute and relative lung weights of 1 mg/m<sup>3</sup> males and 2 mg/m<sup>3</sup> males and females were significantly greater than those of the controls. Increased numbers of alveolar macrophages occurred in all males and females exposed to 0.5 mg/m<sup>3</sup> or greater. Chronic active inflammation and fibrosis occurred in 1 and 2 mg/m<sup>3</sup> males and females. Lymphoid hyperplasia of the bronchial lymph node and atrophy of the olfactory epithelium in the nasal passages were observed in 2 mg/m<sup>3</sup> males and females. Nickel concentration in the lung of 2 mg/m<sup>3</sup> females was significantly greater than in control animals.

## 2-YEAR STUDY IN RATS

Groups of 65 male and 65 female rats were exposed to nickel sulfate hexahydrate by inhalation at concentrations of 0, 0.12, 0.25, or 0.5 mg/m<sup>3</sup> (equivalent to 0, 0.03, 0.06, or 0.11 mg nickel/m<sup>3</sup>). Animals were exposed for 6 hours plus T<sub>90</sub> (8 minutes) five days per week for 112 weeks. Five male and five female rats from each group were evaluated at 7 months for histopathology; as many as seven males and seven females from each group were evaluated at 7 months for nickel tissue burden in the lung and kidney; and five males and five females from each group were evaluated at 15 months for alterations in hematology, nickel tissue burden in the lung and kidney, and histopathology.

### *Survival, Body Weights, Clinical Findings, and Hematology*

Survival rates of all exposed groups of males and females were similar to those of the controls. Mean body weights of 0.5 mg/m<sup>3</sup> female rats were slightly lower (6% to 9%) than those of the controls throughout the second year of the study; final mean body weights of all exposed groups of males and 0.12 and 0.25 mg/m<sup>3</sup> females were similar to those of the controls. There were no clinical findings or hematology differences that were considered to be related to nickel sulfate hexahydrate administration.

### *Pathology Findings*

No exposure-related neoplasms occurred in male or female rats exposed by inhalation to nickel sulfate hexahydrate for 2 years. Increased incidences of inflammatory lung lesions were generally observed in all exposed groups of male and female rats at the end of the study. The incidences of chronic active inflammation, macrophage hyperplasia, alveolar proteinosis, and fibrosis were markedly increased in male and female rats exposed to 0.25 or 0.5 mg/m<sup>3</sup>. Increased incidences of lymphoid hyperplasia in the bronchial lymph nodes occurred in 0.5 mg/m<sup>3</sup> male and female rats at the end of the 2-year study. The incidences of atrophy of the olfactory epithelium in 0.5 mg/m<sup>3</sup> males and females were significantly greater than those in controls at the end of the study.

### *Tissue Burden Analyses*

Lung nickel burdens in exposed male and female rats were greater than those in the controls at the 7- and 15-month interim evaluations, and lung nickel burdens values increased with increasing exposure concentration.

## **2-YEAR STUDY IN MICE**

Groups of 80 male and 80 female mice were exposed to nickel sulfate hexahydrate by inhalation at concentrations of 0, 0.25, 0.5, or 1 mg/m<sup>3</sup> (equivalent to 0, 0.03, 0.06, or 0.11 mg nickel/m<sup>3</sup>). Animals

were exposed for 6 hours plus T<sub>90</sub> (8 minutes) five days per week for 112 weeks. Five male and five female mice from each group were evaluated at 7 months for histopathology; five males and five females from each group were evaluated at 7 months for nickel tissue burden in the lung and kidney; five males and five females from each group were evaluated at 15 months for alterations in hematology and histopathology; and five males and five females from each group were evaluated at 15 months for nickel tissue burden in the lung and kidney.

### *Survival, Body Weights, Clinical Findings, and Hematology*

The survival rates of all exposed groups of males and females were similar to those of the controls. The mean body weights of 1 mg/m<sup>3</sup> males and of all exposed groups of females were lower than those of the controls during the second year of the study. There were no clinical findings or hematology differences considered to be related to chemical exposure.

### *Pathology Findings*

Inflammatory lesions of the lung generally occurred in all exposed groups of male and female mice at the end of the 2-year study. These lesions included macrophage hyperplasia, chronic active inflammation, bronchialization (alveolar epithelial hyperplasia), alveolar proteinosis, and infiltrating cells in the interstitium. Incidences of macrophage hyperplasia and/or lymphoid hyperplasia occurred in the bronchial lymph nodes of most of the 1 mg/m<sup>3</sup> males and females and in some 0.5 mg/m<sup>3</sup> females at the end of the 2-year study. Atrophy of the olfactory epithelium was observed in 0.5 and 1 mg/m<sup>3</sup> males and in all exposed groups of females at the end of the 2-year study.

### *Tissue Burden Analyses*

At the 7- and 15-month interim evaluations, lung nickel burden parameters measured in control and exposed groups were below the limit of detection. Absolute lung weights of 0.5 and 1 mg/m<sup>3</sup> lung burden study females were significantly greater than those of the controls at 15 months.

## GENETIC TOXICOLOGY

Nickel sulfate hexahydrate (500 to 800  $\mu\text{g}/\text{mL}$ ) was tested for induction of trifluorothymidine resistance in L5178Y mouse lymphoma cells. A positive response was observed in the absence of S9. The test was not performed with S9.

## CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *no evidence of carcinogenic activity\** of nickel sulfate hexahydrate in male or female F344/N rats exposed to 0.12, 0.25, or 0.5  $\text{mg}/\text{m}^3$  (0.03, 0.06, or 0.11  $\text{mg}$  nickel/ $\text{m}^3$ ). There was *no evidence of carcinogenic activity* of nickel sulfate hexahydrate in male or female B6C3F<sub>1</sub> mice exposed to 0.25, 0.5, or 1  $\text{mg}/\text{m}^3$  (0.06, 0.11, or 0.22  $\text{mg}$  nickel/ $\text{m}^3$ ).

Exposure of rats to nickel sulfate hexahydrate by inhalation for 2 years resulted in increased incidences of chronic active inflammation, macrophage hyperplasia, alveolar proteinosis, and fibrosis of the lung; lymphoid hyperplasia of the bronchial lymph node; and atrophy of the olfactory epithelium.

Exposure of mice to nickel sulfate hexahydrate by inhalation for 2 years resulted in increased incidences of chronic active inflammation, bronchialization (alveolar epithelial hyperplasia), macrophage hyperplasia, interstitial infiltration, and alveolar proteinosis of the lung; lymphoid and macrophage hyperplasia of the bronchial lymph node; and atrophy of the olfactory epithelium.

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\* Explanation of Levels of Evidence of Carcinogenic Activity is on page 14.

## Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Nickel Sulfate Hexahydrate

	Male F344/N Rats	Female F344/N Rats	Male B6C3F <sub>1</sub> Mice	Female B6C3F <sub>1</sub> Mice
Doses	0, 0.12, 0.25, or 0.5 mg/m <sup>3</sup> (equivalent to 0, 0.03, 0.06, or 0.11 mg nickel/m <sup>3</sup> )	0, 0.12, 0.25, or 0.5 mg/m <sup>3</sup> (equivalent to 0, 0.03, 0.06, or 0.11 mg nickel/m <sup>3</sup> )	0, 0.25, 0.5, or 1 mg/m <sup>3</sup> (equivalent to 0, 0.03, 0.06, or 0.11 mg nickel/m <sup>3</sup> )	0, 0.25, 0.5, or 1 mg/m <sup>3</sup> (equivalent to 0, 0.03, 0.06, or 0.11 mg nickel/m <sup>3</sup> )
Body weights	Exposed groups similar to controls	0.5 mg/m <sup>3</sup> group lower than controls	1 mg/m <sup>3</sup> group lower than controls	Exposed groups lower than controls
2-Year survival rates	16/54, 16/53, 18/53, 21/53	22/53, 17/53, 29/54, 30/55	26/61, 23/61, 24/62, 25/62	34/61, 39/60, 45/60, 37/60
Nonneoplastic effects	<u>Lung</u> : chronic active inflammation (14/54, 11/53, 42/53, 46/53); macrophage hyperplasia (7/54, 9/53, 35/53, 48/53); alveolar proteinosis (0/54, 0/53, 12/53, 41/53); fibrosis (3/54, 6/53, 35/53, 43/53) <u>Bronchial lymph node</u> : lymphoid hyperplasia (0/51, 0/48, 3/47, 10/52) <u>Nose</u> (olfactory epithelium): atrophy (0/54, 0/52, 3/53, 7/53)	<u>Lung</u> : chronic active inflammation (14/52, 13/53, 49/53, 52/54); macrophage hyperplasia (9/52, 10/53, 32/53, 45/54); alveolar proteinosis (1/52, 0/53, 22/53, 49/54); fibrosis (8/52, 7/53, 45/53, 49/54) <u>Bronchial lymph node</u> : lymphoid hyperplasia (2/50, 1/52, 0/51, 11/49) <u>Nose</u> (olfactory epithelium): atrophy (0/51, 1/52, 1/53, 7/54)	<u>Lung</u> : chronic active inflammation (1/61, 2/61, 8/62, 29/61); bronchialization (1/61, 4/61, 19/62, 39/61); macrophage hyperplasia (6/61, 9/61, 35/62, 59/61); interstitial infiltration (1/61, 0/61, 3/62, 17/61); alveolar proteinosis (0/61, 0/61, 0/62, 42/61) <u>Bronchial lymph node</u> : lymphoid hyperplasia (2/46, 4/49, 2/45, 17/54); macrophage hyperplasia (0/46, 0/49, 8/45, 39/54) <u>Nose</u> (olfactory epithelium): atrophy (0/61, 0/61, 12/61, 37/60)	<u>Lung</u> : chronic active inflammation (1/61, 7/60, 14/60, 40/60); bronchialization (0/61, 9/60, 32/60, 45/60); macrophage hyperplasia (7/61, 24/60, 53/60, 59/60); interstitial infiltration (0/61, 4/60, 16/60, 39/60); alveolar proteinosis (0/61, 0/60, 11/60, 45/60) <u>Bronchial lymph node</u> : lymphoid hyperplasia (15/50, 9/54, 16/58, 26/56); macrophage hyperplasia (2/50, 0/54, 14/52, 37/56) <u>Nose</u> (olfactory epithelium): atrophy (3/61, 2/59, 1/60, 17/60)
Neoplastic effects	None	None	None	None
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	No evidence
Genetic toxicology L5178Y Mouse lymphoma cells gene mutations:			Positive without S9.	

## EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (clear evidence and some evidence); one category for uncertain findings (equivocal evidence); one category for no observable effects (no evidence); and one category for experiments that cannot be evaluated because of major flaws (inadequate study). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase.
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

**NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS  
TECHNICAL REPORTS REVIEW SUBCOMMITTEE**

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on nickel sulfate hexahydrate on November 29, 1994, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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**SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS**

**NOTE:** A summary of the Technical Reports Review Subcommittee's remarks will appear in a future draft of this report.

## INTRODUCTION



### NICKEL SULFATE HEXAHYDRATE

CAS No. 10101-97-0

Chemical Formula:  $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$       Molecular Weight: 262.86

**Synonyms:** Blue salt; hexahydrate, nickel (2+) salt; nickel (2+) sulfate hexahydrate; nickel (II) sulfate hexahydrate; nickel monosulfate hexahydrate; nickel sulphate hexahydrate; nickelous sulfate hexahydrate; nickelous sulphate hexahydrate; single nickel salt, sulfuric acid

### CHEMICAL AND PHYSICAL PROPERTIES

Nickel sulfate hexahydrate (a blue-green crystalline powder) is a water-soluble nickel compound with a melting point of 53.3° C and a density of 2.07 g/cm<sup>3</sup> (*Merck Index*, 1989).

### PRODUCTION, USE, AND HUMAN EXPOSURE

Nickel was first isolated in 1751 and is found primarily as an oxide (laterite) or sulfide ore (pentlandite) (NIOSH, 1977; Warner, 1984; U.S. Bureau of Mines, 1984, 1985a). In 1991, the six largest nickel producing countries were the Soviet Union, Canada, Australia, New Caledonia, Indonesia, and Cuba. Approximately 55% of the nickel currently used is extracted from sulfide ore, and the remainder is extracted from oxide ore. The total annual world production of nickel is estimated at 1,000,000 tons (907,000 metric tons) (U.S. Bureau of Mines, 1991).

The United States consumption of nickel is approximately 200,000 tons (180,000 metric tons) annually (U.S. Bureau of Mines, 1991). The United States consumes unwrought nickel (68%), ferronickel

(17.3%), nickel oxide (11.4%), nickel salts (1.2%), and other forms (2.1%) (U.S. Bureau of Mines, 1984, 1985b). The National Occupational Exposure Survey (NIOSH) reported that 56,843 and 18,165 United States workers are potentially exposed to nickel sulfate and nickel oxide, respectively (information on nickel subsulfide exposure not reported) (NIOSH, 1994).

Half of the nickel sold per year is used to make stainless steel (Warner, 1984), which contains up to 8% nickel. The ability of nickel to impart corrosion resistance and strength leads to its wide use in chemicals and allied products and in petroleum refining (24%); in electrical equipment and supplies (11%); in aircraft and parts (10%); in construction (10%); in fabricated metal products (9%); household appliances (8%); machinery (7%); in ship and boat building (4%) and miscellaneous uses (7%) (U.S. Bureau of Mines, 1984).

Nickel constitutes about 0.008% of the earth's crust. Low levels of nickel are found in air, soil, water, food, and household objects. The average concentration of nickel in finished drinking water is less than 10 ppb. Nickel concentration in United States air has been found to range from 1 to 86 ng/m<sup>3</sup>. The most probable nickel species present in the atmosphere include nickel oxide and nickel sulfate, and the most probable species found in water includes nickel sulfate hexahydrate (ATSDR, 1992). The average amount of nickel in mainstream particulate fractions of cigarette smoke is 79 ng/cigarette (Bache *et al.*, 1985). Dietary intake of nickel per person from foods is estimated at 170 µg per day; intake from inhalation is estimated at 0.1 to 1 µg nickel per day (excluding cigarette smoke), and intake from drinking water is estimated at 2 µg per day (ATSDR, 1992). Nickel is a naturally occurring chemical at waste deposit sites in the United States (Fed. Regist.).

The threshold limit values adopted by the American Conference of Government Industrial Hygienists (ACGIH) are 1 mg/m<sup>3</sup> for nickel metal and water-insoluble salts and 0.1 mg/m<sup>3</sup> for water-soluble salts, but the ACGIH published notice of an intended change to 0.05 mg nickel/m<sup>3</sup> for water-soluble and

water-insoluble nickel compounds (ACGIH, 1993). NIOSH recommended that the permissible exposure limit for nickel be reduced to 0.015 mg nickel/m<sup>3</sup> averaged over a work shift of up to 10 hours per day, 40 hours per week (NIOSH, 1977).

Atomic absorption spectroscopy is a widely used method for quantifying nickel in the environment and in the workplace. This method of analysis measures total nickel without discerning the forms of nickel present, and most studies of environmental or industrial exposure report total nickel and not the occurrence of individual nickel species (ATSDR, 1992).

## ABSORPTION, DISTRIBUTION, AND EXCRETION

### *Experimental Animals*

Animal model systems have been used to obtain information on the absorption, distribution, and excretion of nickel after inhalation exposure (water-soluble and water-insoluble forms of nickel), oral exposure (water-soluble forms of nickel), and dermal exposure (water-soluble forms of nickel).

Intratracheal administration of nickel compounds was one method used by several investigators to study the fate of nickel in the lung. English *et al.* (1981) reported on a comparative toxicokinetic study after intratracheal administration of [<sup>63</sup>Ni]-labeled nickel chloride or nickel oxide (low temperature nickel oxide calcined at 250° C) in Wistar rats. Nickel, after nickel chloride administration, was excreted primarily in the urine. After nickel oxide administration, nickel was equally excreted in the feces and urine. Nickel oxide persisted in the lung for more than 90 days, while nickel chloride was rapidly excreted from the lung with greater than 50% of the nickel cleared from the lungs within 3 days.

Nickel chloride administered as an intratracheal dose to Sprague-Dawley rats was excreted primarily in the urine. By day 3, 90% of the instilled chemical was eliminated from the lungs. The lungs retained 29%

of their initial burden at day 1, and this decreased to 0.1% on day 21; 96% of the chemical was excreted in the urine (Carvalho and Ziemer, 1982).

The pulmonary clearance of intratracheally administered nickel subsulfide ( $\text{Ni}_3\text{S}_2$ ) in mice has two distinct components with initial and final biological half-lives corresponding to 1.2 and 12.4 days, respectively. The excretion of the chemical (measured as  $^{63}\text{Ni}$ ) was 60% in the urine and 40% in the feces; 57% of the administered dose was excreted after 3 days with 33% appearing in the urine (Valentine and Fisher, 1984). In another experiment, the calculated clearance times of nickel subsulfide administered intratracheally to mice was also biphasic with a clearance half-life of 2 hours for the first phase and 119 hours for the second phase (Finch *et al.*, 1987).

In F344/N rats administered [ $^{63}\text{Ni}$ ]-labeled nickel oxide (high temperature, green oxide) or nickel subsulfide by pernasal inhalation, the lung half-life was estimated at 120 days for nickel oxide and 5 days for nickel subsulfide (Benson *et al.*, 1994; Appendix P). Benson *et al.* (1994) found that, following nickel oxide exposure, nickel was not distributed to the extrarrespiratory tract tissue, and the material was only excreted in the feces during the first few days after exposure. In contrast, after nickel subsulfide exposure, nickel was detected in extrarrespiratory tract tissue including blood and kidney, and nickel was excreted in the urine and the feces. The half-life of [ $^{63}\text{Ni}$ ]-labeled nickel sulfate administered to F344/N rats by pernasal administration was 1 to 3 days; nickel was present in extrarrespiratory tract tissues (including blood, kidney, and intestine); and urine was the major route for excretion of nickel (Medinsky *et al.*, 1987).

Other studies also indicated that nickel oxide has a relatively long half-life in the rodent lung. Nickel oxide (formed at 550° C; mass median aerodynamic diameter of  $0.15 \mu\text{m} \pm$  geometric standard deviation of 1.5%) given as an aerosol of  $750 \mu\text{g}/\text{m}^3$  to Wistar rats had a bronchial clearance half-life of 1 day and

an alveolar clearance half-life of 36 days (Hochrainer *et al.*, 1980). Hochrainer *et al.* (1980) estimated that with continuous exposure to nickel oxide, a steady state would be reached after 1 year.

In Wistar rats after exposure to 0.6 or 8.0 mg nickel oxide/m<sup>3</sup> (high temperature, green oxide; mass median aerodynamic diameter of 1.2  $\mu\text{m}$   $\pm$  geometric standard deviation of 2.2%) for 6 to 7 hours per day for 1 to 2 months, the lung clearance was estimated to be 100  $\mu\text{g}$  per year. There was no apparent deposition of nickel in the liver, kidney, spleen, heart, brain, or blood (Kodama *et al.*, 1985). Lung clearance half-lives for nickel oxide (high temperature, green oxide) in Wistar rats exposed for 1 month were estimated to be 8, 11, and 21 months for nickel oxide with mass median aerodynamic diameters of 0.6, 1.2, and 4.0  $\mu\text{m}$ , respectively (Tanaka *et al.*, 1985, 1988).

In summary, in absorption and distribution studies for nickel administered intratracheally or by inhalation exposure, the lung half-life was 1 to 3 days for nickel sulfate, 5 days for nickel subsulfide, and greater than 100 days for nickel oxide. Nickel was detected in extrapulmonary tract tissue after exposure to nickel sulfate or nickel subsulfide, but not after exposure to nickel oxide.

The present studies also report findings on the deposition of nickel sulfate hexahydrate, nickel subsulfide, and nickel oxide in the lungs and tissues of rats and mice after 16 days, 13 weeks, and at 7 and 15 months in the 2-year studies. These data show a relatively short half-life in the lung for nickel sulfate hexahydrate, a longer half-life for nickel subsulfide, and the longest half-life for nickel oxide (Benson *et al.*, 1987; Dunnick *et al.*, 1989).

Studies of other routes of nickel exposure in rats, mice, and dogs indicate that 1% to 10% given as nickel sulfate hexahydrate or nickel chloride was absorbed after oral administration, and only a small percentage (<1%) of nickel chloride was absorbed through the skin of guinea pigs within 24 hours (ATSDR, 1992; Nielsen *et al.*, 1993).

### *Humans*

In the industrial setting, a major route of nickel exposure in humans is by inhalation (Sunderman, 1992); it is estimated that 35% of inhaled nickel is absorbed into the blood from the respiratory tract (Bennet, 1984; Grandjean, 1984; Sunderman and Oskarsson, 1991). There is evidence that, in nickel refinery workers, there are storage depots in the body that retain nickel for long periods of time; nickel was excreted in the urine of workers for periods of up to 6 months after facility closing (Morgan and Rouge, 1983). There were elevated nickel concentrations in specimens of urine, plasma, and nasal mucosa biopsies obtained from retired workers years after cessation of employment, although the specific form of nickel to which these workers were exposed was not identified (Torjussen and Andersen, 1979; Boysen *et al.*, 1984).

Andersen and Svenes (1989) found elevated levels of nickel in the lung of nickel refinery workers, although workers who were diagnosed as having lung cancer had the same concentrations of nickel in the lung at autopsy as those who died of other types of cancer. In the workplace setting, exposure to nickel is monitored by analyzing urine, hair, or fingernails for levels of total nickel.

When nickel sulfate was administered to human volunteers, 27% of the administered dose was absorbed when given in drinking water, while only 0.7% was absorbed when administered in food. The elimination half-life for absorbed nickel averaged 28 hours; 100% of the absorbed nickel was eliminated in either the feces or urine within 4 days (Sunderman, 1989, 1992). In studies in humans, reported absorption of radioactive nickel varied from 55% to 77% of nickel sulfate applied to occluded skin to 3% of nickel chloride applied to occluded skin (ATSDR, 1992).

## **TOXICITY**

Studies of nickel toxicity after experimental or industrial exposure have been summarized in various reviews (NAS, 1975; IARC, 1976, 1984, 1987, 1990; NIOSH, 1977; Brown and Sunderman, 1985;

USEPA, 1986; European Chemical Industry, 1989; WHO, 1991; ATSDR, 1992; Nieboer and Nriagu, 1992). In experimental animals and in humans, the primary toxic response to nickel after inhalation occurred in the respiratory system.

Information on the dissolution half-lives for nickel subsulfide and nickel oxide in water and rat serum have been reported. The calculated dissolution half-lives (based on *in vitro* studies) for nickel subsulfide and nickel oxide in water are greater than 7 or 11 years, respectively. In rat serum, the estimated dissolution half-life is 23 days for nickel subsulfide and greater than 11 years for nickel oxide (Sunderman *et al.*, 1987). While nickel subsulfide and nickel oxide are both relatively insoluble in water, nickel subsulfide is more soluble than nickel oxide in biological fluids. Soluble nickel salts (nickel hydroxide) have been shown to be more soluble in human serum than nickel subsulfide (Kasprzak *et al.*, 1983). The comparative toxicity of nickel sulfate hexahydrate, nickel subsulfide, and nickel oxide parallels the solubility of the compounds in biological fluids.

### *Experimental Animals*

The acute toxicity values for selected nickel compounds are summarized in Table 1. Nickel carbonyl ( $\text{NiC}_4\text{O}_4$ ) is the most acutely toxic form of nickel, but the use or formation of this nickel compound in manufacturing processes is limited (NAS, 1975). Exposure to nickel oxide, nickel sulfate hexahydrate, or nickel subsulfide is more common in the workplace.

In animals, after inhalation exposure to water-soluble and water-insoluble nickel compounds, the primary toxic response is seen in the respiratory system. Changes in a variety of parameters, including dose-related reduction in body weight, reduced leukocyte count, increase in urine alkaline phosphatase and alkaline phosphatase, and increased erythrocyte count, were observed in Wistar rats continuously exposed to nickel oxide at 200, 400, or 800  $\mu\text{g}/\text{m}^3$  for 120 days (except for daily cleaning and feeding periods) (Weischer *et al.*, 1980).

**TABLE 1**  
**Toxicity Values for Nickel Carbonyl, Nickel Oxide, Nickel Sulfate Hexahydrate, Nickel Sulfate,**  
**and Nickel Subsulfide<sup>a</sup>**

Nickel Compound	Species	Route	Toxicity Value <sup>b</sup>	
Nickel carbonyl	Rat	Inhalation	35 ppm (LC <sub>50</sub> )	
		Subcutaneous	63 mg/kg (LD <sub>50</sub> )	
		Intravenous	66 mg/kg (LD <sub>50</sub> )	
		Intraperitoneal	39 mg/kg (LD <sub>50</sub> )	
	Mouse	Inhalation	67 mg/m <sup>3</sup> (LC <sub>50</sub> )	
	Dog	Inhalation	360 ppm (LCLo)	
Nickel oxide	Rat	Inhalation	1,890 mg/m <sup>3</sup> (LC <sub>50</sub> )	
		Rabbit	Inhalation	73 g/m <sup>3</sup> (LCLo)
		Mouse	Inhalation	360 ppm (LCLo)
Nickel sulfate hexahydrate	Rat	Subcutaneous	25 mg/kg (LD <sub>50</sub> )	
		Intramuscular	180 mg/kg (TDLo)	
		Intratracheal	90 mg/kg (TDLo)	
	Mouse	Subcutaneous	50 mg/kg (LD <sub>50</sub> )	
		Intraperitoneal	400 mg/kg (TDLo)	
		Dog	Intraperitoneal	400 mg/kg (TDLo)
Nickel sulfate	Dog	Subcutaneous	500 mg/kg (LDLo)	
		Intravenous	89 mg/kg (LDLo)	
	Cat	Subcutaneous	500 mg/kg (LDLo)	
		Intravenous	72 mg/kg (LDLo)	
Rabbit	Subcutaneous	500 mg/kg (LDLo)		
	Intravenous	36 mg/kg (LDLo)		
Guinea pig	Subcutaneous	62 mg/kg (LDLo)		
	Dog	Subcutaneous	62 mg/kg (LDLo)	
Nickel sulfate	Rat	Intraperitoneal	500 mg/kg (LD <sub>50</sub> )	
		Intravenous	500 mg/kg (LD <sub>50</sub> )	
	Mouse	Intraperitoneal	21 mg/kg (LD <sub>50</sub> )	
		Intravenous	7 mg/kg (LDLo)	
	Dog	Subcutaneous	38 mg/kg (LDLo)	
		Intravenous	38 mg/kg (LDLo)	
	Cat	Subcutaneous	24 mg/kg (LDLo)	
	Rabbit	Subcutaneous	33 mg/kg (LDLo)	
		Intravenous	33 mg/kg (LDLo)	

(continued)

**TABLE 1**  
**Toxicity Values for Nickel Carbonyl, Nickel Oxide, Nickel Sulfate Hexahydrate, Nickel Sulfate,**  
**and Nickel Sub sulfide (continued)**

Nickel Compound	Species	Route	Toxicity Value
Nickel subsulfide	Rat	Inhalation	1 mg/kg (TCLo)
		Subcutaneous	125 mg/kg (TDLo)
		Intravenous	10 mg/kg (TDLo)
		Intramuscular	20 mg/kg (TDLo)
	Mouse	Intramuscular	200 mg/kg (TDLo)

<sup>a</sup> From RTECS (1987)

<sup>b</sup> LC<sub>50</sub> = median lethal concentration; LCLo = lowest lethal concentration; LD<sub>50</sub> = median lethal dose; LDLo = lowest lethal dose; TCLo = lowest toxic concentration; TDLo = lowest toxic dose.

Alveolar macrophages from lung lavage fluid from rats exposed to nickel oxide at 120  $\mu\text{g}/\text{m}^3$  for 12 hours per day, 6 days per week for 28 days or by intraperitoneal injection (10 mg NiO/mouse; sacrifice 1 week later) were examined by electron microscopy. Compared to controls, alveolar macrophages from exposed animals were increased in numbers and enlarged. In the cytoplasm of alveolar macrophages, phagosomes contained osmophilic nickel oxide particles as well as membranous and lamellar structures consistent with accumulation of phospholipid material (Migally *et al.*, 1982; Murthy and Niklowitz, 1983).

Respiratory toxicity to F344/Crl rats administered a single dose of either nickel subsulfide, nickel chloride, nickel sulfate, or nickel oxide by intratracheal instillation was evaluated by examining treatment-related changes in lung lavage fluid (Benson *et al.*, 1986). No significant changes in lung lavage fluid were seen after exposure to nickel oxide. After exposure to nickel subsulfide, nickel sulfate hexahydrate, and nickel chloride, there were increases in the following parameters in lung lavage fluid: lactate dehydrogenase,  $\beta$ -glucuronidase, total protein, glutathione reductase, glutathione peroxidase, and sialic acid. This evaluation was continued by exposing rats or mice to nickel oxide, nickel sulfate hexahydrate, or nickel subsulfide for 13 weeks and looking for treatment-related markers of lung toxicity in lung lavage fluid (Benson *et al.*, 1989; Appendix O). Increases in  $\beta$ -glucuronidase, total protein, neutrophil number, and macrophage number were observed in the lavage fluid after exposure of rats and mice to all three

nickel compounds, although there were quantitative differences in the magnitude of the response. Inflammation was observed histologically in the lung of rats and mice exposed to the three nickel compounds. The severity of lung toxicity as measured by the changes in lung lavage fluid paralleled the severity of histologic changes in the lung. Nickel sulfate hexahydrate was the most toxic, and nickel oxide was the least toxic (Benson *et al.*, 1989). Treatment of rats and mice with water-soluble and water-insoluble nickel salts may cause an alteration of local and systemic immunity, and this toxicity has been studied under various conditions and experiments (Table 2).

Toxic responses to the immune system were measured in B6C3F<sub>1</sub> mice after inhalation exposure to nickel subsulfide, nickel oxide, or nickel sulfate hexahydrate for 6 hours per day and 5 days per week for 13 weeks. Exposure concentrations were 0.11, 0.45, and 1.8 mg nickel/m<sup>3</sup> for nickel subsulfide; 0.47, 2.0, and 7.9 mg nickel/m<sup>3</sup> for nickel oxide; and 0.027, 0.11, and 0.45 mg nickel/m<sup>3</sup> for nickel sulfate hexahydrate. Thymic weights in mice exposed to 1.8 mg nickel/m<sup>3</sup> of nickel subsulfide were lower than those of the controls. Lung-associated lymph nodes were increased in size after exposure to all compounds. The number of alveolar macrophages in lavage samples were increased in mice exposed to the highest concentrations of nickel sulfate hexahydrate and nickel oxide and to 0.45 and 1.8 mg nickel/m<sup>3</sup> nickel subsulfide. Numbers of antibody-forming cells in lung-associated lymph nodes of mice exposed to 2.0 and 7.9 mg nickel/m<sup>3</sup> nickel oxide and 1.8 mg nickel/m<sup>3</sup> nickel subsulfide were greater than those in the controls. Low numbers of antibody-forming cells were observed in spleens of mice exposed to nickel oxide and in mice exposed to 1.8 mg nickel/m<sup>3</sup> nickel subsulfide. Only mice exposed to 1.8 mg nickel/m<sup>3</sup> nickel subsulfide had a low mixed lymphocyte response. All concentrations of nickel oxide resulted in low levels of alveolar macrophage phagocytic activity, as did 0.45 and 1.8 mg nickel/m<sup>3</sup> nickel subsulfide. None of the nickel compounds affected the phagocytic activity of peritoneal macrophages. Only 1.8 mg nickel/m<sup>3</sup> nickel subsulfide caused a depressed spleen natural killer cell activity. Results indicate

**TABLE 2**  
**Studies on the Immunologic Effects of Nickel Compounds**

Nickel Compound	Species/Route	Treatment	Response	Reference
<b>Cell-Mediated Immunity</b>				
Nickel chloride	CBA/J mice/ intramuscular	Single injection, 18 mg/kg	Reduced T-lymphocyte proliferation	Smialowicz <i>et al.</i> (1984)
	Guinea pig	<i>In vitro</i> study on spleen cells	Inhibited macrophage migration	Hennighausen and Lange (1980)
Nickel sulfate	B6C3F <sub>1</sub> mice (female)/oral	Up to 4,000 mg/kg/day for 23 weeks	Depressed spleen lymphoproliferative response to LPS (no effect on NK activity; PFC assay; mitogen response in spleen cells; resistance to <i>Listeria</i> challenge)	Dieter <i>et al.</i> (1988)
<b>Humoral Immunity</b>				
Nickel chloride	CBA/J mice/ intramuscular	Single injection, 18 mg/kg	Reduced antibody response to T-cell dependent sheep red blood cells	Smialowicz <i>et al.</i> (1984)
	C57BL/6J spleen cells	<i>In vitro</i> exposure to nickel chloride	Decreased response	Lawrence (1981)
	Swiss albino mice/ intramuscular	3-12 $\mu$ g Ni/kg body weight followed by immunization with sheep red blood cells	Depressed antibody formation	Graham <i>et al.</i> (1975a)
	Swiss mice/ inhalation	2-hour inhalation exposure at 250 $\mu$ g/m <sup>3</sup>	Depressed antibody response to sheep red blood cells	Graham <i>et al.</i> (1978)
Nickel acetate	Sprague-Dawley rats/intraperitoneal	11 mg/kg body weight immunized with <i>E. coli</i> bacteriophage	Depressed circulating antibody response	Figoni and Treagan (1975)
Nickel oxide	Wistar rats/ inhalation	25-800 $\mu$ g/m <sup>3</sup> for 4 weeks to 4 months	Decreased ability to form spleen antibodies to sheep red blood cells	Spiegelberg <i>et al.</i> (1984)
<b>Macrophage Function</b>				
Nickel chloride	CBA/J mice/ intramuscular	Single injection, 18 mg/kg	No effect on phagocytic capacity of peritoneal macrophages	Smialowicz <i>et al.</i> (1984)
	Rabbits	Alveolar macrophage <i>in vitro</i> exposure	Reduced viability of macrophages	Graham <i>et al.</i> (1975b)
Nickel oxide and nickel chloride	Wistar rats/ inhalation	12 hours/day, 6 days/week for 2 weeks at 0.1 mg/m <sup>3</sup>	Increased number of alveolar macrophages after nickel oxide; no change after nickel chloride	Bingham <i>et al.</i> (1972)
Nickel oxide	Wistar rats/ inhalation	800 $\mu$ g/m <sup>3</sup> for 2 weeks	Decrease in alveolar macrophage phagocytic ability	Spiegelberg <i>et al.</i> (1984)
(continued)				

**TABLE 2**  
**Studies on the Immunologic Effects of Nickel Compounds (continued)**

Nickel Compound	Species/Route	Treatment	Response	Reference
<b>Natural Killer Cell Activity</b>				
Nickel chloride	CBA/J and C57BL/6J mice/ intramuscular	Single injection, 18 mg/kg	Depressed NK activity (against Yac-1 murine lymphoma cells)	Smialowicz <i>et al.</i> (1984, 1985, 1986)
<b>Host Resistance</b>				
Nickel chloride and nickel oxide	CD mice and Sprague-Dawley rats/ inhalation	0.5 mg/m <sup>3</sup> for 2 hours	Enhanced respiratory infection to <i>Streptococcus</i>	Adkins <i>et al.</i> (1979)

that inhalation exposure of mice to nickel can have varying effects on the immune system, depending on dose and physicochemical form of the nickel compound, and these effects were observed at occupationally relevant exposure concentrations (Haley *et al.*, 1990; Appendix N).

Administration of nickel sulfate in the drinking water for 180 days (1 to 10 g/L drinking water, estimated to deliver 116 to 396 mg/kg body weight) resulted in a depressed proliferating response in the bone marrow and spleen of B6C3F<sub>1</sub> mice (Dieter *et al.*, 1988).

While experimental studies in animals show the potential of nickel to affect the immune system, the clinical significance of these studies in humans has not been determined (Nicklin and Nielsen, 1992). Further, there are no studies to examine if there is a relationship between effects on the immune system and the carcinogenic effects of nickel.

### *Humans*

Most of the toxicity information on nickel and nickel compounds came from studies of workers in nickel refineries where the primary toxicity is to the respiratory system. In the industrial setting, nickel exposures were associated with rhinitis, sinusitis, and nasal-septal perforations. Hypersensitive allergic

asthmatic reactions to nickel are rare (Nemery, 1990). There were also reports of pulmonary fibrosis in workers inhaling nickel dust (WHO, 1991). While respiratory toxicity has been observed in workers exposed to nickel in the industrial setting, these workers are often exposed to other toxic metals and/or cigarette smoke, and it has not always been possible to conclude that nickel is the sole causative agent of toxicity (ATSDR, 1992).

Nickel contact hypersensitivity has been seen in the general population and in exposed workers. In the general population contact sensitivity to nickel-containing jewelry and/or prosthesis is another form of nickel toxicity (ATSDR, 1992). Other toxic reactions to nickel were reported in humans in isolated cases including: cardiovascular effects in a child ingesting nickel sulfate; and gastrointestinal effects, transient increases in blood reticulocytes, or muscular pain in workers exposed to nickel-contaminated water (ATSDR, 1992).

## CARCINOGENICITY

### *Experimental Animals*

The International Agency for Cancer Research (IARC, 1990) summarized the results of experimental studies which studied the carcinogenic potential of nickel compounds after local injection (e.g., subcutaneous or intramuscular injection). Nickel oxide, nickel subsulfide, nickel carbonyl, and nickel powder cause neoplasms at the injection site, while the soluble nickel salts such as nickel sulfate have generally not been associated with a carcinogenic response at the injection site. A portion of the IARC (1990) listing and tabulation of over 100 experiments on the carcinogenic potential of nickel compounds is presented in Table 3.

**TABLE 3**  
**Summary of Studies Used to Evaluate the Carcinogenicity of Nickel Compounds**  
**in Experimental Animals<sup>a</sup>**

Nickel Compound	Species/Route	Lesion Incidence <sup>b</sup>	Reference	
<b>Nickel oxides and hydroxides</b>				
Nickel monoxide (green)	Rat/inhalation	0.6 mg/m <sup>3</sup> : 0/6 lung lesion 8 mg/m <sup>3</sup> : 1/8 lung lesion	Horie <i>et al.</i> (1985)	
Nickel monoxide	Rat/inhalation	0.06 mg/m <sup>3</sup> : 0/40 lesion 0.2 mg/m <sup>3</sup> : 0/20 lesion	Glaser <i>et al.</i> (1986)	
	Rat/intrapleural	Controls: 0/32 local lesions 31/32 local lesions	Skaug <i>et al.</i> (1985)	
	Rat/intratracheal	Controls: 0/40 lesions 10 × 5 mg: 10/37 lung lesions 10 × 15 mg: 12/38 lung lesions	Pott <i>et al.</i> (1987)	
	Rat/intramuscular	21/32 local lesions	Gilman (1962)	
	Rat/intramuscular	2/20 local lesions	Gilman (1966)	
	Rat/intramuscular	0/20 local lesions	Sosiński (1975)	
	Rat/intramuscular	14/15 local lesions	Sunderman and McCully (1983)	
	Rat/intramuscular	0/20 local lesions	Berry <i>et al.</i> (1984)	
	Rat/subperiosteal	0/20 local lesions	Berry <i>et al.</i> (1984)	
	Rat/intraperitoneal	46/47 local lesions	Pott <i>et al.</i> (1987)	
	Rat/intraperitoneal	25 mg: 12/34 local lesions 100 mg: 15/36 local lesions	Pott <i>et al.</i> (1989, 1992)	
	Nickel monoxide (green)	Rat/intrarenal	0/12 local lesions	Sunderman <i>et al.</i> (1984)
	Nickel monoxide	Mouse/intramuscular	33/50 and 23/52 local lesions	Gilman (1962)
Hamster/inhalation		1/51 osteosarcoma	Wehner <i>et al.</i> (1975, 1979)	
Hamster/intratracheal		Controls: 4/50 lung lesions 1/49 lung lesions	Farrell and Davis (1974)	
Nickel hydroxide	Rat/intramuscular	15/20 local lesions	Gilman (1966)	
	Rat/intramuscular	Dried gel: 5/19 local lesions Crystalline: 3/20 local lesions Colloidal: 0/13 local lesions	Kasprzak <i>et al.</i> (1983)	
	Rat/intramuscular	0/10 local lesions	Judde <i>et al.</i> (1987)	
Nickel trioxide	Rat/intramuscular	0/10 local lesions	Judde <i>et al.</i> (1987)	
	Rat/intracerebral	3/20 local lesions	Sosiński (1975)	

(continued)

**TABLE 3**  
**Summary of Studies Used to Evaluate the Carcinogenicity of Nickel Compounds**  
**in Experimental Animals (continued)**

Nickel Compound	Species/Route	Lesion Incidence	Reference
<b>Nickel sulfides</b>			
Nickel disulfide	Rat/intramuscular	12/14 local lesions	Sunderman (1984)
	Rat/intrarenal	2/10 local lesions	Sunderman <i>et al.</i> (1984)
Nickel sulfide (amorphous)	Rat/intramuscular	5.6 mg: 0/10 local lesions 22.4 mg: 0/10 local lesions	Sunderman and Maenza (1976)
$\beta$ -Nickel sulfide	Rat/intramuscular	14/14 local lesions	Sunderman (1984)
Nickel sulfide (amorphous)	Rat/intramuscular	3/25 local lesions	Sunderman (1984)
Nickel sulfide	Rat/intrarenal	0/18 local lesions	Jasmin and Riopelle (1976)
$\beta$ -Nickel sulfide	Rat/intrarenal	8/14 local lesions	Sunderman <i>et al.</i> (1984)
Nickel sulfide (amorphous)	Rat/intrarenal	0/15 local lesions	Sunderman <i>et al.</i> (1984)
Nickel subsulfide	Rat/inhalation	14/208 malignant lung lesions 15/208 benign lung lesions	Ottolenghi <i>et al.</i> (1975)
	Rat/intratracheal	0.94 mg: 7/47 lung lesions 1.88 mg: 13/45 lung lesions 3.75 mg: 12/40 lung lesions	Pott <i>et al.</i> (1987)
	Rat/intrapleural	28/32 local lesions	Skaug <i>et al.</i> (1985)
	Rat/subcutaneous	3.3 mg: 37/39 local lesions 10 mg: 37/40 local lesions	Mason (1972)
	Rat/subcutaneous	18/19 local lesions	Shibata <i>et al.</i> (1989)
	Rat/intramuscular	25/28 local lesions	Gilman (1962)
	Rat/intramuscular	Controls: 1/19 local lesion 10 mg powder: 19/20 local lesions 10 mg diffusion chamber: 14/17 local lesions 500 mg fragments: 5/7 local lesions 500 mg discs: 14/17 local lesions	Gilman and Herchen (1963)

(continued)

**TABLE 3**  
**Summary of Studies Used to Evaluate the Carcinogenicity of Nickel Compounds**  
**in Experimental Animals (continued)**

Nickel Compound	Species/Route	Lesion Incidence	Reference
Nickel sulfides (continued)			
Nickel subsulfide (disc)	Rat/intramuscular	Removal of disc after 64 days: 4/10 local lesions Removal of disc after 128 days: 7/10 local lesions Removal of disc after 206 days: 10/10 local lesions	Herchen and Gilman (1964)
Nickel subsulfide	Rat/intramuscular	NIH black: 28/28 local lesions Hooded: 14/23 local lesions	Daniel (1966)
	Rat/intramuscular	3.3 mg: 38/39 local lesions 10 mg: 34/40 local lesions	Mason (1972)
	Rat/intramuscular	5 mg: 8/20 local lesions 20 mg: 9/9 local lesions	Sunderman and Maenza (1976)
	Rat/intramuscular	Fischer: 59/63 local lesions Hooded: 11/20 local lesions	Yamashiro <i>et al.</i> (1980)
	Rat/intramuscular	0.6 mg: 7/30 local lesions 1.2 mg: 23/30 local lesions 2.5 mg: 28/30 local lesions 5 mg: 29/30 local lesions	Sunderman <i>et al.</i> (1976)
	Rat/intramuscular	0.63 mg: 7/29 local lesions 20 mg: 9/9 local lesions	Sunderman (1981)
$\alpha$ -Nickel subsulfide	Rat/intramuscular	9/9 local lesions	Sunderman (1984)
Nickel subsulfide	Rat/intramuscular	10/20 local lesions	Berry <i>et al.</i> (1984)
	Rat/intramuscular	2/100 local lesions	Judde <i>et al.</i> (1987)
	Rat/intramuscular	19/20 local lesions	Shibata <i>et al.</i> (1989)
	Rat/intraperitoneal	9/37 local lesions	Gilman (1966)
	Rat/intraperitoneal	27/42 local lesions	Pott <i>et al.</i> (1987)
	Rat/intraperitoneal	6 mg: 20/36 local lesions 12 mg: 25/35 local lesions 25 mg: 25/34 local lesions	Pott <i>et al.</i> (1989, 1992)
	Rat/subperiosteal	0/20 local lesions	Berry <i>et al.</i> (1984)
	Rat/intrafemoral	10/20 local lesions	Berry <i>et al.</i> (1984)
	Rat/intrarenal	In glycerin: 7/16 local lesions In saline: 11/24 local lesions	Jasmin and Riopelle (1976)
(continued)			

**TABLE 3**  
**Summary of Studies Used to Evaluate the Carcinogenicity of Nickel Compounds**  
**in Experimental Animals (continued)**

Nickel Compound	Species/Route	Lesion Incidence	Reference
Nickel sulfides (continued)			
$\alpha$ -Nickel subsulfide	Rat/intrarenal	Wistar Lewis: 7/11 local lesions NIH black: 6/12 local lesions Fischer 344: 9/32 local lesions Long-Evans: 0/12 local lesions	Sunderman <i>et al.</i> (1979)
Nickel subsulfide	Rat/intratesticular	16/19 local lesions	Damjanov <i>et al.</i> (1978)
	Rat/intraocular	14/15 local lesions	Albert <i>et al.</i> (1980); Sunderman (1983a)
	Rat/transplacental	No difference in lesion incidence	Sunderman <i>et al.</i> (1981)
	Rat/pellet implantation into subcutaneous implanted tracheal grafts	5 mg: 9/60 local lesions 15 mg: 45/64 local lesions	Yarita and Nemesheim (1978)
	Rat/intra-articular	16/19 local lesions	Shibata <i>et al.</i> (1989)
	Rat/intra-fat	9/20 local lesions	Shibata <i>et al.</i> (1989)
	Mouse/intratracheal	No increase in lung lesion incidence	Fisher <i>et al.</i> (1986)
	Mouse/subcutaneous	5 mg: 4/8 local lesions 10 mg: 7/8 local lesions	Oskarsson <i>et al.</i> (1979)
	Mouse/intramuscular	Swiss: 27/45 local lesions C3H: 9/18 local lesions	Gilman (1962)
	Mouse/intramuscular	5 mg: 4/8 local lesions 10 mg: 4/8 local lesions	Oskarsson <i>et al.</i> (1979)
$\alpha$ -Nickel subsulfide	Hamster/intratracheal	0/62 lung lesions	Muhle <i>et al.</i> (1992)
Nickel subsulfide	Hamster/intramuscular	Controls: 0/14 local lesions 5 mg: 4/15 local lesions 10 mg: 12/17 local lesions	Sunderman (1983b)
$\alpha$ -Nickel subsulfide	Hamster/topical	54 mg total: 0/6 local lesions 108 mg total: 0/7 local lesions 540 mg total: 0/15 local lesions 1080 mg total: 0/13 local lesions	Sunderman (1983a)
Nickel subsulfide	Rabbit/intramuscular	16 local lesions	Hildebrand and Busene (1979a,b)
(continued)			

**TABLE 3**  
**Summary of Studies Used to Evaluate the Carcinogenicity of Nickel Compounds**  
**in Experimental Animals (continued)**

Nickel Compound	Species/Route	Lesion Incidence	Reference
<b>Nickel sulfides (continued)</b>			
$\alpha$ -Nickel subsulfide	Rabbit/intramuscular	0/4 local lesions	Sunderman (1983b)
Nickel subsulfide	Salamander/intraocular	7/8 local lesions	Okamoto (1987)
Nickel ferrosulfide	Rat/intramuscular	15/15 local lesions	Sunderman (1984)
	Rat/intrarenal	1/12 local lesions	Sunderman <i>et al.</i> (1984)
<b>Nickel salts</b>			
Basic nickel carbonate tetrahydrate	Rat/intraperitoneal	Controls: 1/33 lung lesions	Pott <i>et al.</i> (1989, 1992)
		25 mg: 1/35 lung lesions	
		50 mg: 3/33 lung lesions	
Nickel acetate	Mouse/intraperitoneal	72 mg: 8/18 lung lesions	Stoner <i>et al.</i> (1976)
		180 mg: 7/14 lung lesions	
		360 mg: 12/19 lung lesions	
Nickel acetate tetrahydrate	Rat/intramuscular	1/35 local lesions	Payne (1964)
	Mouse/intraperitoneal	Controls: 0.32 lung lesions/animal	Poirier <i>et al.</i> (1984)
1.5 lung lesions/animal			
Nickel acetate tetrahydrate	Rat/intraperitoneal	Controls: 1/33 lung lesions	Pott <i>et al.</i> (1989, 1992)
		25 mg: 3/35 lung lesions	
		50 mg: 5/31 lung lesions	
Nickel ammonium sulfate	Rat/intramuscular	0/35 local lesions	Payne (1964)
Nickel carbonate	Rat/intramuscular	6/35 local lesions	Payne (1964)
Nickel chloride	Rat/intramuscular	0/35 local lesions	Payne (1964)
Nickel chloride hexahydrate	Rat/intraperitoneal	Controls: 1/33 lung lesions 4/32 lung lesions	Pott <i>et al.</i> (1989, 1992)
Nickel chromate	Rat/intramuscular	1/16 local lesions	Sunderman (1984)
Nickel fluoride	Rat/intramuscular	3/18 local lesions	Gilman (1966)
Nickel sulfate	Rat/intramuscular	1/35 local lesions	Payne (1964)
	Rat/intramuscular	0/20 local lesions	Gilman (1966)
	Rat/intramuscular	0/20 local lesions	Kasprzak <i>et al.</i> (1983)
(continued)			

**TABLE 3**  
**Summary of Studies Used to Evaluate the Carcinogenicity of Nickel Compounds**  
**in Experimental Animals (continued)**

Nickel Compound	Species/Route	Lesion Incidence	Reference
<b>Nickel salts (continued)</b>			
Nickel sulfate hexahydrate	Rat/intramuscular	0/32 local lesions	Gilman (1962)
Nickel sulfate heptahydrate	Rat/intraperitoneal	Controls: 1/33 lung lesions 6/30 lung lesions	Pott <i>et al.</i> (1989, 1992)
<b>Other</b>			
Nickel carbonyl	Rat/inhalation	30 mg/m <sup>3</sup> for 32 weeks: 1/64 pulmonary lesions 60 mg/m <sup>3</sup> for 32 weeks: 1/32 pulmonary lesions 250 mg/m <sup>3</sup> once: 1/80 pulmonary lesion	Sunderman <i>et al.</i> (1957, 1959)
	Rat/inhalation	Controls: 0/32 lung lesions 1/71 lung lesions	Sunderman and Donnelly (1965)
	Rat/intravenous	19/120 lung lesions	Lau <i>et al.</i> (1972)

<sup>a</sup> From IARC (1990)

<sup>b</sup> Number of animals with lesion per effective number

Information for the carcinogenic potential of nickel oxide, nickel subsulfide, and nickel sulfate hexahydrate by inhalation exposure is limited. Ottolenghi *et al.* (1975) reported that nickel subsulfide (70% of particles were smaller than 1  $\mu\text{m}$  in diameter; 25% of particles were between 1 and 1.5  $\mu\text{m}$ ) caused an increased incidence in lung tumors in F344/N rats exposed to 1 mg/m<sup>3</sup> by inhalation (6 hours/day and 5 days/week for 108 weeks). In the exposed groups, 12% to 14% of the animals (208 animals examined histologically) had lung tumors compared to less than 0.5% of control animals (215 animals examined histologically). At the end of the 108-week exposure period, fewer than 5% of the animals in exposed groups were alive compared with a survival of 31% in control groups.

Other experimental studies indicated carcinogenic potential of nickel subsulfide for the respiratory tract mucosa. Yarita and Nettesheim (1978) reported that a single nickel subsulfide intratracheal dose of 1 or

3 mg/kg caused tumors in heterotrophic tracheal transplants in female F344 rats. These authors noted that toxicity might decrease a carcinogenic response resulting in a misleadingly low carcinoma incidence, based on the finding that the more toxic dose (3 mg/kg) caused only a 1.5% incidence of carcinomas (there was a high incidence of tracheal hyperplastic change) versus a 10% carcinoma incidence in the 1 mg/kg group (generally with only a low incidence of toxic lesions).

Hamsters exposed to 53 mg nickel oxide/m<sup>3</sup> (median diameter of 0.3 μm; geometric standard deviation of 2.2) for 2 years did not have an increase in the incidence of lung tumors (Wehner *et al.*, 1975). The hamster may be less sensitive than the rat to the carcinogenic effects of nickel (Furst and Schlauder, 1971).

Sunderman *et al.* (1959) found a low incidence of lung tumors in groups of Wistar rats exposed to nickel carbonyl (0.03 to 0.25 mg/m<sup>3</sup> for 30 minutes 3 times/week for 1 year). Follow-up studies also showed a low incidence of lung tumors in rats exposed to nickel carbonyl (Sunderman and Donnelly, 1965).

Information on the carcinogenic potential of nickel after oral administration is limited (IARC, 1990). Life-long exposure to nickel acetate at low concentrations (5 ppm) induced no lung lesions in Swiss mice (Schroeder *et al.*, 1964; Schroeder and Mitchener, 1975); the maximum tolerated dose was not reached. Ambrose *et al.* (1976) administered nickel sulfate hexahydrate in the diet of Wistar rats or dogs (0, 100, 1,000, 2,500 ppm) for 2 years, and no treatment-related lesions were observed.

### *Humans*

Exposure to nickel in the workplace has been associated with an increase in lung and nasal sinus tumors (IARC, 1976, 1987; Doll, 1990). Based on the finding of lung and/or nasal sinus tumors in nickel refinery workers, IARC classified nickel and nickel compounds as human carcinogens (Group 1), although there was insufficient information available to evaluate the carcinogenic risk for individual nickel

compounds or the risk for cancer based on exposure to different concentrations of nickel compound(s) (IARC, 1987).

Information on the hazards associated with exposure to nickel came from studies on occupational exposure in nickel refineries and mines in Clydach, South Wales; Kristiansand, Norway; the International Nickel Company (INCO) refineries in Ontario, Canada; or from other studies of nickel refineries or mining operations throughout the world (Doll, 1984).

The United States Environmental Protection Agency (USEPA, 1986) and the International Committee on Nickel Carcinogenesis in Man (Doll, 1990) reviewed the epidemiological evidence for cancer after exposure to nickel in mining or refinery operations. A complete analysis on the type of ore mined and the calcining, smelting, and refining operations in 10 different mines or refineries throughout the world can be found in Doll (1990) and in other more recent summaries (Courtin, 1994; McIlveen and Negusante, 1994; Nieboer and Templeton, 1994; Norseth, 1994). Doll (1990) also estimates the type of nickel exposures encountered based on knowledge of the nickel process procedures used and a few relatively recent measurements of total airborne nickel.

The first indication that some form of nickel can give rise to lung and nasal sinus cancers was obtained from refinery workers at Clydach, South Wales (Bridge, 1933; Doll, 1958; Morgan, 1958). The Clydach Nickel Refinery (Mond Nickel Works) opened in 1902 and received nickel sulfide matte primarily from INCO (Port Colborne refinery, Canada). In 1933, nasal sinus and lung tumors were first noted in workers who were employed prior to 1925. After 1925, the copper and sulfate content of the matte was reduced, the arsenic contamination in sulfuric acid used to extract copper was reduced, the use of respirators and masks was introduced, and improvements were made in factory design that reduced exposure to nickel (USEPA, 1986). An increased risk for lung and nasal sinus tumors was particularly noted in refinery work involving roasting, sintering, and calcining processes that converted impure nickel-copper matte to an oxide (Doll, 1990).

Peto *et al.* (1984) analyzed the incidence of lung and nasal sinus cancers found in workers in the Clydach plant and found the highest incidence of cancer in those workers employed in the copper sulfate and furnace areas. There was no increased risk to workers in the reduction area where nickel carbonyl concentrations were highest.

Other evidence for nasal sinus and lung cancer come from studies of workers in the INCO (Ontario, Canada) mines and refineries (Roberts *et al.*, 1989a,b; Muir *et al.*, 1994). Facilities operated include the Sudbury area mines (Copper Cliff Smelter and the Port Colborne refinery) that use an ore that is primarily petlandite (NiFeS<sub>2</sub>). Men working in mining operations in Ontario had a two-fold increase in lung cancer risk, but no nasal sinus cancers (Doll, 1990).

The Falconbridge refinery in Kristiansand, Norway, receives nickel ore (a nickel copper sulfide matte) from Canada and uses an electrolysis process to refine the ore. Workers in roasting and smelting operations are exposed to dry dust containing nickel subsulfide and nickel oxide. Electrolysis workers are also exposed to nickel sulfate and nickel chloride. In this cohort, nasal sinus and lung cancer risks were increased in men working in the electrolysis department, thus implicating the soluble forms of nickel as the cause for the cancer (USEPA, 1986; Doll, 1990). The electrolysis workers had the highest average plasma and urine nickel concentrations (Høgetveit *et al.*, 1978).

Enterline and Marsh (1982) and Goldberg *et al.* (1994) studied cancer rates in a refinery in Huntington, West Virginia, which received nickel sulfide matte from Canada. The Doll Committee reported no clear evidence for an increased incidence in lung cancer in this population, although the data from this cohort provided weak evidence for an increased incidence in lung cancer in men exposed to sulfidic nickel at 4 mg nickel/m<sup>3</sup> for more than a year (Doll, 1990).

Results of epidemiology studies of workers in the nickel mining, smelting, and refinery operations in New Caledonia (French territory in the South Pacific) showed no increased incidence of lung or upper respiratory tract cancers. Nickel at this site is mined from nickel oxide or the silicate form of the ore. The Doll Committee also reported little evidence for an increased incidence in lung or upper respiratory tract cancer in this group of nickel workers (Doll, 1990).

The ten cohorts of nickel workers studied by the Doll Committee include the six cohorts mentioned above (nickel refinery operations, Clydach, South Wales; Falconbridge Nickel Mines, Ontario, Canada; INCO mines and refineries [Copper Cliff and Port Colborne], Ontario, Canada; Falconbridge refinery, Kristiansand, Norway; Huntington Alloys, West Virginia; and New Caledonia mines) as well as the Hanna Nickel Smelting Co., Oregon; Oak Ridge Gaseous Diffusion Plant, Tennessee; Outokumpu Oy nickel refinery, Finland; and Henry Wiggin Alloy Co., England (Doll, 1990).

The results within the individual cohorts varied, but the overall conclusion by the Doll Committee suggested that more than one form of nickel gives rise to lung and nasal sinus cancer. Much of the respiratory cancer risk was attributed to exposure to a mixture of oxidic and sulfidic nickel. Exposure to oxidic nickel in the absence of sulfidic nickel was also associated with increased lung and nasal sinus cancer risks. There was evidence that exposure to soluble nickel salts increased the risk of lung and nasal sinus cancer and that it may enhance risks associated with exposure to less soluble forms of nickel. There was no evidence that metallic nickel was associated with increased lung and nasal sinus cancer risks. There was no evidence to suggest that exposure to metallic nickel or any of its compounds was likely to produce cancers elsewhere than in the lung or nose. These investigators were not able to provide dose-specific estimates of risks for individual nickel species. However, the evidence from these studies suggests that respiratory cancer risks are primarily related to exposure to water-soluble nickel compounds at concentrations in excess of 1 mg nickel/m<sup>3</sup> and to exposure to less soluble forms at concentrations greater than 10 mg nickel/m<sup>3</sup>.

There are no studies evaluating the potential carcinogenic effect in humans specifically after oral exposure to nickel (ATSDR, 1992).

While nickel and nickel compounds are classified by the IARC as Group 1 (human) carcinogens, the mechanism for this carcinogenic activity is not fully understood (Sunderman, 1989; Costa, 1991; Snow, 1992). The mechanisms involved in the induction of cancer by nickel compounds may be related to the ability of nickel ions to interact with chromatin proteins and/or the ability of nickel to generate intracellular oxidants (Costa *et al.*, 1994). Recent studies suggest that nickel generates free radicals, and the subsequent oxidative reactions lead to DNA damage and cancer. Studies show that: 1) incubation of nickel ions with cysteine under aerobic conditions generated hydroxyl radicals and carbon-centered alkyl radicals, suggesting free radicals are generated by nickel (II)-thiol complexes and molecular oxygen (Shi *et al.*, 1993); 2) in forward mutation assays with bacterial DNA, nickel ions produce tandem double CC → TT mutations consistent with damage to DNA by either ultraviolet irradiation or oxygen free radicals (Tkeshelashvili *et al.*, 1993); 3) and, in *in vitro* studies, nickel ions induce increases in 8-hydroxy-2'-deoxyguanosine (8-OH-dG), a biomarker of oxidatively damaged DNA (Littlefield *et al.*, 1991).

After subcutaneous or intramuscular injection of nickel compounds, the water-insoluble nickel compounds are the most potent carcinogens. These findings may be related to the fact that water-insoluble nickel compounds are more readily phagocytized than are the water-soluble nickel salts, which passively diffuse through the cell membrane. Phagocytized nickel particles are internalized in vacuoles whose acidity accelerates the dissolution of nickel ions and results in a higher concentration of nickel than would be achieved by the cellular uptake of water-soluble nickel salts (Costa *et al.*, 1994).

## REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

### *Experimental Animals*

Leonard and Jacquet (1984) reviewed studies which show that water-soluble nickel compounds administered orally or by peritoneal routes have the potential to cause embryotoxicity in rodents. In these studies, the nickel compounds were generally administered at higher doses than humans would be exposed to in drinking water or in the diet.

Studies in rodents have indicated that water-soluble nickel compounds can cross the placenta or be excreted in the milk of lactating animals. When [<sup>63</sup>Ni]-labeled nickel chloride was administered as an oral bolus dose (10  $\mu$ mol or 0.58 mg/kg body weight) to pregnant mice, the label was detected in various fetal tissues including liver, kidney, lung, brain, and heart. In another experiment, when [<sup>63</sup>Ni]-labeled nickel chloride was injected into pregnant mice, nickel was found to cross the placenta, and a marked uptake of nickel was seen in the embryo as measured by whole-body autoradiography (Olsen and Jonsen, 1979). When nickel chloride hexahydrate was given as a single subcutaneous dose (10 to 100  $\mu$ mol NiCl<sub>2</sub> · 6H<sub>2</sub>O/kg body weight or 23 mg/kg) to lactating rats, nickel was excreted in the milk and was found in the plasma of the pups (Dostal *et al.*, 1989). The doses used in these studies are higher than the average concentration of nickel found in drinking water in the United States (48  $\mu$ g/L water) (NAS, 1975).

Nickel chloride administered in the drinking water (50 and 250 ppm, estimated to deliver 7 or 31 mg/kg of nickel compound) to female rats for 11 weeks prior to mating and then during two successive gestation and lactation periods, caused an increase in the proportion of dead pups per litter (Smith *et al.*, 1993).

Other studies in rodents administered nickel chloride by intramuscular or intraperitoneal injection during gestation also showed developmental toxicity or fetal death. Nickel chloride injected intraperitoneally (1, 2, or 4 mg/kg body weight) to pregnant Wistar Porton rats on day 8, 12, or 16 of pregnancy caused

skeletal retardation (poor ossification), hydrocephalus, hydronephrosis, heart defects, and hemorrhage. At these doses, there was an increase in maternal plasma glucose concentration (Mas *et al.*, 1985).

Nickel chloride injected intramuscularly (16 mg/kg) on day 8 of gestation to Fischer rats reduced the mean number of live pups per dam and diminished fetal body weights on day 20 (Sunderman *et al.*, 1978).

Nickel chloride injected into chicken eggs at doses of 0.02 to 0.8 mg per egg on days 0, 1, 2, 3, and 4 after fertilization caused malformations in the embryo examined at day 8 including exencephaly, everted viscera, abnormalities in the limb development, microphthalmia, and reduced body size (Gilani and Marano, 1980).

Groups of pregnant hamsters were exposed to nickel carbonyl by inhalation (0.06 mg/L/15 minutes) on days 4, 5, 6, 7, or 8 of gestation; dams were evaluated on day 15 of gestation. Teratogenic effects observed included cystic lung, exencephaly, cleft palate, and fused ribs. In another series of experiments, dams were allowed to deliver the pups; neonatal mortality was increased in the exposed groups (Sunderman *et al.*, 1980). Nickel carbonyl administered to pregnant dams by intravenous injection (11 mg/kg) on day 7 of gestation caused an increase in fetal mortality, diminished body weight of live pups, and increased incidences of fetal abnormalities including anophthalmia, microphthalmia, cystic lungs, and hydronephrosis (Sunderman *et al.*, 1983).

In a study of nickel oxide, Wistar rats were exposed to 1.6 mg nickel/m<sup>3</sup> by inhalation on gestation days 1 through 20. There was no evidence for embryotoxicity (Weischer *et al.*, 1980).

These and other studies show that water-soluble nickel salts have the potential to cause embryotoxicity in rodents. The metal can cross the fetomaternal barrier and enter the fetus. The embryotoxicity of nickel may be related to several factors including the mutagenic properties of nickel, direct effects on the

mammalian embryo, or indirect effects through maternal toxicity. Further work is needed to understand the mechanisms for these effects (Leonard and Jacquet, 1984).

### *Humans*

Until recently, there have been few studies of reproductive effects in humans after exposure to nickel (ATSDR, 1992). A study of nickel refinery workers in Norway who were exposed to water-soluble nickel salts in electrolysis departments notes a suggested increased risk of pregnancy complications in female workers. The authors point out that the results of their studies should be considered preliminary data, and further investigations are needed (Chashschin *et al.*, 1994).

## GENETIC TOXICITY

Recent detailed reviews of the mutagenicity of nickel compounds and the possible mechanisms involved in the production of these effects were presented by Coogan *et al.* (1989), Christie and Katisifis (1990), Costa (1991), Snow (1992), and Costa *et al.* (1994). Nickel compounds are not typically detected as bacterial mutagens, but they often give positive results in *in vitro* assays designed to identify compounds that induce chromosomal damage in mammalian cells in the form of sister chromatid exchanges, chromosomal aberrations, and DNA strand breaks. Nickel salts have been shown to inhibit DNA replication and to increase replication errors in mammalian cells *in vitro*, possibly by competing with magnesium for essential binding sites on DNA polymerases (Christie *et al.*, 1991). In addition, positive results were demonstrated in mammalian cell forward mutation assays (TK locus in mouse lymphoma cells and hypoxanthine phosphoribosyl transferase locus in hamster V79 cells), although these responses are usually weak (Nishimura and Umeda, 1979; Amacher and Paillet, 1980; Morita *et al.*, 1991; Lee *et al.*, 1993). Insoluble crystalline nickel compounds are more active in genetic toxicity assays than the soluble or amorphous forms of nickel. Presumably, this differential activity derives from the more efficient entry of insoluble nickels into the cell through phagocytosis (Costa, 1991), longer retention of these compounds

within the cell, and the consequent higher intracellular concentration of nickel (II) ions. Soluble nickel salts cannot be efficiently phagocytized, and do not accumulate in high concentration within the cell.

Based on the results of cell transformation studies in cultured rodent cells, Costa (1983) concluded that the nickel sulfide compounds must be in the crystalline, rather than in the amorphous state to be efficiently phagocytized into the cell and cause genetic damage. Particle size (Costa and Mollenhauer, 1980) and surface charge (Costa *et al.*, 1982) are also important factors in the phagocytosis of nickel compounds. Insoluble nickel compounds, once inside the cell, aggregate near the nucleus (Bryan, 1981; Evans *et al.*, 1982) where they are dissolved by lysosomes, releasing nickel (II) ions that proceed to effect DNA damage (Costa *et al.*, 1994).

The induced DNA damage resulting from nickel exposure has been attributed to one or more of the following mechanisms. It may follow the generation of short-lived reactive oxygen species inside the nucleus, produced by the oxidation of  $Ni^{+2}$  to  $Ni^{+3}$  by hydrogen peroxide or other oxidants subsequent to the binding of nickel ions to ligands such as amino acids, glutathione, and amino acid side chains of nuclear proteins (Biggart and Costa, 1986; Inoue and Kawaniski, 1989; Nieboer *et al.*, 1989; Cotelle *et al.*, 1992; Tkeshelashvili *et al.*, 1993; Sugiyama, 1994). The formation of persistent DNA-protein crosslinks is implicated in the generation of nickel (II)-induced DNA damage (Ciccarelli and Wetterhahn, 1982; Lee *et al.*, 1982; Patierno and Costa, 1985; Sen and Costa, 1986a). Factors involved in the binding of nickel ions to DNA, nuclear proteins, and other nuclear structures are reviewed by Coogan *et al.* (1989). The binding affinity of nickel to protein is far greater than its binding affinity to purified DNA (Eichorn and Shin, 1968) and therefore the mutagenic activity of nickel (II) ions probably derives in greater part from the binding of nickel to chromosomal protein rather than directly to DNA (Costa, 1991). Nickel binds preferentially to heterochromatic regions of the chromosomes such as the long arm of the X chromosome in cultured Chinese hamster cells (Sen and Costa, 1986a,b; Sen *et al.*, 1987; Costa, 1991); binding of nickel ions to the long arm of the X chromosome and subsequent deletions in this region were postulated to cause the loss of a gene controlling senescence in cultured Chinese hamster cells and to

promote immortality in transformed cultured Chinese hamster cell lines (Klein *et al.*, 1991). A schematic representation of some of the proposed mechanisms of nickel-induced genotoxicity, based upon the current understanding of the activities of nickel ions within mammalian cells, is presented in Figure 1. The genetic toxicity data for each of the three nickel compounds under study by the NTP are described below.

The mutagenicity data for nickel oxide are limited; however, there are clear indications of genotoxicity in some *in vitro* test systems. Although exposure to nickel oxide did not result in growth inhibition due to DNA damage in repair-deficient strains of *Bacillus subtilis* (Kanematsu *et al.*, 1980), an S-phase block (determined by flow cytometric analysis) was induced in cycling Chinese hamster ovary cells incubated with 5  $\mu\text{g}/\text{mL}$  nickel oxide (Costa *et al.*, 1982). No increase in gene mutations was detected at the ouabain resistance locus in C3H/10T<sub>1/2</sub> mouse embryo cells (Miura *et al.*, 1989) or at the HPRT locus in hamster V79 cells after exposure to nickel oxide (Kargacin *et al.*, 1993). However, positive effects were reported in mutation assays using a different site, the *gpt* gene, in V79 cells as the target for nickel oxide activity (Kargacin *et al.*, 1993). No induction of chromosomal aberrations was detected in human fibroblast or leukocyte cultures exposed to nickel oxide for 24, 48, or 72 hours (Paton and Allison, 1972); however, the experimental protocol used in this test was designed for water soluble compounds and may not have been suitable for testing insoluble nickel oxide. Data from human epidemiology studies indicate that exposure to nickel oxide-containing fumes or smelter dusts may induce chromosomal aberrations (Waksvik *et al.*, 1984) and DNA-crosslinks (Costa *et al.*, 1993) in peripheral blood lymphocytes of workers, but the evidence is weak. The link between nickel oxide and these genetic endpoints is confounded because smelter dusts and welding fumes contain other nickel compounds as well as other metals such as chromium and magnesium. Also, the genetic effects noted were not correlated with nickel concentrations in urine or blood, whereas increased DNA-crosslink frequencies noted after exposure to chromium-containing fumes, for example, were correlated with urine concentrations of the metal (Popp *et al.*, 1992).

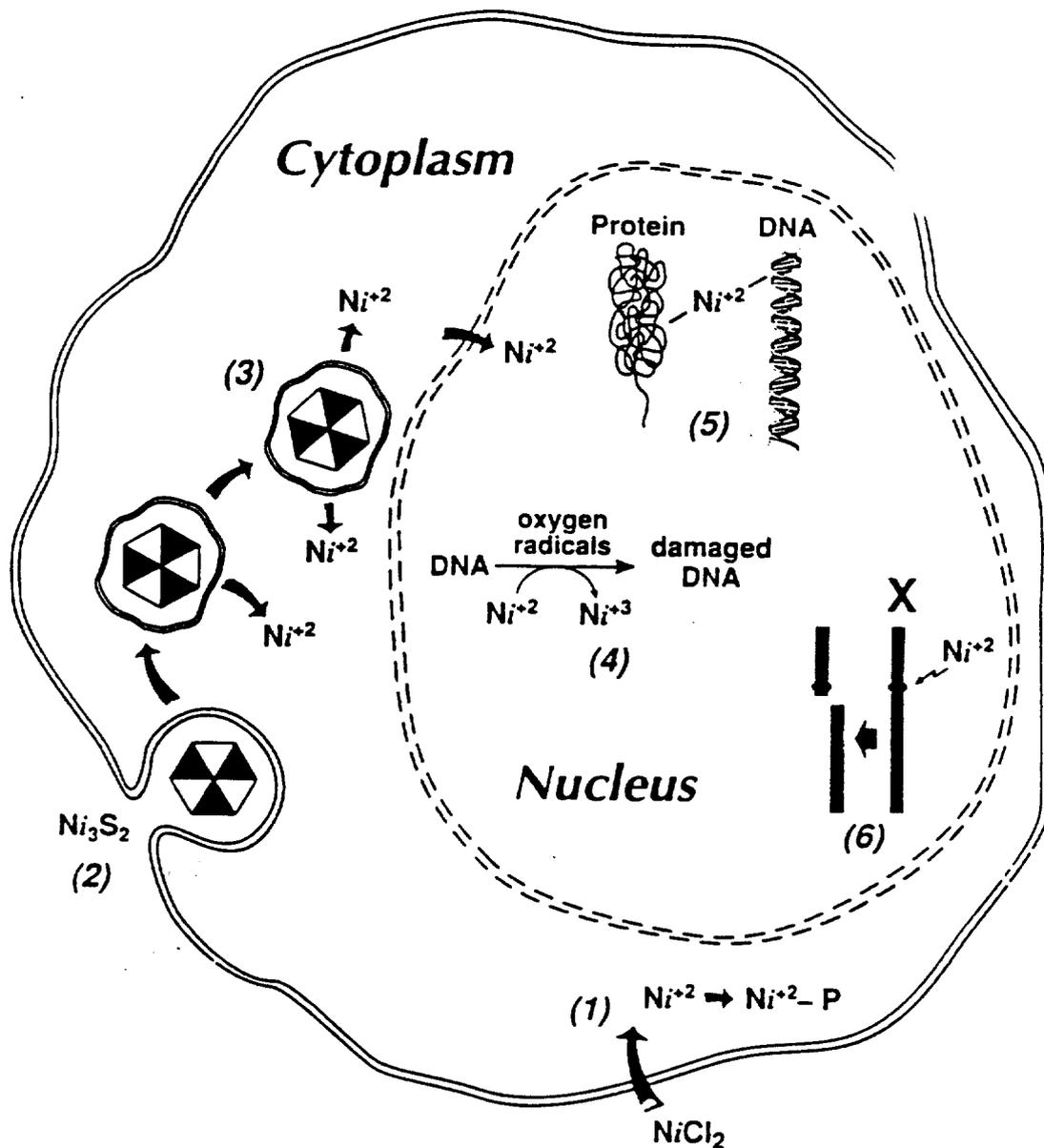
Nickel sulfate hexahydrate did not induce gene mutations in *Escherichia coli* or *Salmonella typhimurium* (Arlauskas *et al.*, 1985), and (in contrast to results reported for nickel oxide) no increases in *gpt* mutants were observed in hamster V79 cells treated with nickel sulfate hexahydrate (Christie, 1989; Lee *et al.*, 1993). However, nickel sulfate hexahydrate did induce mutations in L5178Y mouse lymphoma TK<sup>+</sup> cells, treated with 500 to 1,000 µg/mL in the absence of S9 metabolic activation enzymes (McGregor *et al.*, 1988). In addition, nickel sulfate hexahydrate, administered by injection at doses of 200, 300, and 400 ppm, induced sex-linked recessive lethal mutations in germ cells of male *Drosophila* (Rodriguez-Arnaiz and Ramos, 1986). The pre- and post-meiotic cell stages were affected; the broods obtained from sperm cells undergoing meiosis at the time of treatment showed no evidence of increased lethal mutations. In another test for germ cell effects in male *Drosophila*, the test for sex chromosome loss, only the highest dose of nickel sulfate hexahydrate (400 ppm) resulted in the production of XO males (Rodriguez-Arnaiz and Ramos, 1986). Induction of sister chromatid exchanges and chromosomal aberrations was observed in hamster cells (Larramendy *et al.*, 1981; Ohno *et al.*, 1982), as well as human peripheral lymphocytes (Larramendy *et al.*, 1981) treated with nickel sulfate hexahydrate *in vitro*. However, no induction of DNA single strand breaks was detected in human xeroderma pigmentosum fibroblasts treated with 250 µg/mL nickel sulfate hexahydrate (Fornace, 1982). *In vivo*, no induction of chromosomal aberrations was observed in rat bone marrow or spermatogonial cells after injection of nickel sulfate hexahydrate at doses that provided 3 or 6 mg nickel/kg body weight. Also, no change in the mitotic index of bone marrow cells was noted in treated animals (Mathur *et al.*, 1978).

As with the two nickel compounds discussed above, there are limited published mutagenicity data for the third nickel compound in the present studies, nickel subsulfide. However, results of *in vitro* tests performed with this insoluble nickel compound were mainly positive. In the *Salmonella typhimurium* gene mutation assay, crystalline nickel subsulfide gave equivocal results in one study that used a preincubation protocol (Zeiger *et al.*, 1992) and negative results in a standard plate incorporation assay (Arroujal *et al.*, 1990). It induced lethal mutations in *Paramecium tetraurelia*, without S9 (Smith-Sonneborn *et al.*, 1986).

and unscheduled DNA repair in cultured Syrian hamster embryo cells (Robison *et al.*, 1983). Treatment of cultured Chinese hamster ovary cells for 24 hours with 10  $\mu\text{g}/\text{mL}$  nickel subsulfide resulted in an increase in the number of DNA strand breaks detected by alkaline sucrose gradient techniques (Robison *et al.*, 1982). Nickel subsulfide, in the absence of S9, was a weak inducer of hypoxanthine phosphoribosyl transferase mutations in cultured Chinese hamster ovary cells (Rossetto *et al.*, 1994) and sister chromatid exchanges in cultured human lymphocytes (Saxholm *et al.*, 1981). Nickel subsulfide induced significant dose-related increases in chromosomal aberrations (Arrouijal *et al.*, 1990) and micronuclei (Arrouijal *et al.*, 1992) in human lymphocytes *in vitro*. One reported *in vivo* test with nickel subsulfide, a measure of DNA synthesis inhibition in rats administered 10  $\mu\text{g}/\text{rat}$  (6 mg/100 g body weight) by intrarenal injection, was negative (Hui and Sunderman, 1980). A second *in vivo* study, a mouse bone marrow micronucleus test, reportedly produced positive results (Arrouijal *et al.*, 1990). This second study, however, employed only a single dose (250 mg/kg nickel subsulfide administered by intraperitoneal injection), and no confirmatory study was conducted.

## STUDY RATIONALE

The National Cancer Institute nominated nickel compounds for study because there was little information on the toxic and carcinogenic properties of specific nickel compounds after inhalation exposure. Nickel oxide (NTP, 1994a) and nickel sulfate hexahydrate were selected as compounds that are commonly found in the workplace in the United States. Nickel subsulfide (NTP, 1994b) was selected for study based on a previous study in which lung tumors were observed in rats (Ottolenghi *et al.*, 1975). The NTP toxicity and carcinogenicity studies of nickel oxide, nickel subsulfide, and nickel sulfate hexahydrate were performed to provide comparative toxicology and carcinogenicity information on these nickel compounds. The results of the nickel sulfate hexahydrate studies are presented in this technical report.



**FIGURE 1**  
Possible Mechanisms of Nickel-Induced Genotoxicity

1. Soluble nickel compounds such as nickel chloride diffuse into the cell;  $\text{Ni}^{2+}$  ions are rapidly bound to cytoplasmic proteins (P) (Lee *et al.*, 1993). 2. Insoluble nickel compounds such as nickel subsulfide are phagocytized into the cell and move toward the nucleus (Costa *et al.*, 1982). 3. Lysosomal breakdown of insoluble nickel compounds releases large quantities of  $\text{Ni}^{2+}$  ions which concentrate adjacent to the nuclear membrane (Costa and Heck, 1983). 4. Oxidative damage is induced in DNA by nickel ions bound to nuclear proteins ( $\text{Ni}^{2+} \rightarrow \text{Ni}^{3+}$ ), releasing active oxygen species (Tkeshelashvili *et al.*, 1993; Sugiyama, 1994). 5. DNA-protein crosslinks are produced by  $\text{Ni}^{2+}$  ions binding to heterochromatin (Lee *et al.*, 1982; Paterno and Costa, 1985; Sen and Costa, 1986a). 6. Binding of nickel ions to the heterochromatic regions of the long arm of the X chromosome, which may contain a senescence gene and a tumor suppressor gene, can cause deletion of all or part of this region, leading to an immortalization of the cell and clonal expansion (Conway and Costa, 1989; Klein *et al.*, 1991).

## DISCUSSION AND CONCLUSIONS

Nickel sulfate hexahydrate is a water-soluble nickel compound used in electroplating and exposure may also occur during other mining and refinery operations (Doll, 1990). There have been no studies of nickel sulfate reported in the literature in which inhalation exposure concentration and toxic response relationships have been established or where toxic effects in organs other than the lung have been examined. In these inhalation studies of nickel sulfate hexahydrate in rats and mice, the major toxicity was to the respiratory system.

In the 16-day studies, rats and mice were exposed to nickel sulfate hexahydrate at concentrations of 3.5 to 60 mg/m<sup>3</sup> (equivalent to 0.7 to 12.2 mg nickel/m<sup>3</sup>) (Table 31). Mice were more susceptible than rats to the lethal effects of exposure: two male rats and all female rats exposed to 60 mg/m<sup>3</sup> died before the end of the study, as did all male and female mice exposed to 7 mg/m<sup>3</sup> or greater. Respiratory toxicity, evidenced by labored respiration, occurred in all exposed groups of rats and mice. Histopathologic findings that corresponded to the respiratory toxicity in the lungs of rats and mice included inflammation, degeneration, and necrosis of the respiratory epithelium (Table 32). The severity of the lung lesions increased with exposure concentration. Deaths were considered due to pulmonary inflammation and necrosis.

At the end of the 16-day studies, quantities of nickel were measured in the lungs of rats and mice from special study groups exposed to 0, 3.5, 15, or 30 mg nickel sulfate hexahydrate/m<sup>3</sup> (Table 33). The nickel concentrations in the lung of rats did not increase with exposure concentrations. Lung burdens in mice exposed to 3.5 mg/m<sup>3</sup> were approximately 50% of those in the 3.5 mg/m<sup>3</sup> rats. Early deaths in the 16-day mouse study prevented an evaluation of dose-response effects in that species.

These data indicate that nickel sulfate hexahydrate is rapidly cleared from the lung of rats, a finding supported by toxicokinetic studies of nickel sulfate hexahydrate in which the reported lung half-life is 1 to 3 days (Medinsky *et al.*, 1987). The data also indicate that toxic effects are related to exposure concentration and not nickel lung burden. The relatively constant amount of nickel within the lung over a range of increasing exposure concentrations implies that a small fraction of the delivered nickel may be bound to macromolecules within the lung. Evidence for nickel binding constituents in the lung has been presented by Oskarsson and Tjalve (1979).

The distribution of nickel to other tissues was indicated by the presence of nickel in the kidneys of exposed rats. The quantity of nickel in rat kidneys increased in proportion to exposure concentration, and these results are consistent with the finding that the major route of elimination of soluble nickel is through the urine (Medinsky *et al.*, 1987). Although quantifiable amounts of nickel were found in the kidney of rats at all exposure concentrations, there were no treatment-related gross or histopathologic lesions present in the kidney.

In the 16-day studies, nickel sulfate hexahydrate was more toxic to rats and mice than either nickel subsulfide or nickel oxide (Table 32). Lung inflammatory lesions were observed with all three nickel compounds, but these lesions occurred at lower exposure concentrations in the nickel sulfate hexahydrate 16-day studies. Atrophy of the nasal olfactory epithelium was observed in the nickel sulfate hexahydrate and nickel subsulfide studies, but not in the nickel oxide study.

In the 13-week studies, there were no treatment-related deaths and only minimal effects on body weight changes in rats and mice (Table 34). However, exposure concentrations of 0.25 to 2 mg nickel sulfate hexahydrate/m<sup>3</sup> in rats and 0.5 to 2 mg/m<sup>3</sup> in mice caused treatment-related respiratory toxicity evidenced by increases in lung weights and inflammatory changes in the lung, nose, and bronchial lymph nodes.

The nasal toxicity reported in these studies is characteristic of inhalation exposure to other metal

compounds (cadmium oxide), and is most likely due to direct exposure of the olfactory epithelium to the metal compound, not to systemic exposure. Nickel sulfate hexahydrate had no significant effects on sperm morphology or vaginal cytology in rats or mice.

After 13 weeks of exposure, the amount of nickel present in the lungs of rats and mice reached a steady state (Table 33). The amount of nickel in the lung of male and female rats was similar, although the amount of nickel in the lung of female mice was greater than that in male mice.

Nickel concentrations in the lungs of male rats following 13 weeks of exposure to 0.4 mg nickel/m<sup>3</sup> were approximately 6.5, 7.5, and 80 µg nickel/g lung for nickel sulfate hexahydrate, nickel subsulfide, and nickel oxide, respectively. The very low retention of nickel in the lungs of rats exposed to nickel sulfate hexahydrate and very high retention in those exposed to nickel oxide are consistent with previous intratracheal studies of nickel sulfate hexahydrate and nickel oxide in rats (English *et al.*, 1981). The retention of nickel subsulfide is low because of a relatively rapid clearance of this soluble nickel compound from the lung (Valentine and Fisher, 1984; Benson *et al.*, 1994).

The most significant effect of nickel sulfate hexahydrate exposure observed in the immunologic assays was an increase in the number of cells in the lung-associated lymph nodes (LALN), but this did not affect the ability of the animals to respond to intratracheally deposited antigen. There were no major changes in alveolar macrophage phagocytosis or spleen cell natural killer cell activity. Systemic immunity was not altered by exposure to nickel sulfate hexahydrate. There were no alterations in the antibody-forming cell response following intraperitoneal immunization in female mice exposed to nickel sulfate hexahydrate. In addition, negative results were obtained in the natural killer cell assay, the mixed lymphocyte assay, and the lymphocytic proliferative assay in these female mice (Haley *et al.*, 1990).

At 13 weeks, biochemical changes monitored in the lung lavage fluid were generally similar between male and female rats and mice. Increased numbers of total nucleated cells indicated that some pulmonary inflammation was in progress, and that there had been an influx of inflammatory cells into the pulmonary tissue. Most of these cells were macrophages; however, modestly increased numbers of polymorphonuclear leukocytes were also present. The changes noted in these assays generally paralleled histopathologic findings (Benson *et al.*, 1989).

In the present 13-week studies, the no-effect level for lung alveolar hyperplasia and inflammation and olfactory epithelial atrophy was 0.25 mg/m<sup>3</sup> (equivalent to 0.06 mg nickel/m<sup>3</sup>) in rats and 0.5 mg/m<sup>3</sup> (equivalent to 0.11 mg nickel/m<sup>3</sup>) in mice. The no-effect level for nasal toxicity was approximately 0.25 mg/m<sup>3</sup> for rats and 1.0 mg/m<sup>3</sup> for mice. Respiratory toxicity produced by nickel sulfate hexahydrate in rats and mice occurs at or below the present threshold limit value levels of water soluble nickel salts (0.1 mg nickel/m<sup>3</sup>) (ACGIH, 1993).

Chronic active inflammation of the lung was considered to be potentially life threatening because of the possibility of reduced lung function. The highest exposure concentrations used in the 2-year studies were just below concentrations at which mild chronic active inflammation was observed in the 13-week studies.

Results of the three 13-week studies demonstrate that nickel sulfate hexahydrate was the most toxic and nickel oxide the least toxic (Table 34). The lung and nasal toxicity reflects the relative solubility of the nickel compounds in water and biological fluids, with the most soluble nickel (nickel sulfate hexahydrate) being the most toxic. The soluble nickel compounds are thought to be more toxic than the insoluble nickel compounds because the availability of relatively higher concentrations of free nickel ions for diffusion across the cell membrane and interaction with cytoplasmic proteins, thereby causing toxicity. In contrast, it is thought that the water-insoluble nickel compounds are phagocytized and do not cause extensive

damage to cytoplasmic components of the alveolar/bronchiolar epithelium (Lee *et al.*, 1993; Costa *et al.*, 1994).

In the 2-year studies of nickel sulfate hexahydrate, there were no treatment-related effects on survival (Table 35). Mean body weights of exposed male rats were similar to those of the controls. However, mean body weights of female rats, male mice, and female mice exposed to 0.5 mg/m<sup>3</sup> and those of 0.25 mg/m<sup>3</sup> female rats and female mice were less than those of the controls during most of the last year of the study.

Toxic responses in the lung of rats and mice exposed for 2 years by inhalation to nickel sulfate hexahydrate were less severe than those observed in the lung of rats and mice exposed similarly to nickel oxide or nickel subsulfide (Table 35). The exposure concentrations used in the nickel sulfate hexahydrate 2-year studies were lower than those used in studies of the other nickel compounds (Table 31), primarily because nickel sulfate hexahydrate has a steeper toxic response curve. For example, the highest exposure concentration used in the nickel oxide 2-year studies delivered 2 mg nickel/m<sup>3</sup> to rats and 4 mg nickel/m<sup>3</sup> to mice, and these nickel exposure concentrations were fatal to rats and mice in the 16-day nickel sulfate hexahydrate studies. The highest exposure concentration used in the nickel subsulfide 2-year studies delivered 0.74 mg nickel/m<sup>3</sup> to rats and 0.9 mg nickel/m<sup>3</sup> to mice, and these levels of nickel caused lung toxicity in rats and mice after 12 days of exposure to nickel sulfate hexahydrate.

No exposure-related lung neoplasms occurred in rats or mice exposed to nickel sulfate hexahydrate for 2 years (Table 35). This is consistent with previous studies which have examined the carcinogenic potential of nickel sulfate hexahydrate administered via local injection (Payne, 1964; Gilman, 1962, 1966; Kasprzak *et al.*, 1983; Pott *et al.*, 1989, 1992). In these studies, no treatment-related carcinogenic responses were observed.

Female rats exposed to 0.25 or 0.5 mg/m<sup>3</sup> nickel sulfate hexahydrate during the 2-year study had decreased incidences of spontaneous mammary gland neoplasms. Rao *et al.* (1987) reported that decreases in mammary gland neoplasms in rats are associated with decreased body weights; the mean body weights of 0.25 mg/m<sup>3</sup> female rats were decreased approximately 2% to 6% and those of 0.5 mg/m<sup>3</sup> female rats were decreased approximately 7% to 10% during the last year of the study. Decreases in body weights may affect the development of spontaneous mammary gland neoplasms by decreasing cell proliferation and the development/progression of endogenous mutagenic events.

Although no exposure-related neoplasms were observed in male or female rats or mice in the present studies, the lungs, bronchial lymph nodes, and olfactory epithelium of exposed animals did have significant alterations compared with controls. Both rats and mice exposed to nickel sulfate hexahydrate had a spectrum of inflammatory changes in the lungs similar to those in the nickel oxide and nickel subsulfide studies, but the severity and progression of lesions over exposure time were less in the nickel sulfate-exposed animals.

Respiratory toxicity in the nickel sulfate hexahydrate rat lung occurred for the most part in 0.25 and 0.5 mg/m<sup>3</sup> rats and was characterized by fibrosis, hyperplasia, and alveolar proteinosis; these lesions were considered to be the various components of chronic active inflammation. In mice, treatment-related lung lesions were diagnosed as inflammation, hyperplasia, proteinosis, and cellular infiltration; these lung lesions were observed primarily in 0.5 and 1 mg/m<sup>3</sup> mice.

Some generalities can be made about the comparative lung pathology in rats and mice after 2 years of exposure to nickel sulfate hexahydrate, nickel oxide, and nickel subsulfide. Alveolar/bronchiolar neoplasms in rats and mice exposed to nickel sulfate hexahydrate and in mice exposed to nickel oxide or nickel subsulfide were typical of spontaneously occurring neoplasms. In all three nickel studies, mice were less susceptible to proliferative and fibrotic lung lesions than rats exposed to the same compound.

Of 11 alveolar/bronchiolar carcinomas observed in nickel oxide-exposed rats, five had marked proliferative components with squamous differentiation. Similar squamous differentiation was present in 2 of 21 alveolar/bronchiolar adenomas and in 4 of 14 alveolar/bronchiolar carcinomas in rats exposed to nickel subsulfide. Such proliferative squamous differentiation is not characteristic of spontaneous alveolar/bronchiolar neoplasms in rats.

Incidences of lung neoplasms observed in the three nickel compound studies are not a direct function of the amount of nickel deposited in the lung as measured by atomic absorption spectroscopy at various time points during the course of exposures (Table 33). At the 15-month interim evaluation, less than 10  $\mu\text{g}$  nickel/g of lung was measured in rats and mice in all exposure groups in the nickel sulfate hexahydrate and nickel subsulfide studies, and while nickel subsulfide caused a clear carcinogenic response in the rat lung, nickel sulfate hexahydrate did not. In nickel oxide rats at 15 months, amounts of nickel deposited in the lung were much greater (approximately 300 to 1,100  $\mu\text{g}$  nickel/g lung). However, rats exposed to nickel oxide developed fewer lung neoplasms than did rats exposed to nickel subsulfide.

The results of the present studies with the three nickel compounds showed that water-insoluble nickel compounds (nickel oxide and nickel subsulfide) were carcinogenic to the rat lung, whereas the water-soluble nickel compound (nickel sulfate hexahydrate) was not. Costa *et al.* (1994) has suggested that water-insoluble nickel compounds are capable of causing more critical cancer damage because they are delivered at higher concentrations to the nucleus than are water-soluble nickel compounds such as nickel sulfate hexahydrate.

The incidences of inflammatory lung lesions observed in rats exposed to nickel sulfate hexahydrate occurred with significant positive trends. However, the differences between severities of lung inflammatory lesions observed in exposed and control rats in the nickel oxide and nickel subsulfide studies were greater than the differences observed between severities of exposed and control nickel sulfate

hexahydrate rats. Additionally, rats exposed to nickel oxide or nickel subsulfide had significant parenchymal damage secondary to inflammation. In rats exposed to 1 mg/m<sup>3</sup> nickel subsulfide or 2.5 mg/m<sup>3</sup> nickel oxide, protein accumulations with variable numbers of foamy macrophages were widespread in alveolar spaces. Fibrosis, consolidation, and cellular proliferation apparently secondary to inflammation were multifocally extensive in both nickel subsulfide- and nickel oxide-exposed rats. Foci of necrotic cellular debris, regenerative alveolar epithelial proliferation, and foci of collapse or consolidation were somewhat more prominent in nickel subsulfide-exposed rats. Exposure-related pigment and the condensed appearance of the intraalveolar protein were noteworthy in the nickel oxide-exposed rats. Pigment occurred in the lungs and bronchial lymph nodes of rats and mice exposed to nickel oxide, but was not observed in the nickel subsulfide- or nickel sulfate hexahydrate-exposed animals.

With the exception of the pigment observed in nickel oxide-exposed mice, nonneoplastic lesions in the lungs of exposed mice were similar in all three nickel studies and were composed of various inflammatory reactions, including: intraalveolar protein and macrophages; mononuclear inflammatory cells around vessels; and multifocal intraalveolar aggregates of various combinations of lymphocytes, macrophages, and neutrophils. Inflammatory foci with neutrophils and necrotic cell debris were relatively common in mice exposed to nickel sulfate hexahydrate, while inflammatory foci in mice exposed to nickel oxide or nickel subsulfide were predominantly mononuclear cells with little evidence of necrotic cell debris.

In areas where epidemiology studies were available, Doll (1990) estimated exposures to individual nickel compounds at various refineries or nickel operations throughout the world. In most cases, nickel sulfate hexahydrate, nickel oxide, and nickel subsulfide exposures occurred simultaneously, and workers tended to work in several departments with different nickel exposures throughout their careers. Therefore, it was not possible from the human studies to obtain a risk from exposure to nickel sulfate hexahydrate alone. However, in certain subpopulations such as in the electrolysis departments in the Kristiansand refinery workers (Norway) or the hydrometallurgy departments at the Clydach refinery (Canada), nickel sulfate hexahydrate exposures were particularly high (1 to 5 mg/m<sup>3</sup>), and there was evidence that exposure to

soluble nickel increased the risk of lung cancer in workers also exposed to oxidic, sulfidic, and/or metallic nickel. Soluble nickel was thought to have a synergistic role with oxidic or other forms of nickel in causing lung and nasal neoplasms (Doll, 1990). No exposure-related neoplasms were observed in the nasal cavities of rats or mice exposed to nickel sulfate hexahydrate, nickel subsulfide, or nickel oxide.

Experimental studies have provided evidence to suggest that water soluble nickel salts may enhance the carcinogenic response from exposures to other environmental agents. In *in vitro* studies, water-soluble nickel salts (i.e. nickel chloride) have been shown to enhance the cytotoxicity and mutagenicity of DNA-damaging agents by inhibiting nucleotide excision repair in mammalian cells and repair of ultraviolet-induced photoproducts (Hartwig *et al.*, 1994). These studies suggest that exposure to water-soluble nickel salts may be a factor in the eventual development of cancer when there is concomitant exposure to other agents.

## CONCLUSIONS

Under the conditions of these 2-year inhalation studies, there was *no evidence of carcinogenic activity\** of nickel sulfate hexahydrate in male or female F344/N rats exposed to 0.12, 0.25, or 0.5 mg/m<sup>3</sup> (0.03, 0.06, or 0.11 mg nickel/m<sup>3</sup>). There was *no evidence of carcinogenic activity* of nickel sulfate hexahydrate in male or female B6C3F<sub>1</sub> mice exposed to 0.25, 0.5, or 1 mg/m<sup>3</sup> (0.06, 0.11, or 0.22 mg nickel/m<sup>3</sup>).

Exposure of rats to nickel sulfate hexahydrate by inhalation for 2 years resulted in increased incidences of chronic active inflammation, macrophage hyperplasia, alveolar proteinosis, and fibrosis of the lung; lymphoid hyperplasia of the bronchial lymph node; and atrophy of the olfactory epithelium.

Exposure of mice to nickel sulfate hexahydrate by inhalation for 2 years resulted in increased incidences of chronic active inflammation, bronchialization (alveolar epithelial hyperplasia), macrophage hyperplasia,

interstitial infiltration, and alveolar proteinosis of the lung; lymphoid and macrophage hyperplasia of the bronchial lymph node; and atrophy of the olfactory epithelium.

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\* Explanation of Levels of Evidence of Carcinogenic Activity is on page 14.

**TABLE 31**  
**Comparison of Exposure Concentrations in the 16-Day, 13-Week, and 2-Year Studies**  
**of Nickel Sulfate Hexahydrate, Nickel Sub sulfide, and Nickel Oxide<sup>a</sup>**

	<u>Amount of Compound</u>	<u>Amount of Nickel</u>
<b>16-Day Studies</b>		
Nickel Sulfate Hexahydrate (22.3% Ni)	0, 3.5, 7, 15, 30, 60	0, 0.7, 1.4, 3.1, 6.1, 12.2
Nickel Sub sulfide (73.3% Ni)	0, 0.6, 1.2, 2.5, 5, 10	0, 0.44, 0.88, 1.83, 3.65, 7.33
Nickel Oxide (78.6% Ni)	0, 1.2, 2.5, 5, 10, 30	0, 0.9, 2.0, 3.9, 7.9, 23.6
<b>13-Week Studies</b>		
Nickel Sulfate Hexahydrate (22.3% Ni)	0, 0.12, 0.25, 0.5, 1, 2	0, 0.03, 0.06, 0.11, 0.22, 0.44
Nickel Sub sulfide (73.3% Ni)	0, 0.15, 0.3, 0.6, 1.2, 2.5	0, 0.11, 0.22, 0.44, 0.88, 1.83
Nickel Oxide (78.6% Ni)	0, 0.6, 1.2, 2.5, 5, 10	0, 0.4, 0.9, 2.0, 3.9, 7.9
<b>2-Year Studies</b>		
<b>Nickel Sulfate Hexahydrate (22.3% Ni)</b>		
Rats	0, 0.12, 0.25, 0.5	0, 0.03, 0.06, 0.11
Mice	0, 0.25, 0.5, 1	0, 0.06, 0.11, 0.22
<b>Nickel Sub sulfide (73.3% Ni)</b>		
Rats	0, 0.15, 1	0, 0.11, 0.73
Mice	0, 0.6, 1.2	0, 0.44, 0.88
<b>Nickel Oxide (78.6% Ni)</b>		
Rats	0, 0.62, 1.25, 2.5	0, 0.5, 1.0, 2.0
Mice	0, 1.25, 2.5, 5	0, 1.0, 2.0, 3.9

<sup>a</sup> Amounts of nickel and nickel compounds are expressed in mg/m<sup>3</sup>. Occupational exposure limits in the United States: 1 mg Ni/m<sup>3</sup> for nickel metals, 0.1 mg Ni/m<sup>3</sup> for soluble nickel compounds.

**TABLE 32**  
**Selected Results in the 16-Day Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide<sup>a</sup>**

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate					Nickel Subsulfide					Nickel Oxide							
	0	3.5 (0.7)	7 (1.4)	15 (3.1)	30 (6.1)	60 (12.2)	0	0.6 (0.44)	1.2 (0.88)	2.5 (1.83)	5 (3.65)	10 (7.33)	0	1.2 (0.9)	2.5 (2.0)	5 (3.9)	10 (7.9)	30 (23.6)
<b>Male Rats</b>																		
Survival	5	5	5	5	5	3	5	5	5	5	5	4	5	5	5	5	5	5
Final Mean Body Weights (Relative to Controls)	—	72%	60%	56%	55%	45%	—	109%	105%	92%	72%	52%	—	99%	101%	99%	99%	96%
Absolute Lung Weights <sup>b</sup>	0.98	1.44**	1.45**	1.40*	1.40*	1.62**	1.13	1.41	1.60*	1.59*	1.82**	1.54**	1.06	1.00	1.06	0.96	1.20*	1.36**
<b>Female Rats</b>																		
Survival	5	5	5	5	4	0	5	5	5	5	5	5	5	5	5	5	5	5
Final Mean Body Weights (Relative to Controls)	—	82%	71%	68%	63%	—	—	99%	97%	91%	78%	57%	—	103%	103%	104%	101%	99%
Absolute Lung Weights	0.76	1.28*	1.28*	1.32*	1.40**	1.52**	0.82	1.12**	1.12**	1.36**	1.42**	1.25**	0.78	0.86	0.90	0.82	1.04**	1.12**

(continued)

TABLE 32  
Selected Results in the 16-Day Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide (continued)

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate					Nickel Subsulfide					Nickel Oxide							
	0	3.5 (0.7)	7 (1.4)	15 (3.1)	30 (6.1)	60 (12.2)	0	0.6 (0.44)	1.2 (0.88)	2.5 (1.83)	5 (3.65)	10 (7.33)	0	1.2 (0.9)	2.5 (2.0)	5 (3.9)	10 (7.9)	30 (23.6)
<b>Male Mice</b>																		
Survival	5	5	0	0	0	0	4	5	4	5	5	0	5	5	5	4	5	5
Final Mean Body Weights (Relative to Controls)	—	95%	—	—	—	—	—	99%	90%	92%	86%	—	—	100%	100%	98%	102%	94%
Absolute Lung Weights	0.20	0.24	0.40**	0.36**	0.36**	0.38**	0.22	0.20	0.22	0.28	0.31**	0.38**	0.20	0.16	0.20	0.13**	0.20	0.20
<b>Female Mice</b>																		
Survival	5	5	0	0	0	0	4	5	5	5	5	0	5	5	5	5	5	5
Final Mean Body Weights (Relative to Controls)	—	96%	—	—	—	—	—	106%	104%	101%	99%	—	—	100%	96%	100%	95%	95%
Absolute Lung Weights	0.16	0.22	0.36**	0.36**	0.38**	0.40**	0.20	0.21	0.22	0.27	0.36*	0.25	0.16	0.16	0.14	0.18	0.12	0.20

\* Significantly different (P ≤ 0.05) from the control by Williams' or Dunnett's test  
 \*\* P ≤ 0.01  
 a Survival data indicate number of animals surviving. Five animals initially in group. Final mean body weights are not presented for groups with 100% mortality.  
 b Organ weights are given in grams.

TABLE 33  
Lung Burden Analyses in the 16-Day, 13-Week, and 2-Year Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide<sup>a</sup>

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate (22.3% Ni)			Nickel Subsulfide (73.3% Ni)			Nickel Oxide (78.6% Ni)											
	0	0.12 (0.03)	0.5 (0.06)	2 (0.44)	3.5 (0.7)	15 (3.1)	30 (6.1)	0	0.15 (0.11)	0.6 (0.44)	2.5 (1.83)	10 (7.33)	0	0.6 (0.4)	1.2 (0.9)	2.5 (2.0)	5 (3.9)	10 (7.9)
<b>16-Day Studies</b>																		
Male Rats	- <sup>b</sup>			5	9	8			7	18	67				42	108	267	
Female Rats				8	11	9			9	19	77				54	122	340	
Male Mice				3					10	20	13				32	46	84	
Female Mice				4					8	20	8				31	43	71	
<b>13-Week Studies</b>																		
Male Rats			1	6					5	7	18				80	181	524	
Female Rats			2	7					5	7	17							
Male Mice				1					3	11	17				42	202	736	
Female Mice				4					6	13	23							

(continued)

**TABLE 33**  
**Lung Burden Analyses in the 16-Day, 13-Week, and 2-Year Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide (continued)**

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate (22.3% Ni)			Nickel Subsulfide (73.3% Ni)			Nickel Oxide (78.6% Ni)										
	0	0.12 (0.03)	0.25 (0.06)	0.5 (0.11)	1 (0.22)	2 (0.44)	0	0.15 (0.11)	0.6 (0.44)	1 (0.73)	1.2 (0.88)	0	0.62 (0.5)	1.25 (1.0)	2.5 (2.0)	5 (3.9)	10 (7.9)
<b>7-Month Interim Evaluation</b>																	
Male Rats	-	-	-	1	-	-	6	9	-	175	388	701	-	-	-	-	-
Female Rats	-	-	-	1	-	-	6	9	-	173	477	713	-	-	-	-	-
Male Mice	-	-	1	1	2	-	10	11	-	162	442	1,034	-	-	-	-	-
Female Mice	-	-	1	2	2	-	10	14	-	169	533	861	-	-	-	-	-
<b>15-Month Interim Evaluation</b>																	
Male Rats	-	-	-	1	-	-	4	3	-	328	746	1,116	-	-	-	-	-
Female Rats	-	-	-	2	-	-	4	7	-	262	706	949	-	-	-	-	-
Male Mice	-	-	1	1	2	-	15	26	-	331	959	1,798	-	-	-	-	-
Female Mice	-	-	1	2	2	-	12	20	-	451	1,237	2,258	-	-	-	-	-

<sup>a</sup> Values represent mean amounts of nickel (µg Ni/g lung). Lung burden groups included five to seven animals.

<sup>b</sup> Results were below the limit of detection.

**TABLE 34**  
**Selected Results in the 13-Week Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide<sup>a</sup>**

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate				Nickel Subsulfide				Nickel Oxide									
	0	0.12 (0.03)	0.25 (0.06)	0.5 (0.11)	1 (0.22)	2 (0.44)	0	0.15 (0.11)	0.3 (0.22)	0.6 (0.44)	1.2 (0.88)	2.5 (1.83)	0	0.6 (0.4)	1.2 (0.9)	2.5 (2.0)	5 (3.9)	10 (7.9)
<b>Male Rats</b>																		
Survival	10	10	10	10	10	9	10	10	10	10	10	10	10	10	10	9	10	10
Final Mean Body Weights (Relative to Controls)	—	99%	103%	96%	102%	95%	—	100%	95%	96%	99%	93%	—	103%	104%	99%	102%	100%
Absolute Lung Weights	1.35	1.25	1.51*	1.64**	2.14**	2.22**	1.33	1.74**	1.83**	2.30**	2.63**	2.42**	1.18	1.35**	1.47**	1.70**	1.91**	2.47**
<b>Nonneoplastic Lung Lesions</b>																		
Alveolar Macrophage Hyperplasia (Severity) <sup>b</sup>	0	10	10	10	10	9	0	10	10	10	10	10	0	10	10	9	10	10
Inflammation, Chronic Active (Severity)	0	0	0	2	10	8	0	2	9	10	10	10	0	0	0	2	10	10
Inflammation, Granulomatous (Severity)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	2
Interstitial Infiltrate (Severity)	1	0	1	5	10	9	0	0	1	10	9	8	0	0	1	2	10	10
Pigment (Severity)	0	0	0	0	0	0	0	0	0	0	0	0	0	6	7	9	9	10
Nonneoplastic Nasal Lesions Atrophy, Olfactory Epithelium	0	0	0	1	10	9	0	0	1	5	10	10	0	0	0	0	0	0

(continued)

TABLE 34  
Selected Results in the 13-Week Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide (continued)

	Nickel Sulfate Hexahydrate			Nickel Subsulfide			Nickel Oxide												
	Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	0	0.12 (0.03)	0.25 (0.06)	0.5 (0.11)	1 (0.22)	2 (0.44)	0	0.15 (0.11)	0.3 (0.22)	0.6 (0.44)	1.2 (0.88)	2.5 (1.83)	0	0.6 (0.4)	1.2 (0.9)	2.5 (2.0)	5 (3.9)	10 (7.9)
<b>Female Rats</b>																			
Survival	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
Final Mean Body Weights (Relative to Controls)	—	96%	98%	98%	98%	101%	95%	—	101%	104%	101%	100%	99%	—	101%	101%	98%	98%	100%
Absolute Lung Weights	1.02	1.02	1.16***	1.34***	1.72***	1.72***	1.72***	1.01	1.29***	1.39***	1.82***	1.85***	1.81**	0.98	1.03	1.13*	1.55***	1.61**	2.11**
<b>Nonneoplastic Lung Lesions</b>																			
Alveolar Macrophage, Hyperplasia (Severity)	0	8 (1.0)	10 (1.0)	10 (1.1)	10 (2.2)	10 (3.6)	10	0	10 (1.0)	10 (1.7)	10 (1.8)	10 (2.9)	10 (3.8)	0	10 (1.0)	8 (1.0)	10 (1.0)	10 (1.4)	10 (2.2)
Inflammation, Chronic Active (Severity)	0	0	0	4	10	10	10	0	3	9	10	10	10	0	0	0	1	7	7
Inflammation, Granulomatous (Severity)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4
Interstitial Infiltrate (Severity)	0	0	0	6	10	10	10	0	0	2	9	10	5	0	0	0	2	10	10
Pigment (Severity)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	8	8	10
																(1.0)	(1.0)	(1.0)	(1.2)
<b>Nonneoplastic Nasal Lesions</b>																			
Atrophy, Olfactory Epithelium	0	0	1	2	10	10	10	0	0	0	8	9	10	0	0	0	0	0	0

(continued)

**TABLE 34**  
**Selected Results in the 13-Week Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide (continued)**

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate <sup>2</sup>					Nickel Subsulfide					Nickel Oxide							
	0	0.12 (0.03)	0.25 (0.06)	0.5 (0.11)	1 (0.22)	2 (0.44)	0	0.15 (0.11)	0.3 (0.22)	0.6 (0.44)	1.2 (0.88)	2.5 (1.83)	0	0.6 (0.4)	1.2 (0.9)	2.5 (2.0)	5 (3.9)	10 (7.9)
<b>Male Mice</b>																		
Survival	6	8 <sup>c</sup>	10	10	10	10	8	10	8	9	10	10	10	10	10	10	10	9
Final Mean Body Weights (Relative to Controls)	—	105%	100%	104%	104%	102%	—	102%	106%	103%	101%	97%	—	101%	99%	97%	98%	97%
Absolute Lung Weights	0.20	0.20	0.20	0.21	0.25**	0.31**	0.19	0.20	0.22	0.21	0.23*	0.28**	0.21	0.22	0.21	0.21	0.24	0.29**
<b>Nonneoplastic Lung Lesions</b>																		
Alveolar Macrophage, Hyperplasia (Severity)	0	0	0	10	10	10	0	0	8	9	10	0	10	10	10	10	10	9
Fibrosis, Focal (Severity)	0	0	0	0	2	10	0	0	0	5	10	0	0	0	0	0	0	0
Inflammation, Chronic Active (Severity)	0	0	0	0	2	2	0	0	0	5	7	0	0	0	0	0	0	3
Inflammation, Granulomatous (Severity)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
Interstitial Infiltrate (Severity)	0	0	0	2	8	8	0	1	0	3	2	0	0	0	1	3	8	8
Pigment (Severity)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	9
Nonneoplastic Nasal Lesions Atrophy, Olfactory Epithelium	0	0	0	0	0	10	0	0	0	5	10	0	0	0	0	0	0	0

(continued)

TABLE 34  
Selected Results in the 13-Week Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide (continued)

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate					Nickel Subsulfide					Nickel Oxide							
	0	0.12	0.25	0.5	1	2	0	0.15	0.3	0.6	1.2	2.5	0	0.6	1.2	2.5	5	10
	(0.03)	(0.06)	(0.11)	(0.22)	(0.44)		(0.11)	(0.22)	(0.44)	(0.88)	(1.83)		(0.4)	(0.9)	(2.0)	(3.9)	(7.9)	
<b>Female Mice</b>																		
Survival	7	10	10	10	10	10	10	8	10	9	10	8	9	10	7	10	10	9
Final Mean Body Weights (Relative to Controls)	—	105%	104%	105%	103%	97%	—	101%	100%	101%	101%	99%	—	97%	100%	96%	94%	97%
Absolute Lung Weights	0.20	0.20	0.20	0.20	0.22	0.27**	0.19	0.18	0.20	0.21	0.26**	0.29**	0.20	0.20	0.19	0.21	0.22	0.27**
<b>Nonneoplastic Lung Lesions</b>																		
Alveolar Macrophage, Hyperplasia (Severity)	0	0	0	0	10	10	0	0	4	9	10	10	0	10	7	10	10	9
Fibrosis, Focal (Severity)	0	0	0	0	1	8	0	0	0	0	1	9	0	0	0	0	0	0
Inflammation, Chronic Active (Severity)	0	0	0	0	1	9	0	0	0	0	10	7	0	0	0	0	1	3
Inflammation, Granulomatous (Severity)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Interstitial Infiltrate (Severity)	1	0	0	1	1	8	0	2	3	4	9	8	0	1	0	4	6	8
Pigment (Severity)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	7	10	9
															(1.0)	(1.0)	(1.0)	(1.0)
<b>Nonneoplastic Nasal Lesions</b>																		
Atrophy, Olfactory Epithelium	0	0	0	0	0	5	0	0	0	1	6	10	0	0	0	0	0	0

\* Significantly different (P ≤ 0.05) from the control by Williams' or Dunnett's test

\*\* P ≤ 0.01

a Survival data indicate number of animals surviving. Ten animals initially in group. Final mean body weights are not presented for groups with 100% mortality.

b Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

c Nine animals initially in group.

TABLE 35  
Selected Results in the 2-Year Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide<sup>a</sup>

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate (22.3% Ni)		Nickel Subsulfide (73.3% Ni)		Nickel Oxide (78.6% Ni)		
	0	0.12 (0.03)	0.25 (0.06)	0.5 (0.11)	0	0.15 (0.11)	1 (0.73)
Male Rats							
Survival	16/54	16/55	18/55	21/55	13/53	21/53	18/53
Final Mean Body Weights (Relative to Controls)	—	99%	101%	98%	—	98%	85%
Absolute Lung Weights							
7-Month Interim Evaluation	1.67	1.62	1.65	1.89	1.87	2.38**	3.48**
15-Month Interim Evaluation	2.12	2.48	2.50	3.00**	2.27	3.31**	6.84**
Alveolar/bronchiolar Proliferative Lesions and Neoplasms							
Alveolar Epithelial							
Hyperplasia, Focal or Atypical	3	2	3	2	2	6	11**
Adenoma	0	0	0	2	0	3	6*
Carcinoma	2 <sup>b</sup>	0	1	1	0	3	7*
Adenoma or Carcinoma (Combined)	2 <sup>b</sup>	0	1	3	0	6*	11**
Adrenal Medulla Proliferative Lesions and Neoplasms							
Hyperplasia	28	20	18	26	26	22	10
Benign Pheochromocytoma	16	16	12	11	13	30**	38**
Malignant Pheochromocytoma	0	3	2	1	0	2	10**
Benign or Malignant Pheochromocytoma	16	19	13	12	14	30**	42**
Carcinogenic Activity		No evidence				Clear evidence	Some evidence

(continued)

TABLE 35  
Selected Results in the 2-Year Inhalation Studies of Nickel Sulfate Hexahydrate, Nickel Subsulfide, and Nickel Oxide (continued)

Dose mg/m <sup>3</sup> (mg Ni/m <sup>3</sup> )	Nickel Sulfate Hexahydrate (22.3% Ni)		Nickel Subsulfide (73.3% Ni)		Nickel Oxide (78.6% Ni)						
	0	0.12 (0.03)	0.25 (0.06)	0.5 (0.11)	0	0.15 (0.11)	0	0.62 (0.5)	1.25 (1.0)	2.5 (2.0)	
<b>Female Rats</b>											
Survival	22/52	17/53	28/53	29/54	26/53	25/53	28/52	21/53	26/53	20/53	26/54
Final Mean Body Weights (Relative to Controls)	—	97%	97%	94%	—	96%	78%	—	96%	92%	90%
Absolute Lung Weights											
7-Month Interim Evaluation	1.25	1.22	1.22	1.45*	1.31	1.75**	2.59**	1.14	1.31*	1.65**	1.78**
15-Month Interim Evaluation	1.37	1.57	1.49	1.82**	1.52	2.52**	4.14**	1.56	1.79	2.41**	3.02**
<b>Alveolar/broncholar Proliferative Lesions and Neoplasms</b>											
Alveolar Epithelial											
Hyperplasia, Focal or Atypical	5	3	7	9	2	10*	11**	2	1	6	6
Adenoma	0	0	0	1	2	5	5	1	0	1	4
Carcinoma	0	0	0	0	0	1 <sup>b</sup>	4	0	0	5*	1
Adenoma or Carcinoma (Combined)	0	0	0	1	2	6 <sup>b,d</sup>	9*	1	0	6 <sup>d</sup>	5 <sup>d</sup>
<b>Adrenal Medulla Proliferative Lesions and Neoplasms</b>											
Hyperplasia	6	4	8	8	5	11	16**	8	12	14	22**
Benign Pheochromocytoma	2	4	2	3	2	7	36**	4	7	6	18**
Malignant Pheochromocytoma	0	0	0	0	1	0	1	0	0	0	0
Benign or Malignant Pheochromocytoma	2	4	2	3	3	7	36**	4	7	6	18**
Carcinogenic Activity		No evidence				Clear evidence			Some evidence		

(continued)







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