Toxicological Review of Soluble Nickel Salts

Prepared for:

Metal Finishing Association of Southern California, Inc.,
U. S. Environmental Protection Agency,
and
Health Canada

Prepared by:

Toxicology Excellence for Risk Assessment (*TERA*)

March 1999

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This document does not constitute policy for the Metal Finishing Association of Southern California, the U.S. Environmental Protection Agency, or Health Canada. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

FOREWORD

The purpose of this Toxicological Review is to provide scientific support and rationale for the hazard and dose-response assessment in IRIS pertaining to chronic exposure to soluble nickel salts. It is not intended to be a comprehensive treatise on the chemical or toxicological nature of soluble nickel salts.

Funding for this assessment was provided by the Metal Finishing Association of Southern California, Inc. (MFASC), by the U.S. EPA, and by Health Canada.

In Section 6, the authors have characterized their overall confidence in the quantitative and qualitative aspects of hazard and dose response. Matters considered in this characterization include knowledge gaps, uncertainties, quality of data, and scientific controversies. This characterization is presented in an effort to make apparent the limitations of the assessment and to aid and guide the risk assessor in the ensuing steps of the risk assessment process.

For other general information about this assessment or other questions relating to the document development, please contact the staff of Toxicology Excellence for Risk Assessment (TERA) at 01-513-542-7475 (RISK), 01-513-542-8372 (TERA), 01-513-542-7487 (Fax Line) or tera@tera.org (e-mail).

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This assessment underwent an extensive external peer review, which was sponsored by Health Canada in Cincinnati, Ohio. The peer review panel was selected to include a balanced group of experts from government, academia, industry, and consulting firms. These reviewers provided expertise in a number of areas, including nickel, epidemiology, carcinogenesis, industrial medicine, kidney toxicity, animal toxicology, and risk assessment. Because Toxicology Excellence for Risk Assessment (*TERA*) prepared the document, Health Canada, U.S. EPA, and the Metal Finishing Association of Southern California, requested that reviewers be independently chosen by *TERA*'s trustees, to maintain independence of the review. A summary of the discussions and recommendations from the peer review meetings are found in Appendix D of this report. The peer reviewers were:

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Additional information about *TERA*'s peer review process can be found on the Internet at http://www.tera.org or by contacting:

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LIST OF ABBREVIATIONS

AFC - Antibody forming cell
ATSDR - Agency for Toxic Substances and Disease Registry
$2m - $_2$-microglobulin
BAL - Bronchoalveolar lavage
BMC - Benchmark concentration
$BMCL_{10}$ - Lower bound on concentration corresponding to 10% risk (used to be explicit that the lower bound and not the maximum likelihood estimate is being used)
BMD - Benchmark dose
$BMDL_{10}$ - Lower bound on dose corresponding to 10% risk (used to be explicit that the lower bound and not the maximum likelihood estimate is being used)
BMR - Benchmark response
BW - Body weight
CHO - Chinese hamster ovary
CI - Confidence interval
DNP - Dinitrosopiperazine
DTH - Delayed type hypersensitivity
EHEN - N-ethyl-N-hydroxyethylnitrosamine
FEV1 - Forced expiratory volume in one second
GSD - Geometric standard deviation
HEC - Human equivalent concentration
HSE - Health and Safety Executive
ICNCM - International Committee on Nickel Carcinogenesis in Man
IOM - Institute of Occupational Medicine
IRIS - Integrated Risk Information System
LALN - Lung-associated lymph node
LDH - lactate dehydrogenase
LOAEL - Lowest observed adverse effect level
MLR - Mixed lymphocyte response
MMAD - Mass median aerodynamic diameter
MOE - Margin of exposure
MTD - Maximum tolerated dose
MTE - Maximally tolerated exposure
NAG - N-acetyl-$-D-glucosaminidase
NTP - National Toxicology Program
NK- Natural killer
NOAEL - No observed adverse effect level
P_0 - Parental generation
PAM - Pulmonary alveolar macrophage
PIXE - Proton induced X-ray emission technique

PFC - Plaque-forming cell
Pnd - Postnatal day
ppm - parts per million
RDDR - Regional deposited dose ratio
REL - Reference Exposure Level
RfC - Reference concentration
RfD - Reference dose
RR - Relative risk
Sigma g - Geometric standard deviation
SHE - Syrian hamster embryo
SIR - Standardized incidence ratio
SMR - Standardized mortality ratio
SRBC - Sheep red blood cells
TD - Tumor dose
TLV - Threshold Limit Value
TWA - Time-weighted average
UF - Uncertainty factor

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CONTENTS —TOXICOLOGICAL REVIEW FOR SOLUBLE NICKEL SALTS (CAS No. VARIOUS)

1. INTRODUCTION

1.1 Background on the Risk Assessment Approach Used

This document presents background and justification for the hazard and dose-response assessment for soluble nickel compounds, using methods developed by the U.S. Environmental Protection Agency (U.S. EPA).

The Reference Dose (RfD) and Reference Concentration (RfC) provide quantitative information for noncancer dose-response assessments. The RfD is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis but may not exist for other toxic effects such as some carcinogenic responses. It is expressed in units of mg/kg/day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The inhalation RfC is analogous to the oral RfD. The inhalation RfC considers toxic effects for both the respiratory system (portal-of-entry) and for effects peripheral to the respiratory system (extrarespiratory or systemic effects). It is generally expressed in units of mg/m^3.

The carcinogenicity assessment provides information on the carcinogenic hazard potential of the substance in question and quantitative estimates of risk from oral exposure and inhalation exposure. The information includes a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen and the conditions under which the carcinogenic effects may be expressed. Quantitative risk estimates are presented in three ways. The *slope factor* is the result of application of a low-dose extrapolation procedure and is presented as the risk per mg/kg/day. The *unit risk* is the quantitative estimate in terms of either risk per Fg/L drinking water or risk per Fg/m^3 air breathed. Another form in which risk is presented is a drinking water or air concentration providing cancer risks of 1 in 10,000; 1 in 100,000; or 1 in 1,000,000.

Development of these hazard identification and dose-response assessments for soluble nickel salts has followed the general guidelines for risk assessment as set forth by the National Research Council (1983). EPA guidelines that were used in the development of this assessment may include the following: the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986a), *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991), *Proposed Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998c), *Proposed Guidelines for Carcinogen Risk Assessment* (U.S. EPA 1996a), and *Reproductive Toxicity Risk Assessment Guidelines* (U.S. EPA, 1996b); *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988); (proposed) *Interim Policy*

for Particle Size and Limit Concentration Issues in Inhalation Toxicity (U.S. EPA, 1994a); *Methods for Derivation of Inhalation Reference Concentrations and Application of Inhalation Dosimetry* (U.S. EPA, 1994b); *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994c); *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995b); *Science Policy Council Handbook: Peer Review* (U.S. EPA, 1998a); and texts explaining the Reference Dose (RfD) (Barnes and Dourson, 1988; Dourson, 1994) and memorandum from EPA Administrator, Carol Browner, dated March 21, 1995, Subject: Guidance on Risk Characterization.

The literature search strategies employed for this compound were based on the CASRN and at least one common name. At a minimum, the following databases were searched: RTECS, HSDB, GENETOX, TOXLINE, CANCERLINE, MEDLINE, and MEDLINE backfiles.

1.2 Regulatory Background on Soluble Nickel

Several texts have been developed for the risk assessment of nickel compounds. Each of these texts has a different focus. For example, a text entitled "Toxicological Profile for Nickel (Update)" was prepared by Agency for Toxic Substances and Disease Registry (ATSDR, 1997) for the purpose of protecting public health from exposures to toxic substances at hazardous waste sites. ATSDR looked at exposures and toxicity for a variety of nickel compounds, both soluble and insoluble, for 3 different exposure durations and two routes of exposure. ATSDR set a Minimal Risk Level (MRL) of 2×10^{-4} mg Ni/m^3 for the chronic inhalation exposure to soluble nickel salts. Other MRLs were not derived.

A text entitled "Nickel and Its Compounds" was prepared by Health Canada under the Canadian Environmental Protection Act (Health Canada, 1993). This text looked at exposures to nickel for Canadians and their environment, and at the toxicity to experimental animals and humans for a number of exposure routes and durations. Tumorigenic Doses at the 5% level (TD_{05}s) were estimated for total nickel between 0.04 and 0.98 mg Ni/m^3 for lung cancer studies in humans and between 0.21 and 62 mg Ni/m^3 for nasal cancer studies in humans. Soluble nickel was not addressed separately.

A risk assessment for the chronic oral exposure of soluble nickel [i.e., a Reference Dose (RfD)] was prepared by U.S. Environmental Protection Agency (U.S. EPA), verified 7/16/87, and is available on its Integrated Risk Information System (IRIS) (U.S. EPA, 1998b). The value of the RfD is 2×10^{-2} mg Ni/kg/day. A Reference Concentration (RfC) was not developed, nor was a risk value for potential cancer effects estimated for either route of exposure. U.S. EPA has also developed risk values for several insoluble forms of nickel (U.S. EPA, 1998b).

The Office of Environmental Health Hazard Assessment (OEHHA) of California's Environmental Protection Agency prepared a risk assessment for nickel in 1991 (CalEPA, 1991). This assessment evaluated cancer and noncancer effects, but provided quantitative risk values only for cancer. The unit risk and slope factor for nickel compounds were 2.6 E-4 (Fg $Ni/m^3)^{-1}$ and 9.1 E-1 $(mg\ Ni/kg/day)^{-1}$, respectively. The corresponding air concentration or oral dose associated with an upper limit lifetime excess cancer risk of 1×10^{-6} (i.e., one in one million) is ~ 4 E-6 mg Ni/m^3, or 1 E-6 mg Ni/kg-day, respectively. The assessment considered whether different forms of nickel differ in health effects. The document concluded (p. 143) that

> "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 than 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."

Therefore, they determined not to speciate nickel compounds in the course of their identification as Toxic Air Contaminants. This cancer assessment was reaffirmed in the Draft Air Toxics Hot Spots Program Risk Assessment Guidelines: Technical Support Document for Describing Available Cancer Potency Factors (CalEPA, 1997a). Noncancer quantitation has been prepared more recently, in the Draft Technical Support Document for the Determination of Noncancer Chronic Reference Exposure Limits (CalEPA, 1997b). This text developed a chronic Reference Exposure Level (REL, similar to EPA's RfC) of 5 E-5 mg Ni/m^3 for nickel compounds, based on lung histopathological changes in male rats in the NTP (1996a) study, using an uncertainty factor of 30.

An occupational Threshold Limit Value (TLV) for soluble nickel was prepared by the American Conference of Government and Industrial Hygienists (ACGIH) (ACGIH, 1998). The TLV-TWA (time-weighted average) is listed as 0.1 mg Ni/m^3, as inhalable nickel particulate. The cancer classification for soluble nickel compounds is A4, not classifiable as a human carcinogen. The TLV-TWA for elemental/metal nickel was 1.5 mg Ni/m^3, and the TLV-TWAs of insoluble nickel compounds were 0.2 and 0.1 mg Ni/m^3, respectively. All of the TLVs are in terms of inhalable nickel particulate. The cancer classification of elemental/metal nickel is A5 (not suspected as a human carcinogen), and of insoluble nickel compounds and nickel subsulfide is A1 (confirmed human carcinogen). TLVs are intended for

use in the practice of industrial hygiene as guidelines and are not intended to be used in the evaluation or control of community air pollution. TLVs are believed to be levels to which nearly all workers may be repeatedly exposed day after day without adverse health effects.

A text entitled "Occupational Exposure Limits Criteria Document For Nickel and Nickel Compounds" was prepared by the Nickel Producers Environmental Research Association (NiPERA) (NiPERA, 1996). This document mainly focused on the industrial use of nickel compounds and occupational exposure to nickel, and evaluated the risk of inhalation exposure to nickel compounds. For soluble nickel compounds, the NiPERA document recommended that the 8-hour Time Weighted Average (TWA) Occupational Exposure Limit (OEL) be set at 0.1 mg Ni/m^3. The authors also stated that soluble nickel compound should be classified as a Category 3 (substances for which there is cause for concern owing to possible carcinogenic effects, but for which there is inadequate information). This OEL was based on epidemiological studies reporting an increased risk of lung and nasal cancer associated with exposures to soluble nickel compounds primarily at levels >1 mg Ni/m^3. Carcinogenic effects of insoluble nickel were also assessed. NiPERA (1996) did not suggest any noncancer inhalation risk values, or any cancer or noncancer oral risk values for soluble nickel compounds.

An evaluation of the potential cancer risk of selected nickel compounds has also been developed by Oller et al. (1997). These authors propose a mechanistic model to relate the carcinogenicity of various nickel compounds to either direct or indirect heritable changes induced by nickel compounds, or to the promotion of cell proliferation elicited by certain nickel compounds. They conclude that nickel subsulfide is likely to be carcinogenic to humans; that nickel sulfate hexahydrate is not likely to be carcinogenic to humans, although it may enhance the effect on the carcinogenicity of insoluble nickel compounds; and that green nickel oxide may only be carcinogenic to humans at high doses that result in chronic inflammation.

The purpose of the current document is to present a toxicological review and dose response assessment of *soluble nickel salts*, in light of new studies that show differences in the toxicity of different nickel compounds [e.g., National Toxicology Program (NTP), 1996a, 1996b, 1996c]. Selected occupational studies of mixed soluble and insoluble nickel exposures are also reviewed in detail in an attempt to distinguish the toxicity between these forms. Although this document focuses on soluble nickel salts, some mechanistic and kinetic data for insoluble nickel compounds are also presented in order to address differences in results of studies with the different compounds. Risk values for noncancer toxicity of soluble nickel salts after chronic exposures via the oral or inhalation routes are derived. The carcinogenic potential of soluble nickel salts via the inhalation and oral routes is also assessed.

2. CHEMICAL, PHYSICAL AND OCCUPATIONAL EXPOSURE INFORMATION RELEVANT TO ASSESSMENTS

2.1 Chemical and Physical Properties

Nickel is a group VIII transition metal. Although it can exist in several different oxidation states, the only important oxidation state under environmental conditions is Ni+2. Physical properties of nickel compounds for which toxicity data are available are presented in Table 1. As noted in the table, nickel subsulfide is insoluble in water, but somewhat soluble in biological fluids (Benson et al., 1994; Oller et al., 1997). As used in this document, "insoluble nickel compounds" refers to solubility in water, and includes nickel subsulfide. Furthermore, unless otherwise specified, "insoluble nickel" refers to insoluble nickel compounds, not metallic nickel.

Nickel is used with other metals to form alloys to make such items as coins, jewelry, valves, heat exchangers, and stainless steel. Nickel alloys impart corrosion resistance, heat resistance, hardness and strength. Nickel compounds are also used to color ceramics, in batteries, and as catalysts (ATSDR, 1997).

Table 1. Physical Properties of Soluble Nickel Compounds and Some Insoluble Nickel Compounds Relevant to the Assessment[1]

Name	Formula	CAS	MW	Solubility in water (g/100 ml) (superscript is temperature, ^{E}C)
Nickel Fluoroborate	$Ni(BF_4)_2.6H_2O$	14708-14-6	340.42	Soluble
Nickel Sulfamate	$Ni(SH_2NO_3)_2 \cdot 4H_2O$	13770-89-3	322.95	Soluble
Nickel Formate	$Ni(CHO_2)_2.2H_2O$	3349-06-2	184.76	Soluble
Nickel Nitrate	$Ni(NO_3)_2\ 6H_2O$	13138-45-9	290.79	238.5^{0}
Nickel Chloride	$NiCl_2$	7718-54-9	129.60	64.2^{20}, 87.6^{100}
Nickel Chloride Hexahydrate	$NiCl_2 \cdot 6H_2O$	7791-20-0	237.69	254^{20}, 599^{100}
Nickel Sulfate	$NiSO_4$	7786-81-4	154.75	29.3^{0}, 87.3^{100}

Name	Formula	CAS	MW	Solubility in water (g/100 ml) (superscript is temperature, ^{E}C)
Nickel Sulfate Hexahydrate	$NiSO_4 \cdot 6H_2O$	10101-97-0	262.84	65.5^{0}, 340.7^{100}
Nickel Acetate	$Ni(CH_3CO_2)_2$	373-02-4	176.80	17^{20}
Nickel Acetate Tetrahydrate	$Ni(CH_3CO_2)_2.6H_2O$	6018-89-9	248.9	Soluble
Nickel Ammonium Sulfate	$Ni(NH_3)_2SO_4.6H_2O$	7785-20-8	395.00	2.5^{4}, 39.2^{85}
Nickel Fluoride	NiF_2	10028-18-9	96.69	4^{25}
Nickel Hydroxide	$Ni(OH)_2$	12054-48-7	92.70	0.013
Nickel Carbonate	$NiCO_3$	333-67-3	118.70	0.0093^{25}
Nickel Sulfide	NiS	16812-54-7	90.75	0.00036^{18}
Nickel Subsulfide	Ni_3S_2	12035-72-2	240.19	Insoluble[2]
Nickel Oxide	NiO	1313-99-1	74.69	Insoluble
Nickel	Ni	7440-02-0	58.7	Insoluble

[1]Information was obtained from Lide (1992), NiPERA (1996), ATSDR (1997).
[2]Relatively soluble in biological fluids (Benson et al., 1994; Oller et al., 1997).

2.2 Exposure to Nickel

Exposure to nickel can be through air, water or food. The following discussion presents typical exposure and intake from air, water, and food, and presents all of the data in common units. ATSDR (1997) states that average nickel concentrations in ambient air of U.S. cities range from about 5 to 50 ng Ni/m^3 , leading to an average inhalation of about 1 E-6 to 1 E-5 mg Ni/kg body weight/day (assuming 20 m^3 breathed per day for a 70 kg person, and assuming all inhaled nickel is deposited). Daily average intakes from water fall in the range of 2 Fg Ni/day (or an intake of about 3 E-5 mg Ni/kg/day for a 70 kg person). Average daily intake of nickel in food is much larger at ~170 Fg Ni/day (or an intake of about 2 E-3 mg Ni/kg/day assuming a 70 kg person). It is difficult to compile all of these various intakes into a composite value, however, because of the known differences in absorption between these routes and

media (as discussed in Section 3.1). However, some perspective can be provided by noting that humans absorb nearly 40 times as much nickel from water under fasting conditions as they do from food (Sunderman et al., 1989) (direct food versus water comparisons are not available for unfasted conditions).

Exposures in Canada closely match the U.S. values. For example, Health Canada (1993) states that average nickel concentrations in ambient air of Canadian cities range from 1 to 20 ng/m^3, leading to an average inhalation of about 3 E-7 to 9 E-6 mg Ni/kg/day for different age groups. Average concentrations in drinking water fall in the range of 0.2 to 7.2 Fg/L, leading to intakes of 4 E-6 to 8 E-4 mg Ni/kg/day for different age groups (Health Canada, 1993). This group also states that average daily intake of nickel in food is much larger, at about 4 E-3 to 2 E-2 mg Ni/kg/day for different age groups.

2.3 Occupational Exposure to Nickel

Occupational studies play a key role in the determination of the risk of cancer in humans from exposure to soluble nickel compounds. Use of the occupational studies for quantitation of a risk value, however, requires that the reported exposure data provide an accurate reflection of the concentration of soluble nickel available to reach the target tissue.

Because the reliability of much of the epidemiology data is related to the accuracy of the corresponding exposure assessments, an understanding of the issues related to exposure assessment for nickel workers is important. The following section describes work activities related to nickel exposure, the relative predominance of different nickel species, and exposure assessment issues related to the epidemiology literature for nickel. These issues are described in greater detail in Appendix C.

The report of the Working Group of the International Committee on Nickel Carcinogenesis in Man (ICNCM, 1990) includes estimates of worker exposure and nickel speciation, based on some minimal monitoring, as well as worker recall and process information. The initial processes that involve handling and purification of nickel-containing ores, such as mining, milling, and smelting operations typically involve higher exposures to insoluble than soluble nickel compounds (Warner et al., 1984). The ratio of soluble to insoluble nickel increases at later stages of refining. Nickel refineries often employ a purification process called electrowinning, or electrolytic refining. This process involves purification of crude nickel in an electrolytic bath containing soluble nickel salts. Appropriate electrolytic conditions require constant mixing of the bath, which is usually accomplished by bubbling air through the solution. The agitation of the solution generates aerosols of soluble nickel salts that can enter the plant environment and result in worker exposure. Metal finishing operations, such as electroplating and electroless plating, involve deposition of pure nickel onto a workpiece through a similar process as used in electrowinning. In both electrowinning and nickel plating, similar solutions of

soluble nickel salts are used, and the aerosolization of the soluble nickel salts accounts for the high soluble nickel exposures relative to insoluble forms. For example, in electrowinning operations, soluble nickel has been reported to constitute from 86% to 99.7% of the total nickel content of air samples in various areas of an electrowinning facility (Kiilunen et al. 1997a). Soluble nickel also predominates in electroplating shops, with the percent soluble nickel ranging from 18% to 100% reported for three Finnish shops (Kiilunen et al., 1997b) and 63.7% and 90.3% for two American shops (Tsai et al., 1996).

In addition to knowledge of the primary form of nickel present in the environment, estimating the potential tissue doses also requires knowledge of the air sampling method employed to estimate the worker exposure. Different sampling methods were used in different studies, and the implications for estimating exposure have to be considered. For example, in many cases, area samples are reported, which do not necessarily correlate well with personal exposure data. For this reason, personal monitoring data are preferred. Even when personal exposure monitoring was done, however, the variability was often large and the number of samples insufficient to characterize typical worker exposures. In many cases, mean exposure levels were presented, stratified by job categories or work areas.

The typical air sampling techniques do not differentiate among nickel species or particle size distributions. The particle size selectivity of the air sampling method also plays a key role in estimating the tissue dose, since the particle size largely determines whether a particle enters the respiratory tract and how far it penetrates. In the case of soluble nickel, the industrial hygiene data were generally collected for the “total,” or inhalable particulate fraction, in light of the concerns for a role of soluble nickel in the development of lung, nasal cavity, and stomach cancers. The sampling methods that were used in the different nickel studies, however, do not collect the entire inhalable fraction with equal efficiency. Inhalable particulate samplers generally collect a greater particle mass than a "total" dust sampler placed in the same environment, with greater disparity occurring for large particles (Werner et al., 1996). Exposures measured with “total” particulate samplers can be converted to the equivalent inhalable particulate concentrations by multiplying by a default ratio of 2.0, which is typical of mists (Werner, et al., 1996).

2.4 Nickel Speciation: Relevance to Exposures Through Water

Nickel carried in water can occur as particles of insoluble compounds or in true solution as nickel ion (Ni^{++}). However, in a gavage study, it was found that absorption was less than 1% when rats were administered nickel in the insoluble forms of nickel oxides, nickel subsulfide or metallic nickel, while 9.8-33.8% was absorbed by rats administered soluble forms of nickel (Ishimatsu et al., 1995). Therefore, ingestion of insoluble nickel would not be expected to contribute significantly to nickel toxicity. As described in Section 4, animal toxicity studies and human clinical studies involved exposure to defined soluble nickel compounds, such as nickel

chloride, nickel sulfate, or nickel acetate. In these cases, the animals were exposed to the dissociated divalent nickel cation and its accompanying anion. All doses in this report were calculated in terms of the amount of nickel. By contrast, nickel concentrations in finished drinking water consumed by the general public are typically evaluated in terms of total nickel. EPA test methods permit the differentiation of soluble and insoluble nickel by filtration of the sample through a 0.45 m filter, but available reports do not usually state whether this extra step was included. If the nickel concentration in a drinking water sample is measured using a technique that combines both soluble and insoluble nickel compounds, the risk due to the nickel content will be overestimated, since insoluble nickel compounds appear to have a low toxicity by ingestion.

3. TOXICOKINETICS RELEVANT TO ASSESSMENTS

Absorption of soluble nickel from the lungs is rapid and extensive. Absorption of ingested nickel is less extensive, and is lower when nickel is administered in food than when it is administered in water. Absorbed nickel by either route is rapidly distributed to the kidney and lung, but does not accumulate in the body to a significant extent. Elimination of absorbed nickel is predominantly by the urinary route.

3.1 Absorption

Soluble nickel salts can be absorbed after either inhalation or ingestion exposures. Animal studies suggest that the most of intratracheally instilled soluble nickel is absorbed rapidly into the body. Although absorption of *inhaled* nickel has not been quantified, the absorption kinetics following inhalation exposure would be expected to be similar to the kinetics of intratracheally instilled nickel. In contrast, a smaller proportion of ingested soluble nickel is absorbed even by fasting subjects; the presence of food in the gastrointestinal tract further decreases nickel absorption.

3.1.1 Inhalation

Data on nickel absorption are available for both humans and animals. Among the nickel compounds studied, soluble nickel salts (e.g., nickel sulfate and nickel chloride) were more readily absorbed after inhalation exposure than were less-soluble nickel compounds (nickel subsulfide) and insoluble nickel (nickel oxide). Both animal studies and studies of occupational exposure indicate that inhaled soluble nickel salts are absorbed, based on increases in serum and urine nickel levels shortly after exposure. More quantitative data are available from animal studies than from human studies.

Like all inhaled particulates, inhaled nickel particles are deposited in the upper and lower respiratory tract and are subsequently either absorbed or cleared from the lung. The

region of particle deposition is determined by the particle size. In humans, large particles having an aerodynamic diameter of 5 to 30 Fm are mainly deposited in the nasopharyngeal region by interception and impaction. Smaller particles having an aerodynamic diameter of about 2.5 to 5 Fm pass through the nasopharyngeal region and are deposited in the tracheobronchial regions by sedimentation. At particle sizes smaller than about 4 Fm, deposition in the terminal airways and alveoli becomes increasingly important. At particle sizes smaller than about 0.5 Fm, deposition is primarily by diffusion and electrostatic precipitation (U.S. EPA, 1994b). In addition to the particle size, the breathing pattern can also affect the pattern of particle deposition. No information on particle size distributions for occupational exposure to soluble nickel salts was located, and so the deposition pattern of soluble nickel in the lung after occupational exposure is unknown.

Several human studies indicate that exposure of electroplating workers to soluble nickel salts results in nickel absorption. In studies on electroplating workers (workroom air concentrations ranging from 0.03 to 0.16 mg Ni/m^3), the urinary and plasma nickel concentrations were higher in post-shift samples than in pre-shift ones, and a close positive correlation was found between the air nickel concentrations and the urine and plasma nickel concentrations (Tola et al., 1979; Bernacki et al., 1980; Ghezzi et al., 1989). When the nickel concentration in electroplating workers' urine was measured before, in the middle, and at the end of the work shift, urinary nickel concentration increased over the course of a regular working day (Bernacki et al., 1980).

Workers in nickel roasting/smelting are exposed to less-soluble nickel compounds (such as nickel subsulfide and nickel oxide). Although these compounds are less soluble, they are also absorbed, as evidenced by elevated nickel concentrations in the plasma and urine of exposed workers. These less-soluble compounds were absorbed less efficiently than the soluble nickel salts, since workers exposed to nickel subsulfide and nickel oxide had lower plasma and urinary nickel concentrations than workers exposed to lower concentrations of soluble nickel. The half-life of nickel release from the nasal mucosa was estimated to be about 3.5 years, although there is considerable uncertainty in this estimate. By contrast, higher nickel concentrations in nasal mucosa resulted from exposure to nickel subsulfide and nickel oxide than from exposures to soluble nickel, indicating that the lower plasma levels result from reduced clearance from the lung, rather than from reduced deposition (Torjussen and Andersen, 1979). No other quantitative human data on the amount and rate of absorption are available.

The limited available quantitative data from animal studies indicate that clearance of inhaled soluble nickel is rapid and extensive. Benson et al. (1995) measured clearance of inhaled radiolabeled nickel sulfate ($^{63}NiSO_4$) in rats and mice that also were exposed via inhalation to unlabeled nickel sulfate for 2-6 months. In rats, approximately 99% of the inhaled nickel had a half-time for clearance from the lung of 2-3 days; the rest of the deposited material

cleared over an indefinitely long period. In mice, the half-time for the initial phase was faster, but accounted for less of the material. Approximately 80-90% of the material cleared with a half-time of less than 1 day, and the half-time for the rest of the material was 5-17 days. Although nickel levels in blood and urine were not measured, it is likely that most of the clearance was via absorption, rather than clearance of particulates (since the particulates formed from soluble nickel aerosols rapidly dissolve once they are deposited in the respiratory tract), and thus the clearance rates give some indication of likely absorption kinetics.

Benson and colleagues also evaluated the absorption and clearance of inhaled nickel subsulfide and nickel oxide ($^{63}Ni_3S_2$, ^{63}NiO) (Benson et al., 1994; Benson et al., 1995). They found that inhaled nickel subsulfide had a relatively rapid clearance half-time of 4 days. Thus, although nickel subsulfide is nearly insoluble in water, it is dissolved rapidly in the lung, and behaves like a relatively soluble particle. By contrast, nickel oxide was slowly cleared from the lungs, with a half-time of approximately 120 days. The differences between these clearance times and those of Torjussen and Andersen (1979) may reflect the considerable uncertainty in the human study, the differences in respiratory region (nasal vs. lung), or they may reflect actual interspecies differences.

More data are available from intratracheal instillation studies, which indicate that soluble nickel salts are rapidly and extensively absorbed from the respiratory tract in animals. In Wistar rats, intratracheal instillation of nickel chloride (5.9 Fg Ni in form of $^{63}NiCl_2$) resulted in rapid absorption from the lung and distribution of nickel throughout the body, followed by rapid elimination of nickel from the body. In most tissues, the concentrations of nickel at 0.5 hour after instillation were the highest of the study, indicating that significant absorption had occurred within that time period. By the third day, rats had excreted an average of 70% of the instilled nickel (English et al., 1981).

In another study (Carvalho and Ziemer, 1982), rapid absorption of nickel after intratracheal injection of Sprague-Dawley rats with nickel chloride (1.27 Fg Ni/rat in the form of $^{63}NiCl_2$) was also observed. Nickel in blood was increased 35 minutes after the injection, with smaller increases observed at day 1 and day 3. By days 7 and 21, Ni was not detectable. Within one day of dosing, 72% of the initial body burden was eliminated in the urine, and 78% was eliminated within 3 days. By day 21, an average of 96% of the initial body burden was excreted by the urinary route. The excretion via the fecal route accounted for only 3% of the initial burden from day 1 to day 21. These data show that only a small percentage of the instilled nickel was removed by mucocilliary clearance and excreted in the feces, while most of the nickel was absorbed. This experimental design could not distinguish between nickel absorbed from the lungs and nickel cleared from the lungs, swallowed, and absorbed from the gastrointestinal tract, but gastrointestinal absorption of soluble nickel is low, as described in the next section.

Rapid absorption of injected soluble nickel salts was also seen in guinea pigs. Intratracheal instillation of 1 mg Ni in the form of $^{63}NiCl_2$ caused a rapid increase in the nickel levels in several organs. The nickel concentration measured as radioactivity in kidney, lung, and other tissues was determined, and the highest tissue concentration was observed at 6 hours after the instillation. The nickel concentration was decreased at 24 and 72 hours after instillation (Clary, 1975).

The amount of nickel absorption also depends on the exposure level. When rats were given 17, 190, or 1800 nmoles (1, 11, or 106 Fg) nickel as nickel sulfate ($^{63}NiSO_4$) by intratracheal instillation, the nickel concentration measured as radioactivity in blood was highest 4 hours after the instillation, with blood levels decreasing at 24 and 96 hours after instillation. Urinary excretion accounted for 54%, 56% and 82% of the low, mid, and high doses, respectively, while the corresponding amounts of the administered dose in the feces were 31%, 26%, and 13% (Medinsky et al., 1987). In addition, the absorption and clearance half-time was shorter at the higher doses. Thus, both the extent and rate of absorption and clearance were higher at the higher doses. The study authors attributed the dose-related changes to a decreased percentage of nickel binding to lung proteins at the higher dose, and thus a larger percentage that was available for clearance by diffusion of nickel ions. A possible implication of this finding is that animal inhalation experiments conducted at high exposure levels would be expected to *underestimate* the lung burdens of people exposed at lower levels, where the relative rate of clearance and absorption is greater. However, this is not likely to be an issue for the interpretation of the 2-year bioassay of NTP (1996a), since the maximum lung burden in that study was less than 5 Fg/lung.

3.1.2 Oral

Data from both animals and humans show that only a small proportion (1- 27%) of ingested soluble nickel salts is absorbed; the rest is excreted in the feces (Diamond et al., 1998). As with the inhalation route, absorption of ingested nickel is evaluated by measuring its concentration in serum and urine. Ingested nickel reaches the serum rapidly; the mean transit time of the metal from the lumen of saline-perfused jejunum in the rat to portal blood is much shorter than that of cadmium and is approximately only 3 minutes (Foulkes and McMullen, 1987). After continuing exposure, serum levels reach a maximum within 2.5 to 3 hours. The maximal urinary excretion occurs within 8 hours. The degree of absorption of ingested nickel absorption is higher when given in water than in food.

Ingestion of nickel either in food or on a nonfasted stomach decreases the extent of absorption. Diamond et al. (1998) used a biokinetic model to estimate nickel absorption, based on experimental data from Christensen and Lagesson (1981), Menne et al. (1978), and others. These results are summarized in Table 2, and show that estimated nickel absorption ranged from 12-27% of the dose when nickel was ingested after a fast, to 1-6% when nickel

was administered either in food, in water, or in a capsule during (or in close proximity to) a meal. When fasting subjects ingested nickel sulfate in water, 27% of the dose was absorbed, but nickel absorption in fasting subjects decreased to 0.7% when the nickel sulfate was given with food (Sunderman et al., 1989). Data of Christensen and Lagesson (1981) show that 5.7% of the nickel dose [as estimated by Diamond et al. (1998)] was absorbed after nonfasting subjects ingested nickel sulfate. The results of Solomons et al. (1982) also showed that food inhibited nickel absorption. When fasting subjects ingested nickel sulfate in water, the nickel concentration in plasma was elevated while no increase in plasma nickel concentration was observed when nickel was administered with food. The plasma nickel concentration increased when the nickel was administered in Coca-Cola™, but the increase was diminished when nickel was given with whole cow's milk, coffee, tea, or orange juice. Thus, food and beverage intake can significantly affect absorption of ingested nickel salts. Nielsen et al. (1999) found that the peak serum concentration was 13-fold higher when nickel was ingested prior to a meal, compared to when it was ingested with a meal. Absorption in women was less than that in men, and the authors suggested the difference was due to differences in gastric emptying rates. Absorption measured in a group of nickel-sensitized women did not differ from that in control women.

Several human studies with soluble nickel salts also demonstrated that the rate of absorption after ingestion is rapid (see Table 2). Ingestion of nickel sulfate led to increased nickel concentrations in serum within 1 hour and peaking 1-3 hours after ingestion (Christensen and Lagesson, 1981; Nielsen et al., 1999; Solomons et al., 1982; Sunderman et al., 1989). This rapid absorption led to increased urinary nickel levels within 24 hours (Hindsen et al., 1994), with maximal urinary nickel excretion occurring within the first eight hours after ingestion (Christensen and Lagesson, 1981).

Rapid nickel absorption was also observed in animal studies. When non-fasting Fischer-344 rats were dosed by oral gavage with nickel chloride ($^{63}NiCl_2$), the peak urinary nickel concentration measured as radioactivity was at 4 hours after dosing. The study showed that regardless of the quantity of nickel given orally, the animals eliminated the entire amount within 48 hr. About 3-6% of the nickel dose was excreted in the urine, while the unabsorbed nickel was eliminated in the feces (Ho and Furst, 1973).

The absorption of nickel compounds administered orally is closely related to the solubility of the compound. Male Wistar rats were administered a single dose of a non-radiolabeled nickel compound (10 mg Ni in the form of $NiSO_4$, $NiCl_2$, $Ni(NO_3)_2$, Ni_3S_2 or NiO) in 5% starch saline solution by gavage, and the absorption and distribution of nickel were determined. Nickel absorption was much higher for the soluble nickel compounds nickel sulfate, nickel chloride, and nickel nitrate (11%, 9.8%, and 34%, respectively), compared to 0.47% for slightly soluble nickel subsulfide and 0.01% for insoluble green nickel oxide. Comparison with the solubilities shown in Table 1 for these compounds shows that the relative

percent absorption correlates with the solubility of the different compounds. Similarly, nickel organ levels were higher in rats given soluble nickel compounds [$Ni(NO_3)_2$, $NiCl_2$, or $NiSO_4$] than in rats receiving insoluble compounds (Ishimatsu et al., 1995).

3.2 Distribution

Nickel tissue distribution in animals does not appear to depend significantly on the route of nickel administration. Inhaled nickel is rapidly dissolved and distributed to extrarespiratory tissues, where its distribution is similar to that of ingested nickel. Low levels of accumulation in tissue are observed (generally <1 ppm), but the increases in tissue levels that are observed occur rapidly. A primary site of elevated tissue levels is the kidney. In addition, elevated concentrations of nickel were often found in the lung, even after oral dosing, and in the liver. Elevated nickel levels are less often found in other tissues.

3.2.1 Inhalation

Limited information exists on nickel tissue distribution in humans after inhalation. Autopsy distribution data are only available for lung tissues and not other internal organs. Other human distribution data for inhaled soluble nickel only address tissues that can be sampled less invasively (e.g., blood, hair). Animal studies show that inhaled soluble nickel salts primarily distribute to the lung and kidney, with some distribution to the liver and other tissues.

Elevated nickel concentrations have been observed in the lungs of workers occupationally exposed to nickel, presumably due to deposited nickel that has not been cleared or absorbed. Elevated nickel levels in nasal mucosa, blood, and hair have also been observed after nickel inhalation. Consistent with the observation that inhaled nickel is deposited in the nose and lungs, and that clearance correlates with solubility of the nickel species, workers exposed to soluble nickel compounds had elevated nickel levels in the nasal mucosa, and higher levels were observed in workers exposed to less-soluble nickel compounds (Torjussen and Andersen, 1979). Among manufacturing workers occupationally exposed to nickel salts (species was not stated) by inhalation, the blood plasma nickel concentration was more than five times that in controls, and the nickel content of scalp hair was approximately ten times higher than normal (Spruit and Bongaarts, 1977).

Nickel tissue distribution after exposure was investigated in more detail in animal studies. Tissue levels following inhalation of soluble nickel were generally low, with the nickel distributing primarily to the lung and kidney (Table 2). Lung levels following inhalation exposure to unlabeled nickel sulfate for 2 years were much lower than following exposure under similar conditions to nickel subsulfide or nickel oxide (NTP, 1996a, 1996b, 1996c). Most of the studies of the distribution of inhaled radiolabeled soluble nickel compounds showed that nickel concentrations were highest in the lung, followed by the kidney (Carvalho and Ziemer, 1982;

English et al., 1981; Medinsky et al., 1987). However, one study found a slightly higher nickel concentration (based on radioactivity levels) in the kidney than in the lung 6 hours and 24 hours after intratracheal instillation, although the nickel level in the lung was higher 72 hours after exposure (Clary, 1975). The elevated kidney levels at the earlier time points appear to be due to experimental variability. Other studies (Carvalho and Ziemer, 1982; English et al., 1981; Medinsky et al., 1987) found much lower nickel levels in other tested tissues, such as the ovaries, testes, skin, pancreas, spleen, adrenals, pituitary, liver, and heart. Thus, the animal studies indicate that nickel primarily distributes to the lung and kidney with relatively higher concentrations in the lung shortly after exposure.

NTP (1996a) reported on the lung and kidney burdens of nickel among rats and mice that were exposed by inhalation to nickel sulfate for 16 days, 13 weeks, 7 months, and 15 months. In rats, lung burdens of nickel tended to increase with exposure level, particularly in the 13-week and longer studies. Thus, some degree of tissue accumulation occurred, even with this rather soluble nickel compound. Measurable lung burdens were reported in mice at the high concentration in the 13-week study, but not at lower exposure levels, or in the 7- or 15-month studies. In rats, kidney levels showed some tendency to increase with exposure concentration or duration, but the results were highly variable and not statistically different from background levels. Kidney levels of nickel in mice in the 7- and 15-month studies were not elevated above background levels.

Lung levels of nickel were higher in rats and mice exposed by inhalation to nickel subsulfide or nickel oxide (NTP, 1996b, 1996c). Lung burdens were much higher following exposure to nickel oxide than to similar concentrations of nickel subsulfide, consistent with the slight solubility of nickel subsulfide in biological fluids. (The NTP studies only looked at tissue burdens in the lung and kidney.) The solubility of the nickel compound also affects the distribution of inhaled nickel to tissues beyond the portal of entry. English et al. (1981) evaluated the distribution of nickel in rat tissues 0.5 hours after an intratracheal injection of soluble $^{63}NiCl_2$ or insoluble ^{63}NiO. In both cases the lungs and mediastinal lymph nodes had the greatest concentration (radioactivity) of nickel. In the animals treated with $NiCl_2$, the kidney had the next greatest amount (Ni/g wet tissue), followed in order by the femur, heart, and duodenum. Following NiO treatment, the greatest radioactivity levels after lung and lymph nodes were found in the heart, followed in order by femur, duodenum, and kidney (English et al., 1981). Therefore, the relative nickel distribution varied when animals were exposed to nickel compounds with different solubilities.

3.2.2 Oral

Very limited information exists on the human tissue distribution of nickel after oral exposure. Most of the data on the distribution of nickel following oral exposure are from animal studies, which are summarized in Table 4. These studies show that after the ingestion of soluble

nickel salts, the highest nickel level is observed in the kidney. The absorbed nickel can also cross the placenta and accumulate in fetal tissues.

Data from a case in which workers in an electroplating plant accidentally drank water contaminated with nickel sulfate and chloride (1.63 g Ni/liter) provide information on nickel toxicokinetics in humans at high doses (Sunderman et al., 1988). The estimated oral intake of nickel by the symptomatic workers ranged from 0.5 to 2.5 g. On day 1 postexposure, serum nickel concentrations averaged 0.286 mg Ni/liter, compared with 0.004 mg Ni/liter in the comparison group of nickel plating workers who had not drunk the water. Urinary nickel concentrations in exposed workers averaged 5.8 mg Ni/liter, compared to the control level of 0.050 mg Ni/liter.

In animals, the highest nickel concentration was commonly seen in the kidney after ingestion of soluble nickel salts (Table 4). The sole exception was the study of Schroeder et al. (1964), in which unlabeled nickel (compound not reported) was administered in drinking water in a reproductive toxicity study. This study reported that the nickel concentration was higher in the spleen than in the kidney after mice were chronically exposed to nickel salts. A similar chronic study in rats (Ambrose et al., 1976) showed the kidney as the organ with the highest nickel concentration after oral exposure, but the spleen was not examined in this study. Thus, the significance of the result from the study of Schroeder et al. (1964) is not clear. In studies where the lung was examined, the second highest nickel concentration was observed in this tissue (Jasim and Tjalve, 1986; Ishimatsu et al., 1995; Borg and Tjalve, 1989; Whanger, 1973; Dieter et al., 1988; Ambrose et al., 1976). In addition, nickel concentrations in the placenta and fetus also increased, showing that absorbed nickel is able to cross the placenta (Jasim and Tjalve, 1986; Schroeder et al., 1964). In fetal tissues, the highest nickel concentration was observed in the kidney, a pattern of nickel distribution that was similar to that observed in adults (Jasim and Tjalve, 1986).

3.2.3 Whole-body Aspects of Distribution

Upon entry into the bloodstream, nickel ion is bound to specific serum components and rapidly distributed throughout the body. In blood serum, nickel is present in three forms: as ultrafiltrable material (low molecular weight form), as a complex associated with albumin, and as a complex associated with a nickel-metalloprotein (nickeloplasmin) (Nomoto et al., 1971). In rabbit serum, 16% of the nickel is present as ultrafiltrable material, 40% is associated with albumin, and 44% is associated with nickeloplasmin (Nomoto et al., 1971). The distribution in human serum is somewhat different: 40% in ultrafiltrable material, 34% associated with albumin, and 26% associated with nickeloplasmin [reported by Hausinger (1993a), apparently based on the results of Nomoto and Sunderman (1988)].

The predominant low molecular weight form of nickel in serum is a complex of nickel

with the free amino acid L-histidine (Lucassen and Sarkar, 1979; Sarkar, 1984). At the pH of serum, L-histidine has a greater affinity for Ni(II) than does human serum albumin, and L-histidine is thought to form the $Ni(His)_2$ complex (Glennon and Sarkar, 1982; Sarkar, 1984).

The most important nickel-binding protein in serum is albumin, which binds nickel at the amino terminus. Nickel can exchange between albumin and free histidine via a ternary complex that binds the metal ion very tightly (Glennon and Sarkar, 1982). Interspecies variation in the nickel-binding capacity of albumin has been observed. While albumin from most species tested, such as humans, rats, rabbits, and cows, had a high binding capacity for nickel, the nickel-binding capacity of albumin from dogs and pigs is much lower (Callan and Sunderman, 1973).

The high molecular weight nickeloplasmin serum protein has been identified as alpha-2-macroglobulin (Nomoto et al., 1971; Nomoto and Sunderman, 1988). Nickel is firmly bound to nickeloplasmin, so that it is not removed during successive purification procedures. When pooled serum was dialyzed against solution containing Ni(II), the concentration of nickel associated with nickeloplasmin was the same as in the nondialyzed control sample. Thus, the binding of nickel to nickeloplasmin is not in simple equilibrium (Nomoto et al., 1971). Alpha-2-macroglobulin is also the major zinc-binding protein in the serum, and it has been suggested that nickel and zinc bind at the same site.

The predominant intracellular form of nickel has been observed to vary among tissues. In the lung and liver of NMRI mice, nickel was bound predominantly to a high-molecular-weight protein; in the kidney, it was bound mainly to low-molecular-weight ultrafiltrable ligands (Oskarsson and Tjalve, 1979).

3.2.4 Uptake into Cells

Nickel can enter animal cells by three different mechanisms: uptake via metal ion transport systems, diffusion of lipophilic nickel compounds through the membrane, and phagocytosis. Several different investigators have reviewed the different mechanisms by which cells uptake different nickel compounds (Oller et al., 1997; IARC, 1989; Hausinger, 1993a). As described in this section, differences in cellular uptake of soluble and insoluble forms of nickel are proported to play a major role in the observed differences in nickel carcinogenicity among these compounds. According to this hypothesis, insoluble nickel compounds enter the cell via phagocytosis, while soluble nickel compounds are not phagocytized, but can enter via the magnesium transport system or through membrane diffusion. The latter two processes are much less efficient, so that the same *extracellular* levels of soluble and insoluble nickel compounds lead to lower nickel levels *inside* the cell for soluble nickel (Fletcher et al., 1994). Because of the importance of the difference in handling of soluble and insoluble nickel compounds, this discussion begins with a description of cellular uptake of insoluble nickel

compounds, and then addresses uptake of soluble nickel.

A number of studies have shown that cellular uptake of the insoluble nickel compounds nickel subsulfide and nickel sulfide particles occurs via phagocytosis (Costa and Mollenhauer, 1980; Costa et al., 1981; Abbracchio et al., 1982b; Heck and Costa, 1983). Small amounts of nickel subsulfide may also dissolve outside the cell, and enter the cell as soluble nickel. In a comparison of a number of insoluble nickel compounds (using similar particle sizes), transformation activity in Syrian hamster embryo (SHE) cells correlated with the phagocytic activity of the compound (Costa and Heck, 1982; Abbracchio et al., 1982b). Soluble nickel chloride has lower transforming activity than the well-phagocytised compounds. This difference is attributed to more efficient uptake and better retention of insoluble nickel entering via phagocytosis, compared to soluble nickel entering via diffusion or transport (Costa et al., 1981; NTP, 1996a; Oller et al., 1997).

The process by which soluble nickel enters the cell is less clearly understood. Uptake of soluble nickel into cells may occur as a result of transport or diffusion of nickel complexes through the cell membrane. In enteric cells, nickel interacts with cell membranes in a manner similar to the interactions of cadmium and other heavy metals (i.e., via passive diffusion), without the need for assuming specific transport systems (Foulkes and McMullen, 1986). Similar passive diffusion mechanisms may exist in lung cells. Other data are consistent with the hypothesis that magnesium ion transport system is responsible for the uptake of soluble nickel salts into cells, although this process has not been directly demonstrated in mammalian cells. Uptake of nickel via ion transport systems, particularly via the magnesium transport system, has been reported as a major route in microbial cells (Hausinger, 1993b). The same mechanism may also exist in mammalian cells. In mice injected intraperitoneally with a soluble nickel salt, magnesium inhibited nickel uptake by lung cells, inhibited nuclear and cytosolic uptake of nickel by pulmonary cells, and inhibited nickel binding to pulmonary DNA (Kasprzak et al., 1987). Similarly, in frog embryos, magnesium deprivation enhanced, and magnesium supplementation diminished, nickel-induced embryotoxicity and teratogenicity (Luo et al., 1993). These results are consistent with the hypothesis that cells uptake soluble nickel through a Mg(II) transport system.

Passive diffusion of nickel across cell membranes is markedly reduced under normal physiological conditions, when nickel is bound to proteins or amino acid ligands, forming hydrophilic complexes. As noted above, nickel in serum does not exist in a free ionic state. Instead, it is primarily complexed with albumin, (which is also a major protein composite in airway lining fluid), alpha-2-macroglobulin, or L-histidine. As shown in *in vitro* studies, the presence of L-histidine and human serum albumin at physiological concentrations inhibited the uptake of nickel by rabbit alveolar macrophages, human B-lymphoblasts and human erythrocytes (Nieboer et al., 1984a). In another study, Chinese hamster ovary (CHO) cells maintained in a minimal salts/glucose medium display about a 10-fold higher uptake of soluble

nickel compounds, and greater consequent toxicity, than cells exposed to soluble nickel in a complete cell culture medium supplemented with 10% fetal bovine serum. The inhibitory effect of serum on the uptake of nickel is substantially reduced by dialysis of the serum to remove small molecular weight metal binding ligands such as cysteine and histidine (Abbracchio et al., 1982a). Thus, in cell culture, physiological concentrations of metal-binding amino acids such as cysteine and histidine exert dramatic inhibitory effects on uptake of ionic nickel, and appear to account for the majority of the inhibitory activity of whole serum. Packaging soluble nickel compounds in liposomes increases nickel transport by this mechanism, and increased the resulting DNA damage (Sen and Costa, 1986).

Uptake of nickel via the magnesium transport mechanism is also less efficient than phagocytosis. Magnesium concentrations inside and outside the cell are in the millimolar range, so nickel would not compete effectively with magnesium for uptake under normal exposure conditions, which would result in nickel concentrations well below the millimolar range (Oller et al., 1997).

The differences between the processes by which soluble and insoluble nickel enter the cell are reflected in differences in disposition once the nickel is inside the cell. Soluble nickel directly enters the cytoplasm, where it binds to cellular proteins, decreasing the bioavailability of soluble nickel to enter the nucleus and interact with DNA (Costa et al., 1981). Small amounts of nickel subsulfide may also be dissolved in the extracellular fluid, and be taken up by cells via this pathway. The presence of nickel ion in the cytoplasm increases the potential for cytotoxicity. By contrast, phagocytosed (insoluble) nickel particles are retained in vacuoles, and migrate to the region near the nucleus (Evans et al., 1982). The retention of phagocytosed insoluble nickel particles in vacuoles decreases the opportunity for insoluble nickel to interact with cytosolic macromolecules, thus decreasing the potential for cytotoxicity or for interactions that render the nickel unavailable for interacting with DNA. After congregating near the nucleus, vacuoles containing insoluble nickel interact with lysosomes. This interaction decreases the pH of the vacuole, increasing the rate of dissolution of nickel ion (Evans et al., 1982). Nickel ions are released from the vacuoles in close proximity to the nuclear membrane, where they can interact with DNA. The net result of these differences is that exposure to insoluble nickel compounds results in much higher nuclear nickel concentrations and higher DNA binding than exposure to similar levels of soluble nickel compounds (Harnett et al., 1982).

Thus, soluble forms of nickel interact with the cell in a way that maximizes cytotoxicity and minimizes nickel delivery to the nucleus, while insoluble forms of nickel, such as nickel subsulfide, interact with cells in a way that decreases the cytotoxic potential while increasing the delivery of nickel to the nucleus. Cytotoxicity is important for two reasons. First, in order for cancer to develop, the altered cell must survive and transmit precancerous changes to its daughter cells. Secondly, high levels of cytotoxicity (resulting ultimately in organ toxicity) can prevent a chemical from being tested at high enough doses for cancer to be evident. Thus,

although the observation of DNA damage and chromosome aberrations in cell cultures (Section 4.4.2) suggests a potential for direct genotoxic effects of soluble nickel under certain *in vitro* conditions (e.g., absence of extracellular amino acids and serum proteins), these effects may be prevented or greatly attenuated *in vivo* by extracellular complexation and other elimination mechanisms limiting the availability of extracellular Ni^{2+} to the cell interior and nucleus.

3.3 Metabolism

Nickel is an essential trace element in many animal species, although the exact role of nickel has not been identified (reviewed in Nielsen, 1991). Nickel has been identified as a component of bacterial enzymes, but specific mammalian enzymes that require nickel as a cofactor have not been identified. Instead, evidence for an essential role of nickel derives from larger-scale systems. This evidence indicates that nickel acts in synergy with vitamin B_{12} in stimulating hematopoeisis. Interactions between vitamin B_{12} and nickel affecting growth, kidney:body weight ratio, and tissue levels of other metals have been reported in rats deprived of methionine or methyl groups. Nielsen (1991) suggested that nickel plays a role in the production of a compound that requires vitamin B_{12} for further metabolism. In the absence of sufficient vitamin B_{12}, the substance accumulates, resulting in depressed growth. Conversely, in the presence of low nickel levels, the substance is produced at lower levels, and low vitamin B_{12} is less deleterious. It has been suggested that nickel may be essential in humans, at levels of less than 0.1 mg Ni/day (<0.001 mg Ni/kg/day for a 70 kg adult), although no nickel requirement or allowance has been set (Nielsen, 1991).

3.4 Excretion

Regardless of the route of exposure, absorbed nickel is eliminated predominantly in urine, with some loss of the metal ion in sweat, bile, and hair. Unabsorbed nickel is eliminated in the feces.

3.4.1 Inhalation

When soluble nickel salts are inhaled or injected intratracheally, most of the nickel is absorbed and then excreted through the urinary route, although appreciable fecal excretion has also been observed. Animal data indicate that the rate of urinary nickel excretion increases at higher (when compared to lower) intratracheal doses.

Elevated urinary nickel levels are observed in electroplating workers, who are exposed predominately to soluble forms of nickel compounds. Urinary nickel levels rapidly paralleled recent exposure, increasing at the end of the workday compared to preshift levels, and then dropping again by the next morning. This result suggested that a fraction of the nickel was absorbed and some of the nickel was rapidly eliminated through the urinary route (Ghezzi et al.,

1989; Torjussen and Andersen, 1979). The urinary nickel concentration also increased as the workweek progressed, indicating that some nickel accumulation occurred with continued exposure, and that some of the absorbed nickel was excreted more slowly (Ghezzi et al., 1989; Tola et al., 1979; Bernacki et al., 1980).

No information on the excretion of inhaled soluble nickel salts by animals is available, although several studies did evaluate the excretion of soluble nickel salts following intratracheal instillation of rats (summarized in Table 5). Medinsky et al. (1987) found that the half-time for excretion in the urine decreased and the extent of excretion increased with increasing dose in rats (Table 5). They interpreted these results as indicating that the higher intratracheal doses resulted in saturation or disruption of the processes by which nickel is reabsorbed in the kidneys, or that the nickel complexes that predominate at high plasma nickel concentrations are less likely to be reabsorbed. The half-time for excretion in feces was unaffected by dose, but a higher percentage of the administered dose was excreted in the feces at lower doses. The study authors noted that the dose-related differences could also be due to differing sites of nickel instillation. They suggested that a higher percentage of the dose may have been deposited in the trachea at the lower doses. The dose deposited in the tracheobronchial region might then be eliminated by mucociliary clearance, swallowed, and excreted in the feces, although the test material was administered in solution, rather than as a particulate. Other intratracheal instillation studies with soluble nickel found 58% to 72% excretion in the urine in one day, 61% to 78% in 3 days, and 64% to 96% in 21 and 90 days; fecal excretion accounted for only 3.4-6.4% of the dose (English et al., 1981; Carvalho and Ziemer, 1982). Although these studies are via the intratracheal route, rather than the inhalation route, similar effects would be expected for inhalation exposure.

The half-life of nickel in the lungs after exposure to soluble nickel salts varied with the solubility of the nickel species, indicating that clearance is related to nickel solubility. The half-life of nickel in the lungs of rats administered unlabeled nickel sulfate by nose-only inhalation or intratracheal instillation (either $^{63}NiSO_4$ or unlabeled $NiSO_4$) was about 21 to 36 hours (Hirano et al., 1994; Medinsky et al., 1987). Benson et al. (1994) found that the less soluble nickel compounds nickel subsulfide and nickel oxide had a longer half-life in the lung. In male F344/N rats exposed to nickel subsulfide ($^{63}Ni_3S_2$) or nickel oxide (^{63}NiO), the half-life of nickel in the lungs was 4 days and 120 days, respectively (Benson et al., 1994). Results from these two studies show that the soluble nickel salt was readily absorbed and cleared out from the lung, while insoluble nickel compounds stayed in the lung for longer periods of time.

3.4.2 Oral

In humans, most ingested nickel salts are not absorbed and are excreted in the feces. Absorbed nickel is excreted primarily through the urinary route, with the maximal excretion in the first 8 to 9 hours after ingestion. Food markedly decreased nickel absorption, resulting in

increased fecal and decreased urinary excretion.

Several studies investigated the half-time of elimination of ingested nickel in human subjects. Nickel blood levels peaked 2.5 - 3.0 hours after subjects ingested nickel ($NiSO_4$) in water (Christensen and Lagesson, 1981; Solomons et al., 1982; Sunderman et al., 1989). The half-time of serum nickel was 11 hours (Christensen and Lagesson, 1981). The elimination half-time for absorbed nickel averaged 28 ± 9 hr (Sunderman et al., 1989). In workers who accidentally drank water contaminated with soluble nickel salts, the serum nickel elimination half-time averaged 60 hours. After treatment for 3 days with intravenous fluids to induce diuresis, the elimination half-time decreased substantially to 27 hours (Sunderman et al., 1988).

Sunderman et al. (1989) compared the absorption of nickel in food and water. This study also found that 26% of a dose of soluble nickel given in water was excreted in the urine within 4 days of treatment, while only 2% was excreted in urine when nickel was given in food. During the same time, fecal elimination of nickel averaged 76 ±19% of the dose ingested in water, compared with 102±20% of the dose ingested in food (Sunderman et al., 1989). Similarly, Nielsen et al. (1999) found that urinary excretion accounted for 23% of the nickel administered in water 4 hours after a meal, but only 2-3% of the dose was excreted in urine if the nickel was administered with the meal.

A similar pattern of nickel excretion has been observed in animals. In non-fasting rats receiving $^{63}NiCl_2$ by oral gavage, 3-6% of the initial dose was excreted in the urine within one day after the dosing, with a peak urinary nickel concentration at 4 hours after dosing. Fecal excretion accounted for 94-97% of the initial dose (Ho and Furst, 1973). Among male rats administered unlabeled soluble nickel chloride, nickel nitrate, or nickel sulfate by gavage in a starch-saline solution, urinary excretion within 24 hours of dosing accounted for 94-96% of the absorbed dose (Ishimatsu et al., 1995). The absorbed fraction for the soluble nickel compounds ranged from 9.8% to 33.8%, with the rest presumably excreted in feces.

3.4.3 Other Routes of Nickel Excretion

In addition to excretion through the urinary route, small amounts of absorbed nickel can also be excreted through other routes, such as via sweat, bile, and milk.

Nickel can be released as a metal ion in sweat. After healthy male and female volunteers exercised in a chamber at 37.8°C and 35% relative humidity, whole body sweat (about 850 to 900 ml) was collected during a 90-minute period. An average of 57 Fg/L nickel was detected in the sweat. The nickel concentration in sweat was more than 3-fold higher than the nickel concentration in urine. Based on a total sweat excretion of three to six liters per day, 170 Fg to 340 Fg nickel could be excreted through sweating (Cohn and Emmett, 1978).

A similar nickel concentration in the sweat collected from the arm was observed in healthy subjects during a 15-minute exposure to heat in a sauna bath at 93 °C (Hohnadel et al., 1973). In this study, a reciprocal relationship between nickel concentration and sweat volume was noted, indicating that the amount of nickel excretion in sweat might be more important than the concentration of nickel in sweat. However, this and the previous study were conducted under conditions of heavy sweating and it is not known how much absorbed nickel is excreted in sweat under normal conditions.

Nickel excretion into rat bile has also been noted. Within six hours after subcutaneous injection of $^{63}NiCl_2$ (1.7 µmol/kg), 0.26% (62.7 ng/rat) of the dose was excreted in bile. After receiving sub-lethal doses of unlabeled $NiCl_2$ (125 or 250 µmol/kg), 0.29% to 0.32% of the nickel dose (5.5-12.4 µg/rat) was excreted in the bile within 24 hours post-injection. These results suggest that absorbed nickel could be excreted through the bile. However, Marzouk and Sunderman (1985) stated that biliary excretion is quantitatively unimportant for the elimination of exogenous nickel in rats.

Milk is another route for nickel excretion. The ratio for the concentration of nickel in milk to that in plasma following a single subcutaneous dose of nickel chloride to rats was 0.02 across a range of nickel doses (Dostal et al., 1989). These authors reported a higher milk:plasma ratio of 0.1 in the milk of rats subcutaneously administered nickel chloride on lactation days 12-15, suggesting that nickel can accumulate in the milk. Although the kinetics would differ following oral exposure, these data do show that nickel can be excreted in the milk. An abstract reported that the nickel concentration in milk specimens obtained from 102 American mothers averaged 17±2 and 14±1 µg/kg at 4-7 days postpartum and at 30-45 days postpartum, respectively (Feeley et al., 1983). These nickel concentration were substantially greater than the concentrations of nickel in whole blood or serum (0.34-0.28 µg/L and 0.28-0.24 µg/L, respectively) (Sunderman et al., 1986). It is unclear why Feeley et al. (1983) found higher levels in milk than are reported in blood, while Dostal et al. (1989) found lower concentrations in milk. The difference may be due to species, dose, single dosing versus continuous exposure in diet, or the fact that Feeley et al. (1983) and Sunderman et al. (1986) sampled different human populations. Nonetheless, these data show that nickel excretion into breast milk could be an important route for nickel exposure of infants.

3.5 Toxicokinetic Models

A toxicokinetic model is available that predicts nickel absorption, serum levels, and excretion in humans following oral exposure to nickel in water and food. This model was developed by Sunderman et al. (1989) based on a linear, compartmental, toxicokinetic model that included two inputs of nickel: the single oral dose of nickel sulfate administered in water or food, and the baseline dietary ingestion of nickel. The two compartments were serum and tissues. Also included in the model were the following estimated parameters: a first-order rate

constant for intestinal absorption of nickel from the oral dose of nickel sulfate, a pseudo-zero order rate constant for fractional absorption of dietary nickel, a first-order rate constant for urinary elimination of nickel, two first-order rate constants for transfer of nickel between the compartments, and the mass fraction of nickel absorbed from the oral dose of nickel sulfate. This model resulted in good fit to the data that were used to estimate the parameters, but it has not yet been validated with additional human data. The development of validated models in humans and rats could aid in reducing the uncertainty involved in the extrapolation from rats to humans. However, it should be noted that the Sunderman model is an empirical model using fitted parameters, rather than a true physiologically based pharmacokinetic (PBPK) model.

A toxicokinetic model for soluble nickel salts has also been developed for the inhalation route in rats (Menzel et al., 1987; Menzel, 1988). Deposition rate in the lung was calculated based on the respiratory frequency, tidal volume, and deposition fraction. Clearance was described as following Michaelis-Menten kinetics. Such a model might be useful in evaluating the effect of clearance on nickel lesions in the respiratory tract. Such a model might also aid in the interpretation of the exposure levels that result in lesions following subchronic or chronic exposure (see Section 4.2.2). However, this model has also not been validated, and different optimized kinetic parameters were needed for different exposure durations, so it is unclear whether this model could address the duration issue. Menzel (1988) also described a model for the systemic distribution of nickel following inhalation exposure. By comparing kidney dose following inhalation exposure to the kidney dose associated with functional changes in the Vyskocil et al. (1994b) study (see Section 4.2.1), a systemic model could be used to evaluate the potential for kidney effects following inhalation exposure. (The inhalation bioassay with nickel sulfate [NTP, 1996a] evaluated kidney histopathology, but did not measure sensitive indicators of kidney function.) However, the Menzel model is not fully documented in the available publications, and may not be appropriate for such predictive work.

4. HAZARD IDENTIFICATION

4.1 Studies in Humans —Epidemiology, Case Reports, Clinical Controls

Cancer of the lung and nasal sinuses were observed in nickel refinery workers in England as early as the 1930's. Epidemiological studies of nickel refinery workers in various countries have been designed to determine what manufacturing processes, or levels and types of exposures to nickel, are associated with such cancer increases, and whether other cancer types are also increased. Excess risks of other types of cancer have been reported on occasion, but increases in lung and nasal sinus cancers are a consistent observation. Few health endpoints other than cancer have been monitored, but mortality studies in relatively large cohorts have not consistently suggested that other causes of death are increased.

Only one human study reported on possible cancer effects by the oral route; an

association of higher rates of lung and bladder cancer were seen in males (but not in females) with nickel levels above 0.5 Fg/L of drinking water (Isacson et al., 1985). No epidemiology studies of non-cancer effects in humans following ingestion of nickel were located. However, case reports of poisoning incidents provide information about additional effects of nickel, and indicate that the kidney effects seen in animals also occur in humans.

4.1.1 Inhalation Cancer Studies

In 1990 the International Committee on Nickel Carcinogenesis in Man completed its evaluation of 10 large-scale epidemiological studies. The purpose of this work was to gain understanding of the health risks of nickel exposure. The report of the Working Group of the ICNCM, chaired by Sir Richard Doll of England (ICNCM, 1990) is the most extensive and detailed assessment of the epidemiological data available. The report contains data and original analyses regarding cancer in nickel workers that are not available elsewhere in the published literature. It focuses solely on the epidemiological data, and includes information on exposures.

The primary goal of the Doll report was to assess which forms of nickel were associated with increased risk of cancer of the lung and nasal sinuses. The report authors evaluated large scale epidemiological studies that met several criteria: 1) information on exposure to nickel species in workplaces was either available or could be obtained, 2) the cohort was large enough to provide useful information, 3) adequate follow-up was possible, and 4) good individual work histories could be obtained. Thus, the quality of these studies is generally adequate for assessing major mortality endpoints in the exposed cohort. However, there are differences among the studies, and all share limitations in the quantification of exposures and lack of information on potential confounding factors, particularly smoking habits.

The Doll report provides information that forms a preliminary basis for qualitative issues in cancer risk assessment and some information regarding exposure levels. In order to determine the risks associated with specific forms of nickel, the report compared risks in workplaces where workers had different exposures. Cumulative exposures were determined for each individual based on his work history. In epidemiology, cumulative exposure estimates serve as a surrogate for dose in order to estimate dose-response relationships. Because most workers incurred exposure to more than one species of nickel, categorical exposure levels (high, medium, low) were cross-classified to estimate risks from exposure to different forms of nickel. Although the ranges representing the various levels differed across cohorts, this approach clarifies the way in which exposure to one form of nickel modifies the effect of exposure to another form. The Doll report refers to this effect as “interaction” between forms of nickel, or to the “accentuation” or “enhancement” of the effect of insoluble nickel from exposure to soluble nickel. Because these terms risk confusion between biological and statistical interaction, the epidemiologic term “effect modification” is more appropriate. Effect measure modification refers to variation in the magnitude of an effect of exposure across levels

of another variable. That is, if the effect of exposure A is not uniform across levels of exposure B, exposure B is said to modify the effect of exposure A. Measuring effect modification provides information on causality.

Data from several of the cohorts supported the conclusion that metallic nickel presents no discernible risk for lung and nasal cancers, therefore, most of the Doll report analysis focused on exposure to oxidic, soluble, and sulfidic forms of nickel. (In these cohorts sulfidic refers to nickel subsulfide.) The stratified analysis indicated, for example, that in Clydach, Wales, exposure to soluble nickel has no effect on the lung cancer risk when both sulfidic and oxidic are low. When both sulfidic and oxidic nickel are high, high exposure to soluble nickel increased the risk significantly. High levels of exposure to sulfidic nickel has a significant effect, increasing risk when exposure to oxidic and soluble are low. The analyses were consistent with a carcinogenic effect of both oxidic and sulfidic nickel. The authors inferred that soluble nickel plays an auxiliary role in carcinogenicity, by enhancing risks associated with exposure to other forms of nickel.

In evaluating the nickel epidemiology data, confounding factors must be considered. These factors could affect the health outcome of interest, differ between the control and exposed groups, but are not measured. Confounders have occurred to varying degrees in almost every cohort studied and include effects of smoking, exposure to insoluble forms of nickel, and exposures to other chemicals in the workplace. Data are insufficient to control for the effect of smoking in these workers, and in some cohorts smoking may have been more prevalent than in the general population used as the reference. This confounds the assessment because smoking is a known cause of lung cancer. In addition, the exposure assessments are based largely on process materials and judgement, rather than systematic measurements, bringing considerable uncertainty to the estimates of exposure to all forms of nickel. Co-exposures to insoluble nickel may occur even to workers in electroplating, because of proximity to other processes, or as a result of other jobs the workers have held.

Exposure to other chemicals in the workplace must be considered. For example, electroplating operation can include numerous other chemicals such as organic compounds including aromatic sulphonamides or sulphonimides, formaldehyde and other aldehydes, amines, nitriles, and azo dyes (Dennis and Such, 1972). Sulfuric acid mists, linked to both lung and nasal cancer, have been reported in tankhouses (ICNCM, 1990. Nickel refining processes can also involve exposure to other metals, such as arsenic and cobalt (UNEP, 1987). Any exposure that is presumed to cause lung cancer (e.g. arsenic), or nasal cancer (e.g. sulfuric acid mists) and has not been controlled for will confound the analysis.

4.1.1.1 Selection of Studies for Discussion

An ideal way to consider the question of carcinogenicity of soluble nickel would be to

study a cohort of workers exposed to soluble nickel as the only form of nickel. In addition, it would be useful to compare the risks between workers who were exposed only to soluble nickel and workers exposed only to other forms of nickel. Although such groups are not available, these objectives guided the analysis and the selection of data to evaluate for this assessment.

The current assessment focuses on studies that include information on cancer risks from soluble nickel species. The primary criterion for selecting epidemiological information was that the study specify exposure to soluble nickel, or to processes that indicate such exposure (i.e., electrolysis, electrowinning, or electroplating). All such studies located, published after the Doll report, were reviewed and evaluated to determine the nature of the information each provided regarding the question of soluble nickel exposure and cancer. The occupational environment that is most likely to meet the criterion of exposure to only the soluble form is electroplating in the metal finishing industry. However, only one epidemiology study (Pang et al., 1996) involved nickel platers. Results from that study are not definitive, because exposures for this group were of short duration, and appear to have been to low concentrations. Therefore, the current assessment also evaluated studies in the nickel production industry, where workers are also exposed to insoluble nickel compounds. These exposures result because of the nature of the operation and the materials, and exposures from other processes in the work areas. In addition, workers often hold different jobs during the period of time that they work in a company, and the different nickel operations involve exposure to different nickel species.

The Doll report focused on the nickel production industry, where the process is one of producing nickel metal from raw materials by electrolysis, a process also called electrowinning (see Appendix C). Hydrometallurgy, a related process used in nickel refining, involves exposure to mist and spray containing soluble nickel. Nickel refining generally includes exposures to other species of nickel, particularly if the electrowinning facility is in close proximity to other refinery operations.

Another population of interest, but not addressed in the Doll report, are workers in electroplating plants in the metal finishing industry. These workers apply a surface nickel coating to metal by electrolysis, and are likely to be exposed to nickel compounds primarily as soluble nickel sulfate. One study of electroplaters in the user industry included electroplaters who had no exposure to chromium, thus reducing the confounding from another exposure (Pang et al., 1996).

Five studies, representing five cohorts, were selected as possible key studies for this assessment. Three of the ten studies assessed in the Doll report were selected based on electrolysis work, high exposure to total nickel, or presence of more than extremely low exposure to soluble nickel. The cohorts were in the INCO operation in Clydach, Wales; Falconbridge refinery in Kristiansand, Norway; and the INCO refinery in Port Colborne,

Ontario. Subsequent to the Doll report, additional epidemiological information has been provided regarding the INCO plant in Clydach, Wales (Easton et al., 1992) and the Falconbridge refinery in Kristiansand, Norway (Andersen et al., 1996). Finland's Outokumpu Oy was not evaluated in the Doll report because of its small size and short follow-up time, but a recent report provides information that is reviewed here (Andersen et al, 1996). The Pang et al. (1996) study was also selected for this assessment. Several cohorts evaluated by ICNCM were not considered in this analysis because they did not include exposure to soluble nickel (e.g. Hanna mining and smelting, and smelting operations in Falconbridge, Ontario). Other studies did not provide additional evidence for cancer risk from nickel, usually related to small numbers of workers or limited exposure (e.g. New Caledonia).

Major characteristics of the exposure estimates for each of the five cohorts are summarized in Table 6, and cohort size and cancer risk estimates are summarized in Table 7. A discussion of exposure issues, relevant to the analysis of these studies is presented below. This is followed by a summary of results and commentary for each of the five selected studies.

4.1.1.2 Exposure Issues

It is difficult to find a facility or location in nickel production where exposure was solely to soluble or insoluble nickel. For example, the electrowinning process involves primarily exposure to soluble nickel compounds (see Section 2). However, exposures to oxidic nickel, and to a lesser extent to sulfidic nickel, may also occur in this environment. For most other nickel production exposures, insoluble nickel compounds are a significant confounder. The information provided on exposure and the methods of characterizing exposures in work areas varies among the studies. One approach (which is typical for epidemiology studies) has been to characterize exposure in particular work areas. Each worker's exposure at each job can then be combined to obtain a surrogate estimate of total exposure. However, the methods for characterizing exposures in work areas are generally not precise. In addition, the assessment of exposure is further complicated by mixed exposures in work areas, and workers may hold jobs in different work areas at different times. Work areas are not necessarily physically separated to confine emissions to that area, and workers in other areas may be exposed. For example, tankhouses, in which the electrowinning process occurs, may not be totally isolated from other sources of nickel exposure. And, as further discussed in Section 2, air work area sampling is problematic.

In an ideal evaluation of human exposure data, each substance in the environment would be identified and its concentration in the air that could be inhaled would be measured. However, in real-life situations, the only data available from occupational studies are frequently job titles and general identifications of the processes with which persons in those jobs are involved. Careful evaluations of the processes are necessary to identify the chemical substances to which workers are exposed. For nickel it is important to determine the chemical

forms in which the element occurs: soluble, insoluble, metallic, etc. Where airborne exposure samples have been collected and analyzed, it is necessary to identify the collection and analytical techniques to assure that they can provide accurate quantitative measurements of the form of concern. For example, a measurement of total dust levels provides very little information on nickel exposures, and an analysis for total nickel does not permit independent assessment of exposures to soluble or insoluble nickel compounds. On a more subtle level, it must be recognized that only particles in certain size ranges are retained in the respiratory tract, and only a more restricted range of particle sizes can reach the alveoli.

The data available for risk assessment for airborne nickel rely heavily on workplace exposure in the nickel production industries. However, measurements of exposure have been sparse, considering the size and age of the industry, and measurement procedures can best be described as rudimentary. Much published exposure data are based on qualitative recollections of plant managers who described airborne concentrations as "high", "medium" or "low". A few airborne measurements might have been taken by non-standard techniques to calibrate these qualitative descriptions. The Doll report (ICNCM, 1990) reviewed health outcomes in over 140,000 nickel workers who were employed during the period of 1902-1979. Only a few thousand airborne exposure measurements were taken, and most of these were area samples taken over periods of less than an hour and that were analyzed for total nickel.

In analyzing the data from within a facility, such as the Clydach refinery, the Doll report compares risks for low and high levels of each form or species of nickel, controlling for the levels of other nickel species. It is difficult to reliably compare quantitative levels of exposure across cohorts because of the differences in approaches used to estimate exposure. For example, soluble nickel in Norway's Kristiansand facility included nickel sulfate, chloride, carbonyl and hydroxide, but other places, such as Clydach, included only the sulfate and chloride. Assessment of exposures is based largely on the judgment of personnel experienced with the process, combined with some information from airborne samples (see Section 2 and Appendix C). This information can be used for relative comparisons as long as the limitations are kept in mind. It should also be appreciated that the exposures of each member of a cohort can only be estimated and must frequently be reconstructed from sparse data that had been collected for other purposes (e.g. process control).

When exposures in a particular plant are estimated at a single point in time by a group of experienced plant personnel, the estimates are likely to be internally consistent, although subject to significant random error. However, there are no reports of cross correlations between qualitative exposure estimates in different plants. In addition, the few actual environmental measurements that may have been taken usually used different instruments and sampling techniques. Accordingly, it is necessary to exercise great caution in comparing exposures between different facilities. Even within a given plant, air sampling instruments and techniques are likely to have changed over time, so that temporal changes in exposures may be

the result of instrumental artifacts rather than actual changes in working conditions.

Because both exposure and health outcome data are extremely sparse in the electroplating industry, the present assessment is derived largely from nickel production. Unfortunately, mixed exposures to soluble and insoluble nickel compounds are extremely prevalent in production industries, so that a variety of epidemiological statistical methods must be used to isolate the unique effects of the soluble compounds. Because of ambiguities in exposure assessments, significant inaccuracies in quantitative measurements, and inadequate adjustment for confounding variables, both qualitative and quantitative epidemiological conclusions must be interpreted with great caution.

4.1.1.3 Nickel Refinery in Clydach, Wales

The cohort study of 2521 men who worked at the Clydach nickel refinery provided an opportunity for the Working Group of the ICNCM to gain insight into risks from exposure to different nickel species (ICNCM, 1990). The variety of activities of this refinery resulted in measurable air concentrations of metallic, oxidic, sulfidic and soluble nickel compounds. In Clydach, as in most nickel operations, no category of workers was exposed to only one type of nickel compound. This diversity of exposures, and the long duration of surveillance and study of the refinery, permitted an extensive analysis of risk of lung cancer and nasal cancer cross-classified by relative levels of each of the other species.

The Clydach plant has been operating continuously since 1902. The Doll report (ICNCM, 1990) describes the processes used and changes over time and provides estimates of exposures to the various species of nickel (Tables C1 and 2 of that report). However the report notes that the airborne nickel concentrations and the percentages of nickel species were estimated on the basis of process knowledge, subjective impressions of relative dustiness, and a few measurements of total airborne nickel made with cotton wool as the filter medium. No measurements of actual concentrations of nickel were conducted prior to 1950. An appendix to the Doll report describes the methods used to estimate nickel exposures in most of the plants covered by the report, but no information is provided for the Clydach facility. Because the exposures appear to have been estimated by a single group of experienced plant personnel at a single point in time it is likely that any systematic biases are constant over time. However, the estimates are likely to be subject to significant random error. Because so little data are provided on the methods used to measure actual airborne nickel concentrations, it is impossible to estimate what the concentrations would have been had they been measured using modern instruments and techniques.

Observations from this plant had provided the first indication of the increased risks of lung and nasal sinus cancers in nickel workers. After changes in operations, the total nickel and dust levels decreased substantially after 1930. The leaching operation was phased out after

1936, which would seem to have eliminated the source of exposure to soluble nickel between 1936 and 1948. In addition, by 1929, they had introduced gauze masks, and switched to low arsenic fuel, which would lead to decreased exposures to arsenic (Easton et al., 1992; Draper et al., 1994)[1]. These changes over time in the plant's process caused considerable changes in the airborne concentrations, which permits comparisons of risks under different exposure scenarios. Lung cancer in the Clydach cohort was lower in the groups that were first employed in 1930 and thereafter than it was in those employed prior to 1930. The ICNCM analysis shows that the standardized mortality ratio (SMR), the measure of the risk for lung cancer mortality, decreased nearly four-fold after 1930. Easton et al. (1992) shows a substantial decline by decade of hire, from statistically significant SMRs over 600 before 1920, to a non-significant SMR of 118 in those first employed between 1940 and 1949.

To study the effect of the different nickel species on cancer risk, the ICNCM identified workplace departments by process, such as the nickel plant, calcining, or hydrometallurgy, and estimated the concentration of each species in each department. For example, the hydrometallurgy department is characterized by high levels of exposure to soluble nickel, and the calcining and furnace areas by high levels of oxidic and sulfidic nickel. Estimated concentrations are given as ranges because environmental measurements were limited, scattered in time, and taken with different methods. To estimate cancer risks related to workers' exposure, the data were stratified by duration of employment, or duration of employment within a specific department. Standardized mortality ratios were calculated using the population of England and Wales as a reference. No information on smoking was provided.

The Doll report contains a remarkable analysis of the risks in the Clydach cohort of exposures from each species of nickel, and how exposure to other species modifies the effect.for the Clydach cohort. These are summarized in a series of tables reproduced here (Tables 10, 11, and 12 for lung cancer risk). Low soluble nickel was defined as <10 mg Ni/m^3 x years and high soluble nickel was set at $10 mg Ni/$m^3$ x years. Low exposure to oxidic nickel was <50 mg Ni/m^3 x years , and low exposure to sulfidic nickel was defined as <15 mg Ni/m^3 x years. (As noted before, the criteria for high and low levels differ among the cohorts in the Doll report.)

Table 10 shows the different risks for low and high cumulative exposure to soluble nickel, stratified by degree of exposure to other species. The ICNCM Working Group concluded that increased exposure to soluble nickel increased the SMR for lung cancer only if

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[1]The Doll Report included follow-up through 1984, and Easton through 1985. However, the numbers are not identical, but vary by a few cases for unknown reasons. The differences are small relative to the number of subjects and do not affect the relative magnitudes of the SMRs.

exposure occurred in conjunction with high exposures to oxidic nickel. Table 11 displays risk for low and high cumulative exposure to sulfidic nickel. High soluble nickel exposures did not significantly increase the relatively high risk from high sulfidic nickel. Table 12 shows the different risks for low and high cumulative exposure to soluble nickel, stratified by degree of exposure to the other species. Given high exposure to oxidic nickel, the risks were significantly greater with high soluble nickel exposure than with low soluble nickel exposure. Thus, soluble nickel exhibits discernible effect modification with oxidic nickel forms, but not with sulfidic nickel.

These tables also show the relative effects of individual forms by considering the cell in the table in which one exposure is high and the other two are low. For lung cancers, with all other exposures low, the SMRs were largest for sulfidic (638) followed by oxidic (350) and then soluble nickel (168). These numbers should be considered broad and relative estimates, given the limits of the exposure assessment and epidemiological analyses. Thus, the relative mortality risks for lung cancer are sulfidic>oxidic>soluble.

A similar analysis for nasal cancers to assess the effects of individual species when other forms were low showed increased SMRs only for sulfidic nickel (see Tables 13 and 14). Examining for effect modification, high exposure to soluble nickel significantly increased the SMR only when exposure to sulfidic nickel was high. Thus, in the Clydach cohort, exposure to high concentrations of soluble nickel and low concentrations of oxidic and sulfidic alone was not associated with increased lung or nasal cancer SMRs (ICNCM, 1990), but exposure to soluble nickel did increase the risk seen with exposure to high levels of insoluble nickel.

Roberts et al. (1992) conducted a further analysis of the data summarized in the ICNCM report, and reported declines in cancer risks with time at Clydach. Risks decreased after decreases in exposures to nickel and dusts overall, consistent with the carcinogenicity attributed to the nickel production industry.

In a follow-up study, Easton et al. (1992) list atmospheric concentrations in different work areas by period of operation in terms of total nickel concentrations over time, and percent of each of the various species in the environment. The data presented in that study are of interest for showing quantitatively the distribution of different nickel species in each work area, but they provide limited insight into individual exposures. The total estimated nickel concentration in the nickel sulfate process area is estimated as <5 mg Ni/m^3 in all periods, with 30-40% as soluble species.

The Easton et al. (1992) update includes a few additional cancer cases (in addition to those included in the Doll report) among workers who began work after 1930 (n=2524). An increased risk of lung cancer in men first employed 1930-1939 was observed in “other employees,” but not in those who had 10 or more years of service and average levels of

metallic nickel >1 or soluble nickel >0.1 mg Ni/m^3. Nasal cancer in the Clydach cohort was confined to those first employed before 1930. Follow-up through 1985 identified cases, mostly in workers employed before 1930, one in a worker first employed 1930-39, and none among workers employed thereafter (Kaldor et al., 1986; Easton et al., 1992).

Thus, the excess in both lung and nasal cancer occurs mostly in cohorts exposed prior to 1930 (Table 46.3 of Easton). As best as can be determined, reduction in soluble nickel follows a time frame similar to that of the reduction in dusts, so it is not possible to attribute changes in risk over time to any single exposure. In other words, higher exposures to both soluble nickel and to dusts occurred before 1948. Because the risk reduction was discernible after 1930, it can be interpreted either as a response to the earlier improved overall hygiene (reduction in dust and total nickel) or to the somewhat later reduction in soluble nickel. However, considering the long latency period, particularly that of nasal cancer, the cancer decline seems to begin before the decrease in soluble nickel. A decrease in exposure to arsenic is also likely to have contributed to the decrease in mortality from lung cancer.

One step in the process used at Clydach was to reduce the copper content of the material (the matte) by leaching with sulfuric acid to produce copper sulfate. The site of this process is called the copper plant. This step was followed by leaching and recovering nickel as nickel sulfate in the nickel plant. Both the ICNCM report and Easton et al. (1992) noted that the risks in the copper plant and in the nickel sulfate plant are similar. Both had soluble nickel concentrations estimated to be about 1 mg Ni/m^3, and little subsulfide nickel. Nickel oxide levels were 5- to 10-fold higher in the copper plant (10 mg Ni/m^3) than in the nickel plant. On the surface, this suggests a predominant role of soluble nickel, but these exposure estimates are not precise.

In an earlier document, the authors of Easton et al. (1992) had fitted a model to data on men first employed prior to 1930, which attributed risk to soluble and metallic nickel (Kaldor et al., 1986). Easton et al. (1992) revised the model based on the more recent data. The risk of lung cancer also could be explained in the more recent data by assuming no effect of soluble nickel. For nasal cancer, assuming no effect of soluble nickel on nasal cancer gave a poorer fit.

4.1.1.4 Falconbridge Nickel Refinery, Kristiansand, Norway

The Andersen et al. (1996) update of the cohort studied by ICNCM (1990) includes 125,000 person years, and is the largest cohort study of the five selected for discussion in this report. No working areas with exposure exclusively to soluble or insoluble forms were found.

The analysis was based on a job and exposure matrix described in the ICNCM report. This exposure matrix for work areas was based on the subjective judgements of retired personnel (an “expert group”) supplemented by 127 air samples (13 of which were in

electrolysis areas) taken at one point in time in 1973 (Hågetveit et al., 1978). Both area and personal samples were collected, but there is no information on the types of samplers used, except that they presumably collected "total" particulate. Additional aspects to be considered in assessing risk are: concentrations were estimated by the expert group for 82 work areas, and these were accepted by union and management; and nickel species were assumed to be present in workers' breathing zones in proportion to their presence in the work areas. The concentrations are presented as ranges, from negligible through low, medium and high, and the ranges of each level were the same for all species of nickel.

Workers in calcining and roasting had minimal exposures to soluble nickel and high exposures to nickel oxides. The epidemiological analysis expresses exposure by cumulative estimates in mg Ni/m^3 x year for total nickel, soluble nickel, and nickel oxide. Changes in risk over time were examined by year of first exposure. Exposure to insoluble forms was reduced after 1968, and exposure to soluble forms was reduced after 1978. The process was said to be similar to that used at Clydach, but this plant handled some substances up to 1978 that Clydach handled only up to 1929. A smelter plant nearby also may have affected the exposure assessment. Exposure to soluble nickel occurs mostly in the electrolysis department at 0.5 to 2.0 mg Ni/m^3, with some higher exposures; levels of insoluble forms were estimated to be 0.1 to 0.5 mg Ni/m^3 (ICNCM, 1990).

Risk for lung cancer was highest in the group starting exposure from 1916 to 1944, but risk in workers beginning employment in subsequent decades did not continue to decline. A major purpose of this study was to assess the effect of smoking when combined with these occupational exposures. The data suggest that smoking does modify the effect of exposure substantially, which indicates that studies without information on smoking that report modest risks may be confounded.

Of 32 cases of nasal cancer, 12 had worked in calcining and roasting, areas where soluble nickel is minimal or not present. These 12 cases were in the category of highest cumulative exposure to nickel oxide. Only 2 of the other 20 workers with nasal cancer were presumed to have had no exposure to nickel oxide. All 32 of the workers who had nasal cancer were employed before 1956, after which nickel oxide concentrations in these areas declined from an estimated time weighted average (TWA) of 10 mg Ni/m^3/year to 5 mg Ni/m^3/year. (TWA values were reported as per year here, to help estimate cumulative nickel exposure over time.) The risk increased with cumulative exposure to soluble compounds, provided that there was some exposure to nickel oxides. Thus, as for lung cancer in the Clydach cohort, higher exposure to soluble nickel increased the risk of nasal cancer only when there was co-exposure to nickel oxide. By contrast, high risk was observed in the nickel oxide groups, even with minimal (<1 mg Ni/m^3 x years) exposure to soluble forms. There is no similar cross-classification analysis for lung cancer by Andersen et al. (1996). However, the data provided by Andersen and submitted by NiPERA in comments on a draft of this

assessment (NiPERA, 1998) indicate effect modification; lung cancer is more strongly associated with exposure to soluble nickel in the presence of oxidic nickel than with exposure to soluble nickel alone (Table 8). The lung cancer standard incidence ratios (SIRs) from the Andersen study show an increase in SIR with increasing amounts of soluble nickel that is greater when oxidic nickel exposure is high than when it is low; these results are similar to those for this cohort reported by the ICNCM . Overall, these data are consistent with the conclusion that exposure to both oxidic and soluble nickel results in a higher lung cancer risk, but the data are insufficient to determine the effect of soluble nickel alone.

Table 2 in Andersen's paper shows an imprecise, roughly 2-fold SIR for lung cancer in those with low cumulative nickel exposure for a time period less than 15 years, which is less than the likely latency for lung cancer. (Use of the SIR means that the comparison group or reference population is the entire Norwegian population.) Thus, a plausible alternative explanation for the 2-fold increase in lung cancer in those exposed to soluble nickel at low levels (Table 8) is that there is a higher rate of smoking in the cohort than in the reference group. Similarly, for the lowest levels of both oxidic and soluble nickel, the unpublished NiPERA data (NiPERA, 1998) report an SIR of 1.8 (Table 8). NiPERA (1998) suggested that the elevated SIR for the group with the lowest exposure to both oxidic and soluble nickel is due to higher cigarette smoking in the workers than in the comparison population.

Andersen et al. (1996) analyzed the Kristiansand data using a multivariate regression analysis for soluble nickel, adjusted for oxide, smoking, and age. They reported a three-fold increased risk for lung cancer with cumulative exposure to soluble nickel >15 mg Ni/m^3 x years (mean exposure 28.9 mg Ni/m^3). For exposure to nickel oxide, only a modestly increased risk of no greater than 1.6 was observed. However, the authors did not further describe the model parameters, nor indicate whether they evaluated any interactions among the parameters. There is no way to determine from this analysis whether those with high exposure to soluble nickel also had exposure to oxidic nickel, or to see what these risks were. These data suggest a role for soluble nickel in cancer. However, the statistical method used, adjusting for other exposures, does not directly address the question of the modification of the effect of insoluble nickel by exposure to soluble nickel. The authors also noted that is difficult to identify working areas where exposure is limited to soluble or to insoluble nickel compounds.

The analysis of other cohorts in the ICNCM (1990) report indicated the “interaction” of soluble and oxidic nickel, or the “enhancement” of the effect of insoluble nickel by soluble nickel is important, but ICNCM had reported that this Norwegian cohort provided little evidence that oxidic nickel alone increased the lung cancer risk. The data provided by NiPERA (NiPERA, 1998) provide some support for soluble nickel modifying the effect of exposure to oxidic nickel.

Although exposure to insoluble forms of nickel was decreased in 1968, prior to the

reduction in soluble forms (after 1978), it does not appear that this difference will aid in addressing the contribution of soluble nickel to the cancer risk. At the time of this analysis (Andersen et al., 1996), there was insufficient follow-up time to detect a decrease in cancer from these reductions in exposure. Given the close time frame of the reductions of exposure to both types of nickel, it is not certain that such an analysis in the future will permit distinguishing between effects of soluble and insoluble forms.

4.1.1.5 Outokumpu Oy Nickel Refinery - Finland

The data on this cohort have been updated since the Doll report (ICNCM, 1990), which reported one lung, one nasal and one stomach cancer in this small cohort of 129 men. They had been exposed in the refinery to soluble nickel at concentrations < 1 mg Ni/m^3. The man who had nasal cancer was exposed to other chemicals as well.

Karjalainen et al (1992) and Antilla et al. (1998) reported on the cohort of male workers employed in the Outokumpu Oy nickel refinery in Harjavalta, Finland. The report of incident (newly diagnosed) cases up to 1995 (Antilla et al., 1998) supercedes that of Karjalainen et al. (1992) because of the longer follow-up period. The full cohort, both smelter and refinery workers, now includes 1339 male workers. The process involved electrowinning, where soluble nickel was the primary exposure, and sulfidic nickel is not present. Numerous measurements of airborne exposures to soluble nickel were taken over the period of 1966 through 1993 (Kiilunen et al., 1997a). Both area and personal samplers were used and several measurements were taken inside workers' respirators. As described in Section 2.2 and Appendix C, area measurements ranged from 0.112 to 0.484 mg Ni/m^3, breathing zone measurements were in the range of 0.16 to 0.23 mg Ni/m^3, and exposures inside of respirators were observed to be 0.0005 to 0.0069 mg Ni/m^3. Exposure of refinery workers did not include nickel oxides. Nickel subsulfide was present in the grinding hall (between 0.05 and 0.2 mg Ni/m^3) and is present in the precipitates of the leaching reactors. The matte is ground as part of the refining process; thus it is reasonable to assume that exposure to subsulfides may have occurred. Measurements taken in the 1990s (Kiilunen et al., 1997a) indicated that 86 to 99.7% of the airborne nickel in the electrowinning operations was soluble in hot (70°C) water. Individuals at the refinery may have been exposed to products from the copper/nickel smelter. Even prior to 1970, the arsenic content was considered low.

Antilla et al. (1998) described workers employed from 1953 through 1995, specifying that the nickel operations began in 1960. Thus, workers whose employment ended before 1960 were not exposed to nickel. Worker exposure was estimated by person-years of employment in specific work sites. Measurements in the smelter indicated that nickel exposure was to insoluble forms (sulfides and subsulfide). Prior to 1973 the location of these activities was likely to spread these dusts to the area where electrolysis was performed. After that time, the predominant exposure to workers in the refinery, where electrolysis was performed, was to

nickel sulfate. By 1990, more than 90% of the airborne nickel was water-soluble. No information on smoking was presented.

Region-specific rates in Finland were used as a reference to calculate the standardized incidence ratios (SIR). SIRs were calculated for workers unexposed to nickel (those who terminated before 1960), and workers who began in 1960 or later when the nickel smelter and refinery were operating. The cohort of 1388 included two main groups; 1155 workers (including 49 women) whose employment continued or started after 1960 and thus were considered exposed to nickel, and another 233 workers whose employment ended prior to 1960, and therefore were considered unexposed to nickel. The authors presented various measurements of nickel at different times, but the cancer analysis is based on the individual's workplace location.

Table 9 shows the comparisons among unexposed, exposed, smelter, and refinery workers for lung and nasal cancers. Higher SIRs for lung cancer were reported in refinery workers than in smelter workers. Among the refinery workers, 6 cases of lung cancer occurred; the SIR was higher in the group with 20 or more years of latency. In the smelter workers, the lung cancer SIR was only slightly increased (1.39), but was higher in the group with 20+ years latency, 2.0 (confidence interval [CI], 1.07-3.42). Two cases of cancer listed as nasal cancers occurred in the cohort, both in refinery workers, for which the SIR was calculated to be 67 (CI, 8-242) after 20 years latency. The authors reported that they observed two additional cases after the closing date of the follow-up, one of nasal cancer and one tumor classified as nasopharyngeal. Typically, cases that occur after the cut-off date are excluded from statistical analysis to avoid bias; however, the infrequency of nasal cancer cases minimizes bias in this instance. That is, adjusting the reference population to account for nasal cancer in the reference population occurring in the next time interval would have minimal effect. In addition, examining case-specific data for information on exposure and diagnosis is often informative, particularly when the number of cases is small and other causes of the cancer are recognized.

NiPERA (1998) provided information on the smoking and work history of all 4 cases. Of the 2 diagnosed within the study's time frame, one had both worked in carpentry work and had been a smoker. He began his 25 years of smoking one year before he began work in the nickel refinery. Exposure to wood dusts is a recognized risk factor for nasal cancer (IARC, 1998). The other, a woman, was a non-smoker. The working environment in the refinery included exposure to sulfuric acid mists. Taken together, these data provide additional explanations other than that of soluble nickel as the sole explanation for the nasal cancers. It is also plausible that soluble nickel may modify the effect of other exposures, such as sulfuric acid mists and/or cigarette smoke, or wood dusts from carpentry work. Neither the role of soluble nickel nor these alternative explanations can easily be ruled out.

In addition to lung and nasal cancer, stomach cancer (not shown) was increased in the refinery, but not the smelter group. A total of five cases of stomach cancer were observed, SIR 4.98 (CI, 1.62-11.6). Three of these were in the 20+-year latency group, and the SIR was similar (4.97).

Although exposure to soluble nickel occurs in refinery processes, these cases of lung cancer cannot be attributed solely to soluble nickel for several reasons. First, all 6 cases of lung cancer in the refinery cohort were in the 20+-year latency group. This means that their work began before 1975. Thus, exposure to insoluble nickel was likely, because the grinding and leaching processes, sources of insoluble forms of nickel, were in proximity to the electrolysis process until 1973. Second, two of the six lung cancer cases had worked both in the smelter and in the refinery, and may have incurred exposure to insoluble nickel in the smelter prior to exposure in the refinery. Finally, Antilla et al., (1998) provide no information on the smoking habits, or previous occupations, for the individual cases. Given the small number of cases and strong influence of smoking on these diseases, confounding by smoking cannot be ruled out. This study does provide some support for the role of soluble nickel as an effect modifier for insoluble nickel in lung cancers.

This is a relatively small cohort, and, like the others, lacks information on smoking habits. The maximum follow-up was only 27 years. Given the long latency of lung cancer, cases that occurred after less than 15 years of exposure may be attributable to smoking, rather than to exposure to nickel. However, there is some advantage to the fact that this is a study of newly diagnosed or "incident" cases rather than deaths. The standardized incidence ratio (SIR), can be more reliable that standardized mortality ratios (SMRs) because incident cases are identified sooner, and closer to the time of exposure, than is possible for identifying deaths from the disease. This difference is more important for those types of cancers that respond to treatment, because such cases may not be detected by studying mortality if the individuals die of other causes.

4.1.1.6 INCO Mining, Smelting and Refining Operations - Port Colborne, Ontario Canada

This cohort offers the opportunity to compare cancer risks of men who worked in a sinter plant or at leaching, calcining, and sintering operations, with men who worked only in other operations, mostly involved with electrolysis. The electrolysis work included the activity of pumping and washing anode scrap, and resulted in exposures to soluble nickel that were 3 to10 fold higher than for other tasks. Electrolysis workers had lower exposure to sulfidic and oxidic nickel than did leaching, calcining, and sintering workers.

Airborne exposures to total dust were measured starting in the early 1950s (ICNCM, 1990, Appendix) and samples were analyzed for nickel starting in 1970. High volume samplers

were used through 1979, although personal samples were collected throughout the 1970s. Also during the 1970s, a distinction was usually made between soluble and insoluble nickel in the samples of airborne particulate. Exposures to soluble nickel varied from less than 0.05 mg Ni/m^3 in the anode and foundry additives department to about 1.5 mg Ni/m^3 for pumping anode slimes and washing of anode scrap. Total nickel exposures were higher than soluble nickel, indicating that insoluble nickel compounds were also present in the environment.

High volume samples are normally collected for only five to ten minutes at a time and frequently only in areas and at times when high exposures are anticipated. The samplers will also collect particles that are too large to be inhaled. Accordingly it is not possible to derive eight-hour time weighted average personal exposures to inhalable particulate from short-term samples taken with a high volume sampler.

Lung cancer risk was elevated at the sinter plant, where there was high exposure to soluble and sulfidic nickel. There is little support for a link between work in electrolysis (primarily exposure to soluble nickel alone) and lung cancer. However, nasal cancer increased with duration of exposure to sintering (high soluble and sulfidic nickel exposure), with four nasal cancer deaths observed in the cohort with <5 years in sintering. None of the men who worked in tasks with high exposure to soluble nickel had nasal cancer.

All four of the nasal cancer deaths of electrolysis workers included some sinter plant exposure. Nasal cancers occurred in men with short-term exposure to large amounts of insoluble compounds in leaching, calcining, and sintering, with additional long-term exposure to soluble nickel. This finding is consistent with the hypothesis that sulfidic nickel is carcinogenic and that soluble nickel may enhance or promote carcinogenicity.

4.1.1.7 British Electroplaters

The cancer mortality in the cohort of 284 British electroplaters is much less likely to be confounded by other exposures, particularly insoluble nickel compounds, than some of the cohorts in the nickel producing industry (Pang et al., 1996). Airborne exposures to nickel had not been measured while the plant had been operating, but during the 1970s through the 1990s, several investigators evaluated exposures to nickel in electroplating shops in Italy, France, Finland, the United Kingdom and the United States. These measurements can be used to estimate the range of exposures that might have been experienced in the plant studied by Pang et al. (1996). Table C1 summarizes the exposure data from 26 surveys and presents estimates of exposures to soluble nickel in the inhalable particulate fraction. The median exposure is about 0.020 mg Ni/m^3; 80% of the values are below 0.08 mg Ni/m^3 and fewer than 20% are below 0.01 mg Ni/m^3. Accordingly a reasonable estimate of the exposures in the Pang et al. (1996) cohort is in the range of 0.01 to 0.08 mg Ni/m^3.

These authors report, however, that exposures during the early period when most of the cohort was employed (1945-1961) were higher than those found currently in the industry (concentration not specified). This cohort included all men first employed in 1945-1975 who had worked for at least 3 months in departments that included nickel, but not chromium, in the environment. These men were followed until 1993.

Standardized mortality ratios (SMRs) were calculated for the entire time period based on the population in England and Wales for that time period. Compared to expected deaths, lung cancer was not elevated, and no deaths from nasal cancer were reported. The risk estimate for stomach cancer is elevated, but other cohorts with higher exposures to nickel do not show increases in this cancer site (Table 7). The follow-up time was long (1945-1993), and 89% of workers were followed through age 85. The mean duration of exposure of these workers was short (mean 2.1 years). Using regression analysis, the authors calculated relative risks using the internal group with less than one year of exposure to nickel as the standard. Lung cancer was not significantly elevated in those exposed for more than one year.

This study is potentially significant as the only study of nickel electroplaters in the metal finishing industry, for whom exposure is primarily to soluble nickel. Electroplaters in the nickel production industries, such as the nickel refineries described above, are likely to be exposed to other species of nickel. Although the cohort is relatively small, the long follow-up time increases the power of the study, as ascertainment of death is high. The observation that no increase in lung or nasal cancer occurred over time is consistent with the hypothesis that soluble nickel is not a carcinogen by itself and does not appear to act as an early stage carcinogen. However, many of the workers were exposed for less than 1 year, weakening the conclusions that can be drawn from the absence of an association between nickel exposure and lung cancer in this study.

4.1.1.8 Summary and Conclusions

The relevant epidemiology data includes four cohorts of nickel production workers - Clydach (Wales), Kristiansand (Norway), Port Colborne (Ontario), and Harjavalta (Finland) - and a cohort of British electroplaters. The relevant data for the Clydach and Port Colborne cohorts are primarily those published in 1990 by the ICNCM, although the cohorts in Norway and in Finland have been updated. (Andersen et al., 1996; Antilla et al., 1998, respectively). In these updated cohorts, the approach taken is similar to the analysis used by the ICNCM. The ICNCM evaluated the effects of different species on lung and nasal cancer by cross classifying the risk estimates, usually SMRs, by predominate species in the working environment.

This evaluation by ICNCM indicated an effect of insoluble nickel compounds that increased with estimated exposure levels, and an “enhancement” (increased risk) of the effect of exposure to insoluble compounds when the environment included exposure to soluble forms.

The relative effects of soluble and insoluble nickel compounds were consistent in Clydach and Kristiansand, two of the three cohorts analysed that included exposure to soluble species from nickel refining and were large enough for analysis. A third cohort, Port Colborne, however, did not show evidence of increases in lung cancer in the subset of the cohort who were electrolysis workers, where exposure to soluble nickel occurred. ICNCM interpreted the lack of lung cancer in electrolysis workers at Port Colborne as being due to the ratio of soluble to insoluble compounds being lower at Port Colborne than in the other refineries. In 1990, the ICNCM had reported that the exposure associations from Port Colborne are "in keeping with the evidence from Clydach and Kristiansand that soluble nickel enhances the respiratory cancer risks associated with exposure to other forms of nickel."

Data from the British electroplaters (Pang et al., 1996) are of particular interest because this is the only cohort that was exposed essentially to only soluble nickel salts, without confounding by exposure to insoluble nickel salts. There was no evidence of an increase in lung or nasal cancer in this cohort. Although this study included a long follow-up time, exposures were much lower than in the nickel production facilities, and many of the workers were exposed for less than one year, decreasing the strength of the conclusions.

The following brief summary is an assessment of the weight of the epidemiology evidence for determining whether soluble nickel, alone, is carcinogenic. It provides more detail on the updates than on the cohorts analyzed by ICNCM. This summary describes data that support the link between soluble nickel and cancer, data that bear on these observations and diminish their importance, data that argue against a link between soluble nickel and cancer and their limitations.

The following observations support the link between soluble nickel and cancer:

- The multivariate analysis from the Kristiansand refinery showed increased lung cancer risk with exposure to soluble nickel (Andersen et al, 1996).
- Lung cancer and nasal cancer were increased in the refinery workers at Harjavalta compared to unexposed workers, and compared to workers in the smelter (presumed exposure to insoluble nickel). Risks increased in workers with 20 or more years of exposure (Antilla et al., 1998). Soluble nickel was estimated to make up 90 % of the exposure. Nasal cancer risk ratios were quite high. In addition, even though the risk rations were based on only 2 cases, additional cases occurred after the end of the follow-up period.
- At Clydach, risks were increased similarly in the "copper plant" and the "nickel plant," and both plants had similar soluble nickel concentrations (about 1 mg Ni/m^3). Nickel oxide levels were 5- to 10-fold higher in the copper plant (10 mg Ni/m^3) than in the "nickel plant" (Easton et al., 1992). (These "plants" represent stages in the refining

process). If the observed increases were due only to insoluble nickel oxide, a larger increase in risk would have been observed in the "copper plant."

- Multivariate analysis of the Clydach data (Table 10) shows that lung cancer risk increased with exposure to soluble nickel, although these increases were observed only when exposure to oxidic nickel or sulfidic nickel was high.

The importance of these associations is diminished by the following observations:

- Other than the British electroplaters, none of the cohorts or groups of workers studied were exposed solely to soluble nickel compounds. The concomitant exposure always included insoluble nickel compounds, which appears to be a strong carcinogen. Other lung and nasal carcinogens in the workplace have introduced bias in some of the studies.
- Smoking has been shown to modify the effect of exposure to nickel, so the absence of smoking data introduces bias. This is important particularly in the Kristiansand cohort, because the data suggest that members of the refinery cohort smoked more than the general population, and in the Harjavalta cohort, because the number of cases is so small (6 lung cancers). In a small cohort, the association is suspect because the influence of smoking on lung cancer is strong, and misclassification of a case or two would distort the result.
- At Harjavalta, 2 of the 6 lung cancer cases had worked in the smelter, so prior exposures to insoluble nickel compounds cannot be ruled out. In addition, the lung cancer cases had 20 years of latency, so their exposure included the time in which exposure areas form the refiner and the smelter were not isolated, again meaning that confounding exposures to insoluble nickel compounds may have occurred.
- Additional data from Harjavalta showed that 2 of the 4 individuals with nasal cancer had worked previously in occupations that included carpentry work, a recognized cause of nasal cancers. The ability of soluble nickel to reach the nasal sinuses has been questioned.

Other evidence in these cohorts argues against the hypothesis that soluble nickel alone is a cause of cancer, but these data also have weaknesses:

- No increased risk in the cohort of electroplaters studied by Pang et al. (1996). The researchers successfully traced the vital status of most members. However, despite the completeness of the follow-up, this was a small cohort. In addition, levels of exposure to soluble nickel were estimated many years after the exposures of interest.
- No increased risk of lung cancer was observed in electrolysis workers at Port Colborne, who were exposed to similar soluble nickel levels as the workers at Kristiansand, but to 7-fold lower levels of insoluble nickel compounds than in the latter group. If soluble nickel were responsible for the cancers observed at Kristiansand, an

increased risk of lung cancer also should have been seen among the electrolysis workers at Port Colborne.

Taken together these epidemiologic data suggest a role for soluble nickel in the development of cancer. The evidence is consistent with the hypothesis proffered by ICNCM that soluble nickel modifies (increases) the carcinogenic effect of exposure to insoluble forms of nickel such as nickel oxide. However, evaluation of the role of soluble nickel is complicated by the potentially confounding effects of smoking, a known cause of lung cancer, co-exposure to insoluble forms of nickel as a result of the processes and work environment, and, in some time periods, exposures to other chemicals in the workplace. Any exposure that is presumed to cause lung cancer (e.g. arsenic), or nasal cancer (e.g. sulfuric acid mists) and not controlled in the analysis will confound the analysis. Consequently, the role of soluble nickel *alone* in carcinogenicity to humans cannot be determined from the epidemiologic studies.

4.1.2 Noncancer Effects in Humans Following Inhalation Exposure

4.1.2.1 Respiratory Effects

Muir et al. (1993) evaluated chest radiographs of 745 nickel sinter plant workers at the Copper Cliff plant in Sudbury, Ontario. The plant operated between 1948 and 1963. The exposure data provided by Warner (1985) were very limited. He reported that the total dust concentration in a single 40-hour sample of dusty air on the operating floor was 46.4 mg Ni/m^3 in 1960, but no information on nickel concentration in that sample, or the sampling method used, was provided. A graph showed the concentration of nickel exiting the roof monitors as being approximately 50 mg Ni/m^3 in the late 1950s, and higher at earlier times. Based on this very limited data and additional process information, ICNCM (1990) developed more detailed exposure estimates for the Copper Cliff sinter plant (part of the INCO facilities in Ontario). ICNCM (1990) estimated the total nickel workplace exposure as 40-100 mg Ni/m^3 in 1948-1954, and 8-40 mg Ni/m^3 in 1955-1963. Exposure to sulfidic nickel was reported as 15-35 mg Ni/m^3 and 3-15 mg Ni/m^3 during these two periods, and exposure to soluble nickel was reported only as <4 mg Ni/m^3 and <2 mg Ni/m^3 during these periods, respectively. These estimates were based, in part, on the assumption that the roof monitors could be considered high-volume samples of the work area, since the plant air could leave the building only through the monitors or through windows just below them. However, that assumption is a very crude one, in light of the differences in height between the breathing zone and the emissions stack, and the resulting potential for larger particles to concentrate gravimetrically in the breathing zone. Thus, actual exposure concentrations may have been higher than those measured. Recent work by Werner et al. (1996) suggests that the percentage soluble nickel estimated by ICNCM (1990) may have been underestimated by approximately a factor of 2. This would mean that exposure to soluble nickel would be estimated as <8 mg Ni/m^3 and <4 mg Ni/m^3. It should be noted, however, that no lower bound estimates for these exposures were provided. On the other hand, the assessment described below assumes that all of any observed effect is due to soluble nickel. In light of the relative exposure to soluble and insoluble nickel, and the higher toxicity of soluble nickel sulfate, a better estimate might be that approximately half of the effect was due to soluble nickel, and half may have been due to insoluble nickel.

In the Muir et al. (1993) study, the workers were monitored as part of a voluntary surveillance medical program, beginning in 1973. No information was provided on the percentage of workers who participated in the program, or on how successful the authors were at obtaining radiographs for workers who died or left the program for other reasons. To control for variability in reading the radiographs, five readers were used to classify the two most recent films from each worker, using the International Labour Office (ILO) 1980 protocol. The most recent films were used to maximize the latency and allow detection of inflammatory or fibrotic changes that may have developed after removal from exposure. The authors stated that opacities due to a fibrotic process do not appear to resolve after removal from exposure.

Control films were randomized with the index films, but no data on the incidence of opacities in the control films were reported. Such internal control data would have been very useful, given the wide inter-reader variability observed. Information on the age distribution at the time of the radiograph and time since first exposure (ranging from 0-9 years up to 44 years) was provided. A total of 596 workers were exposed for <5 years, and 149 were exposed for 5 or more years. No other dose-response information was available. The study authors stated that the prevalence of irregular opacities was low, and similar to that observed in studies of smoking populations and of workers exposed to dusts of low fibrogenic potential. There was, however, some evidence of a dose-response, based on prevalences calculated for this assessment. The total prevalence of films with irregular opacities ranged from 3% to 8% among those exposed <5 years, and from 7.4% to 20% for those exposed for those exposed 5 or more years. In addition, for each reader the prevalence was higher among those exposed for the longer duration. These increases suggest that an increase in profusion score with duration of employment occurred. However, most of the opacities were classified as ILO profusion score 0/1 or 1/0, which are relatively common in the general population. There was no clear duration-related increase in the prevalence of profusion score 1/2 and higher. In the absence of appropriate control data, the observed increases also cannot be clearly attributed to nickel. An alternative interpretation of the data is that the increase in opacities with duration of exposure may reflect an age-related increase in opacities, rather than an increase related to nickel. These alternatives cannot be distinguished in the absence of appropriate controls. Lung function tests are sometimes more sensitive measures of lung dysfunction than radiographs, but were not conducted for the study population. In addition, the radiographs would not have detected acute inflammatory changes, although significant inflammation would have resulted in overt clinical findings. No information on smoking prevalence was provided.

In light of the large uncertainties regarding exposure, the mixed exposure to soluble and insoluble forms of nickel, the wide variability among readers, the questions regarding degree of ascertainment, the minimal evaluation of effect, the absence of a control group, and the minimal effect observed, this study is not desirable as a basis for an RfC. If this study were considered as the basis for the RfC, the single exposure level available would be considered a minimal LOAEL, based on the observation of a duration-related increase in the prevalence of findings. Based on the ICNCM (1990) report and the revised (Werner et al., 1996) information on percent soluble nickel, a reasonable estimate of the exposure is 4 mg Ni/m^3. It should be noted, however, that this may be an overestimate, in light of the ICNCM characterization of exposure as *less than* 4-8 mg Ni/m^3. Conversely, an RfC derived from this study would assume that all of the toxicity is due to soluble nickel, while insoluble nickel was also present and would have contributed to any observed toxic effects. Thus, attributing the entirety of any effect to *soluble* nickel would underestimate the RfC. In light of these two opposing factors, a reasonable estimate is that 4 mg Ni/m^3 as soluble nickel is a minimal LOAEL. Adjusting for occupational exposure durations and minute volume, the LOAEL is 1.4 mg Ni/m^3.

An additional adjustment is necessary because of the large differences between particle sizes under occupational and ambient exposure conditions. No information was available on the particle size distribution in the Muir et al. (1993) study, so the particle size was estimated from information in other studies. Unpublished particle size distribution data from Vincent (1996) indicate marked variability of particle size with process worksite at nickel plants. There was also significant variability between different samples taken at a given location within a plant. These data can be used, however, to estimate rough bounds on the particle size distributions, and the resulting pulmonary deposition. For consistency with the use of animal data, the particle size distribution under ambient conditions was estimated as the average of the particle size distributions for nickel sulfate hexahydrate in the chronic NTP (1996a) study. Using these distributions, the pulmonary dose for humans is 7-80 fold higher (depending on the process worksite) when exposure is to the particle size distribution used in the animal studies, compared to the pulmonary dose when exposure is to the particle size distribution found under occupational conditions.[1] The resulting LOAEL (human equivalent concentration), or LOAEL (HEC), is 0.018 to 0.2 mg Ni/m^3.

Other industrial hygiene data presents information on the percent of particles in the respirable fraction. The term "respirable fraction" is used by industrial hygienists, and is defined operationally based on sampler collection efficiency, but corresponds roughly to the fractional pulmonary deposition used in the U.S. EPA (1994b) methodology. A broad range of respirable fractions has been reported in different nickel refineries. Thomassen et al. (1999) characterized worker exposure in a Russian nickel refinery, and found that the respirable fraction was essentially 0%. Werner et al. (1999) evaluated the particle size distributions for

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[1]The HEC was calculated using the RDDR program (U.S. EPA, 1994b) as follows: Deposition in the pulmonary region for humans was estimated using the particle size distribution data in Table 3.8 of Vincent (1996). Data for the matte grinding and roasting/smelting worksites were used, as most representative of the conditions to which the workers in the Muir et al. (1993) study were exposed. Using the distributions for matte grinding, the total deposition fraction was 0.015-0.03. The MMAD values for roasting/smelting were too large for use with the RDDR program, although the large geometric standard deviations suggested there may have been some pulmonary deposition at that site. The Vincent (1996) report also calculated the percent respirable mass, which ranged from approximately 2% for the roasting/smelting area, to 9.2% in the matte grinding area. Ramachandran et al. (1996) provide a default particle size distribution for the respirable fraction of MMAD of 4.25 Fm and geometric standard deviation of 1.5 Fm. Using this distribution and the range of 2-9.2% respirable mass, the fraction pulmonary deposition would be in the range of 0.0027 to 0.012. Combining these two methods of estimating the fractional pulmonary deposition under occupational conditions, the range is estimated at 0.0027-0.03. These fractions were compared with the deposition under the conditions of the NTP study with nickel sulfate hexahydrate (NTP 1996a). For that study, the average MMAD was 2.33 Fm, and the average geometric standard deviation was 2.22 Fm. (Note that these values are slightly different from the values used to calculate the HECs for the NTP study itself, because those calculations used the concentration-specific particle size distributions, not the average values.) The resulting pulmonary deposition fraction in the pulmonary region for humans was 0.22. The ratio of the pulmonary deposition under the NTP conditions versus the occupational exposure conditions ranged from 7 to 80, depending on the worksite. Regardless of worksite, however, deposition was lower under the occupational conditions, due to the overall larger particles.

nickel and other metals in several different process areas of the Kristiansand, Norway, nickel refinery. They found that the respirable fraction ranged from 2% to 6.8%, depending on the process area. In light of the wide range in respirable fraction for refineries between the Thomassen et al.(1999) and Werner et al. (1999) studies, and the considerable range between different worksites at a given plant (Vincent, 1996), there is considerable uncertainty regarding the actual pulmonary deposition under the conditions of the Muir et al. (1993) study, and under the conditions of the plants studied in the epidemiology studies discussed in Section 4.1

A mortality study of nickel platers in an engineering firm in England reported an increased standardized mortality ratio (SMR) for respiratory disease in those with more than one year of exposure (Burges, 1980). This study was in male workers exposed only to soluble nickel, not to chromium or to other nickel compounds. The study size was relatively small (508 workers, 101 deaths) and no other risk factors were assessed. No other epidemiologic studies of non-cancer effect of nickel were located.

A series of surveys were conducted in Norwegian nickel workers to assess the extent of nasal histology and to monitor changes in individual workers over time, particularly those who had developed dysplasia, which was considered a precancerous lesion (Torjussen et al.,1979; Boysen et al., 1982, 1984, 1994). Because the studies were designed to estimate prevalence or to monitor workers over time, and because the sampling was not random or systematic over time, comparative changes over time were not reliably estimated in these studies. In the workers studied, these reports demonstrate similar rates and extent of histopathological changes, including nasal dysplasia, in workers in electrolysis facilities and in roasting or sintering activities. Electrolysis involves exposure primarily to soluble nickel compounds, and roasting and sintering activities expose workers to insoluble compounds. However, the workers were classified by the activity where they worked the majority of the time, and it must be assumed that many workers had experience in both activities. Reliable exposure estimates are not available for these studies.

Nickel salt exposure can cause asthmatic symptoms. Most of the cases were observed in workers in the electroplating industry, where exposure is primarily to soluble nickel salts, (McConnell et al., 1973; Malo et al., 1982; Novey et al., 1983; Malo et al., 1985; Hong et al., 1988). The significance of this apparent specificity is unclear. In these studies, respiratory symptoms, such as cough, wheezing, dyspnea, and chest tightness, developed in patients who were repeatedly exposed to nickel salts. Cessation of the nickel salt occupational exposure eliminated the reoccurrence of the asthmatic attacks. Nickel salts were suggested as a causal factor, based on a positive response in the skin prick test and a decrease in forced expiratory volume in one second (FEV1) following inhalation challenge with nickel sulfate (see Table 28).

A Type I hypersensitivity reaction may be responsible for asthma induced by nickel salts. The nickel (II) cation may act as a hapten which, in combination with human serum albumin, acts as a complete antigen. (When the nickel-specific IgE antibody binds to this

antigen, the antibody can bind to receptors on mast cells and basophils, and activate the release of slow reacting substance and histamine from those cells, resulting in a Type I hypersensitivity response.) Support for this idea comes from serological studies, which indicate that, in addition to the positive response in the skin prick test, IgE

antibodies to nickel sulfate-human serum albumin antigen were also present in those patients (Malo et al., 1982 ; Novey et al., 1983).

A non-antigenic mechanism may also be responsible for some of the nickel salt-induced asthma. In addition to the reports by other authors of an immediate asthmatic attack induced by nickel salts, a late asthmatic reaction due to nickel sulfate was also reported by Malo et al. (1985). In this case, inhalation challenge with nickel sulfate induced a late asthmatic reaction, starting 3 hours after the exposure. Neither a skin prick test nor serological IgE antibody measurement showed a nickel-specific response. Thus, it is possible that nickel sulfate can elicit an asthmatic reaction through a non-antigenic mechanism.

Davies (1986) reported on three cases of occupational asthma in a nickel catalyst plant. An unspeciated atmospheric nickel concentration of 0.013 mg Ni/m^3 to 0.067 mg Ni/m^3 was estimated based on normal running of the plant. However, this study did not provide any information on nickel specificity, such as by the skin prick test, serological test, or inhalation challenge. In all of other studies, the occupational exposure concentrations were not available. Therefore, the concentration response between nickel inhalation exposure and the nickel-specific asthmatic reaction is unknown.

4.1.2.2 Reproductive Effects in Nickel Workers

A cross sectional study of female nickel hydrometallurgy workers in a Russian refinery plant reported increased general health effects and increased rates of congenital malformations and pregnancy complications (Chashschin et al., 1994). No information is provided regarding the nickel species, although the term hydrometallurgy means it was an electrolysis plant, in which exposure is likely to be all, or largely, soluble nickel. The published report includes a statement by the editors that the results are "incompletely documented and must be considered inconclusive," but are presented "because they identify a concern that requires investigation."

Although the study reports increases in reproductive endpoints, these data are unsubstantiated because of the design flaws of the study. Numbers are small, and no statistical measures of variability or tests of hypotheses are used. Chronic diseases are vaguely defined without reference to standard diagnostic criteria, and reported without reference to a control group. Rates for the reproductive endpoints in women are based on extremely small numbers. For example, incidence of malformations is reported as rates per 100 in populations as low as 124. Complications of pregnancy are reported using poorly defined and nonstandard definitions such as "threatened abortions" are used. Rates of spontaneous or "threatened" abortion are reported to be increased in comparison to a control group, but there is no information on or control for age, which is a major risk factor for spontaneous abortion. Other environmental conditions were present that may contribute to reproductive outcome, including heat stress, lifting heavy anodes, and exposure to chlorine gas.

4.1.2.3 Other Toxicities in Nickel Workers

Three studies of renal toxicity in nickel workers provide information on biochemical measures of nephrotoxicity related to long term exposures to airborne nickel species (Sunderman and Horak, 1981; Sanford and Nieboer, 1992; Vyskocil et al., 1994a).

The first of these studies provides clinical data in the context of developing a methodology of biochemical tests for assessing kidney function in exposed workers. Sunderman and Horak (1981) compared data from random urinary samples of 30 nickel refinery workers and 18 electroplaters to a reference group of healthy subjects. Exposure was not discussed. However, based on the occupation described, workers in both places were likely to have been exposed to soluble nickel. The "nickel electroplating facility" was not further described. The reference group consisted of volunteers who did not take medications, but the age of the subjects was not specified (an important consideration, since kidney function declines with age). There was no information on the workers' medications or medical history.

The urinary measurements are not 24-hour voids, but appear to be spot samples. Electroplaters had higher mean and median urinary nickel concentrations than 50 reference healthy subjects, but none had urine β_2 microglobulin (β_2m) greater than the upper reference limit of 240 µg/L, and their mean level was similar to the combined male and female reference group. Nickel refinery workers had higher mean β_2m concentrations than the reference group, particularly in the subgroup that had urinary nickel concentrations greater than 100 µg/L. That subgroup had a significantly greater number of subjects with urinary β_2m greater than 240 µg/L.

Sanford and Nieboer (1992) studied biochemical indices of nephrotoxicity in 26 workers in two electrolytic refining plants. Multivoid 24-hour urine samples were collected from the workers. No reference group was included, so the mid-point of the normal range of reference values was used to assess biochemical indices of nephrotoxicity.

Total daily excretion of urinary creatinine and total protein were not significantly different from the normal range, nor were urinary β_2m or serum creatinine concentrations. Results were compared with those of Sunderman and Horak (1981, described above). Urinary nickel concentrations were 28 and 60 µg/L in the two groups of refinery workers, comparable to the average for the subset of refinery workers with urinary levels lower than 100 µg/L in the Sunderman and Horak (1981) study. The β_2m levels were 62 and 80 µg/L, comparable to the 89 µg/L for the <100 µg Ni/L urine in the Sunderman and Horak study. Based on these biochemical indicators of decreased renal function, presumed to be minimal, Sanford and Nieboer (1992) concluded that nickel is a mild nephrotoxin. These changes were not viewed as indicating the same extent of nephrotoxicity seen with other metals, such as cadmium. The β_2m levels observed were below those typically considered adverse

(approximately 250 Fg/L). Results from the Sanford and Nieboer (1992) study illustrated the importance of the difference between spot urine samples and 24-hour voids. For two donors, elevated levels of $\$_2$m were found in spot urine samples, but the total amount in the 24-hour void was normal.

Vyskocil et al. (1994a) provided information on exposure to soluble nickel and more information on individual characteristics of workers than did the other studies. However, it appears that only spot urine samples were used. Exposure was defined as 4 to 26 times the Threshold Limit Value (TLV) of 0.5 mg Ni/m^3 of nickel as nickel sulfate and nickel chloride. Twenty-four exposed male and female workers from a chemical plant that produced soluble nickel compounds were compared to 22 controls in other plants in the vicinity who had no source of exposure to nephrotoxic compounds. Health, occupational, and demographic data were collected using a questionnaire. Workers who had medical conditions with potential renal effects were excluded. A single urine sample was collected at the end of the fourth day, at the end of the shift.

The exposed workers had significantly increased urinary levels of nickel, and increased average levels of $\$2$m and NAG compared to the control workers. Women had higher increases in urinary nickel, and increases in protein that did not occur in the male subjects. Small increases in the prevalences of "elevated" values (defined as more than two geometric standard deviations above control values) were found for several renal parameters, but the markers with increased prevalences did not completely correspond to markers with increased average values. Albumin and transferrin did not differ between exposed and unexposed workers. The authors concluded that adverse effects of soluble nickel on kidney tubular function occurred at these high exposure levels, which were above the TLV.

Wall and Calnan (1980) provided a case report of severe dermatitis among electroforming process operators in a plant where nickel is electrodeposited on other materials. Exposures were not measured, but presumably involved oral and inhalation exposure. Protective gear was worn, but the authors noted that contamination of protective clothing with nickel sulfide is "impossible to avoid." Dermatitis was reported in 7/16 operators; three affected operators had left the electroforming work, and one of them continued to show evidence of dermatitis. The study reported patch tests on all 17 process workers, all 5 supervisory staff, two cleaners, and one affected fitter. Patch testing revealed sensitivity to nickel chloride in 13 of the tested subjects, 8 of whom were process operators. All 17 process operators were negative for proteinuria in a paper-strip test. This study confirms the sensitization potential of dermal exposure to nickel. The negative proteinuria test is of interest, but in the absence of exposure data, or more specific measures of tubular or glomerular protein markers, these data are insufficient to conclude either that there was indeed no adverse effect on the kidneys, or to determine any exposure levels that are associated with the absence of such an effect.

Thus, the overall epidemiological database regarding potential kidney effects of inhalation exposure to soluble nickel is weak. However, the available data do provide suggestive evidence that the kidney can be affected under exposure conditions below those causing acute toxicity. The positive findings (Sunderman and Horak, 1981; Vyskocil et al., 1994a) are limited by the potential for false positives in spot urine samples, while exposures in the negative study (Sanford and Nieboer, 1992) were lower than in the other studies. Respiratory effects were not evaluated in these studies, so the relative sensitivity of the kidney and respiratory system can not be determined.

4.1.3 Oral Cancer Studies

The only study of cancer in humans exposed to nickel by the oral route compares cancer rates based on the detectable levels of nickel in the municipal drinking water (Isacson et al., 1985). Nickel concentrations in finished drinking water of Iowa towns with populations between 1000 and 10,000 people were measured by the proton induced X-ray emission technique (PIXE) test in 1979. The percentage of towns having detectable nickel concentrations is highest in surface water, and decreases with depth of groundwater. Data were obtained on chlorination, volatile organic compounds, chromium levels, estimated smoking rates by town, and socioeconomic variables including income and education. Cancer incidence rates over a 12-year period were age adjusted to the 1970 Iowa population for several cancer types: lung, bladder, female breast, prostate, rectal, and stomach cancer.

Cancer rates were compared among towns with nickel levels above and below 0.5 Fg/L. Incidence rates of lung and bladder cancer in males, but not in females, were associated with living in a town having higher nickel levels. No significant difference was found for stomach cancer. The association was independent of chlorination status, smoking and chromium concentrations.

This type of ecological, or geographic correlation, study, is limited by the absence of individual exposure assessments. This uncertainty is reduced somewhat because the exposure is assessed on the level of the city rather than aggregated over counties, a larger area used in other studies of drinking water and cancer. This aggregation approach includes uncertainty due to misclassification of exposure. The authors suggest that nickel could be a surrogate for other anthropogenic contamination.

4.1.4 Oral Studies of Noncancer Effects in Humans

4.1.4.1 Systemic Effects

The majority of 32 workers in an electroplating plant who drank water contaminated with a plating bath solution containing nickel sulfate and nickel chloride (1.63 g Ni /L)

developed acute gastrointestinal symptoms, and neurological symptoms including headache and lassitude (Sunderman et al., 1988). The observed symptoms (nausea, diarrhea, vomiting) were described as consistent with the few other reports of acute toxicity. This report includes the greatest number of subjects of studies of acute nickel toxicity, and detailed information on biochemical endpoints. Estimated oral intake in the 20 workers who developed symptoms ranged from 0.5 to 2.5 g nickel consumed over an unspecified time in one evening. Assuming a body weight of 70 kg, these doses correspond to single acute doses of 7-36 mg Ni/kg. The exposure included 20 to 100 mg boron, believed to be too low for boric acid poisoning. Several subjects were hospitalized and treated by intravenous fluids. After 3 days, symptoms subsided. Nickel concentrations in body fluids and the rate of elimination are described in Section 3 above (toxicokinetics). Diagnostic tests focused on nephrotoxicity, and the clinical course was studied for up to 6 weeks post exposure.

Clinical tests indicated mild erythrocytosis and reticulocytosis, which decreased 6 weeks post-exposure. Urine albumin levels above the upper limit of the laboratory's reference range were reported for 3 subjects on day 2 postexposure, and returned to normal by day 5. Transient hyperbilirubinemia was also observed in 2 exposed subjects, but no effects on other clinical chemistry parameters (serum creatinine, creatine kinase, lactate dehydrogenase, aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase) were observed. Five days after exposure, nickel concentrations in serum and urine decreased, but had not returned to the levels observed in control subjects. By 6 weeks after exposure, clinical chemistries indicated no abnormalities.

As part of a study of nickel kinetics in humans (Sunderman et al., 1989), one male subject developed left homonymous hemianopsia after ingesting 0.05 mg Ni/kg in drinking water as nickel sulfate. This condition of blindness in the corresponding vision field of each eye (i.e., the left field is affected in the left eye) was considered nickel-related, because it occurred shortly after the peak serum concentration of nickel. The condition lasted 2 hours, and a transient neurologic disturbance was suspected, which the authors suggested may have reflected acute cerebral vasospasm. Later subjects (4/dose) were dosed with 0.018 or 0.012 mg Ni/kg, and had no adverse effects. It should be noted that the study design maximized nickel absorption. Subjects fasted overnight, and no food was ingested for 3 hours postdosing. Since food inhibits nickel absorption, the prolonged absence of food would have increased the tissue dose for the given ingested amount. In addition, the anecdotal nature of the report of a subjective effect makes this endpoint unreliable for risk assessment. The serum nickel concentration was approximately 35 Fg/L at 3 hours postdosing.

4.1.4.2 Oral Exposure to Nickel and Hypersensitivity

Sensitivity to nickel, also known as allergic contact dermatitis, is more common in women than in men. This allergy is induced by skin contact, and elicited by dermal re-

exposure. No reports were found of contact dermatitis being induced by oral exposure to nickel in food, nickel administered experimentally, or from other sources of oral exposure to nickel.

In Denmark, this allergy was estimated to be present in 10% of women and 2% of men, based on patch tests (Nielsen and Flyvholm, 1983). Santucci et al. (1988) reported that the prevalence of nickel sensitivity is increasing. The higher occurrence in women than in men is attributed to the fact that women more commonly wear nickel-containing jewelry, such as earrings.

The research regarding sensitization effects of oral exposures to nickel addresses three issues: 1) whether oral exposures to nickel can elicit responses in sensitized individuals, a reaction called systemic contact dermatitis; 2) whether low level of exposure to nickel can induce tolerance in those not previously sensitized; and 3) whether long-term exposure to low levels of nickel can reduce sensitivity in those previously sensitized.

4.1.4.3 Systemic Contact Dermatitis

There has been some research and controversy regarding whether oral exposure to nickel can elicit the allergic response in sensitized individuals (Veien, 1997; Burrows, 1992; Nielson et al., 1990). Systemic contact dermatitis has been observed for a number of agents, particularly medicines that can be used both topically and orally, such as corticosteroids (Veien, 1997).

It is difficult to study the question of nickel systemic contact dermatitis, in part because nickel is ubiquitous in food. Nickel is a normal component of the diet, with highly variable average daily intakes in the population, but usually averaging less than 0.5 mg Ni/day (Veien, 1997).

Based on observations of oral challenges eliciting a response in nickel sensitive individuals, some researchers have proposed that reducing dietary nickel may reduce allergic contact dermatitis. However, nickel in the single doses of nickel sulfate generally used in provocation studies is not the same biologically as nickel in the diet, or in drinking water. As noted in Section 3.1, absorption of dietary nickel is much lower than absorption of nickel in drinking water, particularly if the latter is administered on an empty stomach. Furthermore, the bolus doses of nickel used in challenge studies result in higher peak serum nickel concentrations compared to the equivalent dose of nickel in drinking water. Several studies of nickel-sensitive individuals on low-nickel diets report some reduction in dermatitis, but results are equivocal (Veien and Menné, 1990; Nielson et al., 1990; Veien et al., 1993). No studies of nickel ingestion by drinking water have been undertaken to test elicitation of allergic response.

Studies of ingested nickel at low levels that have been conducted to induce tolerance are reviewed in the next section.

Studies of systemic contact dermatitis include oral challenge with single bolus doses of nickel up to 5 mg, usually as nickel sulfate hexahydrate, as well a longer-term exposures to lower doses (e.g., Spiechowicz et al., 1984; Veien et al., 1993). These studies were conducted primarily to investigate the phenomenon of systemic contact dermatitis and for hazard identification, but some dose-response information is available. Other limitations to some of the studies include the absence of controls and not evaluating the results in a double-blind fashion.

Burrows (1992) reviewed 11 trials of oral challenges of nickel-sensitized subjects, including several double blind, placebo-controlled trials, usually of single doses. Three of these studies provide the best dose-response information available on doses that cause a flare-up of eczema in previously-sensitized individuals. Gawkrodger et al. (1986) conducted a randomized double-blind cross-over study of systemic contact dermatitis in sensitized women orally administered 0, 0.4, 2.5, or 5.6 mg nickel as nickel sulfate hexahydrate (approximately 0, 0.006, 0.04, or 0.08 mg Ni/kg). Dosing was in capsules taken before breakfast, and each woman was tested with only one dose and the placebo. All 6 women receiving the high dose reacted to the nickel dose. At the other doses (n=10), the number of subjects responding to nickel alone was comparable to the number responding to both nickel and to the placebo, or to the placebo alone. Cronin et al. (1980) treated subjects (n=5) with a single dose of capsules containing 0.6, 1.2, or 2.5 mg nickel as nickel sulfate hexahydrate (approximately 0.008, 0.02, or 0.08 mg Ni/kg). The percent responders (1/5, 3/5, and 4/5) exhibited a clear dose-related increase, but this study is limited by the absence of blinding or a control group, and the small numbers. Kaaber et al. (1979, as cited in Burrows, 1992) conducted a single-blind, placebo-controlled study of 14 subjects administered 0, 0.6, 1.2, or 2.5 mg nickel as nickel sulfate hexahydrate. One individual responded at both of the low doses, and 9/14 at the high dose. Several other studies of sensitized individuals have reported systemic contact dermatitis, typically with single doses of 2.5 mg (approximately 0.035 mg Ni/kg) via the oral route (Veien et al., 1987; Veien and Menne, 1990; Christensen et al., 1981).

It is difficult to establish a clear NOAEL for oral challenge, due to the small size of the groups tested, the wide variability among individuals, and the high background of individuals who respond to the placebo. However, the data are consistent with the conclusion that most sensitized individuals respond to a single capsule or gavage dose of 5 mg nickel (about 0.08 mg Ni/kg), and few respond to doses of 1.2 mg nickel (about 0.02 mg Ni/kg). These doses are in addition to normal dietary nickel intake.

Induced Tolerance: Oral Exposures to Prevent or Reduce Dermal Contact Sensitivity.

Animal studies have been reported to show that low doses of allergens, including nickel, administered for long periods of time can induce tolerance. To test this concept in humans, Van Hoogstraten et al. (1991) studied nickel allergy and low-level oral exposure presumed to occur from wearing dental braces. (Dental braces are nickel-containing metal alloys, and can release nickel ions.) They interviewed 2176 male and female patients in nine European dermatology clinics who had positive skin patch tests. The prevalence of nickel sensitivity (positive nickel patch test) in men and women was compared in relation to age, sex, pierced ears, and orthodontic treatment. As expected, the percent with nickel sensitivity was higher in women than in men, and higher in men or women who had their ears pierced. Nickel sensitivity occurred less often among individuals who had their ears pierced after wearing braces than in those who had the ear piercing before they wore the dental braces. These results provide indirect evidence of induction of increased tolerance from oral exposure to low levels of nickel among people who had dental braces for at least 6 months prior to ear piercing. The large numbers of patients and the information on age and timing of exposure are important measures of quality. However, a limitation of the study is that group sizes differ, and the data for each category (age, status of braces and ear piercing) are reported in percentages with no measure of the statistical variability of the estimates (i.e., no confidence interval or standard error). The authors reported that no data exist regarding the concentration of nickel ions in the oral cavity from metal dental appliances, but cited *in vitro* corrosion studies that reported releases that vary from 2 - 400 Fg per day depending on the concentration of nickel in the alloy (Newman, 1981, as cited by Van Hoogstraten et al., 1991).

Two studies in humans were designed to determine whether low levels of oral nickel intake reduce the frequency or intensity of existing allergy. In a series of small trials, Sjovall et al. (1987) administered 5 or 0.5 mg nickel per day or a placebo in weekly capsules for 6 weeks to allergic patients. The sum of patch test scores before and after administration of the nickel provided evidence of reduced sensitization after the course of nickel exposure at 5 mg but not at 0.5 mg, although some patients had flare-ups during the treatment. Panzani et al. (1995) administered tablets stated as containing 0.1 ng (sic) nickel sulfate up to 2 tablets a day, for 3 years to 51 patients in an attempt to cure their nickel allergy. The patients were asked to follow a low-nickel diet by avoiding certain foods. Remission of symptoms was reported in 37 of the patients on this diet and regimen (time not specified), 14 stopped treatment, and 7 had reactivation of symptoms. All ten control individuals with chronic skin symptoms who were asked to follow a nickel-free diet for one year also reported remission of symptoms, but symptoms reappeared after reintroduction of prohibited foods. The authors claimed that an overall increase in tolerance was observed, as measured by remission of dermal symptoms, and by results of provocation tests in which a higher oral dose was needed to elicit response after treatment. However, several factors limit the conclusions of this study. Several patients stopped treatment because symptoms were aggravated, patch tests showed no significant difference, and the numbers are small, inconsistent across trials and difficult to interpret.

Some insight into this phenomenon is provided by the study of Santucci et al. (1988). These authors dosed 25 nickel-sensitized women with a single oral challenge dose of 2.2 mg Ni, and found that 22 reacted. After a 15-day rest period, the subjects were given gradually increasing doses under the following schedule: 0.67 mg Ni/day for one month, 1.34 mg Ni/day for the second month, and 2.2 mg Ni/day for the third month. In this phase of testing, 3/17 of the subjects had flare-ups even at the lowest dose. The other 14 subjects, however, did not respond even to the highest dose, even though they had responded to that dose in the initial testing. These patients did have flare-ups, however, when they resumed wearing (presumably nickel-containing) earrings, indicating that this was not strictly a hyposensitization phenomenon. Instead, the authors suggested that nickel absorption may have decreased, but no measurements of absorption were made in this study. By contrast, Nielsen et al. (1999) found no such decrease in absorption among sensitized subjects in a detailed toxicokinetics study.

These small studies in humans provide some limited support that sensitivity can be reduced by long-term, low-level exposure. The single study of nickel sensitivity in people with and without long-term, low-level exposures to nickel from dental braces (Van Hoogtraten et al., 1991) provides evidence in support of the hypothesis that chronic low-level exposures reduce the frequency of allergy. These data suggest no adverse effects on sensitized individuals, and possibly a beneficial effect, from short-term oral exposures to nickel at levels below about 0.5 mg per day (approximately 0.007 mg Ni/kg/day).

4.2 Prechronic and Chronic Studies and Cancer Bioassays in Animals – Oral and Inhalation

4.2.1 Oral Exposure

Systemic studies of the toxicity of soluble nickel have been conducted in rats, mice, and dogs. Primarily nonspecific indications of toxicity, such as decreased body weight, have been observed. However, diminished kidney function has been observed at low doses. Immunosuppressive effects have also been observed. Relevant benchmark doses (BMDs) calculated for each study are briefly presented for each study writeup, and are presented in greater detail in Appendix A. The text and analysis for the development of the BMDs is largely derived from Haber et al. (1998), and from the related EPA report (U.S. EPA, 1997).[2]

As in the rest of this document, all doses are reported in terms of nickel, rather than the nickel compound. For one study (American Biogenics Corporation, 1988), it is unclear whether the authors reported doses in terms of nickel or the nickel compound, and the doses under both conditions are noted. A further complication is related to the

[2]Some doses and calculated BMDs/BMCs presented here differ from those in Haber et al. (1998) or U.S. EPA (1997), due to minor differences in dose conversions.

fact that most nickel compounds are sold primarily as the hydrate, with the anhydrous form available at a premium price. For some studies, the authors did not specify whether they used the hydrate or anhydrous form, but it is reasonable to assume that the hydrate was used, since the material was administered in water. Because the use of the hydrate would affect the nickel dose calculated from the total compound dose, situations of ambiguity are noted. In other situations, the authors reported the dose in terms of nickel

amounts, but may not have reported whether the hydrate was used. In these cases, the doses reported by the authors were used.

Vyskocil et al. (1994b) treated Wistar rats (20/sex/group) with 0 or 100 ppm nickel as nickel sulfate (hydration state not noted) in drinking water for up to 6 months, with an interim sacrifice of 10/sex/group at 3 months. Nickel intake was calculated by the authors based on drinking water consumption. Averaged over the two 3-month periods, males consumed 6.9 mg Ni/kg/day, and females consumed 7.6 mg Ni/kg/day. Urinalysis was conducted after 3 and 6 months of exposure; histopathology was not evaluated. Parameters measured in urine were albumin as a marker of glomerular function, and lactate dehydrogenase (LDH), $\$_2$-microglobulin ($2m), and N-acetyl-$-D-glucosaminidase (NAG) as markers of tubular function, as well as total protein. Kidney and body weights were also determined. There was no effect on body weight gain in either sex. Kidney weights in both sexes at all time points were slightly higher in the exposed groups, and a slight but statistically significant increase in kidney weight was observed in males at 6 months. There was no effect on the markers of tubular function. However, urinary albumin levels, a marker of glomerular function, were significantly increased in females at 6 months (Table 15). Although the increase in urinary albumin in males was not statistically significant, evaluation of the individual animal data showed a clear increase at 6 months; the lack of a statistically significant effect in males was attributable to two control males with abnormally high values. Thus, the single dose in this study, 6.9 mg Ni/kg/day in males and 7.6 mg Ni/kg/day in females, was a LOAEL for decreased kidney function. BMD modeling is not useful for this study, because only one dose of nickel was tested. A limitation of this study is that there was considerable variability in response in both males and females. As part of this assessment, the study authors were contacted in order to obtain the individual animal data and to evaluate the implications of the variability, including a determination of whether individual nickel-exposed animals showed increased albuminuria between the 3- and 6-month analyses (baseline values were not obtained). However, the individual data were no longer available. The results in the males are less reliable than those in the females, in light of the high degree of variability in urinary albumin levels seen in male rats in general, and because the results in the males were not statistically significant (although they did reflect a population shift). Therefore, the study LOAEL is the LOAEL in females, 7.6 mg Ni/kg/day.

A chronic feeding study was conducted in which groups of 25 male and 25 female weanling Wistar rats were fed 0, 100, 1000 or 2500 ppm dietary Ni as nickel sulfate hexahydrate for up to 2 years (Ambrose et al., 1976). Information on the amount of nickel in the basal diet was not reported. Using a reference food factor (amount of food ingested on a body weight basis) of 0.079 kg food/kg body weight/day, based on the strain- and sex-specific food consumption and body weights reported in U.S. EPA (1988), daily intakes are estimated to have been approximately 0, 8, 80, and 200 mg Ni/kg body

weight[3]. Longevity was poor in all exposed groups (68-92% mortality) but not nickel-related, as two-year survival in male and female controls was similarly low (84% and 92% mortality, respectively). Body weights were significantly lower than control values in females at $80 mg Ni/kg/day from week six and in both sexes at 200 mg Ni/kg/day from the beginning of the study. Body weights at 78 weeks (the next longest duration) were considered to be more reflective of toxicity than body weights at study termination, in light of the high mortality during the last 6 months of the study. At 78 weeks, body weights were decreased 18% in mid-dose females and 8% in mid-dose males; corresponding decreases at the high dose were 32% and 35%. The investigators noted that the decreased weight gains, particularly at the high dose, may have been partly due to reduced food consumption (data not reported). Limited clinical pathology analyses found no exposure-related changes in hematology (hemoglobin, hematocrit, and differential leukocyte counts) or urinalysis (reducing substances and protein) endpoints. Relative liver weights were significantly decreased in females at 80 mg Ni/kg/day and relative heart weights were statistically significantly increased in the same group, although there was no clear dose-response. Complete histological examinations showed no exposure-related neoplastic or non-neoplastic lesions in either sex. This study is limited by the high mortality in all groups, resulting in a relatively small number of animals that were exposed for a full 2 years, and thus a small number at risk for developing cancer over the 2-year observation period. Overall, these findings strongly suggest that chronic exposure to dietary nickel sulfate was not carcinogenic, but are not conclusive in this regard. Based on the decreased body weight in females, this study identified a NOAEL of 8 mg Ni/kg/day and a LOAEL of 80 mg Ni/kg/day. BMDs calculated for this study ranged from 11 to 58 mg Ni/kg/day, depending on whether the benchmark response (BMR) was defined as a 10% increased risk of low body weight (lower value) or 10% decrease in the mean body weight (higher value). Low body weight was defined as a 10% decrease in body weight compared to controls. This difference in definition is discussed further in Appendix A. This study is limited by the high mortality in the control and exposed groups, and by the limited reporting of the study design and results.

The carcinogenicity of nickel acetate was tested in three drinking water studies with rats and mice (Schroeder et al., 1964, 1974; Schroeder and Mitchener, 1975). These studies had similar experimental designs, with groups of 50-54 male and 52-54 female Long-Evans rats or Charles River Swiss mice being exposed to 0 or 5 ppm Ni as nickel acetate (hydration state not reported) from the time of weaning until natural death. The diet in the first mouse study (Schroeder et al., 1964) was different from that used in the other studies in that it was devoid of cadmium and low in other trace metals. All three studies used a pathology protocol in which histological examinations were limited to the lungs, heart, liver, kidneys, and spleen.

[3]The NOAEL and LOAEL were reported as 5 mg Ni/kg/day and 50 mg Ni/kg/day in the nickel RfD verified on 07/16/87, due to the use of a generic food factor of 0.05, rather than the strain-specific value used for this assessment.

In the rat study, the reported estimated total daily nickel intakes (water plus food) in the control and exposed groups were 2.6 and 37.6 Fg/rat, respectively (Schroeder et al., 1974). Assuming an average body weight of approximately 300 g for males and 220 g for females (based on the authors' data), the controls received approximately 0.009 mg Ni/kg/day (males) or 0.012 mg Ni/kg/day (females), and the 5 ppm group received approximately 0.13 mg Ni/kg/day (males) or 0.17 mg Ni/kg/day (females). Mean body weights were slightly reduced in males and females at 18 months (10-13% lower than control values). There were no exposure-related effects on longevity or tumor incidences in either sex. The only histological lesion attributable to nickel exposure was a slightly increased incidence of myocardial fibrosis (13.3% higher than controls, $p<0.025$, sex not specified).

Nickel intake data were only reported in the first of the two mouse drinking water studies (Schroeder et al., 1964). The maximum daily nickel intake from water was estimated to be 0.45 to 0.51 mg Ni/kg bw (~20 Fg Ni/mouse), although the actual value may be lower because of some water spillage. No estimate of nickel intake from the low trace metal diet was reported. Body weights were slightly reduced in both sexes after one year (4-13% lower than controls) in the first mouse study, but there were no changes in body weight clearly attributable to exposure in the second study. No adverse exposure-related effects on survival or occurrence of tumors and other lesions occurred in either mouse study. Early mortality occurred in both exposed and control groups in the first study, the number of deaths from all tumor types was significantly lower in exposed females than in controls in the first study, and the lifespan of exposed females was significantly longer than controls in the second study.

None of the studies summarized above (Schroeder et al., 1964, 1974; Schroeder and Mitchener, 1975) provided any indication that nickel acetate in drinking water was carcinogenic in rats or mice. However, due to the single exposure levels and other limitations in the design of these studies, the findings are not conclusively negative. In particular, it is unclear if a maximum tolerated dose was achieved in any of the studies because the decreases in body weights (~10%) were not clearly adverse. In addition, the first study is limited by high (>30%) autolysis, and it is unclear whether non-neoplastic lesions not considered to have caused death were evaluated. Similarly, less than half of the animals in the latter two studies were necropsied, and even fewer were sectioned. Therefore, nonneoplastic lesions and any small tumors could have been missed. These studies are also inadequate for evaluation of noncancer effects, in light of the incomplete evaluation of noncancer endpoints.

Male and female Sprague-Dawley rats were administered 0, 5, 35, or 100 mg Ni/kg/day nickel as nickel chloride hexahydrate by gavage in water for 92 consecutive days (American Biogenics Corporation, 1988)[4]. All high-dose animals died; other toxicology data

[4]The study is rather ambiguous regarding whether the doses were reported as nickel or as nickel chloride. Some other reviews (e.g., ATSDR, 1997 and Health Canada, 1993) have

were not reported for these animals. At the mid dose, 6/30 males and 8/30 females died; deaths of 3/6 males and 5/8 females was attributed to gavage errors, based on histopathological analysis. One male and one female died at the low dose. Salivation, lethargy, and irregular breathing were observed both in rats that died early and in those that survived. Final body weight was significantly decreased in males and females at the mid dose, to 81% and 92% of the corresponding controls, respectively. Food consumption was also decreased in males, but there was no statistically significant decrease in females. Significantly increased relative organ weights (adrenal, brain, testes in males; adrenals and heart in females) were apparently related to the decreased body weight. Similarly, decreased absolute organ weights (kidney, liver, and spleen in males; kidney in females) were probably related to the decreased body weight, in the absence of data indicating that nickel causes atrophy of these organs. Urinalysis evaluated protein, glucose, specific gravity, and pH; no individual proteins in urine were evaluated. The study authors stated that a statistically and clinically significant increase in white blood cells was observed in males at the mid dose at the interim sacrifice, and there was a minor increase in low- and mid-dose females, but there was no effect at the terminal sacrifice. Blood glucose was statistically significantly decreased in females treated with 35 mg Ni/kg/day at the terminal sacrifice, but not at the interim sacrifice or in males at any time point. Pneumonitis, characterized by intra-alveolar accumulation of pulmonary macrophages and degeneration of type II pneumocytes, was reported in mid-dose males (7/25) and females (10/25). However, histopathology was not conducted on low-dose animals. No histopathological effects in other organs were observed. The NOAEL in this study was 5 mg Ni/kg/day, and the LOAEL was 35 mg Ni/kg/day, based on decreased body weight in males and pneumonitis in both sexes. It should be noted, however, that deaths in 10% of the males and 10% of the females were attributed to nickel at this dose. BMDs calculated for this study ranged from 6.8 to 36 mg Ni/kg/day, depending on whether the benchmark response (BMR) was defined as a 10% increased risk of low body weight (lower value) or 10% decrease in the mean body weight (higher value).

In a 28-day study, Weischer et al. (1980) administered drinking water containing 0, 2.5, 5, or 10 ppm Ni as nickel chloride (hydration state not reported) to groups of ten male Wistar rats. These doses correspond to 0, 0.37, 0.7, and 1.5 mg Ni/kg/day, assuming a body weight of 0.22 kg and water consumption of 0.032 L/day, as reported in U.S. EPA (1988). A dose-related, statistically significant increase in serum glucose was observed at all doses. Although this endpoint is supported by several studies via other routes by this and other authors, and by other authors investigating mechanism, the small degree of increase (14-

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interpreted the study as reporting doses as nickel chloride. However, based on the reporting of analytical determinations, it appears that the doses were actually reported as nickel. If the reported doses were as amount of nickel chloride hexahydrate, the nickel doses would have been 0, 2.7, 19, and 55 mg Ni/kg/day, and the study NOAEL would have been 2.7 mg Ni/kg/day, with a LOAEL of 19 mg Ni/kg/day.

28%) is probably pre-adverse. Decreased body weight gain of >10% was observed at all doses, but the body weight at all three doses was similar. The biological significance of this decrease at doses as low as 0.37 mg Ni/kg/day is unclear, in light of the lack of decreasing body weight as doses increased. The response at the high dose is similar to that of some other drinking water studies, but a much higher NOAEL for this endpoint was observed by Ambrose et al. (1976) and other authors for this endpoint for exposures in diet and drinking water.

Dietary exposure of beagle dogs (3/sex/group) to 2500 ppm Ni (about 63 mg Ni/kg/day, assuming a food factor of 0.025) resulted in depressed body weight gain; no effects were seen at either 100 ppm (about 2.5 mg Ni/kg/day) or 1000 ppm Ni (about 25 mg Ni/kg/day) in the diet (Ambrose et al., 1976). The authors noted that the high dose caused vomiting, and required stepwise increases from 1700 ppm to reach the final dose. One high-dose male and female had polyuria when measured at the end of 2 years. This study indicates that rats (see previous description) are more sensitive than dogs, although the dog study is limited by the small sample size.

The effect of nickel on the immune system is complex. As discussed in Section 4.1.4.2, both oral and inhalation exposure to nickel can lead to sensitization, although the immune mechanism for nickel-induced asthma is different from that for dermal sensitization. However, despite this evidence of immune stimulation by nickel, decreased immune response has been observed following oral dosing.

Effects on immune endpoints were investigated in groups of ten female B6C3F1 mice provided drinking water containing 0, 1000, 5000, or 10,000 ppm nickel sulfate (hydration state not reported) for 180 days (Dieter et al., 1988). The measured intake was 0, 115.7, 285.7, and 395.7 mg nickel sulfate/kg/day (0, 44, 108, 150 mg Ni/kg/day). (If the reported doses were really as nickel sulfate hexahydrate, the nickel doses would have been 0, 25, 64, and 88 mg Ni/kg/day.) Immune function assays included measurements of plaque-forming cell (PFC) response to sheep red blood cells (SRBC), lymphoproliferative response, natural killer (NK) cell activity of spleen cells, and resistance to challenge with the bacterium *Listeria monocytogenes*. Myeloproliferative assays included bone marrow cellularity and stem cell proliferative response. Water consumption was markedly decreased at the two highest doses, and the study authors suggested that there may have been some dehydration at the high dose. Histopathology was evaluated in 6/group. Mild tubular nephrosis was observed in all evaluated mid- and high-dose mice, but not at the low dose or in controls. Mild thymic atrophy, characterized by a decrease in size of the lymphocyte-rich thymic cortex, was observed in all treated mice (6/6 in all groups), while minimal atrophy was observed in only 1/6 control. The histologic finding of thymic atrophy was supported by statistically significant decreases in thymus weight at all doses. A significant decrease in the lymphoproliferative response to a B-cell mitogen, but not to a T-cell mitogen, was observed at

all doses. In addition, statistically significant decreases in PFC response and spleen cellularity were observed at the high dose. A statistically significant, dose-related decrease in the granulocyte-macrophage proliferative response was observed at all doses, and a decrease in bone marrow cellularity occurred at the two highest doses. Although the decrease in lymphoproliferative response was observed at the low dose, the study authors considered this effect to be secondary to effects on the myeloid system because other immune function parameters were not affected. However, based on the histologically-observed thymic atrophy and decreased thymic weight, the LOAEL in this study was 44 mg Ni/kg/day.

Inhibition of immune function compared to controls was also observed in female SJL mice fed diets containing 2700 ppm nickel sulfate hexahydrate (600 ppm nickel) for 4 weeks (Schiffer et al., 1991). This concentration corresponds to approximately 120 mg Ni/kg/day, assuming a food factor of 0.20 for a subchronic study. Both *in vivo* and *in vitro* proliferative responses to T cell-dependent antigens, and the *in vitro* proliferative response to a B cell-dependent antigen, were decreased. The impaired response continued even after culture of spleen cells from exposed animals in standard (nickel-free) media for 5 days.

Oral nickel administration has also been reported to overcome the sensitizing effects of dermal exposure to nickel. The delayed type hypersensitivity (DTH) reaction to nickel sulfate was significantly reduced in C3H/He mice (sex not specified) treated for 10 weeks with 0.1, 0.25, or 0.5% nickel as nickel sulfate in drinking water (Ishii et al., 1993). Based on the authors' calculations of the amount of nickel ingested per mouse, and a default mouse body weight of 0.03 kg, the corresponding doses were 178, 155, and 237 mg Ni/kg/day. (Mice at the two highest doses drank less.) Exposure for 7 weeks was insufficient to elicit tolerance at any dose, and treatment with 0.05% nickel (87 mg Ni/kg/day) or less had no effect at either duration. The induction of tolerance was specific for nickel, since there was no effect on DTH to $CrCl_3$. The suppression was attributed to $CD4^-CD8^+$ suppressor T cells, but could be overcome by $CD4^+$ helper T cells. Nickel tolerance resulting from oral exposure to nickel has also been reported in human studies, as discussed in Section 4.1.4.2.

4.2.2 Inhalation Exposure

Data from well-conducted chronic and subchronic inhalation studies in rats and mice with nickel sulfate are available from NTP (1996a). These studies found that the respiratory tract is the primary target of inhaled soluble nickel, with effects occurring in both the lungs and nose. Supporting mechanistic data are available from related studies conducted by the same group (Haley et al., 1990; Benson et al., 1989).

The National Toxicology Program (NTP) performed a comprehensive chronic inhalation bioassay of nickel sulfate hexahydrate aerosol in which groups of F344/N rats (53-55/sex) and

B6C3F1 mice (60-62/sex) were exposed for 6 hours/day[5], 5 days/week for up to 104 weeks (NTP, 1996a). The core studies included an additional 5 animals/sex/group that were sacrificed at 7 months for histopathology exams, and at 15 months for histopathology and hematology analyses. Additional groups of 5-7 animals/sex were sacrificed at 7 and 15 months for tissue burden studies (lungs and kidneys in mice and lungs in rats). The aerosol mass median aerodynamic diameters (MMADs) ranged from 2.27-2.53 µm (Table 16). Endpoints evaluated in the core study were clinical signs, body weight, organ weights (interim sacrifices only), and complete histopathology.

Male and female rats were exposed to 0, 0.12, 0.25, or 0.5 mg compound/m^3 (0, 0.027, 0.056, or 0.11 mg Ni/m^3) for 6 hours/day, 5 days/week for 2 years (duration adjusted to 0.0048, 0.010, and 0.020 mg Ni/m^3). In the rat study, mean body weights of the females were decreased by ~6% at the high exposure level; male body weights were unaffected. No clinical signs of toxicity or biologically significant hematological changes were observed. The primary target of toxicity was the respiratory tract, as summarized in Table 16. Lung nickel burdens were significantly higher than control values in male and female rats at 0.11 mg Ni/m^3 after 7 months and male and female rats at $\geq$0.027 mg Ni/m^3 after 15 months (concentration-related). At the 2-year sacrifice, nonneoplastic inflammatory lesions of the lung were observed at exposure concentrations $\geq$0.056 mg Ni/m^3 in males and females, with no elevation over control incidence at the low concentration. Chronic active inflammation of the lung was described as multifocal, minimal to mild accumulations of macrophages, neutrophils, and cell debris in alveolar spaces. Fibrosis occurred at the same levels, and was described as "increased connective tissue and collagen involving alveolar septae in the parenchyma and subjacent to the pleura and focal sclerotic areas either subjacent to the pleura or at the tips of the lung lobes." Alveolar proteinosis was also observed, at a lower incidence. A lesion described by NTP as "alveolar macrophage hyperplasia" was also reported at a high incidence at $\geq$0.056 mg Ni/m^3. The hyperplasia was described as being of minimal to mild severity, and consisted of "macrophages (usually with abundant pale vacuolated cytoplasm) within alveolar spaces." NTP stated that the macrophages were probably derived from the pool of circulating monocytes. However, it is not possible to definitively identify the source of the macrophages. Nonetheless, "alveolar macrophage accumulation" may be a better
term than "alveolar macrophage hyperplasia," and will be used for the rest of this document, even though the NTP tables refer to hyperplasia. If the macrophages did not originate in the lung, their presence would reflect lung damage that circulating macrophages have been recruited to repair. The lung toxicity was more severe in rats
than in mice as indicated by higher incidences of inflammation and macrophage accumulation in rats at an exposure level of 0.056 mg Ni/m^3 and progression to fibrosis.

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[5]Daily exposure time was actually 6 hrs plus T_{90} (the time for the chamber concentration to reach 90% of target, approximately 8 minutes).

Lymphoid hyperplasia of the bronchial lymph node and atrophy of the olfactory epithelium also occurred at the high exposure level (0.11 mg Ni/m^3) in both sexes of rats.

As shown in Table 18, the incidence in males of chronic active inflammation was significantly increased at the 7-month interim evaluation, and all mid-concentration males at this sacrifice time exhibited macrophage accumulation. Smaller increases that were not statistically significant at the low concentration were also observed in females at this time point. Macrophage accumulation was observed in all males and 4/5 females at 0.056 mg Ni/m^3 in the 7-month evaluation, but the incidence was lower at this concentration in the 15-month evaluation. The lesions in males appear to be a transient, reversible effect, since they were not seen at the low concentration following exposure for 15 months or 2 years. In addition, although alveolar macrophage accumulation was observed at 0.027 mg Ni/m^3 in the subchronic rat study (see below), the lesion termed "chronic active inflammation" was not observed in the subchronic study until the much higher concentration of 0.22 mg Ni/m^3. Therefore, the inflammation observed in males at the 7-month interim sacrifice was not considered to be an appropriate basis for a LOAEL.

Another endpoint that requires additional discussion is macrophage accumulation. Because the evaluation of the potential adversity of this endpoint required an integrated evaluation of the entire database, this discussion addresses results from both the chronic and subchronic rat and mouse studies. In the chronic rat studies, macrophage accumulation in males and females was one of the more sensitive endpoints, but it always occurred in the presence of chronic active inflammation at the same or higher incidence. In the chronic mouse studies, macrophage accumulation was the lung lesion found at the lowest concentration in males and females, or occurred at the same concentration as other lesions, but at a higher incidence. The higher sensitivity of this endpoint was more striking in the subchronic studies. In both males and females in the rat and mouse
studies, alveolar macrophage accumulation was observed at a high incidence at least two exposure levels below the concentration that induced increases in other lesions. The authors noted, however, that only minimal increases in the number of macrophages were observed. As noted above, the macrophage accumulation may be a repair response to tissue damage, and may be an adaptive effect. Alternatively, this accumulation may
reflect part of a continuum of effects, leading to inflammation, and ultimately to fibrosis. However, this progression appears to be one that increases in severity with increasing exposure levels, rather than progressing with increasing exposure duration. The observation of macrophage accumulation at *lower* exposure levels in the subchronic study than any effect was observed in the chronic study indicates that early macrophage accumulation at low concentrations does not lead to more severe lesions at the same concentrations and longer exposure times. Macrophage accumulation can also be a physical response to particulate exposure, although the low exposure level at which it was seen and the differences between responses in rats and mice suggests that the response

was at least partially nickel-specific. A definitive conclusion regarding the adversity of the endpoint is not possible. However, based on the occurrence of macrophage accumulation at concentrations that do not result in other effects, the decreased response at longer exposure periods, and the potential nonspecificity of the response, this endpoint may be on a continuum of more severe effects, but does not appear do progress to these effects with continued exposure. The implications of the alternative interpretation (that macrophage accumulation can form the basis for an equivocal LOAEL) on the development of the RfC is discussed in Section 5.2.

Thus, the NOAEL for lung effects in rats in the chronic study was 0.027 mg Ni/m^3 and the NOAEL(HEC) for the lung effects (all of which were considered to occur in the pulmonary region) was 0.0021 mg Ni/m^3 for males and 0.0024 mg Ni/m^3 for females. The NOAEL for atrophy of the olfactory epithelium was 0.056 mg Ni/m^3, corresponding to a NOAEL(HEC) of 0.0019 mg Ni/m^3 in females and 0.0033 mg Ni/m^3 in males. No exposure-related neoplasms in the lungs or other tissues were observed (Table 19). The lowest BMC(HEC) was 0.0017 mg Ni/m^3, calculated for lung fibrosis in males; a BMC(HEC) of 0.0023 mg Ni/m^3 could be calculated for this endpoint in females. The BMC(HEC) calculated for atrophy of the olfactory epithelium was 0.0025 mg Ni/m^3 for female rats and 0.004 mg Ni/m^3 for male rats. The BMC(HEC) calculated for alveolar proteinosis in female rats was 0.0028 mg Ni/m^3. The overall NOAEL(HEC) for this study was 0.0019 mg Ni/m^3, based on atrophy of the olfactory epithelium in females. The lowest BMC(HEC) was 0.0017 mg Ni/m^3, calculated for lung fibrosis in males. The BMC calculations are documented in further detail in Section 5.2.2 and Appendix A.

In the chronic mouse study (NTP, 1996a), male and female mice were exposed to 0, 0.25, 0.5, or 1 mg compound/m^3 (0, 0.056, 0.11, or 0.22 mg Ni/m^3) using the same protocol as used in the rat study. The duration-adjusted values for the mice were 0, 0.010, 0.020, and 0.040 mg Ni/m^3. Body weights were slightly reduced during most of the second year, with final body weights decreased at the high exposure level by 8.7% in males and by 12% in females. Lung nickel burdens were significantly higher than control values in female mice at 0.22 mg Ni/m^3 after 15 months. As in the rats, histologic lesions were confined to the respiratory tract. Females were more sensitive than males to pulmonary effects of nickel sulfate, but males were more sensitive to nasal effects. In females, chronic active inflammation (intra-alveolar accumulation of inflammatory cells), bronchialization, and alveolar macrophage accumulation were observed at the low exposure level (0.056 mg Ni/m^3) and higher. As noted for the rats, macrophage accumulation is judged not to be an adverse effect. Bronchialization was described as hyperplastic and/or hypertrophic cuboidal epithelial cells extending from the terminal bronchial into the alveolar ducts and proximal alveoli, and thus was considered a thoracic (combined tracheobronchial and pulmonary) effect. The same lesions were observed at $0.11 mg Ni/$m^3$ in males. Interstitial infiltration and alveolar proteinosis were also observed in females at $0.11 mg Ni/$m^3$ and in males at 0.22 mg Ni/m^3. In the bronchial lymph node, macrophage accumulation

occurred in both sexes at $0.11 mg Ni/m^3, and lymphoid hyperplasia was seen in both sexes at the high concentration. Atrophy of the olfactory epithelium was also observed in males at $0.11 mg Ni/m^3 and in females at the high concentration. No exposure-related neoplasms in the lungs or other tissues occurred in male or female mice. The LOAEL(HEC) for the pulmonary effect of inflammation in females was 0.0088 mg Ni/m^3; no NOAEL was identified for this endpoint in females, although a NOAEL(HEC) of 0.0090 mg Ni/m^3 was identified in males. For the tracheobronchial endpoint of bronchialization in females, the LOAEL(HEC) was 0.012 mg Ni/m^3. For the extrathoracic endpoint of olfactory epithelial atrophy, males were more sensitive, with a NOAEL(HEC) of 0.0028 mg Ni/m^3; the NOAEL for females was at the next higher exposure level, with a NOAEL(HEC) of 0.0054 mg Ni/m^3. The BMC(HEC) values for chronic inflammation in females and for bronchialization in females were both approximately 0.006 mg Ni/m^3. The BMC(HEC) for olfactory epithelial atrophy in males was approximately 0.004 mg Ni/m^3.

Overall, NTP (1996a) concluded that there was "no evidence" of nickel sulfate carcinogenicity in male and female rats and mice. In similarly designed inhalation bioassays of poorly soluble nickel compounds, NTP (1996b, 1996c) found "some evidence" of nickel oxide carcinogenicity in male and female rats (based on lung and adrenal tumors, see Table 20) and "clear evidence" of nickel subsulfide carcinogenicity in male and female rats (lung tumors, Table 20). There was no evidence of nickel subsulfide carcinogenicity in male or female mice, no evidence of nickel oxide carcinogenicity in male mice, and "equivocal evidence" of nickel oxide carcinogenicity in female mice, in light of the absence of an increase at the high concentration, and the "marginally increased incidences" at the two lower concentrations.

Some arguments have been raised that the negative evidence from the NTP (1996a) bioassays cannot be considered definitive. The first line of reasoning is that insufficiently high concentrations were tested in the rat bioassay. This line of reasoning is based on the observation of a somewhat higher incidence of lung lesions in rats exposed to 0.11 mg Ni/m^3 for two years as nickel subsulfide than in rats exposed to the same concentration as nickel sulfate (compare Tables 17 and 21). (Nasal lesions, however, occurred at a higher incidence in the rats exposed to nickel sulfate.) Nickel subsulfide at this concentration produced small statistically significant increases in lung adenomas and carcinomas (combined). Larger statistically significant increases were observed at the higher nickel subsulfide concentration tested - 0.73 mg Ni/m^3. According to this argument, the toxicity of nickel sulfate was comparable to, or lower than, the toxicity of nickel subsulfide; nickel sulfate could have been tested at higher concentrations, and may have been carcinogenic at higher concentrations.

The NTP review panel considered whether adequately high concentrations were tested in the nickel sulfate bioassay. The panel members noted that slightly higher concentrations could have been tested, but overall the study was judged to be adequate. NTP (1996a) noted that the high concentration in the nickel sulfate bioassay was chosen based on the observation

of chronic active inflammation in the lung in the 13-week study. This lesion was considered to be potentially life-threatening, because of the possibility of reduced lung function. The high nickel sulfate concentration in the 2-year bioassay was just below the concentration at which mild chronic active inflammation was seen in the 13-week study. The 13-week data also suggest a steeper concentration-response curve for nickel sulfate than for nickel subsulfide. One male rat exposed to 0.44 mg Ni/m^3 as nickel sulfate died.

The second line of reasoning questioning the adequacy of the NTP bioassay relates to questions regarding how informative the mouse bioassay data are considered to be. Nickel subsulfide was classified as having "clear evidence" of carcinogenicity in male and female rats, but "no evidence" of carcinogenicity in male and female mice. Similarly, nickel oxide was classified as having "some evidence" of carcinogenicity in male and female rats, but "no evidence" in male mice and "equivocal evidence" in female mice. In light of either "no" or "equivocal" responses for nickel subsulfide and nickel oxide in mice, it has been argued that the mouse data are not informative for nickel compounds. It should be noted, however, that this argument presumes that nickel sulfate is carcinogenic. Without this presumption, nickel sulfate would be considered to have been adequately tested in two species. Moreover, a search of the NTP bioassay results database found no tendency for mice to be less likely to develop lung tumors than rats, even when considering only inhalation studies or metals (cobalt sulfate, molybdenum trioxide, and selenium sulfide) (NTP, 1999).

A third line of reasoning questions the relevance of the NTP bioassay results, contending that occupational exposures are much higher than the exposures tested in the NTP study. However, while the concentrations of nickel particles may have been higher under occupational conditions (with exposure levels up to 1-10 mg Ni/m^3), the actual dose to the lungs of the workers was smaller than or comparable to that in the bioassays. The animal and human doses can be compared by converting both to the human equivalent concentration (HEC). As noted in Section 4.1.2, an exposure level of 4 mg Ni/m^3 for occupational exposure in a nickel refinery corresponds to an HEC of approximately 0.001 mg Ni/m^3. The BMC(HEC) based on lung effects in the rat bioassay was 0.0017 mg Ni/m^3. There is considerable variability in the particle size distributions observed under occupational exposure conditions, with a larger respirable fraction (which would reach the pulmonary region of the lung) reported in a different refinery (Werner et al., 1999), but the lung dose is much closer to that in the bioassay even under the latter conditions.

The final line of reasoning states that the bioassay with nickel sulfate hexahydrate did not test high enough concentrations, based on the low lung burden in that assay, compared to that observed in the nickel subsulfide and nickel oxide bioassays (NTP, 1996a, 1996b, 1996c). However, the lung burden of nickel reflects the rate of deposition and clearance of each nickel compound. Toxicity data, rather than lung burden results, should be used in evaluating whether sufficiently high concentrations were tested.

Results from the related subchronic studies in mice and rats also show that the respiratory tract is the primary target of inhaled soluble nickel aerosol (NTP, 1996a; Dunnick et al., 1989). F344/N rats (10/group/sex) were exposed to 0, 0.12, 0.25, 0.50, 1, or 2 mg compound/m^3 (0, 0.027, 0.056, 0.11, 0.22, or 0.45 mg Ni/m^3) for 6 hours/day, 5

days/week for 13 weeks (duration adjusted to 0, 0.0048, 0.010, 0.020, 0.040, and 0.080 mg Ni/m^3). The MMAD and sigma g values are presented in Table 22. There were no clinical signs of toxicity. One high-concentration male rat died. Absolute and relative lung weight were statistically significantly increased in a concentration-related manner at all but the low exposure level in females and the lowest two exposure levels in males. Neutrophilia was observed in females at $0.056 mg Ni/$m^3$ and in males at $0.11 mg Ni/$m^3$, and was considered to be consistent with the pulmonary inflammation. Minimal hematological changes (increased hematocrit, hemoglobin concentration, and erythrocyte count) were observed in females at 0.22 and 0.45 mg Ni/m^3, and were attributed to either mild dehydration, or to possible tissue hypoxia. Females exposed to $0.11 mg Ni/$m^3$ had lymphocytosis, which the authors suggested may have been related to the observed lymph node hyperplasia. There were no significant effects on sperm morphology or vaginal cytology. Alveolar macrophage accumulation was observed in almost all of the rats at all exposure levels. The increases in the number of macrophages at the two lowest concentrations was described as minimal, and there was no other accompanying evidence of inflammation at these concentrations. (The number of macrophages increased at higher exposure levels, at which there was additional evidence of inflammation.). Chronic lung inflammation and interstitial infiltrate were observed in female rats at $0.11 mg Ni/$m^3$ and in male rats at $0.22 mg Ni/$m^3$. Atrophy of the olfactory epithelium and hyperplasia of the bronchial and mediastinal lymph nodes occurred in both sexes at $0.22 mg Ni/$m^3$. The NOAEL(HEC) for lung lesions in females (considered to be pulmonary effects) was 0.0047 mg Ni/m^3, and the NOAEL(HEC) for increased lung weight, a thoracic effect, was 0.0032 mg Ni/m^3. The NOAEL(HEC) for olfactory epithelial atrophy in female rats was 0.0016 mg Ni/m^3 and the corresponding NOAEL(HEC) for males was 0.0024 mg Ni/m^3. Thus, the overall NOAEL(HEC) for this study was 0.0016 mg Ni/m^3, based on olfactory epithelial atrophy in female rats; the corresponding BMC(HEC) was 0.0005-0.0007 mg Ni/m^3, depending on the model used.

Mice in the subchronic study were also exposed to 0, 0.027, 0.056, 0.11, 0.22, or 0.45 mg Ni/m^3 for 6 hours/day, 5 days/week for 13 weeks (duration adjusted to 0, 0.0048, 0.010, 0.020, 0.040, and 0.080 mg Ni/m^3). Increased neutrophil counts and lymphocyte counts were observed in females at $0.11 mg Ni/$m^3$, although the authors noted that inflammation and lymph node hyperplasia were not observed until higher concentrations. There were no significant effects on sperm morphology or vaginal cytology. Alveolar macrophage accumulation was the most sensitive endpoint, and was observed in all animals at $0.11 mg Ni/$m^3$. Other histologic lesions were confined to the high exposure level in males and females, and consisted of chronic active inflammation, fibrosis, and interstitial infiltrate of the lungs, bronchial lymph node hyperplasia, and olfactory epithelial atrophy. No histologic lesions were found in any other tissue. The lowest NOAEL(HEC) was 0.007 mg Ni/m^3, for olfactory epithelial atrophy in females.

In a related study, Haley et al. (1990) exposed groups of 40 female $B6C3F_1$ mice to nickel sulfate hexahydrate at actual concentrations of 0, 0.12, 0.52, or 2.01 mg compound/m^3 (0, 0.027, 0.12, and 0.45 mg Ni/m^3) for 6 hours/day, 5 days/week for 13 weeks (duration adjusted to 0, 0.0048, 0.021, and 0.080 mg Ni/m^3). The MMAD was reported as 2.3 µm and the GSD was 2.4 µm. The mice were subjected to a battery of immune function tests, including the antibody forming cell (AFC) response in lung-associated lymph nodes (LALN) and in the spleen, pulmonary alveolar macrophage (PAM) response, mixed lymphocyte response (MLR), spleen cell proliferative response to mitogens, spleen cell natural killer (NK) cell activity, and lung host resistance to tumor challenge. The only statistically significant effects were an increase in the number of nucleated cells recovered in lavage fluid (due to an increase in the percent and numbers of alveolar macrophages and neutrophils) and a decrease in AFC/spleen at the high concentration. The study authors noted that the increased numbers of macrophages and neutrophils in lavage samples were consistent with pulmonary inflammation observed in a parallel study (Dunnick et al., 1989; NTP, 1996a), and that this response is probably associated with nonspecific mediators of inflammation, rather than an altered immune response. The decrease in spleen AFCs was not accompanied by other changes in immune function. Overall, these results show that the immune effects of soluble nickel occur at higher exposure levels than effects on the respiratory system.

The same group also investigated biochemical responses of the lung to inhaled nickel, although this work appears to have been conducted with a separate group of animals. Benson et al. (1989) exposed male and female rats (6/sex/group) and mice (8/sex/group) to 0, 0.02, 0.1, or 0.4 mg Ni/m^3 for 6 hours/day 5 days/week for 13 weeks. Bronchoalveolar lavage was conducted at the end of the study, and the fluid was analyzed for lactate dehydrogenase (LDH), beta-glucuronidase, and total protein, and total and differential cell counts were performed. Histological analyses of the lungs were also conducted. The authors stated that no significant sex-related differences were observed, and so results were reported for both sexes combined. Marked concentration-related and statistically significant increases in LDH and beta-glucuronidase were observed in rats and mice at the mid-and high-exposure levels, and for total protein in rats at the same concentrations. A concentration-related, statistically significant increase in total nucleated cells was observed in rats at 0.02 mg Ni/m^3 and higher, and in mice in mice at 0.1 mg Ni/m^3 and higher. In rats, the percent neutrophils was significantly elevated and the percent macrophages was significantly depressed at the mid and high concentrations. Chronic inflammation and interstitial infiltrates were observed in rats at 0.1 mg Ni/m^3 and higher, and macrophage accumulation was observed at all exposure levels. In mice, macrophage accumulation and interstitial infiltrates were observed at the mid and high concentrations, and chronic inflammation and fibrosis were observed only at the high concentration.

4.3 Reproductive/Developmental Studies – Oral and Inhalation

As noted in Section 3.2, nickel can cross the placenta, and several oral studies have reported increased neonatal mortality at doses below those resulting in maternal toxicity. Several oral multigeneration reproduction studies are available (Research Triangle Institute, 1988; Smith et al., 1993; Ambrose et al., 1976; Schroeder and Mitchener, 1971), and one (Research Triangle Institute, 1988) included teratological evaluation of the F2 generation rats. No multigeneration reproduction study is available via the inhalation route, although evaluation of reproductive structure and function as part of general inhalation toxicity studies has found no evidence of effects at concentrations causing respiratory effects (Dunnick et al., 1989; NTP, 1996a). No standard developmental studies with soluble nickel species via either the oral or inhalation routes were located. There is an inhalation developmental toxicity study in rats exposed to nickel oxide (Weischer et al., 1980), but there was only minimal evaluation of the pups. Although nickel oxide is an insoluble form of nickel, any observed systemic effects would be attributable to absorbed nickel, which presumably would be in a soluble form.

Smith et al. (1993) conducted a 2-generation reproductive toxicity study of Long-Evans rats administered 0, 10, 50, or 250 ppm nickel as nickel chloride hexahydrate in drinking water. Groups of 34 females were administered the nickel for 11 weeks prior to breeding, mated with experienced, unexposed males. Exposure of the females continued during two successive gestation periods (G1 and G2) and lactation periods (L1 and L2). The overall average doses were reported as 0, 1.33, 6.80, and 31.63 mg Ni/kg/day. Average water intake was unaffected except at the high dose. Average doses were about 20% lower during prebreeding and gestation, and about 60% higher during lactation, due to higher water consumption during lactation. Maternal body weight was statistically significantly reduced at the high dose, and a statistically significant decrease in body weight gain was observed at the mid and high doses. A small, but statistically significant decrease in prolactin was observed in high-dose dams. There was no treatment-related effect on reproductive performance indices (mating success, rate of impregnation, pups/litter, gestation length), or on mean pup birth weight or weight gain in either generation. However, the number of litters with dead pups at birth was significantly increased at the high dose in both the first and second breeding. There was no effect on other measures of pup mortality in the first generation, but the total number dead pups and the percentage of dead pups per litter on postnatal day 1 were statistically significantly increased at all doses in the second generation, and the number of litters with dead pups was also borderline significant ($p<0.06$) at the low dose of the second generation. The inconsistency between generations and the absence of a clear dose-response make it difficult to identify a NOAEL or LOAEL for this study. However, the study authors noted that all three measures of pup death were statistically significant or borderline significant at the low dose in the second generation, and stated that the absence of a clear dose-response should not result in a discounting of a response at the low dose. The study authors also suggested that the dose-response curve is shallow in the low- and mid-dose regions, and is steeper at higher doses after a homeostatic mechanism regulating nickel absorption is overcome. Based on the observation

of increases in two measures of pup deaths, an equivocal LOAEL for this study was 1.33 mg Ni/kg/day.

Research Triangle Institute (1988) administered nickel chloride hexahydrate to male and female CD rats (30/sex/dose) at 0, 50, 250, or 500 ppm nickel in drinking water in a 2-generation study. (An additional dose level of 1000 ppm was eliminated after 2 weeks due to excessive toxicity.) The parental animals were exposed beginning 11 weeks before cohabitation, and continued for a total of 24 weeks (males) or 30 weeks (females). Groups of 10 rats/sex comprised a satellite subchronic nonbreeder study. The average nickel consumption reported by the authors varied by more than a factor of 2, with the highest consumption at the beginning of the premating exposure and during the latter part of the lactation period. As a conservative estimate, the average exposure during gestation, which was on the low end of overall exposure levels, was used as the dose level for each group. This choice also takes into account the possibility that gestational exposure alone could have accounted for the observed effects. Thus, the estimated doses were 0, 6.0, 25, and 42 mg Ni/kg/day. At the 500 ppm dose level there was a statistically significant decrease in the Po maternal body weight, along with absolute and relative liver weights; similar effects were observed in males. Thus, 250 ppm (25 mg Ni/kg/day) was a NOAEL for Po breeders. Histopathology was performed for liver, kidney, lungs, heart, pituitary, adrenals and reproductive organs to make this assessment. This maternal NOAEL is higher than the NOAEL derived based on body weight changes in the chronic Ambrose et al. (1976) and subchronic gavage (American Biogenics Corporation, 1988) assays.

In the Research Triangle Institute (1988) F1a generation at the 500 ppm dose level, the number of live pups/litter was significantly decreased, pup mortality was significantly increased, and average pup body weight was significantly decreased in comparison with controls. Although there was no statistically significant effect at 250 ppm, there was some indication of decreased live pups/litter. Similar effects were seen with F1b litters of Po dams exposed to 500 ppm nickel. In the 50 and 250 ppm dose groups, increased pup mortality and decreased live litter size was observed in the F1b litters. However, these effects seen with F1b litters are questionable because the room temperature tended to be 10EF higher than normal at certain times (gestation-postnatal days) along with much lower levels of humidity. As evidenced in the literature, temperatures that are 10EF above normal during fetal development cause adverse effects (Edwards, 1986). Therefore, the above results seen at 50 and 250 ppm cannot be considered to be genuine adverse effects. The study authors also noted that the results could not distinguish between direct effects of nickel on the pups and effects secondary to decreased maternal water consumption, and possibly dehydration, during gestation. A dose-related increase in deaths during delivery was also observed in both the parental females, and in F1 females in the second phase of the study.

F1b males and females of the Research Triangle Institute (1988) study were randomly mated (19-30/sex/group) on postnatal day 70 and their offspring (F2a and F2b) were evaluated through postnatal day 21. This phase included teratological evaluations of F2b fetuses. The average gestational nickel consumption of F1b dams was 0, 6.2, 23, and 42 mg Ni/kg/day. Evaluation of the data indicated that the 500 ppm dose caused significant body weight depression of both mothers and pups, and increased neonatal mortality during the postnatal development period. The intermediate dose, 250 ppm nickel, produced transient depression of maternal weight gain and water intake during gestation of the F2b litters. The 50 ppm nickel exposure caused a statistically significant increase in the incidence of fetuses with short ribs, to 11%. However, since this effect was not seen in both of the higher dose groups, the reported incidence of short ribs in the 50 ppm group is not considered to be biologically significant. Overall, this study supports the results of Smith et al. (1993) that nickel ingestion can cause increased neonatal mortality at doses below those causing maternal toxicity, but a reliable developmental NOAEL cannot be identified in this study.

A 3-generation reproduction study in Wistar rats was briefly described by Ambrose et al. (1976). Groups of 30 weanling rats per sex per group were fed 0, 250, 500, or 1000 ppm nickel as nickel sulfate hexahydrate for 11 weeks (0, 20, 40, and 80 mg Ni/kg/day). Twenty females/group were mated individually with males from the same group for up to three successive 7-day rotations. The number of mated animals, number of pregnancies, alive and dead litters, pups in litter at 1, 5, and 21 days, and total litter weight at weaning was recorded. The F1a pups were sacrificed and necropsied at weaning, and the parental (Po) rats were remated to produce the F1b generation. Mating of the F1b and F2b generations was as for the parental generation (17-20 rats mated/group). A complete histopathology examination was conducted on F3b weanlings (10/sex/group). Body weights of the Fo rats were decreased only at the high dose, with an average decrease of 13% reported for males and 8% reported for females. The fertility index was only ~60% at 250 and 1000 ppm in the F1a generation, and at 1000 ppm in the F2b generation, but this effect was not nickel-related, since fertility was high at the high dose in all other generations. The total number of pups born dead was increased at all nickel doses in the F1a generation and at 500 ppm in the F1b generation, but there was no effect on pup mortality in later generations. The study authors stated that there was no evidence of teratogenicity, based on gross examinations, and no histopathologic effects on the F3b generation, but presented no supporting data. Evaluation of this study is complicated by the lack of statistical analyses and the reporting of results using pups, rather than litters, as the unit of analysis. There was, however, a clear and consistent decrease in live pups/litter on pnd 5 and in mean weanling body weight at the high dose in all generations, although a NOAEL or LOAEL could not be clearly defined.

Schroeder and Mitchener (1971) conducted a 3-generation study of rats administered nickel as an unspecified salt at 5 ppm in drinking water (estimated at 0.43 mg Ni/kg/day), and observed significantly increased neonatal mortality and incidence of runts. This study is

significantly limited, however, by the use of only 5 mated pairs/dose group. In addition, the matings were not randomized and the males were not rotated. This study was conducted in an environmentally controlled facility where rats had access to food and water containing minimal levels of essential trace metals. Because of the interactions of nickel with other trace metals (chromium was estimated as inadequate), the restricted exposure to trace metals may have contributed to the toxicity of nickel. Therefore, this study does not present a reliable estimate of nickel toxicity.

In a study validating the Chernoff-Kavlock short-term test, Seidenberg et al. (1986) found no effect on litter size or weight, or survival on postnatal days 1-3, among the pups of ICR-SIM mice administered nickel chloride (hydration state not reported) at 200 mg Ni/kg/day (90 mg Ni/kg/day) by gavage on gestation days 8-12. This dose did, however, result in a statistically significant decrease in maternal body weight gain.

Reproductive endpoints were also evaluated in the systemic toxicity studies discussed above. No effects on sperm morphology or motility, or on vaginal cytology, were observed in rats or mice exposed to concentrations up to 0.45 mg Ni/m^3 as nickel sulfate hexahydrate for 6 hours/day, 5 days/week for 13 weeks (Dunnick et al., 1989; NTP, 1996a). In addition, no histopathologic effects on reproductive tissue were observed in the chronic studies, with exposures at concentrations up to 0.11 mg Ni/m^3 (rats) or 0.22 mg Ni/m^3 (mice) for 6 hours/day, 5 days/week for 2 years. Degeneration of the germinal epithelium of the testes was observed only at the much higher concentration of 1.6 mg Ni/m^3 in male rats exposed for 6 hours/day for 12 days over a 16-day period (Benson et al., 1988).

In an evaluation of the potential effects of nickel on functional development, Gray et al. (1986) treated pregnant CD1 mice with nickel chloride (hydration state not reported) at 100 mg/kg/day (45 mg Ni/kg/day if as anhydrous nickel chloride, 25 mg Ni/kg/day if as nickel chloride hexahydrate) by gavage on gestation days 8-12. The dams were allowed to deliver, litters were culled, and locomotor activity was evaluated at postnatal days 22, 58, and 200. No effect was observed, although this assay was able to detect effects of other chemicals.

In the only study located that evaluated developmental or reproductive effects of inhaling any nickel compound, Weischer et al. (1980) exposed groups of 10-13 pregnant Wistar rats continuously to NiO at 0.8, 1.6, or 3.2 mg/m^3 NiO (0.6, 1.2, or 2.5 mg Ni/m^3) for 21 days, beginning on gestation day 1. Maternal endpoints evaluated were body weight, organ weights, serum urea, and hematology. The only fetal endpoints evaluated were fetal weight, leukocytes, and serum urea. Maternal body weight gain was statistically significantly reduced in all exposed groups, and statistically significant decreases in fetal body weight were observed at the top two exposure levels. Fetal weight was significantly decreased at the mid- and high-concentration level. Other developmental effects, such as fetal survival, were apparently not evaluated.

Overall, these data indicate that ingested nickel can cause increased neonatal deaths at relatively low doses, but no reliable NOAEL for this endpoint has been identified. An equivocal LOAEL of 1.33 mg Ni/kg/day was observed in a 2-generation study with Long-Evans rats administered nickel chloride in drinking water (Smith et al., 1993). Increased neonatal mortality at doses below those causing maternal toxicity was also observed by Research Triangle Institute (1988) in a 2-generation study of nickel chloride in drinking water with CD rats. However, a reliable developmental NOAEL could not be identified for this study. The Research Triangle Institute (1988) study was also the only oral study that included teratological evaluations; no nickel-related effects were observed. In a 3-generation study of Wistar rats administered nickel sulfate in feed, a clear and consistent decrease in live pups/litter was observed on postnatal day (pnd) 5 and in mean weanling body weight at the high dose in all generations, but a NOAEL or LOAEL could not be clearly defined (Ambrose et al., 1976). Increased neonatal mortality was also observed by Schroeder and Mitchener (1971) in a 3-generation drinking water study in rats, but a reliable LOAEL could not be identified in this study, due to methodological inadequacies. No effect on sperm morphology or vaginal cytology, and no histopathology of reproductive tissue, was observed in the NTP (1996a) subchronic and chronic inhalation studies. In the only inhalation study evaluating reproductive function or developmental effects, Weischer et al. (1980) found decreased fetal weight at concentrations that also caused decreased maternal body weight gain. Neonatal survival was apparently not evaluated.

4.4 Other Studies

4.4.1 Parenteral and Initiation-Promotion Cancer Studies

Parenteral carcinogenicity studies have been conducted in which animals were exposed to soluble nickel compounds by intramuscular or intraperitoneal administration. As summarized below, intramuscular injection or implantation studies in rats consistently found that soluble nickel compounds were not tumorigenic at the site of administration. Other studies provide limited evidence that repeated intraperitoneal injections of nickel chloride, sulfate and/or acetate induced tumors in the peritoneal cavity of rats and lungs of Strain A mice. Offspring of rats that were administered nickel acetate by intraperitoneal injection during pregnancy developed pituitary tumors, suggesting that soluble forms of nickel can act as transplacental carcinogens. Initiation-promotion studies indicate that intraperitoneal injection of nickel acetate can initiate development of kidney tumors in rats and their offspring, and provide suggestive evidence that intraperitoneal injection of nickel chloride or sulfate can promote development of nasopharyngeal tumors in rats.

4.4.1.2 Intramuscular Administration

Groups of 32 Wistar rats (age 2-3 months, sex not reported) were given a single 5 mg injection of nickel sulfate hexahydrate or a single 20 mg injection of nickel subsulfide or nickel oxide as suspensions in one or both thigh muscles (Gilman, 1962). No control group was included in the study. Pathology examinations evaluated both local tumors and metastases. No injection-site or other treatment-related tumors were found in the nickel sulfate hexahydrate-treated rats (54 total injection sites) after #603 days of observation. Examination of the nickel subsulfide and nickel oxide-treated animals showed that local tumors were induced in 25/28 rats (36 total tumors/45 total injection sites) observed for #365 days and 21/32 rats (26 total tumors/64 total injection sites) observed for #595 days, respectively.

A study that compared the solubility and injection-site tumorigenic activity of nickel sulfate and five other nickel compounds in Fischer rats was incompletely reported by Gilman (1966). Doses, treatment schedule, animal numbers, and gender were not specified by the investigator, although it appears that the animals were observed for six months following treatment and IARC (1990) noted that 20 rats/group were tested. No control group was included in the study. Nickel sulfate did not induce any tumors, although tumors were observed with slightly soluble nickel fluoride, and more so with less soluble nickel hydroxide and insoluble nickel oxide and nickel subsulfide. As summarized in Table 23, tumorigenicity generally increased with decreasing compound solubility.

Similar results were reported for groups of 35 NIH Black rats (sex unspecified) administered intramuscular implants of sheep fat pellets containing 0 or 7 mg of various nickel compounds (Payne, 1964; abstract only). Treatment was reported to have been repeated three times (interval unspecified), suggesting that each animal received three implants. The animals were observed for up to 18 months following treatment. There was no evidence of treatment-related tumorigenicity for most of the soluble nickel compounds tested in this study. No local tumors were found in rats treated with nickel chloride, anhydrous nickel acetate, nickel ammonium sulfate, or nickel (III) oxide. Implantation-site sarcomas developed in 1/35, 1/35, 6/35, 4/35, and 12/35 rats treated with nickel sulfate, nickel acetate, nickel carbonate, nickel (II) oxide, and nickel subsulfide, respectively, compared to 0/35 animals in the sheep-fat control group. These latter results should not be over interpreted, since they are from an abstract only.

Groups of 20 male Wistar rats were given an intramuscular injection of 4.4 Fmol (0.26 mg) nickel as anhydrous nickel sulfate every alternate day for a total of 15 injections (3.9 mg Ni/rat total) (Kasprzak et al., 1983). Other groups of 20 rats were given a single 120 Fmol (7 mg) intramuscular injection of nickel as aqueous suspensions of nickel (II) hydroxides (freshly precipitated colloidal gel, air-dried colloidal gel or crystallized) or nickel subsulfide. The nickel subsulfide-treated rats served as a positive control group. An untreated control group was comprised of 20 rats that were administered 15 injections of sodium sulfate on alternate days. Examinations performed 24 months after the start of the experiment showed no injection site

tumors in the nickel sulfate-treated, colloidal nickel hydroxide-treated, or untreated control rats. Local sarcomas were induced in the air-dried colloidal nickel hydroxide, crystallized nickel hydroxide, and nickel subsulfide groups at incidences of 4/19, 3/20, and 15/20, respectively.

Sunderman (1984) investigated the tumorigenicity of 18 nickel compounds in male Fischer rats using standardized experimental conditions and an equivalent intramuscular dose (14 mg Ni). The only soluble nickel compound in the study was nickel chromate, which induced one local sarcoma in 16 rats that were observed for up to two years following intramuscular injection. The tumor incidence (6%) and survival rate (63%) in the nickel chromate-treated rats were not significantly different from vehicle control values. The water-insoluble nickel compounds induced much higher (50-100%) incidences of local sarcomas (e.g., 65, 93, and 100% for nickel metal, nickel oxide, and nickel subsulfide, respectively).

In a study reported as an abstract, Kasprzak (1994) compared the carcinogenic potentials of two forms of the same nickel (II) salt that differ in *in vivo* solubility: nickel sulfate hexahydrate, which is readily soluble in serum (minutes), and anhydrous nickel sulfate, which is very slowly soluble in serum (days). Groups of 30 rats were given a single intramuscular injection of 7.5 or 15 Fmol of $NiSO_4$ hexahydrate or 7.5, 15, 30, or 60 Fmol anhydrous $NiSO_4$ powder (particles $10 Fm), dissolved or suspended in 50% glycerol, in the thigh muscles of both hind limbs. Control rats were similarly treated with 50% glycerol. The dose range of the hexahydrate was limited by acute toxicity. No injection site tumors developed in any of the treated rats and incidences of tumors at other sites were similar in treated and control groups.

4.4.1.3 Intraperitoneal Administration

In contrast to the generally negative results with soluble nickel salts in intramuscular studies, weakly positive results have been seen in a number of intraperitoneal studies.

In an intraperitoneal study with Wistar rats, groups of 18-week-old females were injected once or twice weekly with nickel in various forms, including four soluble salts (Pott et al., 1989; 1990, as cited by IARC, 1990). The animals were sacrificed 30 months after the first injection for assessment of local (non-uterus abdominal) tumor induction. As summarized in Table 24, induction of low incidences of abdominal mesotheliomas and sarcomas was associated with exposure to nickel chloride, nickel sulfate, or nickel acetate.

Two studies tested the lung tumorigenic activity of soluble nickel compounds in Strain A mouse carcinogen screening bioassays (Stoner et al., 1976; Poirier et al., 1984). In the Stoner et al. (1976) study, groups of 10 male and 10 female 6- to 8-week-old mice were intraperitoneally injected with nickel (II) acetate in 0.85% physiological saline three times a

week for 8 weeks at doses of 0, 3, 7.5, and 15 mg compound/dose, yielding total nickel acetate doses of 0, 72, 180, and 360 mg/kg, respectively. These doses correspond to 0, 24, 60, and 120 mg Ni/kg, if the doses were reported in terms of anhydrous nickel acetate, or 0, 17, 42, and 85 mg Ni/kg under the more likely conditions of the doses being reported as nickel acetate tetrahydrate. The total doses represented the maximum tolerated dose (MTD) and 1:2 and 1:5 dilutions of the MTD. Lung tumors (adenomas) were counted in all survivors 30 weeks after the first injection. As summarized in Table 25, the incidence and average number of lung tumors per mouse were significantly increased in the high-dose group compared to the vehicle controls.

In the Poirier et al. (1984) Strain A mouse assay, groups of 30 male and 30 female 6- to 8-week-old mice were injected with 0 or 10.7 mg/kg (MTD) doses of nickel acetate tetrahydrate (2.5 mg Ni/kg) in 0.9% saline three times a week for 8 weeks (total dose 256.8 mg compound/kg, or 61 mg Ni/kg). Examinations 30 weeks after the first injection showed that the mean number of lung adenomas/mouse was significantly higher in 24 treated survivors than in 25 surviving vehicle controls (1.50 ± 0.46 S.E compared to 0.32 ± 0.12, $p<0.05$), but substantially less than the urethane positive control value of 21.6+/- 2.8. Interestingly, co-injection of magnesium acetate with the nickel acetate significantly reduced the number of lung adenomas/mouse to control levels. Tumor incidences were not reported.

The Stoner et al. (1976) and Poirier et al. (1984) bioassays provide a slight indication that nickel acetate was tumorigenic in Strain A mice. However, these findings are insufficient for establishing carcinogenicity of the compound. This bioassay system is a well-validated carcinogen screening test based on a mouse strain that has a high spontaneous lung tumor rate and is particularly sensitive to development of chemically-induced lung tumors. Chemicals that induce a positive response in this test, however, are not necessarily carcinogenic in chronic assays using inhalation and other natural routes of exposure (Shimkin and Stoner, 1975).

4.4.1.4 Initiation-Promotion Studies

A two-stage carcinogenesis study was performed in which nickel acetate was tested as a tumor initiator in male F344 rats using sodium barbital as the promoter (Kasprzak et al., 1990). Two groups of 24 rats were given a single intraperitoneal injection of 90 Fmol/kg of nickel acetate tetrahydrate (5.3 mg Ni/kg) in water, and two groups of 24 rats were given a single intraperitoneal injection of saline. One of the nickel-acetate treated groups and one of the saline-treated groups were subsequently (two weeks later) exposed to drinking water containing 500 ppm of sodium barbital. Rats were observed for up to 96 weeks following the intraperitoneal injections. As summarized in Table 26, incidences of renal cortical adenomas alone and combined adenomas and adenocarcinomas were significantly increased in the initiated/promoted rats compared to those administered nickel without subsequent sodium barbital promotion. The adenocarcinomas were found to have metastasized to the lung, liver

and/or spleen. Most of the initiated/promoted rats developed multiple renal tumors whereas none were found in the nickel-only and saline control groups. Sodium barbital by itself increased the adenoma response ~6-fold higher than nickel alone. No nickel-related tumors were found in the thyroid, prostate, or testes.

A transplacental carcinogenicity study was performed in which an unspecified number of pregnant F344/NCr rats were administered nickel acetate by intraperitoneal injection in a single 90 Fmol/kg (5.3 mg Ni/kg) dose on gestation day 17 (Group 1), 45 Fmol/kg (2.6 mg Ni/kg) doses on gestation days 16 and 18 (Group 2), or 45 Fmol/kg (2.6 mg Ni/kg) doses on gestation days 12, 14, 16, and 18 (Group 3) (Diwan et al., 1992). Control rats were treated with sodium acetate on day 18 of gestation (Group 4). Offspring from each litter that was treated with one or two doses of nickel acetate (Groups 1 and 2) or sodium acetate (Group 4) were distributed into two subgroups which received drinking water that was untreated (offspring in Subgroups 1A, 2A, and 4A), or contained 500 ppm sodium barbital (Subgroups 1B, 2B, and 4B) from 4 weeks of age until the experiment was concluded at 85 weeks of age. Group 3 was deleted from the study due to high perinatal toxicity (all offspring died within 72 hours of birth). Necropsies were performed on remaining offspring that included comprehensive gross examinations and histological examination of kidneys, liver, lungs, spleen, thyroid, pituitary, prostate, and testes. As summarized in Table 26, the incidences of kidney tumors (adenomas or carcinomas of the renal cortex and pelvis) were significantly higher in male offspring that were treated with nickel acetate prenatally and sodium barbital postnatally (Subgroups 1B and 2B) than in control males initiated with sodium acetate (Subgroup 4B) or given prenatal sodium acetate only (Subgroup 4A). No kidney tumors developed in males given prenatal nickel acetate only (Subgroups 1A and 2A) or in any of the female rat groups. There was no indication that the gender-related induction of kidney tumors (males only) was associated with "$_{2\mu}$-globulin nephropathy because careful histologic scrutiny of the kidneys showed no lesions or hyaline droplets compatible with the "$_{2\mu}$-globulin syndrome. Pituitary tumors (combined adenomas and carcinomas) were significantly increased in offspring of both males and females that were exposed to transplacental nickel acetate only (Subgroups 1A and 2A) compared to controls that were transplacentally-exposed to sodium acetate only (Subgroup 4A). Postnatal administration of sodium barbital had no influence on the development of the pituitary tumors. The pituitary tumors in the offspring initiated with nickel acetate had shorter latency periods than those induced by sodium acetate only (mean ~58-74 weeks in Groups 1 and 2, compared to 81-85 weeks in Group 4), and were mixed adenomas and carcinomas, unlike those in the offspring exposed to sodium acetate, which were only adenomas. The results of this study indicate that nickel acetate was a transplacental initiator of kidney tumors and a complete transplacental carcinogen for pituitary tumors.

The tumor-promoting effect of nickel chloride on renal carcinogenesis by N-ethyl-N-hydroxyethylnitrosamine (EHEN) was tested in rats (Kurokawa et al., 1985). One group of 15 male Fischer 344 rats was provided drinking water containing 500 ppm EHEN initiator for two

weeks followed by 600 ppm nickel chloride hexahydrate in the water for 25 weeks. A second group of 15 males received 500 ppm EHEN in drinking water for two weeks followed by untreated water for 25 weeks. Additional groups of 15 males received untreated water for two weeks followed by 600 ppm nickel chloride hexahydrate for 25 weeks, or untreated drinking water for 27 weeks. Mean daily intake of nickel was ~10 mg Ni/kg. Histological examinations of the kidneys at the end of 27 weeks showed that the incidence of renal cell tumors in the group receiving EHEN followed by nickel chloride (8/15) was significantly higher ($p<0.05$) than in the controls given EHEN alone (2/15), nickel chloride alone (0/15), or water alone (0/15). There were no intergroup differences in incidences of liver neoplastic lesions. The authors concluded that nickel chloride exerted a tumor promotion effect on the kidney.

Nickel chloride showed no promoting activity in livers of Fischer 344 rats after initiation with *N*-nitrosodiethylamine, in gastric tissue of Wistar rats after initiation with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, in the pancreas of Syrian golden hamsters after initiation with *N*-nitrosobis(2-oxypropyl)amine, or in the skin of SENCAR mice initiated with 7,12-dimethylbenz[*a*]anthracene (Hayashi et al., 1984, as cited by IARC, 1990,). The method of exposure and other additional data on this study were not available for review.

The tumor-promoting activity of nickel sulfate for nasopharyngeal carcinogenesis has been tested in rats initiated with dinitrosopiperazine (DNP) (Ou et al., 1980, 1983; Liu et al., 1983), as summarized below. Small numbers of animals were tested in these studies; however, the results suggest that nickel sulfate may have had some tumor-promoting effect.

One of the studies (Ou et al., 1980) administered a single subthreshold injection of DNP (9 mg) to groups of 12 rats, followed either six days later by exposure to nickel sulfate in the drinking water for six weeks (3.7 mg Ni/day), or one day later by weekly local applications of nickel sulfate in gelatin solution in the nasopharynx for seven weeks (37 Fg Ni/week). Control groups of 12 rats received DNP alone, nickel sulfate alone by either route, or both vehicles alone. Two nasopharyngeal tumors developed in both groups of 12 rats that received DNP in combination with nickel sulfate (one carcinoma and one fibrosarcoma in the oral promotion group, one papilloma and one carcinoma in the nasopharynx application group), whereas no tumors occurred in any of the five control groups. In a follow-up study, 5/22 rats given an initiating injection of DNP (dose not reported) developed carcinomas (two in the nasopharynx, two in the nasal cavity, one in the hard palate) following oral administration of nickel sulfate in gelatin (Liu et al., 1983). No tumors developed in unspecified numbers of rats treated with DNP followed by aqueous nickel sulfate, nickel sulfate in gelatin alone, or DNP alone.

In the Ou et al. (1983) study, a group of 13 female rats was administered a single subcutaneous injection of DNP (9 mg) on day 18 of gestation. One-month-old pups of the treated dams were fed nickel sulfate (0.05 ml of a 0.05% solution) daily for one month. The

dose of nickel sulfate was subsequently increased by 0.1 ml per month for the next five months. Nasal carcinomas developed in 5/21 pups. The incidence of tumors in a comparison group of untreated pups of DNP-initiated dams was 3/11, but tumor types were different (one nasopharyngeal squamous-cell carcinoma, one neurofibrosarcoma of the peritoneal cavity, one granulosa-thecal-cell carcinoma of the ovary). No tumors developed in groups of seven pups that were treated with nickel sulfate, groups of seven untreated pups, or in any of the dams.

4.4.2 Genotoxicity Studies

Results of representative genotoxicity studies of water soluble nickel compounds are summarized below. Most of these studies tested the activity of nickel as nickel chloride, nickel sulfate, and/or nickel acetate. Evidence for genotoxicity is mixed, although water soluble nickel compounds have been generally consistent in inducing effects in certain kinds of mammalian assays, particularly mutagenic responses and DNA damage *in vitro*, chromosomal effects including aberrations and sister-chromatid exchanges *in vitro* and *in vivo*, and carcinogenic transformation of mammalian cells *in vitro*. Responses in many of these assays were weak and occurred at toxic doses. The genotoxicity of nickel compounds has been reviewed by numerous authors, including Sunderman (1989), Coogan et al. (1989), IARC (1990), Snow (1992), NTP (1996a), and Oller et al. (1997). This discussion is largely based on those previous reviews.

In vitro assays have demonstrated that soluble nickel compounds are almost always non-mutagenic in bacteria and other prokaryotes. Nickel chloride, nickel sulfate, and nickel nitrate did not induce gene mutations in *Salmonella typhimurium* and *Escherichia coli*, even at toxic doses (Arlauskas et al., 1985; Marzin and Phi, 1985; Biggart and Costa, 1986; Wong, 1988). Nickel sulfate was also not mutagenic in the yeast *Saccharyomyces cerevisiae*, although it was weakly positive for gene conversion (Singh, 1984). In contrast to *in vitro* findings in microorganisms, soluble nickel is weakly mutagenic in cultured mammalian cells. Mutagenicity of nickel chloride and nickel sulfate has been expressed in certain mammalian systems, especially assays with mouse L5178Y lymphoma cells (*tk* locus), Chinese hamster V79 (*hgprt* locus) cells, and Chinese hamster G10 and G12 cells (*gpt* bacterial gene integrated into a *hprt* Chinese hamster V79 cell line), although the mutagenicity was often much lower than for insoluble forms of nickel (Miyaki et al, 1979; Amacher and Paillet, 1980; Swierenga and McLean, 1985; McGregor et al., 1988; Hartwig and Beyersmann, 1989; Morita et al., 1991; Kargacin et al., 1993; Lee et al., 1993). *In vivo* tests with *Drosophila* found that nickel chloride was a weak inducer of mutations for wing spots but not eye-color (Rasmuson, 1985; Ogawa et al., 1994), and that nickel sulfate induced sex-linked recessive mutations and sex chromosome loss in male germ cells (Rodriguez-Arnaiz and Ramos, 1986). Intraperitoneal injection of nickel chloride or nitrate did not induce dominant lethal mutations in male mice (DeKnudt and Leonard, 1982).

Soluble nickel compounds can cause DNA and chromosome damage. DNA damage induced by nickel chloride and sulfate *in vitro* included strand breaks and nickel-DNA-protein crosslinks in cultured human and rat gastric mucosal cells, Chinese hamster ovary cell, human osteosarcoma cells, and rat hepatocytes, although no strand breaks were detected in human fibroblasts (Fornace, 1982; Robison et al., 1982; Robison and Costa, 1982; Swierenga and McLean, 1985; Patierno and Costa, 1985; Patierno et al., 1985, 1987; Hamilton-Koch et al., 1986; Conway et al., 1987; Coogan et al., 1989; Pool-Zobel et al., 1994). Other DNA effects of soluble nickel salts observed in mammalian cells *in vitro* and/or *in vivo* include inhibition of DNA replication and transcription, DNA depurination, and structural alteration of DNA from the normal right-handed B-helix to the left-handed Z-helix conformation (Sunderman, 1989; Coogan et al., 1989).

Soluble nickel salts produced chromosomal effects in mammalian cells both *in vitro* or *in vivo*, with damage preferentially occurring in the heterochromatic regions of the chromatin. Nickel chloride and nickel sulfate induced chromosomal aberrations in Chinese hamster ovary and embryo cells, mouse mammary carcinoma cells, and human peripheral lymphocytes *in vitro* (Nishimura and Umeda, 1979; Larramendy et al., 1981; Sen and Costa, 1985, 1986; Conway et al., 1987; Conway and Costa, 1989; Lin et al., 1991; Howard et al., 1991). *In vivo* (intraperitoneal) studies showed induction of chromosomal aberrations in mouse and hamster bone marrow cells by nickel chloride (Chorvatovicova, 1983; Mohanty, 1987), although not in rat bone marrow or spermatogonial cells by nickel sulfate (Mathur et al., 1978). Nickel chloride and nickel sulfate also induced sister-chromatid exchanges in Chinese hamster cells and human peripheral lymphocytes *in vitro* (Larramendy et al., 1981; Ohno et al., 1982; Newman et al., 1982; Conway et al., 1987; Montaldi et al., 1987). Other clastogenic effects of soluble nickel salts include *in vivo* induction of micronuclei in polychromatic erythrocytes (DeKnudt and Leonard, 1982) and sperm head abnormalities (Sobti and Gill, 1989) in mice.

Soluble nickel compounds can cause morphological transformation of mammalian cells *in vitro*. Nickel chloride and nickel sulfate consistently induced transformations in hamster embryo cell cultures (DiPaolo and Casto, 1979; Costa and Heck, 1982; Costa et al., 1982; Conway et al., 1987; Conway and Costa, 1989). Soluble nickel salts also induced transformations in hamster kidney cells and BALB/3T3 mouse clone cells (Hansen and Stern, 1984; Little et al., 1988), although not in mouse embryo or human bronchial epithelial cells (Lechner et al., 1984; Miura et al., 1989). Additionally, there is evidence that nickel chloride and nickel sulfate can inhibit cell-to-cell communication via gap junctions in Chinese hamster V79 and other mammalian cell lines (Loch-Caruso et al., 1986; Miki et al., 1987).

Several *in vitro* studies found that nickel chloride, nickel sulfate, and other soluble nickel salts had synergistic effects with known genotoxic agents, including 9-aminoacridine on mutagenesis in *Salmonella*, UV light on mutagenesis in Chinese hamster V79 cells, and

benzo(a)pyrene on morphological transformation in Syrian hamster embryo cells (Sunderman, 1989; Coogan et al., 1989).

4.4.3 Other Mechanistic Studies

Because chromosome aberrations and other genotoxic effects are observed following exposure to soluble nickel salts, but the nickel ion interacting with isolated DNA does not form premutagenic DNA lesions, a number of studies have investigated the mechanism by which nickel can form mutations and chromosome aberrations.

Several studies have suggested that one way that nickel produces mutations is via the production of reactive oxygen species. However, insufficient dose-response or tissue-specific data are available to demonstrate this mode of action as being responsible for carcinogenesis of any nickel species. The incubation of DNA with hydrogen peroxide and nickel chloride (but not either chemical alone) caused DNA strand breaks, a reaction that was inhibited by singlet oxygen scavengers (Kawanishi et al., 1989). The authors suggested that nickel bound to DNA and reacted with hydrogen peroxide, forming reactive oxygen species. Indirect measurements of the oxidation level in cells also showed an increase following nickel exposure (Costa et al., 1994). The induction of oxidative DNA base modifications was observed in HeLa cells exposed to nickel chloride, although this lesion was restricted to doses causing at least 50% cytotoxicity. Nickel ion inhibition of the repair of glycosylase-sensitive DNA damage induced by visible light was also observed, at much lower concentrations (Dally and Hartwig, 1997). A role of reactive oxygen species in the nickel inhibition of DNA repair has also been suggested (Lynn et al., 1997). However, as noted, the data are insufficient to definitively implicate this mode of action in nickel carcinogenesis, particularly in light of the endogenous background levels of reactive oxygen species, and the body's ability to protect itself from the resulting damage.

A number of studies, primarily on insoluble nickel, have implicated nickel interactions with heterochromatin in the induction of carcinogenesis by these compounds. Heterochromatin is DNA that remains highly condensed beyond mitosis and is transcriptionally inactive (i.e., not expressed genetically). Due to this lack of expression, heterochromatin has generally not been thought to be important in the development of carcinogenesis, but several studies have indicated a specific, direct interaction between nickel and heterochromatin.

Heterochromatin is localized next to the nuclear membrane (Alberts et al., 1994), and thus is more accessible than euchromatin (which is more centrally located in the nucleus) to nickel entering the nucleus. Interaction between nickel and heterochromatin is also favored by the high protein content of heterochromatin and the fact that the affinity of nickel for amino acids is several orders of magnitude higher than the affinity for DNA (Costa et al, 1994). In Chinese hamster embryo cells transformed with soluble (nickel chloride) or insoluble (crystalline

nickel sulfide) forms of nickel, there was a high frequency of chromosome deletions in the long arm (q) of the X chromosome, particularly in the male cultures (Conway and Costa, 1989). Although ionic nickel alone resembled insoluble nickel forms in preferentially targeting chromosome aberrations to heterochromatin, the incidence of aberrations was much lower with soluble nickel (Sen and Costa, 1986). Similarly, the long arm of the X chromosome was fragmented by exposure to nickel sulfide or liposome-encapsulated soluble nickel chloride, but not nickel chloride alone (Sen and Costa, 1985). This part of the X chromosome is largely heterochromatic in Chinese hamster cells. Restoration of an intact X chromosome into the male cells with deletions of portions of the X chromosome resulted in senescence of the previously transformed cells. This senescence was reversed by demethylating the X chromosome (Klein et al., 1991). Increased DNA methylation can inactivate DNA transcription, leading to the formation of facultative heterochromatin. Heterochromatic proteins also enhanced the formation of the oxidized base 7,8-dihydro-8-oxo-2'-deoxyguanosine by nickel chloride and hydrogen peroxide, while euchromatin inhibited the oxidized base formation (Huang et al., 1995).

A hypothesis has been proposed that unifies the oxidative heterochromatin-related mechanisms (Costa et al., 1994; Klein and Costa, 1997). According to this hypothesis, there may be a population of nickel- and magnesium-binding proteins in heterochromatin. Binding of nickel inhibits magnesium binding to these proteins, and results in the generation of oxygen radicals that damage the DNA in heterochromatin. DNA oxidation can also result in altered DNA methylation patterns. In a related model (Huang et al., 1995), nickel interaction with heterochromatin has been observed to enhance chromatin condensation (Lee et al., 1995, as cited in Huang et al., 1995). In this situation, nickel transformation is hypothesized to involve the silencing of a putative X-linked senescence gene by enhancing DNA methylation (Klein and Costa, 1997). If the aberrantly condensed DNA contains genes essential for regulation of normal cell function, as described by Klein et al. (1991), these changes can be a precursor to carcinogenesis.

Thus, several biologically plausible mechanisms for the production of DNA damage by exposure to soluble nickel have been proposed. Several of these models involve some sort of indirect interaction with DNA, suggesting a nonlinear dose-response curve. However, the available data are insufficient to determine the doses at which such nonlinearities occur. Nonetheless, the suggestion of such nonlinearities, together with the lower cellular uptake and nuclear delivery of soluble nickel species (compared with nickel subsulfide), are consistent with the negative animal carcinogenicity studies for soluble nickel.

4.5 Synthesis and Evaluation of Major Noncancer Effects and Mode of Action – Oral and Inhalation

The most sensitive effect of oral exposure to nickel was decreased glomerular function in rats exposed via drinking water for 6 months (Vyskocil et al., 1994b). Increased levels of albumin in urine was observed female rats exposed for 6 months; the increase observed in males was not statistically significant. No effect was seen at the 3-month sacrifice. No statistically significant effects on $2m or on total protein were observed. Although this was the only study that reported kidney effects at such low doses, most chronic studies (e.g., American Biogenics Corporation, 1988; NTP, 1996a) included kidney histopathology analysis, but did not evaluate sensitive measures of kidney function. The chronic rat feeding study of Ambrose et al. (1976) included only semiquantitative tests for reducing substances and protein. Because these studies did not include sensitive measures of kidney function, it is possible that minor effects could have been missed.

Both animal and human studies provide weak support for the kidney as a target organ of nickel toxicity. Dieter et al. (1988) reported mild tubular nephrosis in B6C3F1 mice treated with 108 or 150 mg Ni/kg/day as nickel sulfate in drinking water; no effect was seen at 44 mg Ni/kg/day (no effect at 25 mg Ni/kg/day if the doses were reported as nickel sulfate hexahydrate instead of anhydrous nickel sulfate). It is possible, however, that the nephrosis was related to decreased water consumption, rather than nickel exposure. Among workers who accidentally drank water contaminated with nickel sulfate, nickel chloride, and boric acid, a transient increase in urine albumin was observed in 3/21 exposed workers (Sunderman et al., 1988). It is unclear whether the boric acid would have contributed to the kidney effect. This study suggests that a high bolus dose of nickel can lead to glomerular effects, although it is not clear whether similar effects would be seen at lower doses. Among nickel refinery workers, urinary $2m levels correlated with nickel levels in urine (a measure of exposure) (Sunderman and Horack, 1981). Urinary $2m levels were not elevated in electroplating workers, but urinary nickel levels were also lower. Vyskocil et al. (1994a) found statistically significant effects on markers of tubular function (urinary NAG levels in both sexes, and $2m and RBP in females) in workers exposed to soluble nickel compounds at a chemical plant. Correlations between levels of these proteins and urinary nickel levels were also observed. Urinary albumin was increased in both males and females, but the increase was not statistically significant. It is unclear why tubular damage was reported in most of these studies, while Vyskocil et al. (1994b) reported glomerular damage. It should also be noted that the studies with positive results were based on spot samples, which can lead to false positives or false negatives in comparison with 24-hour samples. Indeed, Sanford and Nieboer (1992) found elevated levels of $_2m in spot urine samples from two subjects, but the total amount in the 24-hour void from these subjects was normal.

The limited available data also indicate that the kidney is a biologically plausible target organ for oral nickel toxicity. As described in Section 3.2, several studies have found that, following gavage, drinking water, or feed administration of soluble nickel, the highest tissue concentrations are in the kidney (Ishimatsu et al., 1995; Jasim and Tjalve, 1986; Borg and

Tjalve, 1989; Whanger 1973; Dieter et al., 1988; Ambrose et al., 1976). Nickel binding to the glomerular basement membrane has been observed, accompanied by a reduction of net charge of ~44% (Templeton, 1987). Moreover, biosynthesis of glomerular basement membrane, requiring the incorporation of a heparin sulfate proteoglycan, was inhibited by nickel chloride at 10 FM. Thus, at least some of the toxic effects of nickel in the kidney appear to be related to surface phenomena. Other kidney effects of nickel may be related to lipid peroxidation, which can result in destruction of membrane lipids by free-radical reactions (Sunderman et al., 1985; Hausinger, 1993a).

Other oral studies investigating systemic effects of nickel primarily reported nonspecific indicators of toxicity, such as decreased body weight (Ambrose et al., 1976; American Biogenics Corporation, 1988). The mechanism for this effect is not known, but it is conceivable that nickel may interfere with general bodily functions by replacing other metals in metalloenzymes. It is also possible that high nickel doses may interfere with the transport of trace element nutrients, such as magnesium.

Several oral studies have reported evidence of increased neonatal mortality or decreased litter size at doses below those resulting in maternal toxicity (Smith et al., 1993; Research Triangle Institute, 1988; Ambrose et al., 1976; Schroeder and Mitchener, 1971), but all of these studies are limited by design, conduct, or reporting problems. The mechanism of effects on progeny is not known, although nickel can cross the placenta (Jasim and Tjalve, 1986; Schroeder et al., 1964). An effect of nickel on placental function remains a possibility. It has also been proposed that the reproductive effects of nickel (neonatal mortality and maternal deaths during delivery) may be due to effects of nickel on prolactin, rather than due to direct reproductive effects of nickel. (Prolactin is a hormone that causes the initiation and maintenance of lactation, and maintains the corpus luteum in rodents.) Nickel has been found to inhibit the release of prolactin from the pituitary *in vitro* at doses that had no effect or stimulated the levels of other hormones (LaBella et al., 1973). The authors also found that nickel may be part of a prolactin inhibiting factor in hypothalamus extracts. This hormonal effect of nickel may explain the lack of a clear dose-response seen multiple reproductive studies.

Although kidney effects have been observed following inhalation exposure to nickel, the primary target of inhalation exposure to soluble or insoluble nickel is the respiratory tract. Effects have been observed at all levels of the respiratory tract, including the nose, tracheobronchial region, and pulmonary region (NTP, 1996a; Dunnick et al., 1989). These effects were attributed to the direct action of nickel, rather than to systemic exposure. Chronic active inflammation of the lungs was a primary effect, with inflammation-related lesions ranging from alveolar macrophage accumulation to fibrosis. On the molecular level, exposure of rats or mice to soluble nickel ($NiSO_4$) resulted in increases in lactate dehydrogenase and $-glucuronidase activities, and in total protein in bronchoalveolar lavage (BAL) fluid (Benson et

al., 1989). Lung effects of nickel may also be attributable to lipid peroxidation (Sunderman et al., 1985; Hausinger, 1993a).

Only limited data are available on noncancer effects in humans of inhalation exposure to nickel compounds. One study (Muir et al., 1993) evaluated x-rays of workers in a nickel sinter plant, and found only minimal effects. However, when the particle size distributions are taken into account, exposure was comparable to the NOAEL in the chronic NTP study.

Peroxidative damage to membrane lipids has been observed in the lung, liver, and kidney following subcutaneous injection of nickel chloride, suggesting that such damage may result in cell injury, and ultimately lung inflammation or kidney injury (Sunderman et al., 1985). Possible mechanisms for nickel-induced lipid peroxidation could be the displacement of ferrous ions by nickel from cellular proteins, nickel ion participation in radical-generating reactions similar to those catalyzed by iron, or nickel ion interference with the normal cellular machinery that protects the cell against endogenous peroxidative damage. Other aspects of nickel-related cell damage may be attributable to altered protein function related to direct or indirect interactions between nickel and metabolic enzymes or structural proteins (Sunderman et al., 1985).

4.6 Weight of Evidence Evaluation and Cancer Classification

4.6.1 Epidemiology Data

The epidemiologic observations of nickel workers provide substantial qualitative information, and information regarding relative levels of exposure. All of the nickel industry data are mixed exposures to soluble and insoluble nickel compounds, but data from work areas where one species or another predominates are informative. Co-exposure to insoluble nickel compounds is of concern because of the known carcinogenicity of this form of nickel. The epidemiologic observations are substantially consistent with the comparative carcinogenicity of the different species indicated by the animal data discussed below.

At Clydach, calciners had higher risks of both lung and nasal cancer than the hydrometallurgy workers. Calciners were exposed primarily to oxidic and sulfidic nickel, and had lower exposure to soluble nickel than hydrometallurgy workers. Exposure was highest for those calciners who worked prior to 1936, and this group had the highest risk. Cleaners, who had low exposure to soluble nickel but high exposures to oxidic and sulfidic nickel, had remarkably high lung cancer rates. Most informative are the Clydach data summarized in tables 33-38 of ICNCM (1990), and reproduced as Tables 10-14 of this document. Table 10 indicates that high exposure to soluble nickel has no effect on the lung cancer risk when both sulfidic and oxidic nickel (both insoluble forms) are low. When both sulfidic and oxidic nickel are high, high exposure to soluble nickel doubles the lung cancer risk relative to low soluble

nickel exposure (Table 10). High exposure to sulfidic nickel has a significant effect on lung cancer, increasing risk when exposure to oxidic and soluble nickel are low (see Tables 11, 12). High exposure to oxidic nickel increases risk primarily when exposure to soluble nickel is also high, which is presumed by ICNCM to indicate that soluble nickel acts synergistically with oxidic nickel. These effects are consistent with a carcinogenic effect of both oxidic and sulfidic nickel, with soluble nickel increasing the risk when insoluble nickel is present at high levels. For nasal cancers, as for lung cancers, high exposure to sulfidic nickel increases risk even when oxidic and soluble nickel are low (Table 13). High exposure to soluble nickel increases nasal cancer risk most strongly when both sulfidic and oxidic nickel are high (Table 14). Similarly, excess lung cancers were observed in Kristiansand electrolysis workers, but not in Port Colborne electrolysis workers. These two groups were exposed to similar levels of soluble nickel, but the exposures to insoluble nickel compounds were substantially higher for the Kristiansand workers. Again, this observation is consistent with the suggestion that soluble nickel enhances the effect of exposure to other nickel compounds.

Additional data from Kristiansand (Andersen et al., 1996) and Harjavalta (Antilla et al., 1998) update the ICNCM report. These data indicate increases in lung cancer and nasal cancer in workers exposed to soluble nickel. However, these increases in lung cancer were not clearly attributable to soluble nickel alone, either because most soluble nickel exposures occurred in conjunction with exposure to insoluble nickel, primarily nickel oxide, or because of potential confounding factors that could not be accounted for. These sources of confounding include smoking (presumed to be high in these workers), as well as concomitant exposures during some periods to potential lung carcinogens such as arsenic (in the case of Kristiansand) and sulfuric acid mists (in the case of Harjavalta). Nasal cancer increases occurred in both operations, but effects of prior occupational exposures to other forms of nickel in both cohorts cannot be ruled out. In the case of Kristiansand, no new nasal cancers have been seen in workers first employed since 1957 and careful examination of the data reveals a predominant association of the earlier nasal cancers with exposure to oxidic nickel. In the case of Harjavalta, concomitant exposures to sulfuric acid mist, and prior occupational work in carpentry in at least two of the observed nasal cancer cases, further confounds any conclusions that might be reached with respect to soluble nickel alone acting as the putative carcinogenic agent.

Overall, the epidemiology data suggest a contribution of soluble nickel to cancers observed in workers exposed to both soluble and insoluble nickel compounds. These data are consistent with soluble nickel acting to enhance the carcinogenicity of insoluble nickel compounds, or otherwise differing from insoluble forms in the mechanism of carcinogenicity. However, the epidemiology data are insufficient to determine whether soluble nickel alone is carcinogenic. If it is, however, the data indicate it would be a much weaker carcinogen than insoluble nickel species. The lack of sufficient quantitative exposure data, and confounding by smoking and other potential factors, preclude a more definitive conclusion regarding the carcinogenic potential of soluble nickel or its putative mode of action.

4.6.2 Animal Bioassays

Standard animal bioassays of soluble nickel compounds administered to rats or mice by the oral (Ambrose et al., 1976; Schroeder et al., 1964, 1974; Schroeder and Mitchener, 1975) or inhalation (NTP, 1996a) routes have not shown soluble nickel compounds to be carcinogenic. Of these studies, NTP (1996a) is considered to be the highest quality cancer bioassay, in that the study assayed all major organs and included chronic exposure concentrations at and below the maximum tolerated exposure (MTE) concentration for soluble nickel (nickel sulfate hexahydrate) in air. NTP (1996a) found no evidence of carcinogenicity of nickel sulfate hexahydrate in rats or mice, while similar studies of insoluble nickel compounds (nickel subsulfide and nickel oxide) provided evidence for carcinogenicity of these compounds in rats, and there was equivocal evidence for nickel oxide carcinogenicity in female mice (NTP 1996b, 1996c). There are, however, two interpretations to the negative mouse data for nickel sulfate. One interpretation is that the mouse bioassay constitutes a valid test in a second species (in addition to the rat), and that the negative result in the mouse study (together with the negative result in the rat bioassay) suggests that soluble nickel is not carcinogenic. A second interpretation is that, based on the negative and equivocal results for nickel subsulfide and nickel oxide, respectively, in mice, the mouse is not a suitable species for studying nickel carcinogenesis. The mouse is, however, considered an appropriate model for inhalation carcinogenesis of metals. Nonetheless, it is clear that the mouse data do not provide a basis for distinguishing between the effects of soluble and insoluble nickel compounds, whereas the data in rats do allow such a distinction. Concerns have also been raised about workers being subject to higher exposure levels than were used in the NTP study. However, the particle sizes under occupational conditions are much larger, resulting in much lower doses penetrating to the lung. The results after normalization by converting to human equivalent concentrations (HECs) varies with the occupational particle size distribution used, but the resulting occupational tissue doses are much closer to (but still higher than) the animal dose (Werner et al, 1999), or comparable to the animal doses (Yu et al., 1998).

Since the key data implicating soluble nickel in carcinogenesis come from epidemiology studies showing that co-exposure to soluble nickel increases the cancer risk from exposure to insoluble nickel, the question of interactions between soluble and insoluble forms of nickel is important. As described below, several mechanisms have been proposed that could explain such interactions. However, the animal bioassay data provide no insight about the potential carcinogenicity of mixed exposures to insoluble and soluble forms of nickel, since cancer bioassays of mixed exposures have not been reported.

Parenteral studies of nickel compounds of differing solubilities follow a similar pattern to that seen after experimental animal inhalation exposures. The parenteral data show some slight evidence of carcinogenicity of soluble nickel, although the carcinogenic activity is much lower than that of insoluble nickel. This is in contrast to the clear negative results for soluble nickel

compounds obtained via the inhalation route and in partially deficient studies via the oral route. Following intramuscular injection, the tumor response appears to be lower or absent for nickel compounds of higher solubility (Gilman, 1966; Table 22). Tumors at the site of intraperitoneal injection also show a similar trend. Four of five soluble nickel forms were weakly positive and one was negative, in contrast with metallic nickel, which is dramatically positive (Pott et al., 1989; 1990, Table 23). Intraperitoneal exposure of nickel acetate (a soluble nickel form) shows only a weak response in the mouse lung adenoma system, but far below the standard positive control of urethane (Stoner et al., 1976; Table 24). Nickel acetate also shows evidence of carcinogenicity in initiation-promotion studies and demonstrates transplacental initiation (Diwan et al., 1992; Kasprzak et al., 1990; Tables 25 and 26) and of promotion of carcinogenesis (Kurokawa et al., 1985; Liu et al., 1980, 1983; Ou et al., 1980). (Insoluble nickel forms were not testable in these latter systems.)

4.6.3 Mode of Action Considerations

Consideration of the results discussed in the previous two sections raises the question of whether soluble forms of nickel differ from insoluble nickel in carcinogenic *potential* (i.e., the qualitative description of carcinogenicity), or only in potency (i.e., the quantitative description of carcinogenicity, the size of the risk). Because the NTP bioassays had only one overlapping exposure level between the nickel subsulfide and nickel sulfate studies, the negative result in the nickel sulfate bioassay cannot be attributed specifically to differences in potential. However, a number of mechanistic studies address this question, and point to a difference in carcinogenic potential between soluble and insoluble forms of nickel.

An extensive body of evidence argues that crystalline nickel subsulfide readily enters cells and interacts with DNA, but soluble forms of nickel are much less effective at entering mammalian cells. Furthermore, the soluble nickel that does enter the cell is much more likely to interact with cytoplasmic constituents, causing toxicity, but not interacting with DNA.

Nickel subsulfide is readily taken up by phagocytosis (Costa and Mollenhauer, 1980; Costa et al., 1981; Abbracchio et al., 1982b; Heck and Costa, 1983). Nickel subsulfide particles are taken up in vacuoles that aggregate near the nucleus, where they interact with lysosomes (Costa et al., 1981). This interaction then leads to the release of nickel ions that mostly localized near the nucleus, and can interact with DNA.

By contrast, particles of soluble nickel readily dissolve when deposited on lung surfaces, and so do not remain in the particulate form long enough to be phagocytized. Instead, the nickel ions can enter the cell via either of two inefficient uptake mechanisms. Nickel can enter cells by passive diffusion, but the efficiency of this process decreases markedly when nickel is complexed with amino acids or protein, the normal form of nickel under physiological conditions (Nieboer et al., 1984a; Abbracchio et al., 1982). Nickel ions may also enter cells

via the magnesium transport system, but nickel is present in extracellular fluid at much lower concentrations than magnesium, and would be able to compete for uptake only at high nickel doses (Oller et al., 1997; Hausinger, 1993b). The competition for uptake of soluble nickel is reflected in decreases in the incidence of colony transformation of CHO cells when the cells are incubated with comparable concentrations of nickel sulphate in a complete medium rather than one devoid of amino acids and protein (DiPaolo and Casto, 1979). Relatively rapid lung clearance of soluble nickel salts (elimination half-time ~ 20-40 hours, Hirano et al., 1994; Medinsky et al., 1987) can remove soluble nickel salts from the extracellular space before the nickel is taken up intracellularly. Therefore, the intracellular concentration of nickel ion is much lower following exposure to a given concentration of a soluble nickel compound than the same concentration of an insoluble nickel compound. Furthermore, while insoluble nickel is protected from cytoplasmic proteins, soluble nickel is taken up directly into the cytoplasm, where it can react with cytoplasmic proteins and cause cytotoxicity. This reduces the nickel dose to the nucleus, and results in competing cytotoxicity.

Thus, soluble forms of nickel interact with the cell in a way that maximizes cytotoxicity and minimizes nickel delivery to the nucleus, while insoluble forms of nickel, such as nickel subsulfide, interact with cells in a way that decreases the cytotoxic potential while increasing the delivery of nickel to the nucleus. Cytotoxicity is important for two reasons. First, in order for cancer to develop, the altered cell must survive and transmit precancerous changes to its daughter cells. Secondly, high levels of cytotoxicity (resulting ultimately in organ toxicity) can prevent a chemical from being tested at high enough doses for cancer to be evident. Thus, although the observation of DNA damage and chromosome aberrations in cell cultures suggests a potential for direct genotoxic effects of soluble nickel under certain *in vitro* conditions (e.g., absence of extracellular amino acids and serum proteins), these effects may be prevented or greatly attenuated *in vivo* by extracellular complexation and other elimination mechanisms limiting the availability of extracellular Ni^{2+} to the cell interior and nucleus.

Water-soluble nickel compounds, in general, have consistently induced effects in certain kinds of mammalian assays, DNA damage *in vitro*, chromosomal effects such as aberrations and sister-chromatid exchanges *in vitro* and *in vivo*, and carcinogenic transformation of mammalian cells *in vitro*. Production of point mutations appears to occur with much lower efficiency. Responses in many of these assays were weak and occurred at toxic doses.

Single strand breaks, DNA-protein cross-links and chromosome aberrations (gaps, breaks, exchanges, dicentrics and fragments) have been shown to occur in Chinese hamster ovary (CHO) cells when cultures are incubated with nickel chloride in a salts/glucose medium that enhances nickel uptake (Patierno and Costa, 1985; Sen and Costa, 1985, 1986). Various mechanisms have been proposed for these effects, including binding of Ni^{2+} to histone protein (Huang et al., 1995), redox cycling of nickel-histone complexes (Datta et al., 1992; Misra et al., 1993) and oxidative damage to heterochromatin and possibly of DNA bases (e.g.,

production of 8-oxo-deoxyguanosine) (Huang et al., 1995; Kasprzak et al., 1992; Nackerdien et al., 1991;
Costa et al., 1994; Klein et al., 1991). Note that many of these mechanisms involve indirect interactions between the nickel ion and DNA, rather than a direct DNA interaction.

In addition to causing DNA damage in a number of ways, there are a number of means by which soluble nickel could increase the carcinogenicity of other chemicals. In light of the low efficiency for soluble nickel uptake by cells, these activities of soluble nickel may explain the effect modification observed in epidemiology studies of workers exposed to soluble and insoluble forms of nickel.

Inhaled soluble nickel produces an inflammatory response and enhances cell proliferation (e.g., epithelial hyperplasia) in the respiratory tract of rats and mice (NTP 1996a). There is some evidence for similar effects in humans, based on evidence of dysplasia of the nasal epithelial mucosa in humans exposed to nickel (Boysen et al., 1982,1984). The severity and frequency of the dysplasia were similar in electrolysis workers (who are only exposed to soluble nickel) and in workers in roasting/sintering (who are less likely to be exposed to soluble nickel). Neither the species nor the levels of nickel that would lead to hyperplasia or other changes indicative of overt toxicity in humans are known. Epithelial hyperplasia and dysplasia are of interest for the development of cancer, since in humans, head and neck cancer generally develop in an area of diffuse epithelial injury, such as that caused by tobacco or alcohol (Benner et al., 1995). Local generation of oxygen radicals by activated macrophages and polymorphonuclear cells, and enhanced cell proliferation associated with the inflammatory response could contribute to DNA damage or promotion of DNA damage into heritable mutations (Cerutti, 1985; Driscoll et al, 1996; Oberdörster, 1995; Swenberg, 1995). Soluble nickel also may act directly on alveolar epithelial cells to enhance cell proliferation. Limited evidence for this comes from studies of *in vitro* cell cultures. Enhanced cell proliferation has been observed in cultures of rat tracheal epithelial cells incubated in the presence of nickel sulfate (Patierno et al., 1993). Nickel sulfate has been shown to induce expression of the proliferin gene family, a class of mitogen-regulated genes involved in cell proliferation, in cultured C3H cells (Parfett, 1992).

Soluble nickel may also impair clearance of insoluble particles from the lung, which, in turn, may enhance inflammation and cell proliferation associated with the accumulation of insoluble nickel particles in the lung. Evidence regarding this potential mechanism is mixed. An *in vitro* study found that nickel chloride has been shown to inhibit phagocytosis of nickel sulfide particles by cultured CHO cells (Heck and Costa, 1983). However, *in vivo* results on inhibition of clearance are equivocal. Inhalation of nickel sulfate for 6 months had no effect in mice, while in rats clearance was reduced after 2 months of exposure, but not after 6 months, even though mild respiratory lesions were observed (Benson et al., 1995).

4.6.4 Summary and Narrative Statements

Overall, inhalation bioassays in rats and mice found "no evidence" of carcinogenicity, although questions have been raised about how informative the mouse data are for nickel compounds. However, a search of the NTP bioassay results database found no tendency for mice to be less likely to develop lung tumors than rats, even when considering only inhalation studies or metals. The epidemiology data indicate that exposure to soluble nickel increases the risk of cancer resulting from the highest cumulative exposures to insoluble forms of nickel. However, coexposures and unaccounted-for confounding factors prevent the use of the epidemiology data by itself to determine the carcinogenicity of exposure to soluble nickel alone. Mechanistic studies indicate that cellular uptake of soluble nickel and potential for interaction with DNA is much lower than that of nickel subsulfide, a clear carcinogen. Mechanistic studies also suggest several means by which soluble nickel can enhance the carcinogenicity of other chemicals, but none of these studies provide sufficient dose-response information to be useful in risk assessment or to fully explain the epidemiological observations. Therefore, the overall weight of evidence, based on the negative results in the various bioassays in experimental animals, the epidemiological data complicated by confounding, and the available data on mode of action, suggest the following weight of evidence statement under the proposed Guidelines for Carcinogen Risk Assessment of EPA (U.S. EPA, 1996a):

> The carcinogenicity of soluble nickel via the inhalation route *cannot be determined*. According to EPA's 1996 draft cancer guidelines, the following subdescriptor applies: The carcinogenic potential of inhalation exposure to soluble nickel *"cannot be determined* because the existing evidence is composed of *conflicting* data (e.g., some evidence is suggestive of carcinogenic effects, but other equally pertinent evidence does not confirm a concern)." Epidemiology studies have demonstrated an association with increased cancer only when co-exposure or prior exposure to insoluble forms of nickel was likely. Thus, data from epidemiology studies are insufficient to determine whether exposure to soluble nickel *alone* causes cancer. In animal studies, response to exposure to soluble nickel is negative in well-conducted 2-year bioassays in both male and female rats, and male and female mice. Several parenteral studies have been conducted with soluble nickel and results from these studies are either negative or weakly positive. Results from parenteral studies make definitive statements regarding inhalation difficult.
>
> The carcinogenicity of soluble nickel compounds for oral exposures *cannot be determined* at this time. According to EPA's 1996 draft cancer guidelines, the following subdescriptor applies: The carcinogenic potential of oral exposure to soluble nickel "*cannot be determined* because there are *inadequate data* to perform an assessment". Several negative oral experimental animal studies exist, but each of them has a deficiency that makes conclusive statements difficult. Moreover, the available

parenteral and initiation/promotion studies, which have indirect relevance to tumor formation after oral (or inhalation) exposure, suggest some tumorigenic activity for some soluble nickel compounds in some assays.

Under EPA's 1996 proposed cancer guidelines, the category "*cannot be determined*" is appropriate "when available tumor effects or other key data are suggestive or conflicting or limited in quantity and, thus, are not adequate to convincingly demonstrate carcinogenic potential for humans." Note that, although the same major descriptor of "cannot be determined" applies to both the oral and inhalation routes, the reasons for this descriptor and the associated subdescriptors differ for the two routes.

Under the current EPA cancer guidelines (U.S. EPA, 1986a), exposure to soluble nickel compounds via both the oral and inhalation routes would be classified as "D", not classifiable as to human carcinogenicity. This is the classification most closely corresponding to the narrative statements under the 1996 proposed guidelines. EPA does not classify the carcinogenicity of chemicals for parenteral routes of exposure.

In contrast, insoluble nickel compounds appear to be associated with tumor responses after inhalation by humans. Moreover, in experimental animals insoluble nickel forms are unequivocally positive for carcinogenicity by the inhalation and parenteral routes. The mechanisms for the apparent difference in carcinogenic potential between water soluble and insoluble nickel compounds are not completely understood and may be related to differences in the whole animal and/or cellular pharmacokinetics and/or bioavailability and clearance of nickel when administered in soluble and insoluble forms.

4.7 Other Hazard Identification Issues

4.7.1 Possible Childhood Susceptibility

Soluble nickel has been tested in newborn and young animals in a number of 2- or 3-generation studies. Effects were seen in these animals at doses that were maternally toxic in many cases, and in some cases at lower doses. Each of these studies had design flaws that preclude definitive analysis regarding the effects in neonates or the reproductive effects of soluble nickel in the parental and succeeding generations. Thus, the available data do not unequivocally rule out the development of adverse effects in newborn and young animals at doses lower than that seen in adult animals. This scientific uncertainty, along with that fact that reproductive and developmental endpoints have not been fully explored, supports the use of a database uncertainty factor in the development of both the RfD and the RfC.

4.7.2 Possible Gender Differences

Human studies are unavailable to assess whether the sexes differ in toxic response to soluble nickel compounds. Clear data are also absent regarding the relative sensitivity of males and females to the kidney effects of soluble nickel, the critical effect after oral exposure (Vyskocil et al., 1994b). However, a human toxicokinetics study found that nickel absorption was lower in women than in men (Nielsen et al., 1999). Nickel accumulation in the kidney of rats that inhaled nickel sulfate aerosols for up to 13 weeks was markedly higher in males than in females, but variability was very high and the measured levels were not statistically significantly higher than background levels (NTP, 1996a). Because inhaled nickel is efficiently absorbed, these results are applicable to ingestion exposure. However, the applicability of these data is limited, due to the wide variability and lack of clear concentration-response relationship observed.

The results from the inhalation studies of soluble nickel salts do not suggest a sex difference in response to the toxicity of these compounds.

5. DOSE RESPONSE ASSESSMENTS

5.1 Oral Reference Dose (RfD)

5.1.1 Choice of Principal Study and Critical Effect - with Rationale and Justification

The studies considered as the basis for the RfD for soluble nickel salts are summarized in Table 29. The most sensitive endpoint was increased albuminuria (indicating renal glomerular dysfunction) in male and female rats exposed to nickel in drinking water for 6 months (Vyskocil et al., 1994b). Effects were seen at 6 months at the only dose tested (6.9 mg Ni/kg/day for males and 7.6 mg Ni/kg/day for females) but not after 3 months of exposure. The study evaluated sensitive functional endpoints, and included both group-level and some individual animal data. This minimal LOAEL was closely supported by several other studies. For example, based on decreased body weight, the NOAEL was 8 mg Ni/kg/day in rats administered nickel sulfate in feed for 2 years (Ambrose et al., 1976). The corresponding $BMDL_{10}$ was estimated as 6.8-36 mg Ni/kg/day, depending on whether the BMR was defined as a 10% increased risk of low body weight (lower value) or a 10% decrease in the mean body weight (higher value). This study, however, is limited by minimal reporting and high mortality in both the control and exposed groups. American Biogenics Corporation (1988) administered nickel by gavage in drinking water to male and female rats, and found that decreased body weight in males was also the most sensitive endpoint, along with pneumonitis in both sexes. The NOAEL in that study was 5 mg Ni/kg/day (assuming that the doses were reported as nickel amounts, or 2.7 mg Ni/kg/day, assuming the doses were reported as amounts of nickel chloride hexahydrate). The utility of this study for risk assessment is limited, however, by uncertainties regarding the dose (i.e., whether the doses were reported as nickel or as nickel chloride hexahydrate).

Neither the Vyskocil et al. (1994b) nor the Ambrose et al. (1976) studies are ideally suited for selection as the principal study for the development of the RfD. The former study was conducted for only 6 months, tested only one dose, did not provide a comparison to baseline values, and evaluated only 10 rats/sex/time point. Only one measure of renal function was clearly affected (and the increases were not large even for that endpoint), and the interpretation of the results was complicated by considerable variability in response in both the control and exposed groups. Although it is biologically plausible that the kidney is *a target organ*, the supporting data are weak and it is not clear whether the kidney is *the most sensitive target organ*. Kidney histopathology has not been observed in chronic studies testing to lethal doses, although no other study included evaluation of sensitive measures of kidney function (i.e., individual protein markers of glomerular and tubular function). Finally, although occupational studies have reported elevated levels of urinary protein markers, these elevations have been observed only in spot urine samples (Sunderman and Horak, 1981; Vyskocil et al., 1994a), which are subject to false positives in comparison to 24-hour multi-void samples (Sanford and Nieboer, 1992).

Two significant deficiencies apply to the Ambrose et al. (1976) study. First, mortality was high in both the control and exposed groups, markedly decreasing the sensitivity of the study. Second, reporting of the methods and results is rather minimal, limiting the degree to which an independent evaluation of the data are possible.

A continuing issue related to the assessment of an RfD for soluble nickel compounds is the inconsistent results in the reproductive studies. This is discussed at length in Section 4.3, but summarized here. In brief, soluble nickel compounds can cause reproductive toxicity, but the doses at which such effects occur generally fall above those which cause kidney toxicity. Thus, the critical effect, increased albuminuria in rats, should be protective of the reproductive endpoints as well. Uncertainties in this judgment are addressed in the section on uncertainty factors. It should be noted, however, that the Metals Subcommittee of the U.S. EPA Science Advisory Board reviewed the reproductive toxicity data in 1991, and concluded that the "most cogent" of the reproductive toxicity studies "failed to yield an RfD that was substantially different" from that derived from the Ambrose et al. (1976) study (SAB, 1991) (and thus the RfD derived from the Vyskocil et al. study would also be protective from reproductive effects).

After consideration of these issues, the Vyskocil et al. (1994b) study was chosen as the principal study. Although there are uncertainties associated with this study, they are insufficient to discount the observed effects, and the RfD based on the Vyskocil study would be more health-protective than an RfD derived based on Ambrose et al. (1976).

Although it can be useful to compare risk values prepared by different agencies using similar methods, ATSDR (1997) did not develop any oral minimal risk levels (MRLs) for nickel. As part of the rationale for not developing an MRL, ATSDR noted the report by Sunderman et al. (1989) that a male volunteer developed left homonymous hemianopsia following a single dose of 0.05 mg Ni/kg in drinking water. It should be noted, however, that the conditions of this study maximized the potential for a toxic effect. Nickel was administered in drinking water, resulting in higher absorption than if it had been administered in food. The use of a single, bolus dose administered to a fasting subject also meant that peak serum concentrations were rapidly reached, and were much higher than if the same dose were administered over a 24-hour time span. Finally, the anecdotal nature of the report of a subjective effect also makes this endpoint unreliable for risk assessment. Finally, it should be noted that the RfD derived in Section 5.1.3 is less than 1/10 the dose in this study. Taking into account the high peak serum levels in this study, and that serum nickel levels would be much lower in the general population continuously exposed to nickel in drinking water at that dose, the RfD is protective of this effect. The American Biogenics (1988) study also evaluated histopathology in the eyes of rats administered nickel as nickel chloride by gavage at doses up to 35 mg Ni/kg, and found no abnormalities, suggesting that eye effects are not of significant concern.

A second reason that ATSDR (1997) provided for not deriving oral MRLs was due to concerns about oral exposure causing dermal reactions in sensitized individuals. Although the RfD is designed to protect sensitive populations, and to protect against sensitization to a chemical, it is not designed to protect people who have already been sensitized to the substance, in light of the vastly greater sensitivity of such individuals. Therefore, the RfD developed here may not necessarily protect sensitized individuals. The limited dose-response data on protection of sensitized individuals was presented in Section 4.1.4.2. Although these studies are limited by relatively small numbers and, in some cases, the absence of adequate controls, the data are fairly consistent that a bolus dose (in water or capsule) of approximately 0.08 mg Ni/kg elicits a response in most sensitized individuals, and few individuals respond to a dose of approximately 0.02 mg Ni/kg (Burrows, 1992; Cronin et al., 1980; Gawkrodger et al., 1986; Kaaber et al., 1979). No data were located on the dose that first produces sensitization.

Even if it were desired to develop an RfD to protect sensitized individuals, the available data are insufficient. As discussed in Section 4.1.4.2, there is no clear dose-response relationship for the development of nickel sensitization. Similarly, although there have been reports of oral nickel exposure resulting in dermal reactions, moderate nickel doses have also resulted in at least short-term desensitization. The data are also insufficient to develop an RfD based on the sensitizing dose.

An alternative endpoint considered as a potential basis for the RfD was the equivocal LOAEL of 1.3 mg Ni/kg/day for increased postnatal deaths in the study of Smith et al. (1993). This equivocal LOAEL is approximately a factor of 5 below the LOAEL for kidney effects in the study by Vyskocil et al. (1994b). This study was not chosen as the principal study, due to the equivocal nature of the response (in the absence of a clear dose-response) and the absence of reproductive effects in other reproductive toxicity studies at this dose. The results of Smith et al. (1993) were, however, considered in the choice of uncertainty factors in Section 5.1.3.

5.1.2 Method of Analysis-No Observed Adverse Effect Level and Lowest Observed Adverse Effect Level

In the Vyskocil et al. (1994b) study, increased urinary albumin levels of were observed at the only dose tested, 6.9 mg Ni/kg/day in males and 7.6 mg Ni/kg/day in females. Because small, but biologically meaningful, changes in sensitive measures of kidney function were observed, this result is considered a minimal LOAEL. In light of the large degree of variability for albuminuria in male rats, and the absence of a statistically significant response in males, the LOAEL of 7.6 mg Ni/kg/day in females was considered the study LOAEL. Meaningful benchmark dose modeling could not be conducted for this study, because only one dose was tested.

5.1.3 RfD Derivation

The nickel intake was calculated separately for males and females by the study authors based on drinking water consumption and body weight. The resulting LOAEL was 7.6 mg Ni/kg/day.

The composite uncertainty factor (UF) to use with a given database for developing RfDs is a case-by-case judgment by experts. U.S. EPA describes its choice of composite UF and subcomponents for individual assessments on its IRIS database (U.S. EPA, 1998b). For soluble nickel, a total uncertainty factor of 1000 was used, resulting in an RfD of 0.008 mg Ni/kg/day. The following discussion addresses each of the areas of uncertainty considered in the development of the RfD, and the selection of uncertainty factors for the soluble nickel RfD.

Human Variability (H)

This factor addresses the question: Do existing data account for sensitive individuals?

If yes, this suggests an uncertainty factor other than a default value of 10---as low as a value of 1 in some instances [see for example, the description of the uncertainty factor for nitrates on U.S. EPA's IRIS (U.S. EPA, 1998b) where a NOAEL of a sensitive population was used as the basis of the RfD]. Scientists familiar with this area have considered this default factor to be composed of roughly equal parts for toxicodynamic and toxicokinetic differences among humans. Some recent work has attempted to quantify these distinctions (Renwick, 1993).

The critical effect for the soluble nickel RfD is increased urinary albumin levels, a marker of decreased glomerular function of the kidney. Other than people who have been dermally sensitized, sensitive populations have not been specifically identified for nickel. However, based on the critical effect and the observed concentration of nickel in the kidney of animals and humans, it is reasonable to expect that people with kidney dysfunction would be more sensitive to ingested nickel. In addition, such individuals would likely have decreased urinary excretion of nickel, and thus might have higher nickel levels in the kidney. Particular groups with decreased kidney function include dialysis patients and diabetics. The default UF of 10 is considered appropriate for protecting these populations, in the absence of data to address the variability of individuals in toxicokinetics and toxicodynamics of soluble nickel.

Inter-Species Variability (A)

This UF addresses the following questions: Do existing data allow for a quantifiable extrapolation of animal dose to the expected human equivalent dose for effects of similar magnitude? Or, as is more likely the case, for NOAELs?

If yes, this suggests an uncertainty factor other than a default value of 10 --- with inhalation RfCs, for example, a value of 3 is often used when dosimetric adjustments are used in the determination of the HEC [see the RfC methods document (U.S. EPA, 1994b) and numerous examples in U.S. EPA's IRIS (U.S. EPA, 1998b)]. Scientists familiar with this area have considered this default factor also to be composed of roughly equal parts for toxicodynamic and toxicokinetic differences between experimental animals and humans. In addition, they recognize that some overlap with the uncertainty factor for intra-species variability exists. Some recent work has attempted to quantify these distinctions in general (Renwick, 1993).

Although some data on absorption and excretion of ingested soluble nickel compounds are available for both humans and rats, the data are not detailed enough to quantitatively compare the rate of nickel absorption or excretion. It does appear that the absorption of ingested soluble nickel is higher in rats than in humans, although the rat data are limited. Ishimatsu et al (1995) found that nonfasted male Wistar rats absorbed 9.8-34% of a single gavage dose of soluble nickel salts administered in a 5% starch saline solution. By contrast, absorption by nonfasted human subjects of soluble nickel salts was only 5.7% (Christensen and Lagesson, 1981, as calculated by Diamond et al., 1998). It is not clear if this difference is due to interspecies differences or to the differences in dosing vehicles. Thus, the default UF of 10 for interspecies extrapolation is appropriate in the absence of data to specifically address the extrapolation from experimental animals to humans on toxicokinetics and toxicodynamics.

Subchronic-to-Chronic Extrapolation (S)

This UF addresses the following questions: Do existing data allow for a quantifiable extrapolation of the critical effect after subchronic exposure to that after chronic exposure? Will NOAELs of different critical effects from subchronic and chronic exposure differ quantitatively?

U.S. EPA has occasionally used values less than 10 (nearly always 3-fold) for less-than-chronic exposures when data were available to support such a reduction [for example, see the RfD for arsine on U.S. EPA's IRIS (1998b)]. Scientists familiar with this area also recognize that this factor overlaps somewhat with the database uncertainty factor.

The principal study (Vyskocil et al., 1994b) reported albuminuria after 6 months of exposure in drinking water, but only minimal increases that were not biologically or statistically significant after 3 months of exposure. Single-dose studies found that absorbed nickel is rapidly and extensively excreted. NTP (1996a) measured the kidney nickel levels in male and female rats and mice that inhaled nickel sulfate aerosols for up to 13 weeks (rats) or 15 months (mice). Because soluble nickel is absorbed from the lung, this study could provide information on the accumulation of nickel in the kidney over time. The data provide some indication of higher

nickel concentrations at later time points, but are too inconsistent for a clear conclusion. Based on the rapid excretion of nickel, but taking into account the suggestive evidence of increased severity of effects between a 3- and 6-month exposure, a partial UF is judged to be appropriate and is discussed further in the text on composite UF below.

Insufficient Database (D)

This UF addresses the questions: Do existing data allow for a reasoned judgment of likely critical effect, given that any one toxicity study is unable to adequately address all possible outcomes?

If data exist from at least five studies (two chronic standard toxicity bioassays in different species, one two-generation reproductive bioassay and two developmental toxicity studies in different species), an uncertainty factor of 1 is generally appropriate. U.S. EPA has occasionally used values less than 10 (nearly always 3-fold) when data were available on several, but not all 5 studies [for example, see the RfD for acetaldehyde on U.S. EPA's IRIS (U.S. EPA, 1998b)], and factors of 10 (generally) when data were only available from a single study. Scientists familiar with this area also recognize that some overlap occurs with the subchronic to chronic uncertainty factor. The general approach when subchronic studies are available in two species, is to assign the uncertainty to the subchronic-to-chronic factor, and not to the database factor.

Two chronic studies that evaluated nonneoplastic lesions are available in rats (Ambrose et al., 1976; American Biogenics Corporation, 1988). Ambrose et al. (1976) also evaluated nonneoplastic effects in dogs exposed for 2 years, and Dieter et al. (1988) evaluated mice exposed in drinking water for 180 days. Several multigeneration studies have been conducted with ingested nickel (Research Triangle Institute, 1988; Smith et al., 1993; Ambrose et al., 1976; Schroeder and Mitchener, 1971), and one (Research Triangle Institute, 1988) included teratological evaluation of the F2 generation rats. These studies found increased neonatal pup death, but no evidence of teratological effects. A clear reproductive NOAEL could not be established for any of these studies, however, due to inconsistent dose-response results, incomplete reporting, and technical experimental difficulties. In light of these issues, some uncertainty remains regarding the reproductive toxicity of nickel compounds. In addition, no developmental toxicity study has been conducted in a second species. Based on these considerations, a partial UF for database deficiencies is appropriate for nickel and is discussed in the text on composite UF below.

LOAEL to NOAEL (L) Extrapolation

This UF addresses the question: Do existing data allow for the use of a LOAEL, rather than a NOAEL for the estimation of an RfD?

If a well-defined NOAEL does not exist, a factor of 10 is often used. However, U.S. EPA has often used values less than 10 (nearly always 3-fold) when data suggest that the LOAEL is for a minimally adverse effect, since the hypothesized NOAEL would likely be closer to a minimal LOAEL then to a LOAEL with greater severity. For example, compare the RfCs for acrylonitrile and 1,2-epoxybutane on U.S. EPA's IRIS (U.S. EPA, 1998b). The former RfC uses a 3-fold factor with degeneration and inflammation of nasal respiratory epithelium; the latter RfC uses a 10-fold factor with more severe degenerative lesions of the epithelium.

The principal study identifies a LOAEL of 6.9 mg Ni/kg/day, based on a sensitive biochemical measure of kidney function in rats (albuminuria) administered nickel in drinking water for 6 months; histopathology was not evaluated in this study (Vyskocil et al., 1994b). However, no histological changes were observed by Dieter et al. (1988) at 44 mg Ni/kg/day in a 180-day mouse study, but mild tubular nephrosis was observed at 108 mg Ni/kg/day and higher. Based on the minimal adversity of the critical effect, a partial UF is appropriate for nickel and is discussed in the text on composite UF below.

Modifying Factor

A modifying factor is not considered necessary with this database because the outstanding uncertainties are adequately addressed with the standard uncertainty factors above. U.S. EPA only occasionally uses a modifying factor. For example, see the RfC for methyl ethyl ketone on U.S. EPA's IRIS (U.S. EPA, 1998b). The default value of 1 is appropriate for soluble nickel salts.

Composite Uncertainty and Modifying Factors

For soluble nickel salts, two full factors of 10 (for intrahuman variability and interspecies extrapolation) and a combined factor of 10 (for subchronic-to-chronic extrapolation, an insufficient database, and use of a minimal LOAEL) were used. A composite factor of 1000 results.[6]

A combined factor of 10 is appropriate for subchronic-to-chronic extrapolation, use of a minimal LOAEL, and for an incomplete database, based on four points:

- the minimal severity of the adverse effect;

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[6]It is of note that standard EPA practice is to condense four full UFs of 10 to a composite UF of 3000 (due to overlap of UFs) (Dourson, 1994). Thus, even if two full UFs and three partial UFs were used for the nickel RfD, the composite UF could be 1000 or 3000, depending on the overall judgement of the database (also standard U.S. EPA practice).

- the availability of chronic studies that do not identify a lower LOAEL than in the critical study;
- although soluble nickel did have an effect in reproductive studies, the studies do not collectively indicate that reproductive effects occur at a lower dose than the critical effect (As noted in Section 5.1.1, even if the RfD were based on the equivocal LOAEL of 1.3 mg Ni/kg/day for reproductive effects in Smith et al. (1993), the RfD would change by only a factor of approximately 2-6, depending on a potential different choice of UFs); and
- the toxicokinetic data suggest that absorption of ingested soluble nickel is higher in rats (9.8-34% of a gavage dose; Ishimatsu et al., 1995) than in humans (5.7% for soluble nickel salts with nonfasted subjects; Christensen and Lagesson, 1981, as calculated by Diamond et al., 1998); it is not clear if this difference is due to interspecies differences or to the differences in dosing vehicles, but lower absorption by humans would mean a lower human tissue dose for a given ingested amount, and therefore that the use of the rat LOAEL as the basis of the RfD is conservative.

Based on these considerations, a composite UF of 1000 is considered sufficient. Thus, the RfD is derived as:

7.6 mg Ni/kg/day ÷1000 = 0.0076 mg Ni/kg/day = 8 E-3 mg Ni/kg/day

The nickel doses in the animal studies did not include the nickel in the diet. Therefore, the RfD presented here represents the dose of nickel *in addition* to the amount in food.

The RfD based on the minimal LOAEL from Vyskocil et al. (1994b) can be seen as consistent with the existing soluble nickel RfD described on IRIS (U.S. EPA, 1998b). (In brief, IRIS lists a chronic NOAEL of 5 mg Ni/kg/day for body weight decrease in the Ambrose et al. (1976) study, and a 300-fold UF: 10-fold for within human variability, 10-fold for animal to human extrapolation, and 3-fold for uncertainties in the database on reproductive endpoints.) First, the current RfD is based on total nickel, while the RfD proposed here is based on nickel in addition to diet, and so would be expected to be lower than an RfD based on total nickel. Second, the RfD on IRIS of 2E-2 mg Ni/kg/day is within a factor of 2 of the RfD proposed here, a difference well within the inherent uncertainty of the RfD.

5.2 Inhalation Reference Concentration (RfC)

5.2.1 Choice of Principal Study and Critical Effect - with Rationale and Justification

Reliable inhalation toxicity studies with soluble nickel compounds are limited to the chronic and subchronic NTP (1996a) studies with rats and mice, the related analysis by Benson et al. (1989) of biochemical effects on the lung, and the study by the same laboratory group

(Haley et al., 1990), showing that immune effects occurred at higher exposure levels than respiratory effects. Rats were more sensitive than mice. Several different lesions were observed at similar exposure levels, with slightly differing response incidences. The most sensitive endpoints were lung fibrosis and chronic active inflammation in male rats, with a NOAEL(HEC) of 0.0021 mg Ni/m^3, and olfactory epithelial atrophy in female rats, with a NOAEL(HEC) of 0.0019 mg Ni/m^3, both in the chronic study (Table 30). The principal study (NTP, 1996a) was a well-designed and executed study of chronic duration that used a fairly large number of rodents of both sexes and two species, and measured multiple endpoints. A well-defined NOAEL and LOAEL were determined and the responses were concentration related.

As noted in Section 4.2, chronic active inflammation was observed in the lungs of male rats at a lower concentration at the 7-month interim evaluation than in the chronic evaluation. This lesion was transient and reversible, was not seen at the same concentration at a shorter duration (in the 13-week study) or at longer durations (15-month or 2 year) in the same study, and is not supported by other lung effects at the same concentration. Macrophage accumulation was also observed at lower concentrations in the subchronic study, but was a transient and possibly nonspecific response, and not supported by other lesions at similar exposure levels. In addition, although macrophage accumulation may be part of a continuum of effects that includes inflammation at higher exposure levels, the macrophage lesion does not appear to be a precursor with increased exposure duration. This is because no effects were seen in the chronic study at concentrations that did result in macrophage accumulation in the subchronic study. As noted in the next section, using either of these endpoints as the critical effect would not have a large impact on the RfC.

It is interesting to note that, although the exposure levels used in the chronic study were the same as the lowest three exposure levels used in the subchronic study, the NOAEL(HEC) for olfactory epithelial atrophy in females in the subchronic rat study (0.0016 mg Ni/m^3) was slightly *lower* than the NOAEL(HEC) for this endpoint in the chronic study (0.0019 mg Ni/m^3). Similarly, the BMC(HEC) of 0.0005-0.0007 mg Ni/m^3 in the subchronic study was markedly lower than the value of 0.0025 mg Ni/m^3 calculated for the chronic study, due to the high response at the subchronic LOAEL. The low BMC is not due solely to the smaller sample size in the subchronic study, since the maximum likelihood estimate (MLE), which does not take sample size into account, was also smaller for the subchronic than the chronic study. On the surface, these estimates suggest some sort of adaptation, with longer exposure resulting in less severe effects. However, these estimates are for the human equivalent concentrations (HECs). When the actual animal exposure concentration alone is considered, the exposure duration-response has the expected relationship. No effect was seen at 0.11 mg Ni/m^3 in the subchronic study, while that concentration was the LOAEL in the chronic study. These results suggest that the HEC may not completely adjust for animal/human differences in the dosimetry of inhaled nickel sulfate. In particular, clearance is not taken into account in the HEC calculations. As

described in Section 3.1, clearance of inhaled soluble nickel is rapid and extensive in rats and mice, but clearance in humans has not been quantified. Based on these considerations, it was not considered appropriate to derive the RfC from this endpoint in the subchronic study, even though it did correspond to the lowest BMC(HEC).

Only one human study was located that examined noncancer effects of inhalation exposure to soluble nickel and included effects on the respiratory tract (Muir et al., 1993). In this study, radiographs from nickel workers exhibited an increase in the prevalence of irregular opacities with duration of exposure. However, there was no control group, and the increase was largely attributable to ILO profusion scores of minimal severity, a severity comparable to values seen in the general public. Exposure measurements were also highly approximate. Other uncertainties in the study include mixed exposure to soluble and insoluble forms of nickel, wide variability among radiograph readers, and questions regarding degree of ascertainment. In light of these uncertainties, this study is not desirable as a basis for an RfC. However, because this study does provide some insight regarding the inhalation toxicity of soluble nickel in humans, an RfC can be derived from this study for comparison to the more reliable RfC derived from the animal data. If this study were considered as the basis for the RfC, the single exposure level available would be considered a minimal LOAEL, based on the observation of a duration-related increase in the prevalence of findings. Thus, this study identifies a minimal LOAEL of 4 mg Ni/m^3.

5.2.2 Method of Analysis - Benchmark Concentration

NTP (1996a) reported lesions in the extrathoracic (nose) and pulmonary (lesions such as inflammation and fibrosis) regions of the rat and mouse, and in the thoracic region (bronchialization, a combined tracheobronchial and pulmonary lesion) of mice. Human equivalent concentrations (HECs) for particulates are calculated by multiplying the exposure concentration by the Regional Deposited Dose Ratio (RDDR). The RDDR is calculated using the RDDR program (U.S. EPA, 1994b), and takes into account the relative regional deposition in humans and the animal species of interest, the species-specific minute volume (the volume of air inhaled per minute), and the surface area in humans and the experimental animal species of the affected region of the respiratory tract. Inputs to the calculation of the RDDR are the mass median aerodynamic diameter (MMAD) and geometric standard deviation (sigma g) that describe the particle size distribution for the experimental animal study, and the body weight of the experimental animal. NTP (1996a) provided concentration-specific values for the MMAD and sigma g, and these values, rather than average values, were used in the calculations. The concentration-specific MMAD and sigma g values, the corresponding RDDRs, and the resulting HECs are presented in Appendix B. In light of the marked effect of the respiratory tract region on the calculated RDDR, it was necessary to calculate the HECs for lesions in different regions of the respiratory tract in order to determine which endpoint was most sensitive.

Quantal data from NTP (1996a) on the incidence of respiratory tract lesions were fit to a polynomial mean response regression model (THRESH, ICF Kaiser, 1997a) and a Weibull power mean response regression model (THRESHW, ICF Kaiser, 1997b) by the maximum likelihood method. The lower bounds on the concentrations corresponding to 5% and 10% risk ($BMCL_5$ and ($BMCL_{10}$) were calculated. The 10% benchmark response level (BMR) was used, based on two considerations. First, BMC values for both the 5% and 10% BMRs for several upper respiratory tract toxicants were found in an EPA review to generally fall between the corresponding NOAEL and the LOAEL (Gift, 1996). Second, results of Allen et al. (1994) with developmental toxicity endpoints found that the 10% response level is only slightly conservative, on average, compared to the corresponding NOAELs. For several of the datasets, there were one or more exposure levels with no response above background, and high response at the higher exposure levels. Such concentration-response patterns are poorly fit when the threshold is set to zero in the modeling. However, improved fit in many cases was obtained by allowing the program to calculate the threshold value. (Note that this is a mathematical threshold, does not necessarily correspond to a true biological threshold.) In other cases, the concentration-response data showed a plateau at high concentrations. Because it was not desirable to have high exposure levels that were not informative with regard to the shape of the concentration-response curve drive the modeling, these endpoints were also modeled with the high exposure level dropped. These results are described in more detail in Appendix A.

Based on the results of the BMC modeling, the most sensitive endpoint was lung fibrosis in male rats chronically exposed to nickel sulfate (response 3/54, 6/53, 35/53, and 43/53). An unacceptable fit ($p<0.01$) was obtained with the threshold set to zero for this endpoint, but the fit was markedly improved by allowing a threshold to be calculated by the program. Although the overall goodness-of-fit p value for this endpoint was still low when the threshold parameter was included ($p=0.032$), a good visual fit was obtained in the low-concentration region (see Figure 1). The resulting $BMCL_{10}$(HEC) of 0.0017 mg Ni/m^3 was only slightly lower than the NOAEL(HEC) of 0.0021 mg Ni/m^3 for this endpoint. The same result was obtained with the polynomial and Weibull models.

Acceptable, but slightly higher $BMCL_{10}$(HEC) values were calculated for several other endpoints from NTP (1996a). No acceptable fit could be obtained for chronic active inflammation in male rats when all of the data were used or when a threshold was allowed, but a $BMCL_{10}$(HEC) of 0.0020 (Weibull model) mg Ni/m^3 was obtained ($p=0.53$) when the high exposure level was dropped; no acceptable fit could be obtained with the polynomial model. Similarly, no acceptable fit could be obtained for lung fibrosis in female rats when all of the data were used, when a threshold was allowed, or with the polynomial model under any conditions, but a $BMCL_{10}$(HEC) of 0.0024 (Weibull model) mg Ni/m^3 was obtained ($p=0.75$) when the high exposure level was dropped. A $BMCL_{10}$(HEC) of 0.0025 (Weibull model) or 0.0026 (polynomial model) mg Ni/m^3 was obtained for atrophy of the olfactory epithelium in female

rats. The same result was obtained whether or not the model was allowed to estimate a threshold, and a good fit was obtained (p=0.73 with the polynomial model and p=0.32 with the Weibull model).

The effect of several scientific judgements made in deriving the RfC was investigated by determining the effect of alternative choices on the RfC. Macrophage accumulation was considered to be a possible precursor to adverse effects for the purposes of this assessment, based on the possibility for this effect to fall on a continuum of effects including inflammation and fibrosis. Evidence opposing this determination include the lack of other supporting evidence of damage at the low concentrations in the subchronic studies, and the potential for hyperplasia to be a physical response to particulate exposure. Nonetheless, $BMCL_{10}$(HEC) values of 0.0012-0.0016 (male rats) and 0.0013-0.0019 (female rats) mg Ni/m^3 were calculated for this endpoint in the chronic study. Acceptable fits could not be calculated with all of the data without a threshold, but dropping the high concentration (males and females) or allowing a threshold (females only) resulted in an acceptable fit. Better fits (indeed, perfect fits) were obtained with the Weibull model, but the estimates obtained had no degrees of freedom. The $BMCL_{10}$(HEC) values calculated based on macrophage accumulation were within a factor of 2 of the value used as the basis for the RfC. This difference is well within the uncertainty associated with the RfC.

For comparison with the animal data, an RfC based on the Muir et al. (1993) study was considered. A minimal LOAEL of 4 mg Ni/m^3 as soluble nickel can be estimated for this study, based on increased prevalence of opacities with exposure duration. After adjusting for occupational exposure durations and minute volumes, the LOAEL is 1.4 mg Ni/m^3. A further adjustment was used to account for differences between particle sizes under occupational and ambient exposure conditions. Using the data of Vincent (1996) on particle sizes at nickel plants, and using the average particle size distribution in the NTP (1996a) chronic rat study with nickel sulfate hexahydrate to estimate the distribution under ambient conditions, the pulmonary dose for humans is approximately 7-80 fold higher when exposure is to the particle size distribution used in the animal studies, compared to the pulmonary dose when exposure is to the particle size distribution found under occupational conditions. The resulting LOAEL(HEC) is 0.018 to 0.2 mg Ni/m^3.

5.2.3 Chronic RfC Derivation

The $BMCL_{10}$(HEC) for lung fibrosis in male rats in the chronic NTP (1996a) study was 0.0017 mg Ni/m^3. The scientific bases for the uncertainty factors are described in greater detail in Section 5.1.3, and are not repeated here. In the absence of sufficient data on sensitive human subpopulations, an uncertainty factor of 10 was applied to account for intrahuman variability. Scientists familiar with this area have considered the default factor of 10 for animal to human extrapolation to be composed of roughly equal parts for toxicodynamic and

toxicokinetic differences between animals and humans. Because dosimetric adjustments were used to account for interspecies differences in toxicokinetics, a factor of 1 was used for the toxicokinetic portion of the factor. In light of the minimal effects seen under occupational exposure conditions (Muir et al., 1993) at exposure concentrations 10 to 100-fold higher than those that resulted in significant respiratory toxicity in the chronic rat bioassay (NTP, 1996a) (based on comparison of HECs, after duration adjustments and accounting for dosimetric differences, including the particle size appropriate for the different exposure conditions) humans appear to be less sensitive than rats to the respiratory effects of nickel. The minimal LOAEL in the
Muir et al. (1993) study is 10 to 100-fold higher than the $BMCL_{10}$(HEC) used as the basis for the RfC. Additional support for the lower sensitivity of humans comes from the lack of worker complaints of respiratory problems (Mastromatteo, personal communication). Thus, the data support a toxicodynamic factor of 1, resulting in a total factor of 1 for interspecies extrapolation. Because a $BMCL_{10}$ from a chronic study was used, no additional uncertainty factors are needed for subchronic to chronic extrapolation or for extrapolation from a LOAEL to a NOAEL.

Although a database uncertainty factor was included in the development of the RfD, it was not considered necessary to include such a factor in the development of the RfC, because the critical effect for inhalation exposure occurs at an exposure level well below that which would be expected to result in reproductive effects, based on the oral data. This conclusion is based on the following estimation of corresponding absorbed doses. The study with a reproductive effect at the lowest dose was that of Smith et al. (1993), who found an equivocal LOAEL of 1.3 mg Ni/kg/day, based on increased pup death. Absorption of ingested soluble nickel in rats has been reported to range from 9.8% to 34% (Ishimatsu et al., 1995). Using 9.8%, to be on the conservative end, results in an absorbed dose of 0.13 mg Ni/kg/day associated with the equivocal LOAEL. The absorbed dose corresponding to inhalation exposure at the NOAEL of 0.027 mg Ni/m^3 was calculated as follows. Because the aim was to identify the rat inhalation exposure level that corresponds to a specified absorbed dose in rats, the duration-adjusted rat value of 0.0048 mg Ni/m^3 was used, rather than the HEC. Based on a daily inhalation rate for male rats of 0.36 m^3/day and a body weight of 0.38 kg (U.S. EPA, 1988), and making the conservative assumption that all inhaled soluble nickel is absorbed, the duration-adjusted exposure level corresponds to an absorbed dose of 0.0045 mg Ni/kg/day. This value is approximately 1/30 the absorbed dose corresponding to the reproductive equivocal LOAEL of Smith et al. (1993), and so no additional uncertainty factor is needed. No modifying factor was applied to the estimation of this RfC. A composite uncertainty factor of 10 results. Thus, the RfC is derived as:

RfC = 0.0017 mg Ni/m^3 ÷ 10 = 0.00017 mg Ni/m^3 = 2 E-4 mg Ni/m^3

An RfC based on the Muir et al. (1993) study can be derived for comparison with this value. In that study, a minimal LOAEL(HEC) of 0.018 to 0.2 mg Ni/m^3 was identified. The following uncertainty factors would be used for this study: An uncertainty factor of 10 would be used for intrahuman variability, since sensitive subpopulations may not have been included in this occupational cohort. A partial uncertainty factor of 3 would be used to account for the minimal LOAEL, based on the minimal effects observed. Because exposure was only described as ">5 years," and the comparison group was exposed for <5 years, a partial uncertainty factor of 3 is used to account for less-than-lifetime exposure. Using reasoning similar to that in the previous paragraph, no database uncertainty factor to account for the potential for reproductive effects is needed. Thus, a composite UF of 100 is suggested. The resulting RfC based on human data is:

RfC = 0.018 to 0.2 mg Ni/m^3 ÷ 100 = 0.00018 to 0.002 mg Ni/m^3 = 2E-4 to 2E-3 mg Ni/m^3 [7]

This RfC is not recommended, in light of the numerous uncertainties associated with the human study. However, it is of interest that the range of RfCs is comparable to, or an order of magnitude higher than, the RfC based on the animal data.

5.3 Cancer Assessment

5.3.1 Choice of Study/Data

The database on the oral carcinogenicity of soluble nickel salts is inadequate for assessing carcinogenic potential. Several negative oral experimental animal studies exist (Ambrose et al., 1976; Schroeder et al., 1964, 1974; Schroeder and Mitchener, 1975), but each of these studies has a deficiency that precludes definitive conclusions.

No appropriate data exist from occupational exposures to support development of a quantitative estimate for excess cancer risk from exposure to soluble nickel compounds. Moreover, available animal inhalation studies for soluble nickel are negative for the cancer endpoint after inhalation exposure, and equivocally negative after oral exposure. Parenteral administration of some soluble nickel compounds yielded slightly positive results in some bioassays and negative results in others. The parenteral route is not appropriate for quantitative risk determination for the oral or inhalation routes. Based on the existing data and the weight of evidence determination, the development of a cancer dose-response curve for soluble nickel for either the oral or inhalation routes is not recommended. Because there is no clear evidence of carcinogenicity, no dose-response calculations can be conducted.

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[7] The calculations for the RfC based on the human data have changed somewhat since the peer review meeting, due to improved particle size distribution information.

6.0 MAJOR CONCLUSIONS IN THE CHARACTERIZATION OF HAZARD AND DOSE RESPONSE

6.1 Hazard Identification

6.1.1 Exposure

Soluble nickel compounds are used in a variety of plating processes and in metal alloys. Occupational exposure also occurs in nickel refinery workers. The general public is exposed to nickel as a normal constituent in food, in contaminated drinking water, and to insoluble and soluble forms of nickel emitted into the air from combustion, incineration, refining, and other industries (ATSDR, 1997).

Ambient concentrations of nickel are seldom reported as soluble or insoluble forms; rather, an estimation of total nickel is given. ATSDR (1997) and Health Canada (1993) both give estimations of total nickel in ambient air. ATSDR (1997) states that average nickel concentrations in ambient air of U.S. cities range from about 0.5 to 5 E-5 mg Ni/m^3. Health Canada (1993) states that average nickel concentrations in ambient air of Canadian cities range from 0.1 to 2 E-5 mg Ni/m^3. For comparison, the estimated RfC for *soluble* nickel of 2 E-4 mg Ni/m^3. It should be noted that nickel in ambient air includes both soluble and insoluble forms (although the relative amounts cannot be quantified with the available data), and that noncancer effects of nickel occur at lower inhalation exposure levels with soluble forms of nickel than with insoluble forms (NTP, 1996a, 1996b, 1996c). Thus, the exposure to soluble nickel in ambient air is likely to be lower than the RfC by a larger margin than is apparent from the exposure values listed above.

ATSDR (1997) and Health Canada (1993) also give estimations of total nickel in food and water. ATSDR (1997) states that daily average U.S. intakes from water or food are about 3 E-5 mg Ni/kg/day or 2 E-3 mg Ni/kg/day, respectively. Health Canada (1993) states that average intakes from drinking water or food fall in the range 4 E-6 to 8 E-4 mg Ni/kg/day or 4 E-3 to 2 E-2 mg Ni/kg/day for different age groups, respectively. All of these estimated intakes are at about or below the estimated RfD for *soluble* nickel of 7 E-3 mg Ni/kg/day. It should be noted, however, that the nickel doses in the animal studies did not include the nickel in the diet. Therefore, the RfD presented here represents the dose of nickel *in addition* to the amount in food. The absorption of nickel from food by humans is approximately 1/30 that from water (Sunderman et al., 1989).

As noted in Section 3.3, nickel is also an essential trace element for many animal species. It has been suggested that nickel may be essential in humans, at levels of less than 0.1 mg Ni/day (<1E-3 mg Ni/kg/day for a 70 kg adult), although no nickel requirement or allowance has been set (Nielson, 1991). This value is approximately 8 times lower than the

RfD of 7E-3 mg Ni/kg/day developed in this assessment.

6.1.2 Noncancer Effects

Minimal information is available on the noncancer effects in humans of inhaled soluble nickel, although there is one study (Muir et al., 1993) of radiographic evidence of lung effects in exposed workers, and kidney effects have been reported (Sunderman and Horak, 1981; Sanford and Nieboer, 1992; Vyskocil et al., 1994a). Inhaled soluble nickel compounds are rapidly absorbed and are quickly excreted by the urinary route (Benson et al., 1995). In contrast, absorption of ingested nickel is lower. Oral absorption is reduced when nickel is ingested in food or with food, compared to absorption of nickel ingested in water (Sunderman et al., 1989; Christensen and Lagesson, 1981). Unabsorbed ingested nickel is eliminated in the feces. Nickel absorbed via both the inhalation and oral routes distributes primarily to the kidneys and lungs. Some interspecies variation may exist in the toxicokinetics of soluble nickel.

The target organ for noncancer effects of inhalation exposure to nickel is the respiratory tract, with effects seen in both the lungs and the nose (NTP, 1996a; Dunnick et al., 1989). A variety of inflammatory lesions have been identified in the lungs of rats and mice following subchronic and chronic exposures. Atrophy of the olfactory epithelium was observed at similar HECs. These findings are supported by biochemical evidence of lung damage, based on increased enzyme levels in BAL fluid (Benson et al., 1989). The critical effect for the RfC was lung fibrosis in male rats exposed for 2 years. The observed lung effects are attributed to the direct action of nickel, rather than to systemic exposure.

Although the critical effect was not in the nose, it is interesting to note that the NTP (1996a) study found degeneration of the olfactory epithelium in rats and mice. This lesion is consistent with anecdotal reports of anosmia in nickel workers (Mastromatteo, 1995).

Only one study was located of respiratory effects in humans exposed to soluble nickel (Muir et al., 1993). Radiographs from nickel workers were evaluated, and there was a duration-related increase in the prevalence of irregular opacities. Numerous uncertainties limit the use of this study for risk assessment, including very crude exposure measurements, the absence of a control group (particularly important because the observed lesions were similar in severity to those seen in the general public), mixed exposure to soluble and insoluble forms of nickel, wide variability among readers, and questions regarding degree of ascertainment. However, this study, together with the absence of reports of significant respiratory effects of nickel in the nickel industry, does provide some insight regarding the inhalation toxicity of soluble nickel in humans.

Areas of scientific uncertainty in the development of the RfC relate to dosimetric considerations and the interpretation of alveolar macrophage accumulation. The

NOAEL(HEC) for olfactory epithelial atrophy in females in the subchronic rat study was slightly *lower* than the NOAEL(HEC) for this endpoint in the chronic study, even though the subchronic NOAEL (i.e., based on exposure concentration) was higher than the chronic NOAEL. Thus, response increased with exposure duration when only exposure concentration was considered, but appeared to decrease with exposure duration after dosimetric adjustments were made. This suggests that the dosimetric conversion used may not completely adjust for animal/human differences in the dosimetry of inhaled nickel sulfate, possibly because clearance is not taken into account in the adjustment. Alternatively, the observed difference may be due to inter-experiment variability. The effect of this area of uncertainty is not easily quantified, but use of the lung endpoint from the chronic study appears to provide a reasonable estimate of the critical effect. The chronic study is preferred as the basis for the RfC, because it was conducted with a higher number of animals and included a longer exposure and observation time.

A second area of uncertainty is the adversity of alveolar macrophage accumulation observed in the subchronic and chronic inhalation mouse and rat studies. In the subchronic studies, this lesion occurred at two exposure levels lower than all other lesions, although in the chronic studies this lesion was of comparable or lower sensitivity than the critical effect of fibrosis. Macrophage accumulation can both be a cause of tissue damage, and can reflect tissue damage. This lesion may be on a continuum of effects that includes inflammation and fibrosis, but the macrophage accumulation does not appear to progress with time. Macrophage accumulation was observed in the subchronic study at concentrations below those at which any lesion was observed in the chronic study. Furthermore, as noted in Section 5.2, considering macrophage accumulation to be adverse would result in only a relatively small quantitative effect on the RfC.

The most sensitive target for noncancer effects of oral exposure is the kidney, specifically decreased glomerular function in rats exposed via drinking water for 6 months (Vyskocil et al., 1994b). This is the only animal study that evaluated sensitive biochemical indicators of kidney function. However, this endpoint is supported by histological evidence of kidney damage in animals at higher doses (Dieter et al., 1988) and evidence of nickel distribution to the kidney (Ishimatsu et al., 1995; Dieter et al., 1988; Ambrose et al., 1976). There is some indication of support for kidney damage following occupational inhalation exposure to nickel (Sunderman and Horack, 1981; Vyskocil et al., 1994a), but these studies were done using spot urine samples, which can result in false positives. Sanford and Nieboer (1992) found two individuals with elevated $2m values in spot samples did not have elevated values based on 24-hour sampling. These authors did not find nickel-related increases, but exposure levels were lower than for the other two studies. It is unclear why tubular damage was reported in the human inhalation studies, while Vyskocil et al. (1994b) reported glomerular damage. Sunderman et al. (1988) reported a transient increase in urinary albumin among workers who accidentally drank water contaminated with a nickel plating solution containing

nickel sulfate, nickel chloride, and boric acid. Although this study represents unusual exposure conditions, it does provide support for the target organ. Estimated doses in this acute bolus exposure were 7-36 mg Ni/kg body weight.

Several deficiencies in the principal study limit the strength of the conclusions that can be drawn from it. The study duration was only 6 months, and only one dose was tested, with only 10 rats/sex evaluated at each sacrifice time. In addition, there was considerable variability within groups (decreasing the confidence that the increase was exposure-related), and the response was not very strong. In light of these limitations, additional studies designed to examine sensitive indicators of kidney toxicity would be useful for confirming the critical effect. If such studies are conducted, the hamster would be the experimental species of choice (see Appendix D).

Other oral studies have reported primarily nonspecific indicators of toxicity at low doses, such as decreased body weight (Ambrose et al., 1976; American Biogenics Corporation, 1988). This decreased body weight may reflect nickel interfering with metals binding to metalloenzymes, or possibly to effects on nutrient transport.

Complex effects of nickel on the immune system have been observed. Oral administration of high nickel doses to animals has resulted in decreased immune function (Dieter et al., 1988; Schiffer et al., 1991), and to decrease the delayed type hypersensitivity reaction (a measure of cellular immunity) (Ishii et al., 1993). Sensitivity to nickel (contact dermatitis) results from dermal contact with nickel. Oral exposure of sensitized individuals may also elicit dermatitis reactions (termed systemic contact dermatitis). On the other hand, the data also suggest that low levels of oral exposure may induce tolerance, either preventing or reducing dermal contact sensitivity. Either way, there is no evidence regarding whether oral exposure to nickel can cause the initial sensitization. Because the level of nickel in drinking water varies among municipal water supplies, it may be possible to obtain information regarding the effects of low level exposures on the frequency of nickel allergy, and whether low-level exposures do decrease the occurrence of nickel sensitivity. The feasibility of epidemiological studies regarding nickel sensitivity and drinking water concentrations should be explored. The RfD is designed to protect people from sensitization, but may not necessarily be protective for sensitized individuals. No data were available on the dose that produces sensitization. However, the limited dose-response data on systemic contact dermatitis indicates that few sensitized individuals respond to a bolus dose of approximately 0.02 mg Ni/kg, while a dose of approximately 0.08 mg Ni/kg elicits a response in most sensitized individuals (Burrows, 1992; Cronin et al., 1980; Gawkrodger et al., 1986; Kaaber et al., 1979). These doses were on top of dietary nickel intake.

An area of uncertainty for both the oral and inhalation noncancer assessments relates to the potential reproductive effects of nickel. Several multigenerational studies have been

conducted (Smith et al., 1993; Research Triangle Institute, 1988; Ambrose et al., 1976), and have found evidence of decreased pup viability, but no clear NOAEL or LOAEL could be established. In particular, several of these studies were limited by inconsistent dose-response data. Teratogenicity data have been obtained in only one species (rats), via the oral route (Research Triangle Institute, 1988). Nonetheless, the available data indicate that reproductive effects would occur above the levels causing kidney or lung effects via the oral or inhalation routes, respectively.

Sensitive populations specifically related to nickel exposure have not been identified. Based on the target organs of the kidney and the lung following oral and inhalation exposure, respectively, people with compromised kidney and lung function would be expected to be more sensitive to the adverse effects of nickel. Certain renal conditions may also inhibit the body's ability to eliminate soluble nickel. In such cases, higher toxicity might be expected for a given dose when compared to healthy people.

6.1.3 Cancer

Extensive epidemiology data are available that show that inhalation exposure to mixed soluble and insoluble nickel salts causes the development of lung and nasal cancer in humans. However, the possible contribution of soluble nickel exposure to the observed cancer deaths has long been debated and is difficult to ascertain. Interpretation of the data is complicated by co-exposure to soluble and insoluble forms of nickel and by limitations in exposure measurements. Other confounding factors include include smoking (presumed to be high in these workers), as well as concomitant exposures during some periods to potential lung carcinogens such as arsenic (in the case of Kristiansand) and sulfuric acid mists (in the case of Harjavalta). Although electrowinning and electroplating have been described as operations for which airborne nickel exposures are exclusively to soluble salts (and thus would form the basis for the cleanest epidemiology data), estimates of water-soluble nickel exposure in these industries varied from as low as 18% to as high as 100%, with most estimates falling in the 60 to 90% range (Kiilunen et al., 1997b; Tsai et al., 1995). Exposure estimates were often based on worker recollections or reconstructions from sparse data, and so limit both quantitative and qualitative epidemiological analysis. Electroplaters constitute the workers exposed to the highest percentage of nickel as soluble nickel, with the lowest likelihood of confounding by exposure to insoluble nickel compounds. However, only one study (Pang et al., 1996) investigated cancer mortality in electroplaters, and this study investigated a relatively small cohort, most of whom were exposed for less than 1 year, with negative results.

Substantially more information is available for nickel refinery workers, who are exposed to soluble and insoluble forms of nickel, with clearly higher exposures to insoluble nickel than for electrowinning and electroplating. In particular, a semiquantitative analysis of cancer risk among groups at the Clydach refinery exposed to low or high levels of soluble, oxidic, or

sulfidic nickel found that respiratory cancer risk was higher in groups exposed to soluble and insoluble forms of nickel together than in those exposed to the same level of insoluble nickel or soluble nickel alone (ICNCM, 1990). The epidemiology data are insufficient to determine whether soluble nickel *alone* is a human carcinogen. Overall, the evidence reviewed in this assessment suggests that soluble nickel is not carcinogenic by itself, but rather acts as an effect modifier, increasing the lung cancer risk associated with exposure to other forms of nickel. Soluble nickel also appears to contribute to the risk of nasal cancer only with concomitant exposure to insoluble nickel. This conclusion does not differ substantively from that reached by ICNCM (1990).

In contrast, when nickel compounds of different solubilities are tested separately in experimental animal systems, differences in the ability to evoke tumors by inhaled soluble and insoluble nickel are readily apparent. For example, in inhalation studies for nickel sulfate (a soluble form of nickel) no evidence for tumorigenicity was found (NTP, 1996a). By contrast, for nickel oxide (an insoluble form of nickel) there was some evidence for tumorigenicity in male and female rats, no evidence in male mice and equivocal evidence in female mice (NTP, 1996b). For nickel subsulfide (a form of nickel that is insoluble in water, but slightly soluble in biological fluids), there was clear evidence for tumorigenicity in male and female rats, and no evidence in male and female mice (NTP, 1996c). An animal carcinogenesis study involving co-exposure by inhalation

to soluble and insoluble forms of nickel would be very useful in addressing the potential interaction between these forms.

An area of uncertainty is in the comparison between tissue doses in the animals in the NTP (1996a) study and under occupational conditions. Typical particle sizes under occupational conditions are much larger than those used in the bioassay, resulting in lower doses. However, widely varying particle size distributions have been reported. Reports of the respirable fraction in nickel refineries (corresponding approximately to the percent pulmonary deposition) range from essentially 0% in a Russian refinery (Thomassen et al., 1999) to 2-6.8% in the Kristiansand, Norway, refinery (Werner et al., 1999). Using the data of Vincent (1996) on particle sizes at nickel plants, the pulmonary dose for humans is approximately 7-80 fold higher when exposure is to the particle size distribution used in the animal studies, compared to the pulmonary dose when exposure is to the particle size distribution found under occupational conditions.

Parenteral exposures of animals to nickel compounds of different solubilities follow a similar pattern to that seen after inhalation exposure of experimental animals. The parenteral data, however, provide evidence of weak carcinogenicity of soluble nickel salts. Tumors at the site of intramuscular injection appear to be invoked in an inverse manner with solubility (the greater the solubility, the less the response, or the response is negative). Tumors at the site of intraperitoneal injection show a similar trend, with soluble nickel forms being either negative or slightly positive (depending on the compound), in contrast with metallic nickel, which is dramatically positive. Intraperitoneal exposure to nickel acetate (a soluble nickel form) results in only a weak response in the mouse lung adenoma system, far below the standard positive control of urethane. Nickel acetate also shows some slight evidence of initiation in initiation-promotion studies and demonstrates transplacental initiation. (Insoluble nickel forms were not testable in these latter systems.) A direct test of the hypothesis that soluble nickel enhances the carcinogenicity of insoluble nickel compounds might be to evaluate soluble and insoluble forms together in an initiation-promotion study.

Multiple oral studies of soluble nickel compounds in different experimental animal species (Ambrose et al., 1976; Schroeder et al., 1964, 1974; Schroeder and Mitchener, 1975) did not indicate any cancer-causing potential. (Oral exposures of nickel have only tested soluble forms.) However, each of these oral studies had design flaws that preclude a definitive conclusion that soluble nickel is not carcinogenic after oral exposure. Even though flawed, the results of this oral testing are not inconsistent with those seen after inhalation and parenteral administration of nickel compounds; inhalation animal studies were negative, and the parenteral studies were negative or at most slightly positive. Nonetheless, the lack of definitive oral carcinogenicity data for soluble nickel, and absence of oral studies investigating potential interactions between soluble and insoluble forms of nickel via the oral route, are areas of scientific uncertainty for this assessment.

Evidence for genotoxicity of water soluble nickel compounds is mixed. Water soluble nickel compounds have been generally consistent in inducing effects in certain kinds of mammalian assays. In particular, these effects include mutagenic responses and DNA damage *in vitro*, chromosomal effects including aberrations and sister-chromatid exchanges *in vitro* and *in vivo*, and carcinogenic transformation of mammalian cells *in vitro*. Responses in many of these assays were weak and occurred at toxic doses. However, standard animal carcinogenicity studies conducted at high dose are negative. This inconsistency may be due to differences in the nickel clearance in the test system. In animal studies, soluble nickel compounds are rapidly cleared from the lung after exposure, compared with no clearance mechanism in *in vitro* genotoxicity assays. In addition, cell culture conditions allow high concentrations of nickel salt treatment, while this is precluded in animal studies due to the toxic effects of soluble nickel compounds. Thus, *in vitro* assays subject cells to constant high nickel exposure that could eventually lead to a high concentration of nickel in the nucleus and cause the genotoxic effects.

Kinetic factors also indicate that exposure to soluble nickel alone has a low carcinogenic potential. Unlike insoluble forms of nickel, which can enter the cell via phagocytosis, soluble forms are inefficiently taken up by cells by passive diffusion via the magnesium transport system. The soluble nickel that does enter cells binds preferentially to proteins over DNA, further reducing the amount of soluble nickel ion available to react with DNA. Combined, these factors tend to increase the cytotoxic potential and decrease the potential for DNA damage. There are a number of means by which soluble nickel could increase the carcinogenicity of other chemicals, including the production of an inflammatory response and associated enhanced cell proliferation, impaired clearance, and local generation of oxygen radicals. Overall, these studies suggest that soluble nickel can enhance the carcinogenicity of other chemicals, but the data are insufficient to provide sufficient dose-response information to be useful in risk assessment.

Taken together, these results suggest the following:

- human studies suggest a secondary role for soluble nickel in occupational carcinogenicity, whereas water-soluble nickel salts are not carcinogenic in experimental animals exposed by the inhalation or oral routes;
- water-soluble salts of nickel are distinctly different from water-insoluble nickel compounds with respect to carcinogenic potential, as demonstrated by data from both the inhalation and parenteral routes;
- assays of the carcinogenic activity of water-insoluble nickel compounds should not be used to predict the carcinogenic potential of water-soluble nickel salts.

Thus, under the 1996 proposed Guidelines for Carcinogen Risk Assessment, <u>inhaled soluble nickel</u> compounds would be classified as *cannot be determined*, because the existing

evidence is composed of *conflicting data.*

The carcinogenicity of soluble nickel compounds following oral exposures *cannot be determined* because there are *inadequate data* to perform an assessment. Several negative oral experimental animal studies exist, but each of them has a deficiency that makes conclusive statements difficult. Under the current (1986) guidelines, classification of the carcinogenic response of soluble nickel compounds would be classified as "D", not classifiable as to human carcinogenicity, for both the oral and inhalation routes of exposure.

The mechanisms for the apparent difference in carcinogenic potential between water-soluble and insoluble nickel compounds are not completely understood and may be related to differences in the whole animal and/or cellular pharmacokinetics and/or bioavailability and clearance of nickel when administered in soluble and insoluble forms.

6.2 Dose Response

Mechanistic data are not sufficiently developed in order to generate a biologically-based, or case specific, model for soluble nickel's toxicity for either cancer or noncancer toxicity, for either inhalation or oral exposure.

6.2.1 Noncancer

In the absence of adequate human data, a Reference Concentration (RfC) of 0.0002 mg Ni/m^3 (2E-4 mg Ni/m^3) is recommended based on fibrosis in male rats exposed to soluble nickel sulfate for 2 years (NTP, 1996a). The RfC was calculated from a Benchmark Concentration (BMC)-Human Equivalent Concentration [BMC(HEC)] of 0.0017 mg Ni/m^3 and an overall uncertainty factor of 10. This uncertainty factor is composed of a 10-fold factor for the potential sensitivity of certain populations of humans to the toxicity of soluble nickel (in the absence of data to change the default). Because comparisons of occupational exposure data and data from animal studies indicate that humans are less sensitive than rodents to the respiratory effects of inhaled soluble nickel sulfate, a factor of 1 was used to address potential toxicodynamic differences between the rat and the human. Because dosimetry adjustments were used to extrapolate between the rat and humans, a 3-fold factor for kinetic differences is not needed. Therefore, the total uncertainty factor for animal to human extrapolation is 1, and the composite uncertainty factor for the calculation of the RfC is 10.

Confidence in the critical study used as a basis of the RfC is considered high. This study is of chronic duration, used a fairly large number of animals, measured multiple endpoints, and included an extensive evaluation of the respiratory tract. Multiple BMCs using several different models and endpoints have been evaluated in the selection of this critical effect (see Appendix A). Confidence in the supporting database is considered medium to high. A second chronic bioassay in a different species supports the choice of study and critical effect, but supporting developmental and reproductive toxicity studies are from oral exposure and have

several inadequacies that preclude a

statement of high confidence in the database. Overall confidence in the RfC is also considered medium to high. This means that additional data may more likely change the value of this RfC when compared to a high confidence RfC (for another chemical).

For comparison purposes, an RfC was also developed from the limited human data (Muir et al., 1993), a study of radiographic findings in the lung of nickel workers. Considering the exposure duration-related increase in the prevalence of lung opacities to be a minimal LOAEL, based on the low severity of the lesions, the study identified a minimal LOAEL of 4 mg Ni/m^3, or 1.4 mg Ni/m^3, after accounting for occupational exposure conditions and minute volumes. After taking into account differences in the particle size under occupational and ambient exposure conditions, a LOAEL(HEC) of 0.018 to 0.2 mg Ni/m^3 can be estimated. The range results from uncertainty in estimation of the particle size distribution to which the workers in the Muir study were exposed, due to variability in the particle size distribution between different workstations at a given nickel plant. A total uncertainty factor of 100 would be appropriate with this study. This factor is composed of a full factor of 10 for intrahuman variability, a partial factor of 3 for the use of a minimal LOAEL, and a partial factor of 3 for the use of a less-than-lifetime study. The resulting RfC based on the human data would be 0.00018 to 0.002 mg Ni/m^3 (2E-4 to 2E-3 mg Ni/m^3). These RfCs range from comparable to the RfC based on the animal data, to an order of magnitude higher than the RfC based on the animal data. Use of the RfC derived from the animal study is preferred, in light of the numerous uncertainties in the human study.

A Reference Dose (RfD) of 0.008 mg Ni/kg/day is recommended, based on a minimal LOAEL of 7.6 mg Ni/kg/day for decreased glomerular function (the critical effect) found in rats exposed via drinking water (Vyskocil et al., 1994b), and an overall uncertainty factor of 1000. This uncertainty factor includes a 10-fold factor for the potential sensitivity of certain human subpopulations to the toxicity of soluble nickel (in the absence of data to change the default) and a 10-fold factor to address the potential toxicokinetic and toxicodynamic differences between the rat and the human. An additional factor of 10 was used to address remaining uncertainties in the reproductive studies, the use of a minimal LOAEL, and the progression of the critical effect in the critical study between the 3- and 6-month sacrifice times.

Confidence in the critical study is considered low. The critical study was of 6 months duration and measured multiple sensitive endpoints for kidney function. However, the study only tested one dose and only tested ten animals/sex/exposure duration. Confidence in the supporting database is considered medium. Although the critical study was the only one that reported kidney effects at such low doses, kidney effects have been seen in human studies (Sunderman et al., 1988; Sunderman and Horack, 1981; Vyskocil et al., 1994a). However, a well-conducted human study at lower exposures did not observe any kidney effects (Sanford and Nieboer, 1992). Kidney histopathology has generally not been seen at higher doses in animal studies (American Biogenics Corporation, 1988; Ambrose et al., 1976), and may have been associated with low water intake in the one study in which it was seen (Dieter et al.,

1988). However, no other studies included sensitive measures of kidney function. Uncertainties in the developmental and reproductive toxicity studies also preclude a higher rating for database confidence. Overall confidence in the RfD is considered low, meaning that additional data may more likely change the value of this RfD when compared to a high confidence RfD (for another chemical).

The experimental animal studies on which the RfC and RfD are based are from long-term exposures and continuous dosing, in which careful attention has been paid to determining the doses associated with effects of soluble nickel. Exposure routes in these studies closely match the expected human exposure routes from environmental exposure, thereby increasing the confidence in the resulting estimates of risk.

The data on the toxicity, kinetics, and dynamics of soluble nickel are sufficient to support the development of an RfC and an RfD from the available experimental animal data. Standard assumptions were used in their development, and include:

- the use of experimental animal data as a surrogate for humans;
- the use of lung effects in rats (for inhalation noncancer effects) and kidney response in rats (for oral noncancer effects) as meaningful for extrapolating to human disease;
- the use of factors based on a logarithmic scale (10, 3 or 1) with either the RfC or RfD that address additional scientific uncertainties in the overall database; and
- the use of 1 digit of arithmetic precision for the RfC and RfD, because our understanding of the underlying biology is unlikely to be more precise than this.

The use of these and similar assumptions is common practice in conducting dose response assessments by other environmental and health agencies throughout the world.

6.2.2 Cancer

Specific data from occupational exposures do not exist for soluble nickel compounds from which a quantitative estimate for excess cancer risk could be developed. Moreover, available animal inhalation studies for soluble nickel are unequivocally negative for the cancer endpoint (in contrast to such data on insoluble forms of nickel). Thus, quantitative estimates of cancer risk from the inhalation of soluble nickel compounds, either from the occupational studies or animal bioassays, are not recommended. Furthermore, quantitative estimation of cancer risk from the inhalation of soluble nickel compounds based on the animal bioassay results for insoluble nickel compounds or based on the human occupational data for mixed soluble and insoluble exposures is also not recommended.

Chronic oral bioassays found no indications of carcinogenicity in experimental animals. However, these studies had design limitations that preclude drawing definitive conclusions

regarding the oral route of exposure. Human studies from which to make this determination are not available. Parenteral administration of soluble nickel compounds has yielded slightly positive results in some bioassays, but are not appropriate for quantitative risk determination. Thus, quantitative estimates of cancer risk from the oral exposure of soluble nickel compounds are not recommended.

Several biologically plausible mechanisms for the production of DNA damage by exposure to soluble nickel have been proposed, many of which would indicate a nonlinear dose-response curve. However, the available data are insufficient to determine the doses at which such nonlinearities might occur. Nonetheless, the suggestion of such nonlinearities, together with the lower cellular uptake and nuclear delivery of soluble nickel species (compared with nickel subsulfide), are consistent with the negative animal carcinogenicity studies for soluble nickel. If sufficient quantitative mode of action data were available, soluble nickel *might* be classified as *"unlikely to be carcinogenic at low concentrations but may be carcinogenic at high concentrations*," but the data are not sufficient to support such a classification.

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APPENDIX A. BENCHMARK CONCENTRATION ANALYSES[8]

1. Methods for Modeling

The data sets available for analysis include both quantal and continuous endpoints. The quantal endpoints were modeled using the Weibull mean response regression model (THRESH, ICF Kaiser, 1997a) and polynomial mean response regression model (THRESHW, ICF Kaiser, 1997b):

Weibull model: $P(d) = 1 - \exp\{-\alpha - \beta*(d-d_0)^{\gamma}\}$, Eq. 1

where P(d) is the probability of response at dose d and the four unknown parameters, α, β, d_0, and γ are estimated by maximum likelihood methods. The parameter γ is not constrained to be an integer, but it is constrained to be greater than or equal to 1. A "threshold" (intercept) parameter was included in the modeling only when a sufficient number of dose groups were available (at least 4) and when the models without a threshold provided a relatively poor fit to the data.

The polynomial model can be described as:

$P(d) = 1- \exp\{-q_0 - q_1*(d-d_0) - q_2*(d-d_0)^2 - ... - q_k*(d-d_0)^k\}$, Eq. 2

where the parameters, the q_i's and d_0, are estimated by maximum likelihood methods. The degree of the polynomial was restricted to be no greater than the number of dose groups minus one. The same restrictions on estimation of the threshold parameter, d_0, were applied here as with the Weibull model. In the case of the polynomial model, the total number of parameters estimated was constrained to be no greater than the number of dose groups. Both models are fit by methods of maximum likelihood.

For the continuous endpoint of body weight, several different modeling approaches were used. First, we used the "hybrid" modeling approach described by Gaylor and Slikker (1990) and elaborated by Crump (1995). This approach uses all of the information contained in the original observations, by modeling changes in mean response as a function of dose, but defines BMDs in terms of probability of response.

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[8]The modeling presented here was conducted by ICF Kaiser, K.S. Crump Group. Technical contributions were made by Bruce Allen and Harvey Clewell. Expert modeling assistance was provided by S. Eric Brooks, Holly Bartow, and Cynthia Van Landingham.

For the hybrid modeling approach, the two models used to describe how the probability of response is assumed to vary with dose are the Weibull model and the power model. The Weibull model,

$$P(d) = p_0 + (1-p_0)\,[1 - \exp\{-(\$*d)^{(}\}, \qquad \text{Eq. 3}$$

is identical to the Weibull model used for the quantal endpoints. Maximum likelihood methods are used to estimate the parameters, $ and (, as well as a background mean response level, m(0), and a fixed standard deviation estimate for all dose groups, F. If a normal distribution for the continuous measure around the dose-specific means is assumed, the Weibull model can be expressed as the change in mean as a function of dose:

$$m(d) = m(0) + F[N^{-1}(1-p_0) - N^{-1}((1-p_0)\exp\{-(\$*d)^{(}\})], \qquad \text{Eq. 4}$$

where N^{-1} is the inverse normal function.

The power model was also used to model continuous endpoints:

$$m(d) = " + (\$*d)^{k}, \qquad \text{Eq. 5}$$

where m(d) is the mean response at dose d and the three unknown parameters, ", $, and k, as well as the dose group standard deviations, are estimated by maximum likelihood methods. The dose group standard deviations estimated by the model account for both the variation in the observed data and for any difference between the observed mean and the mean estimated by the model. The parameter k is not constrained to be an integer, but it is constrained to be greater than or equal to 1. The underlying change in probability of response as a function of dose induced by Equation 5 (assuming normal variation around the dose-specific means and a constant variance) is

$$P(d) = 1 - N[N^{-1}(1-p_0) - (\$*d)^{k}/F], \qquad \text{Eq. 6}$$

where N is the cumulative normal function, N^{-1} is its inverse, and F is the standard deviation assumed for all dose levels. This form is for those cases in which increased values of the endpoint are adverse; a similar equation holds for those cases in which decreased values of the endpoint are considered adverse. The Weibull and power models for continuous endpoints were fit using the software program Bench C (ICF Kaiser, 1996a).

Use of the Weibull or power models for continuous endpoints requires definition of a

background incidence of abnormality, p_0, or the specification of a level of response that can be considered the cut-point between normal and abnormal responses, x_0. Specification of p_0 (and of the type of distribution -- assumed here to be normal for all endpoints) implicitly defines a cut-point, x_0, when the parameters for the background variability are estimated as part of the modeling. Similarly, specification of a cut-point determines the background incidence once the background variability is estimated (Crump, 1995). The BMD is then defined as the lower bound on dose at which the increased probability of an abnormal response is equal to 10% (see below). As described below, the cut-point, x_0, was specified in the case of models applied to body weights (data from Ambrose et al., 1976, and American Biogenics Corporation, 1988).

The continuous form of the Weibull model used here assumes that the standard deviation is constant for all dose groups. The power model was run either assuming a constant variance or allowing dose-specific standard deviations. Although the standard deviations do not appear explicitly in the power model (Equation 5), they are also estimated and affect estimates of the probability of response (see Equation 6).

In keeping with EPA practice, extra risk was generally used for these analyses as the more conservative choice in the absence of information on whether the background response occurs via the same mechanism as the chemical-induced response. The dose corresponding to a given extra risk will always be the same or lower than that corresponding to the same percent additional risk. There is very little difference between additional risk and extra risk for low values of P(0), but a background response of 20-50% was observed for some of the endpoints modeled. The exceptions to this rule were the following. The software that is currently in use for applying the Weibull or power models for probability of response for continuous endpoints will only calculate additional risk. In addition, for the body weight change endpoints, only a specific "relative" change level (10%) was considered as the BMR. This choice corresponds to the historical and commonly accepted practice of considering a 10% change in mean body weight to represent an adverse effect.

In order to mimic a commonly accepted practice with respect to body weight, the power model (Equation 5) and a corresponding continuous polynomial model

$$m(d) = \beta + \beta_1 d + \beta_2 d^2 + \ldots + \beta_k d^k \qquad \text{Eq. 7}$$

were applied to the body weight endpoints and used to predict the doses for which there would be a 10% change in mean weight. This approach was implemented using the THC and THWC programs (ICF Kaiser, 1996b, 1996c). The BMDs derived in this approach do not correspond to specified changes in the probability of response. Rather, they correspond to

doses for which the relative change in mean response ([m(d)-m(0)]/m(0)) is 0.10, regardless of variability around the means. In addition, because individual body weight data were available from one study (American Biogenics Corporation, 1988), the body weights were quantized (considered abnormal if they were below a chosen x_0 and normal otherwise), and the counts of abnormal body weights were modeled using the quantal Weibull and polynomial models.

For the quantal models, goodness of fit was determined using the chi-square test. For the continuous models, goodness of fit was determined using an F test that normalizes the differences between the observed and predicted means (using variabilities observed within dose groups) and accounts for the degrees of freedom associated with the predictions and those associated with the within-group variability.

2. Results

2.1 Inhalation

All inhalation endpoints modeled were from NTP (1996a), and, except where otherwise noted, were for chronic exposure. Results of the benchmark concentration modeling for inhalation exposure are presented in Table A1. For several of the endpoints modeled, there were one or more exposure levels with no response above background, and high response at the higher exposure levels. Such concentration-response patterns are poorly fit when the threshold is set to zero in the modeling. However, improved fit in many cases was obtained by allowing the program to calculate the threshold value. (Note that this is a mathematical threshold, not a true biological threshold.) In other cases, the concentration-response data showed a plateau at high concentrations. Because it was not desirable to have high exposure levels that were not informative with regard to the shape of the concentration-response curve drive the modeling, these endpoints were also modeled with the high exposure level dropped. For all of the modeled endpoints, the results of the modeling of all of the data with no threshold are presented in Table A1. The choice of whether it was necessary to try an alternative model (i.e., allow a threshold or drop the high concentration), and which alternative was more appropriate, was based on an evaluation of the goodness of fit of the initial modeling, and a qualitative assessment of the shape of the concentration-response curve. When adequate fits were obtained with both of the alternative approaches, the BMC was in close agreement with (or slightly higher than) the BMC for the alternative shown. All acceptable modeling alternatives were within a factor of two or less.

Because use of the threshold parameter often resulted in the use of an additional parameter (in other cases, it meant that some of the parameters were zero), and dropping the

high-concentration group resulted in one fewer dose group, there were concerns about the potential for overparameterizing the modeling. Indeed, there were several cases for which the resulting estimates had no degrees of freedom (indicated with a # in Table A1). However, in all such cases, a perfect fit was obtained. Because the BMCs for these endpoints were all at least 50% above that for the critical effect, or the effect was considered nonadverse (macrophage accumulation), and because these results were obtained with an alternative modeling approach (i.e., allowing a threshold or dropping a dose group), no attempt was made to further refine the modeling by requiring the modeling to be linear.

The endpoints modeled were those with the highest responses at the lowest human equivalent concentrations (HECs). Macrophage accumulation was modeled even though it was not considered an adverse lesion, in order to facilitate a quantitative discussion of the implications of the alternative judgement (i.e., that it is an adverse lesion). The incidence data from the 7-month interim sacrifice were not modeled, because the high response made such modeling less informative, and because the wide confidence limits related to the small sample size would not be reflective of the overall results from the 2-year study.

The most sensitive endpoint was lung fibrosis in male rats chronically exposed to nickel sulfate, for which a visually acceptable fit was obtained only when a threshold was calculated by the program (Figure A1). The calculated $BMCL_{10}$(HEC) was 0.0017 mg Ni/m^3. A slightly higher $BMCL_{10}$(HEC) of 0.0020 mg Ni/m^3 was obtained with the Weibull model for chronic active inflammation in male rats when the high concentration was dropped; no acceptable fit could be obtained with the polynomial model (Figure A2). A $BMCL_{10}$(HEC) of 0.0021 mg Ni/m^3 was obtained for chronic active inflammation in female rats, but an acceptable fit was obtained only for the Weibull model with the high concentration dropped, and there were no degrees of freedom in the estimate. The $BMCL_{10}$(HEC) values calculated for lung fibrosis in female rats and for alveolar proteinosis in female rats were of similar magnitude (Table A1). As noted in the main document, the $BMCL_{10}$(HEC) values calculated for olfactory epithelial atrophy in female rats following chronic and subchronic exposure did not follow the expected exposure duration-response relationship. As shown in Table A1, an excellent fit was obtained for subchronic exposure, and with the polynomial model for the chronic data. These results are shown graphically in Figures A3 and A4, respectively. The lines shown are the maximum likelihood estimates (MLEs), and so do not take into account differences in sample size. The smaller sample size for the subchronic study means that the difference between the $BMCL_{10}$(HEC) and the MLE is larger for the subchronic study than for the chronic study.

Table A1 also presents modeling results for macrophage accumulation, an endpoint judged to be nonadverse. For all of the sex/species combinations modeled, an acceptable fit

could be obtained only by dropping the high-concentration group, and the resulting estimate had no degrees of freedom. Figure A5 presents the graphical results for the sex/species combination for macrophage accumulation with the lowest $BMCL_{10}$(HEC), 0.0012 to 0.0016 mg Ni/m^3 for male rats.

2.2 Oral

The only oral endpoints modeled were the body weight changes reported by Ambrose et al. (1976) and American Biogenics Corporation (1988). Results of the modeling are shown in Table A2. This table presents the results of modeling using the hybrid continuous model, with the BMR defined as a 10% additional risk of a 10% decrease in the mean, and the results of modeling using THC/THWC, with the BMR defined as a 10% decrease in the mean. The decreased body weight compared to controls in the American Biogenics Corporation, (1988) study could be well represented by the continuous endpoint models (Figure A6). The Weibull model allows a "plateau-ing" that appears to be more consistent with the data, but, with only three dose groups, the linear representation provided by the power model cannot be ruled out. Satisfactory fits for both models were also obtained for decreased body weight data following chronic exposure to nickel sulfate (Ambrose et al., 1976) (Table 6).

Because individual animal data were available for the American Biogenics Corporation (1988) study, the data were also quantized using the same cut-point as used in the hybrid modeling, and modeled as quantal data. This allowed a direct comparison between modeling based on the actual number of low-body-weight animals, and modeling using the hybrid approach that predicts the probability of low-body-weight animals based on the variability around the mean. As shown, the results are very similar, with some small differences attributable to differences in the method used to maximize the likelihood for the two approaches. The BMD estimates (representing lower bounds on the maximum likelihood estimates) show much closer agreement than do the MLEs.

Haber et al. (1998) presented a discussion of the choice of the appropriate benchmark response for the body weight endpoint. They stated:

> Draft U.S. EPA guidance (U.S. EPA, 1996c) and a peer review of that guidance (U.S. EPA, 1996d) have recommended that biological significance should be the preferred basis in the choice of the BMR. The body weight results provide a particularly relevant basis for starting to carefully consider some of the issues associated with specifying biologically significant changes, where the concept of biological significance relates to the identification of a

certain degree of change in a continuous measure that is considered indicative of abnormality or adverse response. At issue are questions concerning whether biological significance should be based on group-level observations (corresponding, in our analyses, to BMDs based on a 10% decrease in the *mean* body weight), which appears to be the commonly accepted practice, or on changes in incidences of *individual* adverse responses (corresponding to the hybrid approach).

The difference between these two approaches can be illustrated simply by assuming a symmetrical distribution of body weight and that an effect on body weight is evenly distributed in the population. In this case, a 10% decrease in the mean corresponds to a 10% decrease in body weight for *50%* of the animals. A dose that affects 50% of the animals is almost certainly greater than a dose that causes the 10% increased risk that is the basis for the BMR in the hybrid approach. As expected from this analysis, BMDs based on current toxicological practice (i.e, a 10% decrease in the *mean* response being adverse) resulted in higher MLEs and BMDs than those estimated based on a 10% increase in the incidence of "low-weight" animals (Table A2). (For the purposes of comparison, "low-weight" was defined to be 10% below the control group mean.) A BMD of 1.5-4.9 mg Ni/kg/day was calculated for the American Biogenics Corporation data when the BMR was defined in terms of a 10% increase in incidence of low weights. In contrast, the BMD was 17 mg Ni/kg/day (power model or polynomial model) based on a 10% decrease in the mean. While a 10% decrease in the mean body weight may be an adequate cutpoint for determining when a *group* of animals can be considered adversely affected, it may be a poor indicator of a biologically significant change for an *individual* animal. Although this point is illustrated here using body weight data, similar problems are likely to arise in other attempts to use biological significance as the basis for the choice of BMR, because biological significance in animals is often defined in terms of changes at the group level, while the probability of an effect is modeled for an individual.

Haber et al. (1998) also noted that the current definition of adversity for body weight is consistent only as an indicator of LOAELs, and that, therefore, the 10% decrease in mean weight identifies when *adversity* (on a group level) has become apparent. This means that the defining the BMR as a 10% decrease in mean weight may result in estimates that are more like LOAELs than NOAELs.

Because the body weight BMDs calculated using the hybrid model were based on an endpoint (10% increased risk of a 10% decrease in body weight) that is clearly more sensitive than the standard definition of adversity of a 10% decrease in mean weight, these BMDs were not considered appropriate as the basis for the RfD. The BMDs based on a 10% decrease in a mean body weight were well above the LOAEL used as the basis for the RfD, and thus were not of further relevance to the assessment.

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Table A1. Summary of Results of Modeling Quantal Data for Nickel Sulfate Inhalation[1]

		Polynomial			Weibull		
Endpoint	Compute Threshold	MLE	BMC	G-O-F P-value	MLE	BMC	G-O-F P-value
M rat Lung fibrosis	No	0.0012	0.00069	0.00075	0.0014	0.00084	0.0017
	Yes	0.0022	**0.0017**	0.032	0.0022	0.0017	0.032
M rat Chronic Active Inflammation	No; High dose dropped	0.0015	0.0011	0.031	0.004	0.0020	0.53
	No	0.0012	0.00059	0.000065	0.0013	0.00073	0.00014
F rat Chronic Active Inflammation	No; High dose dropped	0.0013	0.0011	0.00055	0.0044	0.0021	1#
	No	0.0016	0.00081	0.000007	0.0013	0.00087	0.000009
F rat Lung fibrosis	No; High dose dropped	0.0015	0.0012	0.0004	0.0045	0.00240	0.1
	No	0.0016	0.00078	0.0	0.0015	0.00093	0.0
F rat Alveolar proteinosis	No	0.0033	0.0025	0.010	0.0032	0.0026	0.016
	Yes	0.0032	0.0028	1#	0.0036	0.0028	1#
F rat Olfactory epithelial atrophy, subchronic exposure	No	0.0011	0.00048	0.96	0.0011	0.00073	0.72
	Yes	0.00098	0.00053	0.99	0.0011	0.00073	0.72
F rat Atrophy of olfactory epithelium	No	0.0035	0.0026	0.73	0.0035	0.0025	0.32
	Yes	0.0035	0.0026	0.73	0.0035	0.0025	0.32
M rat Atrophy of Olfactory Epithelium	Yes	0.005	0.0038	0.79	0.0056	0.0042	0.79
	No	0.0056	0.0043	0.58	0.0056	0.0042	0.33
M mouse atrophy of olfactory epithelium	No	0.0051	0.0036	0.11	0.0049	0.0039	0.14
	Yes	0.0045	0.0039	1#	0.0047	0.0037	1#
F mouse Chronic active inflammation	No High dose dropped	0.0086	0.0054	1#	0.0086	0.0054	1#
	No	0.0095	0.0060	0.7	0.010	0.0070	0.35
F mouse Bronchialization	No; High dose dropped	0.0094	0.0058	0.71	0.010	0.0064	1#

		Polynomial			Weibull		
Endpoint	Compute Threshold	MLE	BMC	G-O-F P-value	MLE	BMC	G-O-F P-value
	No	0.0065	0.0039	0.061	0.0074	0.0045	0.096
F mouse Macrophage accumulation	No; High dose dropped	0.0042	0.0024	0.42	0.0051	0.0032	1#
	No	0.0035	0.0017	0.024	0.0037	0.0022	0.058
M rat Macrophage accumulation	No; High dose dropped	0.0016	0.0012	0.10	0.0026	0.0016	1#
	No	0.0017	0.00088	0.019	0.0016	0.0010	0.029
	Yes	0.0023	0.00180	0.52	0.0023	0.0018	0.52
F rat Macrophage accumulation	No; High dose dropped	0.0021	0.0013	0.15	0.003	0.0019	1#
	No	0.0019	0.00091	0.026	0.0019	0.0010	0.029
	Yes	0.0028	0.0019	0.35	0.0028	0.0019	0.039

[1]All modeled data from NTP 1996a, BMR of 10%, chronic exposure unless otherwise stated.
No degrees of freedom in the estimate

Table A2. Decreased Body Weight Following Oral Exposure to Nickel

BMR Definition	Power			Weibull			Polynomial		
	MLE	BMDL	G-O-F P-value	MLE	BMDL	G-O-F P-value	MLE	BMDL	G-O-F P-value
American Biogenics Corporation 1988, Decreased body weight at 13 weeks in male rats exposed via gavage to nickel chloride									
10% decrease in mean	21	17	0.15	N/D[1]	N/D	N/D	21	17	0.15
10% additional risk of 10% decrease, quantal modeling	N/D	N/D	N/D	4.4	1.5	1.0	4.4	1.5	1.0
10% additional risk of 10% decrease, continuous modeling, "hybrid approach"	5.9	4.9	0.17	2.4	1.5	N/A[2]	N/D	N/D	N/D
Ambrose et al. 1976, Decreased body weight at 78 weeks in female rats exposed in diet to nickel sulfate [3,4]									
10% decrease in mean	67	58	0.60	N/D	N/D	N/D	67	58	0.60
10% additional risk of 10% decrease, continuous modeling, "hybrid approach"	30	24	0.68	16	11	0.95	N/D	N/D	N/D

[1]N/D = Not Done; this form of the model does not exist

[2]N/A = Not available. Goodness-of-fit p values could not be calculated for some models due to insufficient degrees of freedom.

[3]Quantal modeling could not be conducted for Ambrose et al. (1976), because individual animal data were not available.

[4]The MLE and BMD values shown differ from those shown in Haber et al. (1998), due to the use of a generic food factor of 0.05, rather than the strain-specific value used for this assessment.

APPENDIX B. DOCUMENTATION OF PARTICLE SIZE DISTRIBUTIONS AND CALCULATION OF HECS FOR NTP 1996A

The attached tables present the exposure concentrations reported by NTP (1996a) for nickel sulfate hexahydrate, the calculated nickel exposure levels, and the duration-adjusted values. In addition, the concentration-specific particle size distributions and the corresponding region-specific RDDR values are presented, as well as the calculated HEC values.

Table B1. Calculation of the HEC Values for the Chronic Mouse and Rat Studies with Nickel Sulfate Hexahydrate (NTP, 1996a)

Mouse Data																
$NiSO_4 \cdot 6H_2O$	Ni conc	Duration adj	MMAD	GSD	RDDR ET F	RDDR ET M	RDDR PU F	RDDR PU M	RDDR Thoracic F	RDDR Thoracic M	HEC F ET	HEC M ET	HEC F PU	HEC M PU	HEC Thoracic F	HEC Thoracic M
0.25	0.056	0.010	2.3	2.2	0.26	0.28	0.88	0.90	1.2	1.3	0.0026	0.0028	0.0088	0.0090	0.012	0.013
0.5	0.11	0.020	2.3	2.1	0.27	0.29	0.88	0.90	1.3	1.3	0.0054	0.0058	0.018	0.018	0.025	0.026
1	0.22	0.040	2.5	2.0	0.26	0.28	0.85	0.87	1.2	1.2	0.010	0.011	0.034	0.035	0.047	0.049
Rat Data																
$NiSO_4 \cdot 6H_2O$	Ni conc	Duration adj	MMAD	GSD	RDDR ET F	RDDR ET M	RDDR PU F	RDDR PU M	RDDR Thoracic F	RDDR Thoracic M	HEC F ET	HEC M ET	HEC F PU	HEC M PU	HEC Thoracic F	HEC Thoracic M
0.12	0.027	0.0048	2.5	2.4	0.18	0.31	0.51	0.45	0.64	0.62	0.00084	0.0015	0.0024	0.0021	0.0031	0.0030
0.25	0.056	0.0100	2.2	2.2	0.19	0.33	0.53	0.47	0.68	0.66	0.0019	0.0033	0.0053	0.0046	0.0068	0.0066
0.5	0.11	0.020	2.3	2.1	0.20	0.34	0.54	0.48	0.69	0.66	0.0039	0.0068	0.011	0.0095	0.014	0.013

Table B2. Calculation of the HEC Values for the Subchronic Mouse and Rat Studies with Nickel Sulfate Hexahydrate (NTP, 1996a)

Rat Data																
$NiSO_4 \cdot 6H_2O$	Ni conc	Duration adj	MMAD	GSD	RDDR ET F	RDDR ET M	RDDR Pu F	RDDR Pu M	RDDR Thoracic F	RDDR Thoracic M	HEC ET F	HEC ET M	HEC F PU	HEC M PU	HEC Thoracic F	HEC Thoracic M
0.12	0.027	0.0048	2.3	2.1	0.092	0.15	0.56	0.56	0.66	0.68	0.00044	0.00069	0.0027	0.0027	0.0031	0.0033
0.25	0.056	0.0100	2.1	2.7	0.084	0.13	0.47	0.48	0.61	0.65	0.00084	0.0013	0.0047	0.0048	0.0061	0.0065
0.5	0.11	0.020	3.1	2.9	0.079	0.12	0.48	0.48	0.56	0.58	0.0016	0.0024	0.0096	0.0096	0.011	0.012
1	0.22	0.040	1.8	2.2	0.090	0.15	0.52	0.53	0.68	0.73	0.0036	0.0058	0.021	0.021	0.027	0.029
2	0.45	0.080	2.0	2	0.094	0.15	0.57	0.57	0.70	0.74	0.0075	0.012	0.045	0.046	0.056	0.059
Mouse Data																
$NiSO_4 \cdot 6H_2O$	Ni conc	Duration adj	MMAD	GSD	RDDR ET F	RDDR ET M	RDDR PU F	RDDR PU M	RDDR Thoracic F	RDDR Thoracic M	HEC ET F	HEC ET M	HEC F PU	HEC M PU	HEC Thoracic F	HEC Thoracic M
0.12	0.027	0.0048	2.3	2.1	0.17	0.23	0.74	0.83	1.0	1.2	0.00079	0.0011	0.0035	0.0040	0.0050	0.0057
0.25	0.056	0.0100	2.1	2.7	0.16	0.22	0.74	0.85	1.1	1.2	0.0016	0.0022	0.0073	0.0085	0.011	0.012
0.5	0.11	0.020	3.1	2.9	0.13	0.18	0.71	0.81	0.97	1.1	0.0027	0.0037	0.014	0.016	0.019	0.022
1	0.22	0.040	1.8	2.2	0.18	0.26	0.76	0.88	1.1	1.3	0.0073	0.010	0.030	0.035	0.045	0.051
2	0.45	0.080	2.0	2	0.18	0.26	0.75	0.86	1.1	1.3	0.015	0.020	0.060	0.069	0.088	0.100

APPENDIX C. ANALYSIS OF OCCUPATIONAL EXPOSURE TO NICKEL

Information on the human toxicity of nickel is gleaned primarily from occupational exposures where many epidemiologic observations of nickel workers have been conducted. Most of the data that form the basis of these observations are from exposures to both soluble and insoluble nickel compounds. Due to these mixed exposures, it has not been possible to distinguish between the contributions of insoluble and soluble nickel to observed cancers.

Because the reliability of much of the epidemiology data is related to the accuracy of the exposure assessments, an understanding of the issues related to exposure assessment for nickel workers is important. The following section describes processes and work activities related to nickel exposure, the relative predominance of different nickel species for various processes, air sampling considerations, and some exposure assessment issues related to the electrowinning studies.

1. Occupational Process and Work Activities: Implications for Nickel Speciation

There are many occupational processes that involve potential exposures to nickel. Mining, milling, and smelting processes involve the purification of nickel from nickel ores. Impure nickel can undergo further purification during refining. Metal finishing involves solutions of soluble nickel salts. Three operations in the nickel industry have been identified in which airborne nickel exposures have been reported to be almost exclusively to soluble salts: electroplating, electrowinning, and nickel chemicals industry segment.

Metal finishing is a series of different processes in which the characteristics of the surfaces of metal objects are modified to provide desirable physical properties, such as hardness, corrosion resistance, and reflectivity (shininess). One form of metal finishing involves the application of a surface coating of nickel by several different processes. The most common method of applying a nickel coating is electroplating, although a similar process called electroless plating is widely used. In electroplating, an object to be plated (the workpiece) and a bar of nickel metal are connected to a source of direct current such that the workpiece is negatively charged with respect to the nickel bar. The workpiece is frequently called the cathode and the nickel bar is called the anode. The workpiece and the nickel anode are immersed in a solution of nickel salts and other chemicals called the plating bath. In the solution, the nickel exists as divalent ions (Ni^{++}) complexed with water molecules or other chemical species also present in the solution. The nickel ions migrate to the cathode, where they receive electrons and are converted to nickel metal:

$Ni^{++} + 2e^- \rightarrow Ni$

In order to maintain electrical neutrality, electrons are removed from the nickel anode to form nickel ions:

$Ni \rightarrow Ni^{++} + 2e^-$

In essence, nickel is transferred from the metal anode through the aqueous plating bath to the workpiece.

The salts commonly used in electroplating, listed in order of decreasing solubility, are:

Nickel fluoroborate
Nickel sulfamate
Nickel formate
Nickel chloride
Nickel sulfate
Nickel ammonium sulfate
Nickel acetate

Plating baths also can contain boric acid, phosphoric acid, phosphorous acid, ammonium chloride, fluoroboric acid, sodium thiocyanate and a variety of proprietary ingredients that help in the formation of a nickel coating with the desired physical characteristics. These proprietary ingredients include a variety of organic compounds, including aromatic sulphonamides or sulphonimides, formaldehyde and other aldehydes, amines, nitriles, and azo dyes (Dennis and Such, 1972).

The process known as electroless plating also deposits metallic nickel onto workpieces, but the conversion of nickel ion in solution to the metal is mediated by a chemical reducing agent rather than through electrolysis. The nickel salts used in electroless plating are among those also used in electroplating baths.

During the deposition of nickel metal onto the workpiece, nickel ions are removed from the solution around the workpiece. Although nickel ions are migrating into this area from the bulk of the plating bath, nickel ions may plate onto the workpiece faster than they are replenished by migration. The resultant decrease in the concentration of nickel around the workpiece may adversely affect the quality of the plated coating or decrease the efficiency of the process. To counter this depletion, plating baths are agitated, commonly by bubbling air

through them. As the air bubbles rise to the surface and break, small droplets of the plating solution are released into the air. Larger droplets fall back into the tank or deposit on surfaces nearby. Smaller droplets become airborne and drift into the general plant air where the workers can inhale them.

Small droplets rapidly lose their water by evaporation at a rate dependent on humidity, leaving behind solid particles made up of crystals of nickel salts and the other components of the plating bath. Because of the way airborne nickel is sampled from the environment, it is not usually possible to determine the proportions of solid particles and liquid droplets in the air. In sampling airborne contaminants, air is drawn through a filter by a small suction pump. Airborne particles deposit on the filter and remain there while air is drawn over them continuously. The water in deposited droplets evaporates, leaving solid particles behind on the filter. Microscopic examination of the deposit would show only those particles.

Electrowinning, an electrolytic process in nickel refining, is a process similar to that of electroplating and employs similar aqueous solutions of nickel salts. Different degrees of electrical current are used, and impure forms of nickel are present during electrowinning, since this is a refining process. The pure metallic nickel can then be used in metal finishing operations. As with the plating operations, the aerosolization of the soluble nickel salts accounts for thehigh soluble nickel exposures relative to insoluble forms.

A third nickel operation with relatively high soluble nickel exposures is the nickel chemicals industry which includes the use of many different compounds. The manufacture and processing of nickel sulfate and nickel chloride, as well as the other soluble salts used in electroplating are included in this category. Facilities that manufacture nickel pigments (insoluble), coatings for welding rods, and miscellaneous substances such as nickel carbonate and oxide are also usually included. The soluble nickel salts are handled as crystalline solids as well as solutions. However, no human health observations have been published for workers in this industry segment.

As noted above, electrowinning and electroplating baths are both aqueous solutions of nickel salts. Since the airborne nickel in these operations is derived from droplets of the solutions, it has generally been assumed that exposures in electrowinning and electroplating operations are to soluble nickel compounds with no exposure to insoluble compounds. Four investigations have examined the proportions of soluble and insoluble nickel in airborne nickel in these environments.

Kiilunen et al. (1997a) measured exposure to soluble nickel in an electrowinning

operation at the Outokumpu Oy, Finland refinery. Area and personal air samples were collected on mixed cellulose ester filters from which soluble nickel was extracted with hot water (70EC for one hour). After decanting the extract, the filters were rinsed with water three times and dried overnight at 100EC. The dry filters and back-up pads were dissolved in concentrated nitric acid followed by hydrochloric acid to identify the insoluble nickel fraction. A total of 140 samples were collected in 50 locations in six identified areas of the plant, as well as several other sites. Water-soluble nickel constituted 86% of airborne nickel in the leaching area, 96% in the solution purification area, 99.7% in the tankhouses, and 92% in other areas.

Kiilunen et al. (1997b) also measured soluble and total airborne nickel exposures in three electroplating shops drawn from a larger survey of 38 Finnish plating shops. The shops were selected to represent what the authors felt were clean, intermediate and dirty shops. Soluble and insoluble nickel was identified by hot water and acid extraction as in the previous study. Airborne nickel exposures are reported for the clean and dirty shops. The soluble fraction of the airborne nickel varied from 18% to 100%. Similar results were found in two American electroplating shops where the soluble fraction of the airborne nickel was 90.3% and 63.7% (Tsai et al., 1996). This study identified the various nickel species in the airborne particulate through a complex leaching procedure (Zatka et al., 1992). Although specific chemical compounds were not identified, sulfidic, oxidic and metallic nickel were found in the airborne particulate in both shops. In another study, soluble forms of nickel predominated in two electroplating facilities. Oxidic and metallic forms of nickel were present, but no sulfidic nickel was found (Vincent et al., 1995). The origin of the insoluble nickel is not clear. However, it has been hypothesized that these other nickel forms result may have been formed from additives in the plating bath.

Unlike electrowinning and electroplating, nickel mining, milling and smelting operations have been thought to primarily involve exposure to insoluble forms of nickel. The Doll report (ICNCM, 1990) and the NiPERA Criteria Document (NiPERA, 1996) both report that in mining, milling and smelting operations, exposures were to insoluble nickel compounds. Considering that the nickel salts used in electroplating had been purified extensively, it appears to be unlikely that the insoluble airborne nickel contains the same chemical compounds as are observed in nickel mines, mills and smelters. However, small exposures to soluble nickel may have occurred.. For example, Warner reports that an analysis of airborne dust at INCO's Sudbury, Ontario smelter showed that about 5-25% of the nickel was soluble in a solution buffered at pH 5. About 10% of ore dust was similarly soluble (Warner, 1984).

2. Air Sampling Considerations

In calculating risk estimates, it is important to recognize that different methods of measuring aerosol concentrations can produce significantly different exposure estimates. Estimating the potential tissue does requires knowledge and understanding of the air sampling method employed to estimate the worker exposure. Different sampling methods were used in different studies. There are a number of factors to consider, including the location of the sampling instrument relative to the breathing zone, the areas the worker is in during the course of a day, the size distribution of the particulates, and the design of the sampler used to measure the exposure.

One of the significant parameters to consider is the location of a sampling instrument with respect to the breathing zone of workers in the environment being evaluated (i.e. the contrast between area and personal samples). Before the general availability of personal air sampling pumps, air contamination was commonly measured using samplers placed in fixed locations in areas of a plant where exposures were considered to be "typical." However, contaminant concentrations commonly vary during a workday, and workers move around a workplace between areas of different contaminant levels, and are thus exposed to varying concentrations of contaminants. Therefore, when assessing worker exposure, it is preferred to take personal breathing zone air samples rather than area samples in fixed locations. Current industrial hygiene practice is to attach small battery-operated vacuum pumps to a worker's clothing and connect these to a sampling head placed in the worker's breathing zone. The sampler collects the airborne contaminant throughout the exposure period and integrates the concentration changes in the process as the worker moves in and out of areas of different concentrations. The measured exposure is thus a time weighted average (TWA) for the whole period that the sampler was running.

Industrial hygiene experience has shown that there is no uniform relationship between work area and personal measurements taken in the same workplace on the same day. The amount of time that a worker spends in an area where airborne contamination is being measured will depend on the process and the work routine at the facility. Where a worker's activities bring him or her close to a point of contaminant generation, the exposure will be higher than the concentration in the general work area. Conversely, when a process is largely automatic, a worker may spend a large fraction of the workday in a control room or other relatively clean area. Reports of exposure surveys often do not describe work routines and do not provide enough information to estimate personal exposures from area samples. When using reported workplace exposures as the dose metric in developing estimates of risk, it is advisable to restrict consideration to personal exposure measurements. Incorporating area sampling

measurements into a risk analysis will increase the uncertainty of the calculated risk values.

Another key determinant for the risk of disease from inhaled toxic material is the actual dose of the substance to the target organ or to a site which can influence the disease outcome (Vincent, 1995). While this tissue dose is related the concentration of the toxicant in the air, the particle size also plays a key role in determining the tissue dose, since the particle size largely determines whether a particle enters the respiratory tract and how far it penetrates. For industrial hygiene data, three particle size fractions are defined corresponding to the probability of deposition in the nasopharyngeal region, in the bronchial region, or in the gas-exchange region. These fractions are described as inhalable, thoracic, and respirable particulate, respectively. Generally, the smaller the particle, the deeper it can penetrate into the respiratory system. For example, for particles with an aerodynamic diameter of 1 Fm, 97% of the particulate mass would be deposited in the gas-exchange region. In contrast, 77% of 10 Fm particles would deposit in the nasopharyngeal region, but only 1% would deposit in the gas-exchange region. Particles as large as 100 Fm can enter the nasopharyngeal region, but would not appreciably penetrate to the respiratory region (ACGIH, 1998).

Industrial hygiene air-sampling methods call for measurement of the inhalable particulate if the toxicant can exert its effect anywhere in the respiratory tract, while the respiratory particulate fraction is often collected for toxicants that specifically damage the gas-exchange region. In the case of soluble nickel studies, the industrial hygiene data were generally collected so as to measure the inhalable fraction, in light of the concerns for a role of soluble nickel in the development of lung, nasal cavity, and stomach cancers. The sampling methods that were used in the different nickel studies, however, do not collect the entire inhalable fraction with equal efficiency.

Since the 1960s, the most common sampler for airborne particulate has been a 37 mm filter mounted in a plastic cassette with a 4 mm orifice through which contaminated air entered. Samples collected with this instrument were designated as "total dust" or "total particulate". Experiments have found that this sampler actually collected less particulate than could be inhaled through the nose and/or mouth (Vincent, 1995). The fraction collected was nearly 100% for small particles but was less than 50% for particles larger than 10 Fm. The Institute of Occupational Medicine (IOM) in Edinburgh, Scotland developed a sampler that closely mimics the size selection characteristics of the nose and mouth, and since that time several other instruments have been developed that also follow these selection characteristics (Kenny et al., 1997). In Great Britain, samplers using 25 mm filters have been used that have been found by some investigators to follow the inhalable criterion quite well (Kenny et al., 1997), but not by others (Mark and Vincent, 1986; Terry and Hewson, 1996).

Table 6-28 of Volume II of NiPERA (1996) lists the type of sampler used for various studies. The published studies all used 37-mm cassettes, while 25-mm closed-face cassettes were used in the Health and Safety Executive (HSE) surveys. The table identifies the aerosol fraction collected by the samplers used in the HSE surveys as "total". However, the units are likely to be the same as those described by Kenny et al. (1997), which may actually collect inhalable particulate. The study by Kiilunen, et al. (1997a) used 37-mm cassettes, which collect "total" particulate, while the Tsai et al. (1996) study and the University of Minnesota study referenced in tables 6-27 and 6-28 of the NiPERA document both used the IOM inhalable particulate sampler.

When an inhalable particulate sampler and a "total" dust sampler are placed side-by-side in the same environment, the inhalable sampler will usually collect more sample (Werner et al., 1996), and the difference is more pronounced with larger particles. In environments where all the airborne particles are small (less than 1 Fm in diameter), the two types of samples will collect the same amount of material, but the ratio of total sample collected can be as high as 4.0 in environments that contain a high proportion of particles larger than about 20 Fm. The ratio between the amounts of total sample collected by the two types of samplers are also affected by wind velocity, the position of the samplers on the worker and other unknown factors. Tsai et al. (1996) found that there were differences in this ratio in the two electroplating shops that they investigated. In a more comprehensive study of an electrolytic refining operation, the ratio of inhalable to "total" particulate varied from 1.2 to 4.0 in different parts of the facility (Tsai et al., 1995). In developing risk assessments that incorporate airborne exposure data measured with different instruments, Werner et al. (1996) recommend a set of default values for converting "total" particulate measurements to inhalable. For mists (such as are generated in electroplating) they suggest that a conversion factor of 2.0 should be used.

The data in tables 6-26, 6-27 and 6-28 of the NiPERA Criteria Document (1996) for personal samples collected in areas of electroplating shops where exposures were reported as being predominantly to soluble nickel are collated in Table C-1. This table includes a column that provides an estimate (in mg/m^3) of the inhalable concentration of soluble nickel. Where the original studies used 37-mm filters, the reported values are multiplied by a factor of two as recommended by Werner et al. (1996). For the 25 measurements, the median exposure is about 0.020 mg/m^3; 80% of the exposures are less than 0.080 mg/m^3 and fewer than 20% are below 0.010 mg/m^3.

3. Soluble Nickel Exposures in Electrolytic Refining (Electrowinning)

The Doll report (ICNCM, 1990) identifies three facilities in which nickel metal is produced by an electrowinning process: an INCO plant in Port Colborne, Ontario; the Falconbridge refinery in Kristiansand, Norway; and the Outokumpu Oy refinery in Finland. The report provides estimates of exposure to soluble nickel and subsequent papers provide additional data for the INCO and Outokumpu Oy plants.

No information is provided in the Doll report on the sampling or analytical methodology used at the Port Colborne refinery, and only ranges and upper limits are identified for the exposures. Assuming that the data represent area samples, taken over relatively short time spans, exposures in electrolytic workplaces were <1.0 mg/m^3 for total nickel and <0.3 mg/m^3 for soluble nickel. Pumping anode slimes and washing anode scrap generated exposures of 1.0-3.0 mg/m^3 of soluble nickel. Recent exposures (i.e. in the mid 1980s) were stated to be <1.0 mg/m^3 and <0.20 mg/m^3 for total and soluble nickel respectively (ICNCM, 1990).

Warner, who worked for INCO, Ltd., reported personal exposures to soluble nickel in electrowinning tankhouses, presumably at the Port Colborne plant (Warner, 1984). Tankman exposures ranged from 0.012 to 0.071 mg/m^3 with an average concentration of 0.030 mg/m^3; the range for anode scrapmen was 0.001-0.236 mg/m^3 with the average being 0.052 mg/m^3. There is no indication of potential exposures to other forms of nickel.

Nickel exposures at the Kristiansand refinery are provided in the Doll report and also repeated with slight modification by Andersen et al. (1996). Table 6 in the Doll report provides qualitative estimates of nickel exposures classified by operation, time period, and nickel species (ICNCM, 1990). Exposure levels were identified as negligible, low, medium, or high. The corresponding ranges were <0.5 mg/m^3 for low exposures, 0.5-2.0 mg/m^3 for medium and >2.0 mg/m^3 for high exposures. In the period from 1946-1977, the concentrations of oxidic and sulfidic nickel (both insoluble) were noted as being low (<0.5 mg/m^3) in the nickel tankhouse, while the concentration of soluble nickel was medium (0.5-2.0 mg/m^3). From 1978-1984 concentrations of oxidic and sulfidic nickel were negligible, while exposure to soluble nickel was low. As stated in the Doll report, estimated concentrations were based largely on the subjective judgments of retired personnel. Airborne samples were analyzed only for total nickel, with estimates for the different species being assumed to be the same as in the materials being handled. Nonstandard air sampling instruments were apparently used. The Doll report states that the results were arrived at on the understanding that they were the best estimates of what the readings would have been if they had been taken as "total dust" obtained with standard personal gravimetric sampling equipment. In contrast, Appendix B of the

NiPERA Criteria Document states that good sampling data had been obtained at the plant during the period of 1985-1993 and was summarized in the tables at the end of the appendix (NiPERA, 1996). However, the tables do not contain the indicated data.

Kiilunen et al. (1997b) report an extensive series of historical and current exposure measurements to soluble nickel at the Outokumpu Oy refinery with measurements dating back to 1966. Area samples, which were collected on 37-mm filters at 20 liters per minute, ranged from 0.26 to 0.76 mg/m^3 in the tankhouses. During the period of 1991-1993, area measurements were in the range of 0.112 to 0.484 mg/m^3. Mean breathing zone exposures from 1979 through 1981 were found to be 0.16 to 0.23 mg/m^3. The authors report that workers did not wear respiratory protection during the 1979 through 1981 period. Measurements taken during 1991 to 1993 illustrate the complication in exposure assessment introduced by the use of respirators. Workers did not wear respirators when they were performing tasks deemed to have low exposures, but they did wear respirators when high exposures were anticipated. The authors measured nickel concentrations inside respirators when they were being worn and found exposures from 0.0005 mg/m^3 to 0.0069 mg/m^3. When no respirators were in use, exposures were 0.0013 to 0.0021 mg/m^3. As noted above, more than 90% of the airborne nickel was in soluble form, although the nature of the insoluble material was not identified.

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Table C1. Nickel Personal Exposure Data for Electroplating[1]

Reference or Company Code[2]	Location, Plant, or Process	Number of Samples (Workers)[7]	Type of Sampler[3]	Aerosol Fraction[4]	Sampler	Range of Exposures (mg Ni/m^3)	Mean Aerosol Exposure Concentrations (mg Ni/m^3)	Inhalable Ni (mg/m^3)[12]	Year(s) Samples Taken	Study Quality[5]
Ghezzi et al., 1989	Italy (6 plants)	92(23)	P	T	37-mm	U	0.004-0.19[6]	0.182	1989	S
Hery et al., 1990	France (5 plants)	102(U)	P	T	37-mm	0.0001-0.80	0.004-0.079[6]	0.082	pre-1990	S
Mahieu et al., 1990	France (several plants)	U(U)	S	T	37-mm	0.001-1.77	U	0.08	1990	G
Tola et al., 1979	Finland	20(4)	P	T	37-mm	0.03-0.16	0.091	0.182	1979	S
EIS-05, 1993	Electroless plating	4(U)	P	T	NG	0.01-0.05	<0.023	0.04	1991-93	G
EIS-05	Electrolytic plating	4(U)	P	T	NG	0.02-0.19	0.1	0.2	1991-93	G
HSE-02, 1985	Electroplating	6(6)	P	I	25-mm	U	Trace	--	1985	G
HSE-03, 1985	Electroplating	10(6)	P	I	25-mm	U	0.01	0.01	1985	G
HSE-15, 1985	Electroless plating	U(1)	P	I	25-mm	0.051-0.357	0.12	0.12	1985	G/S
HSE-15, 1985	Ancillary operations	U(30)	P	I	25-mm	0.004-0.015	0.01	0.01	1985	G/S
HSE-17, 1985	Electro-arming	U(3)	P	I	25-mm	U	<0.10	0.1	1985	G
HSE-28, 1985	Electroplating	U(U)	P	I	25-mm	0.001-0.10	0.01	0.01	1985	G
HSE-52, 1985	Electroforming	U(U)[8]	P	I	25-mm	0.001-0.070	0.020[10]	0.029	1985	G/S
HSE-53, 1985	Electroforming	2(19)[9]	S	I	25-mm	0.013-0.019	0.01	0.010	1985	G/S
Bernacki et al., 1978	North America (several plants)	11(11)	P	T	37-mm	0.00004-0.0021	0.008	0.016	1978	S
Bernacki et al., 1980	USA	20,980	P	T	37-mm	0.005-0.021	0.009	0.018	1980	G
Bicknell et al., 1989	USA	9(9)	P	T	37-mm	0.003-0.051	0.018	0.036	1987-88	G
Daniels & Gunter, 1987	USA	7(7)	P& S	T	37-mm	<0.001-0.0045	0.0025[11]	0.005	1986	G
Daniels et al., 1988	USA	10(10)	P&S	T	37-mm	<0.001-0.039	0.006	0.012	1987	S
Mortimer, 1982	USA	4(4)	P	T	37-mm	0.003-0.006	0.004	0.008	1982	S

Reference or Company Code[2]	Location, Plant, or Process	Number of Samples (Workers)[7]	Type of Sampler[3]	Aerosol Fraction[4]	Sampler	Range of Exposures (mg Ni/m^3)	Mean Aerosol Exposure Concentrations (mg Ni/m^3)	Inhalable Ni (mg/m^3)[12]	Year(s) Samples Taken	Study Quality[5]
UM, 1995	USA, electroless plating	29(NG[5])	P	T	37-mm	0.001-0.022	0.005	0.01	1993-94	S
UM, 1995	electroless plating	29(NG)	P	I	IOM	0.001-0.037	0.01	0.01	1993-94	I
UM, 1995	electroless plating	26(NG)	P	T	37-mm	0.002-0.165	0.043	0.086	1993-94	S
UM, 1995	electroless plating	26(NG)	P	I	IOM	0.008-0.182	0.069	0.069	1993-94	I
Kiilunen et al., 1997	electroplating	3(3)	P	I(?)	25-mm	0.0005-0.0007	0.0006	0.0006	U	
Kiilunen et al., 1997	electroplating	6(U)	P	I(?)	25-mm	0.0056-0.0783	U	0.021	U	

Notes

[1] All data except Kiilunen et al. (1997) are drawn from Tables 6-26 and 6-27 in the NiPERA Criteria Document (NiPERA, 1996). All exposures are to soluble nickel. In the UM and Kiilunen et al. studies, some insoluble nickel was present, as shown by analysis of airborne particulate.

[2] EIS: Drawn from a European Industry Survey conducted by NiPERA from 1989-1993.
HSE: Collated from an internal unpublished survey conducted by the British Health and Safety Executive in 1985
UM: Results from a research project at the University of Minnesota

[3] P=Personal; S=Static(area)

[4] T="Total" particulate; I=Inhalable particulate

[5] The quality of the exposure data as assessed by the authors of the NiPERA Criteria Document. G=Good; S=Superior; I=Ideal (generally where inhalable samples were collected)

[6] The range of geometric mean exposures found in the different facilities is listed.

[7] U=Unknown; NG=Not given

[8] Plant had at least 17 workers.

[9] A total of 19 workers were employed; it is unclear how many worked in electroplating.

[10] Range of exposures based on personal and static sampling; mean based on personal sampling.

[11] Geometric mean.

[12] "Total" particulate converted to “inhalable” for 37 mm samplers by multiplying by 2.0 as recommended by Werner et al. (1996).

APPENDIX D. SUMMARY OF THE DISCUSSIONS AND RECOMMENDATIONS FROM THE PEER REVIEW MEETING

The attached document presents the results of the independent peer review of the July 1998 draft of this document.