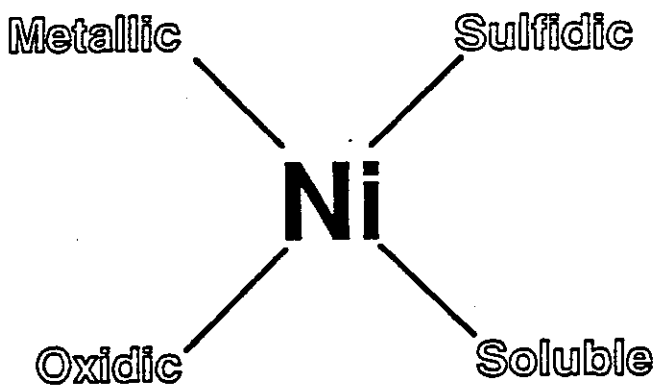
INITIAL STATEMENT OF REASONS FOR RULEMAKING

PROPOSED IDENTIFICATION OF NICKEL AS A TOXIC AIR CONTAMINANT



TECHNICAL SUPPORT DOCUMENT

Part B

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HEALTH RISK ASSESSMENT FOR NICKEL

California Department of Health Services

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1. Summary

Based on the findings of nickel-induced carcinogenesis in humans and animals, as well as the results of this risk assessment, the staff of DHS finds that nickel compounds are air pollutants which may cause or contribute to an increase in mortality or an increase in serious illness, or which may pose a present or potential hazard to human health.

A. Noncancer Health Effects

The nickel compounds usually associated with toxicity and carcinogenicity contain nickel in the zero or +2 valence state. Nickel compounds may be absorbed from the lungs and somewhat from the gastrointestinal tract; however, the less soluble forms of nickel[1] are not readily absorbed. Some nickel compounds may be absorbed slowly through the skin, but this route of exposure does not significantly contribute to nickel levels in other organs.

Inhalation of 210 mg/m^3 nickel carbonyl (30 ppm) for 30 minutes may be lethal to humans. However, nickel carbonyl is one of the few nickel compounds recognized as a cause of acute inhalation systemic toxicity. In welders, irritation of nose, nasopharynx and bronchi, eye irritation, headache and tiredness, have been attributed to nickel metal aerosol from high-nickel alloys. Acute nickel toxicity has not been associated with ambient air exposure. Noncancer effects associated with chronic occupational exposure to high concentrations of airborne nickel include increased risk for chronic respiratory tract infection and asthma. Nickel may provoke a variety of sensitization reactions.

The most sensitive noncancer endpoint in humans is allergic sensitization. Several cases of occupational asthma have been attributed to nickel salts in a catalyst production plant where nickel concentrations were normally between 0.013 and 0.067 mg Ni/m^3. Ingestion of 2.5 mg (approximately 0.04 mg/kg body weight) nickel as nickel sulfate has been shown to aggravate some cases of chronic dermatitis. In one study, approximately 10% of tested populations were found to be potentially allergic to nickel. In addition to dermal effects and asthma, nickel sensitization may also cause conjunctivitis, eosinophilic pneumonitis, and local or systemic reaction to nickel-containing prostheses.

Immune suppression is the most sensitive noncancer endpoint reported from animal studies. Acute exposure to nickel chloride at 0.25 mg Ni/m^3 produced this effect in mice, whereas 0.11 mg Ni/m^3 was reported to be a no observed effect level (NOEL). A four week exposure to nickel oxide at 0.08 mg Ni/m^3 caused significant alterations in rat alveolar macrophage populations. Chronic exposure of rats to nickel oxide at 0.06 mg Ni/m^3 has caused alveolar proteinosis, lung congestion, weight depression and decreased survival time. In addition, one study indicated immune system suppression may occur above 0.025 mg Ni/m^3 (as nickel oxide).

These noncancer effects occur at concentrations greater than three orders of magnitude above a 24-hour maximum (0.000024 mg Ni/m^3) measured near an industrial source. Therefore, the staff of the California Department of Health Services (DHS) concludes that it is unlikely that noncancer adverse

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1. Throughout this document, if not otherwise indicated, the word "nickel" generally refers to metallic nickel or inorganic compounds of nickel. In discussions of nutritional essentiality, "nickel" refers to the element and/or its compounds.

health effects would be caused by the levels of nickel compounds currently found in the ambient air. However, "hot spots" have not been fully evaluated.

B. Mutagenicity

Nickel compounds have reportedly produced mutagenic responses in mammalian assays, but have tested negative for mutagenicity in bacterial cell lines. Nickel compounds have produced morphological transformations in mammalian cells, inhibited DNA repair, altered DNA structure, and induced chromosome damage. The genotoxic responses have been reported from nickel compounds that are soluble in water as well as those that are insoluble in water. Genotoxic responses have been reported from nickel compounds in the 0, +1, +2 and +3 valence states.

C. Reproductive and Developmental Toxicity

Animal studies by routes of exposure other than inhalation demonstrate that nickel adversely affects spermatogenesis, litter size and pup body weight. The lowest observed effect level (LOEL) in an inhalation study of reproductive function was 3.6 mg Ni/m^3 (testicular degeneration in rats and mice from nickel subsulfide). The LOEL for reproductive effects using oral administration is 0.4 mg Ni/kg/day (from an unspecified nickel salt) in a multigenerational study using rats. The LOEL exposure of 3.6 mg Ni/m^3 in rats, assuming 100% absorption and continuous exposure, would correspond to a daily intake of 0.3 mg Ni/kg-day. This is comparable to 0.4 mg Ni/kg-day as an oral LOEL. No teratogenic effects have been clearly demonstrated for compounds other than nickel carbonyl. There are insufficient data to assess nickel's effect on reproductive functions in humans.

D. Carcinogenicity

The International Agency for Research on Cancer (IARC) reviewed nickel and nickel compounds in 1990 and concluded that there is sufficient evidence in humans for the carcinogenicity of nickel sulfate, and of the combinations of nickel sulfides and oxides encountered in the nickel refining industry; there is inadequate evidence in humans for the carcinogenicity of metallic nickel and nickel alloys; there is sufficient evidence in experimental animals for the carcinogenicity of metallic nickel, nickel monoxides, nickel hydroxides and crystalline nickel sulfides; there is limited evidence in experimental animals for the carcinogenicity of nickel alloys, nickelocene, nickel carbonyl, nickel salts, nickel arsenides, nickel antimonide, nickel selenides and nickel telluride; and there is inadequate evidence in experimental animals for the carcinogenicity of nickel trioxide, amorphous nickel sulfide and nickel titanate. IARC concluded that nickel compounds are carcinogenic to humans (Group 1) and that metallic nickel is possibly carcinogenic to humans (Group 2B). IARC made the overall evaluation on nickel compounds as a group on the basis of the combined results of epidemiological studies, carcinogenicity studies in experimental animals, and several types of other relevant data, supported by the underlying concept that nickel compounds can generate nickel ions at critical sites in their target cells. The U.S. Environmental Protection Agency (EPA) concluded that there is sufficient evidence that nickel refinery dust and nickel subsulfide are carcinogenic to humans. The EPA concluded that there is sufficient evidence that nickel carbonyl is carcinogenic to animals, but that there is inadequate epidemiologic evidence of its carcinogenicity in humans.

The carcinogenic effects of nickel in humans are well-documented in the epidemiologic literature. Several studies of workers employed in nickel refining have found associations between respiratory cancer mortality and nickel exposure. Four cohort studies have been evaluated. The respiratory cancer standardized mortality ratios (SMRs) for the four study cohorts occupationally exposed to nickel range from 1.12 to 8.71. These results were unlikely to have been due to any systematic error in the occupational studies.

The Ontario cohort involved 495 workers and 37 lung cancer deaths. The average exposure concentrations ranged from 46 to 400 mg Ni/m^3. The SMR reported from this cohort was 8.71. The Welsh cohort involved 967 refinery workers and 137 lung cancer deaths. The exposure concentration reported ranged from 8 to 42 mg Ni/m^3. The SMR reported for the total cohort was 6.23. The Norwegian cohort involved 2247 refinery workers and 82 lung cancer deaths. EPA estimated the exposure ranged from 3 to 35 mg Ni/m^3. The SMR for the total cohort was 3.73. The West Virgina cohort consisted of 1855 workers and 8 lung cancer deaths were reported. Nickel exposure ranged from 0.01 to 5.0 mg/m^3 and the SMR was 1.12.

The available data are inadequate to evaluate the possibility of interactions between nickel and other chemicals. Interaction with smoking has been characterized; the effect of combined smoking and nickel exposure on respiratory cancer rates is consistent with additivity rather than a multiplicative relationship. These findings have been confirmed and added to by further followup studies of each cohort.

E. Mechanism of Action for Carcinogenicity

No single mechanism has been postulated for nickel-related carcinogenicity. The mechanism of nickel carcinogenesis may be the result of direct genotoxicity (e.g., mutations or chromosomal damage), conformation transition of B-DNA to Z-DNA, inhibition of excision-repair, or a combination of these actions. Differences in carcinogenic potencies of nickel compounds may be partly the result of variation in their ability to provide Ni^{2+} at critical sites within the target cells. Since soluble nickel salts do not remain in the lung as long as insoluble nickel compounds, their potency might be lower than that of insoluble nickel compounds, but a recent analysis of human studies concluded there was evidence of carcinogenicity of soluble compounds also and total nickel should be considered when evaluating the risk by inhalation.

While some of nickel's effects may be governed by a threshold, there is at present no way to quantitatively determine a carcinogenic threshold. Furthermore, there is no evidence that nickel acts by one mechanism only. The staff of DHS concludes that neither the epidemiologic evidence nor the toxicologic evidence supports a threshold-mediated mechanism for nickel's carcinogenicity. In the absence of compelling evidence of a threshold, the staff of DHS treats the mechanism of nickel's carcinogenicity as a nonthreshold process.

F. DHS Conclusions on Nickel Compounds

The staff of DHS finds the evidence for human carcinogenicity due to inhaled nickel to be strong. This conclusion is based on (1) the high relative risks (mortality ratios) seen in occupational studies, (2) the high statistical significance of these findings, (3) the evidence of a dose-response effect, and (4) the demonstration of a nickel-related effect among cohorts which are geographically dispersed (Canada, Wales, Norway).

IARC (1990) and the International Committee on Nickel Carcinogenesis in Man (ICNCM, 1990) indicated that the epidemiological evidence points to insoluble and soluble nickel compounds as contributing to the cancers seen in occupationally exposed persons. Both insoluble and soluble nickel compounds have produced tumors in animals by a variety of routes, primarily injection. Both soluble and insoluble nickel compounds are genotoxic in a wide variety of assays. Evidence is available indicating that the Ni 2+ ion is the genotoxic agent and probably the carcinogenic agent as well. DHS staff conclude that based on available genotoxicity data, carcinogenicity data and physicochemical properties of nickel compounds, all nickel compounds should be considered potentially carcinogenic to humans by inhalation and total nickel should be considered when evaluating the risk by inhalation.

G. Quantitative Risk Assessment

A risk assessment to quantify the risk posed by ambient atmospheric levels of nickel in California is presented herein. A multistage model, fit to adjusted[2] data from an inhalation bioassay of nickel subsulfide in rats yielded a maximum likelihood estimate of carcinogenic potency of 2.01 per mg Ni_3S_2 (2.76 per mg Ni/m^3). The upper 95% confidence limit of the estimated carcinogenic potency is 2.75 per mg/m^3 Ni_3S_2 (or approximately 3.71 per mg Ni/m^3). The

Risk quantification was also conducted using epidemiological data from worker studies. The Ontario cohort study was determined to be the most appropriate for quantitative cancer risk assessment due primarily to the fact that it was the only cohort study with exposure measurements available for a sufficiently early time period. The West Virginia cohort was rejected for use in the quantitative risk assessment due to the imprecision associated with the low SMR reported, which could have been due to confounding factors. The Norwegian cohort was deemed unsuitable for risk assessment due to the absence of nickel exposure data from the refinery. The Welsh cohort was also not used in the quantitative risk assessment because exposure measurements were not available for a relevant time period.

A relative risk model was chosen as the most appropriate method for linear extrapolation to low dose lifetime exposure. The excess risk from nickel exposure among smokers was assumed to be the same as among non-smokers. SMR values were plotted against cumulative exposure, and the slope of the linear regression of the data was 9.22. The 95% confidence limit of the slope was 11.26. This upper limit was corrected to 11.85 for the fraction of the study group lost to follow-up. The exposure was adjusted for an equivalent lifetime exposure [(8/24) x (5/7) x (48/52)]. The excess relative risk estimate for lifetime exposure at 1 mg/m^3 was 5.04. Considering the background lifetime mortality risk of 0.051 for Ontario at the time of the cohort study followup, the upperbound for lifetime added risk for exposure to 1 mg/m^3 was 2.57×10^{-1} (or 2.57×10^{-4} per ug/m^3).

Using the human and animal data, the range of cancer risks is from 2.1 to 37×10^{-4} per ug/m^3. The best value for the upperbound of risk is 2.6×10^{-4} per ug/m^3 derived from the human study. The unit risk is the lifetime number of excess cancer deaths predicted to result from continuous exposure to nickel compounds at a concentration of 1 ug/m3. The estimated unit risk of lung cancer ranges from 210 to 3700 per million persons, from the human average estimate to the 95% upper confidence bound for the animal study, with the best value being 260.

The risk to residents of California from inhalation of atmospheric nickel was estimated by applying the unit risk estimate to the average total nickel concentrations measured by Air Resources Board (ARB) staff. ARB staff

reported a range of mean nickel concentrations of 0.0028 to 0.0231 ug Ni/m^3. This range of risk is estimated to be from 0.6 (0.0028 ug/m^3 x 210 x 10^{-6}) to 85 (0.0231 ug/m^3 x 3700 x 10^{-6}) lung cancer deaths per million. The lower figure is derived from an average value of unit risk and the higher figure is derived from a 95% upperbound using animal studies. The statewide population weighted exposure is 7.3 ng Ni/m^3. Using this value and the best potency value leads to an upper estimate of 2 lung cancer deaths per million people exposed.

The staff of DHS emphasizes that the risk estimates derived in conducting a risk assessment are not exact predictions, but rather represent plausible estimates based on current scientific knowledge and methods. Uncertainty in this risk assessment stems primarily from the relative carcinogenicity of the various nickel compounds, and the proportion of various nickel compounds in the Ontario cohort and ambient air. This estimate of risk represents an upperbound which can be adjusted if information on the carcinogenicity of specific nickel compounds is improved.

1.1. Risk Assessment Highlights

I. National and International Evaluations

A. U.S. Environmental Protection Agency (EPA)
1. Short term tests: Demonstrated ability of nickel compounds to induce genotoxic effects.
2. Animal carcinogenicity assays: Sufficient evidence of carcinogenicity for nickel carbonyl. Classified in Group B2.
3. Human carcinogenicity: Nickel refinery dust and nickel subsulfide are classified as known human carcinogens, Group A.
4. Conclusions: Only 2 nickel compounds and nickel refinery dust have been classified.

B. International Agency for Research on Cancer (IARC)
1. Animal carcinogenicity assays: Sufficient evidence in animals for carcinogenicity of metallic nickel, nickel monoxides, nickel hydroxides and crystaline nickel sulfides.
2. Human carcinogenicity: Sufficient evidence of carcinogenicity in humans from exposure to nickel and nickel compounds, particularly nickel sulfate, nickel sulfides and nickel oxides. Classified in Group 1.
3. Conclusion: Nickel compounds treated as a group in terms of carcinogenicity.

II. Carcinogenic Mechanism

A. Shape of the dose-response curve.
1. Animal: Insufficient information.
2. Human: Data from occupational studies are consistent with a linear relationship.

B. Pharmacokinetic information: Qualitative in nature. Extensive absorption by inhalation, and poor absorption by dermal and gastrointestinal routes.

C. Short-term tests: Genotoxicity not related to valence state or solubility.

D. Animal bioassays: Insoluble compounds appear more likely to be carcinogenic.

E. Conclusion: Genotoxic mechanism is unclear. Threshold for carcinogeniciity was not identified.

III. Exposure Sources

A. Air levels
1. Ambient mean levels of total nickel measured throughout California range from 2.8 to 23.1 x 10^{-3} ug/m^3 (2.8 to 23.1 ng/m^3)
2. Indoor Air: Insufficient information.

B. Water levels
1. National data: Surface waters range from 5 ug/L to 600 ug/L. Ground water levels range from 3 to 4430 ug/L.
2. California data: Not available.

C. Levels in food: Plant levels range from < 1 to 100 ppm. Food levels range from 0.02 to 9.8 ppm.

IV. Quantitative Risk Assessment

A. Range of extrapolation
Animal study: 960 ug/m^3 nickel subsulfide to ambient levels 0.02 ug/m^3 of total nickel (4 orders of magnitude).
Human studies: From 46000 ug Ni/m^3 in human studies to 0.02 ug/m^3 in ambient levels (6 to 7 orders of magnitude).

B. Range of Unit Risks
Animal study: Estimated unit risk for continuous lifetime exposure to nickel subsulfide per ug Ni/m^3 is 2.8 x 10^{-3} for the maximum likelihood estimate and 3.7 x 10^{-3} for the upper 95% confidence limit.

Human study: Estimated unit risk for continuous lifetime exposure to nickel compounds at 1 ug/m^3 ranges from 2.1 x 10^{-4} for the maximum likelihood estimate to 2.57 x 10^{-4} for the upper 95% confidence limit.

C. Conclusion: The unit risk recommended is 95% upper confidence limit developed from the human studies. Based on ambient levels, the range of risks is from 0.5 to 6 lung cancer deaths per million. Using the population-weighted exposure of 7.3 x 10^{-3} μg Ni/m^3, the upperbound estimate for lung cancer deaths is 2 per million persons exposed.

2. Introduction

The prime focus of this risk assessment concerns nickel in ambient air. However nickel, along with other constituents of airborne particulates, is deposited on ground surfaces, including soil, vegetables and waterways. Thus airborne nickel can result in human exposure by various pathways including inhalation, and ingestion of food and water. This risk assessment therefore also considers food sources. The issue of the essentiality of nickel is also relevant to assessing low level exposure to nickel, so this topic is reviewed.

At this stage, various considerations suggest that cancer risks from airborne nickel can be appropriately assessed by considering inhalation risks alone. In contrast to other effects, cancer risks may not be associated with a threshold. Cancer risks were therefore the main focus of low exposure risk estimation. However, the report also considers other health effects. The topics of chemical and physical properties of nickel, its presence in the environment, and its presence in food was covered in the Part A exposure assessment of nickel.

3. Pharmacokinetics

The major routes of nickel exposure and subsequent absorption in humans are inhalation and ingestion. Percutaneous absorption is of minor consideration for systemic effects of nickel, but is important for dermal effects. Inhalation absorption of nickel compounds have been reported as high as 60%. Ingestion absorption generally range from 1 to 10%, but have been reported as high 22%. However, absorption appears to be dependent on the specific compound studied and the experimental design, and the information available is for the most part indirect in nature. Experimental studies employing parenteral administration of nickel have limited relevance to environmental exposure. However, these studies are helpful in assessing the kinetics of nickel once it enters the body (i.e., transport, distribution, excretion). Parenteral exposure of humans to nickel (e.g., medications, hemodialysis, prostheses, etc.) does occur in subsectors of the population. Transplacental transfer of nickel from pregnant animals to the fetus does occur and will be addressed in Section 3.5.

The pharmacokinetics of nickel can be influenced by the form of nickel, as will be discussed below. For one particular form of nickel, nickel oxide (NiO), the conditions under which it is formed may also affect pharmacokinetics and biological activity. The temperature of formation of NiO (up to 1045^{o}) determines the color of the crystals, the crystalline surface area and the nickel (III) content (< 0.03 to 0.81% by weight) (IARC, 1990). The temperature of formation may also affect the crystalline structure and the incidence of defects within it.

The dissolution half-times of differently prepared samples of NiO in water were longer than 11 years. However, in rat serum and renal cytosol, the half-time decreased to approximately 1 year for low-temperature NiO while the high temperature NiO retained the greater than 11 year value (Sunderman et al., 1987). Two preparations of NiO obtained at temperatures $\leq$ 735^{o}C appeared to be phagocytized by C3H/10T1/2 cells more actively than the other nickel oxides (Sunderman et al., 1987). It has been noted (see Section 6) that NiO prepared at different temperatures exhibit different cell transformation

ability. In general, the lower the temperature at which the NiO was formed the more potent they were.

Although temperature of formation is a critical parameter to consider while reviewing the NiO literature it is also important to note that the conditions under which environmental NiO is formed are not known at this time.

3.1. Absorption

3.1.1. Inhalation

Respiratory absorption of various forms of nickel is the major route of nickel entry under occupational conditions, whereas ingestion is the major route of nickel intake under environmental conditions. Qualitative studies have been conducted on lung clearance of inhaled nickel.

The term "lung clearance" utilized in this section refers to the removal of nickel from the lung itself. Removal can be the result of: (1) mucociliary clearance and coughing which results in translocation from the lung to the gastrointestinal tract; (2) exhalation; and/or (3) absorption from the lung into the body compartment. Experimentally, actual absorption from the lung is very difficult to separate from other routes of removal. Therefore, lung clearance (e.g., changes in lung concentration over time) is often utilized as a yardstick to estimate potential absorption.

Once absorbed into the body, nickel is mainly excreted via the urine. In the absence of lung burden information, nickel urinary excretion is also utilized as an indicator of absorption following inhalation exposure. Torjussen and Anderson (1979) reported that nickel accumulation in the nasal mucosa of nickel workers was highest with inhalation of particulate subsulfide and oxide forms as compared to inhalation of nickel chloride/sulfate aerosols. Workers exposed to aerosols of nickel chloride and sulfate, on the other hand, had higher plasma and urine nickel levels. This finding would be expected on the basis of the relative water solubility of the respective compounds. Nasal mucosal nickel underwent extremely slow clearance having a removal half-life of about 3.5 years.

Numerous animal studies provide quantitative information on the deposition and potential absorption rates of the various forms of nickel in the lung. See Table 3.1 for summaries of the inhalation and intratracheal studies. The MMAD (mass median aerodynamic diameter) of the nickel particles is provided when available.

3.1.1.1. Deposition

Kodama and coworkers have conducted a study examining the deposition rate of various particulate sizes of nickel oxide (NiO) (Kodama *et al.*, 1985). In calculating the deposition fraction of NiO in the lung, minute volumes were estimated from the average body weights of the animals during the exposure period. The total volume of air inhaled during the exposure period was then multiplied by the NiO concentration to yield the total amount of inhaled NiO. The apparent deposition fraction was obtained by dividing the measured lung burden (ug NiO) by the estimated inhaled amount (ug NiO). Deposition was observed to be inversely related to particulate diameter (MMAD). Exposure to nickel oxide particulates with MMAD of 0.6-0.7, 1.2 and 4.0 um resulted in deposition of 18-23.4%, 14.5-19.8%, and 2.3 - 3.9%, respectively. The rate of deposition appeared to be relatively independent of

Table 3.1 Summary of Experimental Pulmonary Kinetic Studies of Nickel and Nickel Compounds Following Inhalation or Intratracheal Administration.

Exposure	Animal Species	Effects/Observation	Reference
Nickel Carbonyl, $Ni(CO)_4$:			
Exposed to radiolabeled $Ni(CO)_4$ 200 mg Ni/m^3 for 15 months (authors estimated exposure of 120 ug Ni/rat) (MMAD not reported)	Sprague Dawley Rats	Very high air concentration leading to additional exposure via ingestion from oral grooming of fur. At 24 hours postexposure lung radiolabeled nickel was calculated to be 18.3% of the body burden. Amount of nickel initially deposited, removed by exhalation, mucociliary transport, and/or existing as unabsorbed nickel within the intestinal tract is not known. Based on authors' estimate dose approximately 26.3% of dose was excreted in urine over 4 day postexposure period.	Sunderman and Selin, Tox Appl Pharmacol 12:207-218, 1968
Nickel Chloride, $NiCl_2$:			
Head only exposure to 0.644 mg Ni/m^3 as $NiCl_2$ for 2 hours (MMAD $\leq$ 3 um)	Swiss Mice	Lung nickel concentrations (mg Ni/g lung) were measured immediately after exposure, 1, 2, 3, and 4 days postexposure. Lung burdens indicated removal of 34%, 50%, 53% and 72% of the original lung burden by postexposure day 1, 2, 3, and 4, respectively. (removal half-time of approximately 2 days)	Graham et al, Env Res 16:77-87, 1978
Exposed for 2 hr/day to 0.09 or 0.4 mg Ni/m^3 as $NiCl_2$ for up to 14 days. (MMAD 0.7 - 0.9 um) During 22 hrs between exposure and up to 3 days postexposure rats were kept in clean air.	Sprague-Dawley male Rats	0.09 mg Ni/m^3: steady state lung burden was observed within the study period 0.4 mg Ni/m^3: lung burdens continued to increase with repeated exposure The percentage of submicron $NiCl_2$ aerosols retained in the lung was 6.9%. Data support a saturable clearance mechanism. Clearance half-time approximately 2 to 2.5 days	Henzel et al, Toxicol Lett 38:33-43, 1987

Table 3.1 (cont)

Exposure	Animal Species	Effects/Observation	Reference
Intratracheal administration of 1.27 ug Ni/animal as radiolabeled $NiCl_2$	Sprague Dawley Rats	By 24 hours postexposure 72% of initial body burden was eliminated in the urine and 29% was retained in the lungs. By day 21 postexposure 96.5% of body burden was excreted in the urine and only 0.1% remained in the lung. Indicating lung removal half-time of < 24 hours.	Carvalho and Ziemer, Arch Env Contam Tox 11:245-248, 1982
Intratracheal administration of 0, 0.01, 0.1, or 1 umol Ni/rat (0, 0.6, 6 or 60 ug Ni/rat) as $NiCl_2.6H_2O$	F344/Crl Rats	Lung nickel concentrations were measured at 1 and 7 days postexposure. On day 7 only 5% of the day 1 lung burden remained.	Benson et al, Fund Appl Tox 7:340-347, 1986
Intratracheal administration of radiolabeled 100 nmol $NiCl_2$/rat (13 ug $NiCl_2$/rat; 5.9 ug Ni/rat)	Wistar Rats	Radiolabel just detectable at 90 days postexposure, < 0.01% of initial lung burden. (Value estimated from Figure 2). Total urinary excretion was 64.3% of dose by 90 days of postexposure.	English et al, Am Ind Hyg Assoc J 42:486-492, 1981
Intratracheal administration of 1, 10 or 127 nmol $NiCl_2$/rat (0.13, 1.3 or 16.5 ug $NiCl_2$/rat; 0.059, 0.59 or 7.5 ug Ni/rat)	Sprague Dawley Rats	Rate of removal from lung was independent of dose. Suggesting that removal was by a process of diffusion. Estimated lung removal half-time of 6.6 hours. However, lung burden only measured up to 2 hours postexposure. Study period too short to accurately determine half-time.	Williams et al, Tox Appl Pharm 55:85-93, 1980
<u>Nickel Oxide, NiO:</u>			
Exposure to 73.5 mg NiO/m^3 for 7 hours on 2 consecutive days (57.7 mg Ni/m^3) (MMAD 1.25 um) Method of NiO generation - not specified.	Golden Syrian Hamsters	By day 6 postexposure > 70% of initial NiO burden (i.e. burden immediately after exposure) was still present in lungs. Even after 50 days postexposure approximately 50% of the original burden was still present. No significant quantity of nickel found in carcass, liver, or kidneys at any time point (i.e. from immediately after exposure up to 155 days postexposure).	Wehner and Craig, Am Ind Hyg Assoc J 33:146-155, 1972

Table 3.1 (cont)

Exposure	Animal Species	Effects/Observation	Reference
Exposed for 6-7 hour/day, 5 day/wk for up to 3 months. Group 1: 6.9 mg NiO/m^3 for 1 month (5.4 mg Ni/m^3) (MMAD 4.0 um) Group 2: 70 mg NiO/m^3 for 1 month (55 mg Ni/m^3) (MMAD 4.0 um) Group 3: 1.4 mg NiO/m^3 for 1 month (1.1 mg Ni/m^3) (MMAD 1.2 um) Group 4: 7.0 mg NiO/m^3 for 1 month (5.5 mg Ni/m^3) (MMAD 1.2 um) Group 5: 6.5 mg NiO/m^3 for 2 month (5.1 mg Ni/m^3) (MMAD 1.2 um) Group 6: 0.4 mg NiO/m^3 for 3 month (0.3 mg Ni/m^3) (MMAD 0.7 um) Group 7: 1.1 mg NiO/m^3 for 3 month (0.86 mg Ni/m^3) (MMAD 0.6 um) Method of NiO generation - pyrolysis of nickel acetate (as cited in IARC 1990)	Wistar Rats	Group 1: Measured lung burden of 220 ug NiO. Estimated deposition fraction of 3.9%. Group 2: Measured lung burden of 2130 ug NiO. Estimated deposition fraction of 2.3%. Group 3: Measured lung burden of 360 ug NiO. Estimated deposition fraction of 19.8%. Group 4: Measured lung burden of 1300 ug NiO. Estimated deposition fraction of 14.5%. Group 5: Measured lung burden of 2350 ug NiO. Estimated deposition fraction of 14.5%. Group 6: Measured lung burden of 330 ug NiO. Estimated deposition fraction of 18%. Group 7: Measured lung burden of 1200 ug NiO. Estimated deposition fraction of 23.4%. No significant difference in Ni concentration in body organs, except lungs, between exposed and unexposed animals.	Kodama et al, In: Proceedings in Nickel Toxicology - Proceedings of the Third International Conference on Nickel Metabolism and Toxicology. Eds: Brown and Sunderman, pp. 81-84, 1985
Exposed 6-7 hour/day, 5 day/wk for 1 month to either 0.6 or 8.0 mg NiO/m^3 (0.47 or 6.3 mg Ni/m^3) MMAD 1.2 um) Method of NiO generation - same as above	Wistar Rats	0.6 mg NiO/m^3: Estimated lung deposition fraction of 19.8%. Lung burden just after last exposure and at 1 year- 140 and 50 ug, respectively. 8.0 mg NiO/m^3: Estimated lung deposition fraction of 14.5%. Lung burden just after last exposure and at 1 year- 1370 and 1250 ug, respectively. No significant difference in nickel concentrations in body organs, except the lungs, between exposed and unexposed animals.	ibid

Table 3.1 (cont)

Exposure	Animal Species	Effects/Observation	Reference
Exposed for 7 hour/day, 5 day/wk for 1 month to 0.6, 8.0 or 70 mg NiO/m^3. (MMAD 1.2 um for 0.6 and 8.0 mg NiO/m^3 and 4.0 um for 70 mg NiO/m^3) Method of NiO generation - same as above.	Male Wistar rats	No apparent deposition of nickel in liver, kidney, spleen, and blood immediately after exposure. However, in case of the two higher exposure groups, the nickel concentration in liver, spleen and blood slightly increased with the increasing time of clearance. The biological half-time of NiO deposited in the lungs was estimated by the assumption that the amount of clearance is proportional to the amount of the NiO deposited. This resulted in a biological half-time of 11.5 and 21 months for 1.2 and 4.0 um MMAD, respectively.	Tanaka, et al, Biol Trace Elem Res 8:203-10, 1985
Exposed for 7 hour/day, 5 day/wk for 3, 6, or 12 months to 0.3 or 1.2 mg NiO/m^3 (MMAD 0.6 um) Method of NiO generation same as above.	Wistar Rats	The nickel concentrations in liver, kidney, spleen and blood slightly increased with increasing exposure time. However, there were no significant differences between the exposed and unexposed group organ weights, except the lung. The clearance half-time was very slow and the biological half-time was determined to be 7.7 months. The results showed that the clearance rate increased with decreasing particle diameter (see above study).	Tanaka et al, Biol Trace Elem Res 9:187-195, 1986 and Tanaka et al, Biol Trace Elem Res 16:19-26, 1988
Intratracheal administration of 100 nmol "NiO"/rat (7.5 ug "NiO"/rat; 5.9 ug Ni/rat). See text for method of "NiO" generation.	Wistar Rats	By day 30 and day 90 postexposure approximately 42% and 11% of initial lung burden remained (values estimated from Figure 2). Urinary excretion by day 90 postexposure was approximately 29% of dose. Fecal excretion was virtually equivalent to urinary excretion, however, this was most likely due to translocation of particles from lung to the gastrointestinal tract via coughing and ciliary removal. Authors did report an increase in nickel levels in the body organs.	English et al, Am Ind Hyg Assoc J 42:486-492, 1981

Table 3.1 (cont)

Exposure	Animal Species	Effects/Observation	Reference
Intratracheal administration of 0, 0.01, 0.1 and 1 umol Ni/rat as NiO (0, 0.6, 6 or 60 ug Ni/rat)	F344/Crl Rats	Lung nickel concentrations were measured at 1 and 7 days postexposure. On day 7 approximately 71% of the day 1 lung burden remained (i.e. 29% had been removed).	Benson et al, Fund Appl Toxicol 7:340-347, 1986
Nickel Subsulfide, Ni_3S_2:			
Exposed for 6 hr/day, 5 day/wk for 6 months. Some animals were killed 24 hr after last exposure and remaining rats were kept for a 12 month clearance period. Exposure concentration 0.5 mg/m^3 (MMAD 2.6 um)	Wistar rats	Nickel concentrations in the liver, kidney, and especially the lung increased during exposure. Blood nickel levels were not significantly different from controls. After 12 month clearance period there were no significant differences in nickel organ concentrations. Apparent lung deposition was 0.5 $\pm$ 0.1%. After 12 month clearance period approximately 98% of the amount deposited during exposure had been cleared.	Kodama et al, unpublished report to NIPERA, 1989
Intratracheal administration of 11.7 ug Ni_3S_2/mouse (8.6 ug Ni/mouse) (MMAD 1.66 um)	A/J Mice	Biphasic lung removal observed over the 35 day postexposure period. Total lung burden was 85% of instilled dose at 4 hr postexposure; 30% at 7 days; and within 35 days 10% of the dose was retained in the lungs. Approximately 60% of the total dose was excreted in the urine by day 35 postexposure.	Valentine and Fischer, Env Res 34:328-334, 1984
Intratracheal administration of 11.8 ug Ni_3S_2/mouse (8.65 ug Ni/mouse) (MMAD 1.65-1.83 um)	BALB/c BYJ Mice	25% of instilled dose was cleared from the lungs within 15 minutes due to coughing. Between the 15 min. and 7 day postexposure time points the amount of instilled dose decreased from 75% to 20.5%.	Finch et al, Env Res 42:83-93, 1987
Intratracheal administration of 0, 0.01, 0.1, or 1 umol Ni/rat as Ni_3S_2 (0, 0.6, 6, or 60 ug Ni/rat)	F344/Crl Rats	Lung nickel concentrations were measured at 1 and 7 days postexposure. On day 7 approximately 90% of the day 1 lung burden remained (i.e. 10% had been removed).	Benson et al, Fund Appl Tox 7:340-347, 1986
Nickel Sulfate, $NiSO_4$:			

Exposure	Animal Species	Effects/Observation	Reference
Intratracheal administration of 0, 0.01, 0.1 or 1 umol Ni/rat as $NiSO_4.6H_2O$ (0, 0.6, 6 or 60 ug Ni/rat)	F344/Crl Rats	Lung nickel concentrations were measured at 1 and 7 days postexposure. On day 7 approximately 21% of the day 1 lung burden remained (i.e. 79% had been removed)	Ibid
Exposed to 0, 0.017, 0.190 or 1.8 umole Ni in saline/rat (0, 1.02, 11.4 or 108 ug Ni/rat) by intratracheal administration.	F344 female rats	Urine was the major route of excretion accounting for 50% of the dose after 0.017 and 0.19 umole Ni/rat and 80% of the dose after 1.8 umole Ni. The half-time for urinary excretion was 23 hr, 12 hr and 4.6 hr in the 0.017, 0.19 and 1.8 umole dose groups, respectively. Fecal excretion accounted for 30% (0.017 and 0.190 umole) or 13% (1.8 umole) of initial dose. Of the nickel remaining in the body at the end of 96 hr (4 days) > 50% was in the lungs. The long-term half-time for clearance of Ni from the lungs was 36 hr, 35 hr, and 21 hr in the 0.017, 0.19 and 1.8 umole dose groups.	Medinsky et al Env Res 43:168-178, 1987
Nickel Containing Fly Ash:			
Exposure to nickel enriched fly ash aerosol (NEFA) (9% nickel) or control fly ash (0.03% nickel). Single 6 hour exposure, respirable aerosol concentration 200 mg/m^3	Syrian Hamster	Lung nickel burden at 7 days postexposure was 7 ug Ni/lung. Lung nickel burden at 30 days postexposure was 6.8 ug Ni/lung. Study duration was too short to determine removal half-time.	Wehner et al, Env Res 19:355-370, 1979
Exposure to coal-fired power plant fly ash 6 hour/day, for 15 days. Chamber concentration maintained between 200 to 400 mg/m^3.	Wistar Rats	Lung nickel concentrations were measured at 1, 7, 15, and 30 days after exposure. The measured concentrations were 3.4, 2.7, 1.0, and 1.2 ug/g lung, respectively. Increased nickel concentrations were observed in liver, heart, kidney, and small intestine at day 1 and/or day 7 postexposure but not at the later time points. Nickel concentration in the serum was not different from the control value.	Srivastava et al, J Env Sci Health A(19)663-677, 1984

Table 3.1 (cont)

Exposure	Animal Species	Effects/Observation	Reference
Nickel Containing Welding Fume:			
Exposure to 45 mg/m^3 of manual metal arc (MMA) or metal inert-gas (MIG) welding fumes for 4 weeks (1 hr/day, 5 day/week). Concentration of nickel was 0.4 - 1.0 and 3.1-6.5% by weight for MMA and MIG, respectively.	Wistar Rats	Animals were killed at 1 hr, 3 hr, 8 hr and 1, 4, 7, 14, 28, 56, and 106 days after exposure. Measured lung nickel burdens at end of exposure period were 7.1 and 130 ug/g for MMA and MIG, respectively. Lung burdens on day 7, 14, and 106 postexposure indicate removal of 25% (MMA and MIG), 38% (MMA) and 32% (MIG), and 82% (MMA) and 66% (MIG), respectively, from the lung. (Values estimated from Figure 2). Concentration of nickel in other organs and blood were below the detection limit.	Kalliomaki and Olkinuora, IARC Sci Rubl No. 53 Nickel in the Human Environment pp 385-393, 1984
Intratracheal administration of 8 ug MMA and 80 ug MIG welding fumes. Concentration of nickel was 0.4% and 4% by weight in MMA and MIG welding fumes, respectively.	Wistar Rats	Lung nickel concentrations were measured at 1 hr, 1, 4, 8, 14, 30, 60, and 103 days postexposure. Measurements indicated removal of: MMA - 53, 59, 65, 70.5, 84, and 90% by day 1, 4, 8, 14, 30, 60 and 103 postexposure, respectively. Approximately 25% of the nickel eliminated in the first 3 days was through translocation to the gastrointestinal tract not absorption from the lungs, therefore corrected removal rates would be 28, 34, 40, 45.5, 59, and 65%. MIG - Due to variability, values fluctuated between 0 and 19% between days 1 to 60 postexposure. At 103 days postexposure apparent removal was 37%.	Kalliomaki et al, Brit J Ind Med 43:112-119, 1986

the length of exposure or concentration. The method utilized for NiO generation was pyrolysis of nickel acetate (IARC, 1990). The importance of the method(s) utilized for NiO generation has been discussed above.

3.1.1.2. Absorption

The degree of absorption depends, in part, on chemical form. Absorption may also be influenced by the amount inhaled and breathing rate. The available information on the various forms of nickel is summarized in the following section. See Table 3.1 for additional details of studies.

Nickel carbonyl, $Ni(CO)_4$, has not been extensively studied. This form of nickel is somewhat unique in that it is lipid soluble. Based on the limited information, the major route of removal is exhalation (Sunderman and Selin, 1968). Based on urinary excretion data, a comparison of kinetics following inhalation and intravenous exposure to $Ni(CO)_4$ suggests that up to approximately 30% of the inhaled dose may be absorbed.

Several studies have been conducted on the removal of nickel chloride from the lungs. All but two of the studies utilized intratracheal administration. Following the intratracheal administration of a bolus, a biphasic curve of removal from the lung is often observed. This is due to the translocation of part of the bolus within the first two hours postexposure to the gastrointestinal tract via coughing, ciliary action, etc. As a result, an increase in fecal nickel concentration is also seen during this time. This phenomenon is particularly true if a relatively large bolus is administered.

Nickel chloride ($NiCl_2$) is one of the most soluble forms of nickel. Studies suggest that the lung removal half-time is approximately 2 days. Within one week, potentially all of an acutely administered dose is absorbed into the body (Graham *et al.*, 1978; Benson *et al.*, 1986). Menzel *et al.*(1987) utilized a repeated exposure protocol. Rats were exposed to 0.09 or 0.4 mg Ni/m^3 as $NiCl_2$ hr/d for up to 14 days. The data indicated a saturable clearance mechanism in the higher dose group.

In contrast to nickel chloride, nickel oxide (NiO) is one of the least soluble forms of nickel. Long term inhalation studies indicate that the lung removal half-time is at least on the order of months, possibly 12 months or more, and absorption into the body is low (Wehner and Craig, 1972; Kodama *et al.*, 1985; Tanaka *et al.*, 1985, 1986, 1988). The series of studies by Tanaka *et al.*(1985, 1986 and 1988) suggest that the clearance rate increases with decreasing particle diameter.

Intratracheal studies of NiO suggest shorter removal half-times (i.e., 2-3 weeks), however this is most likely due to a relatively rapid translocation of a bolus of the dose to the gastrointestinal tract (English *et al.*, 1981). In addition, increases in body organ nickel concentrations and urinary excretion were reported by only one group of investigators, who suggested a potential absorption of nearly 30% following intratracheal exposure (English *et al.*, 1981). One should note that the suspension of "nickel oxide" was made by combining the carrier and radionuclide $^{63}NiCl_2$ and adding NaOH. The resulting green gel-like suspension of $Ni(OH)_2$ was heated at 250°C for 45 minutes producing a black solid. Chemical analyses of this solid indicated that although 90% of the nickel was in the insoluble form the reaction from $Ni(OH)_2$ was not complete. The fraction of the insoluble preparation which was nickel oxide was not stated, however, it was referred to as "NiO" throughout the publication. The importance of the method utilized for generation of NiO is discussed in Section 3 and 6.

Nickel subsulfide (Ni_3S_2) lung removal following inhalation exposure has only been evaluated by one group of investigators (Kodama *et al.*, 1989). The results of this study are only available in an unpublished report. However, since it is the only inhalation study available it has been included here. Rats were exposed to 0.5 mg/m^3 for 6 hr/d, 5 d/wk for 6 months. Exposed animals were killed either after the last exposure or after a 12 month clearance period. Nickel concentrations increased in the liver, kidney and especially the lungs during exposure, whereas blood levels did not. After 12 months no significant differences in organ nickel concentration were apparent. Approximately 98% of the amount deposited in the lungs had been cleared, suggesting a half-time of approximately 2 months.

The intratracheal studies demonstrate an early rapid removal phase, with up to 25% of the dose cleared to the gastrointestinal tract. The studies conducted in mice indicated a lung removal half-time of 6-13 days, and potential absorption of approximately 60% based on urinary excretion data (Valentine and Fisher, 1984; Finch *et al.*, 1987). However, the study conducted on rats by Benson *et al.* suggested a much longer removal half-time (approximately 40 days) (Benson *et al.*, 1986). Neither urinary excretion nor body tissue nickel levels were measured, so the percent absorption could not be estimated from this particular study.

Only two groups of investigators have examined lung removal of nickel sulfate (Benson *et al.*, 1986; Medinsky *et al.*, 1987). Intratracheal administration was the route of exposure utilized in both studies. Benson *et al.*(1986) reported a lung removal half-time of approximately 3 days. However since neither urinary excretion nor body tissue nickel levels were measured, absorption from the lungs could not be determined from this study.

Medinsky *et al.*(1987) did evaluate urinary excretion following administration. Urinary excretion was found to be the major route, accounting for 50% of the dose-after 0.017 and 0.19 umole Ni/rat and 80% of the dose after 1.8 umole Ni/rat. Fecal excretion accounted for 30% (0.017) and 0.19 umole dose) or 13% (1.8 umole) of the initial dose. The authors also found that as the dose instilled decreased, the fraction of the instilled dose which cleared the lung with a slow clearing rate-constant increased. In addition, as the amount instilled decreased, the half-time for the slow clearing rate-constant increased from 21 to 36 hours. The authors suggested that potential binding sites for nickel in lung tissue or carrier-mediated clearance mechanisms for nickel may exist and were saturated at the higher doses, resulting in a more rapid clearance of nickel at the higher doses due to passive diffusion of nickel ions.

Retention of nickel-containing flyash and welding fumes have been examined. The form of nickel contained in the ash or fumes was not known. The two studies evaluating nickel containing flyash present conflicting results (Wehner *et al.*, 1979; Srivastava *et al.*, 1984). Wehner *et al.* (1979) reported that nickel leaching from nickel enriched flyash did not occur (Wehner *et al.*, 1979). Virtually no change in lung nickel concentration was observed during the study period, i.e., 30 days postexposure, suggesting no absorption in the body. However, Srivastava *et al.* reported that nickel does leach from coal-fired power plant flyash (Srivastava *et al.*, 1984). Increases in nickel levels in various body organs were observed. Unfortunately, the data reported do not permit the estimation of absorption.

In a later study examining pulmonary clearance of flyash, Wehner *et al.*(1980) stated that the results reported in Wehner *et al.*(1979) were only based on 2 animals and may not be correct. The more recent results indicated a biological half-life of 2.6 and 34.5 days for flyash deposited in the

airways and deep lung, respectively. Since one would anticipate that the pulmonary clearance of flyash particles and Ni-enriched flyash particles would be similar and given the larger data base for the results the authors suggested that the clearance half-time values of 2-3 days for airways and approximately 35 days for the deep lung were probably more valid.

The inconsistencies between these studies could be due to a variety of factors, such as differences in flyash composition and/or size, species, exposure protocols, etc. Thus, specific conclusions regarding absorption of nickel from flyash cannot be made at this time.

The lung removal of nickel following inhalation or intratracheal exposure to nickel-containing welding fumes has been examined by Kalliomaki and coworkers (Kalliomaki *et al.*, 1984; Kalliomaki *et al.*, 1986). Two types of welding fumes were assessed: manual metal arc (MMA) and metal-inert gas (MIG). The lung removal pattern was different for each type of welding fume.

Following inhalation exposure to MMA welding fumes a lung removal pattern was difficult to determine due to the low nickel concentration in the lungs (Kalliomaki *et al.*, 1984). A single exponential model was fitted to the measured concentration; the half-time calculated in this manner was 30 days. Concentrations of nickel in body tissue and blood were below the detection limit.

Following intratracheal administration of MMA a lung removal half-time of 49 days was reported (Kalliomaki *et al.*, 1986). Nickel blood levels were below the detection limit, however, based on increases in liver and kidney concentrations, some absorption had occurred.

Inhalation exposure to MIG welding fumes produced a biphasic lung removal curve. The half-times of each phase were 3 and 85 days, respectively (Kalliomaki *et al.*, 1984). Blood and body tissue levels remained below the detection limit. Apparent lung removal was much slower following intratracheal administration (Kalliomaki *et al.*, 1986). Due to extensive fluctuations in the measured lung burdens at different post-exposure time points a removal half-time could not be estimated. However, by day 103 postexposure nearly 60% of the initial burden still remained.

3.1.2. Gastrointestinal Absorption

As was the case in the inhalation studies, absorption following oral exposure has not been measured directly. Although not an ideal method, urinary excretion of nickel is used as a method to estimate absorption.

Results of animal studies indicate poor absorption of nickel from the gastrointestinal tract. Following oral administration of radiolabeled nickel chloride most of the label remains unabsorbed and is simply passed in the feces (Sunderman, 1986a). Absorbed nickel is eliminated mainly via the urine. Recent data from Kirchgessner *et al.* provide evidence of an absorption regulatory mechanism (Kirchgessner *et al.*, 1985). Rats were fed diets containing 0.06, 0.11, 0.22, 1, 10, 15, 20, 25 50, 100, 200, 400 and 600 ppm nickel (Unspecified form). Concentrations over 200 ppm were toxic to the animals. The absolute amount of intake retained (i.e., actual amount absorbed) maintained a plateau, until dietary levels reached 100 ppm or more.

The relative absorption (% of intake) of nickel was high, approximately 22%, if the dietary level was very low (0.06 ppm). The percent absorbed decreased rapidly, resulting in less than 1% absorption when dietary levels were >1 ppm. Dietary levels of 0.11 and 0.22 ppm resulted in 6.6 and 2.7% of the intake absorbed, respectively. The data suggest that nickel absorption in

relation to nickel supply may be regulated in order to compensate for an insufficient supply by increased absorption and an elevated supply by decreased absorption. The increase in amount absorbed at dietary levels of 100 ppm or greater may indicate an overloading of the regulatory mechanism.

To date the human data indicate that up to 27 percent nickel is absorbed following oral exposure. Sunderman *et al.* (1989) has recently evaluated the gastrointestinal absorption of nickel. Healthy volunteers ingested 12, 18 or 50 ug Ni/kg as nickel sulfate in drinking water or added to food. The subjects fasted 12 hours before drinking or eating the nickel containing water or food. Absorbed nickel averaged 27 $\pm$ 17% (mean $\pm$ SD) of the dose in water vs. 0.7 $\pm$ 0.4% of the same dose ingested in food. The elimination half-time of absorbed nickel averaged 28 $\pm$ 9 hours.

Solomons and coworkers have also shown that bioavailability of nickel was dependent on dietary composition (Solomons *et al.*, 1982). Adult human volunteers ingested 5 mg of nickel as the soluble sulfate in water and the resulting blood nickel concentration profiles were compared to those obtained when the same amount of nickel was given in beverages and two test meals. Except for soft drinks, the beverages and the test meals suppressed absorption. The actual rate of absorption was not estimated.

In an earlier study by Christensen and Lagesson (1981) adult human volunteers ingested, without fasting, a single dose of 5.6 mg nickel as nickel sulfate. Over the three days after ingestion, urinary nickel levels rose to a peak and then decreased. The cumulative excretion over this short time period was 176 ug, indicating a minimal gastrointestinal absorption rate of approximately 3 percent. Great individual variation of nickel excretion in urine was seen, with apparent minimal absorption ranging from <1 to 8%. The volunteers had been instructed to drink and eat about the same amount and type of liquids and foods at the same period during the study. Compliance with these instructions was not reported. The nickel half-time, based on serum levels during the study period, was calculated by the authors to be 11 hours. However, since the urine and serum nickel levels were still significantly higher than baseline values at the last time point assessed, the duration of the study may have been inadequate, resulting in an underestimation of total urinary excretion, and hence the absorption and the half-life.

3.1.3. Skin Absorption

Percutaneous absorption of nickel is considered to be mainly restricted to the passage of nickel past the outermost layers of skin, deep enough to bind with apoantigenic factors. Sensitization to nickel has to be preceded by permeation of the nickel into the dermis. Nickel penetrates deeper at sweat ducts and hair-follicle ostia and has a special affinity for keratin (Spruit *et al.*, 1965).

Spruit *et al.*, utilizing human cadaver skin from which the epidermis was removed, have shown that the dermis absorbs nickel (Spruit *et al.*, 1965). Recently, Fullerton (Fullerton *et al.*, 1986) also examined the permeation of nickel salts through human skin in vitro. The nickel salts examined were nickel sulfate hexahydrate and nickel chloride hexahydrate. Human breast or loose skin operation tissues were exposed to 250 11 of nickel salt solution resulting in a dose of 184 Mg Ni/cm^2. In the first experiment the effect of occlusion on the permeation rate of nickel from $NiCl_2$ was examined. Occlusion resulted in a significantly higher permeation rate (approximately 3.6 percent of applied dose) compared with non-occluded exposure (approximately 0.23 percent) after 144 hours.

In the second experiment the effect of the type of nickel salt was examined. This experiment was carried out under occlusive conditions to maximize the permeation rate. Nickel ions from a chloride solution passed through the skin about 50 times faster than nickel ions from a sulfate solution. The amount of permeation of $NiCl_2$ was much higher (16 percent at 144 hours) than $NiSO_4$ (0.3 percent). The authors concluded that nickel was capable of penetrating the skin barrier, but the process was slow, having a lag-time of about 50 hours.

As one can see, the occlusion permeation rate of $NiCl_2$ is much higher in experiment 2 than in experiment 1 (9-16% vs 3.6%). This difference was apparently due to the fact that the skin was supplied by different donors.

Dermal application of 60 mg Ni/kg or higher (as nickel sulfate) resulted in microscopic changes in the liver and testis of rats indicating that absorption can occur from the intact skin (Mathur *et al.*, 1977). However, it should be noted that under these experimental conditions, skin injury did occur and therefore may have allowed easier passage of nickel through injured tissue. Unfortunately, it was not possible to estimate the level of absorption from the data.

3.2. Distribution

3.2.1. Transport of Nickel in the Blood

Blood is the main route by which absorbed nickel is delivered to other tissues. In individuals with no known exposure, serum nickel values are approximately 0.2 to 0.3 Mg/dl (EPA, Sept. 1986a).

Once absorbed, nickel is transported in the blood bound to one of three fractions (Sarkar, 1984): 1) an amino-acid-bound fraction which is mostly in the form of Ni-L-histidine complex; 2) nickel bound to albumin; and 3) nickel bound to alpha-2-macroglobulin. L-histidine has been shown to have a greater affinity for nickel than albumin. However, under in vivo conditions the concentration of albumin is much higher than the concentration of L-histidine and for this reason, it appears that most of the nickel in blood becomes associated with albumin.

3.2.2. Tissue Distribution

Studies utilizing autopsy data indicated generally low tissue nickel levels. The major tissues with detectable nickel, in decreasing order of frequency of detection, were lung, thyroid, adrenals, kidney heart, liver, brain, spleen, and pancreas (Rezuke *et al.*, 1987). The only tissue exhibiting age-dependent accumulation appeared to be the lungs (Mushak, 1980). Such accumulation was believed to be associated with less soluble forms of inhaled nickel.

Based on data from experimental animals, tissue distribution appears to be affected by route of exposure. Sunderman and Selin (1968) administered radiolabeled nickel carbonyl by intravenous injection (22 mg Ni/kg) and inhalation (200 mg/m^3 for 15 min). Twenty-four hours after exposure the tissue distribution patterns were: following intravenous administration 41.2 percent in muscle and fat, 31.1 percent in bone and connective tissue, 26.6 percent in viscera and blood, and 1.1 percent in brain and spinal cord; and following inhalation 30.4 percent in muscle and fat, 15.6 percent in bone and connective tissue, 49.7 percent in viscera and blood, and 4.1 percent in brain and spinal cord. This altered distribution was attributed, in part, to label retained in

the thoracic viscera (from inhalation) and in the abdominal viscera (from ingestion of nickel on fur). It is interesting to note the significant increase in levels found in the brain and spinal cord following inhalation. A more recent study (Oskarsson and Tjalve, 1979) also found higher concentrations of radiolabeled nickel in the brain, spinal cord, and heart after inhalation of nickel carbonyl than after injection. Nickel carbonyl is somewhat unique in that it is soluble in lipids and therefore could easily pass through biological membranes. Once within the cell nickel carbonyl undergoes a slow decomposition to non-ionized nickel and carbon monoxide; the released nickel is then oxidized to Ni^{++}.

A number of reports in the literature describe the tissue distribution of divalent nickel following parenteral administration of nickel salts (EPA, Sept. 1986a; Smith and Hackley, 1968). It can generally be stated that nickel administered in this way leads to highest accumulation in the kidney, followed by the endocrine glands and lungs, the liver, and heart. Relatively little nickel was found in bone or neural tissue.

3.3. Subcellular Distribution of Nickel

Nickel toxicity to organelles is associated with specific patterns of subcellular distribution, particularly with respect to carcinogenicity. Subcellular partitioning of nickel differs markedly between soluble and insoluble nickel compounds. In vitro cellular studies by Costa *et al.* indicate that carcinogenic nickel subsulfide (Ni_3S_2), crystalline nickel sulfide (NiS), and crystalline nickel subselenide (Ni_3Se_2) are all actively phagocytized with subsequent transfer of nickel to the cell nuclei (Costa *et al.*, 1981). However, particles of similar size consisting of amorphous nickel sulfide (NiS) and metallic nickel were not significantly phagocytized despite long exposure periods to high concentrations.

Harnett *et al.* compared the differential binding of insoluble crystalline nickel sulfide and soluble nickel chloride in cultured cells (Harnett *et al.*, 1982). RNA and DNA binding of nickel following sulfide exposure was 300 to 2000 times greater than with nickel chloride. The subcellular distribution of nickel from nickel oxide has not been studied.

Nickel binds to chromatin, nuclei and nuclear proteins. The amount of nickel bound to the different protein fractions is not the same in all tissues. Studies by Ciccarelli and Wetterhahn demonstrated that the relative amount of nickel bound to whole chromatin was greater for the kidney than for the liver and was directly related to nuclear nickel content (Ciccarelli and Wetterhahn, 1984). In addition, much higher levels of nickel were found in the DNA-histone complex from kidney as compared to liver.

3.4. Retention and Excretion

As with other kinetic parameters, the extent of retention and excretion depends on the form of nickel.

3.4.1. Animal Studies

Nickel chloride is rapidly excreted resulting in virtually no retention regardless of route of administration (Smith and Hackley, 1968; Carvalho and Ziemer, 1982; English *et al.*, 1981). The major excretion route of absorbed nickel is via the urine (greater than 90%) with the remaining 10 percent

excreted in the feces. Nickel is also excreted in the bile. However, this route of elimination is minor.

Four days after the administration of radiolabeled nickel carbonyl by intravenous injection 38.4 percent had been eliminated in the expired air, 31.2 percent in the urine and 2.4 percent in the feces, i.e., a total of 72 percent (Sunderman and Selin, 1968). Unfortunately, the study did not determine where the remaining 28% of radiolabel was located. One should note that all of the radiolabel exhaled (i.e., 38.4%) was exhaled within 6 hours postexposure.

Intratracheal administration of nickel subsulfide and "nickel oxide" results in significant fecal as well as urinary excretion (English *et al.*, 1981, please see Section 3.1.1.2 for method of generation; Valentine and Fisher, 1984). This, in part, is due to the translocation of insoluble nickel to the gastrointestinal tract rather than absorption into the body as is the case with soluble forms of nickel. Thirty-five days after exposure approximately 60 percent and 40 percent of administered nickel subsulfide was eliminated in the urine and feces, respectively. Potential loss through exhalation was not examined. Ninety days after administration of nickel oxide 25 percent and 21 percent were eliminated in the urine and feces, respectively.

3.4.2. Human Data

Urinary excretion is usually the major excretory route of absorbed nickel. Reported urinary levels vary, but normal values in the range of 2 to 4 Mg/l have been suggested (EPA, Sept. 1986a). On the basis of air, plasma and urine nickel concentrations in 4 nickel platers during one working week, Tossavanainen *et al.* computed a plasma nickel biological half-time of 20 to 34 hours and in urine of 17 to 39 hours (Tossavainen *et al.*, 1980). The four electroplaters were exposed to soluble nickel sulfate and nickel chloride. A one-compartment model was assumed. Biliary excretion of nickel is known to occur in several animal species but constitutes a minor route of excretion. Based on autopsy information, from non-occupationally exposed individuals, nickel is found in human bile (Rezuke *et al.*, 1987). The average concentration was 2.3 Mg/l, range 1.5 to 3.3 Mg/l. Whether biliary nickel is reabsorbed from the gastrointestinal tract is not known.

Sweat can constitute a major route of nickel excretion under specific circumstances (e.g., heat stress; long, hard, manual labor, etc.). Hohnadel *et al.* determined nickel concentrations (Mg/l) in sweat of healthy subjects during sauna bathing for short periods of time (i.e., 15 minutes) (Hohnadel *et al.*, 1973). The average concentrations were 52 Mg/l for men and 131 Mg/l for women. The men sweated more profusely than the women (average 23 ml vs 7 ml) thereby exhibiting a lower concentration. One should note that a portion of the nickel in the sweat could have come from the skin itself. Concentrations of nickel in other biological fluids (e.g. blood, serum) has been summarized by the National Academy of Sciences (1975).

3.5. Transplacental and Body Distribution During Pregnancy

Transplacental transfer and fetal uptake of nickel does occur. To date the only nickel compound which has been examined is nickel chloride (Olsen and Jonsen, 1979; Lu *et al.*, 1981; Sunderman *et al.*, 1978a). Nearly all the studies utilized radiolabeled nickel and autoradiography, therefore, the data

are mainly of a qualitative nature. See Table 3.2 for a detailed summary of studies.

Within the uterus, retention of radiolabel was particularly seen in the yolk sac and placenta with a smaller portion actually entering the fetus. Accumulation of radiolabel was observed until late gestation. This may be related to the fact that it is not until day 17-18 of gestation that the kidney is able to function and thereby excrete nickel. Distribution of radiolabel early in fetal development was relatively homogeneous. With increasing gestational age fetal distribution eventually resembles that of the mother.

The relative distribution of nickel in major maternal tissues and fluids after injection were: kidney >> blood > placenta > lung > fetuses/liver > spleen. Blood and placental peak concentrations were seen at 2 hours postexposure. The remaining tissue peak levels were reached at 4 hours postexposure or later. Peak fetal levels were seen at 8 hours postexposure. Between 8 and 24 hours postexposure fetal levels remained fairly constant and then began to drop rapidly.

One interesting observation regarding maternal distribution should be noted. In addition to the tissues listed above, the pituitary glands from exposed pregnant and nonpregnant rats were compared (Sunderman *et al.*, 1978a). The nickel concentrations in the pregnant females were 7 to 8 times higher than nonpregnant animals ($p<0.001$).

Compounds which are able to form lipophilic complexes with nickel have been shown to alter the distribution of nickel in pregnant and nonpregnant mice (Jasim and Tjalve, 1984). Oral administration of radiolabeled nickel, as $NiCl_2$, together with thiuram sulfides or sodium diethyldithiocarbamate resulted in highly increased levels of nickel in several tissues of mice compared with animals given nickel alone. In pregnant animals administration of these compounds also resulted in increased nickel levels in the fetuses. In particular, the uptake of nickel in the brains of adults and fetuses was markedly enhanced. Dipentamethylenethiuram monosulfide and tetraethylthiuram disulfide were the most efficient compounds in this respect.

Nickel has been detected in human fetuses at levels comparable to those in adults indicating that transplacental passage does occur (EPA, Sept. 1986a).

3.6. Summary of Pharmacokinetics

Inhalation and intratracheal studies allow for more qualitative than quantitative estimations of absorption. A variety of nickel compounds have been studied following these two routes of exposure.

The water soluble forms of nickel ($NiCl_2$ and $NiSO_4$), appear to be extensively absorbed and exhibit lung removal half-times in the order of days. Nickel chloride, $NiCl_2$, has been the most extensively studied soluble form. The data for $NiCl_2$ suggests potentially 100% absorption and a lung removal half-time of 2 days or less.

Nickel carbonyl, $Ni(CO)_4$, is somewhat unique in chemistry (i.e., lipid soluble). However, it also exhibits short lung removal half-timers with nearly 40% apparentlyeeliminated by exhalation.

The insoluble forms of nickel (NiO and Ni_3S_2) appear to have very limited absorption and exhibit lung removal half-times of months or longer. Nickel oxide (NiO) has been more extensively evaluated than nickel subsulfide (Ni_3S_2). Absorption following inhalation appears to be negligible for nickel oxide. Absorption following intratracheal administration appears to be much

Table 3.2 Summary of Experimental Studies of Trasplacental Transfer and Body Distribution of Nickel Chloride During Pregnancy.

Exposure	Animal Species	Effects/Observation	References
Intraperitoneal (i.p.) injection of radiolabel NiCl2 on various days of gestation (from 5 to 20). Distribution determined by whole-body autoradiography. Mothers were killed at 2 hrs postinjection.	NMRI/BOM Mice	Retention of radiolabel was particularly seen in the yolk sac and placenta with a much smaller portion actually entering the fetus. Rabiolabel crossed the placenta throughout gestation. Uptake of rabiolabel was seen already in day 5 embryos. Fetal accumulation occurred up to day 16. Radiolabel distribution in early embryos was relatively homogeneous, whereas the distribution was more differentiated with increasing gestation age, eventually resembling the distribution pattern seen in the mother.	Olsen and Jonsen Toxicology 12:165-172,1979
I.P. injection of NiCl2 (4.6 mg Ni/kg) on day 16 of gestation. Animals killed at various time points postexposure (2 to 48 hrs)	ICR Mice	Fetal uptake and elimination exhibited an upward-convex three phase curve. Maximum concentration was reached 8 hrs after injection. Between 8 and 24 hrs nickel concentrations remained stable, then decreased rapidly from 24 to 48 hrs postexposure. Maternal distribution: at 24 hrs - kidney > blood > placenta > fetuses/liver > spleen. Blood and placental maximum concentrations were seen at 2 hrs post-injection. Remaining tissue maximums were seen at 4 hrs postinjection At 48 hrs- kidneys > blood > placenta > liver/spleen/ fetuses.	Lu et al Tox Appl Pharmacol 59:409-413,1981

Exposure	Animal Species	Effects/Observation	References
Intramuscular (i.m.) injection of radiolabel NiCl2 (12 mg Ni/kg) to nonpregnant and day 8 or day 18 pregnant rats. Animals were killed 24 hrs after injection.	Fischer Rats	Distribution in nonpregnant animals: kidney >> serum > adrenal > lung/ovary/uterus > spleen/ heart > liver > skeletal muscle/pituitary. Distribution in day 8 pregnant animals: kidney >> serum > fetuses and membranes/adrenal/lung/ovary > pituitary > spleen/liver/heart > skeletal muscle. Distribution in day 18 pregnant animals: kidney >> placenta > lung/serum > adrenal > fetuses/ovary/ pituitary > spleen/heart/amniotic fluid > liver > skeletal muscle.	Sunderman et al Tox Appl Pharmacol 43:381-390,1978
Oral administration of radiolabel NiCl2 (0.59 mg Ni/kg) alone, or in combination with thiuram sulfides or sodium diethyl-dithiocarbamate (SDC) on day 18 of gestation. Animals were killed 24 hrs post-exposure.	C57BL Mice	Whole body autoradiography demonstrated that the coadministration of thiuram sulfides or SDC resulted in highly increased levels of radiolabel nickel in maternal body tissues and fetuses. In particular, the uptake of radiolabled nickel in the brains of both mothers and fetuses was markedly enhanced. Significant increases were also seen in fetal kidney, lung and liver.	Jasim and Tjalve Toxicology 32:297-313,1984

higher than following inhalation exposure. However, this apparent increase in lung clearance could be due, in part, to translocation of a fraction of the dose to the gastrointestinal tract by coughing. Based on urinary excretion data, a maximum of 30% and 60% intratracheally instilled NiO and Ni_3S_2, respectively, is absorbed.

Other sources of airborne nickel, such as nickel- containing flyash and welding fumes, have been examined. The chemical form(s) of nickel contained within these sources was not known. The experimental data produced in these studies indicate that absorption of nickel from these sources can occur.

Nickel is generally regarded as being poorly absorbed from the gastrointestinal tract. Nonfasting human volunteers exhibit a mean minimum absorption of 3 percent (range from <1 to 8 percent) (Christensen and Lagesson, 1981). The absorption rate is presented as a minimum value for two reasons. First, since the urine and serum nickel levels were still significantly higher than baseline values at the last time point examined, the study period appears to have been too short to accurately determine the extent of urinary excretion and therefore the absorption rate. Secondly, the study design did not account for possible fecal excretion.

Data from animal experiments provide evidence for a gastrointestinal absorption regulatory mechanism (Kirchgessner *et al.*, 1985). The relative absorption (percent of intake) of nickel was high, approximately 22 percent, if the dietary level was very low (0.06 ppm). The percent absorbed decreased rapidly, resulting in less than 1 percent absorption when dietary levels exceeded 1 ppm. Dietary levels of 0.11 and 0.22 ppm resulted in 6.6 and 2.7 percent absorbed, respectively.

The currently available information on dermal absorption is inadequate to determine the extent of absorption through the skin. However, recent studies by Fullerton (Fullerton *et al.*, 1986) indicate that non-occluded exposure results in very little permeation (<0.25%).

With the exception of the lungs, nickel does not accumulate in tissue. Once absorbed, nickel appears to distribute in a relatively uniform manner in the body. In vitro cell culture studies have demonstrated subcellular binding of insoluble forms of nickel.

4. Essentiality of Nickel in the Human Diet

An element is considered essential if a dietary deficiency of that element consistently results in a suboptimal biological function that is preventable or reversible by physiological amounts of the element. Nickel deficiency in humans has not been described, however, nickel has recently been established as an essential trace metal in a number of microorganisms, plants and experimental animals. The specific form(s) of required nickel has not been determined. Based on the animal experiments, levels of 100 ppb (120 Mg) should be adequate to prevent deficiency if nickel is an essential element; average daily ingestion of nickel has been estimated to be 156 Mg (see Section 4.4). Several reviews on the nutritional value of nickel have been published (Spears, 1984a; Nielsen, 1984a; Nielsen, 1974a; Nielsen, 1985; Nielsen, 1984b; Anke *et al.*, 1984).

4.1. Experimental Studies on Essentiality in Animals

Because of the chemical and physical relationship between iron, cobalt and nickel investigators have attempted to demonstrate biological essentiality of nickel since the 1950's. From 1950 to the beginning of the 1970's studies

were unsuccessful or produced inconsistent results. A major difficulty in the production of nickel deficiency in animals is the preparation of a diet low in nickel. Nickel is a ubiquitous element. The nickel content in purified proteins, amino acids and minerals is often 0.1 - 1.0 Mg/g (Nielsen, 1984b). Therefore, the conventional methods of diet preparation are not suitable.

Studies with improved diets and environmental controls have consistently shown impairment in physiological function when nickel is deficient in the diet (Nielsen, 1974a). Signs of nickel deprivation have been described for 5 species--chicks, rats, pigs, sheep, and goats. Brief summaries of the findings in each species are given in the following section. The results of animal studies are summarized in Table 4.1. The most prominent and consistent signs include depressed growth, depressed hematopoiesis, and changes in the level of iron, copper and zinc in the liver (Nielsen, 1984a).

4.1.1. Chicks

Chicks fed a nickel deficient diet (44 ppb or less) exhibited suboptimal liver function as evidenced by ultrastructural degeneration, reduced oxidative ability, increased lipid and a decreased phospholipid fraction. The basal diet of control chicks contained 3 ppm (Nielsen and Ollerich, 1974b).

4.1.2. Rats

A three-generation study of rats under nickel deficient (2-15 ppb) and normal (3 ppm) conditions has been conducted throughout fetal, neonatal and adult life. Rats maintained on nickel deficient diets exhibited: 1) increased perinatal mortality; 2) inability of young rats to thrive; 3) increased rate of alpha-glycerophosphate oxidation by liver homogenates; 4) decreased liver cholesterol and 5) gross and ultrastructural changes in the liver. Nickel deficiency also tended to decrease growth, hematocrit and total liver lipids and phospholipids. Nickel deficient rats were also more lethargic (Nielsen and Ollerich, 1974b; Nielsen, 1974a; Nielsen *et al.*, 1975).

4.1.3. Pigs

Pigs fed diets of 100 ppb exhibited significant decreases in rate of weight gain and retarded development resulting in delayed sexual maturation compared to control animals (10 ppm) (Anke *et al.*, 1974). Return of estrus after mating was also delayed. Sows fed low nickel diets farrowed later and had smaller litters than controls. Neonatal mortality in the nickel deficient litters was nearly twice that exhibited in controls.

Studies by Spears *et al.* indicate that dietary levels of 120 to 160 ppb are adequate for growth in neonatal pigs (Spears *et al.*, 1984b). However, additional nickel appeared to further improve the iron and zinc status of young pigs.

4.1.4. Sheep

Lambs fed a low-Ni diet containing 30 ppb exhibited the following effects: 1) decreased growth rate; 2) increased mortality (40 percent vs 0); 3) decreased serum protein concentrations; and 4) decreased total liver lipid and cholesterol (Spears *et al.*, 1978a). In lambs fed 65 ppb weight gain was not greatly different from controls (Spears *et al.*, 1978b). The efficiency of converting feed to weight gain was slightly higher in controls.

TABLE 4.1. Summary of Experimental Studies on Nickel Essentiality in Animals.

Exposure	Animal Species	Response/Observations	Reference
12 chicks: basal diet 3.4 ppm Ni; low-Ni diet 44 ppb, for 30 days.	Chicks	Ultrastructural changes in hepatocytes - possible subclinical Ni deficiency syndrome.	Sunderman, 1972
Basal diet 33 ppm; deficient diet: 0 - 44 ppb for 3.5 weeks.	Chicks	Suboptimal liver function: ultrastructural and physiological changes.	Nielsen, 1974a
3 successive generations on low Ni (2 - 15 ppb) or normal diet (3 ppm)	Rats	Increased perinatal mortality: rough coat; uneven hair development; altered gross appearance and biochemical and ultrastructure changes in liver; decreased growth rate; decreased hematocrits; decreased serum urea, ATP, glucose; and lethargy.	Nielsen, 1974b, 1974a, 1975
Diet containing 15 ppb Ni	Rats	Decreased growth; decreased hematocrit, hemoglobin concentration and erythrocyte count.	Schnegg, 1979
Diet containing 100 ppb Ni	Pigs	Decreased rate of weight gain; retarded development; delayed sexual maturity; (sows farrowed on average 44 days later and had 1 piglet less than controls); at 28 days post-partum 37% of piglets alive vs 65% in controls; piglets weighed 15% less than controls; skin changes in piglets; decreased calcium/phosphate ratio in deficient piglets' bones.	Anke, 1974

Exposure	Animal Species	Response/Observations	Reference
Dietary levels of 120 - 160 ppb	Neonatal pigs	Found to be adequate for growth.	Spears, 1984
Low-Ni diet, 65 ppb; basal diet, 5 ppm for 97 days.	Lambs	Showed slightly lower efficiency in converting feed to weight gain; decreased serum transaminase and total serum proteins at 56 days.	Spears, 1978
Twins or triplets separated soon after birth; some fed low-Ni diet (30 ppb), controls fed 5 ppm.	Lambs	Decreased growth rate; increased mortality (40% vs 0%); decreased serum protein; decreased total liver lipid and cholesterol.	Spears, 1978
Nickel deprivation of nannies over several generations and of kids through nannies' milk and synthetic feed.	Goats	Kids: lower birth weight; and higher mortality. Nannies: highest mortality rates during lactation; skin eruptions; brittle hair; fissures on mouth and legs during lactation period.	Anke, 1984

4.1.5. Goats

Goats appear to be one of the species more sensitive to nickel deficiency, requiring >100 ppb in their diet. Nickel requirements may be higher in ruminants due, in part, to the microbiological digestion processes in the rumen. The nickel dependence of urease is particularly important (Anke *et al.*, 1984) for ruminants, which obtain some of their nitrogen as urea. The urease-dependent release rate of ammonia in the rumen fluid of nickel deficient goats was ten times lower than controls. The restricted urease activity in the rumen of Ni-deprived ruminants can affect the protein metabolism of the animals and this may explain the more sensitive reaction of goats to nickel deprivation than monogastric animals.

4.2. Interaction of Nickel with Other Trace Metals During States of Deficiency

4.2.1. Nickel and Calcium

The bones of nickel deficient pigs contained significantly less calcium than those of control animals (Anke *et al.*, 1974). Early in the experimental time period Ni deficient animals excreted more calcium through the kidneys than control animals. Their phosphorus concentrations were virtually unchanged. Kirchgessner and Schnegg (as cited by Anke *et al.*, 1984) found that thirty-day old rats raised on a nickel deficient diet incorporated more magnesium into bones instead of calcium.

4.2.2. Nickel and Copper

Studies in rats (Nielsen *et al.*, 1980; Nielsen and Zimmerman, 1981) indicate that signs of copper deficiency become more severe in rats with moderate copper deprivation with supplemental nickel than without. The exacerbation of effects was greater when dietary nickel was higher (50 vs 5 ppm).

4.2.3. Nickel and Iron

Nielsen (Nielsen, 1984a; Nielsen, 1985) has found this interaction to be affected by the form and level of dietary iron. Nickel deficiency decreased hematocrit levels and whole body retention of an oral dose of radiolabeled iron only when iron was provided in the ferric form at a slightly deficient level. When iron was supplied either at an adequate level or as a 60:40 mixture of ferric and ferrous iron, nickel had no effect on hematocrit or iron retention. Nickel may function as a biological cofactor facilitating the intestinal absorption of Fe^{+3} (ferric)

4.2.4. Nickel and Zinc

A reduction in the rate of incorporation of zinc into blood serum, milk and body organs has been demonstrated utilizing radiolabeled zinc (Anke et al., 1984). Only the zinc content of the skeleton and pancreas was similar to control animals. The rumen and gastrointestinal tract of nickel deficient goats, on the other hand, contained about 30 percent more radiolabeled zinc than control animals, indicating a possible reduction in zinc resorption.

4.3. Nickel Function

Several hypotheses of nickel function have been put forth in the literature:

(1) the ultrastructural changes noted in hepatocytes during nickel deficiency suggest that nickel may play a role in membrane structure and function (Nielsen, 1974a). Nickel binds more strongly to membranes than calcium and in isolated tissues has been reported to behave like calcium in nerve excitability.

(2) significant concentrations of nickel are present in DNA and RNA. It has been suggested that nickel may contribute to the stabilization of the structure of nucleic acids (Nielsen and Ollerich, 1974b).

(3) several nickel-containing enzymes function in plants and microorganisms. Nickel can activate many enzymes in vitro but its role as a specific cofactor or component for any animal enzyme has not been demonstrated (Nielsen, 1984a). Nickel has been found to have a structural role in a metalloprotein (nickeloplasmin) in human serum, but the function of this protein is unclear.

(4) at high concentrations nickel has been reported to stimulate or inhibit the release of various hormones (e.g., thyroid hormone, adrenocorticotropic hormones, LH, FSH) (Spears, 1984a). However, the response may not reflect functions at typical biological levels.

4.4. Nickel in Humans

Based on the animal data nickel may be required by humans. Animal requirement estimates can be utilized to give a general idea of the amount of nickel that may be required by humans. For rats and chicks the nickel requirement is apparently approximately 50 ppb in the diet. For pigs it is >100 ppb and for sheep it is >65 ppb. For goats the requirement is higher (>100-500 ppb), probably because some rumen bacteria use nickel as part of their enzyme urease. Nielsen, based on data from monogastric animals, stated a dietary nickel requirement for humans of 35-50 Mg (30 - 42 ppb) daily (Nielsen, 1984a; Nielsen, 1984b), but did not document the assumptions nor the calculations utilized. The average daily intake of nickel is approximately 155 Mg (see ARB section on food sources and human intake.)

Based on the limited data available from monogastric animals the nickel requirements of swine may be higher than that reported for other species examined to date. The dietary requirement for swine appears to be between 100 and 120 to 160 ppb based on the studies by Anke *et al.* and Spears. The studies by Anke *et al.* on swine appear to be the most appropriate for extrapolating to humans.

Bennett has reported the mean concentration of nickel in food to be 130 ppb (Bennett, 1986). A daily consumption rate of 1.2 kg of food (Bennett, 1986) would result in a daily intake of 156 Mg in an adult. If one extrapolates the required dietary concentration in swine, i.e., 100 ppb, to humans, the required daily human intake would be 120 Mg. This value is surprisingly close to the estimated average intake, considering that nickel deficiency in humans has not been documented. It is possible that human requirements may be lower than estimates from swine. However, based on a review of the pertinent literature it does not appear that nickel deficiency has been investigated in humans.

5. Noncancer Health Effects in Animals and Humans

The following section is basically divided into two areas: (1) general non-cancer health effects; (2) reproductive effects. The first area discussed, general non-cancer health effects, is subdivided according to length of exposure. Information on human toxicity will be included where it is available. Data concerning human exposure to nickel and subsequent toxicity have come largely from the industrial setting. The two types of exposure seen occupationally are acute, due to accidental spills or leaks, and long term or chronic exposure. Therefore, the available human exposure data will be discussed in the acute and chronic exposure subsections.

For acute toxicity, the lowest concentration which produced a noncarcinogenic effect is 0.25 mg Ni/m^3, for a 2-hour exposure of mice to $NiCl_2$. The adverse effect reported was immune suppression. For subchronic or chronic exposures, the lowest concentration associated with a noncarcinogenic effect is 0.02 mg Ni/m^3. Effects noted near or above this concentration were alterations in macrophage populations and effects on the immune system in rats. Occupational asthma has been reported in humans exposed to 0.013 - 0.067 mg Ni/m^3. Thus, noncarcinogenic effects are not expected from exposure to ambient levels of nickel (0.000001 mg to 0.00002 mg Ni/m^3).

5.1. Acute Exposure Toxicity

Acute nickel toxicity has occurred at high doses within the industrial context; it has not been associated with environmental exposure to nickel (EPA, Sept. 1986a). Recent studies describe the effects on humans from occupational exposure to nickel aerosol and nickel carbonyl ($Ni(CO)_4$) (Akesson and Skerfving, 1985; Zhicheng, 1986). Nickel carbonyl is the only nickel compound that has been recognized as a cause of human acute inhalation systemic toxicity (National Research Council, 1975). Inorganic nickel salts have a relatively low degree of acute toxicity to humans because of their poor absorption. At high levels they may cause local discomfort and interfere with food intake owing to their astringent properties (Leonard et al., 1981). A single case study has been reported by Daldrop et al. (1983) involving the fatal ingestion of nickel sulfate by a 2 1/2 year old girl. Death was by cardiac arrest approximately 8 hours after ingestion. It was estimated that roughly 15 g of nickel sulfate crystals were ingested (approximately 3.3 g Ni) (ATSDR, 1988).

5.1.1. Human Studies

Individuals exposed to nickel carbonyl have exhibited an immediate reaction followed by an asymptomatic interval, then a delayed reaction. Nickel carbonyl intoxication in the most severe cases may cause death after 4-11 days. Exposure to 30 ppm for 30 minutes (~210 mg nickel carbonyl/m^3) may be lethal (Leonard et al., 1981).

Zhicheng studied 179 people exposed to air concentrations over 50 mg/m^3 from under 30 minutes to over 2 hours (Zhicheng, 1986). Symptoms appeared within 30 minutes to 4 hours in 79% of those exposed; in 21%, onset was delayed for a week. The immediate toxic effects were manifested predominantly in neurological symptoms (dizziness, headache, sleeplessness, blurred vision, numbness, dysphoria) and upper respiratory tract irritation (throat dryness, cough, sore throat, dyspnea, hemoptysis, expectoration). Individuals who developed only immediate symptoms were considered mild cases. The delayed

stage was characterized by chest pain, cough, dyspnea, vomiting, palpitations and cold intolerance. Six severe cases developed pulmonary edema or pneumonitis and three suffered toxic myocarditis (Zhicheng, 1986).

Pathological pulmonary changes due to nickel carbonyl poisoning include pulmonary hemorrhage and edema, derangement of alveolar cells, degeneration of bronchial epithelium and formation of fibrinous intraalveolar exudate (EPA, Sept. 1986a). Nickel carbonyl may also be nephrotoxic in humans, resulting in renal edema, hyperemia and parenchymatous degeneration.

Akesson and Skerfving (1985) found that men engaged in high nickel alloy welding experienced more symptoms than those welding ordinary stainless steel materials. Eleven welders who had not welded high nickel alloy for 4 weeks before the study began were then followed over 4 days (Akesson and Skerfving, 1985). Symptoms included irritation of the nose, nasopharynx and bronchi, with eye irritation, headache and tiredness. However, no effect on lung function was found. The authors speculated nickel aerosols were the cause of the reactions.

5.1.2. Animal Studies

A review of the acute toxic effects of nickel was prepared by the National Academy of Sciences in 1975 (National Research Council, 1975). Nickel carbonyl was the most acutely toxic form of nickel in animals, as in humans. Exposure to 50 mg/m^3 for 30 minutes was reported to produce 80% mortality in mice (National Research Council, 1975). A summary of the acute exposure (i.e., a single exposure) studies conducted is presented in Table 5.1. The table includes studies which employed intratracheal and injection routes of administration, but only studies utilizing inhalation and oral administration will be discussed in depth since these routes of exposure relate to environmental exposure pathways. However, it is interesting to note that the toxicity of nickel salts, administered by injection, can be influenced by age and pregnancy. Older mice appear more sensitive to the lethal action of nickel acetate than younger mice (Hogan, 1985). Pregnant female rats appear to be more sensitive to the toxic effects of nickel than nonpregnant females (Mas et al., 1985). This change in toxicity could not be explained by the dependence of the dose on total body weight.

5.1.2.1. Inhalation Exposure

Nickel carbonyl ($Ni(CO)_4$) and nickel chloride ($NiCl_2$) have been evaluated following single exposures. Studies which examined the acute effects of nickel carbonyl utilized very high exposure levels, 250 - 600 mg $Ni(CO)_4/m^3$, and reported poor survival (Sunderman et al., 1959; Sunderman and Donnelly, 1965). The study evaluating nickel chloride toxicity utilized much lower exposure levels and reported a NOEL of 0.25 mg $NiCl_2/m^3$ (i.e., 0.11 mg Ni/m^3) and a LOAEL of 0.55 mg $NiCl_2/m^3$ (i.e., 0.25 mg Ni/m^3) for a 2-hour exposure (Graham et al., 1978). The effect observed at 0.55 mg $NiCl_2/m^3$ was immune suppression.

Table 5.1. Summary of Experimental Acute Toxicity Test Conducted on Nickel and Nickel Compounds

Exposure	Animal Species	Effects/Observations	Reference
Inhalation Exposure:			
Single 2 hour exposure to nickel chloride ($NiCl_2$) at 0, 0.11, 0.25, 0.375 or 0.485 mg Ni/m^3 (values estimated from graph) (MMAD $\leq$ 3 um) (0, 0.24, 0.55, 0.83, or 1.07 mg $NiCl_2/m^3$)	Swiss Mice	0.11 mg Ni/m^3: NOEL 0.25 mg Ni/m^3: Lowest effective dose. Exposure to 0.25 mg Ni/m^3 or greater resulted in suppression of the primary humoral immune response.	Graham et al Env Res 16:77-87, 1978
Single 6 hour exposure to nickel-enriched fly ash (9% Ni, chemical form unknown). Respirable concentration 200 mg/m^3 (MMAD 2.8 um)	Hamsters	Slight accumulation of dark brown granular pigment. Pigment was primarily phagocytized in macrophages located in areas of bronchiolization of alveolar epithelium. No other treatment related changes were observed.	Wehner et al Env Res 19:355-370, 1979
Single 30 minute exposure to nickel carbonyl (Ni $(CO)_4$) at 0 or 250 mg/m^3. (0 or 86 mg Ni/M^3)	Rats	86 mg Ni/m^3: 65% of exposed rats died within 1 week of exposure. Other signs of toxicity were not reported.	Sunderman et al AMA Arch Ind Hlth 20:36-41, 1959
Single 30 minute exposure to 0 or 600 mg Ni $(CO)_4/m^3$ (0 or 200 mg Ni/m^3)	Wistar Rats	200 mg Ni/m^3: 75% of exposed rats died within 3 weeks of exposure. Signs of other toxicity were not reported.	Sunderman and Donnelly Am J Clin Pathol 46:1027-1038, 1965

Table 5.1. (cont)

Exposure	Animal Species	Effects/Observations	Reference
Intratracheal Exposure:			
Single intratracheal instillation of 0, 0.01, 0.1, or 1.0 umol Ni (i.e. 0, 0.6, 6, or 60 ug Ni) as Ni_3S_2, $NiCl_2$, $NiSO_4$ or NiO.	F344 Rats	Monitored: LDH (extracellular lactate dehydrogenase). BG (beta glucuronidase), TP (total protein), GR (glutathione reductase), SA (sialic acid) and total nucleated cells. Significant increases in all parameters following 1.0 umol Ni as Ni_3S_2, $NiSO_4$ of $NiCl_2$, but greatest changes occurred in Ni_3S_2 group. Increases in LDH, TP, and SA also occurred at 0.1 umol Ni as Ni_3S_2, $NiSO_4$ or $NiCl_2$. Changes in the total and differential cell count- 1.0 umol Ni as Ni_3S_2 and at 0.1 umol Ni as $NiSO_4$ or $NiCl_2$. Alveolitis at $\geq$ 0.1 umol Ni as $NiSO_4$ or $NiCl_2$ and 1.0 umol Ni as Ni_3S_2. NiO exposure did not result in any significant changes in any measured parameter.	Benson et al Fund Appl Toxicol 7:340-347, 1986
Single intratracheal instillation of saline or 11.8 ug Ni_3S_2/mouse (MMAD 1.65 to 1.83 μm) (0 or 8.6 ug Ni per mouse)	BALB/c Mice	Animals were lethargic of at least 3 days after exposure. Pulmonary hemorrhaging. Decreased body weight. Alterations in lavaged cell population.	Finch et al Env Res 42:83-93, 1987
Single intratracheal instillation of 0 or 0.06 umol Ni_3S_2 per gram lung (10.56 ug Ni/gm lung)	Cynomolgus Monkeys	Macrophage phagocytic activity was significantly decreased accompanied by a secondary increase in natural killer cell mediated killing of target cells.	Haley et al Toxicol Appl Pharm 88:1-12, 1987
Intramuscular Injection:			
Single i.m. injection of saline or 10 to 30 mg Ni/kg as $NiCl_2$ to nonpregnant or timed pregnant rats. (0, 22 to 66 mg $NiCl_2$/kg)	Fischer Rats	Nonpregnant LD50 was approx. 23 mg Ni/kg. 8 day pregnant LD50 was approx. 22 mg Ni/kg. 18 day pregnant LD50 was approx. 16 mg Ni/kg.	Sunderman et al Toxicol Appl Pharm 43:381-390, 1978

Exposure	Animal Species	Effects/Observations	Reference
Single i.m. injection of 0, 3.09, 6.17, 9.25, or 12.34 mg Ni/kg as $NiCl_2$, $NiSO_4$ or NiO (0, 6.8, 13.6, 20.4, or 27 mg $NiCl_2$/kg; 0, 8.1, 16.3, 24.4, or 32.5 mg $NiSO_4$/kg; 0, 3.9, 7.9, 11.8, or 15.7 mg NiO/kg)	Swiss Mice	Significant immunosuppression was observed at: $\geq$ 3.09 mg Ni/kg as $NiSO_4$ (note this is lowest dose tested) and $\geq$ 9.25 mg Ni/kg as $NiCl_2$. Did not see an effect on primary immune function following NiO exposure.	Graham et al Env Res 16:77-87, 1978
Single i.m. injection of 0, 9.1, CBA/J Mice 18.3 or 36.6 mg $NiCl_2$/kg (0, 4.1, 8.3 or 16.6 mg Ni/kg)	CBA/J Mice	At 18.3 mg $NiCl_2$/kg or greater: significant suppression of T-cell mediated immune response and natural killer cells activity. Decrease in LD50 of endotoxin. Immune suppressive effects of $NiCl_2$ were found to be transient with response returning to normal within a few days.	Smialowicz et al Env Res 33:413-427, 1984
Single i.m. injection of saline, 9.1, 13.7, 18.3, 27.5 or 36.6 mg $NiCl_2$/kg (0, 4.1, 6.2, 8.3, 12.4, or 16.6 mg Ni/kg)	CBA/J and C57BL/6J Mice	Exposures of $\geq$ 18.3 mg $NiCl_2$/kg resulted in significant depression of splenic natural killer cell activity, and a significant increase in the number of lung tumors in mice which had been injected (i.v.) with the B16-F10 melanoma.	Smialowicz et al Env Res 36:56-66, 1985
Single i.m. injection of saline, 10, 15 or 20 mg $NiCl_2$/kg (0, 4.5, 6.8, or 9.1 mg Ni/kg)	Fischer 344 rats	$\geq$ 10 mg $NiCl_2$/kg: Suppression of natural killer cell activity ($p < 0.05$). Level of activity did return to control values by 3 days post-treatment. $\geq$ 15 mg $NiCl_2$/kg: Decrease in body weight ($P < 0.05$). 20 mg $NiCl_2$/kg: Decrease in spleen weight ($p < 0.05$).	Smialowicz et al Toxicology 44:271-281, 1987
Single i.m. injection of saline or 20 mg $NiCl_2$/kg (0 or 9.1 mg Ni/kg) in combination with injection of MADB106 mammary adenocarcinoma cells.	Fischer 344 Rats	20 mg $NiCl_2$/kg: Significant increase in mortality due to lung tumors compared with saline injected rats ($p < 0.01$).	Smialowicz ibid

Table 5.1. (cont)

Exposure	Animal Species	Effects/Observations	Reference
Subcutaneous Injection:			
Single s.c. injection of saline, 10 or 20 mg $NiCl_2.6H_2O$/kg (0, 2.5 or 4.9 mg Ni/kg)	Sprague-Dawley Rats	Both exposure levels resulted in increased circulating prolactin (PRL) levels up to final observation time point (i.e. 7 days post-treatment), increased insulin levels at 1 and 2 days post-treatment, and increased glucose serum levels up to 7 days post-treatment. No significant effects on GH, FSH, LH, TSH or thyroxine.	Clemons and Garcia Toxicol Appl Pharmacol 61:343-348, 1981
Monitored exhalation rates of ethylene or thane following single s.c. injection of 0, 0.5 or 0.75 mmol $NiCl_2$/kg (0, 29.3 or 44 mg Ni/kg)	Fischer 344 Rats	Evidence of increased lipid peroxidation at both treatment levels.	Knight et al Ann Clin Lab Sci 16(5)386-394, 1986
Single s.c. injection of saline or 0.5 mmol $NiCl_2$/kg (0 or 29.3 mg Ni/kg)	Fischer 344 Rats	The mean thymic weight was significantly decreased by 24 hrs post-injection ($p < 0.01$). By 72 hrs post-injection thymic weight was 24% of control values. The thymic weight to body weight ratio was decreased ($p < 0.01$). Moderate to profound depletion of cortical lymphocytes. Cortical lymphocytes present showed extensive degenerative changes. Significant increase in lipoperoxides in thymus ($p < 0.01$).	Knight et al Res Commun Chem Pathol Pharmacol 55(3)291-302, 1987
Intraperitoneal Injection:			
Injection of nickel acetate: 0, 56, 75, 100 or 133 mg/kg to 3 weeks old mice and 0, 24, 36, 54 or 81 mg/kg to 9 and 14 week old mice.	ICR Mice	LD50 in 3 week old mice: 90-100 mg/kg LD50 in 9 week old mice: 50-54 mg/kg LD50 in 14 week old mice: 40-54 mg/kg	Hogan Bull Environ Contam Toxicol 34: 446-450, 1985
Single i.p. injection of various of $NiCl_2$ to virgin, 12 or 19 day pregnant rats.	Fischer Rats	Calculated LD50 for virgin rat: 9.33 mg/kg Calculated LD50 for 12 day pregnant rat: 6.3 mg/kg Calculated LD50 for 19 day pregnant rat: 6 mg/kg	Mas et al Toxicology 35: 47-57, 1985

5.1.2.2. Oral Exposure

There are no available studies on effects other than lethality, i.e., LD 50 studies. See the Reproductive Section for effects of acute exposure during organogenesis.

5.2. Repeated Exposure Toxicity

This section is divided into two parts: (1) short-term exposure studies (i.e., exposure duration of up to 1 month) and (2) subchronic exposure studies (i.e., exposure duration from 2-4 months). A summary of the studies is given in Tables 5.2 and 5.3, respectively.

5.2.1. Short-Term Exposure

5.2.1.1. Inhalation Exposure

A variety of nickel forms have been evaluated, i.e., nickel chloride ($NiCl_2$), nickel oxide (NiO), nickel subsulfide (Ni_3S_2), nickel sulfate ($NiSO_4.6H_2O$) and metallic nickel dust. The study by Spiegelberg and co-workers (1984) demonstrated the lowest NOEL and LOEL values. Wistar rats were exposed to 0, 0.05, 0.1, 0.2, 0.4 or 0.8 mg NiO/m^3 continuously for 4 weeks. NiO aerosols were generated by atomization of an aqueous Ni-acetate solution and subsequent pyrolysis of the particles at 550°C. The aerosol particles then contained 78.1% Ni, which was very close to the Ni content of NiO (i.e. 78.6%). No effects were observed at the lowest dose, 0.05 mg/m^3 (i.e., 0.04 mg Ni/m^3). Significant alterations in the alveolar macrophage population were seen at the next dose level, 0.1 mg/m^3 (i.e., 0.08 mg Ni/m^3). Definite adverse effects, changes in the humoral immune system, were reported at 0.2 mg/m^3 (0.16 mg Ni/m^3).

Benson et al. (1987) and Dunnick et al. (1988) both reported health effects in rats at all nickel subsulfide exposure levels evaluated (i.e. 0.6 to 10 mg/m^3). However, these same investigators reported a NOEL of 0.6 and a LOEL of 1.2 mg/m^3 nickel subsulfide (i.e. 0.4 and 0.9 mg Ni/m3, respectively) in mice.

The remaining nickel compounds assessed exhibited effects at the lowest dose tested. Therefore, NOELs cannot be estimated for these compounds. The lowest dose tested for each of the compounds was: 0.23 mg/m^3 nickel chloride (0.109 Ni/m^3); ; 3.5 mg/m^3 nickel sulfate (0.84 mg Ni/m^3); and 0.45 mg/m^3 metallic nickel dust (Bingham et al., 1972; Benson et al., 1988; and Dunnick et al., 1988 and Camner et al., 1978, respectively).

The lung and immune systems appear to be the most sensitive target organs. The nickel oxide dose levels evaluated were the lowest concentrations examined among the various compounds assessed. Nickel chloride and metallic nickel dust also exhibited effects on lung alveolar macrophage populations as well as alterations in lung lysozyme levels. However, the levels which produced these effects were higher than those required for nickel oxide.

5.2.1.2. Oral Exposure

Two short-term studies have been conducted in rats utilizing drinking water containing either nickel chloride or nickel sulfate (Weischer et al., 1980a; Kadiiska et al., 1985). Weischer et al. (1980a) exposed male Wistar Rats to drinking water containing 0, 2.5, 5 or 10 ug Ni/ml as nickel chloride

Table 5.2. Summary of Experimental Short Term (i.e. up to 1 month exposure) Toxicity Test Conducted on Nickel and Nickel Compounds

Exposure	Animal Species	Effects/Observations	Reference
Inhalation Exposure:			
Exposed to 0.109 mg Ni/m3 as NiCl2 (MMAD 0.32 um) or 0.120 mg Ni/m3 as NiO (MMAD 0.25 um) for 12 hr/day, 6 d/wk for at least 2 weeks. Method of NiO generation - unknown.	Wistar Rats	0.109 mg Ni/m3 as NiCl2 - bronchial epithelium was hyperplastic with evidence of marked mucus secretion. 0.120 mg Ni/m3 as NiO - marked accumulation of macrophages, hypersecretion in the bronchial epithelium and thickening of the alveolar walls.	Bingham et al Arch Env Health 25:406-414, 1972
Exposed to 0, 0.2, 0.4 or 0.8 mg NiO/m3 continuously for 28 days (MMAD 0.58 um) (0, 0.16, 0.3, or 0.6 mg NiO/m3). Method of NiO generation - pyrolysis of nickel acetate at 550oC.	Male Wistar Rats	> 0.16 mg Ni/m3 - decreased hematocrit, decreased serum urea > 0.3 mg Ni/m3 - increased lung weight, increased serum glucose, increase serum bilirubin, decreased serum alkaline phosphate 0.6 mg Ni/m3 - decrease in liver weight and decrease in leukocyte count	Weischer et al Zbl Bakt Hyg I Abt Orig B 171:336-351, 1980
Exposed to 0, 0.8, 1.6 or 3.2 mg NiO/m3 to pregnant and non-pregnant females continuously for 21 days (throughout gestation) (MMAD 0.58 um) (0, 0.6, 1.25, or 2.5 mg NiO/m3). Method of NiO generation - pyrolysis of nickel acetate at 550oC.	Female Wistar Rats	> 0.6 mg Ni/m3 - decreased serum glucose in non-pregnant rats, decreased body weight, increased lung weight in pregnant and non-pregnant rats > 1.25 mg Ni/m3 - decreased fetal body weight, marked macrocytosis, decreased kidney weight, increased hematocrit, decreased red blood cell count in pregnant and non-pregnant females 2.5 mg Ni/m3 - decrease serum urea in pregnant rats	Weischer et al Zbl Bakt Hyg I Abt Orig B 171:336-351, 1980
Exposed to 0, 0.24 or 0.5 mg Ni/m3 as nickel chloride (NiCl2), 6 hr/d, 5 day/wk for 4 weeks (MMAD 0.5 - 1.0 um) (0, 0.53, 1.1 mg NiCl2/m3)	Rabbits	> 0.24 mg Ni/m3: Significant decrease in lysozyme levels in lavage fluid, macrophages and in culture medium from incubated macrophages. Lysozyme levels remained unchanged in the mucous membrane.	Lundberg and Camner Environ Res 34:335-342, 1984

Exposure	Animal Species	Effects/Observations	Reference
Exposure to 0, 0.05, 0.1, 0.2, 0.4 or 0.8 mg NiO/m3 continously for 4 weeks (MMAD 0.4 - 0.5 um) (0, 0.04, 0.08, 0.16, 0.31, 0.31 or 0.63 mg Ni/m3) Method of generation of NiO - pyrolysis of nickel acetate at 550oC.	Wistar Rats	0.04 mg Ni/m3: NOEL > 0.08 mg Ni/m3: Significant alterations in alveolar macrophages. > 0.16 mg Ni/m3: Significant alterations in the humoral immune system.	Spiegelberg et al Ecotox Env Safety 8:516-525, 1984
Exposure to 0, 0.6, 1.4, 7 or 8 mg NiO/m3 6 hr/d, 5 day/wk for 1 month (MMAD 1.2 um) (0, 0.47, 1.1, 5.5 or 6.3 mg Ni/m3). Method of green NiO generation - not stated.	Wistar Rats	Animals were assessed at three time points: at end of exposure (1.1 or 5.5 mg Ni/m3), 12, or 24 months post-exposure (0.47 or 6.3 mg Ni/m3). Localized interstitial pneumonia, hyperplastic and pre-neoplastic lesions were seen in animals maintained 12 months or longer in both groups so maintained (i.e. 0.47 or 6.3 mg Ni/m3)	Horie Biol Trace Elem Res 7:223-239, 1985
0, 0.6, 1.2, 2.5, 5, or 10 mg/m3 nickel subsulfide (Ni_2S_3) 6 hr/day, 5 day/week for 12 days. (MMAD 2.8 um) (0, 0.4, 0.9, 1.8, 3.6 or 7.3 mg Ni/m3)	B6C3F1 Mice	0.4 mg Ni/m3: NOEL > 0.9 mg Ni/m3: lung and nasal epithelium lesions > 1.8 mg Ni/m3: emaciation occurred. > 3.6 mg Ni/m3: testicular lesions, atrophy of spleen and thymus. 7.3 mg Ni/m3: 100% mortality.	Benson et al Fund Appl Toxicol 9:251-265, 1987
Same exposure groups and study design as directly above.	F344/N Rats	> 0.4 mg Ni/m3: nasal and lung lesions (note effects at lowest dose tested). > 3.6 mg Ni/m3: decreased weight gain, atrophy of the spleen and thymus, testicular degeneration and labored breathing. 7.3 mg Ni/m3: high mortality	Benson et al ibid
0, 3.5, 7, 15, 30 or 60 mg ($NiSO_4.6H_2O$)/m3, 6 hr/day, 5 day/wk for 12 days (MMAD 1.9 um) (0, 0.84, 1.7, 3.3, 6.7 or 13.4 mg Ni/m3)	B6C3F1 Mice	0.84 mg Ni/m3: no effect on resistance to tumor cells as determined by spleen natural killer cell activity. > 0.84 mg Ni/m3: labored breathing, decreased weight gain and lethargy, inflammation of the lung, atrophy of olfactory epithelium (note: changes seen at lowest dose tested). > 1.7 mg Ni/m3: 100% mortality.	Benson et al

Table 5.2. (cont)

Exposure	Animal Species	Effects/Observations	Reference
		Fund Appl Toxicol 10:164-178, 1988	
Exposure groups and study design as study directly above.	F334/N Rats	> 0.84 mg Ni/m3: labored breathing, decreased weight gain and lethargy, red discharge around nose and inflammation (note: effects seen at lowest dose tested). > 3.3 mg Ni/m3: 10-20% mortality.	Benson et al ibid
Exposed to 0, 1.2, 2.5, 5, 10 or 30 mg NiO/m3 for 6 hr/day, 5 d/wk for 12 days (MMAD 3 um) (0, 0.9, 2.0, 3.9, 7.9, or 23.6 mg Ni/m3) . NiO generated at 1350oC, > 99% pure.	F344/N rats	> 0.9 mg Ni/m3 (i.e. all dose groups): lung hyperplasia > 7.9 mg Ni/m3: lung inflammation 23.6 mg Ni/m3: atrophy of the nasal olfactory epithelium	Dunnick et al. Toxicology 50:145-156, 1988
Same exposure as above	B6C3F1 mice	3.9 mg Ni/m3: NOEL > 7.9 mg Ni/m3: lung hyperplasia 23.6 mg Ni/m3: lung inflammation	ibid
Exposed to 0, 3.5, 7.0, 15.0, 30.0, or 60.0 mg NiSO4.6H2O/m3 (MMAD 1.9 um) 6 hr/d, 5 d/wk for 12 days. (0, 0.8, 1.6, 3.3, 6.7, or 13.3 mg Ni/m3)	F344/N rats	> 0.8 mg Ni/m3 (i.e. all exposure levels): decreased body weight, lung inflammation, atrophy of the nasal olfactory epithelium > 6.7 mg Ni/m3: labored breathing 13.3 mg Ni/m3: increased mortality	ibid
Same exposure as above	B6C3F1 mice	0.8 mg Ni/m3: only group with enough survivors to examine. Lung inflammation, atrophy of the nasal olfactory epithelium > 1.6 mg Ni/m3: increased mortality	ibid
Exposed to 0, 0.6, 1.2, 2.5, 5 or 10 mg Ni3S2/m3 (MMAD 2.8 um) using same exposure protocol as above. (0, 0.4, 0.9, 1.8, 3.6 or 7.3 mg Ni/m3)	F344/n rats	> 0.4 mg Ni/m3 (i.e. all exposure levels): lung inflammation > 0.9 mg Ni/m3: atrophy of the nasal olfactory epithelium > 1.8 mg Ni/m3: decreased body weight, emphysema 7.3 mg Ni/m3: labored breathing	ibid

Exposure	Animal Species	Effects/Observations	Reference
Same exposure as above.	B6C3F1 mice	> 0.4 mg Ni/m3: NOEL > 0.9 mg Ni/m3: lung inflammation and atrophy of the nasal olfactory epithelium > 1.8 mg Ni/m3: decreased body weight 3.6 mg Ni/m3: fibrosis 7.3 mg Ni/m3: increased mortality and labored breathing	ibid
Exposure to 0, 0.45 or 2.04 mg metallic nickel dust/m3, 6 hr/day, 5 day/wk for 4 weeks	New Zealand Rabbits	Significant effects on lung weight, lung density, phagocytic activity, size distribution and ultra-structure of alveolar macrophages observed in both exposure groups.	Camner et al Env Res 16:226-235, 1978
Exposure to 0 or 1.7 mg metallic nickel dust/m3, for 1 month (approximately 40% respirable)	Rabbits	Increased lung weight; increased lung lipid concentration; increased number, size, and size variation of lavaged macrophages.	Casarett-Bruce et al Env Res 26:353-362, 1981
Intratracheal Exposure:			
Instillation of 0, 0.024, 0.056, 0.156, 0.412 or 1.1 mg nickel subsulfide/kg once per week for 4 weeks (Ni3S2) (0, 0.01, 0.02, 0.06, 0.156, or 0.417 mg Ni/m3)	B6C3F1 Mice	Animals maintained for approximately 20 months post-exposure. Lung morphology appeared normal in all groups.	Fisher et al Env Res 40:313-320, 1986
Oral Exposure:			
Exposed to 0, 2.5, 5, or 10 mg Ni/l as NiCl2 for 28 days. Authors estimated intakes of 6.08, 11.7 and 23.7 mg Ni/kg rat/28 days or approximately 0.2, 0.4, and 0.8 mg/kg-day for the 2.5, 5, and 10 ppm Ni treatment groups, respectively.	Male Wistar Rats	> 2.5 mg Ni/l - decreased body weight, decreased serum urea, decreased water consumption, increased urine urea and increased serum glucose > 5.0 mg Ni/l - increased leukocyte count 10 mg Ni/l - decreased wet weight of liver and kidney	Weischer et al Zbl Bakt Hyg I Abt Orig B 171:336-351, 1980

Table 5.2. (cont)

Exposure	Animal Species	Effects/Observations	Reference
Exposed to 0 or 20 mg NiSO4/kg/day in drinking water for 30 days (0 or 7.6 mg Ni/kg/day)	Wistar Rats	Shortened duration of hexabarbital sleeping time ($p < 0.05$). Increased the activity of ethylmorphine-N-demethylase (EMD) ($p < 0.05$).	Kadiiska et al Arch Toxicol Suppl 8:313-315, 1985
Administration by Injection:			
i.m. injection of 0, 1.8, 3.7 or 7.3 mg nickel chloride/kg ($NiCl_2$), 5 days/wk for 2 weeks (0, 0.8, 1.7, or 3.3 mg Ni/kg)	CBA/J Mice	At all dose levels: significant decrease in mitogen resonse of spleen cells and significant decrease in thymus to body weight ratio. No effect on body weight or spleen to body weight ratio.	Smialowicz et al Env Res 33:413-427, 1984
i.m. injection of 0, 1.8, 3.6 or 7.2 mg $NiCl_2$/kg, 5 days/wk for 2 weeks (0, 0.8, 1.6, or 3.3 mg Ni/kg)	CBA/J Mice	Significant decrease in natural killer cell activity. Reduction of activity was comparable at all 3 treatment levels.	Smialowicz et al Env Res 36:56-66, 1985

Table 5.3. Summary of Experimental Subchronic (i.e. ≥ 1 - 4 month exposure) Toxicity Test Conducted on Nickel and Nickel Compounds

Exposure	Animal Species	Effects/Observations	Reference
Inhalation Exposure:			
Exposed to 0.001, 0.005, 0.05 or 0.5 mg NiO/m^3 continuously for 3 months (0.0008, 0.004, 0.04, or 0.4 mg Ni/m^3). Method of NiO generation - not stated.	Rats	0.0008 mg Ni/m^3: NOEL ≥ 0.004 mg Ni/m^3: changes in the total amount of nucleic acids (RNA, DNA) in the blood (all groups became normal after 2.5 month recovery period); and alterations in serum catalase and cholinesterase activity ≥ 0.04 mg Ni/m^3: decreased number of erythrocytes in peripheral blood; increase serum protein levels 0.4 mg Ni/m^3: transient decrease in body weight	Yelfimova et al Gig Sanit No 12:18-22, 1977
Exposed to nickel enriched fly ash aerosol, 9% nickel (actual chemical form unknown), 6 hr/d, 5 day/wk for 2 months. Respirable concentration 185 mg/m^3 (MMAD 2.8 um)	Syrian Hamsters	Accumulation of dark brown granular pigment. Increased number of macrophages with phagocytized dust. Minimal inflammatory reaction. No noticeable toxic effects.	Wehner et al Env Res 19:355-370, 1979
Exposed to 0, 0.025 or 0.15 mg NiO/m^3, continuously for 4 months (0, 0.02 or 0.118 mg Ni/m^3). Method of NiO generation - pyrolysis of nickel acetate at 550°C.	Wistar Rats	≥ 0.02 mg Ni/m^3: Significant alterations in alveolar macrophage populations at both treatment levels 0.118 mg Ni/m^3: Significant decrease in humoral immune response	Spiegelberg et al Ecotox Environ Safety 8:516-525, 1984
Exposed to 0, 0.6, 1.2, 2.5, 5 or 10 mg NiO/m^3 for 6 hr/day, 5 day/wk for 13 weeks (MMAD 2.8 um) (0, 0.4, 0.9, 2.0, 3.9, or 7.9 mg Ni/m^3). NiO generated at 1350°C.	B6CBF1 Mice	≥ 0.4 mg Ni/m^3: alveolar macrophage hyperplasia. (Note: effects seen at lowest level examined) 7.9 mg Ni/m3: Body weight depression and lung inflammation.	Dunnick et al. Fund Appl Toxicol 12:589-94, 1989

Table 5.3. (cont)

Exposure	Animal Species	Effects/Observations	Reference
Same exposure groups and study design as study directly above.	F344/N Rats	No overt clinical signs of toxicity. $\geq$ 0.4 mg Ni/m^3: alveolar macrophage hyperplasia and perivascular lymphocytic infiltrates. $\geq$ 2.0 mg Ni/m^3: lung inflammation and cortical hyperplasia of bronchial lymph nodes.	Dunnick et al ibid
Exposed to 0, 0.12, 0.25, 0.5, 1 or 2 mg $NiSO_4.6H_2O/m^3$, 6 hr/day, 5 day/wk for 13 weeks (MMAD 1.9 um) (0, 0.02, 0.05, 0.1, 0.2, or 0.4 mg Ni/m^3).	F344/N Rats	$\geq$ 0.02 mg Ni/m^3: alveolar macrophage hyperplasia. $\geq$ 0.1 mg Ni/m^3: chronic lung inflammation, atrophy of the olfactory epithelium of the nose and cortical hyperplasia of the bronchial lymph nodes.	Dunnick et al ibid
Same exposure groups and study design as study directly above.	B6CBF1 Mice	0.05 mg Ni/m^3: NOEL $\geq$ 0.1 mg Ni/m^3: alveolar macrophage hyperplasia. $\geq$ 0.2 mg Ni/m^3: chronic lung inflammation and fibrosis. 0.4 mg Ni/m^3: olfactory epithelial atrophy.	Dunnick et al ibid
Exposed to 0, 0.15, 0.3, 0.6 1.2 or 2.5 mg Ni_3S_2/m^3, 6 hr/d, 5 day/wk for 13 weeks (MMAD 1.9 um) (0, 0.11, 0.2, 0.4, 0.9, or 1.8 mg Ni/m^3)	F344/N Rats	$\geq$ 0.11 mg Ni/m^3: decreased body weight in males, chronic lung inflammation, alveolar macrophage hyperplasia and cortical hyperplasia of the bronchial lymph nodes (Note: effects seen at lowest level tested). $\geq$ 0.2 mg Ni/m^3: interstitial infiltrate and atrophy of the olfactory epithelium of the nose.	Dunnick et al ibid
Same exposure groups and study design as study directly above.	B6CB1 Mice	0.11 mg Ni/m^3: NOEL $\geq$ 0.2 mg Ni/m^3: alveolar macrophage hyperplasia. $\geq$ 0.4 mg Ni/m^3: olfactory epithelial atrophy. $\geq$ 0.9 mg Ni/m^3: chronic lung inflammation and fibrosis.	Dunnick et al ibid
Oral Exposure:			
Exposed to 100, 500 or 1,000 ppm Ni as nickel acetate in diet for 6 weeks. Basal diet contained 0.21 ppm Ni.	OSU Brown Rat	100 ppm Ni: NOEL $\geq$ 500 ppm Ni: decreased weight gain, decreased hemoglobin, decreased plasma alkaline phosphatase activity	Whanger Toxicol Appl Pharm 25:323-331, 1973

for 28 days. The authors monitored body weights and water consumption and estimated intakes of 0.2, 0.4 and 0.8 mg Ni/kg-day for the 2.5, 5 and 10 ug Ni/ml treatment groups, respectively. Health effects were reported in all treatment groups. The lowest exposure evaluated, 0.2 mg Ni/kg-day, resulted in decreased body weight, decreased serum urea, decreased water consumption, increased urine urea and increased serum glucose.

Kadiiska et al. (1985) exposed Wistar rats to 20 mg/kg/day nickel sulfate (7.6 mg Ni/kg/day) for 30 days resulted in alterations in liver enzyme activity. Exposed rats exhibited decreased hexabarbital sleeping time and an increase in ethylmorphine-N-demethylase activity. Unfortunately, only one dose level was evaluated, 20 mg/kg/day Nickel sulfate (i.e., 7.6 mg Ni/kg/day). However, the effects observed were indicative of only mild toxicity and may represent a LOEL for nickel sulfate in rats.

5.2.2. Subchronic Exposure

5.2.2.1. Inhalation Exposure

Nickel oxide (NiO), nickel sulfate ($NiSO_4.6H_2O$), and nickel subsulfide (Ni_3S_2) have been investigated following inhalation exposure. When air levels are expressed in terms of elemental nickel, changes in alveolar macrophage populations were produced at 0.02 mg Ni/m^3 in rats exposed to nickel oxide and nickel sulfate (Spiegelberg et al., 1984; Dunnick et al., 1989). Nickel levels of approximately 0.11 mg Ni/m^3, regardless of whether or not it was in the form of nickel oxide, sulfate or subsulfide, produced relatively severe lung and immune effects. These effects included suppression of humoral immune response, lung inflammation, and hyperplasia of the bronchial lymph nodes. The order of toxicity to the lung and nasal cavity was nickel sulfate > nickel subsulfide > nickel oxide.

Elfimova et al. (1977) have investigated the effects of very low, continuous exposure to nickel oxide. White rats were exposed continuously for 3 months to 0, 0.001, 0.005, 0.05 or 0.5 mg NiO/m^3 (i.e. 0, 0.0008, 0.004, 0.04 or 0.4 mg Ni/m^3). Animals were evaluated for weight gain changes, changes in the morphological composition of the blood, and enzyme activity changes. Many changes followed a dose-response pattern. The highest dose, 0.4 mg Ni/m^3, produced significant changes in almost all parameters evaluated. Exposure levels of 0.004 and 0.04 mg Ni/m^3 produced changes in several parameters, e.g. serum catalase activity, however, it was not clear whether these changes represented an adverse effect. The lowest exposure group, 0.0008 mg Ni/m^3, did not produce any significant effects. The 0.004 mg Ni/m^3 dose level may represent an effect level, however, it does not appear to be an adverse effect level.

All of the other studies conducted found adverse effects in rats at the lowest exposure level evaluated. Therefore, a subchronic NOEL could not be determined for this species. Mice, however, appear to be less sensitive to the effects of nickel sulfate and nickel subsulfide (Dunnick et al., 1989). NOELS of 0.05 mg Ni/m^3 and 0.11 mg Ni/m^3 were found for nickel sulfate and nickel subsulfide, respectively.

5.2.2.2. Oral Exposure

Only one investigator has evaluated the effects of subchronic ingestion of nickel (Whanger, 1973). Weanling DSU brown rats were fed either a basal ration (0.21 ppm Ni), 100 ppm Ni, 500 ppm, or 1000 ppm Ni as nickel

acetate for 6 weeks. Dietary levels of 100 ppm Ni (i.e. 100 mg Ni/kg diet) apparently did not result in any significant adverse effects. Higher dose levels, i.e. $\geq$ 500 ppm Ni, resulted in decreased weight gain, decreased hemoglobin, and decreased plasma alkeline phosphate activity. Utilizing the body weight information provided by the author and EPA's recommended methodology for estimating food consumption (EPA, 1988). A NOEL and LOEL can be estimated. The NOEL dietary level, 100 ppm, would represent a daily intake of approximately 11 mg Ni/kg/day.

5.3. Chronic Exposure Toxicity

5.3.1. Animal Studies

See Table 5.4

5.3.1.1. Inhalation Exposure

Nickel subsulfide, nickel oxide and nickel carbonyl have been evaluated following chronic exposure. These studies were designed to assess tumorigenicity and therefore only limited non-cancer effects were reported.

As in shorter term exposure studies, nickel oxide produced effects at the lowest exposure conditions for insoluble forms of nickel. Exposure to 0.076 mg NiO/m^3 (i.e., 0.06 mg Ni/m^3) continuously for 18 months resulted in weight depression, decreased survival time, lung congestion and alveolar proteinosis (Takenaka et al., 1985; Glaser et al., 1986). In an earlier, unpublished study by researchers at the same institution (Fraunhofer-Institut fur Toxicology and Aerosol Research), rats were chronically exposed to lower levels of NiO (Rittmann et al., June 1981). The report, written in German, contained an English abstract. Based on information contained in the abstract the immune system appeared to be the most sensitive to toxic insult. The authors reported that ''A diminution of the immunosystem is manifest above a concentration of 0.025 mg/m^3.''

Itskova et al. (1978) have evaluated the effects of very low, continuous exposure to nickel chloride hydroaerosol. Female white rats were exposed continuously for 6 months to 0, 0.0002, 0.001, 0.005, and 0.05 mg Ni/m^3. No effects were noted at the lowest dose level (0.0002 mg Ni/m^3). At exposure concentrations of $\geq$ 0.001 mg Ni/m^3 a decrease in the iodine fixing function of the thyroid gland and in blood catalase activity was observed.

5.3.1.2. Oral Exposure

Two investigations have evaluated the chronic effects of nickel following oral ingestion (Schroeder et al., 1974; Ambrose et al., 1976). The lowest exposure level was examined by Schroeder et al. Rats were exposed to 5 ppm nickel in drinking water for their lifetime. No toxic effects were reported at this low exposure level. In fact, a slight growth enhancement was reported.

Higher exposure levels have been examined for nickel sulfate in water and food (Dieter et al., 1987; Ambrose et al., 1976). Dieter et al. (1987) exposed mice to 0, 0.4, 1.9 or 4 g Ni/l as nickel sulfate in drinking water for 180 days. The authors estimated daily intakes of 0, 44, 108 or 150 mg Ni/kg, respectively. Health effects were observed at the lowest dose, 44 mg Ni/kg. Therefore, an oral NOEL can not be determined in this species. The

Table 5.4. Summary of Experimental Chronic Noncancer Toxicity Test Conducted on Nickel and Nickel Compounds

Exposure	Animal Species	Effects/Observations	Reference
Inhalation Exposure:			
Exposed to 0 or 0.97 mg Ni_3S_2/m3, 6 hr/d, 5 day/wk for 78-80 weeks.(0 or 0.71 mg Ni/m3)	F344 Rats	0.71 mg Ni/m3: Depressed body weight, hyperplasia, metaplasia, and neoplasia of the lungs in exposed group.	Ottolenghi et al JNCI 54:1165-1172, 1974
Exposed to 0 or 53.2 mg NiO/m3, 7 hr/d, 5 day/wk for up to 2 years (0 or 42 mg Ni/m3). Method of generating NiO - not specified.	Syrian Hamsters	42 mg Ni/m3: Heavy pulmonary nickel oxide burden resulted in pneumoconiosis but did not result in specific toxicity.	Wehner et al Am Indus Hyg Assoc J 36:801-809, 1975
Exposed to 0.0002, 0.001, 0.005 or 0.05 mg Ni/m3 as $NiCl_2$ continuously for 6 months	female White Rats	0.0002 mg Ni/m3: NOEL > 0.001 mg Ni/m3: decreased iodine fixing function of the thyroid gland; decreased blood catalase activity > 0.005 mg Ni/m3: decreased body weight gain	Itskova et al Gig Sanit No 3:8-11,1978
Exposed to 0, 0.06 or 0.2 mg Ni/m3 as NiO, continuously (23 hr/d, 7 day/wk for 18 months (0, 0.076 or 0.25 mg NiO/m3). Method of NiO generation - pyrolysis of nickel acetate.	Wistar Rats	> 0.06 mg Ni/m3: After 13 months of exposure exposed groups exhibited weight loss, significant decrease in mean survival time, and marked lung enlargement accompanied by congestion and alveolar proteinosis.	Takenaka et al In: Progress in Nickel Toxicology-Proceeding of the 3rd International Conference on Nickel Metabolism and Toxicology. Eds: Brown and Sunderman. 89-92, 1985 and Glaser et al Int Congr Ser-Excerpta Med 676:325-28, 1986

Table 5.4. (cont)

Exposure	Animal Species	Effects/Observations	Reference
Exposed to 0, 30 or 60 mg Ni (CO)4/m3, 30 min., 3 times per week for 1 year (approx. 10 and 20 mg Ni/m3, respectively).	Rats	Control: 27/41 (65.8%) mortality rate within 12 months 10 mg Ni/m3: increased mortality within 12 months (48/64 or 75%) 20 mg Ni/m3: increased mortality within 12 months (28/32 or 87.5%) Other signs of toxicity were not reported.	Sunderman et al AMA Arch Ind Hlth 20:36-41, 1959
Exposed to 0 or 30 mg Ni(CO)4/m3, 30 min., 3 times per week till death (approx. 10 mg Ni/m3)	Wistar Rats	10 mg Ni/m3: Increased mortality (25% at end of 1 year vs 7% in controls). Weight suppression evident in exposed animals throughout study.	Sunderman and Sunderman Am J Clin Pathol 46:1027-1038, 1965
Oral Exposure:			
Exposed to 0, 0.0005, 0.005, 0.05, 0.5, or 5 mg/kg NiCl2 by gavage for 7 months	Rats	5 mg/kg - depressed weight gain	Itskova et al Pharmacol Toxicol 32:102-105, 1969
Exposed to background or 5 ppm Ni as nickel acetate in drinking water for lifetime (estimated daily intake 2.6 ug Ni/rat in control and 37.6 ug Ni/rat in exposed)	Rats	5 ppm Ni: NOEL (Overall slight growth enhancement. No effect on longevity or tumor incidence)	Schroeder et al J Nutr 104:239-243, 1974
Exposed to 0, 100, 1000 or 2500 ppm Ni in diet as nickel sulfate (NiSO4.6H2O) for 2 years.	Wistar Rats	100 ppm Ni: NOEL > 1000 ppm Ni: body weight depression, increased heart to body weight ratio and decreased liver to body weight ratio. Histopathology showed no treatment related lesions.	Ambrose et al J Food Sci Tech 13:181-187, 1976
Same exposure groups and study design as study directly above.	Beagle Dogs	1000 ppm Ni: NOEL 2500 ppm Ni: Depressed body weight, lowered hematocrit and hemoglobin values, induced polyuria, and increased kidney- to- and liver-to-body weight ratios. Histopathology revealed lung lesions and 33% (2/6) of exposed dogs with granulocytic hyperplasia of the bone marrow. Note: study duration may not have been sufficiently long to determine chronic effects in this species.	Ambrose et al ibid

Exposure	Animal Species	Effects/Observations	Reference
Exposed to 0, 1, 5, or 10 g/L nickel sulfate in drinking water (0, 0.4, 1.9, or 4 g Ni/L) for 180 days. Authors estimated daily intakes of 0, 44, 108, or 150 mg Ni/kg, respectively.	B6C3F1 Mice	> 0.4 g Ni/L (44 mg Ni/kg-d): treatment resulted in a dose-related decrease in thymus weight and a reduction in spleen lymphoproliferative response.	Dieter et al National Toxicology Program (in press, 1987 as cited by ATSDR)

observed effects included dose-related decreases in thymus weight and a reduction in spleen lymphoproliferative response.

Ambrose et al. (1976) have reported a NOEL and LOEL in rats. Rats were fed diets containing 0, 100, 1000 or 2500 ppm nickel for 2 years. No effects were reported in the lowest nickel group, i.e., 100 ppm. Levels of 1000 ppm or greater produced body weight suppression and alterations in heart- and liver-to-body weight ratios. Unfortunately, the authors did not monitor food intake. However, using the body weight information by the authors and EPA's recommended methodology for estimating food consumption (EPA, 1988) a daily intake NOEL and LOEL can be estimated. The dietary level which produced no effects (NOEL) was 100 ppm and represents a daily intake of approximately 7.5 mg Ni/kg/day in rats. The lowest dietary level evaluated which resulted in toxic effects (LOEL) was 1000 ppm or 77.6 mg Ni/kg/day.

Itskova et al. (1969) reported a LOEL of 5 mg/kg and a NOEL of 0.5 mg/kg for decreased weight gain in rats exposed to nickel chloride by oral gavage. The animals were dosed for 7 months at concentrations ranging from 0.0005 to 5.0 mg/kg.

5.3.2. Human Studies

Occupational studies are the major source of information concerning the chronic effects of nickel in humans. Some additional information comes from patients treated for non-occupational dermatitis. The effects produced following long-term exposure include dermatitis, respiratory tract irritation and immune alterations. Nickel dermatitis and asthma involve sensitization of tissue and therefore may result, in part, from the effects on the immune system.

The principal hazard of nickel to humans, besides its potential carcinogenicity, is its ability to provoke sensitization reactions (Leonard et al., 1981). In addition to dermal effects, nickel sensitization may also cause conjunctivitis, eosinophilic pneumonitis, asthma and local or systemic reaction to nickel-containing prostheses (Sunderman, 1986a).

Among various subgroups of the US population who may be at special risk for adverse effects of nickel are those who have nickel hypersensitivity and suffer chronic flare-ups of skin disorders. Nickel dermatitis usually begins as itching or burning papular erythema in the webs of fingers and spreads to the fingers, wrists and forearms (EPA, Sept. 1986a). Clinically, the condition is usually manifested as a papular or papulovesicular dermatitis with a tendency toward lichenification, having the characteristics of atopic, rather than eczematous dermatitis.

"Nickel itch" dermatitis can be caused by jewelry worn in contact with the skin or by occupational exposure in nickel industries, and it represents about 5% of all eczema in humans (Leonard et al., 1981). Approximately 10% of tested populations were found to be potentially allergic to nickel (Leonard et al., 1981).

The exposure level required to produce dermal sensitization is not known. Several investigations have reported positive allergic skin reactions following the application of nickel solutions directly to the skin of hypersensitive individuals. Wahlberg (EPA, Sept. 1986a) reported that 5 out of 14 positive reactors were sensitive to concentrations of less than 0.039% nickel sulfate. Kaaber (EPA, Sept. 1986a) demonstrated that ingestion of 2.5 mg nickel as nickel sulfate aggravated chronic dermatitis in 17 of 28 patients. When placed on a low nickel diet, 9 of the 17 patients improved.

The reason for the sensitizing actions of nickel remains to be elucidated. It has been hypothesized that after penetrating the epidermis, that nickel binds to a protein, resulting in the production of an antigen complex (Leonard et al., 1981).

Asthma and increased risk for chronic respiratory tract infections may also result from nickel exposure. Asthmatic lung disease has been seen in nickel-plating workers and in welders. Five cases of occupational asthma caused by nickel salts were found among 53 workers employed in the production of nickel catalyst (Davies, 1986). The plant atmospheric concentrations normally varied between 0.013 and 0.067 mg/m^3.

Bencko et al. have recently reported alterations in the immunochemical parameters of a group of production workers (Bencko et al., 1986). Unfortunately, neither exposure levels nor nickel form(s) were described in this study. Significant increases in serum proteins and immunoglobulins IgG, IgA, IgM and IgI were observed in exposed workers. Significant decreases in IgE were also reported. The direct health effect of these alterations is not known. However, they reflect changes in immunoreactivity and potentially may have an impact on susceptibility to infection.

Akesson and Skerfving (1985) examined the lung function of eleven welders who utilized high -Ni alloys. All eleven welders reported one or more symptoms (irritation of upper airways, headaches, tiredness) as occurring more often ($p < 0.006$) during high -Ni welding than when welding ordinary stainless steel. Nickel levels in air during welding of high -Ni alloy were high (mean 0.44 mg Ni/m^3, range 0.07-1.1 mg Ni/m^3). Lung-function studies conducted on these welders produced normal results.

Killburn et al. (1990) also examined welders. The authors reported that 11 years of stainless-steel welding resulted in reduced vital capacities and expiratory flows. Unfortunately, exposure levels were not available. It is also important to note that these welders were also exposed to other toxic agents (e.g. chromium, oxidant gases). Chronic oral exposure studies in humans were not available.

5.4. Summary of Noncancer Health Effects

Limited data are available on the non-carcinogenic systemic effects of nickel. The key oral and inhalation studies of exposure to nickel and its compounds in humans and animals are discussed in the above sections. Additional experimental studies are presented in Tables 5.1, 5.2, 5.3, and 5.4.

The studies of the effects of inhalation of nickel compounds have evaluated several forms of nickel. Nickel metal and metal salts are relatively non-toxic when ingested, requiring large doses to elicit toxic responses. This in part is due to the poor gastrointestinal absorption of these forms of nickel. When administered by injection (e.g., subcutaneously, intravenously, etc.) nickel salts are much more toxic.

Unfortunately the post-exposure observation period for most of the short-term studies were very short or nonexistent. Therefore, the reversibility or exacerbation of the effects exhibited is not known.

Based on the available data discussed and/or presented in tabular form, the following general conclusions can be made concerning systemic nickel toxicity:

(1) Primary target of toxicity

(i) Inhalation--lung and immune system (including sensitization reactions).

(ii) Oral--liver function and intestinal irritation.

(iii) Other organ systems affected at higher doses and/or under parenteral administration--endocrine system, hematological and hemopoietic systems, and possibly the cardiovascular system.

(2) Species sensitivity

(i) Experimental animals--rat > mouse > hamster.

(ii) Humans appear to be particularly prone to the development of hypersensitivity.

(3) Health effect levels (NOEL and LOEL values)

(i) Acute exposure (i.e., 1 dose):

Humans:

Inhalation: --Human data clearly establishing a NOEL or LOEL are insufficient. Overt toxicity has been observed following single exposure to air concentration over 50 mg/m^3 (Zhicheng, 1986).

Oral:--Human data clearly establishing a NOEL or LOEL are insufficient. Death has been reported in one case involving the ingestion of approximately 3.3 g Ni, as nickel sulfate, and a 2 1/2 year old child (Daldrup et al., 1983).

Animals:

Inhalation --NOEL--0.11 mg Ni/m^3; and LOEL--0.25 mg Ni/m^3 (based on single 2 hour exposure) (Graham et al., 1978).

Oral --Data are insufficient to clearly establish an oral NOEL or LOEL in .

(ii) Short-term repeated exposure (up to 1 month):

Humans:

A NOEL or LOEL has not been reported. Animals:

Inhalation--NOEL--0.04 mg Ni/m^3 and LOEL--0.08 mg Ni/m^3 (based on 4 weeks of continuous exposure) (Spiegelberg et al., 1984).

Oral--insufficient data. Lowest dose examined, 0.2 mg Ni/kg/day, resulted in decreased body weight, decreased serum urea, decreased water consumption, increased urine urea and increased serum glucose (based on 30 day drinking water study) (Weischer et al., 1980).

(iii) Subchronic exposure (2-4 months):

Humans:

NOEL and LOELS have not been reported.

Animals:

Inhalation--Data are insufficient to clearly establish NOEL or LOEL values. The lowest dose resulting in adverse alterations was 0.02 mg Ni/m^3. The effect was alterations in alveolar macrophage populations. Since this is a rather mild adverse effect the LOEL may be near this exposure level (based on 4 month continuous exposure study) (Spiegelberg et al., 1984).

Oral-- NOEL 11 mg Ni/kg/day; LOEL 62 mg Ni/kg/day (based on exposure to 100 and 500 ppm nickel in diet for 6 weeks) (Whanger, 1973).

(iv) Chronic exposure:

Humans:

insufficient data to calculate NOEL or LOEL. However, occupational asthma reported in a plant with levels between 0.013 and 0.067 mg Ni/m^3 (Davies, 1986).

Animals:

Inhalation--NOEL 0.0002 mg Ni/m^3 and LOEL of $\geq$ 0.001 mg Ni/m^3 (6 months continuous exposure) (Itskova et al. 1978).
Oral--NOEL 7.5 mg Ni/kg/day (100 ppm nickel in diet for 2 years) (Ambrose et al., 1976); LOEL 5 mg Ni/kg/day and a NOEL of 0.5 mg Ni/kg/day (7 months) (Itskova et al. 1969).
LOEL 44 mg Ni/kg/day (based on exposure to 0.4 g Ni/l in drinking water for 180 days)(Dieter et al., 1987).

5.5. Reproductive Effects

5.5.1. Male Reproductive Effects

Nickel compounds assessed to date include nickel sulfate, nickel subsulfide, nickel chloride, nickel nitrate and nickel carbonyl (Sunderman et al., 1983; Deknudt and Leonard, 1982; Jacquet and Mayence, 1982; Benson et al., 1987; Hoey, 1966; Waltschewa et al., 1972; Mathur et al., 1977). Several of these compounds have been demonstrated to result in testicular toxicity. A detailed summary of the studies is presented in Table 5.5. The following discussion is an overview of the currently available information.

Several forms of nickel appear to be male reproductive toxins. The second stage of spermatogenesis, spermiogenesis, appears to be particularly sensitive to insult. Spermiogenesis is the phase in which morphological transformation from spermatids to spermatozoa occurs. Only one study utilized a sufficient length of exposure to assess effects on all stages of spermatogenesis (Waltschewa et al., 1972). Unfortunately, fertility (i.e., number of pregnant females) was the only reproductive parameter evaluated.

Dominant lethal tests examine implantation loss as well as the incidence of pregnancy over various post-exposure time periods. However, none of the tests reported were of sufficient length to assess all stages of spermatogenesis, particularly the earlier stages (i.e., epithelial cycle through the meiotic phases). In order to sample all stages of germ cell development 8 weeks of mating are required in the mouse and 10 weeks in the rat. No effects on testicular function of workers in the nickel industry have been reported in the literature.

All forms of nickel examined to date have exhibited adverse effects on the male reproductive function. The available data to date are somewhat limited, however, when the dose is expressed in terms of elemental nickel the most potent form of nickel following oral administration, appears to be nickel sulfate, $NiSO_4$. Rats fed 25 mg $NiSO_4$/kg (i.e., 9.5 mg Ni/kg) for 120 days exhibited inhibition of spermatogenesis and subsequent infertility (Wattschewa et al, 1972). Nickel sulfate has also been shown to have adverse effects following injection and dermal applications.

Nadeenko et al. (1979) has apparently examined the reproductive toxicity of nickel chloride. Male rats were exposed to nickel in drinking water for 4 months prior to mating. Unfortunately study design (e.g. exposure protocol)

Table 5.5. Summary of Experimental Studies of Male Reproductive Toxicity of Nickel and Nickel Compounds

Exposure	Animal Species	Effects/Observations	Reference
Nickel Carbonyl ($Ni(CO)_4$):			
Dominant lethal assay in which males were exposed to air or 160 mg $Ni(CO)_4/m^3$ for 15 min. (50 mg Ni/m^3). Males were then bred weekly from 2nd to 6th week. (N=10/group)	Rats	Note: no positive control group included in study design. 50 mg Ni/m^3: Treatment produced respiratory symptoms (dyspnea, tachypnea) lasting 7 to 10 days. No apparent effect on fertilization rates or reproductive outcome.	Sunderman et al In: Reproductive and Developmental Toxicity of Metals. Eds: Clarkson, Nordberg and Sager. pp 399-416, 1983
Dominant lethal assay in which males were exposed to vehicle or 22 mg Ni/kg as $Ni(CO)_4$ by single i.v. injection. Males were bred weekly from 2 to 6 weeks. (N= 8 exposed; 12 control)	Rats	22 mg Ni/kg: Treatment produced respiratory symptoms (dyspnea, tachypnea) lasting 7 to 10 days. Diminished fertilization in 2nd week, although this may reflect failure to copulate due to acute toxic effects. The number of pregnant females per time point was very small, 3 to 9. Decrease in number of live pups was significantly different at week 5. The ratio of dead fetuses per implantation site (i.e., postimplantation loss) was significantly higher at week 3, 4, 5 and 6. The largest fetal loss appeared during the 3rd to 5th week suggesting that toxic insult occurred after the meiotic stage of spermatogenesis (i.e., morphological maturation phase). No positive control included.	Sunderman, et al ibid

Table 5.5. (cont)

Exposure	Animal Species	Effects/Observations	Reference
Nickel Chloride ($NiCl_2$):			
Dominant lethal assay in which males were exposed to 0 to 25 mg $NiCl_2$/kg by single i.p. injection (11.3 mg Ni/kg). Males were bred weekly for 5 weeks.	Bal/c Mice	Study included a negative and positive (cyclophosphamide) control groups. Number of males per treatment group not reported. 11.3 mg Ni/kg: Incidence of pregnant females was significantly decreased from week 1 through 4. The mean number of implanted embryos per female was significantly depressed from week 2 through 4. No difference from negative control in incidence of postimplantation loss. Results indicate toxic effect on morphological maturation phase of spermatogenesis.	Deknudt and Leonard Toxicology 25:289-292, 1982
Nickel Nitrate ($Ni(NO_3)_2.6H_2O$):			
Dominant lethal assay in which males were exposed to 0 or 56 mg/kg by single i.p. injection (11.3 mg Ni/kg). Males were bred weekly for 5 weeks.	Bal/c Mice	Study included negative and positive (cyclophosphamide) control groups. Number of males per treatment group assessed was no stated. 11.3 mg Ni/kg: Incidence of pregnant females significantly decreased at all mating intervals examined. The mean number of implanted embryos per female was significantly depressed from week 2 through 4. No effect on post-implantation loss was seen. Results indicate that toxic effect on second stage of spermatogenesis (i.e., morphological maturation phase).	Deknudt and Leonard ibid
Dominant lethal assay in which males were exposed to 0, 40 or 56 mg/kg by single i.p. injection (0, 8.1 or 11.3 mg Ni/kg). Males were bred weekly for 5 weeks.	Bal/c Mice	No positive control group. 8.1 mgNi/kg: No effect on reproductive parameters assessed. 11.3 mg Ni/kg: Significant decrease in fertilization rate in week 3 and 4 and somewhat at week 5. Results indicate toxic effect on later stages of germ cell development, spermatids and spermatocytes, whose morphological transformation to spermatozoa requires 3 to 4 weeks.	Jacquet and Nayence Toxicology Letters 11:193-197, 1982

Table 5.5. (cont)

Exposure	Animal Species	Effects/Observations	Reference
Nickel Subsulfide (Ni_3S_2):			
Short-term toxicity test in which males were exposed to 0, 0.6, 1.2, 2.5, 5.0 or 10.0 mg Ni_3S_2/m^3, 6 hr/day, 5 day/wk for 12 days. (0, 0.4, 0.9, 1.8, 3.6 or 7.3 mg Ni/m^3) (N=5/group)	F344/N Rats	7.3 mg Ni/m^3: 2/5 animals died (40%). 3.6 mg Ni/m^3: 2/5 animals exhibited testicular degeneration (40%). 1.8 mg Ni/m^3: 1/5 animals exhibited testicular generation (20%). < 0.9 mg Ni/m^3: No testicular effects.	Benson et al Tox Appl Pharm 9:251-265, 1987
Same exposure groups as above study.	B6C3F1 Mice	7.3 mg Ni/m^3: 100% mortality. > 3.6 mg Ni/m^3: 4/5 animals exhibited testicular generation (80%). > 1.8 mg Ni/m^3: emaciation	Benson et al ibid
Nickel Sulfate ($NiSO_4$):			
Males were exposed to 6.2 mg/kg/day by single s.c. injection or multiple injections (1 per day for 1 to 30 days) (2.35 mg Ni/kg)	Rats	No control group. Only 1 animal assessed at each time point. Incomplete description of study design and results. Limited recovery after discontinuation of exposure. 2.35 mg Ni/kg: degeneration of spermatogenesis.	Hoey J Reprod Fertil 12:461-471, 1966
Males exposed to daily oral doses of 0 or 25 mg/kg for 120 days (0 or 9.5 mg Ni/kg). (N=10 controls; 30 exposed)	Rats	9.5 mg Ni/kg: infertility and severe lesions in the germ cells, particularly the second stage of spermatogenesis, spermiogenesis, in which morphological transformation from spermatids to spermatozoa occurs, were observed. Changes in the liver and kidney were scarcely observed indicating a selective toxic effect on the testes.	Waltschewa et al Exp Pathol 6:116-120, 1972

Exposure	Animal Species	Effects/Observations	Reference
Males exposed to 0, 40, 60, or 100 mg Ni/kg/day as $NiSO_4.6H_2O$ dermally for 30 days. (N=8 per group)	Albino Rats	No statistical analyses of data. Animals may have ingested nickel by licking fur. Four animals per group were killed and assessed on day 15 and 30 of exposure. No clinical signs of toxicity. 40 mg Ni/kg/day: NOEL > 60 mg Ni/kg/day: By day 15 observed liver effects (swollen hepatocytes and feathery degeneration) were seen. By day 30 testis, skin and liver all exhibited toxic effects. The kidneys did not exhibit effects.	Mathur et al Bull Environ Contam Toxicol 17:241-247, 1977

and data were not presented, therefore it is impossible to comment on this study.

Nickel subsulfide, Ni_3S_2, has only been assessed under inhalation exposure conditions. Adverse effects have been clearly demonstrated at 5 mg Ni_3S_2/m^3 (i.e., 3.6 mg Ni/m^3)(Benson et al., 1987). Due, in part, to the small number of animals in each exposure group it was unclear whether significant effects were produced at the lower exposure of 2.5 mg Ni_3S_2/m^3 (i.e., 1.8 mg Ni/m^3). No effects were observed at exposure levels of 1.2 mg Ni_3S_2/m^3 (0.9 mg Ni/m^3) or less.

5.5.2. Female Reproductive Toxicity

Very little information regarding female reproductive toxicity of nickel exists. The current data are inadequate to determine whether nickel is a female reproductive toxin. Nickel chloride has been shown to be a potent short-term inhibitor of prolactin secretion from the rat and bovine pituitaries (LaBella et al., 1973; Carlson, 1984). Basal prolactin release as well as the stimulation of prolactin secretion were suppressed. Hormone release of TSH, FSH, LH, ACTH and GH were not affected. After the initial depression of prolactin the levels increased after 1 day and remained significantly elevated at least up to 7 days after exposure (Clemons and Garcia, 1981). All studies were conducted in male rats and therefore the possible effects of prolactin suppression on female reproduction is not certain. However, it is noteworthy that uptake of radiolabeled nickel by the pituitary gland is significantly higher in pregnant than in nonpregnant rats (Sunderman et al., 1978a).

As part of a study to examine the effects of nickel on the developing organism Nadeenko et al. (1979) exposed female rats prior to and during mating as well as during gestation. Animals receiving 10 mg Ni/l as $NiCl_2$ (i.e. 0.5 mg Ni/kg as calculated by the authors) exhibited a decreased pregnancy rate (only 4 of 8 females became pregnant). An increase in the resorption rate was reported in the pregnant females. Females receiving 0.1 mg Ni/l (0.005 mg Ni/kg as calculated by the authors) exhibited increased embryo mortality. The lowest dose evaluated 0.01 mg Ni/l (0.0005 mg Ni/kg) was reported to be a NOEL. However, only group means and probability values were reported.

As part of a series of experiments, Sunderman exposed female rats to nickel subsulfide prior to breeding to study the effects of polycythemia (Sunderman et al., 1983). Seven females received a single intrarenal injection of Ni_3S_2 equivalent to 30 mg Ni/kg. A control group was also studied. Each female was caged with a male 7 days after injection. Although erythrocytosis (an increase in the number of circulating red blood cells) developed in the nickel treated dams no effects were seen on fertility or numbers of live pups.

5.5.3. Developmental Toxicity

Five forms of nickel have been tested for teratogenic effect: nickel acetate, nickel carbonyl, nickel chloride, nickel oxide and nickel subsulfide (Ferm, 1972; Sunderman et al., 1978b; Sunderman et al., 1979; Weischer et al., 1980; Sunderman et al., 1980; Sunderman et al., 1983; Sunderman et al., 1978a; Lu et al., 1979; Storeng and Jonsen, 1981; Chernoff and Kavlock, 1982; Mas et al., 1985; Smialowicz et al., 1986).

Unfortunately, the large majority of these studies utilized injection as the route of administration. This route is not particularly relevant or useful in estimating human health risks resulting from environmental exposure.

A detailed summary of the studies is presented in Table 5.6. When conducting and comparing the results of teratogenicity studies there are several points one must keep in mind (Palmer, 1978; Gaylor, 1978). Reproductive and developmental parameters exhibit high variability and the data are usually skewed, not normally distributed. Because of these problems, certain components of experimental design are recommended: 1) adequate numbers of litters should be examined--generally 15 to 20 per group are suggested; 2) non-parametric statistical tests should be used; and 3) since pups within a litter are part of the same "experimental unit" it is also suggested that the litter, not the individual pup, be used as the statistical unit. It is believed that adherence to these recommendations will lead to the identification of true developmental toxic agents and minimize false positives. Teratogenic effects should be separately noted for dead and live fetuses. The dosage regimen should not be excessively toxic to the pregnant mothers. The available studies should be evaluated on these parameters as well as the effects of nickel.

The data provided by the studies conducted to date are insufficient to accurately estimate a development LOEL or NOEL for nickel. In general, it appears that exposure to nickel has resulted in fetotoxicity (e.g., lethality, growth inhibition, etc.) rather than gross malformation. The exception to this statement is nickel carbonyl, $Ni(CO)_4$.

Exposure of Fisher 344 rats to 60 mg $Ni(CO)_4/m^3$ (20.4 mg Ni/m^3) for 15 min resulted in a significant increase in malformations, specifically opthalmic malformations (Sunderman et al., 1978b). Hamsters exposed to the same level of nickel carbonyl appeared to be more sensitive to the lethal effects of this form of nickel (Sunderman et al., 1980). Exposed hamsters exhibited a 25% maternal mortality incidence. Litter size and fetal weight were not affected in surviving hamster mothers, however, significant increases in malformations were observed. The lowest dose of nickel carbonyl examined to date is 60 mg/m^3 for 15 min. Lower doses need to be examined to determine LOEL and NOEL dose levels.

Nickel subsulfide (Ni_3S_2) has been examined utilizing injection administration and relatively high dose levels (Sunderman et al., 1978a; Sunderman et al., 1983). A single i.m. injection of 80 mg Ni/kg or an intrarenal injection of 30 mg Ni/kg did result in decreases in fetal viability and pup body weight. No teratogenic effects were reported. Lower doses need to be examined to determine LOEL and NOEL values. Nickel chloride ($NiCl_2$) has been the most extensively studied nickel compound. The majority of the studies conducted have utilized injection or subcutaneous implantation. These studies have been summarized in Table 5.6. It is interesting to note that Storeng and Jonsen (1981) have demonstrated that $NiCl_2$ influences mouse embryos prior to implantation (i.e., gestation day 1-5) and subsequently results in decreased litter size and fetal body weight.

Berman and Rehnberg (1983 as cited by EPA 1986 and ATSDR, 1988) administered 0, 500 or 1000 ppm $NiCl_2$ in drinking water to pregnant mice from day 2 through 17 of gestation. The authors reported a NOEL level of 500 ppm (ATSDR estimated daily dose of 100 mg Ni/kg). The higher dose level, 1000 ppm, resulted in an increased incidence of spontaneous abortion, material weight suppression, and reduced birth weight in pups.

Nadeenko et al. (1979) exposed rats to 0, 0.01, 0.1 or 10 mg Ni/l as $NiCl_2$ for 7 months. The authors estimated that these treatment levels

Table 5.6. Summary of Experimental Studies of Developmental Toxicity of Nickel and Nickel Compounds

Exposure	Animal Species	Effects/Observations	Reference
Nickel Acetate ($NiCH_3COOH$):			
Exposed by i.v. injection on day 8 of gestation to doses of 2, 5, 10, 20, 25, or 30 mg/kg (1, 2.5, 4.9, 10, 12.4 or 14.8 mg Ni/kg)	Hamsters	No mention of controls or statistical analyses. ≥ 4.9 mg Ni/kg: increased perinatal death (increasing to 100% at 14.8 mg Ni/kg). ≥ 12.4 mg Ni/kg: increased number of abnormal live embryos, however, embryo mortality at this dose was 87%. Types of abnormalities were not specified.	Ferm Adv Teratol 5:51-75, 1972
Nickel Carbonyl ($Ni(CO)_4$):			
Exposed to 0, 60 or 120 mg/m^3 for 15 min on day 8 of gestation. (N=12 control; 6-7/treatment group) (0, 20.4, or 41.3 mg Ni/m^3)	Fischer 344 Rats	Fetuses examined on day 20 of gestation. 41.3 mg Ni/m^3: significant effects on the number of live fetuses per mother, postimplantation loss and fetal body weight. ≥ 20.4 mg Ni/m^3: significant increase in ophthalmic malformations	Sunderman et al Tox Appl Pharm 45:345, 1978 (abstract only)
Experiment 1: 160 or 300 mg/m^3 for 15 min on day 7 of gestation (N=14/group) (54.4 or 102 mg Ni/m^3)	Fischer 344 Rats	> 54.4 mg Ni/m3: significant decrease in number live fetuses, significant decrease in fetal weights and significant increase in ophthalmic malformations. 102 mg Ni/m3: 47% maternal mortality.	Sunderman et al Science 203:550-552, 1979
Experiment 2: 0, 80, or 160 mg/m^3 for 15 min on day 8 of gestation (N= 12-16/grp) (0, 27.2 or 54.4 mg Ni/m^3)		27.2 mg Ni/m^3: nonsignificant increase in opthalmic malformations and significant decrease in number of live fetuses. 54.4 mg Ni/m^3: significant decrease in number live fetuses, significant decrease in fetal weight, significant increase in ophthalmic malformations, and 13% maternal mortality.	

Table 5.6. (cont)

Exposure	Animal Species	Effects/Observations	Reference
Experiment 3: 160 mg/m^3 for 15 min on gestation day 9 (N=13) (54.4 mg Ni/m^3)		54.4 mg Ni/m^3: Significant decrease in fetal weight and number of live fetuses. No malformations observed.	
Exposed to 60 mg/m^3 for 15 min on day 4, 5, 6, 7, or 8 of gestation (20.4 mg Ni/m^3) Sham control exposed day 6	Syrian Hamsters	20.4 mg Ni/m^3: Day 4: maternal mortality of 25%. Day 5, 6, 7, or 8: maternal mortality 50 to 60%. In surviving mothers the number of fetuses per litter and fetal weight were not significantly different from controls. The number of litters with malformed fetuses was significantly increased in day 4 and day 5 exposure groups, but not day 6, 7 or 8 exposure groups.	Sunderman et al Teratol Carcinogen Mutagen 1:223-233, 1980
Exposed to 0 or 11 mg Ni/kg by i.v. injection on day 7 of gestation (N=5-8/group) (32.3 mg $Ni(CO)_4$/kg)	Fischer 344 Rats	11 mg Ni/kg: Significant depression of fetal weight and significant increase in incidence of fetal malformations (anophthalmia, microphthalmia, cystic lungs and hydronephrosis).	Sunderman et al In: Reproductive and Developmental Toxicity of Metals. Eds: Clarkson, Nordberg and Sager. 1983 pp 316-416.
<u>Nickel Chloride ($NiCl_2$):</u>			
Experiment 1: 0, 8, 12 or 16 mg Ni/kg by single i.m. injection on day 8 of gestation. (N=11-13/group)	Fischer 344 Rats	No maternal mortality reported. No anomalies observed. A dose response relationship in number of live fetuses per litter and fetal body weight was observed. 8 mg Ni/kg: NOEL $\geq$ 12 mg Ni/kg: significant decrease in number of live fetuses per litter. 16 mg Ni/kg: significant depression of fetal body weight.	Sunderman et al Tox Appl Pharm 43:381-390, 1978
Experiment 2: 0, 1.5, or 2 mg Ni/kg by i.m. injection twice a day on gestation days 6, 7, 8, 9, and 10 (i.e. 3 or 4 mg Ni/kg/treatment day) (N=12-13/group)		No maternal mortality reported. No anomalies observed. 3 mg Ni/kg/day: NOEL 4 mg Ni/kg/day: significant decrease in number of fetuses per litter. No significant effect on body weight.	

Table 5.6. (cont)

Exposure	Animal Species	Effects/Observations	Reference
0, 1.2, 2.3 , 3.5, 4.6, 5.7 or 6.9 mg Ni/kg by single i.p. injection on gestation day 7, 8, 9, 10, or 11 (N=8-10 group)	ICR Mice	6.9 mg Ni/kg: Toxic to mothers at all exposure time points. Both dose level and time of administration influence extent of effects. ≥ 3.5 mg Ni/kg on day 10 or 11 and ≥ 4.6 mg Ni/kg on day 7, 8 or 9 of gestation: significantly lower fetal weight and placental weight ≥ 2.3 mg Ni/kg: No statistical analysis of fetal mortality however, a time related increase was observed, e.g., 2.3 mg Ni/kg on day 7-10% fetal death whereas 2.3 mg Ni/kg on day 11-28% fetal death rate. Mortality was also related to dose level, e.g., on average 10% of fetuses from 2.3 mg Ni/kg groups died whereas almost 100% of those from 6.9 mg Ni/kg groups died. No statistical analysis of incidence of abnormalities were conducted, however, increases occurred only at dose levels where fetal death also occurred. Largest incidences occurred on days 8 and 9 and were elevated at doses of ≥ 2.3 mg Ni/kg. 1.2 mg Ni/kg: ???	Lu et al Teratology 19:137-142, 1979
Daily intraperitoneal injections of 1 ug Ni/day as $NiCl_2$ for 3 consecutive days and then everyother day for a total of 11 doses during pregnancy. (1 ug/mouse = ?)	CFW Mice	Offspring were tested for reflex development and behavior from day of birth to 16 days of age. At weaning offspring were divided and placed in either enriched or non-enriched environments. At 5 weeks of age offspring were selected for maze learning test. Early reflex response - slower in Ni-treated offspring, however, by age 12 days no difference was seen. Maze learning - significant increase in errors and running time in treated female offspring reared in enriched environment	Tsujii and Hoshishima, Shinshu Daigaku Nogakubu Kiyo 16:13-28, 1979

Exposure	Animal Species	Effects/Observations	Reference
Exposed to 0, 0.01, 0.1 or 10 mg Ni/l as $NiCl_2$ for 7 months to male and female for 8 weeks prior to mating and throughout gestation. (authors estimated doses at 0.5, 5, or 500 ug/kg)	Rats	0.5 ug Ni/kg: NOEL 5 ug Ni/kg: increased embryo lethality (increased pre- and post-implantation loss) 500 ug Ni/kg: decreased pregnancy rate	Nadeenko et al Gig Sanit 6:86-88, 1979
Examined effect of exposure during the preimplantation period. Exposed by single i.p. injection of 0 or 20 mg $NiCl_2.6H_2O$/kg (4.9 mg Ni/kg) on gestation day 1, 2, 3, 4, 5, or 6 (N=23-30/group)	NMRI/Bom Mice	4.9 mg Ni/kg: significant decrease in implantation frequency in treatment groups on day 1 only. Number of normal live fetuses per litter was significantly different from controls on day 1, 3 and 5. Decreased fetal weight and fetal resorptions in treated mice on all exposure days. Also observed increased frequency of perinatal death and abnormalities in treated groups but no statistical analyses. Incidence of abnormalities was only 2% in treated groups which is not an unusual incidence in mice.	Storeng and Jonsen Toxicology 20:45-51, 1981
Exposed by single i.p. injection of 0 or 30 mg/kg on gestation day 8 (N=30/treated group; 25 control) (13.6 mg Ni/kg)	CD-1 Mice	13.6 mg Ni/kg: significantly decreased number of live pups born, no effect on fetal weight, and significant decrease in maternal weight gain.	Chernoff and Kavlock Env Health 10:541-550, 1982
Exposed by single i.p. injection of 0, 1, 2 or 4 mg Ni/kg on gestation day 8, 12, or 16. (N=1-6/group)	Wistar Rats	Inadequate number of mothers per group. 1 mg Ni/kg: NOEL $\geq$ 2 mg Ni/kg: decrease in fetal weight on day 12.	Mas et al Toxicology 35:47-47-57, 1985
Exposed by implanted mini-pumps from day 5 through day 19 of gestation. Experiment 1: 0, 0.65, 1.3 or 2.6 mg $NiCl_2$/kg-day (0, 0.03, 0.6 or 1.2 mg Ni/kg)	C57BL/6J Mice	0.6 mg Ni/kg-day: NOEL 1.2 mg Ni/kg-day: significant decrease in litter size.	Smialowicz et al Toxicology 8:293-303, 1986

Table 5.6. (cont)

Exposure	Animal Species	Effects/Observations	Reference
Experiment 2: 0, 2.6 or 5.2 mg $NiCl_2$/kg-day (0, 1.2 or 2.4 mg Ni/kg-day) (N=4-10 litters/group)		2.4 mg Ni/kg: NOEL, no significant difference in litter size. Reason for discrepancy between Experiment 1 and 2 not clear, however, the number of litters examined was small and the size of litters was also small (3-9) pups/litter).	
Administered 0, 500, or 1000 ppm $NiCl_2$ in drinking water to pregnant mice from gestation day 2 through day 17 (doses estimated by ATSDR (1988) 100 and 200 mg Ni/kg-day)	CD-1 Mice	100 mg Ni/kg-day: NOEL 200 mg Ni/kg-day: maternal weight suppression, reduced mean birth weight in pups and increased incidence of spontaneous abortion.	Berman and Rehnberg, EPA report 1983 as cited by EPA 1986 and ATSDR 1988
Nickel Oxide (NiO):			
Exposed to NiO at 0, 0.8, 1.6, or 3.2 mg/m^3 (0, 0.6, 1.3, and 2.5 mg Ni/m^3) throughout pregnancy, i.e. continuously for 21 days. (MMAD 0.58 um) Method of NiO generation - pyrolysis of nickel acetate at 550°C.	Wistar Rats	$\geq$ 0.6 mg Ni/m^3: decreased maternal body weight $\geq$ 1.3 mg Ni/m^3: decreased fetal body weight	Weischer et al Zbl Bakt Hyg I Abt Orig B 171:336-351, 1980
Nickel Subsulfide (Ni_3S_2):			
Exposed to 0 or 80 mg Ni/kg by single i.m. injection on day 6 of gestation (N= 12-14/group)	Fischer 344 Rats	80 mg Ni/kg: No maternal mortality, significant decrease in mean number of live fetuses per mother, and no effect on fetal body weight or incidence of anomalies.	Sunderman et al Tox Appl Pharm 43:381-390, 1978
Exposed by intrarenal injection of 0 or 30 mg Ni/kg. Animals were mated 7 days post-injection.	Fischer 344 Rats	Mothers were allowed to litter and raise offspring until 4 weeks after birth. 30 mg Ni/kg: mothers exhibited polycythemia and erythrocytosis and pups from treated mothers exhibited a decrease in weight throughout the 4 weeks.	Sunderman et al In: Reproductive and Developmental Toxicity of Metals. Eds: Clarkson, Nordberg and Sager, 1983 pp 399-416

resulted in daily intakes of 0.5, 5 or 500 ug Ni/kg, respectively. The exposure period encompassed pre-mating as well as throughout gestation. Female rats receiving 10 mg Ni/l exhibited decreased pregnancy rates, whereas, the 0.1 mg Ni/l exposed females exhibited increased embryo mortality. The lowest dose evaluated, 0.01 mg Ni/l was reported to be a NOEL. Data was reported in the form of group means and probability values only. The description of study design and methodology utilized to assess reproductive parameters was also incomplete making it difficult to evaluate the scientific validity of this study.

Only one study has evaluated the developmental effects of nickel oxide (NiO). Utilizing inhalation exposure. Weischer et al. (1980) exposed rats to 0, 0.8, 1.6 or 3.2 mg NiO/m^3 throughout pregnancy, i.e. continuously for 21 days. Suppression of fetal weight was reported at $\geq$ 1.6 mg NiO/m^3 (i.e.1.3 mg Ni/m^3). Maternal weight suppression was reported at the lowest exposure concentration (0.8 mg NiO/m^3 or 0.6 mg Ni/m^3), however, significant effects on fetal parameters were not reported.

5.5.4. Multigenerational Reproductive Studies

Several multigenerational studies have been conducted (see Table 5.7). Schroeder and Mitchener (1971) exposed Long-Evans rats to low doses of nickel in drinking water through three generations. Approximately 5 ppm nickel as soluble nickel salt (specific salt unspecified) was added to the drinking water. The diet contained a basal level of 0.31 ppm wet weight. Five pairs of rats were randomly selected for study. Rats were allowed to breed as often as they wanted up to 9 months of age or longer. The study was terminated when three generations had been weaned. The numbers of neonatal deaths were 9.1, 10.2 and 21.0 percent in the first, second and third generations, respectively. The numbers of runts (defined as animals with large heads and small bodies) were 30.6, 5.1 and 6.2 percent in the first, second and third generations, respectively. The size of the litters decreased with each generation. The number of litters produced in each generation ranged from 10 to 15. The male to female offspring ratio was approximately 1 in the first and second generation but in the third generation it was 0.44.

Several years later Ambrose et al. (1976) conducted a three-generation study in which nickel sulfate hexahydrate was added to the diet of rats in amounts yielding dietary concentrations of 0, 250, 500 and 1000 ppm as nickel. The number of litters examined ranged from 14 to 19 per group per generation. The following indices were calculated: fertility (pregnancies/matings); gestation (number of litters/pregnancies); viability (live pups at day 5/live pups born) and lactation (weaned/live pups on day 5). Histopathological studies were performed on 10 male and 10 female third generation offspring from each diet level. Statistical analysis was not discussed or reported. In addition, the pup rather than the litter was used as the unit of comparison.

No effects on fertility, gestation, viability and lactation indices were apparent at any of the dietary levels of nickel. Data on the number of pups born dead showed higher incidence of stillborn in the first generation at all levels of nickel, however, this was not observed in subsequent generations. An effect on average weaning body weight was apparent in weanlings on 1000 ppm diet in all generations. The average number of pups per litter decreased with increasing dose, resulting in a 9% and 12.5% reduction from controls in the 500 and 1000 ppm dose groups, respectively. The average number of pups weaned per litter also decreased with increasing dose, resulting in a 11%, 16% and 21% reduction compared to controls in the 250, 500 and 1000 ppm dose groups,

Table 5.7. Summary of Multigenerational Reproductive Experimental Studies of Nickel and Nickel Compounds

Exposure	Animal Species	Effects/Observations	Reference
Exposed to 5 ppm Ni in drinking water (unspecified nickel salt) for 3 generations. (N=10-15 litters/generation)	Long-Evans Rats	Exposed animals exhibited: increased number of runts in 1st and 3rd generation. Number of fetal deaths increased with each generation (9.1, 10.2* and 21.0*, respectively) ($p < 0.025$) Male to female offspring ratio decreased with each generation (approximately 1 in 1st and 2nd generation but 0.44 in 3rd generation).	Schroeder and Mitchener Arch Env Hlth 23:102-106, 1971
Exposed to 0, 250, 500 or 1000 ppm Ni as nickel sulfate hexahydrate ($NiSO_4.6H_2O$) for 3 generations (N=14-19 litters/group/generation)	Wistar Rats	Statistical analyses and p-values were not discussed or reported. Increased number of stillborn in all exposed groups in 1st generation. Decreased weanling weight in 1000 ppm group in all generations. No malformations. Average number of pups per litter decreased with increasing dose (10.3, 10.6, 9.8 and 9.0 in 0, 250, 500 and 1000 ppm groups, respectively). Average number of pups weaned per litter decreased with increasing dose (8.1, 7.2, 6.8 and 6.4 in 0, 250, 500 and 1000 ppm groups, respectively).	Ambrose et al J Food Sci Tech 13:181-187, 1976
Exposed to nickel chloride ($NiCl_2$) in drinking water for 2 generations. Dose levels 0, 50, 250 and 500 ppm (0, 73, 30.8 and 51.8 mg/kg/day) (N=30/sex/group)	CD Rats	500 ppm: significant decreased maternal body weight; decreased litter size; and increased pup mortality. $>$ 250 ppm: significant decrease in water intake producing dehydration as a confounding factor. 50 ppm: some skeletal malformations but no statistically significant.	Rubenstein et al 1988 Society of Toxicology Annual Meeting Presentation, Dallas, Texas, Abstract: Toxicologist 8 192, 1988.

respectively. Since no statistical analyses were performed it is not known whether the dose-related decreases were significantly different from control values. Gross observations on offspring at all dietary levels throughout the three generations showed no teratogenic effects. Histopathological findings in the third generation weanlings were entirely negative.

Recently, a two-generation reproductive study has been completed by the EPA (Rubenstein et al., 1988). In this study CD rats (30/sex/group) were administered nickel chloride at concentrations of 0, 50, 250 or 500 ppm nickel in drinking water (author estimated intake: 0, 7.3, 30.8 and 51.6 mg Ni/kg/day). Maternal toxicity was observed in the highest dose group, 500 ppm. No maternal effects were noted at 250 ppm. Toxic effects noted in offspring from the 500 ppm group were increased mortality, decreased number of live pups per litter and depression of body weight. In the F_1 generation, increased pup mortality and decreased live offspring per litter were also observed in 50 and 250 ppm groups. The EPA conducted an independent statistical evaluation which indicated that the increased pup mortality was not statistically different between the 50 or 250 ppm groups and controls. The decreased number of live offspring per litter was statistically significant when compared to concurrent controls. The concurrent controls exhibited large litter sizes and very low standard deviations. When treatment group values were compared to historic control values no statistical significance was seen. The EPA labeled the concurrent controls as unusual and as a confounding factor.

There was an increase in skeletal abnormalities, i.e., short ribs, in the F_2 offspring at the 50 ppm treatment level but not at higher doses. The investigators dismissed this effect as spurious since no dose-response trend was observed. One should note that the water intake in the 250 and 500 ppm groups was much lower than in the controls and 50 ppm groups. Therefore, water deprivation and dehydration could be a confounding factor. The EPA has labeled the 50 ppm dose (7.3 mg/kg/day) as a NOEL, however, several questions regarding data interpretation still remain.

The multigenerational studies indicate the possibility of toxic effects on reproduction and development at approximately 7-12 mg Ni/kg/day (Ambrose et al., 1976; Rubenstein et al., 1988). The study by Schroeder and Mitchener (1971), however, suggests much lower levels of nickel, i.e., less than 1 mg/kg/day. Given the problems associated with these studies, (e.g., small numbers of animals, lack of statistical analysis, confounding factors, etc.) it is not possible to accurately estimate LOEL or NOEL values.

The potential reproductive effects of nickel on humans are not known (Rubenstein et al., 1988).

5.5.5. Summary of Reproductive Effects

The multigenerational reproductive studies offer the most complete information. The exposure is from conception until the end of the reproductive age for several generations and both males and females are exposed. The three multigenerational studies all employed oral administration of a soluble nickel salt to rats. Unfortunately, the results of the available studies are inconsistent.

The multigenerational drinking water study by Schroeder and Mitchener (1971) suggests effects at the lowest level (5 ppm Ni). Decreases in litter size, increased fetal death and decreased male:female ratios were observed with each successive generation. Based on earlier chronic nickel dietary studies conducted by the same authors (Schroeder et al., 1974) on the same

animal species (Long-Evans rats) and employing a similar study design, the daily intake was estimated to be approximately 0.4 mg Ni/kg/day (Schroeder et al., 1974). The authors' intake calculations were based on daily food and water consumption rates of 6 g/100 g body weight and 7 ml/100 g body weight. Utilizing the EPA's recommended methodology for estimating consumption rates of 12 g/100 g and 18 ml/100 g body weight, respectively, were calculated (EPA, 1988). Utilizing these estimated values, a daily intake of roughly 1 mg Ni/kg/day is calculated. Only one dose level was examined in this study.

The multigenerational dietary rat study by Ambrose et al. (1976) demonstrated dose-related effects. The most sensitive parameter was the average number of pups weaned per litter. Exposure to 250, 500 and 1000 ppm Ni resulted in an 11, 16 and 21% decrease compared to control values. Utilizing the information on body weight provided by the authors and the EPA's recommended methodology for estimating food intake (EPA, 1988), these dietary levels correspond to daily intake levels of approximately 12, 24 and 50 mg Ni/kg/day, respectively. The recent EPA drinking water study (Rubenstein et al., 1988) suggests dose-effect values 7.3 - 30.8 mg Ni/kg/day similar to those reported by Ambrose et al. However, as stated above, several questions regarding data interpretation and experimental control still remain.

Given the problems associated with each of these studies, the most health protective approach would be to accept the study by Schroeder and Mitchener (1971) as demonstrating the lowest effect level, utilizing oral administration, to date. The lowest effect level observed would therefore be approximately 0.4 - 1 mg Ni/kg/day in the rat. This value is lower than the lowest effect level exhibited in male reproductive studies. However, Nadeenko et al. (1979) have reported a developmental NOEL and LOEL of 0.5 and 5 ug Ni/kg, respectively. This LOEL value is at least 80 times lower than the LOEL based on the multigenerational study by Schroeder and Mitchener (1971). Since the study design and methodology utilized by Nadeenko et al. could not be adequately evaluated, as noted earlier, and multigenerational studies are more comprehensive, the Schroeder and Mitchener (1971) study will be considered to be the lowest effect level.

An exposure of 24 ng Ni/m^3 per day (is the maximum level reported by ARB), would result in 0.007 ug Ni/kg-day if a 70-kg person breathed 20 m^3/day and absorbed 100% of the nickel compound:

[(24 ng Ni/m^3 x 20 m^3/day)(20 m3/day)]/[(70 kg) (1000 ug)] = 0.007 ug Ni/kg.

This maximum level, 24 mg Ni/m^3, level is 1000 times lower than the non-cancer chronic inhalation exposure LOEL (25 ug Ni/m^3, see section 5.4) in rats. The maximum daily intake (0.007 ug Ni/kg) is nearly 6 to 7 orders of magnitude lower than the non-cancer chronic oral exposure NOEL (7.5 mg Ni/kg) and LOEL (44 mg Ni/kg), respectively, in the rat. Considering the public health oriented nature in defining the most sensitive endpoint, the margin of safety appears to be more than adequate for ambient levels. Thus, typical ambient exposures would not be expected to be associated with any reproductive or developmental effects. However, "hot spot" levels still need to be evaluated.

Data from inhalation studies are insufficient to accurately estimate a reproductive NOEL for airborne nickel. However, the available data on the toxicity of airborne nickel suggest that the most sensitive target organs following this route of exposure are the lung and immune system, not the reproductive system.

6. Genotoxicity and Mechanisms of Carcinogenicity

Positive genotoxic responses have been reported with 11 nickel compounds, both soluble and insoluble. Mammalian cells exposed *in vitro* have exhibited mutations, chromosome aberrations, increased sister chromatid exchange (SCE) rates and cellular transformations.

Nickel sulfate was positive when tested in the *Drosophila Melanogaster* assay. Chromosomal damage has been reported in workers employed at nickel refineries and nickel plating facilities. The genotoxicity of nickel compounds appears dependent upon intracellular bioavailability of Ni^{+2}. Nickel ions produce highly selective damage to the heterochromatic region of chromosomes. Thus, nickel compounds appear to produce direct genotoxic effects on DNA and DNA replication.

Sunderman (1989), Coogan et al., (1989) and the IARC Monograph series (IARC, 1987a) have recently published reviews on the genotoxic and carcinogenic mechanisms of nickel and nickel compounds. The following section is largely a summary of these recent reviews. Many of the frequently cited studies and the most recent studies have been summarized in Tables 6.1, 6.2 and 6.3.

6.1 Genotoxic Activity of Nickel Compounds

Nickel is mutagenic in some microorganisms but not in others. *In vitro* exposure of mammalian cells to nickel may result in mutations, chromosome aberrations, increased sister chromatid exchange (SCE) rates and cellular transformation, and some of these effects have been caused by insoluble or sparingly soluble as well as easily soluble nickel compounds (Grandjean, 1986).

In general, nickel compounds have not tested positive for mutagenesis in the bacterial tester strains Salmonella typhimirium, Escherichia coli and Bacillus subtillis; however, they have produced positive effects in Corynebacterium. The ciliated protazoan Paramecium has been used to quantitate the genotoxic effects of nickel (Smith-Sonneborn et al., 1986). Genotoxicity was indicated by the significant increases in the fraction of nonviable offspring which was presumed to be an index of lethal mutations.

Mutagenesis tests of nickel compounds (nickel chloride, $NiCl_2$ and nickel subsulfide, Ni_3S_2) in cultured mammalian cells have encountered difficulty due to cell toxicity, i.e., mutagenesis apparently required Ni^{2+} concentrations sufficient to inhibit growth and kill most cells. Recently however, several studies utilizing alternative cell lines have reported nickel-induced mutagenesis at non-toxic or subtoxic concentrations. Exposure to nickel chloride resulted in increased reversions in rat kidney cells infected with murine sarcoma virus mutant "ts110". Exposure to crystalline nickel sulfide, NiS, significantly increased the mutation frequency in Chinese hamster g12-V-79 cells.

Rodriguez-Arnaiz and Ramos (1986) have reported mutational effects *in vivo* utilizing Drosophila melanogaster as a model. Significant increases in sex-linked recessive lethal mutations were observed at all nickel sulfate concentrations (200 to 400 ppm).

Morphological transformation of mammalian cells by nickel varies between nickel compounds (Table 6.2). The transforming potencies appear to be determined, in part, by rate of cellular uptake and intracellular bioavailability of Ni^{2+}. Utilizing BHK-21 (baby hamster kidney) cells Hansen and Stern (1984) determined the relative transformation potency of nickel

Table 6.1. Gene Mutation Studies

Test System	Exposure	Results/Comments	Reference
T4 Bacteriophage	Nickel sulfate ($NiSO_4$) 300 ug/ml (67 ug Ni/ml)	No activity	Corbett et al 1970 as cited by Grandjean 1986
Bacillus subtilis (Rec system)	0.05 M nickel chloride ($NiCl_2$) (2.9 mg Ni/ml)	No activity	Nishioka 1975 as cited by Grandjean 1986
Bacillus subtilis (Rec. system)	0.005 to 0.5 M $NiCl_2$ (0.3 to 29 mg Ni/ml); NiO (0.3 to 29 mg Ni/ml); and Ni_2O_3 (0.6 to 59 mg Ni/ml)	No activity	Kanematsu et al 1980 as cited by Grandjean 1986
Escherichia coli WP2 fluctuation test	5 to 25 ug/ml $NiCl_2$ (1.2 to 6.2 ug Ni/ml)	No activity	Green et al 1976 as cited by Grandjean 1986
Salmonella typhimurium (reverse mutation)	$NiCl_2$ and $NiSO_4$ (concentration not stated)	No activity	Arlauskas et al 1985 as cited by Grandjean 1986
Escherichia coli (repair-deficient strain)	0 to 1000 ug/ml $NiCl_2$ (0 to 247 ug Ni/ml)	Positive activity in differential killing assay	Tweats et al 1981 as cited by Grandjean 1986
Saccaromyces cerevisiae	0.005 to 0.04 M $NiSO_4$ (0.3 to 2.3 mg/ml)	Mixed results - cytoxic at concentrations tested	Singh 1984 as cited by Grandjean 1986
Salmonella test strains	0, 1.0, 5.0, or 10.0 mM $NiCl_2$(130, 650, or 1300 ug/ml; 59, 294, or 590 ug Ni/ml)	Inspite of substantial uptake of nickel into the cell mutagenic activity was not apparent.	Biggart and Costa (1986) Mutat Res 175:209-215.
Cornebacterium Homoserine-dependent	0.031 to 10 ug/ml $NiCl_2$ (0.014 to 4.5 ug Ni/ml)	Preliminary study. Dose-related increases in revertants only seen at levels $\geq$ 0.5 ug/ml .	Pikalek and Necasek (1983) Folia Microbiol (Prague) 26:17-21 as cited by (1) and (3)

Table 6.1.

Test System	Exposure	Results/Comments	Reference
Ciliated protozoan Paramecium	Ni powder and Ni_3S_2	Ni_3S_2 - 5 and 50 ug/ml (3.7 and 36.7 ug Ni/ml) produced significant genotoxicity based on percent of dead progeny (authors suggested lethal mutation)	Smith-Sonneborn et al 1986 Environ Mutagen 8:621-626
In Vitro			
Chinese hamster V79 cells	0.4 or 0.8 mM $NiCl_2$(52 or 104 ug/ml; 23 or 47 ug Ni/ml)	At lower concentrations results were similar to controls. At higher concentrations the cell survival was too low to realistically estimate mutation rate.	Miyaki et al (1980) In: Genetic and environmental factors in environmental and human cancer. Ed: HV Gelboin, pp.201-213 as cited by (1), (2) and (3)
House lymphoma L5178Y	40 to 127 ug/ml $NiCl_2$ (18 to 57.5 ug Ni/ml)	Dose-response relationship was exhibited	Amacher and Paillet (1980) Mutat Res 78:279-288 as cited by (1), (2), and (3)
Rat kidney cells infected with murine sarcoma virus mutant ts110 (MuSVts110) retrovirus	Cells were treated with 20 - 160 uM$NiCl_2$ (2.6-20.7 ug/ml; 1.2-9.4 ug Ni/ml) for 24 hours, washed and examined 2 to 3 weeks later for reversion to transformed phenotype.	Transformed foci were detected within 14 days. Average number of foci per 5 x 10^5 cells: Control - 2 $NiCl_2$: 20 uM - 3 40 uM - 11 80 uM - 14 120 uM - 15 160 uM - 7	Biggart et al, (1987). J Virol 61(8)2378-2388
CHO cells	0 - 10 ug Ni/ml as $NiCl_2$ (i.e. 0 - 22.1 ug/ml)	No. mutants/No. dishes and Mutant fraction (x10^{-5}) Control - 144/30 (ave. 4.8); 4.2 1 and 2 ug Ni/ml - 117/20 (5.85); 4.4 5 and 10 ug Ni/ml - 93/20 (4.65); 5.3	Little et al 1988 Teratogen Carcinogen Mutagen 8:287-292

Table 6.1.

Test System	Exposure	Results/Comments	Reference
Chinese hamster V79 cells using HGPRT assay	Cells were treated with 0 to 2.0 mM $NiCl_2$ (0 - 259.2 ug/ml; 117 ug Ni/ml)	Mutants/10^6 cells: 0 - 4.1 0.5 mM - 16.3 1.5 mM - 17.3 2.0 mM - 35.2	Hartwig and Beyersmann 1989 Mutat Res 217:65-73
In Vivo			
Drosophila melanogaster	Males were injected intraperitoneally (i.t.) with vehicle or 200, 300 or 400 ppm nickel sulfate ($NiSO_4$)	Sex-linked recessive lethal (SLRL) - In 0-2 day and 7-10 day broods at all nickel treatment concentrations ($p<0.05$). Data exhibited a dose-effect relationship. Sex-chromosome loss (SCL) - nickel sulfate did not significantly increase the number of XXY females at any dose level. A positive response was found a the highest concentration for induction of XO males. This concentration also represented the LD50.	Rodriguez-Arnaiz and Ramos (1986) Mutat Res 140:115-117.

Sources: Original articles, EPA 1986 (1), Sunderman 1989 Arch Toxicol Suppl 13:40-47 (2), and Grandjean (3).

Table 6.2. Mammalian Cell Transformation Studies

Test System	Exposure	Results/Comments	Reference
In Vitro			
Syrian Hamster Embyo (SHE) cells	2 or 5 ug/ml of Ni_3S_2 (1.5 or 3.7 ug Ni/ml) or amorphous NiS (1.3 or 3.2 ug Ni/ml) for 6-8 days. Cells were then washed and incubated for 2 weeks and examined for altered morphology.	Disordered colonies/total colonies: Control - 0/323 NiS: 2 ug/ml (1.5 ug Ni/ml) - 0/125 5 ug/ml (3.7 ug Ni/ml) - 1/317 Ni_3S_2: 2 ug/ml (1.3 ug Ni/ml) - 15/174 ($p<0.0005$) 5 ug/ml (3.2 ug Ni/ml) - 19/174 ($p<0.0005$) Ni_3S_2 was also significantly greater ($p<0.005$) than NiS treated cells.	Costa et al (1978) Ann Clin Lab Sci 8:502.
SHE cells	0 to 5 ug/ml Ni_3S_2 (0 to 3.7 ug Ni/ml), 0 to 10 ug/ml $NiSO_4.6H_2O$ (0 to 2.23 ug Ni/ml) and 0 to 20 ug/ml amorphous NiS (0 to 12.9 ug Ni/ml)	Both Ni_3S_2 and $NiSO_4.6H_2O$ compounds produced high incidence of transformed foci. Amorphous NiS did not exhibit activity. Control - 0% transformed colonies/total colonies Ni_3S_2: 1.0 ug/ml - 1.51% 2.5 ug/ml - 5.17% 5.0 ug/ml - 11.5% $NiSO_4.6H_2O$: 2.5 ug/ml - 1.25% 5 ug/ml - 1.44% 10 ug/ml - 5.45%	DiPaolo and Castro (1979) Cancer Res 39:1008-1013
SHE cells	10 ug/ml of crystalline NiS (6.5 ug Ni/ml), crystalline Ni_3S_2 (7.3 ug Ni/ml), amorphous NiS (6.5 ug Ni/ml), metallic nickel, Ni_3O_2 (8.5 ug Ni/ml), NiO (7.8 ug Ni/ml), and $NiCl_2$ (4.5 ug Ni/ml)	Incidence of transformation (% relative to crystalline NiS): crystalline NiS - 100% crystalline Ni_3S_2 - 118% amorphous NiS - 8% metallic nickel - 18% Ni_3O_2 - 17% NiO - 9% $NiCl_2$ 41%	Costa and Heck (1982) TIPS 408-410.

Table 6.2.

Test System	Exposure	Results/Comments	Reference
SHE cells	1 to 10 ug/ml amorphous NiS, Ni_3S_2 and crystalline NiS	Transformation incidence: am-NiS: 1 ug/ml - 0.39 5 ug/ml - 0.41 10 ug/ml - 0.48 cry-NiS: 1 ug/ml - ND 5 ug/ml - 1.47 10 ug/ml - 2.67 Ni_3S_2: 1 ug/ml - ND 5 ug/ml - 6.4 10 ug/ml - 11.5	Costa 1983 Biol Trace Elem Res 5:285-295
SHE cells	$NiCl_2$, crystalline NiS and amorphous NiS	$NiCl_2$ was approximately one-third as potent as crystalline NiS. Amorphous NiS did not exhibit such activity.	Robinson et al (1982) Carcinogenesis 3:657 "as cited by Costa (1983) Biol Trace Elem Res 5:285-295.
BHK-21 (baby hamster kidney) cells in culture	Cells were incubated with nickel oxides (NiO and $NiO_{1.4}\cdot 3H_2O$), Ni_3S_2, nickel dust, nickel welding fume or $Ni(CH_3COO)_2\cdot 4H_2O$ for 6 or 24 hours. Cells were then washed and incubated for 3 weeks after which the number of transformed colonies were counted.	Range of transformation potency was found to be a function of the dose, all nickel compounds produced approximately the same number of transformed colonies at the same degree of toxicity (e.g. 50% survival): NiO - 100 ug/ml (78.7 ug Ni/ml) $NiO_{1.4}\cdot 3H_2O$ - 14 ug/ml (6.2 ug Ni/ml) Ni_3S_2 - 10 ug/ml (7.3 ug Ni/ml) Nickel dust - 200 ug/ml welding fume - 300 ug/ml $Ni(CH_3COO)_2\cdot 4H_2O$ - 225 ug/ml (53.1 ug Ni/ml) (Values taken from Table 1 of publication)	Hansen and Stern (1984) In: Nickel in the human environment, Ed. Sunderman. pp.193-200

Table 6.2.

Test System	Exposure	Results/Comments	Reference
Human diploid foreskin fibroblasts (HFC) cells	NiO, Ni_3S_2, $NiSO_4$, and $Ni(CH_3COO)_2$	Nickel compounds caused significant ($p<0.000001$), dose-dependent anchorage independence of fibroblasts but did not induce other <u>in vitro</u> markers of morphological transformations. Frequency of anchorage independence/10^5 survivors: Vehicle - 18 50 uM NiO (3.7 ug/ml; 2.9 ug Ni/ml) - 194 10 uM $NiSO_4$ (1.5 ug/ml; 0.59 ug Ni/ml) - 607 10 uM Ni_3S_2 (2.4 ug/ml; 1.76 ug Ni/ml) - 611 10 uM $Ni(CH_3CO_2)_2$ (1.8 ug/ml; 0.6 ug Ni/ml) - 233	Biedeman and Landolph (1987) Cancer Res 47:3815-3823
SHE cells	5 or 10 ug/ml of 6 nickel oxides, 4 nickel-copper oxides, or alfa NiS	alfa NiS, 'INCO black NiO', and compounds B, H, and I exhibited cell transforming activity. In general the lower the temperature at which the compounds were calcined, the more potent they were.	Sunderman et al (1987) Carcinogenesis 8(2)305-313
SHE and CHO cells	500 and 1000 uM (65 and 130 ug/ml; 29 or 59 ug Ni/ml) $NiCl_2$	Decrease in survival and increase in the incidence of morphological transformation	Conway et al (1987) Carcinogenesis 8(8)1115-1121
mouse BALB/3T3 cells	1 ug Ni/ml as NiCl2 (2.2 ug/ml)	Total foci/total dishes, mean number of foci/dish; fraction of dish with foci: control - 7/175, 0.04, 0.04 1 ug Ni/ml (note: 91% survival): 21/135, 0.16, 0.13 ($p<0.02$)	Little et al 1988 Teratogen Carcinogen Mutagen 8:287-292
Normal human bronchial epithelial (NHBE) cells	5 to 20 ug/ml $NiSO_4$ (1.9 to 7.6 ug Ni/ml)	Reduced colony-forming effeciency by 30 to 80%. 1 focus per 10^5 cells arose in cultures exposed to > 5 ug/ml. None of the Ni-transformed cell cultures were tumorigenic upon injection into athymic nude mice.	Pfeifer et al 1989 Environ Health Perspec 80:209-220
Chinese hamster embryo cells	10 ug/ml crystalline NiS (6.5 ug Ni/ml) and 1 mM NiCl2 (130 ug/ml) (59 ug Ni/ml)	NiCl2 was more toxic than NiS. Transformation was similar between compounds tested. Male cells were 2-3 fold more sensitive to transformation than female cells. Female cells were much more sensitive to NiCl2 induced cytotoxicity than male cells.	Conway and Costa 1989 Cancer Res 49:6032-6038

Table 6.2.

Test System	Exposure	Results/Comments	Reference
C3H/10T1/2 Cl 8 mouse embryo fibroblasts (10T1/2)	various concentrations ranging from 0.5 to 400 uM NiS, Ni3S2, NiO, NiSO4 and NiCl2	Insoluble Ni3S2, NiS and NiO caused dose-dependent cytotoxicity and low, dose-dependent frequency of morphological transformation in the concentration rages 0.5-40 uM, 5-50 uM, and 50-400 uM, respectively. Soluble NiSO4 and NiCl2 caused dose-dependent cytotoxicity at concentrations > 0.5 uM, but neither compound induced morphological transformation even at concentrations causing up to 94% cytotoxicity.	Miura et al 1989 Env Molec Mutagen 14:65-78.
In Vivo			
SHE cells following transplacental exposure in vivo	Pregnant hamsters received intraperitoneal (i.p.) injections of 2.5 or 5 mg nickel sulfate ($NiSO_4$)/100 g body weight on day 11 of gestation. Embryos were excised 48 to 60 hours later. Embryo cells were cultured and evaluated for transformation.	Transformation was observed in nickel treated cells cloned from the third subpassage. Specific data was not reported.	DiPaolo and Castro (1979) Cancer Res 39:1008-1013

Table 6.3. Chromosomal Effects (e.g. aberrations, SCE, etc.)

Test System	Exposure	Results/Comments	Reference
In Vitro			
Mammary carcinoma cells from C3H mouse	Exposure to 0.1 - 1 mM nickel chloride ($NiCl_2$) (13-130 ug/ml; 5.9-58.7 ug Ni/ml), nickel acetate ($Ni(CH_3CO_2)_2$) (17.7-177 ug/ml; 5.9-58.7 ug Ni/ml), potassium cyanonickelate ($K_2Ni(CN)_4$) (24.1-241 ug/ml; 5.9-58.7 ug Ni/ml), and nickel sulfide (NiS) (9.1-91 ug/ml; 5.9-58.7 ug Ni/ml). Measured cellular uptake; leucine (^{3}H-leu), uridine (^{3}H-UR) and thymidine (^{3}H-TdR) uptake; and chromosomal aberrations.	Similar cellular concentrations were attained regardless of nickel form in media. Incorporation of ^{3}H-TdR and ^{3}H-leu was inhibited at concentrations > 6 x 10-4 for $NiCl_2$, $Ni(CH_3CO_2)_2$, and NiS. With $K_2Ni(CN)_4$ the uptake of ^{3}HTdR, ^{3}H-leu and ^{3}H-UR were inhibited. Chromosomal aberrations - similar across all four nickel compounds.	Nishimura and Umeda (1979) Mutat Res 68:337-349.
FM3A cells from C3H mouse mammary carcinoma	0.2 to 1 mM $NiCl_2$ (25.9 to 130 ug/ml; 11.7 to 58.7 ug Ni/ml), $Ni(CH_3COO)_2$ (35.3 to 176.7 ug/ml; 11.7 to 58.7 ug/Ni/ml),or 0.32 to 2 mM NiS (29 to 181.5 ug/ml; 18.8 to 117.4 ug Ni/ml) for 24 or 48 hours	$NiCl_2$ and Ni-acet induced few aberrations; NiS induced low but a definite increase in aberrations.	Umeda and Nishimura 1979 Mutat Res 67:221-229
Human lymphocytes	2.33 to 2,330 uM $NiSO_4$ (0.36 to 360.6 ug/ml; 0.137 to 136.8 ug Ni/ml)	Positive response reported. Dose response reported with student T-test	Wulf (1980) Dan Med Bull 27:40-42 as cited in (1) and (2)

Table 6.3.

Test System	Exposure	Results/Comments	Reference
Chinese hamster Don cells	50 ug/ml $NiSO_4$ or 32 ug/ml NiCl2 (14.5 ug Ni/ml)	Positive response reported. No dose response studied.	Ohno et al (1982) Mutat Res 104:141-145 as cited in (1) and (2)
Human lymphocytes	nickel sulfide (concentrations not reported)	Positive response reported. No data presented.	Anderson (1983) Environ Health Perspect 47:239-253 as cited in (1)
Human lymphocytes	0.001 to 100 ug/ml nickel sulfide	Positive response reported. No dose response.	Saxholm et al (1981) Cancer Res 41:4136-4139 as cited in (1) and (2)
Human lymphocytes	1 to 100 uM $NiCl_2$ (0.13 to 13 ug/ml; 0.06 to 6 ug Ni/ml)	Positive reponse reported.	Newman et al (1982) Mutat Res 101:67-74 as cited in (1)
Human lymphocytes	9.5 uM (2.5 ug/ml) and 19 uM $NiSO_4$ (5 ug/ml)	Positive response was reported. Low concentrations used. The results would probably be more dramatic at higher concentrations.	Larramendy et al (1981) Environ Mutagen 3:597-606 as cited in (1) and (2)
Syrian hamster cells	3.8, 9.5 or 19 uM $NiSO_4$ (1, 2.5 or 5 ug/ml, respectively)	Positive response reported.	ibid
CHO cells	10 and 100 uM $NiCl_2$ (1.3 or 13 ug/ml; 0.6 and 5.9 ug Ni/ml), 10 ug/ml crystalline NiS (6.5 ug Ni/ml), and 10 ug/ml amorphous NiS (6.5 ug Ni/ml)	Single strand breaks - 100 uM NiCl2 and 10 ug/ml cr-NiS induced strand breaks. 10 ug/ml am-NiS did not induce strand breaks.	Robinson et al (1982) Carcinogenesis 3:657

Table 6.3.

Test System	Exposure	Results/Comments	Reference
SHE and CHO cells	NiCl2, amorphous NiS, crystalline NiS, and Ni3S2	Induction of DNA repair: CHO cell studies - NiCl2: 1 mM - considerable repair (slightly less than 5 ug/ml cry-NiS) cry-NiS: > 1 ug/ml - considerable repair am-NiS: 10 ug/ml - very little repair SHE cell studies - NiCl2: 10 uM - no significant repair 100 uM - significant repair Ni3S2: 10 ug/ml signficant repair (approx. 1.35 times that seen at 100 uM NiCl2) am-NiS: 5 or 10 ug/ml - no significant repair	Robinson et al 1983 Cancer Lett 17:273-279
SHE cells and CHO cells	1 - 10 ug/ml amorphous NiS (0.65-6.5 ug Ni/ml), crystalline NiS, and crystalline Ni_3S_2 (0.73-7.3 ug Ni/ml), 10-100 uM $NiCl_2$ (1.3 to 13 ug/ml; 0.6-6 ug Ni/ml)	SHE and CHO cells following exposure for 24 hours to crystalline NiS and Ni_3S_2 exhibited substantial DNA repair activity. $NiCl_2$ activity was evaluated in SHE cells only following 3 hours of incubation - some repair activity was evident. Unfortunately, two different test protocols were utilized so direct comparison of insoluble and soluble nickel forms can not be done.	Costa (1983) Biol Trace Elem Res 5:285-295.
CHO cells	0.1, 0.5, 1 or 2.5 mM NiCl2 (13, 65, 130 or 324 ug/ml; 6, 29, 59 or 146.8 ug Ni/ml)	Rate of DNA synthesis: NiCl2: 0.1 mM - no effect 0.5, 1.0 and 2.5 mM - suppression of DNA synthesis. 2.5 mM - DNA-protein cross-links were observed.	Conway et al 1986 J Biochem Toxicol 1(2)11-26
Chinese hamster ovary (CHO) cells	$NiCl_2$ and crystalline NiS. Measured sister-chromatid exchange (SCE)	Similar number of SCE per cell and SCE per chromosome at equi-molar concentrations (0-100 uM range) of $NiCl_2$ (0-13 ug/ml; 0-6 ug Ni/ml) and NiS (0-9.1 ug/ml; 0-5.9 ug Ni/ml). $NiCl_2$ blocked cells in their first mitosis at concentrations > 100 uM.	Sen and Costa (1986) Carcinogenesis 7(9)1527-1533.

Test System	Exposure	Results/Comments	Reference
CHO cells	0 to 1000 uM $NiCl_2$ (0 to 130 ug/ml; 0 to 59 ug Ni/ml) for 2 hours; 0 to 20 ug/ml NiS (0 to 12.9 ug Ni/ml) for 6, 24 or 48 hours	% Cells with chromosomal damage: $NiCl_2$ in simple salt medium: 0 - 10.25% 1 uM - 15.7% 100 uM - 33.1% 500 uM - 58.7% 1000 uM - 42.5% $NiCl_2$ in minimal essential medium: 0 - 7.05% 100 uM - 17.8% 250 uM - 15.0% 500 uM - 20.1% 1000 uM - 19.2% NiS: 0 - 17% 5 ug/ml - 22.8, 20 or 34% (for 6, 24, or 48 hours, respectively) 10 ug/ml - 21.6, 16.1 or 36.6% 20 ug/ml - 21.5, 40.3, 61.3%	Sen and Costa 1985 Cancer Res 45:2320-2315
CHO and HOS (human osteocarcoma) cells	250 to 1000 uM $NiCl_2$ (32.4 to 130 ug/ml; 14.7 to 59 ug Ni/ml)	Increased DNA-protein crosslinks and single strand breaks. HOS cells appeared to be more sensitive to the induction of DNA damage by nickel than the CHO cells.	Conway et al (1987) Carcinogenesis 8(8)1115-1121.
CHO cells	300 to 1000 uM $NiCl_2$ (38.9 to 130 ug/ml; 17.6 to 59 ug Ni/ml)	Increase in SCE and chromosomal aberrations, particularly in the heterochromatin regions.	ibid
Chinese hamster V79 cells	0, 0.3, 0.4 or 0.5 mM Ni(II)	Enhanced the number of background SCE's from 6.0 to 8.4 per cell. Even though the elevation was slight it was reproducible and significant ($p<0.01$)	Hartwig and Beyersmann 1989 Mutat Res 217:65-73
Double stranded poly[d(G-C)]	Nickel chloride ($NiCl_2$), nickel sulfate ($NiSO_4$), nickel carbonyl ($NiCO_4$) and nickel subsulfide (Ni_3S_2).	B to Z helical transition at 40 mM $NiCl_2$. Did not report additional data.	Liquier et al (1984) Anticaner Res 4:41-47

Table 6.3.

Test System	Exposure	Results/Comments	Reference
	NiCl2, NiSO4, Ni3S2 and nickel carbonate (NiCO3)	Transition of B-DNA to Z-DNA at concentrations of 0.4 mM (24 ug Ni/ml)	Bourtayre et al 1984 as cited by (2)
Double stranded poly[d(G-C)]	0 to 5 mM (0-648 ug/ml; 0-293.5 ug Ni/ml)	Transformation from B to Z-type conformation	Hacques and Marion 1986 In: Biopolymers 25:2281-2293
Double stranded poly[d(A-T)]	NiCl2	up to 85 mM NiCl2 unchanged spectrum (i.e. B conformation). > 85 mM NiCl2 induces a rapid modification of the spectrum (altered conformation - probably Z conformation)	Bourtayre et al 1987 J Biomolec Struc Dynam 5(1)97-104
In Vivo			
Rat bone marrow and spermatogonial cells	3 or 6 mg/kg nickel sulfate (NiSO4) by i.p. injection (1.14 or 2.28 mg Ni/kg) for 7 or 14 days	Negative response reported	Mathur et al (1978) Toxicology 10:105-113 as cited by (1) and (2)
Rat - kidney, liver, lung and thymus evaluated following i.p. administration	5 - 40 mg/kg NiCO3 (2.5 to 20 mg Ni/kg)	Single-strand breaks in lung and kidney nuclei as well as DNA-protein and DNA interstrand cross-links in kidney nuclei	Ciccarelli et al 1981; Ciccarelli and Wetterhahn 1982 as cited by (2)
House bone marrow cells (micronucleus test)	25 mg/kg $NiCl_2$ (11.3 mg Ni/kg) (50% LD50) or 56 mg/kg nickel nitrate ($Ni(NO_3)_2 \cdot 6H_2O$) (11.3 mg Ni/kg) (50% LD50) administered i.p. injection.	Negative response reported. Dose response not studied.	Deknudt and Leonard (1982) Toxicology 25:289-292
House - dominant lethal test	25 mg/kg $NiCl_2$ (11.3 mg Ni/kg) (50% LD50) or 56 mg/kg $Ni(CH_3COO)_2$ (18.6 mg Ni/kg) (50% LD50) administered i.p. injection.	Negative response reported. Dose response not studied. Not clastogenic but induced preimplantation failure.	Ibid

Table 6.3.

Test System	Exposure	Results/Comments	Reference
Mouse - embryonic cells derived from treated male germ cells	40 or 56 mg/kg $Ni(NO_3)_2$ (8.1 to 11.3 mg Ni/kg)	Not clastogenic but reduced fertilizing capacity of sperm.	Jacquet and Mayence (1982) Toxicology Letters 11:193-197
Swiss albino mice bone marrow cells	Single i.p. injection of 6, 12, or 24 mg/kg $NiCl_2$ (2.7, 5.4 or 10.9 mg Ni/kg) for 6, 24, or 48 hours. Repeated i.p. injections (5 injections) of 4.8 mg/kg (24 mg/kg total)	Chromosomal breaks and SCE: control - 0.66% aberration $NiCl_2$: 6 hour: 6 mg/kg - 2.33%; 12 mg/kg - 3.0%; and 24 mg/kg 2.66% 24 hour: 6 mg/kg - 3.33% ($p<0.01$); 12 mg/kg - 4.33% ($p<0.01$); and 24 mg/kg - 4.0% ($p<0.01$) 48 hour: 6 mg/kg - ND; 12 mg/kg - 3.66%; 24 mg/kg - 3.33% Repeated doses: 4.66%	Mohanty (1987) Current Science 56:1154-1157
Peripheral blood lymphocytes from nickel-exposed workers	0.5 mg Ni/m3 (range 0.1 - 1.0 mg Ni/m3)	Significant increase in chromosomal gaps but no difference in the number of chromosomal breaks.	Waksvik and Boysen (1982) Mutat Res 103:185-190 "as cited by (2)
Peripheral blood lymphocytes from nickel-exposed workers	Exposed to inhalation of furnace dust of Ni_3S_2 or NiO or aerosols of $NiCl_2$ and $NiSO_4$ > 1 mg/m3 for > 25 years.	Increased incidence of breaks ($p<0.001$) and gaps ($p<0.05$) but no difference in incidence of SCEs	Waksvik et al (1984) Carcinogenesis 5:1525-1527
Peripheral blood lymphocytes from welders		Statistically significant increase in chromosomal aberrations were found in welders who used a welding process involving cored wire containing nickel. These workers had signficantly higher concentrations of seum and urine nickel than other welders. However, no significant correlation between nickel levels and frequency of chromosomal aberrations was found. There was a significant correlation between length of employment and frequency.	Elias et al 1989 J Occup Med 31(5)477-483

Sources: Original articles, EPA 1986 (1), and Grandjean 1986 (2).

metal and a number of nickel compounds. These included relatively insoluble particulates, nickel subsulfide and several oxides and a soluble salt, nickel acetate. Although a wide range of transformation potency was found as a function of the dose, all substances produced the same number of transformed colonies at the same degree of toxicity. If toxicity is a direct measure of net available nickel, then apparently nickel or nickel ion *per se* is the ultimate transforming agent, independent of source or uptake mechanism (Hansen and Stern, 1984). The effect of the insoluble particulate compounds was not time dependent; however, that of the nickel acetate solution was time dependent. This indicated a difference in the rates associated with different uptake mechanisms for insoluble particulates and for soluble, dissolved material. The authors suggested that the crucial property which determines the potency of various nickel compounds is the availability of nickel critical to a given bioassay *in vitro*, and presumably *in vivo* as well. Costa and Mollenhauer (1980) have demonstrated that morphological transformation of Syrian hamster embryo (SHE) cells by various particulate nickel compounds is proportional to phagocytic uptake of the particles by Chinese hamster ovary (CHO) cells.

Morphological transformation of Syrian hamster embryo (SHE) cells and Chinese hamster ovary (CHO) cells has been reported following *in vitro* exposures to nickel subsulfide, nickel oxide (NiO), nickel sulfate ($NiSO_4$), metallic nickel, nickel selenide (Ni_3Se_2), nickelic oxide (Ni_2O_3), nickel chloride, nickel acetate and crystalline nickel sulfide, whereas similar experimental conditions yielded negative results for amorphous nickel sulfide.

Genotoxicity tests utilizing nickel oxide have produced varying results. Sunderman et al., (1987) evaluated 10 nickel oxides and nickel-copper oxides, which all contained NiO (bunsenite) as the predominant crystalline phase. The oxides were prepared under a variety of metallurgical conditions and varied significantly in transformation ability. In general the lower the temperature at which the compounds were calcined the more potent they were.

Depending on the specific assay and transformational endpoint examined the transforming potencies of the various nickel compounds relative to nickel subsulfide, which appears to be the most potent, range from roughly equal to 13 times less potent. Based on the limited available data the potencies, relative to Ni_3S_2 range from equivalent to 8 times less potent for nickel sulfate, from equivalent to 4 times less potent for crystalline nickel sulfide, from 5 to 13 times less potent for nickel oxides (NiO or Ni3O2), from 2 to 3 times less potent for nickel chloride, and approximately 2 times less potent for nickel acetate. When the effective concentration levels are corrected for nickel content the disparity in potencies lessens.

Human cell cultures do not appear to be as susceptible to morphological transformation. Human fetal kidney explants and human diploid foreskin fibroblasts developed anchorage independence following *in vitro* exposure to nickel oxide, nickel sulfate, nickel subsulfide and nickel acetate ($Ni(C_2H_3O_2)_2$). However, other markers of morphological transformations, e.g., immortality, were not present. *In vitro* exposure of human bronchial epithelial cells to nickel sulfate did not result in significant levels of morphological transformation.

Nickel sulfate has been evaluated for transformational potency *in vivo* (DiPaolo and Castro, 1979). Pregnant Syrian hamsters were injected i.p. with nickel sulfate on day 11 of gestation. Embryos were excised 48 to 60 hours later, the cells were cultured and subsequently examined for transformation. Transformation was observed in the nickel sulfate treated cells cloned from the third subpassage.

Nickel compounds are known to induce chromosomal damage, e.g., mitotic aberrations, sister chromatid exchanges (SCE), chromosome breaks and chromosome gaps (Table 6.3). Chromosomal damage has been reported in rodent and human cells following *in vitro* exposure to nickel acetate, nickel chloride, potassium cyanonickelate, nickel subsulfide, nickel sulfate, and crystalline nickel sulfide. *In vivo* exposure to nickel chloride and nickel carbonate ($NiCO_3$) has resulted in chromosomal effects in mice and rats. Lymphocytes from nickel refinery workers and nickel platers also exhibit increases in chromosomal damage.

Sen and co-workers have noted that chromosome aberrations induced by *in vitro* nickel exposure frequently occur in the protein-rich heterochromatic regions of chromosomes, and especially include decondensation or fragmentation of the heterochromatic long arm of the X-chromosome. Sen and Costa (1985) evaluated the induction of chromosomal aberrations in CHO cells treated with crystalline NiS and $NiCl_2$. They observed that $NiCl_2$ was more potent in inducing chromosomal aberrations in cells that were maintained with a salts/glucose medium (SGM) during nickel treatment than when cells were treated in a culture medium. This was due to the higher uptake of nickel into the cells maintained in SGM. Nickel chloride was not readily taken up by cells in culture growth medium due to the inhibitory effects of nickel binding amino acids present in the culture medium. Serum proteins and other compounds of culture medium do not reduce the phagocytosis of crystalline nickel sulfides (Heck and Costa, 1982).

The mechanism of metal delivery also influenced the nature of the chromosomal lesion observed. Chromosomal aberrations induced by $NiCl_2$ occurred randomly among the autosomal arms; however, the heterochromatic centromeric regions of the chromosomes were preferentially damaged. In addition to inducing the same type of aberrations found with $NiCl_2$, crystalline NiS also caused the selective fragmentation of the heterochromatic long arms of the X-chromosomes. This fragmentation was attributed to the difference in the mechanism of delivery of nickel ions from phagocytized crystalline NiS particles which aggregate around the nuclear membrane and release large amounts of nickel ions. In contrast, ionic nickel from $NiCl_2$, which enters the cell less readily, is distributed throughout the cell, interacting with numerous ligands in addition to DNA. When cells are treated with high levels of $NiCl_2$ in an attempt to achieve the cellular levels of nickel produced by NiS, this overloading resulted in cytotoxic responses rather than the preferential fragmentation of heterochromatin. However, liposome-mediated delivery of $NiCl_2$ did result in fragmentation of the long arm of the X-chromosome, therefore, the selective breakage of heterochromatin by crystalline NiS may be the result of the mechanism of Ni^{+2} delivery in cells.

Chromosomal aberrations have also been reported following *in vivo* nickel carbonate and nickel sulfate exposure in experimental animals. Ciccarelli et al., (1981; 1982 as cited by Grandjean 1986 and Sunderman 1989) noted DNA strand breaks in lung and renal nuclei from rats injected i.p. with nickel carbonate ($NiCO_3$). In addition to DNA strand breaks, DNA-protein complexes and DNA interstrand cross-links in renal nuclei were also reported. Mohanty (1987) has also reported chromosomal damage following *in vivo* exposure. A significant increase in chromosomal breaks and SCE's in mice bone marrow cells were observed following i.p. injection of $NiCl_2$. Investigators evaluating chromosomal damage following *in vivo* exposure to nickel chloride or nickel nitrate have thus far reported negative responses (Deknudt and Leonard, 1982; Jacquet an Mayence, 1982).

Waksvik and coworkers have examined peripheral lymphocytes from nickel exposed workers for evidence of chromosomal damage (Waksvik and Boysen, 1982; Waksvik et al., 1984). In the earlier study, involving workers from the Falconbridge nickel refinery in Norway, peripheral lymphocytes showed a statistically significant increase in chromosome gaps in two groups of exposed workers. No statistically significant relationship was found between the increase of gaps and plasma concentration of nickel, length of exposure or age of the workers. In the latter study, a group of retired nickel refinery workers was matched with controls who were never occupationally exposed to nickel. A statistically significant increase in both chromosome breaks and gaps was found. The exposed group had been exposed for more than 25 years to nickel concentrations in the air above 1 mg/m3, levels which were higher than in the previous study (Grandjean, 1986).

Recently Elias and co-workers have reported statistically significant increases in chromosomal aberrations (mainly chromatid gaps) in one of three analyzed groups of welders (Elias et al., 1989). These welders employed a welding process which involves cored wire containing nickel. This group had significantly higher concentrations of serum and urine nickel unlike the other groups of welders. However, no significant correlations between nickel and frequency of chromosomal aberrations were found. There was a significant correlation between the length of welding employment of these welders and the frequency of chromosomal aberrations, although there was no significant correlation between age and the frequency of chromosomal aberrations. These results indicate that certain welding processes may generate fumes that seem to have a clastogenic activity.

The influence of various nickel compounds on the repair of DNA damage has been examined in mammalian cells *in vitro*. Some of the studies indicated that cells exposed to nickel compounds retain the ability to repair DNA strandbreaks when repair primarily involves the action of DNA ligases. Robinson et al., (1983) studied the influence of various nickel compounds on the repair of DNA strand-breaks in CHO and SHE cells. Cells exposed *in vitro* to $NiCl_2$, Ni_3S_2 and crystalline NiS (but not amorphous NiS) appeared to have enhanced DNA repair activity, even at concentrations of nickel compounds that did not cause detectable DNA strand-breaks. Other studies indicated that cells exposed to nickel compounds lose the ability to repair damaged DNA via the excision-repair pathway, which involves the coordinated actions of endonucleases and DNA polymerases. Hartwig and Beyersmann (1989) have shown that $NiCl_2$ inhibits the excision repair of mutations induced at the HGPRT locus in V-79 Chinese hamster cells by exposure to ultraviolet light.

Double-stranded DNA is normally found as a right-handed double-helix (B-DNA), however, under certain conditions it can adopt a left-handed double-helix formation (Z-DNA). Submillimolar concentrations of nickel chloride, nickel sulfate, nickel carbonate ($NiCO_3$) and nickel subsulfide induce B to Z helical transition of double-stranded poly-d(G-C) suggesting that intracellular Ni^{+2} favors formation of the left-handed double-helix. It has been speculated that transition between B and Z forms distort transcriptional activity. Nickel-induced disruption of DNA synthesis has been suggested by recent data showing that Ni^{+2} interacts with DNA *in vitro* and promotes deoxyadenosine hydrolysis by cleaving bonds between the purine base and deoxyribose leading to the release of adenine. The resulting apurinic sites of DNA are believed to induce mutations during subsequent replication by DNA polymerase, mediated by the misincorporation of purines, particularly deoxyadenosine, in the daughter strand. The chemical mechanism whereby Ni^{+2}

induces DNA depurination has not been established, but it may involve free-radical reaction similar to Ni^{+2} mediated lipid peroxidation.

6.2. Synergistic Interactions

Several soluble nickel compounds have been shown to interact synergistically with other known mutagens and carcinogens under *in vitro* and *in vivo* conditions. The only insoluble nickel compound evaluated thus far is nickel subsulfide (Ni_3S_2). Rivedal and Sanner (1981) described a promotion-like effect of $NiSO_4$ on *in vitro* transformation of SHE cells that had been exposed to BaP. The promotion-like effect was apparent when the cells were treated sequentially as well as concurrently with BaP and $NiSO_4$. When cells were first exposed to BaP, NiSO4 showed a promotion-like effect similar to that obtained with the tumor promoter 12-*O*-tetradecanoylphorbol-13-acetate (TPA). When TPA or BaP were used as promoting agents, $NiSO_4$ was able to initiate morphological transformation. Nickel sulfate was also tested in the presence of the carcinogens NQO (4-nitroquinoline 1-oxide) and N-OH-AAF (N-hydroxy-2-acetylaminofluorene) (Rivedal and Sanner 1981). These carcinogens alone gave a transformation frequency of less than 0.5%. When cells were exposed to $NiSO_4$ and NQO or N-OH-AAF in combination, a large enhancement of the transformation frequency was observed (approximately 5 times higher than expected based on individual compound results). Since NQO and N-OH-AAF are activated by different mechanisms, the results would suggest that nickel sulfate does not affect metabolism but rather the carcinogenic process itself.

Nickel chloride has been found to significantly enhance the mutagenic effects of ultraviolet light, cis-$PtCl2(NH3)_2$, and methyl methanesulphonate (Hartwig and Beyersmann, 1989). The authors suggested that Ni^{+2} enhanced the cytotoxicity and/or mutagenicity of all compounds causing DNA damage which is repaired by the long-patch excision repair system.

Soluble nickel compounds (nickel sulfate and nickel chloride) have been shown to act synergistically *in vivo* in rats when given in conjunction with the carcinogens dinitrosopiperazine (DNP), N-ethyl-N-hydroxyethylnitrosamine (EHEN), and urethan (Ou et al., 1980 and Kurokawa et al., 1985 as cited by Sunderman 1989; Blakely, 1987; IARC 1990). It is worth noting that in these experimental studies the nickel was administered in drinking water. Ling-Wei et al., (1988, as cited by Sunderman, 1989) found a highly positive correlation between nickel concentrations in drinking water and nasopharyngeal cancer morbidity among residents of the Xiangxi region of Hunan, China, and Isacson et al., (1985 as cited by Sunderman, 1989) noted an association between nickel concentration in drinking water and morbidity from lung and bladder cancer among residents of towns in Iowa.

[Note: the original references have not arrived, the following is based on information from IARC 1990 and for INCO's commentor]

Two groups of investigators have examined the synergistic effect of nickel subsulfide (Ni_3S_2) and BaP (Maenza et al., 1971; Kasprzak et al., 1973; both as cited IARC 1990). Maenza et al. (1971) administered 10 mg Ni_3S_2, 10 mg BaP, 20 mg Ni_3S_2 and 10 mg BaP, or vehicle by intramuscular injection to Fischer rats. The local tumor incidences were 24/30 (80%), 4/30 (13%), 28/30 (93%) or 0%, respectively. Although an increase was observed in the animals receiving both Ni_3S_2 and BaP a higher dose of Ni_3S_2 was utilized in this group than in the Ni_3S_2 only group. Therefore it is not clear whether the increase in tumor incidence or the decrease in tumor latency, also reported by the authors, was due to a synergistic effect or simply the higher Ni_3S_2 dose.

Kasprzak et al. (1973) administered a single intratracheal injection of 5 mg Ni_3S_2, 2 mg BaP or 5 mg Ni_3S_2 and 2 mg BaP, to Wistar rats. The animals were then observed for 15 months. Tumor formation was not significantly different, however, a significant difference in the incidence of preneoplastic lesions was observed, Ni_3S_2 and BaP > BaP > Ni_3S_2.

6.3 Mechanism(s) of Action

The mechanism of nickel carcinogenic activity may be the result of direct (e.g., mutations, chromosomal damage, etc.) or indirect (e.g., inhibition of excision-repair, immunosuppression, cell-to-cell communication, etc.) genotoxicity or by a combination of these actions. Differences in the carcinogenic potencies of nickel compounds may be, in part, the result of variations in their ability to provide Ni^{+2} at critical sites within the target cells. Nickel compounds, when given in combination with other carcinogenic agents, have also been shown to act synergistically or promote carcinogenic activity.

6.3.1. Direct Effects on DNA

Various forms of nickel have been reported to affect the rate of DNA synthesis. Conway et al., (1986) reported a significant suppression of DNA synthesis in CHO cells incubated *in vitro* in the presence of nickel chloride. Nickel chloride promoted DNA-protein cross-linking which may have contributed to the inhibition of DNA replication. A partial inhibition of DNA replication in the presence of a persistent lesion, such as a DNA-protein cross-link, could result in the fixation of a mutation in the DNA or in chromosomal aberrations. The addition of magnesium chloride to the medium completely reversed the inhibition of replication by nickel chloride. This effect did not appear to be due to an antagonism of the cellular uptake of nickel (Ni^{+2}) by magnesium (Mg^{+2}) since the maximally effective dose of Mg^{+2} reduced Ni^{+2} uptake by no more than 25%.

Sen and Costa (1985) reported that the DNA replication phase was more susceptible to the induction of chromosomal aberrations by nickel chloride. It is interesting to note that most of the aberrations were caused during the late S phase, the cell cycle period when heterochromatic DNA replicates. Costa and co-workers have been investigating the mechanism of nickel genotoxicity and carcinogenicity for several years. The available evidence on the mechanism of nickel carcinogenesis based on *in vitro* models has been recently summarized and reviewed by Costa (1989). The following presents an overview.

Nickel ions produce highly selective chromosome damage in heterochromatic regions of mouse and CHO cell chromosomes. The initial interaction of nickel with heterochromatin may cause a change in chromatin organization. This change in organization may have effects during replication of heterochromatic DNA. This may come about in two ways: 1) the disruption of chromatin structure caused by the initial effects of nickel ions leading to errors during DNA replication; or 2) nickel ions cause inhibition of DNA polymerase by interacting with magnesium sites on the enzyme as it replicates heterochromatic DNA.

At the chromosome level, the DNA lesions induced by nickel in heterochromatin are inhibited to a greater extent by excess magnesium than those induced in euchromatin. This was one of the first indications that DNA damage induced in heterochromatin was related to nickel carcinogenesis.

Additional evidence includes the observation that male Chinese hamster embryo cells undergo neoplastic transformation at a much higher frequency than the female Chinese hamster embryo cells (Conway and Costa, 1989). In male Chinese hamster cells, there is only one X-chromosome and the long arm of the chromosome is entirely heterchromatic, while the female cells have two X-chromosomes. To determine whether there might be a tumor suppressor gene located on the long arm of the X-chromosome, that was deleted during nickel-induced transformation, Costa and co-workers utilized the technique of microcell fusion (Costa, 1990). A normal Chinese hamster X-chromosome was placed into one of the nickel-transformed lines with a complete X_q deletion. This resulted in senescence of the nickel-transformed clones. This data suggests that nickel treatment may induce deletions of regions of the X-chromosome which result in the loss of a senescence/tumor suppresor gene.

The incidence of chromosomal effects in early passage male and female cultures was similar following treatment with either nickel chloride or crystalline nickel sulfide, which suggested that the dose of Ni^{+2} delivered to the nuclei of male and female cells was similar (Conway and Costa, 1989). The concentrations of nickel chloride and nickel sulfide utilized were 1 mM (130 ug/ml, 59 ug Ni/ml) and 10 ug/ml (6.5 ug Ni/ml), respectively.

6.3.2. Indirect Effects

Recently, nickel compounds (e.g. Ni3S2, NiCl2) have been shown to enhance lipid peroxidation resulting in the production of superoxide anion radicals, hydroxyl radicals and singlet oxygen (Kasprzak and Hernandez, 1989; Inoue and Kawanishi, 1989). In the presence of very low concentrations of H_2O_2, which normally would not induce DNA damage, Ni^{+2} causes base alteration(s) and/or liberation(s), and sugar phosphate backbone breakage (Kawanishi et al., 1989).

Suppression of the natural killer (NK) cell function may be an important property of carcinogens, contributing to carcinogenicity by providing a favorable environment for transformed cells. Injection of 20 mg Ni (in the form of metallic Ni, Ni2O3, or Ni3S2) produced a long-lasting depression of NK cell activity (Judde et al., 1987). Prospective analysis of individual NK cell responses demonstrated that a persistent depression of basal NK cell activity was restricted to rats that subsequently developed a tumor. In these animals the time between carcinogen treatment and clinical detection of the primary tumor was positively correlated with the mean level of NK cell activity. Admixture of manganese to nickel inhibited the development of tumors and also prevented the depression of NK cell activity produced by nickel treatment.

Smialowicz et al., (1985) reported that NK cell activity was inhibited in $NiCl_2$-treated mice. The $NiCl_2$ inhibition of NK cell activity resulted in enhancement of the susceptibility of exposed animals to develop lung tumors following the injection of syngenetic tumor cells.

Intracellular communication is an important mechanism for controlling cell growth and replication; disruption of gap junctional communication occurs during the neoplastic process and may be a prerequisite for tumor development. Nickel sulfate has been shown to disrupt cell-to-cell communication in a dose related manner from 98% of the base value at 0.5 mM to 2% at 5 mM (Miki et al., 1987). Cell viability was not affected at these concentrations. The inhibitor action of nickel sulfate could be partially prevented by concurrent exposure to MgSO4.

6.4. Conclusions

Potential genotoxicity of nickel compounds is not limited to insoluble nickel compounds such as nickel subsulfide and crystalline nickel sulfide. Instead, potential genotoxic responses have been reported with 11 nickel compounds in one or more assays. These positive responses include water-soluble nickel compounds (nickel acetate, nickel chloride and nickel sulfate), and relatively water insoluble nickel compounds (nickel carbonate, nickel oxide, nickel sulfide, and nickel subsulfide).

At the present time evidence points to ionic nickel (Ni^{+2}) as the ultimate toxic agent of the various nickel compounds. Formation of ionic nickel species by dissolution of a phagocytized particle in the cell cytoplasm may differ qualitatively and quantitatively in terms of cellular distribution from that resulting from extracellular exposure to soluble forms such as NiCl2 and NiSO4. Higher cellular concentrations and more intensive effects are often seen following exposure to phagocytized insoluble forms of nickel. However, exposure of animals *in vivo* and treatment of cells in culture with water soluble nickel compounds has produced DNA strand breaks and DNA-protein crosslings (Robinson et al., 1982; Ciccarelli et al., 1981; Ciccarelli and Wetterhahn, 1982). Additionally, both $NiCl_2$ and particulate nickel compounds (e.g. crystalline NiS and Ni_3S_2) are very potent inducing DNA repair activity (Robinson et al., 1983).

Nickel compounds can initiate carcinogenesis, possibly by direct genotoxic effects on DNA and DNA replication. Nickel compounds can also enhance tumor development and/or tumor progression, i.e. serve as a promoter, by blocking cell-to-cell communication, stimulating lipid peroxidation, and/or by inhibiting NK cell activity.

7. Carcinogenic Effects on Animals and Humans

7.1. Carcinogenic Effects on Animals

The carcinogenic effects of nickel have been shown to vary with the chemical form and physical state of nickel, the route of administration, the animal species and the exposure level. Several extensive reviews have been published within the last few years (Sunderman, 1986b; Sunderman, 1984b; Sky-Peck, 1985; EPA, Sept. 1986a; Sunderman, 1986c; Sunderman Jr, 1984). A review of the current literature indicates that no significant studies have been published since the publication of the EPA Health Assessment Document for Nickel, 1986. The National Toxicology Program is sponsoring inhalation carcinogenicity studies of nickel oxide, nickel sulfate and nickel subsulfide. However, study results are not available at this time.

The following sections will discuss the results of animal studies involving various routes of exposure. The inhalation and oral routes of administration will be discussed more fully as these are the most relevant to potential human exposure. The final section will address the selection of the animal study for quantitative risk assessment.

7.1.1. Inhalation or Intratracheal Administration

See Table 7.1 for summary of inhalation studies.

Table 7.1 Summary of Experimental Carcinogenicity Studies of Nickel and Nickel Compounds Following Inhalation or Intratracheal Exposure.

Exposure	Animal Species	2 Year Survival	Responses/Observations	Reference
Metallic Nickel Powder:				
15 mg/m3, 6 hr/day, 4-5 day/week for up to 21 months (99% pure, ≤ 4 um)	Guinea Pigs	0/42 (all dead at 21 months)	No control data reported. 37 animals were histologically evaluated; all animals exhibited adenomatoid formations, animal exhibited a multicentric anaplastic carcinoma of the lung.	Hueper Arch Pathol 65:600-607, 1958
Same exposure as above	Wistar Rats and Bethesda Black Rats	2/160 (1%)	No control data reported. Several (number not stated) neoplasms originating from and involving abdominal and mediastinal lymph nodes. Not believed to be related to nickel exposure. 50 animals were histologically examined; 15 exhibited adenomatoid lesions.	Hueper ibid
Same exposure as above	C57 Mice	0/20 (all dead at 15 months)	No control data reported. Two animals with lymphosarcomas; these were not believed to be related to nickel exposure. Hyperemic and hemorrhagic lungs.	Hueper ibid
Metallic nickel powder + limestone + SO_2 (Nickel particles 1-3 um)	Rats	0/120	No control data reported. No lung tumors. Other unspecified sites: 7 malignant and 6 benign tumors. Chronic inflammation, fibrosing changes with bronchiectasis, squamous cell metaplasia and peribronchial adenomatosis.	Hueper and Payne Arch Env Hlth 5:445-462, 1962
Same exposure as above	Hamsters	0/100	No control data reported. No lung tumors. Other unspecified sites: 8 malignant and 6 benign tumors. Diffuse hemorrhages into alveolar lumina.	Hueper and Payne ibid

Table 7.1 (cont)

Exposure	Animal Species	2 Year Survival	Responses/Observations	Reference
Administered weekly intratracheal instillations for 10 to 12 weeks. Surviving animals were killed at approximately 2.5 years post-treatment.	Female Wistar Rats	> 50%, no difference between treated and control	20 x 0.3 mg Ni (total dose 6 mg Ni): 10/39 (25.6%) tumor incidence ($p<0.05$) 10 x 0.9 mg Ni (total dose 9 mg Ni): 8/32 (25%) tumor incidence ($p<0.05$) Control: 0/40	Pott et al. Exp Pathol 32:129-152, 1987
Nickel Carbonyl ($Ni(CO)_4$): ($Ni(CO)^4$) Group 1: 0.03 mg/l (28 mg/m^3; 9.6 mg Ni/m^3) 30 min/day, 3 day/week for 12 months Group 2: 0.06 mg/l (56 mg/m^3; 19 mg Ni/m^3) 30 min/day, 3 day/wk for 12 months Group 3: 0.25 mg/l (233 mg/m^3: 80 mg Ni/m^3) for a single 30 min exposure. Group 4: Controls (ethanol/ether solvent vehicle)	Wistar Rats	Group 1: 5/64 (8%) Group 2: 1/32 (3%) Group 3: 3/80 (4%) Group 4: 3/41 (7%)	Group 1: 1 of 5 survivors at 27 months developed a squamous cell carcinoma. Group 2: Sole survivor at 24 months developed clear-cell carcinoma with adenocarcinomatous pattern. Group 3: 2 or 3 survivors: 1 at approximately 2 months developed anaplastic carcinoma; 1 at 30 months developed two small papillary adenomas. Group 4: No pulmonary tumors in any of the surviving animals.	Sunderman et al AMA Arch Ind Hlth 20: 36-41, 1959
Group 1: 0.6 mg/l (560 mg/m^3; 192 mg Ni/m^3) for a single 30 min exposure Group 2: same as Group 1 + 50 mg/kg dithiocarb Group 3: ethanol/ether solvent for a single 30 min exposure Group 4: same as Group 3 + 50 mg/kg dithiocarb Group 5: 0.03 mg/l (28 mg/m^3; 9.6 mg Ni/m^3) 30 min/day, 3 day/wk for life. Group 6: ethanol/ether solvent 30 min/day, 3 day/wk for life.	Wistar Rats	Group 1: approx. 43/285 (15%) Group 2: approx. 29/60 (48%) Group 3 and 4: 22/38 (58%) Group 5: 8/64 (12.5%) Group 6: approx. 22/32 (69%)	Group 1: 1 of 43 survivors at 24 months was found to have a pulmonary papillary adenocarcinoma. Renal metastases. Hepatic metastases. 9 malignant lymphomas. Group 2: 1 of 29 survivors at 26 months had anaplastic carcinoma. Spleen and hepatic metastases. Eight malignant lymphomas. Group 3 and 4: 7 malignant lymphomas. No pulmonary tumors. Group 5: 5 malignant lymphomas. 1 of 8 survivors at 26 months was found to have a pulmonary adenocarcinoma. Renal metastases. Group 6: 1 malignant lymphoma. No pulmonary tumors	Sunderman and Donnelly Am J Pathol 46:1027-38, 1965

Exposure	Animal Species	2 Year Survival	Responses/Observations	Reference
Nickel Oxide: (NiO)				
Group 1: 53.2 mg/m^3) 7 hr/day, 5 day/wk for up to 24 months. Method of NiO generation - not specified. Group 2: same as Group 1 + cigarette smoke Group 3: sham-smoke + sham-dust exposure Group 4: smoke + sham-dust.	Hamsters	Group 1: 0/51 Group 2: approx. 4/51 (8%) Group 3: 0/51 Group 4: approx. 4/51 (8%)	Group 1: No pulmonary tumors. Heavy pulmonary nickel oxide burden produced pneumoconiosis. Significant increase in laryngeal lesions. Group 2: Same as Group 1. Three malignant muscoloskeletal tumors in Group 1 and 2 combined. Group 3: No pulmonary tumors. Group 4: No pulmonary tumors.	Wehner et al Am Ind Hyg Assoc J Nov:801-810, 1975
Group 1: 0.25 mg NiO/m^3 (0.2 mg Ni/m^3) continuously for 18 mos. (N=20) (MMAD < 0.3 um) Group 2: 0.076 mg NiO/m^3 (0.06 mg Ni/m^3)continuously for 18 months (N=40) Group 3: filtered air (N=40) Method of NiO generation - pyrolysis of nickel acetate.	Wistar Rats	Group 1: 25% Group 2: 15% Group 3: 90% (Values estimated from figure 1)	Group 1: No pulmonary tumors. Severe pulmonary alveolar proteinosis and lung fibrosis. Group 2: Same effects as Group 1 Group 3: No pulmonary tumors	Takenaka et al In: Progress in Nickel Toxicology- Proceedings of the 3rd International Conference on Nickel Metabolism and Toxicology. Eds: Brown and Sunderman. pp 89-92, 1985 and Glaser et al Int Congr Ser-Excerpta Med 676: 325-28, 1986

Table 7.1 (cont)

Exposure	Animal Species	2 Year Survival	Responses/Observations	Reference
Group 1: 8.0 mg/m^3 (6.3 mg Ni/m^3) 6 hr/day, 5 day/wk for 1 month (N=8) Group 2: 0.6 mg/m^3 (0.47 mg Ni/m^3) 6 hr/day, 5 day/wk for 1 month (N-6) Group 3: filtered air for 6 hr/day, 5 day/wk for 1 month (N=5)	Wistar Rats	all animals killed at 20 months	Group 1: Lung changes--broncial gland hyperplasia and squamous metaplasia. 1 rat developed pulmonary adenomatosis. Group 2: Lung changes-bronchial gland hyperplasia and squamous metaplasia. 1 rat developed a papillary adenocarcinoma, 1 rat developed pulmonary adenomatosis. Group 3: No pulmonary tumors.	Horie et al Biol Trace Elem Res 7:223-239, 1985
Administered weekly intratracheal instillations for 10 to 12 weeks. Surviving animals were killed at approximately 2.5 years post-treatment. Method of NiO generation - not specified.	Female Wistar Rats	> 50%, no difference between treated and control	10 x 5 mg Ni (total dose 50 mg Ni): 10/37 tumor incidence (27%) 10 x 15 mg Ni (total dose 150 mg Ni): 12/38 tumor incidence (31.6%) Control: 0/40	Pott et al. Exp Pathol 32:129-152, 1987
<u>Nickel Subsulfide (Ni_3S_2):</u>				
0 or 0.97 mg/m^3 (0.71 mg Ni/m^3) 6 hr/day, 5 day/wk for 78-80 weeks	Fischer 344 Rats	0 mg/m^3: aprox. 97/241 (40%) 0.97 mg/m^3: approx. 24/226 (11%)	Control: Of 215 animals evaluated, 2 had neoplastic changes in the lungs (1 adenoma and 1 adenocarcinoma) 0.97 mg/m^3: Of 208 animals evaluated, 29 had neoplastic changes in the lungs (adenomas, adenocarcinomas, squamous cell carcinomas and fibrosarcomas)	Ottolenghi et al JNCI 54:1165-72, 1974
Adminsitered weekly intratracheal instillations for 4 weeks Group 1: controls Group 2: 0.024 mg/kg Group 3: 0.056 mg/kg Group 4: 0.156 mg/kg Group 5: 0.412 mg/kg Group 6: 1.1 mg/kg Study terminated at 27 months.	B6C3F1 Mice	Group 1: 9/20 (45%) Group 2: 7/20 (35%) Group 3: 14/20 (70%) Group 4: 9/20 (45%) Group 5: 14/20 (70%) Group 6: 6/20 (30%)	Group 1: No pulmonary tumors. Groups 2 -6: No pulmonary tumors or evidence of respiratory damage in any exposed group.	Fisher et al Env Res 4:313-320, 1986

Table 7.1 (cont)

Exposure	Animal Species	2 Year Survival	Responses/Observations	Reference
Administered weekly intratracheal instillations for 10 to 12 weeks. Surviving animals were killed at approximately 2.5 years post-treatment.	Female Wistar Rats		15 x 0.063 mg Ni (total dose 0.94 mg Ni): 7/47 (14.9%) tumor incidence 15 x 0.125 mg Ni (total dose 1.88 mg Ni): 13/45 (28.9%) tumor incidence 15 x 0.25 mg Ni (total dose 3.75 mg Ni): 12/40 (30%) tumor incidence	Pott et al. Exp Pathol 32:129-152, 1987

7.1.1.1. Metallic Nickel

The first attempts to demonstrate the carcinogenic potential of airborne nickel in laboratory animals are the studies by Hueper (1958) and Hueper and Payne (1962). In the earlier study young guinea pigs (2 to 3 months of age) (N=42), Wistar rats (N=100), Bethesda black rats (N=60), and C57 black mice (N=20) were exposed to powdered metallic nickel (Hueper, 1958). The experimental animals were exposed to 99 percent pure nickel at an average concentration of 15 mg/m^3 for 6 hr/day, 4-5 days/week, for a maximal period of 21 months. None of the test animals were killed for evaluation.

Very high mortality rates were exhibited in all species. By the end of 12 months 45 percent of the guinea pigs, 64 percent of the Wistar rats, 52 percent of the Bethesda black rats and 85 percent of the mice had died. At 21 months all exposed animals had died.

Thirty-seven of the 42 guinea pigs were evaluated histopathologically. (The remaining 5 animals were too decomposed for reliable histological study.) In virtually all animals examined, abnormal multicentric adenomatoid formation and atypical proliferations of the epithelial lining were present. In 6 of the animals, the author reported "the intra-alveolar and intrabronchiolar epithelial proliferations assumed a degree of atypia in circumscribed areas approaching the character of microcarcinomas."

Fifteen of the 50 rats examined histologically had adenomatoid alveolar formations similar to those in guinea pigs. Mice evaluated histologically exhibited hyperemic and hemorrhagic lungs but no abnormalities of the bronchial mucosa or adenomatoid formations were observed.

The author concluded that the lung lesions in the guinea pigs and rats were "equivalents of the respiratory neoplastic reactions seen in copper-nickel matte smelter workers." However, due to the poor survival, limited histological description and absence of control groups the results of this study are not adequate for cancer risk assessment.

Hueper and Payne in a subsequent study with rats and hamsters attempted to confirm the carcinogenic potential of nickel (Hueper and Payne, 1962). Powdered metallic nickel was administered with sulfur dioxide and powdered limestone. The limestone was added to dilute the nickel and to decrease the mortality observed in the previous study. Sulfur dioxide was added to test its potential as a cocarcinogen. Exposure chamber concentrations of nickel were not specified, nor were control animals included.

Lung tumors were not observed in the exposed animals. Lungs of exposed rats exhibited chronic inflammation, fibrosing changes with bronchiectasis, squamous cell metaplasia and peribronchial adenomatosis. The lungs of the hamsters showed scarcely any chronic effects due to exposure, suggesting that this species may be non-responsive to this form of nickel.

Metallic nickel powder has also been evaluated by intratracheal administration. Pott et al. (1987) administered a total intratracheal dose of 6 or 9 mg Ni/animal nickel powder to female Wistar rats. Following treatment, animals were maintained for up to an additional 2.5 years. The lung tumor incidence in the saline control (20 x 0.3 ml saline), 6 (0.3 mg Ni x 20), and 9 mg Ni (0.9 mg x 10) treatment groups were 0%, 25.6% and 25%, respectively.

7.1.1.2. Nickel Carbonyl, $Ni(CO)_4$

Nickel carbonyl has been tested by inhalation for carcinogenic potential by one group of investigators (Sunderman et al., 1959; Sunderman and Donnelly, 1965). Sunderman, et al., (1959) exposed three groups of male Wistar rats to

nickel carbonyl: Group I--64 rats were exposed to 0.03 mg/l (i.e., approximately 28 mg $Ni(CO)_4/m^3$ or 9.6 mg Ni/m^3) three times weekly for one year; Group II--32 rats were exposed to 0.06 mg/l (i.e., approximately 56 mg $Ni(CO)_4/m^3$ or 19.3 mg Ni/m^3) three times weekly for one year; and Group III--80 rats were exposed once to 0.25 mg/l (i.e., 233 mg $Ni(CO)_4/m^3$ or 80 mg Ni/m^3). Exposure was for 30 minute periods. Control animals (N=41) were exposed to a vapor of 5 percent ethanol/ether, the solvent for nickel carbonyl.

Survival times were poor for all groups. At the end of one year only 14 control rats, 16 Group I rats, 4 Group II rats and 8 Group III rats survived. At the end of 24 months the survivors included 3 control rats, 5 Group I rats, 1 Group II rat and 3 Group III rats. Of the nine nickel- exposed animals surviving four were found to have tumors: one from Group I, one from Group II, and two from Group III.

The first death of a rat bearing a pulmonary tumor was reported in Group II at 24 months. In the lungs of the first tumor bearing rat the lesion consisted of multiple masses of clear-cell carcinoma having an adenocarcinomatous pattern. Metastastic tumor masses were also found in one kidney and near the heart. The second rat (Group III) found to have a pulmonary neoplasm died shortly after the first. The pulmonary tumor was morphologically the same as that found in the first tumor bearing rat, but some areas gave evidence of more anaplastic malignancy. The third rat (Group I) which died at 27 months had a relatively small tumor classified as a squamous cell carcinoma. The fourth rat (Group III) was killed at 30 months while in a moribund state. At autopsy no pulmonary tumors were observed; however, histologic studies discovered two small papillary bronchial adenomas. No pulmonary tumors were observed in the surviving control animals.

Sunderman and Donnelly again in 1965 exposed three groups of male Wistar rats to nickel carbonyl. Three control groups were also maintained. The six experimental groups were: Group I--285 rats exposed to 0.6 mg/l (i.e., 559 mg $Ni(CO)_4/m^3$ or approximately 192 mg Ni/m^3) once for 30 minutes; Group II--60 rats exposed as Group I, however, in addition each rat received 50 mg/kg of a nickel chelating agent (Dithiocarb); Group III--19 rats exposed to the solvent vehicle (alcohol/ether vapor) once for 30 minutes (i.e., control for Group I); Group IV--19 rats were exposed as Group III but in addition received Dithiocarb (i.e., control group for Group II); Group V - 64 rats were exposed to 0.03 mg/l (i.e., 28 mg $Ni(CO)_4/m^3$ or 9.6 mg Ni/m^3) for 30 minutes three time per week for the remainder of their lives; Group VI - 32 rats exposed as Group V but only to the solvent vehicle (i.e., control group for Group V). Necropsies were made on all animals that were found dead or sacrificed during the 3 year study period.

Poor survival was seen in the nickel carbonyl exposed groups. By the end of the second year, 329 of 409 exposed rats had died compared to 26 of 70 in the controls. Of the 285 Group I rats approximately 43 survived until 24 months. One of the 43 surviving animals from this exposure group died at 24 months and was found to have a papillary adenocarcinoma in the right lung. Renal and hepatic metastases were also present.

Of the 60 Group II rats approximately 29 survived until 24 months. One animal from this group dying at 26 months was found to have a pulmonary anaplastic carcinoma. Anaplastic metastases were also found in the spleen and liver.

Of the 64 chronically exposed Group V rats approximately 8 survived until 24 months. One animal was sacrificed, due to its moribund state, at 26

months and found to have a pulmonary adenocarcinoma. Renal metastases were also found.

Of the total 70 control rats approximately 44 survived until 24 months. No pulmonary lesions considered neoplastic were found among the control animals.

When the results of the two studies cited above are combined the following is revealed: 1) of 72 rats surviving 24 to 26 months following a single high exposure to 0.6 mg/l $Ni(CO)_4$ (i.e., approximately 559 mg/m^3) 2 developed pulmonary neoplasms (1 papillary adenocarcinoma; 1 anaplastic carcinoma); 2) of 3 rats surviving 24 to 30 months following a single exposure to 0.25 mg/l $Ni(CO)_4$ (i.e., 233 mg/m^3) 2 developed pulmonary neoplasms (1 anaplastic carcinoma; 1 papillary bronchial adenoma); 3) the only rat to survive 24 months following exposure to 0.06 mg/l $Ni(CO)_4$ (i.e., 56 mg/m^3) developed a clear-cell adenocarcinoma; and 4) of the 13 rats surviving 26 to 27 months following chronic exposure to 0.03 mg/l $Ni(CO)_4$ (i.e., 28 mg/m^3) 2 animals developed neoplasms (1 adenocarcinoma; 1 squamous cell carcinoma). It should be noted that overall 4 of the 7 lung neoplasms had metastasized to other organs. In the second study all had metastasized. Although the number of survivors is too small to draw definite conclusions, the results of these studies suggest carcinogenic potential. It is also important to note that tumors only appeared after 24 months or more.

7.1.1.3. Nickel Oxide, NiO

The importance of the method utilized for generating NiO has been discussed in Sections 3 and 6. The manner in which NiO was generated in various studies has been noted in Table 7.1.

The carcinogenicity of nickel (II) oxide has not been well studied in experimental animals. Wehner et al. exposed 102 Syrian hamsters to 53.2 mg/m^3 nickel oxide (i.e., 42 mg Ni/m^3) for 7 hr/day, 5 days/wk for up to 2 years (Wehner et al., 1975). Half of these animals were also exposed to cigarette smoke twice before and once after the 7 hour exposure period. A control group was maintained for each treatment regimen.

Heavy pulmonary nickel oxide burdens resulted in pneumoconiosis but did not result in specific toxicity, mortality or carcinogenic activity (Wehner et al., 1984). A significant increase in laryngeal lesions was reported in nickel exposed groups. It appears that Wehner et al., like Hueper and Payne, observed a lack of pathological response of hamsters to airborne nickel. Three malignant musculoskeletal tumors were found among the nickel-exposed animals. No such tumors were observed among the control animals.

Wehner et al. also investigated the effects of chronic inhalation of nickel-enriched fly ash (NEFA, 6 percent nickel) in Syrian hamsters (Wehner et al., 1981). Four groups of 120 male hamsters were exposed to 70 Mg/l NEFA, 17 Mg/l NEFA, 70 Mg/l fly ash (FA, 0.3 percent nickel) or to filtered air (sham-exposed controls). The exposures were for 6 hr/day, 5 days/wk for up to 20 months. Actual nickel forms and concentrations were not specified. Five animals from each group were killed after 4, 8, 12, and 16 months of exposure. An additional 5 animals were removed from exposure at the same time points and monitored without exposure until the end of the study although heavy deposits of NEFA in the lungs were demonstrated. Exposures had no significant effect on body weight or life span. Therefore, a maximum tolerated dose was not utilized. The severity of the interstitial reaction and bronchiolization was greatest in the high-NEFA and FA-exposed groups as compared to the low-NEFA group, suggesting that these effects were related more to the actual dust

concentrations than to the nickel levels. While two malignant primary thorax tumors were found in two hamsters of the high-NEFA group, no statistically significant carcinogenic response was evident.

Takenaka and co-workers assessed the pulmonary effects of nickel oxide aerosols in Wistar rats (Takenaka et al., 1985; Glaser et al., 1986). Twenty to 40 male Wistar rats were continuously exposed to NiO aerosols at concentrations of 0.076 or 0.25 mg/m^3 (i.e., 0.06 and 0.2 mg Ni/m^3, respectively) for 18 months. Control animals were also maintained. The exposure period was reportedly followed for an additional 13 month observation period, however, the survival times were significantly reduced in the exposed animals. Although microscopic evaluation revealed severe pulmonary alveolar proteinosis and lung fibrosis no primary lung tumors were induced. The authors stated that the survival of the animals was too short to adequately evaluate carcinogenicity (Glaser et al., 1986).

Horie et al. also presented limited information on the results of a study in which male Wistar rats were exposed to nickel oxide (Horie et al., 1985). The rats were exposed for 6 hr/day, 5 day/wk for 1 month to NiO concentrations of 0 (filtered air, N=5), 0.6 mg/m^3 (N=6), or 8.0 mg/m^3 (N=8) and subsequently monitored for 20 months. Most of the lungs from exposed rats exhibited hyperplastic and metaplastic changes. Pulmonary adenomatosis was detected in 2 rats (1 from each of the Ni-exposed groups) and a papillary adenocarcinoma occurred in 1 rat (0.6 mg/m^3 group). The results reported from this study may again be suggestive but in light of the small number of animals employed and the absence of details regarding the experimental methods and results (survival time, etc.), definitive conclusions cannot be drawn.

The results of studies to date assessing the carcinogenicity of NiO following inhalation exposure are negative in hamsters and inconclusive in rats. These and other studies, indicate that hamsters may be unresponsive to the carcinogenic effects of nickel. The rat studies suffer from either a small number of animals studied or a less than lifetime exposure. However, studies utilizing lower exposure levels which hopefully would result in lower mortality rates need to be conducted.

Pott et al. (1987) have evaluated the carcinogenic potential of NiO in the respiratory tract following intratracheal administration. Female Wistar rats received a total dose of 0, 50 or 150 mg Ni/animal. Animals were maintained for up to an additional 2.5 years. The lung tumor rats in the saline control, 50 (10 x 5 mg Ni) and 150 mg Ni (10 x 15 mg Ni) were 0, 27 and 31.6%, respectively.

7.1.1.4. Nickel Subsulfide, Ni_3S_2

Three studies have been conducted to evaluate the pulmonary tumorigenicity of nickel subsulfide (Ottolenghi et al., 1974; Fisher et al., 1986; Pott et al., 1987). One study utilized inhalation exposure and two utilized intratracheal administration.)

Ottolenghi et al. exposed Fischer 344 rats to filtered air or 0.97 mg Ni_3S_2/m^3 (i.e., 0.71 mg Ni/m^3) 6 hr/day, 5 day/wk for 78-80 weeks (Ottolenghi et al., 1974). The animals were observed for an additional 30 weeks. The study design included two subtreatments in a 2^4 factorial arrangement. The two subtreatments were: 1) pre-exposure for one month to 0.97 mg/m^3 Ni_3S_2 6 hr/day, 5 day/week; and 2) injection of the pulmonary infarction agent, hexachlorotetrafluorobutane (HTFB). The design resulted in 16 subgroups, allowing simultaneous estimation of the effect of each of the four factors (i.e., pre- treatment, HTFB, sex and Ni_3S_2 exposure). See Table 7.2.

Table 7.2. Factorial Design of Nickel Subsulfide Rat Inhalation Study by Ottlenghi et al, 1974.

Treatment	Subtreatment							
	Pre-Exposure Injection		No Injection		No Pre-Exposure Injection		No Injection	
	Male	Female	Male	Female	Male	Female	Male	Female
Control	29	28	28	30	32	32	31	31
Nickel Subsulfide (Ni_3S_2)	29	28	22	26	39	24	32	26

Sources: Ottolenghi et al, 1974 and EPA Health Assessment Document for Nickel and Nickel Compounds, Sept. 1986 (EPA/600/8-83/012FF).

Mortality, body weight, and lung effects were not significantly affected by pre-treatment, HTFB injection or sex. Mortality during the first year of the study was essentially the same in control and Ni_3S_2 exposed animals. Higher mortality was exhibited in the exposed group during the last 26 weeks of the exposure (i.e., week 52 to 78). By the end of the exposure period (i.e., 78 weeks) the mortality rates were approximately 18 percent in the control group and 29 percent in the nickel- exposed group. The difference in mortality rates was more pronounced by the end of the observation period (i.e 108-110 weeks), 94 percent in Ni-exposed vs 68 percent for controls. Body weight changes paralleled the mortality effects.

The lungs were significantly affected by Ni_3S_2 treatment. Exposure caused hyperplasia, metaplasia and neoplasia (adenoma, adenocarcinoma, squamous cell carcinoma, and fibroma). Furthermore, these changes and tumors were in both the bronchiolar and alveolar regions. The incidence of hyperplastic and neoplastic changes is shown in Table 7.3. The numerical discrepancy between Tables 7.2 and 7.3 is explained by the fact that several animals in each group were not examined. Among the 226 exposed animals post-mortem examinations of 208 were conducted; the remaining 18 were not examined due to advanced autolysis or cannibalism. Likewise, of 241 control animals post-mortem examinations were conducted on 215 animals.

Two groups of investigators evaluated the carcinogenic activity of intratracheally administered Ni_3S_2 (Fischer et al., 1986; Pott et al., 1987). Fischer et al. (1986) utilized mice and administered 0,0.024, 0.056, 0.156, 0.412 or 1.1 mg Ni_3S_2/kg, weekly for 4 weeks. Following exposure the animals were observed for lung tumor formation for up to 27 months. No pulmonary tumors were observed in the control or treated groups.

Pott et al. (1987) utilized rats and slightly higher dose levels. Female Wistar rats received a total dose of 0, 0.94, 1.88 or 3.75 mg Ni/animal. Animals were maintained for up to an additional 2.5 years. The lung tumor rates in the controls, 0.94 (15 x 0.063 mg Ni), 1.88 (15 x 0.125 mg Ni), and 3.75 mg Ni (15 x 0.25 mg) were 0, 14.9, 28.9 and 30%, respectively.

7.1.2. Oral Administration

See Table 7.4 for a summary of the studies performed by oral administration.

Schroeder and coworkers have conducted several studies of the carcinogenic potential of nickel salts in drinking water (Schroeder et al., 1964; Schroeder et al., 1974; Schroeder and Mitchener, 1975). In the first study (1964), conducted on mice, the diet was considered to be chromium-deficient and therefore the study was repeated by Schroeder and Mitchener in 1975 (Schroeder and Mitchener, 1975). Only the results of the latter study on mice will be discussed here. Male and female Swiss mice (N=108) were given 5 ppm nickel as nickel acetate in their drinking water throughout their lifetime. The nickel treated animals exhibited a longer lifespan than the controls. Thus a maximum tolerated dose was not utilized in this study. Compared to the control group and other trace metal exposure groups (V, Ti, Pb, Ba, Be, and Hg) the nickel treated group exhibited the fewest number of tumors. Tumors were found in 14 of 81 nickel treated animals and in 19 of 88 controls.

In the third study (Schroeder et al., 1974) male and female Long-Evans rats (N=104) were also exposed to 5 ppm nickel as nickel acetate in their drinking water throughout their lifetimes. The average daily nickel intake was estimated by the authors to be 2.6 Mg/rat for the control animals and 37.5

Table 7.3. Hyperplastic and Neoplastic Changes in Lungs of Rats Exposed to Nickel Sulfide[a]

Pathological Changes	Controls		Nickel Subsulfide	
	Males (108)[b]	Females (107)[b]	Males (110)[b]	Females (98)[b]
Typical hyperplasia	26 (24)	20 (19)	68 (62)[c]	65 (66)[c]
Atypical hyperplasia	17 (16)	11 (10)	58 (53)[c]	48 (49)[c]
Squamous metaplasia	6 (6)	4 (4)	20 (18)[d]	18 (18)[d]
Tumors:				
Adenoma	0 (0)	1 (1)	8 (7)[e]	7 (7)[d]
Adenocarcinoma	1 (1)	0 (0)	6 (5)[d]	4 (4)[e]
Squamous cell carcinoma	0 (0)	0 (0)	2 (2)	1 (1)
Fibrosarcoma	0 (0)	0 (0)	1 (1)	0 (0)

[a] Values represent the number of affected animals in each group. Percent of affected animals is given in parentheses.

[b] Number of animals examined.

[c] $p < 10^{-5}$, Fisher's Exact Test

[d] $p < 0.01$, Fisher's Exact Test

[e] $p < 0.05$, Fisher's Exact Test

Sources: Ottolenghi et al, 1974 and EPA Health Assessment Document for Nickel and Nickel Compounds, Sept. 1986 (EPA/600/8-83/012FF).

Table 7.4. Summary of Experimental Carcinogenicity Studies of Nickel and Nickel Compounds Following Oral Exposure.

Exposure	Animal Species	2 Year Survival	Responses/Observations	Reference
Nickel Acetate ($NiCH_3COOH$):				
Exposed to 0 or 5 ppm Ni in drinking water for life (N=52 of each sex)	Swiss Mice	Controls: 10% (each sex) Treated: 10% of males; 10-25% in females	Control: 10/43 (23.3%) tumor incidence in males (4 lung tumors, 2 lymphoma leukemia); 9/45 (20%) tumor incidence in females (1 lung and 8 lymphoma leukemia) Treated: 4/37 (10.8%) tumor incidence in males (2 lung and 0 lymphoma leukemia); 10/44 (22.7%) tumor incidence in females (1 lung and 12 lymphoma leukemias)	Schroeder and Mitchener J Nutr 105:452-458, 1975
Exposed to 0 or 5 ppm Ni in drinking water for lifetime (N=52 of each sex)	Long-Evans Rats	Control: > 50% Treated: > 50%	Control: tumor incidence - 13/40 (32.5%, 4 were malignant) in males; 18/35 (51.4%,7 were malignant) in females. Treated: tumor incidence - 10/26 (38.5%, 2 were malignant) in males; 19/36 (52.8%, 3 were malignant) in females. Tumor sites were not specified.	Schroeder et al J Nutr 104:239-243, 1974
Nickel Sulfate ($NiSO_4$):				
0, 100, 1000, or 2500 ppm Ni for 2 years in diet.	Wistar Rats	0 ppm: 6/50 100 ppm: 15/50 1000 ppm: 14/50 2500 ppm: 9/50	Depressed body weight in 1000 and 2500 ppm groups. Increased heart/body weight ratio and decreased liver/body weight ratio in females on 1000 and 2500 ppm diets.	Ambrose et al J Food Sci Technol 13:181-187, 1976
Same exposure as above	Beagle Dogs	6/6 for all groups	Effects seen only in 2500 ppm group: depressed body weight, decreased hematocrit and hemoglobin, polyuria, increased kidney and liver/body weight ratios, histological changes in lungs, hyperplasia of bone marrow in 2/6 animals.	Ambrose et al, ibid

Mg/rat for the exposed animals. Treated animals did not significantly differ from controls in survival, longevity, specific lesions or tumor incidence. The tumor site(s) were not specified. The tumor incidence was 31/57 in controls and 29/62 in exposed.

All three studies by Schroeder and coworkers produced negative results. However, only one exposure level (5 ppm) was investigated and no signs of toxicity were seen. Note that a dietary level of 100 ppm in food was found to be a NOEL (see Section 5.4), therefore, it is highly unlikely that 5 ppm is near a maximum tolerated dose. In addition, site-specific tumor incidence was not reported, therefore, these studies can only be considered inconclusive with respect to the carcinogenic potential of soluble nickel salts in drinking water of rats and mice.

A chronic study of nickel sulfate in the diet of experimental animals has also been reported (Ambrose et al., 1976). Nickel sulfate hexahydrate ($NiSO_4.6H_2O$) containing 22.3 percent nickel, was added to the diet of Wistar-derived rats and beagle dogs for two years. The dietary concentrations were 0, 100, 1000, and 2500 ppm nickel. Fifty rats (25 of each sex) and 6 beagle dogs (3 of each sex) were assigned to each of the four exposure groups.

By the end of the exposure period (104 weeks) approximately 75 percent of the female and 80 percent of the male rats had died. The survival rates were similar across all groups. Body weight was depressed in rats on the 1000 and 2500 ppm diets. Histologic findings were essentially negative. The authors stated that the distribution of lesions found did not appear to be exposure related.

All the dogs survived the two year exposure period and only the highest exposure group appeared to be affected. No characteristic histopathologic lesions were found in dogs receiving 100 and 1000 ppm nickel. All dogs on 2500 ppm exhibited histological changes in their lungs. Lung pathology included multiple subpleural peripheral cholesterol granulomas, bronchiolectasis, emphysema, and focal cholesterol pneumonia. The only other change observed consisted of granulocytic hyperplasia of the bone marrow in 2 of the 6 dogs on 2500 ppm diets.

The results of the dietary rat study (Ambrose et al., 1976) appear adequate to detect the carcinogenic potential of nickel sulfate in rats following ingestion. The dog study may not be adequate to detect cancer induction potential due to the relatively short duration of exposure and small number of animals.

7.1.3. Administration by Injection

Numerous injection studies have shown various nickel compounds to be potent carcinogens by injection. Nickel subsulfide (Ni_3S_2) has been the most extensively studied nickel compound and has been found to consistently exhibit carcinogenic activity when administered at various injection sites. Nickel metal (powder) and nickel refinery flue dust have also been found to exhibit carcinogenic activity following injection (see table 7.5a).

Sunderman (1984a), Skaug et al., (1984), and Gilman (1962; 1966) have compared the carcinogenic potency of nickel subsulfide (Ni_3S_2) and nickel oxide (NiO) following injection. Nickel oxide exhibited a tumor incidence rate very similar to that of Ni_3S_2.

The only other insoluble or sparingly soluble nickel compound which has been evaluated for carcinogenic activity following injection is amorphous nickel sulfide (NiS). No carcinogenic activity was exhibited by this form of nickel (Sunderman and Maenza, 1976). Unfortunately, crystalline NiS has not

Table 7.5a. Experimental Carcinogenicity Studies of Nickel Compounds Following Injection

Exposure	Animal Species	Responses/Observations	Reference
Nickel Acetate ($Ni(CH_3COO)_2$)			
Intramuscular injection:			
35 mg/kg monthly for 4-6 months	Fischer rats	22% rats with sarcomas	Haro et al 1968 as reviewed by Riguat, 1983
Intraperitoneal injection:			
24 injections 3/week at 72, 180 or 360 mg/kg	Strain A mice	untreated control: 31% tumor incidence, 0.28 tumors/animal saline control: 37%, 0.42/animal 72 mg: 44%, 0.67/animal 180 mg: 50%, 0.71/animal 360 mg: 63%, 1.26/animal	Stoner et al 1976
25 x 1 mg or 50 x 1 mg	Wistar rats	25 x 1 mg: 3/35 (8.6%) 50 x 1 mg: 5/31 (16%) control: 1/67 (1.5%)	Pott et al (1989, 1990) as cited by IARC 1990
Nickel carbonyl ($Ni(CO)_4$)			
Intravenous injection:			
6 x 9 mg Ni/kg	Sprague-Dawley rats	19/121 (15.7%) rats with malignant tumors at various sites, 2/47 rats with pulmonary lymphomas ($p<0.05$)	Lau et al 1972
Nickel Chloride ($NiCl_2$)			
Intraperitoneal injection:			
50 x 1 mg	Wistar rats	50 x 1 mg: 4/32 (12.5%) Control: 1/67 (1.5%)	Pott et al (1989, 1990) as cited by IARC 1990

Table 7.5a (cont)

Exposure	Animal Species	Responses/Observations	Reference
Nickel Hydroxide ($Ni(OH)_2$)			
Intramuscular injection:			
5 mg/thigh of unspecified form	Wistar rats Fischer rats	Wistar rats: 48% local sarcomas (19/40) Fischer rats: 75% local sarcomas (15/20)	Gilman 1966, 1965 as reviewed by Rigaut, 1983 or Gilman and Yamashiro 1985
120 umol in air dried gel, cyrstalline, or colloidal form	Wistar rats	air-dried gel: 5/19 (26.3%) with sarcomas (2 metastasis to lung) crystalline: 3/20 (15%) with sarcomas (1 metastasis to lung) colloidal: 0/20	Kasprzak and Poirier, 1985
Intraperitoneal injection:			
50 x 1 mg	Wistar rats	50 x 1 mg: 3/33 (9%) control: 1/67 (1.5%)	Pott et al (1989, 1990) as cited by IARC 1990
Nickel metal (powder)			
Intramuscular injection:			
0.02 ml of a 0.05% Ni suspension in 2.5% gelatin	C57BL mice	No tumors	Hueper 1955 as cited by EPA 1986
28.3 mg in 0.4 ml serum	Hooded female rats	10/10 (100%) rats with local rhabdomyosarcomas Historic control - 0%	Heath and Daniel 1964 as cited by EPA 1986 and IARC 1990
50 mg	Fischer rats	66% rats with sarcomas	Haro et al 1968 as cited by EPA 1986 and Kasprzak et al 1983
5 monthly injections of 5 mg Ni in 0.2 ml trioctanoin	Fischer rats	38/50 (76%) rats with fibrosarcomas control - 0/50	Furst and Schlauder, 1971 as cited by EPA 1986 and IARC 1990

Table 7.5a (cont)

Exposure	Animal Species	Responses/Observations	Reference
same as above	Hamsters	2/50 hamsters with fibrosarcomas control - 0/50	ibid
20 mg	rat	17/20 (85%) developed local sarcomas control - 0/56	Berry et al 1984 as cited by IARC 1990
20 mg in paraffin oil	rat	20 mg: 14/30 (47%) local tumors control: 0/60	Judd et al 1987 as cited by IARC 1990
3.6 or 14.4 mg/rat	Fischer rats	3.6 mg - 0/10 14.4 mg - 2/10 (20%) with local tumors control - 0/20	Sunderman and Maenza 1976 as cited by EPA 1986; Grandjean 1986; and IARC 1990
14 mg	Fischer rat	13/20 (65%) with sarcomas penicillin control - 0/44 glycerol control - 0/40	Sunderman 1984a as cited by EPA 1986 and IARC 1990
Intraperitoneal injection:			
10 x 7.5 mg (total 75 mg)	Wistar rat	46/48 (95.8%) abdominal tumors	Pott et al 1987 Exp Pathol 32:129-152
1 x 6 mg, 2 x 6 mg, or 25 x 1 mg	rat	6 mg - 4/34 (11.7%) local tumors 6 mg x 2 - 5/34 (14.7%) 1 mg x 25 - 25/35 (71.4%)	Pott et al 1990 as cited by IARC 1990
Intrapleural injection:			
5 monthly injections of 0.5 ml of 12.5% (v/v) suspension (total dose 31.25 mg)	Osborne-Mendal (female) rats	4/12 rats with injection site sarcomas vehicle - 0/70	Hueper 1952 as cited by EPA 1986 and IARC 1990
0.02 ml of a 0.05% Ni suspension in 2.5% gelatin	C57BL mice	No tumors	Hueper 1955 as cited by EPA 1986
5 monthly injections of 5 mg Ni	Fischer rats	2/10 (20%) with pleural mesotheliomas 0/20 in control animals	Furst et al 1973 as cited by EOA 1986 and IARC 1990

Table 7.5a (cont)

Exposure	Animal Species	Responses/Observations	Reference
Intrafemoral injection:			
21 mg	Osborne-Mendal rats	4/17 (23.5%) rats with tumors, 1 squamous cell carcinoma, 3 osteosarcomas	Hueper 1952 as cited by EPA 1986 and IARC 1990
unspecified	rat	9/20 (45%) local tumors	Berry et al 1984 as cited by IARC 1990
Intravenous injection:			
weekly for 2 weeks 0.05 ml of a 0.005% Ni in 2.5% gelatin	C57BL mice	No tumors No control group	Hueper 1955 as cited by EPA 1986
0.5 ml/kg x 6 of a 1% Ni suspension in 2.5% gelatin	Rabbits	No tumors	Ibid
0.5 ml/kg x 6 of a 0.5% Ni suspension	Rats	7/25 (28%) rats with tumors No control group	Ibid
Intrarenal injection:			
5 or 10 mg	Sprague-Dawley rats	No cancer of the kidney	Jasmin et al , 1979
7 mg	Fischer rats	0/18 rats with renal tumors	Sunderman et al 1984
Subcuteneous injection:			
5 mg/mouse	mice	No tumors	Wang et al 1989 Hua Hsi I Ko Ta Hsueh Hsueh Poa 20(3)307-310
4 implants of metallic nickel pellets	rats	Ni treated: 5/10 (50%) control: 0	Mitchell et al 1960 as cited in IARC 1990
Nickel Oxide (NiO)			
Intramuscular injection:			
14 mg Ni/rat	Fischer 344 male rats	14/15 rats with sarcomas Control: 0/40	Sunderman 1984a

Table 7.5a (cont)

Exposure	Animal Species	Responses/Observations	Reference
20-30 mg	Wistar rats	65% with sarcomas	Gilman 1966
5 mg	Swiss mice	66% with sarcomas	ibid
20-30 mg	Fischer rats	5% with sarcomas	ibid
20 mg	Wistar rats	21/32 (65.6%) local tumors	Gilman 1962
5 mg	Swiss mice	33/50 (66%) with tumors	ibid
5 mg	C3H mice	23/52 (44%) with tumors	ibid
Intraperitoneal injection:			
2 x 500 mg (total 1000 mg)	Wistar rats	46/47 (97.9%)	Pott et al Exp Pathol 32:129-152, 1987
single injection of 25 or 100 mg	Wistar rats	25 mg: 12/34 (35.3%) 100 mg: 15/36 (41.7%)	Pott et al (1989, 1990) as cited by IARC 1990
Intrapleural injection:			
1 x 10 mg in 0.4 mg saline	male Wistar rats	1 x 10 mg: 31/32 (96.%) rats with sarcomas Control: 0/32 rats with tumors	Skaug et al, 1985 In: Progress in nickel toxicology, Eds: SS Brown and FW Sunderman, pp 37-40
unspecified dose	rats	25/32 (78%) tumor incidence	Skaug et al 1984 Ann Clin Lab Sci 14:400
Intrarenal injection:			
7 mg Ni/rat	Fischer 344 male rats	No tumors observed in 12 rats	Sunderman et al 1984

Table 7.5a (cont)

Exposure	Animal Species	Responses/Observations	Reference
Nickel refinery flue dust (20% $NiSO_4$, 57% Ni_3S_2, 6.3% NiO)			
Intramuscular injection:			
20 mg injection in thigh	Hooded rats	19/27 (70.3%) rats with sarcomas controls: 0/30	Gilman and Ruckerbauer, 1962
10 mg each thigh	mice	20/36 (55.6%) mice with sarcomas Controls: 0/47	ibid
Nickel Subsulfide (Ni_3S_2)			
Intramuscular injection:			
20 mg/thigh	Wistar rat	25/28 (89.3%) rats with tumors - significant increase in tumors and decrease in latency ($p<0.01$). 95% metastases rate.	Gilman, 1962, Cancer Res 22:158-162
5 mg/thigh	Swiss and C3H mice	Swiss - 27/45 (60%) C3H - 9/18 (50%)	ibid
10 mg powder, 500 mg fragments, 500 mg discs, or 10 mg in diffusion chamber	Fischer rat	10 mg - 19/20 (95%) 500 mg fragments - 5/7 (71.4%) 500 mg discs - 14/17 (82.3%) 10 mg in diffusion chamber - 14/17 (82.3%) control 1/19 (5.3%)	Gilman and Hercher, 1963 as cited by IARC 19990
unspecified dose	NIH black or Hooded rat	NIH - 28/28 (100%) Hooded - 14/23 (60.9%)	Daniel 1966 as cited by IARC 1990
0.6, 1.2, 2.5, or 5 mg	rat	0.6 - 7/30 (23.3%) 1.2 - 23/30 (76.7%) 2.5 - 28/30 (93.3%) 5 mg - 29/30 (96.7%)	Sunderman et al 1976 as cited by IARC 1990

Table 7.5a (cont)

Exposure	Animal Species	Responses/Observations	Reference
5 or 10 mg	Syrian hamster	Local sarcomas 5 mg - 4/15 (26.7%) 10 mg - 12/17 (70.6%) control - 0/14	Sunderman et al, 1983 as cited by IARC 1990
3.3 or 10 mg	Fischer rats	3.3 mg: 85% 10 mg: 97% control: 0%	Mason 1972
10 mg	NMRI mice	Local sarcomas 10 mg - 4/8 (50%) in males and 4/8 (50%) in females	Oskarsson et al, 1979 as cited by IARC 1990
20 mg/rat on day 6 of gestation	pregnant rats	Local sarcomas in all dams, no excess tumors in progeny	Sunderman and McCully, 1983, as cited by Grandjean, 1986
10 mg/rat	Fischer and Hooded rats	Fischer - 59/63 (94%) (25.4% metastesized) Hooded - 11/20 (55%) (81.9% metastasized)	Yamashiro et al, 1980 as cited by Grandjean, 1986 and IARC 1990
40 umol (3.2 mg/rat)	Wistar rats	16/20 (80%) tumor incidence rate	Kasprzak et al, 1983 Carcinogenesis 4:275-279 and as cited by Grandjean, 1986
2.5 mg (1.8 mg Ni)	male Fischer rats	100% incidence of sarcomas	Kasprzak et al 1987 Carcinogenesis 8(7)1005-1011
Intraperitoneal injection:			
25 mg	Wistar female rats	64.3%	Pott et al Exp Pathol 32:129-152, 1987
1 x 6 mg, 2 x 6 mg, or 25 x 1 mg	Wistar female rats	1 x 6 mg: 20/36 (55.6%) 2 x 6 mg: 23/35 (65.7%) 25 x 1 mg: 25/34 (73.5%)	Pott et al (1989, 1990) as cited by IARC 1990

Table 7.5a (cont)

Exposure	Animal Species	Responses/Observations	Reference
Subcutaneous injection:			
5 mg	NMRI mice	Local tumors 5 mg - 4/8 (50%) in female and 7/8 (87.5%) in males	Oskarsson et al, 1979 as cited by IARC 1990
3.3 or 10 mg	Rat	Incidence of local tumors: control: 0% 3.3 mg - 37/39 (94.9%) 10 mg - 37/40 (92.5%)	Mason 1972 as cited by IARC 1990
5 mg	mice	36% incidence of tumors at 62 weeks post-injection. Tumors were mainly fibrosarcomas, metastasis to lungs and/or liver were reported.	Wang et al 1989 Hua Hsi I Ko Ta Hsueh Hsueh Pao 20(3)307-310 (Eng abstract)
Intrapleural injection:			
single injection of 10 mg	rats	28/32 (87.5%) tumor incidence controls: 0/32	Skaug et al, 1985
Nickel Sulfate ($NiSO_4$)			
Intramuscular injection:			
5 mg	Wistar rats	No tumors	Gilman 1962
66 umole/rat (15 x 4.4 umole doses)	Wistar rats	0/20	Kasprzak et al 1983
Intraperitoneal injection:			
50 x 1 mg	Wistar rats	50 x 1 mg: 6/30 (20%) control: 1/67 (1.5%)	Pott et al (1989, 1990) as cited in IARC 1990
Nickel sulfide (amorphous, NiS)			
Intramuscular injection:			
approximately 5 or 20 mg/animal	Fischer rats	No tumors	Sunderman and Maenza, 1976

Sources: Original articles, EPA 1986 (1), ATSDR, 1988 (2)

been evaluated. Based on the genotoxicity data crystalline NiS would be expected to be the "active" form of NiS. Amophous NiS does not exhibit genotoxicity, however, crystalline NiS exhibited genotoxic potential approximately equivalent to Ni_3S_2.

Nickel carbonyl is a lipid soluble form of nickel and has been shown to exhibit carcinogenic potential following inhalation (see Section 7.1.1.2) and injection (see Table 7.5a).

A limited amount of data on the carcinogenic potential of a variety of soluble nickel compounds administered by injection are also available (see Table 7.5a). Nickel acetate ($Ni(CH_3COO)_2$) and nickel hydroxide ($Ni(OH)_2$) have been shown to be carcinogenic (Haro et al., 1968; Stoner et al., 1976; and Gilman 1966; Kasprzak et al., 1983, respectively). Nickel chloride ($NiCl_2$) has only been evaluated by one group of investigators and no treatment related tumors were observed (Wang et al., 1989). Nickel sulfate ($NiSO_4$) has been studied by two groups of investigators (Gilman 1962; Kasprzak et al., 1983). Both investigators reported negative results. It is worth noting that the dose levels utilized in the nickel chloride and nickel sulfate studies were relatively low compared to many of the other injection studies and therefore carcinogenic activity may be produced at higher exposures.

To date, injection administration is the only route of exposure for which data are available for direct comparison of carcinogenic potential of various nickel compounds. In an extensive series of studies Sunderman and co-workers tested 18 nickel compounds for carcinogenicity in 414 male Fischer rats by a single intramuscular injection at equivalent dosages (14 mg Ni/rat) (Sunderman, 1984a). The animals were then monitored for 2 years.

Based on the incidence of sarcomas produced at the injection site the nickel compounds were classified into groups (see Table 7.5): Group A: nickel subsulfide (Ni_3S_2), crystalline nickel monosulfide (beta-NiS), and nickel ferrosulfide (referred to as Ni_4FeS_4 by the authors) induced sarcomas in 100 percent of the rats; Group B: nickel oxide (NiO), nickel subselenide (Ni_3S_2), nickel subarsenide (NiAsS), nickel disulfide (NiS_2) and nickel subarsenide (Ni_5As_2) induced sarcomas in 85 to 93 percent of the rats; Group C: nickel dust, nickel antimonide (NiSb), nickel telluride (NiTe), nickel monoselenide (NiSe) and nickel subarsenide ($Ni_{11}As_8$) induced sarcomas in 50 to 65 percent of the rats. Group D: amorphous nickel monosulfide (NiS) and nickel chromate ($NiCrO_4$) induced local sarcomas in 6 to 12 percent of the rats; Group E: nickel monoarsenide (NiAs), nickel titanate ($NiTiO_3$), and ferronickel alloy ($NiFe_{1.6}$) did not induce sarcomas.

The nickel-induced sarcomas included rhabdomyosarcomas, 52 percent; fibrosarcomas, 18 percent; undifferentiated sarcomas, 13 percent; osteosarcomas, 8 percent; and miscellaneous and unclassified sarcomas, 9 percent. Distant metastases were found in 109 of 180 sarcoma-bearing rats. No sarcomas occurred at the injection site of 84 control rats receiving injections of the vehicle. No significant differences were observed among the test groups in the proportions of histological types of sarcomas or in frequencies of metastases. Nickel carcinogenic activity was found to be correlated to: 1) nickel mass fraction in the respective compounds (nickel mass fraction represents the proportional weight of nickel per unit weight of the compound); and 2) nickel stimulation of erythropoiesis (Sunderman, 1984a).

Sunderman et al. (1979) have also reported induction of renal cancers in 18 of 24 Fischer rats by intrarenal injection of 10 mg nickel subsulfide (as cited by Sunderman, 1984b; EPA, Sept. 1986a).

Albert et al. (1982, as cited by Sunderman, 1984b; EPA, Sept. 1986a) demonstrated the induction of malignant ocular tumors in 14 of 15 Fischer rats

Table 7.5. Summary of Survival Data and Sarcoma Incidence in Carcinogenesis Tests of 18 Nickel Compounds.

Category	Test Substance	Survivors at 2 years/ total number of rats	Rats with sarcomas/ total number of rats	Median tumor latency (weeks)	Median survival period (weeks)	Rats with metastases/ number with sarcomas
Controls	Glycerol vehicle	25/40 (63%)	0/40 (0%)	-	> 100	-
	Penicillian vehicle	24/44 (55%)	0/44 (0%)	-	> 100	-
	All Controls	49/84 (58%)	0/84 (0%)	-	> 100	-
Class A	Nickel Subsulfide (Ni_3S_2)	0/9 (0%)[c]	9/9 (100%)[c]	30	39[b]	5/9 (56%)
	Nickel Monosulfide (NiS)	0/14 (0%)[c]	14/14 (100%)[c]	40	48[b]	10/14 (71%)
	Nickel Ferrosulfide (Ni_4FeS_4)	0/15 (0%)[c]	15/15 (100%)[c]	16	32[b]	10/15 (67%)
Class B	Nickel Oxide (NiO)	0/15 (0%)[c]	14/15 (93%)[c]	49	58[b]	4/14 (29%)
	Nickel Subselenide (Ni_3Se_2)	0/23 (0%)[c]	21/23 (91%)[c]	28	38[b]	18/21 (86%)
	Nickel Sulfarsenide (NiAsS)	0/16 (0%)[c]	14/16 (88%)[c]	40	57[b]	10/14 (71%)
	Nickel Disulfide (NiS_2)	0/14 (0%)[c]	12/14 (86%)[c]	36	47[b]	6/12 (50%)
	Nickel Subarsenide (Ni_5As_2)	0/20 (0%)[c]	17/20 (85%)[c]	22	44[b]	9/17 (53%)
Class C	Nickel Dust	4/20 (20%)[b]	13/20 (65%)[c]	34	42[b]	6/13 (40%)
	Nickel Antimonide (NiSb)	9/29 (31%)[b]	17/29 (59%)[c]	20	66[b]	10/17 (59%)
	Nickel Telluride (NiTe)	12/26 (46%)	14/26 (54%)[c]	17	80[b]	8/14 (57%)
	Nickel Monoselenide (NiSe)	7/16 (44%)	8/16 (50%)[c]	56	72[b]	3/8 (38%)
	Nickel Subarsenide ($Ni_{11}As_8$)	5/16 (31%)[a]	8/16 (50%)[c]	33	88[b]	6/8 (75%)
Class D	Amorphous Nickel Monosulfide (NiS)	5/25 (20%)[b]	3/25 (12%)[b]	41	71[b]	3/3 (100%)
	Nickel Chromate ($NiCrO_4$)	10/16 (63%)	1/16 (6%)	72	>100	1/1 (100%)
Class E	Nickel Monoarsenide (NiAs)	13/20 (65%)	0/20 (0%)	-	>100	-
	Nickel Titanate ($NiTiO_3$)	11/20 (55%)	0/20 (0%)	-	>100	-
	Ferronickel Alloy ($NiFe_{1.6}$)	11/16 (75%)	0/16 (0%)	-	>100	-

[a] $p < 0.05$ versus corresponding vehicle control.
[b] $p < 0.01$ versus corresponding vehicle control.
[c] $p < 0.001$ versus corresponding vehicle control.

Source: FW Sunderman, 1984, IARC Scientific Publications, vol 53.

within eight months after a single intraocular injection of 0.5 mg nickel subsulfide.

Skaug et al. compared the carcinogenic potency of nickel subsulfide and nickel oxide by injection into the pleural cavity of rats (Skaug et al., 1984). Two years after the injection, 27 of the 32 rats (approximately 84 percent) injected with Ni_3S_2 had developed tumors in the chest cavity. The average latency was 183 days. Similarly, by two years 25 of 32 rats (approximately 78 percent) injected with NiO had developed tumors in the chest cavity. However, the average latency was longer, 277 days. All tumors were malignant and most were classified as rhabdomyosarcomas.

7.1.4. Comparative Potencies of Various Nickel Compounds

Inhalation exposure cancer bioassays to date have only examined insoluble forms of nickel for their carcinogenic potential. Likewise, only soluble forms of nickel have been evaluated following oral administration. Evidence of carcinogenic potential following inhalation exposure has been shown for nickel subsulfide (Ni_3S_2) and suggested for nickel carbonyl. Inhalation exposure to metallic nickel resulted in very poor survival, too short to properly evaluate carcinogenicity. Many rats exposed to metallic nickel by inhalation did develop squamous metaplasia and peribronchial adenomatoses. Exposure to metallic nickel by intratracheal instillation did produce a significant increase in lung tumor incidence. The results from the nickel oxide (NiO) studies utilizing inhalation exposure are negative or inconclusive. These studies were also plagued by very poor survival rates. Intratracheal instillation of nickel oxide did result in tumor production.

None of the studies utilizing oral exposure has produced evidence of carcinogenic potential. However, one should note that only nickel sulfate ($NiSO_4$) has been adequately evaluated in rats by this route.

To date, injection administration is the only route of exposure from which data are available for comparison of carcinogenic potency of a variety of nickel compounds. Injection studies by Sunderman (1984a) and Skaug et al. (Skaug et al., 1984) demonstrate that based on the incidence of sarcomas produced at the injection site, nickel subsulfide and nickel oxide possess approximately equal potency following this route of exposure. The average latency was longer and the frequency of metastases was lower following nickel oxide exposure. In light of the limited data and in order to protect public health it would be prudent to regard nickel oxide to be as potent as nickel subsulfide.

When one considers all routes of exposure, 11 nickel compounds (nickel carbonyl, nickel oxide, nickel subsulfide, nickel sulfide, nickel ferrosulfide, nickel subselenide, nickel subarsenide, nickel disulfide, nickel dust, nickel antimonide, nickel telluride, nickel monoselenide) have exhibited at least one positive response in a carcinogenic assay. These positive responses include slightly water-soluble compounds (nickel carbonyl) and water-insoluble compounds (nickel subsulfide, nickel sulfide).

Water-soluble nickel compounds have limited carcinogenic activity in whole animal cancer bioassay studies. One reason for this is believed to be the poor bioavailability of nickel ions *in vivo*. *In vitro*, water-soluble nickel compounds have produced chromosomal aberrations and have displayed cell-transforming activity, however, this may be due in part because the extracellular concentration of nickel can be maintained at a high level, making the nickel ions more bioavailable to the cell. The evidence to date suggests that the bioavailability of nickel is the key to understanding

carcinogenic potential of specific nickel compounds. Once nickel compounds have entered the cell, their transforming potential may depend only on the concentration available and be independent of the original source (Coogan et al., 1989).

The soluble compounds probably enter the cell by active transport whereas nickel compounds of low solubility are probably phagocytized. Phagocytosis of compounds such as Ni_3S_2 does not require prior solubilization (Coogan et al., 1989). Heck and Costa (1982) observed that serum proteins and other compounds of complex culture medium did not reduce the phagocytosis of insoluble crystalline nickel sulfide. Components of complex culture medium have been shown to inhibit uptake of soluble nickel compounds. Cells maintained in a minimal salts/glucose medium displayed about a 10-fold higher uptake of soluble nickel compounds and greater consequent toxicity than cells maintained in regular cell culture medium.

Interestingly, not all insoluble, particulate nickel compounds demonstrate similar carcinogenic activity. The basis of this difference is also believed to be related to differences in bioavailability as the result of selective phagocytosis. Crystalline structure, surface charge and particle size seem to be factors in phagocytic activity. Active phagocytosis represents a highly efficient mechanism for delivering large quantities of nickel ions into the cells (Sen and Costa, 1985). Following phagocytosis, the particles are solubilized in the cell, possibly as a result of the lysosomal acidification. This intracellular solubilization is important for the nuclear uptake of ionic nickel since particles of nickel cannot enter the nucleus. The nuclear levels of nickel have been shown to be higher in cells treated with crystalline nickel sulfide particles compared to cells treated with equivalent concentrations of nickel chloride (Costa, 1983; Costa and Heck, 1982).

Highly water-soluble nickel compounds are taken up only to a limited extent by cells, and the contact time of soluble nickel with cells *in vivo* is short. Exposures to water soluble nickel salts are characterized by rapid excretion (half-life approximately 1 day) compared with exposures to solid intermediates such as NiO and NiS (half-life estimated up to 3 years) (Coogan et al., 1989). Soluble particles deposited in the alveoli may be cleared rapidly, whereas particles of low solubility are cleared by a slower biphasic mechanisms. Therefore, the bioavailability of Ni^{+2} appear to be much greater for insoluble crystalline nickel compounds than for other nickel compounds. It is worth noting that according to Oskarsson and Tjalve, Herlant-Peers et al. and Muhle et al. (all as cited in Knight et al. 1988) rodent lungs contain specific nickel-binding proteins that are responsible for the avid pulmonary uptake and retention of nickel. Parenteral administration of Ni^{+2} (administered as $NiCl_2$) for 3 to 6 weeks lead to nickel accumulation in the lung (Knight et al., 1988). The accumulation was greater in the lung than in the other major organs.

Until the *in vivo* bioavailability and its relation to the carcinogenicity of nickel is better understood, there is insufficient data to quantitatively predict the relative carcinogenic potency of specific nickel compounds.

7.1.5. Selection of Animal Study for Quantitative Risk Assessment

Experimental animal studies indicate that many nickel compounds induce cancer at the site of injection. However, this route is not useful in the assessment of potential human risk from environmental exposure. The rat

dietary study which was of acceptable study design in which nickel sulfate was administered showed no evidence of cancer induction. Inhalation studies have shown evidence of carcinogenicity for nickel subsulfide and nickel carbonyl. The results from the nickel oxide studies conducted to date are inconclusive.

The study results of Ottolenghi et al. for nickel subsulfide were selected for the risk assessment for two reasons: 1) since the acute and chronically $Ni(CO)_4$ exposed animal data cannot be combined, the number of lung tumors observed is too small for an incremental unit risk to be estimated; 2) nickel subsulfide has been assessed by other routes of administration and has been shown to be a potent carcinogenic agent. All routes of administration employed, with the exception of buccal brushing of Syrian hamsters, submaxillary implantation into Fischer rats (Sunderman et al., 1978) and intrahepatic injection of Sprague-Dawley rats (Jasmin and Solymoss, 1978) and Fischer rats (Sunderman et al., 1978), have led to positive tumor response (as cited by EPA, Sept. 1986a).

In the Ottolenghi et al. study 122 male and 104 female Fischer 344 rats were exposed to 970 Mg/m^3 nickel subsulfide via inhalation for 78-80 weeks (6 hr/day, 5 day/wk). The control group, 120 male and 121 female rats, was exposed to clean air. Animals found moribund or succumbing during the study and those killed at the end of the observation period (i.e., 30 weeks) were necropsied. A small number of animals (18 in the exposed and 26 in the control groups) were not examined because of autolysis or cannibalism.

The results demonstrated a significant increase in lung tumors (adenomas, adenocarcinomas, squamous cell carcinomas, and fibrosarcomas) in the exposed 110 male and 98 female rats examined as compared with the control 108 male and 107 female rats examined. These results have been shown in Table 7.5.

Three areas of concern must be addressed before proceeding with risk extrapolation: 1) the effects of subtreatment on tumor incidence; 2) the survival rate of the control group; and 3) the survival rate of the treated vs control group. The two subtreatments (HTFB injection and pre-treatment) did not alter the effects produced by nickel subsulfide treatment. Sex of the exposed animal also did not appear to alter the effects of nickel subsulfide. Therefore, the tumor incidence will be combined across subtreatments and sex.

The normal 104 week survival rate for many bioassays utilizing the Fischer 344 rat is 50 to 60 percent (EPA, Sept. 1986a). The survival rate of the control group at the end of the study was only approximately 32 percent, however, after adjusting for the 4 weeks of subtreatment (i.e., examine mortality at 100 wk) the cumulative mortality graph in Ottolenghi et al. shows a comparable survival rate (approximately 48 percent) at this time in the control animals. Therefore, it is not necessary to adjust for control survival.

In addressing the low survival rate of the nickel subsulfide exposed group it is imperative to examine the time frame of mortality (see Table 7.6). As mentioned above, mortality during the first year of study was essentially the same in control and nickel-exposed groups. In the 76th week of the study the first observed tumor occurred. At approximately this time the mortality rates were 17 and 23 percent in the control and nickel-exposed groups, respectively. Therefore, by 76 weeks there had already been approximately 23 percent nontumor mortality in the Ni-treated group (vs. 17 percent in the control group). Taking this into account, the subsequent mortality [i.e., (0.94 (exposed group mortality) - 0.23] x 208 (animals examined) = 148 animals) in the nickel treated group due to tumors was 29/148 or approximately 20 percent. Based on these assumptions, nearly all of the difference (0.32 -

Table 7.6. Cumulative Mortality in Rats Exposed to Nickel Sulfide[a]

Time Point	Control Group	Exposed Group
52 weeks	< 5 %	< 5 %
76 weeks	17 %	23 %
104 weeks	62 %	92 %
end of study (approx. 108 weeks)	68 %	94 %

a) Source: Ottolenghi et al, 1974 - values estimated from Figure 1.

0.06 - 0.26) in the survival between the control animals (32%) and the nickel treated animals (6%), can be explained
by lung tumor mortality. Therefore, no adjustment for survival rate in the exposed animals appears necessary.

7.1.5.1. Calculation of Human Equivalent Dosage from the Ottolenghi et al. Study.

Following the EPA methodology for calculating equivalent doses we have assumed that mg/surface area/day is an equivalent dose between species (EPA,1980). Since the surface area is proportional to the 2/3rds power of body weight (W), the exposure in $mg/W^{2/3}/day$ is similarly considered to be an equivalent exposure.

$$M_r/W_r^{\ 2/3} = M_h/W_h^{\ 2/3}$$

or

$$M_r\ (W^h/W_r)^{2/3} = M_h$$

where r = rat
h = human
M = average dose per day (mg/day)
W = average body weight

When exposure is via inhalation, compounds in the form of particulate matter, such as nickel subsulfide, can be expected to be absorbed in proportion to the amount inhaled. In this case the exposure, M (mg/day), is equivalent to (I x V x R), where I is inhalation rate (m^3/day), V is exposure concentration (mg/m^3) and R is the absorption fraction. Therefore, assuming equal tumor response in both rats and humans an equivalent exposure can be derived from air intake data. Since the lung is the only organ affected (i.e., the only site of primary tumors) in the rat and nickel pharmacokinetics are uncertain, metabolic factors will be assumed to be equal across species.

The inhalation rate, I, for rats can be calculated from the recommended methodology for estimating inhalation rates (EPA, 1988). The estimated inhalation rate for the rats exposed in the Ottolenghi et al. study:

$$I = 0.80\ W_r^{0.8206}\ m^3/day$$

$$= 0.80(0.3)^{0.8206}\ m^3/day$$

$$= 0.298\ m^3/day$$

For humans, the value of 20 m^3/day for a 70 kg man is adopted as a standard breathing rate (EPA, 1985).

Before calculating an equivalent exposure level for humans the exposure level utilized in the rat study must first be adjusted for continuous lifetime exposure. The continuous lifetime exposure is:

$$(970\ ug/m^3)(6/24\ hr)(5/7\ day)(78/110\ weeks) = 122.8\ ug/m^3$$

This exposure level can also be expressed as dose in mg/day and utilized in calculating equivalent human exposure.

As stated on the previous page, the dose in mg/day is proportional to the amount inhaled (i.e., M = Ivr). Thus based on the relationships and equations stated above the equivalent exposure is:

$$(IVR)_r\ (W_h/W_r)^{2/3} = (IVR)_h$$

In the absence of experimental evidence to the contrary the fraction absorbed, R is assumed to be the same for all species. Therefore, no adjustment for absorption shall be made.

Therefore:

$$(0.298m^3/day)(122.8\ ug/m^3)(70/0.3)^{2/3} = (20m^3/day)V_h$$

Rearranging the above equation to solve for V_h:

$[(0.298m^3/day)(122.8\ ug/m^3)(70/0.3)^{2/3}]\ /\ (20m^3/day) = 70\ ugNi_3S_2/m^3$ or $51\ ugNi/m^3$

7.1.5.2. Calculation of the Incremental Unit Risk Estimation

The unit risk, from the human equivalent exposures, was calculated using GLOBAL86 and is presented in Table 7.7. The maximum likelihood estimate is $2.01 \times 10^{-3}\ (ug\ Ni_3S_2/m^3)^{-1}$ or $2.76 \times 10^{-3}\ (ug\ Ni/m^3)^{-1}$ and the upper 95% confidence limit on a linear slope is $2.75 \times 10^{-3} (ug\ Ni_3S_2)^{-1}$ or 3.77×10^{-3} $(ug\ Ni/m^3)^{-1}$.

The EPA unit risk, based on a different method for estimating inhalation rate for the rat, is also presented in Table 7.7 (see footnote for explanation of recent recalculation).

7.2. Carcinogenic Effects in Humans

Epidemiologic studies of the carcinogenic effects of nickel generally center around cohort studies of refinery workers which have found increased risks of lung cancer and nasal sinus cancer. While other types of studies have been done, cohort studies of nickel refinery workers warrant special consideration for use in quantitative risk assessment analysis in view of the high cancer risks detected and the availability of at least some exposure information. There are many studies of welders, for example, but exposure data are limited and the increase in lung cancer risks may have been more attributable to chromium than to nickel (Stern, 1983). The most comprehensive quantitative risk analyses to date were undertaken by the U.S. Environmental Protection Agency for the 1986 Health Assessment Document (EPA, Sept. 1986a). There have been no new cohort studies published since the EPA risk assessment, although further follow-up has been undertaken for several cohorts (ICNCM, 1990). The following sections review the lung cancer findings for the cohort studies used in the EPA risk assessment, and their subsequent follow-up by the International Committee on Nickel Carcinogenesis in man. These studies also report increased risk of nasal cancer. However the excess number of lung cancers were generally much greater than the excess numbers of nasal cancers. For this reason, this review and the subsequent risk analyses focus on lung cancer.

Table 7.7. Incremental Maximum Likelihood and Upper-Limit Unit Risk Estimates for Rat-to-Human Exptrapolation Using the Ottenghi et al (1974) Rat Inhalation Study of Nickel Subsulfide.

Human Equivalent Continuous Exposure	Incremental Unit Risk Estimates		
	Maximum Likelihood $(ug/m^3)^{-1}$	Upper 95% $(ug/m^3)^{-1}$	Upper 99% $(ug/m^3)^{-1}$
70 ug Ni_3S_2/m^3 (GLOBAL86)[a]	2.01×10^{-3}	2.75×10^{-3}	3.09×10^{-3}
50 ug Ni/m^3 (GLOBAL86)[a]	2.76×10^{-3}	3.77×10^{-3}	4.24×10^{-3}
46.1 ug Ni_3S_2/m^3 (EPA Potency Value)[b]	3.1×10^{-3}	4.2×10^{-3}	NA

[a] Input assumptions: 1) background responses estimated from control animal data; 2) test-type 0 (Chi-square goodness of fit test used both for selecting model and in testing the selected multistage model); 3) poly-degree set to 1; and 4) risk type - extra risk [P(d) - P(0)]/[1-P(0)], where P = lifetime probability of effect and d = dose.

[b] Corrected value differs from those listed in EPA Health Assessment Document for Nickel and Nickel Compounds (EPA/600/8-83/012FF). Original values were calculated by experimental program, GLOBAL83. (Source: personal communication April 22, 1988, from Steven Bayard).

7.2.1. Cohort Studies Considered for Quantitative Risk Assessment

7.2.1.1. The West Virginia Cohort

The first cohort, which will be referred to as the West Virginia cohort (Enterline and Marsh, 1982), consisted of 1855 workers employed by the International Nickel Company (INCO) at a nickel refinery and alloy manufacturing plant in Huntington, West Virginia. Cohort members had at least one year's service prior to 1948 when the calcining operations ceased. The cohort was followed to the end of 1977 and 99.4% of the workers were traced. Death certificates were obtained for 789 of the 791 workers who had died. Among the subset of 266 men that worked in the nickel refinery there were 113 deaths, eight of which were from lung cancer.The corresponding lung cancer standardized mortality ratio for 20 or more years after first exposure was only 1.12 (90% confidence limits of 0.56 and 2.02). Air levels of nickel were in the range of 0.01-5.0 mg/m^3. Cumulative nickel exposure was calculated by multiplying air levels of nickel by the number of months worked, and ranged from means of 4.2 to 563.8 mg Ni/m^3-months over 6 exposure groups.

The ICNCM (1990) reported a further 5 years of follow-up of this cohort up to 1982. Among workers hired before 1947 and who had 15 or more years since first exposure in calcining, there were 8 lung cancer deaths yielding an SMR estimate of 1.15 (90% confidence limits of 0.57 - 2.07). Two nasal cancer deaths were reported with 0.9 expected.

7.2.1.2. The Ontario Cohort

The second cohort under consideration will be referred to as the Ontario cohort and involved refinery workers at Copper Cliff, Ontario (Chovil et al., 1981; Roberts et al., 1984; Muir et al., 1985). A subcohort with high exposure to nickel consisted of 495 workers with five or more years' work history at a sinter plant operated by the International Nickel Company (INCO) between 1948 and 1963. Workers were followed up from 1963 to the end of 1978, a minimum of 15 years.

Eighty-five cohort members died during the follow-up period, of which 37 were lung cancer deaths. The SMR for lung cancer was 8.71, with 90% confidence limits of 6.49 and 11.45.

One criticism of Chovil et al. has been that the 25% of the cohort lost to follow-up were counted as survivors to the end of the study in 1978, thereby leading to a potential underestimation of the SMR. In addition, the EPA has voiced the concern that a large proportion of those lost to follow-up were likely to be those workers who were exposed earliest, and hence to the dustiest conditions (Section 8.1.2.1.4 of EPA, Sept. 1986a). However, Roberts et al., (1989) investigated loss to follow-up by further follow-up of a subset of 1000 workers of unknown status. They estimated that there might have been under ascertainment of total deaths by about 5%. A correction can therefore be made to the SMR's presented by Chovil et al. to account for this.

The EPA also stated a concern that the derivation of expected incident cases may be a problem in the study by Chovil et al., due to the selection process, in which a sample stratified by year of first employment was used. However, as long as the members of each stratum of the cohort were chosen in a random manner, the selection process should not affect the number of expected cases calculated.

The ICNCM (1990) reported a further follow-up of this cohort up to 1984. There were a total of 63 lung cancer deaths and 6 nasal cancer deaths among

the Copper Cliff sinter plant workers with 15 or more years since first exposure. Of the 63 lung cancer deaths, 33 had five or more years of exposure and yielded an SMR of 7.89 (90% confidence limits 5.78 - 10.56). For those who commenced work prior to 1952, the SMR estimate was 8.55 (90% confidence limits 6.15 - 11.59). These estimates are close to the SMR of 8.71 obtained from the earlier report by Chovil et al. presented above.

Exposure data were given by Roberts et al. (1984) who estimated a level of 400 mg/cubic meter in 1950, falling to 100 mg/cubic meter "toward the end of the plant's productive life in 1958." The data were based on results of high-volume exhaust-air samples and measurement of nickel sulfide. Levels of nickel in air escaping from the roof monitors of this plant are available for the period 1948 to 1962 (Warner, 1984) (See Figure 1). Average levels ranged from over 200 mg/m3 around 1950 to around 50 mg/m3 in the 1960s. Calculations based on the data presented in Figure 1 of Warner (1984) give an average of 158 mg/m3 for measurements made before 1952 and an average level of 73 mg/m3 for measurements made after 1952. The cancer mortality data presented by Chovil et al. (1981) involve the assumption that exposures before 1952 were twice the average of exposures after 1952. Based on the data in Figure 1, we have assumed an average level of 150 mg/m3 before 1952, and 75 mg/m3 from 1952 to 1962. The ICNCM report does not present data allowing estimation of risk by cumulative exposure and it was not, therefore, used for the risk analyses.

Warner noted that since nearly all the air exited the plant through the monitors, the monitor samples may be used as estimates of the work environment. He commented that they were probably underestimates as the dusty air leaving the monitors was often diluted with clean air from the windows above the operating floor. While it is possible that the workers were breathing air containing nickel at higher concentrations than in the exit monitors, there are two reasons which suggest that the use of the roof monitor data is reasonable:

1. A 40-hour sample of dusty air on the operating floor gave a concentration of 46.4 mg/m3 in 1960. During the same year, there were 4 measurements from the roof monitors, and their average was a little in excess of 50 mg/m3 based on a graph given by Warner. Thus the operating floor level measurement was actually lower than that for the roof monitors. At the very least, this suggests that roof monitor data would not greatly over-estimate operating floor levels.

2. Coarser dust would be more likely to settle, and less likely to exit via the roof monitors. Thus it is coarse dust concentrations which are likely to be higher at the level of worker breathing zones than at the roof exits. However lung cancer risks would be affected by the fine respirable fraction of dust particulates since large particles are cleared in the upper respiratory tract. The fine particulates are likely to be well represented in dust at the roof exits.

For the above reasons, data from the roof monitors have been used in exposure assessment for cancer risk estimation.

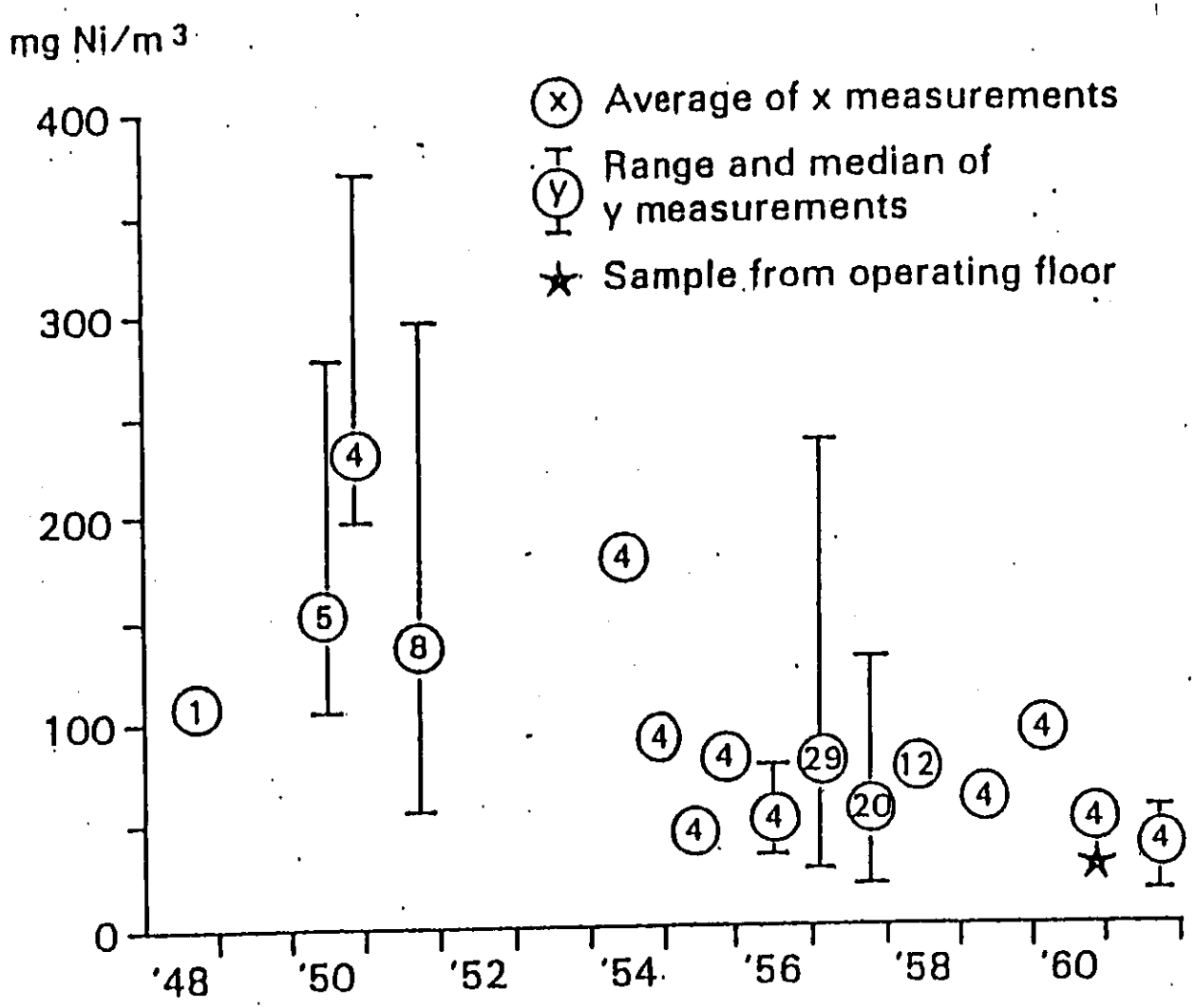


FIG 1 Concentrations of nickel in dusty air leaving the roof monitors of the Copper Cliff Sinter Plant

7.2.1.3. The Welsh Cohort

The third cohort, which will be referred to as the Welsh cohort, involved 967 refinery workers, some of whom started working as early as 1910. The cohort was identified from two or more pay-sheets from the first week of April in the years 1929, 1934, 1939, 1944 and 1949, at a nickel refinery in Clydach, Wales, operated by the International Nickel Company. Several publications provide some form of dose-response data. These studies are Doll et al. (1977) in which lung cancer mortality is presented by year of first employment, the Peto et al. study (Peto et al., 1984) which categorized the cohort members by duration of exposure in the calcining furnaces, and analyses by various exposure variables by Breslow and Day (1987) and Kaldor et al. (1986).

The Doll et al. (1977) study followed the employees up until the end of 1971 and calculated man-years at risk from 1934-1971. There were 689 total deaths in the cohort, including 145 lung cancer deaths, yielding a lung cancer SMR of 5.28 (90% CI: 4.58 - 6.03) The risk of lung cancer was increased in workers exposed before 1930. As of 1972, total deaths in the cohort exposed before 1930 were 612, including 137 lung cancer deaths. The SMR for this cohort was 6.23, with 90% confidence limits of 5.38 and 7.18. Expected deaths were calculated based on national mortality rates during each 5-year calendar period. No exposure measurements were given.

The Peto et al. study updated dates of first employment in cases where additional information had come to light since the publication of the Doll et al. paper (1977). Employees were classified into low and high exposure groups based on the number of years each employee spent at the furnaces or in the copper sulfate work areas. The lung cancer SMR for the low exposure group was 3.7 while that for the high exposure group was 14.0. Again, this paper provided no nickel exposure data.

The analyses by Breslow and Day (1987) and Kaldor et al. (1986) involve mathematical modeling using additive and relative risk models with categorical variables representing age at first employment, year of first employment, time since first employment, and an exposure index based on the number of years of work in certain jobs involving exposure.

The ICNCM reported further follow-up of the cohort to 1984. There were 172 lung cancer deaths and 74 nasal cancer deaths among those hired prior to 1930. The overall lung cancer SMR was 3.93 (90% confidence limits 3.45 - 4.46). For those men hired before 1920, the SMR was 5.49 (90% confidence limits 4.57 - 6.60). This is the only cohort in which the excess numbers of nasal cancer deaths (73.6) was significant in number compared to the excess number of lung cancer deaths (128.2). In the other cohorts reviewed, the excess number of lung cancer cases swamp the excess number of nasal cancers.

Exposure data are available for the Welsh cohort from a paper by Morgan (1985) in which a historical description of nickel monitoring at Clydach was presented. The first record was dated in 1932. Levels ranged between 8 and 42 mg/cubic meter in 1932, although these levels were thought to be lower limits of true exposure as the plant was not operating at peak production during the monitoring period. Particle dust counting in 1945-49 yielded estimates of 1.7-19.1 million particles per cubic foot, where the only particles counted were those less than 10 microns in diameter.

The main problem with the exposure data for this cohort is that the earliest data are for 1932. There were no measurements prior to 1930 when exposures were said to be higher. This is corroborated by the fact that lung cancer SMRs show a strong relationship with year of first employment. For

lung cancer, the SMR estimates for first employment prior to 1915 were around 10. For all workers first employed prior to 1930, the SMR was 6.2, whereas for workers first employed after 1930 the SMR was 1.5 (Doll et al., 1977). While these data are partly confounded by duration worked, follow-up was to 1972 so latency was not a problem. Hence it must be noted that the exposure data for 1932 and later are probably not relevant to the lung cancer risks in the cohort as a whole. In addition, the numbers of workers in the cohort starting after 1930 are too few to lend precision to the findings for this subset of workers.

The ICNCM report gives lung cancer for various species of nickel and noted that "overall, the evidence leans toward soluble nickel as the form that contributed most heavily to risk." These analyses were based on estimates of what exposure might have been in the past, rather than actual measurements.

7.2.1.4. The Norwegian Cohort

The fourth and last cohort considered, which will be referred to as the Norwegian cohort, was described in two papers (Magnus et al., 1982; Pedersen et al., 1973). It involved 2247 refinery workers employed for at least three years prior to 1969 in Kristiansand, Norway. Follow-up was accomplished to the end of 1979, for a minimum of 10 years. Eighty-two lung cancer cases were observed, giving an SMR of 3.73 and 90% confidence limits of 3.08 and 4.48. SMRs unadjusted for smoking habits ranged from 2.28 for those followed up for 3-14 years, to 4.30 for those followed up for more than 35 years. Exposure data for this site were estimated by the EPA based on International Nickel Company (INCO) estimates of exposure at the Clydach Wales plant. No exposure data were available from the Norwegian smelter itself. The EPA took as their exposure estimate the range from 3 to 35 mg Ni/m^3 assuming air levels would be similar to those in the Welsh smelter, and further assumed exposure over one quarter of a lifetime as no record of number of years worked was provided for the Norwegian cohort.

The ICNCM report presented further follow-up of this cohort to 1984. There were 77 lung cancer cases among the refinery workers with an SMR estimate of 2.62 (90% confidence limits 2.15 -3.16). Three deaths from nasal cancer were reported with 0.66 expected. It was thought that the excess lung cancer risk among electrolysis workers should be attributed to soluble nickel exposure. No actual measurements of exposure were presented.

7.2.2. Choice of Cohort Study for Risk Assessment

The question of which studies to choose for risk assessment rests on the precision and accuracy of the relative risk estimates and the precision and accuracy of the exposure estimates. Table 7.8 presents a range of exposure estimates and confidence limits around the SMRs for the four studies which could be used for quantitative risk assessment. (The ICNCM further follow-up of these cohorts does not alter the relative magnitude of the precision estimates). It is apparent that the West Virginia cohort study is the weakest using this criterion. In fact a relative risk of only 1.12 could be due to confounding or to a variety of minor biases. There were only four lung cancer cases in the highest exposure category, which reached a relative risk of 1.61.

The weakness of this study for risk assessment becomes even more evident when one considers the imprecision of the risk estimates. Quantitative cancer risk estimates based on a relative risk model are driven by the excess relative risk (i.e., the relative risk minus 1). In the case of the West

Table 7.8 Studies Used by the EPA

Study	N	Lung Ca Deaths	Lung Ca SMR	90% Conf. Limits	Ratio of uppper CI of SMR-1 to (SMR-1)*
W. VA	1855	8	1.12	0.56, 2.02	8.5
Ontario	495	37	8.71	6.49, 11.45	1.4
Wal s	967	145	5.28	4.58 - 6.03	1.2
Norway	2247	82	3.73	3.08, 4.48	1.3

*Note: the (SMR-1) is an estimate of excess relative risk

Virginia study, the overall excess relative risk is 0.12. The excess relative risk at the upper confidence limit was 2.02 - 1 or 1.02. A quantitative cancer risk estimate based on the upper confidence limit would result in an estimate 1.02/0.12 or 8.5 times higher. Of course, a risk estimate based on the lower confidence limit of 0.56 would lead to a lower cancer risk estimate. This range of uncertainty is very much larger for this study than for the three other cohorts (see Table 7.8).

The West Virginia cohort study was therefore rejected for further consideration for quantitative risk assessment in view of the imprecision of its relative risk estimate. (It might be noted that the EPA health risk assessment placed considerable reliance on this study in their risk assessment. This appears to be because they decided a study with low exposures would be most informative in the context of environmental risk assessment. The problems in precision of low exposure estimates, and particularly in precision of the relative risk estimate, override such a consideration. The only value of a low exposure cohort such as this might be to consider consistency with the findings from higher exposure cohorts. However, the range of estimates which could come from this study are such that they would encompass any estimates based on much more heavily exposed cohorts.)

The Norwegian cohort study was also unsuitable for quantitative risk assessment. It has an important advantage in that it was the only cohort study incorporating smoking data. However, there were no nickel exposure levels available for this cohort.

Although the Welsh cohort study was the best on the basis of the precision of the risk estimates (Table 7.8) it was also unsuitable for quantitative risk assessment. The major problem relates to the exposure data. It was noted that there were exposure measures from the refinery involved, but that the measures were first recorded for 1932, whereas the relevant exposure period for the increased lung cancer risks was predominantly before 1932. This is corroborated by the fact that lung cancer SMRs show a strong relationship with year of first employment. In addition, the numbers of workers in the cohort starting after 1930 was too few to lend precision to the findings for this subset of workers.

The Ontario cohort study was determined to be the most appropriate for quantitative cancer risk assessment. The lung cancer risks were significantly elevated, as for the Norwegian and Welsh cohorts. Most importantly, exposure measures were available for the period from 1948 on, and this is a relevant period for the lung cancer deaths which were ascertained between 1963 and 1978. The precision of the relative risk estimates as assessed by the upper confidence limit of the excess relative risk estimate divided by the point estimate (Table 7.8) was very much better than the estimate for the West Virginia cohort. Our quantitative cancer risk calculations were therefore based on this cohort study, the main reason being that it was the only cohort with actual measurements of exposure associated with a relevant causal period with sufficient latency.

7.2.3. Models for Risk Assessment Analysis using Epidemiological Data

The EPA used both additive risk and relative risk models in their risk assessment analyses. Two types of relative risk estimation procedures were used, a maximum likelihood estimation procedure and what was termed an average relative risk approach. This section reviews additive and relative risk

models and the methods used by the EPA in their Health Assessment Document for Nickel (1986a).

7.2.3.1. Additive Risk Model

The additive model suggests that the risk difference or excess risk over background will depend on cumulative exposure, while the relative risk model is based on the assumption that the relative risk is related to cumulative exposure.

The incidence of virtually all adult cancers rises steeply with age and may be fitted with various power functions for different cancers (Armitage and Doll, 1954). A linear relationship between excess risk and cumulative dose is thus most unlikely. If an additive model is to be used, then it needs to incorporate a power function on age or time to generate the type of incidence curves expected. Such models may be needed when the background rates of a cancer are minimal from exposures other than the one being considered, as is the case for mesothelioma, where asbestos is the major cause. However, a linear additive model for lung cancer risks from exposure to nickel is not such a situation since the major causes of background rates of lung cancer is cigarette smoking, and not exposure to nickel.

7.2.3.2. Relative Risk Model

The relative risk model follows from two possible assumptions or a combination of them. It is usually thought that the agent of interest has to act multiplicatively with the background causes of the disease. In other words, the cause of interest would have to act synergistically with the background causes in a manner which results in multiplication of the background rates. However this assumption is unnecessarily restrictive.

The relative risk model will also result if one merely assumes that the relationship between age and cancer incidence is the same for the cause of interest and for the background causes of a disease. Thus, if the background cancer risk and the risk for a particular cause both rise as the 5th power of age, the relative risk will remain constant. If age factors are related to cancer incidence in such a manner, then the relative risk model (i.e., multiplicative model) will fit even though the cause of interest might be acting quite independently of the background causes. In general then, the relative risk model is both a simple model and one which has empirical and theoretical validity based on the known relationships between age and cancer incidence.

The EPA used two methods to estimate a dose-response relationship from the relative risk model. The first involved a Maximum Likelihood Estimate (MLE) using observed and expected numbers of lung cancer deaths for seven levels of cumulative exposure. The slope, DELTA, was calculated from the following equation:

$$(d \ln L)/\Delta d = \sum_{j=1}^{k} - E_{oj}X_j + (O_jX_j)/(1 + \Delta X_j)$$

where L is the likelihood, k the number of exposure groups, E_{oj} is the number of lung cancer deaths expected due to background, X_j is the cumulative exposure, and O_j is the number of lung cancer deaths observed.

A simpler method for implementing a relative risk model was also used by the EPA:

$$B_H = [P_o(R - 1)]/X$$

where B_H is the estimate of incremental unit risk, P_0 is the background lifetime risk for lung cancer, R is the overall relative risk calculated as observed divided by the expected lung cancer deaths, and X is the average lifetime continuous exposure for the refinery cohort. The EPA refers to this method as the average relative risk method.

This method of calculating risk estimates has several advantages. The calculations are straightforward and easily replicated. Indeed, the EPA cancer risk estimates for nickel presented in Table 7.9 using this method were easily checked, whereas it was not possible to replicate the calculations of the maximum likelihood estimate method (see next section).

One potential problem with either relative risk model is that results depend strongly on the estimate selected for the background lung cancer rate, P_0. Comparison of the draft and final EPA average relative risk assessments for nickel illustrates this point. In the draft (EPA, 1985), the EPA selected 0.026 as the estimate for P_0 for all four cohorts, i.e., the lifetime background risk for death from lung cancer. In the final risk assessment (EPA, Sept. 1986a), the EPA modified the risk assessments for Copper Cliff, Clydach and Kristiansand to better reflect background rates in those countries. The new estimates, 0.026 for Copper Cliff, 0.016 for Kristiansand, and 0.029 for Clydach, considerably changed the risk estimates. The background lifetime lung cancer mortality rates for males in Ontario ranged from 0.036 (i.e. 36 per thousand) in 1963 to 0.068 (i.e. 68 per thousand) in 1978 (Ontario Cancer Foundation) . The estimate for the entire period, 1963 to 1978, was approximately 0.051 or 51 per thousand. This issue will be discussed further in a later section.

7.2.3.3. Discrepancies between EPA's Relative Risk Models

The EPA's results using the two relative risk models on the Ontario cohort study differed considerably. The maximum likelihood estimate for lifetime risk associated with exposure to 1 ug/m^3 was 1.1×10^{-5}. The average relative risk model gave an estimate of 8.9×10^{-5}, 8-fold higher than that based on the above maximum likelihood estimate. The EPA states that the magnitude of the difference in estimates between the two models probably reflects greater sensitivity of the likelihood model to the lower exposure response data. This explanation is unlikely in view of the clearly linear relationship between exposure and the SMR shown in Figure 2 (section 7.2.5).

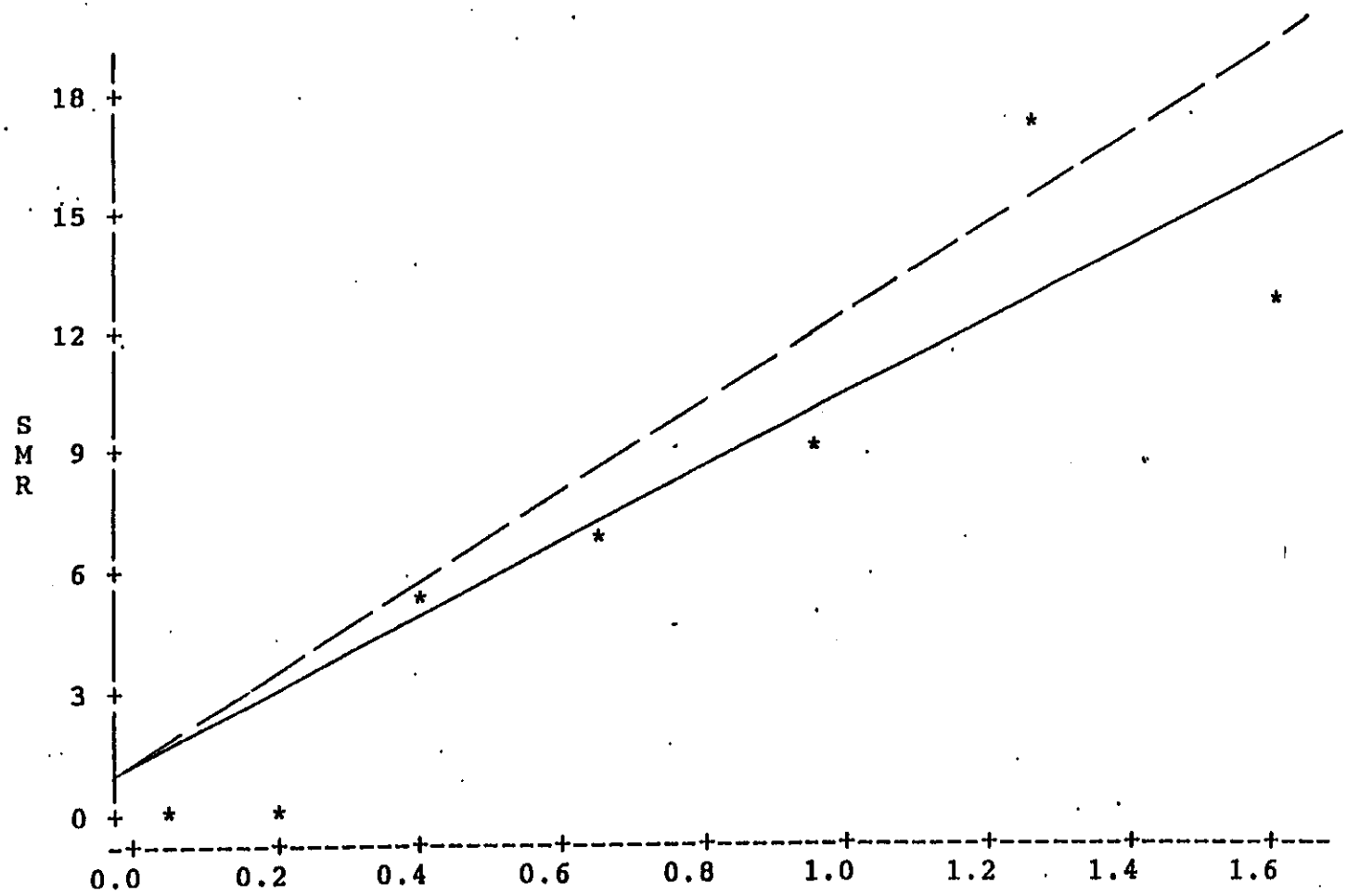
From Table 8-53 in the EPA report, one can calculate that the average duration worked was about 7.2 years (taking into account a weighting of 2 for years prior to 1951). In section 7.2.5 we have calculated a regression slope of 9.22 for the SMR versus a measure weighted cumulative exposure. Expressed in terms of years of work in the smelter, the regression slope would be 0.922. The predicted SMR for a duration of work of 7.2 years is therefore $0.922 \times 7.2 + 1 = 7.64$, which agrees quite well with the overall SMR of 8.7. Thus our regression analysis provides results which agree with the EPA average relative risk method. As noted above, the EPA maximum likelihood estimate was nearly 10 times lower. Based on simple observation of Figure 2, it is apparent that there must be an error in the linear model which predicts a risk 8-fold lower.

Table 7.9 Lifetime Risks (ug/m^3) for Lung Cancer due to Exposure to Nickel, Based on EPA Extrapolations from Epidemiologic Studies

Study	Additive Risk Model	Relative Risk Model	Relative Risk (Average)
West VA (refinery workers)	2.8×10^{-4}	1.5×10^{-5}	3.1×10^{-5}
West VA (non-refinery workers)	1.8×10^{-4}	9.5×10^{-6}	2.1×10^{-5}
Ontario	--	1.1×10^{-5}	8.9×10^{-5}
Norway high expos.	--	--	1.9×10^{-5}
low expos.	--	--	1.9×10^{-4}
Wales high expos.	--	--	8.1×10^{-5}
low expos.	--	--	4.6×10^{-4}

Note: High and low refer to the estimate of exposure for the workers in the study. These values are "best estimates" and not 95% upper confidence limit estimates.

Figure 2: Ontario Nickel Refinery Cohort - SMR's by cumulative exposure



Solid line: Weighted least squares linear regression
Broken line: Upper 95% confidence limit

It is difficult to reconcile the differences between EPA's two relative risk model results. One minor difference is that the first model assumes 240 days per year of exposure and the second assumes 280. However, calculation for the average relative risk model, using the 240 days per year figure, magnifies the discrepancy further since the potency estimate becomes 10.4×10^{-5}. Other reasons for a discrepancy between the two methods were not identified. Since the average relative risk results have been verified as above, it is likely that there are errors in EPA's maximum likelihood estimate results.

7.2.3.4. Choice of Relative Risk or Additive Risk Model

It was noted above that additive risk models require incorporating complex functions involving age and/or time to be used for risk assessment purposes. Thus, relative risk models are generally advantageous provided there exists a substantial background rate of the disease in question.

One can also consider an empirical approach based on which model best fits the data. Kaldor et al. (1986) and Breslow and Day (1987) have reported similar analyses based on the Welsh cohort involving both additive and relative risk models. Breslow and Day found almost identical goodness of fit for the two models. Thus it would appear that, based on empirical model fitting, the two models are equally appropriate. However, on closer examination, this is not the case. The reason both models fit the data equally well was due to the incorporation of 14 binary variables to represent the simultaneous effects of four factors. The four factors were age at first employment, year of first employment, an exposure index which involved duration of exposure, and time since first employment. Inclusion of so many time-related factors obscures the age relationship of increased risks with continuous exposure.

Furthermore, part of the time relationships in this analysis were confused with latency. Finally, the exposure index was very crude, merely duration in certain types of jobs. The implicit assumption was that exposure was independent of calendar time. Since this was known not to be the case, some of the exposure information would be subsumed by the other time-related variables. In addition, exposure variation between different exposed jobs was ignored. This empirical analysis, while interesting in itself, was of little value in considering use of a relative risk or additive risk model for risk assessment.

It might also be noted that Breslow and Day (1987) reviewed the problems of empirical discrimination of additive and multiplicative models (p. 124). They note "Unless the data are quite extensive and the effect of exposure pronounced.., random sampling errors may make such discrimination difficult. Furthermore, errors of misclassification of the exposure variable may operate to distort the true relationship. In view of such uncertainties, the choice of model is legitimately based as much on *a priori* considerations as it is on goodness-of-fit tests, unless of course these show one or other model to be markedly superior." We contend that *a priori* considerations clearly favor a relative risk model for quantitative risk assessment for nickel and lung cancer.

As pointed out in a previous section, use of a relative risk model does not necessarily imply that an exposure of interest has a multiplicative effect with background exposure causing the disease under investigation. For example, it does not imply that effects of nickel are multiplicative with those of cigarette smoking. Kaldor et al. (Kaldor et al., 1986) noted that

population risk estimates for non-smokers and smokers would differ between using a relative risk model or additive risk model. This is correct and has been addressed in detail in a later section. In fact we have shown that the evidence supports additive risks of nickel and smoking, but this issue of interaction is a secondary issue to choosing between a relative risk model or an excess risk model to represent the relationship between lung cancer risk and cumulative exposure to nickel.

7.2.3.5. Proposed Method for Risk Assessment Calculations

As has been noted, the EPA used a variety of methods for estimating cancer risks. We concluded from the above review that a relative risk model was the most appropriate for linear extrapolation to low dose lifetime exposures. It might also be noted that age-specific person years at risk which would be required to apply an additive risk model are not available from the publications on this cohort study. A relative risk model was therefore applied to the Ontario cohort study. Before presenting the calculations involved, the issue of incorporation of background rates into a risk assessment will be addressed.

7.2.4. Incorporation of Background Rates

Application of a relative risk model necessitates a decision as to the background cancer rates to be used when applying cancer risks generated from one study to another population. The SMR values for the Ontario cohort study were based on expected lung cancer deaths derived using Ontario lung cancer rates for the years 1963 to 1978. The relevant background rates to use in risk assessment for Ontarians exposed to nickel in the same calendar period as workers in the study would therefore be Ontario mortality rates for the same period.

A question arises when one considers what to do in assessing current cancer risks in Ontario, since lung cancer mortality rates among Ontarians may have increased due to changes in smoking habits. In addition, a question arises as to what to do when considering the risks from nickel exposure for some other population such as Californians.

The relevant biological question involves interaction. If nickel acts to increase lung cancer risks in an additive manner which is independent of the local population's background lung cancer risks, then rates of lung cancer in the population under consideration would not enter the calculations. Rather, the rates of lung cancer in the population used to calculate the expected value for the cohort lung cancer cases would be involved since they are required to get from extrapolated relative risk to the actual increase in lifetime risks of lung cancer attributable to nickel.

On the other hand, if nickel acts in a multiplicative manner with the background causes of lung cancer in a population, then the background rates of lung cancer in a population will have a direct bearing on the risks one would attribute to nickel. For example, if the background rates were twice that of the population in which the study occurred then the risks attributable to nickel would be doubled.

The additive approach identifies risks from nickel which would not change over time or from place to place with background incidence of cancer.

The following should be noted with regard to the multiplicative approach. Estimated risks from nickel exposure would vary over time and from place to place with variation in background rates of lung cancer. Since

smoking is the main determinant of lung cancer incidence in the general population, and since smoking rates are now falling over time, this means that calculated risks for nickel exposure would also fall without any change in nickel exposure levels. The implication is that estimated risks for ambient nickel, using the most recent lung cancer rates, would need to be adjusted periodically with falling background rates for lung cancer. Acceptable levels for nickel exposure would increase over time if the same lifetime cancer risk yardstick was maintained.

There are obvious policy disadvantages to this approach. However, the critical question is biological validity since, if the real risk from exposure to nickel were falling over time due to reduction in background lung cancer rates, this is relevant to risk management decisions.

As indicated above, the question is one of interaction. In particular, since smoking accounts for 80-90% of the incidence of lung cancer in most countries, the question concerns the interaction between nickel and cigarette smoking in causing lung cancer.

7.2.4.1. Consideration of the Interaction between Nickel and Smoking

Some experimental studies in animals indicate that nickel could act synergistically with benzo(a)pyrene. (See Section 6.1). Rivedal and Sanner (1981) observed a comutagenic effect between nickel sulfate and benzo(a)pyrene (BaP) *in vitro*. In hamster embryo cells, nickel sulfate combined with benzo(a)pyrene produced morphological transformation in 9.6% of cells versus 0.3% and 0.7% for nickel and benzo(a)pyrene alone respectively (Rivedal and Sanner, 1981). Maenza et al. (1971; as cited in IARC 1990) and Kasprzak et al. (1973) have reported some suggestive but inconclusive evidence of synergism between nickel subsulfide and BaP *in vivo*.

One in vivo animal study conducted by Wehner et al. examined the potential synergistic effect of nickel oxide (NiO) and cigarette smoke in hamsters (Wehner et al., 1975). Chronic inhalation of NiO and cigarette smoke eventually caused pneumoconiosis, whose development was unaffected by cigarette smoke. No carcinogenic or cocarcinogenic effect of NiO and cigarette smoke was observed. Exposure to cigarette smoke did not produce pulmonary tumors although an increased incidence of laryngeal lesions was observed. In view of the fact that cigarette smoke did not produce pulmonary tumors in this study, the study is of limited value in assessing interaction with NiO.

Most investigators have not considered smoking in assessing incidence rates of lung cancer in human studies of nickel exposure. However, first Kreyberg (Kreyberg, 1978), and then Magnus et al. (Magnus et al., 1982), investigated a possible interaction between smoking and nickel among workers at the Falconbridge nickel refinery in southern Norway. The cohort, which was discussed in a previous section, consisted of 2247 men who were employed for at least three years in the refinery and began employment prior to 1966. The cohort was followed up from 1953 to 1979. The cohort was divided into those who had never smoked and those who were present or past smokers.

Data on smoking habits were derived from medical records compiled by the plant medical officer for 90% of the cohort (Magnus et al., 1982). Smoking status was based on information from family members or other workers, for 7% of the cohort. For 3% of the cohort, no data on smoking were available.

Incidence rates of lung cancer among smokers and nonsmokers in the cohort were compared to those derived from a survey of a sample from the general Norwegian population. In this survey, more than 12,000 men were

interviewed in 1964-65 about their personal habits, including smoking, and were followed up for cancer incidence from 1966-1977. Since the nickel cohort was followed up for a different period (1953-1979), the authors recalculated the lung cancer rates for refinery workers for a follow-up period of 1966 to 1977 by excluding cases and person-time outside this period. In this manner, the lung cancer incidence rates for smokers and nonsmokers in the work cohort could be compared to those for the general population sample. These comparisons are shown in Table 7.10.

Confidence limits were not given in the Magnus et al. paper, but have been calculated for this document by the following methods. The paper by Magnus et al. presented the number of cases and the person-time at risk for each category. The age-adjusted rate ratios were also given. In order to estimate confidence limits, the person-time in each cell was adjusted in the following manner. Adjusted person-time = (number of cases/age-adjusted rate per 1,000 person-years) x 1,000. For example, there were 116 lung cancer cases among smokers in the general population sample. The age-adjusted rate per 1000 person-years was 1.13. The adjusted person-time was therefore 116/1.13 x 1000 = 102,655. It was then possible to obtain confidence limits with good approximation since their main determinants are the numerator lung cancer numbers, which are not affected by the adjustment. These adjusted person-time values were also used in testing for the additive and multiplicative effects.

Using an additive model, one would expect a rate of 2.54 cases per 1,000 person-years among those nickel workers who smoke (0.19 + (1.13 - 0.19) + (1.6-0.19)). The actual rate of 3.27 is a little higher, suggesting a slightly higher than additive rate. Statistical testing (Rothman, 1986) reveals little evidence for departure from additivity (p=0.67).

Using a multiplicative model in which

$$RR_{11} = RR_{10} \times RR_{01}$$

where

RR_{11} = 3.27/0.19 = 17.21, RR_{10} = 1.13/0.19 = 5.95, RR_{01} = 1.60/0.19 = 8.42;

the expected rate equals

$$RR_{01} \times RR_{10} = 5.95 \times 8.42 = 50.11$$

Thus the observed incidence rate of 3.27 per 1000 person-years was very much lower than what would be expected for multiplicative effects. If rates were multiplicative, the relative risk from nickel exposure among smokers would be identical to that among non-smokers. Statistical testing for heterogeneity (Rothman, 1986) of the stratum relative risk estimates in Table 7.10 indicates it was likely that the effects were less than multiplicative (p=0.03).

Consideration was also given to the possibility that effects were multiplicative, but that this had been masked due to various biases. If nickel workers were lighter and shorter duration smokers than smokers in the general population, then the combined effect of smoking and nickel would be underestimated. However, we do not know of any reason why this would be the case; indeed, industrial workers tend to be heavier smokers than the general population. In addition, the magnitude of bias from this source, if present, would be small.

Evidence for multiplicative interaction might also be missed if workers who had the highest levels of exposure to nickel (i.e., pre-WWII) were significantly less likely to be smokers than workers who were first employed after WWII (and therefore had lower levels of nickel exposure). This seems

Table 7.10
Lung Cancer Incidence Rates per 1000 Person-Years and Relative Risk Estimates for Smokers and Nonsmokers in the Norwegian Refinery Study

Exposure	No smoking	Smoking	Relative risk	90% CI
No nickel	0.19	1.13	5.95	(3.61-9.81)
Nickel	1.60	3.27	2.04	(0.95-4.39)
Relative risk	8.42	2.89		
90% CI	(3.92-18.09)	(2.17-3.87)		

possible since smoking became increasingly popular after the war. However, examination of Table I in the paper by Magnus et al. (1982) reveals that non-smoking nickel workers had only a small tendency to be employed earlier than smokers. For example, 20.8% of the non-smokers were first employed in the period 1916-1939, compared to the 15.8% smokers. Small differences such as this are not likely to confound a truly multiplicative effect. Since the evidence is consistent with additivity of effects, it was assumed for purposes of risk assessment that excess risk from nickel exposure among smokers would be the same as among non-smokers. This is a health-protective assumption since it results in fixed added risks for nickel exposure in spite of current reductions in background lung cancer incidence.

7.2.4.2. Conclusion Regarding Background Rates

Since the evidence indicates effects much closer to additive than multiplicative, this suggests that the estimates for lung cancer risks from nickel exposure should be treated as independent of background rates of lung cancer. It is therefore appropriate to incorporate lung cancer rates for the Ontario population at the time of the lung cancer deaths among the nickel refinery workers, rather than using background lung cancer rates for a population being considered in the risk assessment. Lung cancer mortality statistics for Ontario were therefore used to determine the background lifetime risk of lung cancer at the time of follow-up of the nickel sinter plant workers, as explained in the next section.

7.2.5. Risk Assessment Calculations for the Ontario Cohort Study

The Ontario cohort study reported both incident lung cancer cases and deaths. The quantitative risk assessment presented here was based on deaths because the authors did not have direct estimates of the expected numbers of incident cases in each age group. Instead they obtained them indirectly by multiplying the expected number of deaths by an arbitrary factor of 1.5. In view of this crude estimation method for incidence cases, the quantitative assessment which follows uses lung cancer mortality data from this study. This is appropriate in view of the fact that lung cancer has high fatality, with only about 10% surviving more than 5 years after diagnosis.

The SMR data for the Ontario cohort study are presented in Table 7.11. The expected numbers of deaths were calculated by Chovil et al. from age-specific rates for males in Ontario, which were averaged over the follow-up period (Chovil et al., 1981). The cumulative exposure data in the first column of Table 7.11 were derived by the EPA (EPA, Sept. 1986a) from exposure duration data (Chovil et al., 1981) incorporating a level of 200 mg/m^3 for work prior to 1952, and 100 mg/m^3 for 1952 and thereafter.

The SMR values from Table 7.11 were plotted against cumulative exposure as shown in Figure 2. A weighted least squares linear regression analysis using the expected numbers of lung cancer deaths as weights for each exposure category, and forcing an intercept of 1, produced a slope of 9.22 and standard error of 1.02 (upper 95% confidence limit for the slope = 11.26). The resultant best fit and upper 95% confidence limit lines are also plotted in Figure 2. It can be seen that the data are consistent with a linear relationship between the SMR and exposure.

A weighted least squares regression analysis without forcing the intercept through an SMR of 1 resulted in an intercept of 0.29 (95% confidence limits -3.91 to 4.49). These confidence limits encompass 1, and do not

Table 7.11
Observed and Expected Numbers of Deaths and Standardized Mortality Ratios for the Ontario Nickel Refinery Cohort by Cumulative Exposure

Cumulative exposure (mg/m3) x years/1000	Observed lung cancer deaths	Expected lung cancer deaths	Standardized mortality ratios (SMR)
0.05	0	0.47	0.0
0.20	0	0.36	0.0
0.40	3	0.54	5.6
0.65	4	0.60	6.7
0.95	6	0.68	8.8
1.25	13	0.76	17.1
1.60	11	0.84	13.1
Overall	37	4.25	8.71

Note: The cumulative exposure measure was derived by multiplying person years of exposure by 200 mg/m3 for work prior to 1952, and by 100 mg/m3 for work from 1952 on, and dividing the sum by 1000.

provide evidence supporting a threshold response. A regression analysis with an intercept corresponding to an SMR of 1 was used, since when exposure is zero the SMR must be 1.

The subsequent calculations are presented in Table 7.12. The first line gives the weighted least squares estimate of the slope, and the second line the upper 95% confidence limit. The next few lines involve a correction for the loss to follow-up in this cohort. There were 495 workers in the cohort, and 124 were untraced at the end of follow-up. Chovil et al. assumed that none of those untraced had died of lung cancer, since they could not be found in searching death records. It was noted earlier that it could not be assumed that all those untraced were alive. For this reason Table 7.12 includes a correction for loss to follow-up. Roberts et al. (1989) estimated that there was under ascertainment of deaths for this cohort by about 5%. The regression slope was therefore corrected by multiplying by 1.0/0.95.

The second section of the table involves the exposure calculations. The units for the cumulative exposures given in Figure 2 were 1000 mg/m^3 times years, which corresponds to 1 on the horizontal axis. The next steps involve calculating an equivalent lifetime environmental exposure. In other words, the calculations derive a level of exposure which, if experienced over a lifetime, would give the same unit cumulative lifetime exposure as experienced by workers in the plant. The first step involves adjusting for an 8 hour workday versus a 24 hour environmental exposure day. The second step adjusts for a 5 day work week versus 7 days per week environmental exposure, and the next step for a 48 week workyear versus 52 weeks environmental exposure. Finally, examination of Figure 1 (see section 7.2.1.2) suggests that exposures subsequent to 1952 were lower than the 100 mg/m^3 initially assumed. In addition, exposures prior to 1952 may have been lower than 200 mg/m^3. The measurements shown were obtained from roof monitors. While it is possible exposure levels experienced by workers were higher, the only measurement from the plant giving a level at the operating floor level was actually lower than the average level reported for that year from the roof monitor. It was therefore decided to adjust the exposure level with the assumption that the average level was 75 mg/m^3 after 1952 (and implicitly 150 mg/m^3 before 1952). This involves a further adjustment of 0.75. The overall adjustment factor was therefore 0.165, obtained by multiplying all the adjustment factors together. Thus the cumulative environmental exposure corresponding to the unit cumulative exposure experienced in the refinery was $0.165 \times 1000 = 165$ mg/m^3 years. In other words, the slope considered above would correspond to a unit cumulative exposure experienced 24 hours per day, 7 days per week, and 52 weeks per year of 165 mg/m^3 years. The annual average level of environmental exposure which would result in achieving this cumulative exposure in a 70 year lifetime is therefore $165/70 = 2.35$ mg/m^3.

The final steps shown in Table 7.12 present an estimate of the lifetime added lung cancer risk for continuous environmental exposure at a level of 1 mg/m^3. The excess relative risk corresponding to lifetime exposure at 2.35 mg/m^3 was 10.85. Linear extrapolation of a level of 1 mg/m^3 yields an excess relative risk estimate of 4.61.

The background lifetime lung cancer mortality rates for males in Ontario ranged from 0.036 (i.e. 36 per thousand) in 1963 to 0.068 (i.e. 68 per thousand) in 1978. The estimate for the entire period, 1963 to 1978, was approximately 0.051 or 51 per thousand for males. This overall rate was obtained by dividing the total number of lung cancer deaths in Ontario for the years 1963 to 1978 (26,655) by the total number of deaths from all causes combined in those years (521,376). In effect, this estimation method gives a

Table 7.12: Risk assessment calculations for lung cancer mortality based on the Ontario Nickel Refinery Sinter Plant workers.

Excess relative risk estimates (SMR-1)		
Slope of lung cancer SMR versus exposure (Figure 2)	9.22	
Upper 95% confidence limit of the slope	11.26	
Estimated under-ascertainment of deaths	5%	
Corrected upper 95% limit of slope (11.26 / (1-0.05))	11.85	
Adjustment to equivalent lifetime continuous exposure		
Units of cumulative exposure in Figure 2	1000	mg/m3.yrs
Adjustment for lower exposure than initial estimate (average 75 mg/m3 after 1952 rather than 100 mg/m3)	0.75	
Exposure adjustment for 24 hours per day (8/24)	0.33	
Adjustment for days per week (5/7)	0.71	
Adjustment for weeks per year (48/52)	0.92	
Combined exposure adjustment factor (0.75 x (8/24) x (5/7) x (48/52))	0.165	
Adjusted units of cumulative exposure (0.165 x 1000)	165	mg/m3.yrs
Corresponding exposure level for 70 year lifetime (165/70)	2.35	mg/m3
Calculation of potential lifetime risks from continuous exposure		
Excess relative risk (RR-1) estimate for above unit exposure (this is the upper 95% confidence limit of the slope)	11.85	
Excess RR estimate for lifetime exposure at 1 mg/m3 (11.85 / 2.35)	5.03	
Background lifetime lung cancer mortality risk (Estimated for Ontario for the time of followup of the sinter plant workers)	0.051	
Lifetime added risk for exposure to 1 mg/m3 (5.03 x 0.051)	0.257	
Lifetime added risk for exposure to 1 ug/m3 (1 mg = 1,000 ug)	0.257	per 1,000

Note: The calculations for the above table are explained in the text.

composite estimate of the lifetime risk of dying from lung cancer for cohorts experiencing the mortality rates from lung cancer and from all other causes which occurred in the period 1963-1978 (Smith, 1988).

There is no ideal method for calculation of lifetime risks of death from lung cancer. At any one point in time, age specific mortality rates apply to different birth cohorts who may experience different mortality from the cause of interest and from competing risks. Thus a lifetable estimate of lifetime risks from a particular cause involves many assumptions. There are two main advantages of the method used above. Firstly, the estimate is a composite based on actual mortality rates in a population at the time. Secondly, it is very easy to estimate.

Using this background risk of 0.051 and an excess relative risk of 4.61 yields a risk estimate of 0.257 for lifetime exposure to 1 mg/m^3 (table 7.12). Extrapolating to 1 ug/m^3 gives an estimate of 0.257 per 1000 or 257 per million. The so called average of the unit risk is 210 per million (257 x 9.22/11.26) using the actual SMR rather than the upper confidence limit (see first line Table 7.12). Thus based on the human studies the range of unit risk is approximately 210-257 per million per ug/m^3.

8. Nickel Speciation and Quantitative Cancer Risks

The quantitative cancer risk calculations for the Ontario refinery study given in the previous section were based on measures of nickel dust, without consideration of the form of nickel. Thus the risk estimates and their extrapolation apply to whatever combination of nickel species the refinery workers were exposed.

The main source of information concerning the form of nickel in the air at the refinery was a paper by Warner presented at the Third International Conference on Nickel Metabolism and Toxicology (Warner, 1984). The process involved various sulfur-containing forms of nickel which were oxidized to nickel oxide on traveling grate sintering machines. The principal feeds were concentrates of nickel subsulfide. A chemical analysis of airborne dust samples obtained from the operating floor in 1960 found nickel oxide, nickel subsulfide and nickel sulfate. The actual concentrations were not given, but it was stated that nickel sulfate was the least concentrated. Since the main nickel constituents were therefore nickel subsulfide and nickel oxide, the increased cancer risks may be due to one or the other, or to the combination of both of them, or possibly the total impact of all carcinogenic nickel compounds present.

The determination of carcinogenic potential of nickel species has been evaluated to a limited extent in animal studies. These studies have been reviewed in section 7.1. The carcinogenicity of nickel subsulfide has been evaluated in only one inhalation study. Two intratracheal studies have also been conducted. The negative intratracheal study involved only four doses and a small number of animals and does not therefore provide evidence against carcinogenicity of inhaled nickel subsulfide. The other intratracheal study did demonstrate carcinogenic activity. The potency estimate (upper 95% confidence limit) from the nickel subsulfide inhalation study was 2.01×10^{-3} $(1\ ug\ Ni^3S^2\ /m^3)^{-1}$ or 2.76×10^{-3} $(1\ ug\ Ni/m^3)^{-1}$. This estimate is about 10 times higher than the estimate (upper 95% confidence limit) of 2.57×10^{-4} based on the smelter study. Part of this difference could be due to nickel subsulfide being more potent than the mixture containing nickel subsulfide and nickel oxide at the smelter. It is also possible that these animals are more

susceptible to nickel carcinogenicity, or that differences are attributable to the uncertainties involved in quantitative risk estimates.

Animal inhalation studies of nickel oxide were reviewed in section 7.1.1.3. None of the inhalation studies found increased lung cancer incidence, but definite conclusions could not be drawn due to various problems with the studies. An intratracheal instillation study did produce a positive carcinogenic response. In addition, it was noted in section 7.1.4 that nickel oxide and nickel subsulfide have approximately equal carcinogenic potency when given by injection. Nickel oxide has also given positive responses in some genotoxicity studies. In the absence of good evidence to the contrary, it would therefore be prudent to consider that both nickel oxide and nickel subsulfide might be carcinogenic.

Consider first the possibility that the carcinogenic effect in the Ontario refinery study was solely due to nickel subsulfide. If this were the case then the actual potency for nickel subsulfide would be greater per unit dose than that estimated by a factor which would be the inverse of the proportion of the nickel exposure which was nickel subsulfide. Thus, the potency calculated would be underestimated. The overall effect of this in risk assessment of inhalation of mixtures of nickel compounds would be to underestimate cancer risks when the mixture contained a higher fraction of nickel subsulfide than in the refinery, and to overestimate risks when a mixture contained a lower fraction of nickel subsulfide than in the refinery. However, since environmental nickel exposure involves low proportions of nickel subsulfide, the general effect would be to slightly overestimate cancer risks.

The second possibility is that both nickel oxide and nickel subsulfide are carcinogenic by inhalation. If they were equipotent, then it would be appropriate to sum the nickel oxide and nickel subsulfide components of a mixture and then estimate cancer risks using the potency estimate as calculated from the refinery study. The question would remain concerning other species of nickel. It is unlikely that nickel oxide could be more potent than nickel subsulfide, so the range of possibilities would lie between the extremes of the previous paragraph, and the possibility presented here that the two forms of nickel are equipotent.

The final species consideration concerns soluble nickel salts. Both water soluble and water insoluble nickel compounds have exhibited positive responses in genotoxicity assays. However, insoluble nickel compounds tend to exhibit greater carcinogenicity in bioassays. Consideration of target organ doses suggests that, even if soluble nickel salts were carcinogenic by inhalation, their potency would likely be lower than for the insoluble nickel compounds. This follows from the fact that soluble nickel salts appear to have a lower intracellular bioavailability then insoluble forms (See Section 7.1.4).

On the basis of the above considerations regarding the genotoxicity and carcinogenicity of various nickel compounds, it is protective of public health to calculate the human cancer risks from inhalation of nickel compounds by estimating the total concentration of nickel compounds and using the potency estimate derived from the Ontario refinery study calculated in section 6.2. Since nickel subsulfide does not predominate in environmental exposures to nickel, the potency estimate for nickel subsulfide itself, derived from animals studies in section 7.1, would not seem to be particularly relevant. Thus, DHS staff conclude that all nickel compounds should be considered potentially carcinogenic to humans by inhalation.

9. Carcinogenicity to Humans by Other Routes of Exposure

The potency estimate presented relates to inhalation of nickel compounds. The following reasons suggest that inhalation is the only route of human exposure which needs to be considered when calculating cancer risks. There are no animal or human studies which provide sufficient scientific evidence that nickel is carcinogenic when ingested. However, only nickel acetate and nickel sulfate have been tested, in a limited manner, in animals for carcinogenicity following oral ingestion. Although untested, insoluble nickel compounds such as nickel oxide and nickel subsulfide would not be expected to be absorbed from the alimentary tract. Absorption of nickel as soluble compounds is unlikely to result in increased cancer risks for reasons outlined in Section 7.1.4. Finally, the evidence suggests that some nickel in the diet may be essential. In the absence of greater supportive evidence, it is not possible to make conclusions regarding the carcinogenicity following ingestion of nickel compounds. However, it is clear that further studies are necessary to ascertain the carcinogenic potential of nickel compounds from exposure by the oral route.

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