Hazard Identification and Dose-response of

Inhaled Nickel Soluble Salts

L.T. Haber*,[1], L. ErdreichH, G.L. Diamond&, A.M. Maier*, R. Ratney-, Q. Zhao*, and M.L. Dourson*

Toxicology Excellence for Risk Assessment, 1757 Chase Ave., Cincinnati, OH, 45223; HBailey

Research Associates; & Syracuse Research Corporation; -Mabbett & Associates, Inc.

[1]To whom correspondence should be addressed

Running title - Assessment of Nickel Soluble Salts -- Inhalation Exposure

ABSTRACT

A substantial body of occupational epidemiology data has shown that exposure to mixed soluble and insoluble nickel causes the development of lung and nasal cancer. However, due to coexposure of these populations to soluble and insoluble forms of nickel, and limitations in exposure measurements, the contribution of soluble nickel is difficult to determine. Soluble nickel was negative in an NTP inhalation bioassay, while there was some evidence for tumorigenicity in rats for less soluble nickel oxide, and there was clear evidence for tumorigenicity of insoluble nickel subsulfide in rats. Results of parenteral assays follow a similar pattern, but provide evidence of weak carcinogenicity of soluble nickel. Kinetic factors also indicate that exposure to soluble nickel alone has a low carcinogenic potential. Overall, we conclude that the carcinogenic activity of insoluble nickel compounds should not be used to predict the carcinogenic potential of water-soluble nickel salts. The overall data suggest a nonlinear dose-response relationship for carcinogenicity, but the data are insufficient to determine the doses at which such nonlinearities occur. Under the U.S. EPA's 1996 proposed Guidelines for Carcinogen Risk Assessment, inhaled soluble nickel compounds would be classified as "*cannot be determined,*" because the existing evidence is composed of conflicting data. A Reference Concentration (RfC) of 2E-4 mg Ni/cu.m was calculated, based on lung fibrosis in male rats observed in the NTP study.

INTRODUCTION

Nickel is used with other metals to form alloys to make items such as coins, jewelry, valves, heat exchangers, and stainless steel. Nickel alloys and nickel plating are used to impart corrosion resistance, heat resistance, hardness and strength. Nickel compounds are also used to color ceramics, in batteries, and as catalysts (ATSDR, 1997). Nickel compounds are prevalent in the environment and exposure to nickel can occur through air, water, or food. ATSDR (1997) reports 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 1×10^{-6} to 1×10^{-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 in the respiratory tract). 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. All doses and concentrations reported here are expressed in terms of the amount of nickel.

Several agencies have developed risk assessments for nickel compounds, although not all have addressed soluble nickel specifically (Table 1). This paper presents the findings of *Toxicological Review* of the human health effects of environmental exposure to soluble nickel compounds in air, in particular for compounds such as nickel sulfate, nickel acetate, and nickel chloride (*TERA*, 1999). This work was performed under contract to the Metal Finishing Association of Southern California, Inc., the U.S. Environmental Protection Agency (EPA), and Health Canada. The assessment evaluated the data on soluble nickel in light of new studies that show differences in the toxicity of different nickel compounds [e.g., National Toxicology

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Program (NTP), 1996a, 1996b, 1996c]. The available oral and inhalation data were critically reviewed and summarized, and used as the basis for conclusions regarding hazard identification and dose response relationships. The conclusions of the *Toxicological Review* and opinions expressed in that document and this paper, are those of the authors, and do not necessarily represent the views of the sponsors. After U.S. EPA review, the conclusions of the assessment will also be summarized for EPA's Integrated Risk Information System (http://www.epa.gov/iris), which will also make available the revised *Toxicological Review*.

The main issue in assessing the human health risk from soluble nickel salts is how to evaluate the human occupational data, based on worker exposures to mixtures of chemicals and different forms of nickel, in light of the laboratory animal data. To reach an overall weight of evidence for carcinogenicity, this assessment examined information on the effect of solubility and chemical composition of nickel compounds on the toxicity and toxicokinetics of the compound, the resulting impact on carcinogenicity evaluations, and the relevance of the laboratory animal data to assessing human carcinogenicity. In addition, an inhalation reference concentration (RfC) for noncancer effects on the lung was derived, based on data in rats from NTP (1996a); this RfC is compared here with that calculated from a human study (Muir et al., 1993). An assessment of soluble nickel following oral exposure is discussed in a companion paper (Haber et al., 2000). Development of this hazard identification and dose-response assessment for soluble nickel salts has followed the general guidelines for risk assessment as set forth by the National Research Council (1983), and U.S. EPA risk assessment guidelines for carcinogenicity (US EPA, 1986; 1996) and derivation of inhalation reference concentrations (U.S. EPA, 1994).

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SUMMARY OF TOXICITY DATA

Human Carcinogenicity Data

The carcinogenicity of nickel compounds has been examined in numerous epidemiological studies. However, most of these studies have focussed on workers in the nickel production industry where the exposures were primarily to insoluble nickel compounds. Because exposure to the mixture of insoluble nickel subsulfide and nickel oxide is clearly associated with cancer in human cohorts (ICNCM, 1990), and there is also "clear evidence" (nickel subsulfide) or "some evidence" (nickel oxide) of carcinogenicity in rats (NTP, 1996b, 1996c), it is difficult to separate out the effects of soluble nickel under such co-exposure conditions. This assessment focuses on the cancer risk posed by soluble nickel salts. Therefore, studies on five cohorts with exposure to soluble nickel salts were selected as possible key studies. Studies reviewed by ICNCM (1990) and included in this report were characterized by high exposure to total nickel or the presence of meaningful exposures to soluble nickel (e.g., the cohort included workers exposed to at least 1 mg Ni/m^3 or at least 10 mg Ni/m^3 x years). Studies that were published since the ICNCM (1990) report and included workers exposed to soluble nickel were also included. These cohorts include the INCO facility in Clydach Wales, the Falconbridge refinery in Kristiansand, Norway, the INCO refinery in Port Colborne, Ontario, the Outokumpu Oy facility in Harjavalta, Finland, and a group of British electroplaters. Except for the electroplaters, who were exposed primarily to soluble nickel compounds, exposures were to varying degrees of mixed soluble and insoluble nickel compounds.

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The International Committee on Carcinogenesis in Man (ICNCM, 1990) conducted a detailed evaluation of the exposure and epidemiology data for these occupational cohorts. The ICNCM report (also referred to as the Doll report, after its chair, Sir Richard Doll) contains data and original analyses of cancer incidence and mortality in nickel workers that are not available elsewhere in the published literature. The ICNCM report (ICNCM, 1990) is the most thorough evaluation of the epidemiology data on nickel exposure and cancer. While the ICNCM report forms the foundation for the assessment described here, additional epidemiological information has been published subsequent to the ICNCM report regarding the INCO nickel refinery in Clydach, Wales (Easton et al., 1992), the Falconbridge refinery in Kristiansand, Norway (Andersen et al., 1996), and the Outokumpu Oy refinery in Harjavalta, Finland (Karjalainen et al., 1992; Anttila et al., 1998). A cohort of British electroplaters (Pang et al., 1996) was also evaluated for this assessment.

Exposure estimates for each of the five cohorts are summarized in Table 2, and major characteristics and results for each cohort are summarized in Table 3. For more complete descriptions of these studies, the reader is referred to the complete assessment document (available on the Internet at http://www.tera.org/vera), and the ICNCM report (ICNCM, 1990). A discussion of exposure issues relevant to the analysis of these studies is briefly presented below, followed by a presentation of some key epidemiology data. An integrated assessment of the epidemiology data is presented in the Weight of Evidence discussion, below.

The exposure assessments are critical in determining the reliability of the epidemiology studies. Because it is difficult to find a cohort that was exposed to soluble nickel, much of the assessment of effects of soluble nickel compounds is based on a quantitative comparison of cancer risks at different levels of exposure to soluble and insoluble nickel compounds. For most nickel production exposures, insoluble nickel compounds are a significant confounder in estimating cancer risk from exposure to soluble nickel. Mischaracterization of the exposure levels and characteristics could alter the conclusions.

A complete exposure assessment for nickel also needs to specify the chemical form of the nickel (soluble, insoluble, metallic, etc). This can be difficult, because typical air sampling techniques do not differentiate among nickel species, and so estimates are made based on process knowledge and limited data on nickel speciation in other facilities with similar processes. Particle size distributions are often not determined, even though particle size is an important determinant of particle distribution in the respiratory tract. Instead, studies often report only total dust levels or levels of total nickel in air.

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, there may be mixed exposures in work areas, workers may hold different jobs at different times, and work areas are not necessarily physically separated to confine emissions to that area. For example, three of the

cohorts included subjects who worked in the electrowinning (electrolytic refining) process, which involves exposure primarily to soluble nickel compounds. However, the tankhouses in which electrowinning occurs may not be totally isolated from other sources of nickel exposure, which can result in the exposure of electrowinning workers to insoluble forms of nickel.

Exposure measurements for the industries of interest have been sparse, 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 ICNCM 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 analyzed for total nickel and were area samples taken over periods of less than an hour.

In analyzing the data from within a facility, such as the Clydach refinery, the ICNCM 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. Often the data were

collected for process control purposes, rather than as part of worker exposure estimates. This information can be used for relative comparisons as long as the limitations are recognized.

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. 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.

The Clydach cohort of workers in a nickel sulfate plant provides the best data to evaluate the effects of soluble versus insoluble nickel. The ICNCM report (ICNCM, 1990) 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 tables reproduced here (Table 4 for lung cancer risk and Table 5 for nasal cancer risk). For Clydach, ICNCM (1990) defined low soluble nickel as <10 mg Ni/m^3 x years and high soluble nickel 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.

Table 4 shows the different risks for low and high cumulative exposure to soluble nickel, stratified by degree of exposure to other nickel species. The ICNCM Working Group concluded

that increased exposure to soluble nickel increased the SMR for lung cancer only if exposure

occurred in conjunction with high exposures to oxidic nickel. 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 a discernible effect modification with

oxidic nickel forms, but not with sulfidic nickel. This table also shows 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 Table 5). Examining for

effect modification, high exposure to soluble nickel significantly increased the SMR only when

exposure to sulfidic nickel was high, although a marginal effect was seen with high oxidic

nickel. 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 compounds.

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.

Human Noncancer Data

Only one study was located of respiratory effects in humans exposed to soluble nickel (Muir et al., 1993). Muir et al. (1993) evaluated chest radiographs of 745 nickel sinter plant workers at the Copper Cliff plant in Sudbury, Ontario. Limited exposure estimates are available, but based on exposure levels reported for this site by ICNCM (1990) and work by Werner et al. (1996) on sampling methods, exposure to soluble nickel in this study would be estimated as <8 mg Ni/m^3 and <4 mg Ni/m^3, depending on the time period. No lower bound for the exposures were provided. 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 latency; the authors stated that opacities due to a fibrotic process do not appear to resolve after removal from exposure. 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.

Muir et al. (1993) reported 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

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fibrogenic potential. There was, however, some evidence for a relationship between opacity

prevalence and exposure duration. 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 5 or

more years. In addition, for each reader, the prevalence was higher among those exposed for the

longer duration. 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.

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 radiograph readers, and

uncertainties regarding the degree of ascertainment. However, this study, together with the

absence of anecdotal reports of significant respiratory effects of nickel in the nickel industry,

does provide some insight regarding the inhalation toxicity of soluble nickel in humans.

Derivation of a RfC based on this study is discussed below.

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.,

1986). 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 occupational exposure to the nickel salt 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.

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. 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). Neither a skin prick test nor serological IgE antibody measurement showed a nickel-specific response.

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 the 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.

Laboratory Animal Data

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 (e.g., chronic inflammation, interstitial infiltrates) have been

identified in the lungs of rats and mice following subchronic and chronic exposures. Atrophy of

the olfactory epithelium was also observed at similar human equivalent concentrations. (This

lesion is consistent with anecdotal reports of anosmia in nickel workers [Mastromatteo, 1995]).

The histopathology data are supported by biochemical evidence of lung damage, based on

increased enzyme levels in BAL fluid (Benson et al., 1989).

The National Toxicology Program (NTP) performed a chronic inhalation bioassay of

nickel sulfate hexahydrate aerosol with groups of F344/N rats (53-55 rats/sex) and B6C3F1 mice

(60-62 mice/sex) (NTP, 1996a; Dunnick et al., 1995). 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 measurements of nickel concentrations in tissue in the

respiratory tract (mice and rats) and kidney (mice). The aerosol mass median aerodynamic

diameters (MMADs) ranged from 2.27-2.53 μm, and the geometric standard deviation from 2.02-2.38 μm. Endpoints evaluated in the core study were clinical signs, body weight, organ weights (interim sacrifices only), and complete histopathology.

In NTP (1996a), 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). 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, with lung fibrosis and inflammation, and olfactory epithelial atrophy, observed at the two highest concentrations. There were also small but statistically significant increases in lung nickel burdens at 0.11 mg Ni/m^3 after 7 months and at all concentrations after 15 months (exposure concentration-related). No exposure-related neoplasms were observed in the lungs or other tissues in rats.

At the 2-year sacrifice, non-neoplastic inflammatory lesions of the lung were observed at exposure concentrations ≥0.056 mg Ni/m^3 in males and females, with no elevation over control incidence at the low concentration. 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. 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

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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. Bronchial lymph node lymphoid hyperplasia and olfactory epithelium atrophy were also observed in males and females exposed to the high concentration. 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.

NTP reported "alveolar macrophage hyperplasia" in all males and 4/5 females at 0.056 mg Ni/m^3 in the 7-month evaluation. In the 15-month evaluation, the incidence at this concentration (2/5 males, 3/5 females) was not statistically different from controls (0/5 males, 1/5 females). The incidence of "alveolar macrophage hyperplasia" at the low concentration (0.027 mg Ni/m^3) was not statistically significant following exposure for 15 months or 2 years. A definitive conclusion regarding the adversity of the endpoint is not possible. The macrophage accumulation may be a secondary response to tissue damage and/or may contribute to inflammation, which could progress to fibrosis. However, such a progression was not clearly supported or refuted by the results of the chronic bioassay; therefore, derivation of the RfC was based on more definitively adverse endpoints (e.g., fibrosis).

The No Observed Adverse Effect Level (NOAEL) for lung effects in rats in the chronic study was 0.027 mg Ni/m^3 and the NOAEL (Human Equivalent Concentration [HEC]) (after adjustment for intermittent exposure) 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. 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. Table 6 summarizes the NOAELs and LOAELs (Lowest Observed Adverse Effect Levels) with corresponding human equivalent levels and BMCL$_{10}$(HEC)s. These results are described in more detail in the *Toxicological Review* (*TERA*, 1999) and in Haber et al. (1998). As shown in Table 6, the lowest BMCL$_{10}$(HEC) was 0.0017 mg Ni/m^3, for lung fibrosis in male rats (excluding the BMCL$_{10}$(HEC) values for macrophage hyperplasia).

In a 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. No exposure-related neoplasms occurred in the lungs or other tissues of mice. 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. 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. 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 $BMCL_{10}$(HEC) values for chronic inflammation in females and for bronchialization in females were both approximately 0.006 mg Ni/m^3. The $BMCL_{10}$(HEC) for olfactory epithelial atrophy in males was approximately 0.004 mg Ni/m^3.

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 mice were subjected to a battery of immune function tests, but 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 antibody forming cells (AFC)/spleen at the high concentration. These effects were consistent with the inflammation reported in NTP

(1996a). Overall, these results show that the immune effects of soluble nickel occur at higher exposure levels than effects on the respiratory system.

In a related study by the same group, 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 (BAL) and histological analyses were conducted at the end of the study. The authors stated that no significant sex-related differences were observed, and so results were reported for both sexes combined. Statistically significant increases in nucleated cells in BAL fluid, and alveolar macrophage accumulation (based on the histological analysis) were observed at all concentrations in rats, and in mice at the mid and high concentrations. Effects at higher concentrations progressed to include increases in lactate dehydrogenase (LDH), beta-glucuronidase, and total protein in BAL fluid. Histological findings at the high concentrations included chronic inflammation in the central acinus, interstitial infiltrates, and, (in mice only), fibrosis.

No multigeneration reproduction study of the inhalation exposure route is available, 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). 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 the only study located that evaluated effects on developmental or reproductive function 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 compound/m^3 (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.

Conclusions on Carcinogenicity Based on the Animal Data

The inhalation NTP bioassay (NTP, 1996a; Dunnick et al., 1995) did not show soluble nickel compounds to be carcinogenic; however, there are some scientists who have questioned the conclusions regarding carcinogenicity from the NTP bioassay and their appropriateness for human evaluation. Their concerns include interpretation of the negative mouse data, whether the NTP (1996a) tested sufficiently high concentrations in the rat, whether the mouse bioassay data

are informative, whether the results are relevant given higher occupational exposures, and the differences in lung burdens among the various nickel compounds assayed (NTP, 1996a, 1996b, 1996c).

The NTP (1996a) study is considered to be a high-quality cancer bioassay, in that the study included assays of all major organs and included chronic exposure concentrations at and below the maximum tolerated exposure concentration for soluble nickel (nickel sulfate hexahydrate) in air. No evidence of carcinogenicity of nickel sulfate hexahydrate was found in rats or mice. In similar studies, 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 (NTP, 1996c). 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 (NTP, 1996b). 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. According to this argument, results in mice provide no information about nickel carcinogenicity. 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). Thus, while the mouse is generally considered an appropriate model for inhalation

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carcinogenesis of metals, it is clear that the mouse data in this set of studies do not provide a firm basis for distinguishing between the effects of soluble and insoluble nickel compounds, whereas the data in rats do allow such a distinction.

The concern regarding whether sufficiently high concentrations were tested in the rat bioassay 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. Nasal lesions, however, occurred at a higher incidence in the rats exposed to nickel sulfate. Nickel subsulfide at 0.11 mg Ni/m^3 produced small statistically significant increases in lung adenomas and carcinomas (combined). Larger statistically significant increases were observed at the only other 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 shown evidence of carcinogenicity at higher concentrations.

Another argument that insufficiently high concentrations of nickel sulfate hexahydrate were tested is 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. Following a single acute inhalation exposure, the half-time in the lungs of rats for nickel sulfate is about 32 hours (Hirano et al., 1994), while those for nickel subsulfide and nickel oxide are approximately 4 days, and 120 days, respectively (Benson et al., 1994). When clearance was evaluated in rats exposed via inhalation for 2-6 months, and then receiving a single exposure to radiolabeled

compound, the half-time in the lung was approximately 2-3 days for nickel sulfate, and 10-346

days for nickel oxide (depending on the exposure concentration and whether exposure had been

for 2 or 6 months) (Benson et al., 1995). Clearance half-times for nickel subsulfide following

subchronic exposure are not available. Toxicity data, rather than lung burden results, should be

used in evaluating whether sufficiently high concentrations were tested.

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.

It has also been contended that occupation exposures are much higher than the exposure

levels used in the NTP (1996a) study. 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

particle sizes under occupational conditions are much larger. The results after normalization by

converting to human equivalent concentrations (HECs) vary with the occupational particle size

distribution used. An exposure level of 4 mg Ni/m^3 for occupational exposure in one nickel

refinery was estimated to correspond to a HEC of approximately 0.018 to 0.2 mg Ni/m^3. (See the discussion below of an RfC based on the Muir et al. (1993) study.) The BMCL$_{10}$(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 in nickel refineries, with estimates of the respirable fraction (which would reach the pulmonary region of the lung) ranging from essentially 0% in a Russian refinery (Thomassen et al., 1999) to 2-6.8% in the Kristiansand, Norway, refinery (Werner et al., 1999). Nonetheless, for all occupational particle size distributions located, the lung dose received under occupational exposure conditions is much closer to that in the bioassay than would be apparent by comparing exposure levels.

Based on this analysis, we concur with NTP that their study used appropriate animal models and sufficiently high exposure levels. The conclusions of that study, that there was "no evidence" of nickel sulfate carcinogenicity in male and female rats and mice, are valid.

WEIGHT OF EVIDENCE AND DOSE RESPONSE FOR CARCINOGENICITY OF SOLUBLE NICKEL COMPOUNDS

The database regarding the carcinogenicity of soluble nickel compounds is particularly rich, and includes extensive epidemiology data, animal bioassays, parenteral studies, and mechanistic studies. The complete evaluation of the weight of evidence must consider all of these data.

Evaluation of the Epidemiology Evidence

As discussed earlier, numerous epidemiological studies have been conducted evaluating the potential carcinogenicity of nickel compounds. Most of these studies however, involve exposures that are primarily to insoluble nickel compounds, or to both soluble and insoluble compounds, making it difficult to separate out the effects of soluble nickel under such co-exposure conditions. An evaluation by ICNCM (ICNCM, 1990) indicated an effect of insoluble nickel compounds that increased with estimated exposure levels, and an 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 analyzed that included exposure to soluble species from nickel refining and were large enough for analysis. (An analysis of the Clydach cohort was discussed in more detail earlier in this paper.) However, lung cancer was not increased among electrolysis workers (exposed to soluble nickel) in Port Colborne. ICNCM (1990) interpreted the lack of effect at Port Colborne as being due to a lower ratio of soluble to insoluble compounds, compared to 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 summary is an assessment of the weight of the epidemiology evidence for determining whether soluble nickel, alone, is carcinogenic. It contains more detail on the update studies than on the cohorts as originally analyzed by ICNCM (1990). This summary describes data that provide support for and argue against the link between soluble nickel compounds and cancer, and the limitations of the data on both sides of the argument.

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 (Anttila 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 ratios were based on only two 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" than in the "nickel plant."

- Multivariate analysis of the Clydach data (Table 4) 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 appear to be strong carcinogens. 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

(six 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, two of the six 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, and so could have included confounding exposures to insoluble nickel compounds, due to inadequate industrial hygiene isolation of the refiner and the smelter during early exposure periods.

- Additional data from Harjavalta showed that two of the four 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 was observed 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 low and 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, particularly in the presence of insoluble nickel compounds. 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 nickel compounds, 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 is 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.

Evaluation of the Animal Data

In contrast to the human data, 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).

However, 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. As noted above, a number of

concerns have been raised about the conclusions from the NTP (1996a) bioassay with nickel

sulfate, but, after a detailed consideration of these concerns, we concurred with the NTP

conclusions.

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) (Sunderman, 1984;

Gilman, 1962, 1966; Kasprzak et al., 1983). Tumors at the site of intraperitoneal injection show

a similar trend (Pott et al., 1989, 1990, as cited by IARC, 1990), 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 (Stoner et al., 1976; Poirier et al., 1984). Initiation-

promotion studies show some light evidence that intraperitoneal injection of nickel acetate can initiate development of kidney tumors in rats (Kasprzak et al., 1990) and their offspring (Diwan et al., 1992). In addition, intraperitoneal injection of nickel chloride promoted the development of kidney tumors (Kurokawa et al., 1985), and intraperitoneal injection of nickel sulfate promoted development of nasopharyngeal tumors in rats (Ou et al., 1980, 1983; Liu et al., 1983). (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.

The animal data also suggest a number of ways in which soluble nickel could increase the carcinogenicity of other chemicals, possibly explaining the effect modification seen in epidemiology studies of workers exposed to soluble and insoluble forms of nickel. For example, the inflammation seen in NTP (1996a) could result in enhanced cell proliferation and local generation of oxygen radicals.

Evaluation of Genotoxicity and Mode of Action Data

Consideration of the overall database for soluble and insoluble nickel compounds 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

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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.

Evidence for genotoxicity of water soluble nickel compounds is mixed. Numerous authors, including Sunderman (1989), Coogan et al. (1989), IARC (1990), Snow (1992), NTP (1996a), and Oller et al. (1997) have reviewed the genotoxicity of nickel compounds. 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. Soluble nickel compounds are almost always non-mutagenic in bacteria.

Because chromosome aberrations and other genotoxic are observed after exposure to soluble nickel salts, but the nickel ion alone interacting with isolated DNA does not form premutagenic DNA lesions, alternative mechanisms for the production of DNA damage have been proposed. Perhaps the most plausible is the hypothesis that nickel inhibits the binding of magnesium to proteins in heterochromatin (a highly condensed and genetically inactive form of DNA), leading to DNA damage and expression of genes that are normally silent (Costa et al., 1994). This and other proposed models involve some sort of indirect interaction with DNA, suggesting a nonlinear dose-response relationship. However, the available data are insufficient to determine the doses at which such nonlinearities occur (i.e., where the background human

exposure is relative to the linear portion of the curve.) Nonetheless, the suggestion of such nonlinearities is consistent with the negative animal carcinogenicity studies for soluble nickel.

Differences in nickel clearance have also been proposed to explain the contrast between the generally weak, but positive, results with soluble nickel salts in certain types of genotoxicity assays, and the negative results in standard animal carcinogenicity studies (NTP, 1996a), as well as the contrast between the carcinogenicity with soluble and insoluble nickel compounds (NTP, 1996b, 1996c). 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 and mechanistic differences among nickel compounds may also contribute to and explain the differences observed in the bioassay results (NTP, 1996a, 1996b, 1996c). Several authors (Dunnick et al., 1995; NTP, 1996a; Oller et al., 1997) have hypothesized that crystalline nickel subsulfide readily enters cells and interacts with DNA, but soluble forms of nickel are much less effective at entering mammalian cells under physiological conditions. Furthermore, Oller et al. (1997) suggest that the soluble nickel that does enter the cell is much more likely to interact with cytoplasmic constituents, causing toxicity, but not interacting with DNA.

Support for this hypothesis is as follows. A number of studies have found that crystalline nickel subsulfide is readily taken up by phagocytosis (Costa and Mollenhauer, 1980; Costa et al., 1981; Abbracchio et al., 1982b; Heck and Costa, 1983). The phagocytosed vacuoles have been observed to aggregate near the nucleus, where they interact with lysosomes (Evans et al. 1982), leading to the release of nickel ions that are mostly localized near the nucleus, and can interact with DNA (Costa et al., 1981). Although the mechanism by which nickel sulfate enters the cells is not known, phagocytosis (which acts on particles) would not be expected to play an important role, based on nickel sulfate's high water solubility. Regardless of the mechanism by which soluble nickel salts enter cells, uptake by rabbit alveolar macrophages, human B-lymphocytes, human erythrocytes and Chinese hamster ovary (CHO) cells) is markedly lower in the presence of physiological levels of amino acids and proteins (Nieboer et al., 1984a; Abbracchio et al., 1982a). Under these conditions, the nickel ions are complexed with amino acids or protein, the normal form of nickel under physiological conditions (Lucassen and Sarkar, 1979; Glennon and Sarkar, 1982; Nomoto et al., 1971; Nomoto and Sunderman, 1988; Nieboer et al., 1984). Thus, effects that are observed *in vitro* in minimal salts/glucose medium may occur to a much lower degree under physiological conditions. For example, *in vitro* nickel cytotoxicity to CHO cells is much higher in salts-glucose medium than in complete medium (Abbracchio et al., 1982a). By contrast, when soluble nickel compounds are packaged in liposomes, they are subject to phagocytosis, and the level of DNA damage produced is increased compared to cells incubated with soluble nickel compounds in a salts glucose medium, but without liposomes (Sen and Costa, 1986).

The differences in the way insoluble and soluble nickel compounds are taken up by the cell may result in different doses of nickel to the nucleus, depending on whether exposure is to soluble or insoluble nickel. Soluble nickel, because it is taken up directly into the cytoplasm, may react with cytoplasmic proteins and cause cytotoxicity, making it less available for uptake into the nucleus. On the other hand, insoluble nickel that is sequestered in vacuoles or lysosomes may be less available to cytoplasmic proteins. Lysosomal transport to the perinuclear region and dissolution of nickel particles in the lysosome may give rise to locally high concentrations of nickel ion in the perinuclear region which could, in turn, result in a higher dose of nickel ion to the nucleus (Costa and Heck, 1982). This suggests that soluble forms of nickel may interact with the cell in a way that increases cytotoxicity and decreases nickel delivery to the nucleus, while insoluble forms of nickel, such as nickel subsulfide, may 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, the carcinogenic potential of nickel compounds depends on a number of competing rates and probabilities, including removal of the nickel from the lung, uptake of the nickel into the cell, the probability of the nickel to cause a mutation, and the probability of the cell to be killed by nickel cytotoxicity or another cause before a tumor is initiated. For soluble nickel salts, the relatively rapid clearance from the lung (clearance half-time of ~32 hours after acute exposure [Hirano et al., 1994]; relatively low cellular uptake under physiological conditions; and

uptake processes that tend to deliver nickel to the general cytoplasm (where it can complex with proteins) rather than to the nucleus tend to decrease the carcinogenic potential. Quantitative evaluation of these relative rates was not conducted for this assessment. However, in light of the negative results in the NTP (1996a) study, it is reasonable to conclude that the relative rates are such that any carcinogenic potential of soluble nickel salts is not detectable under standard animal bioassay conditions. By contrast, the slower lung clearance of nickel subsulfide (Benson et al., 1994), coupled with its phagocytic uptake, enhance its carcinogenic potential.

Overall, these studies suggest that soluble nickel can enhance the carcinogenicity of other chemicals, but the data are insufficient to provide adequate 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; and,

- assays of the carcinogenic activity of water-insoluble nickel compounds should not be used to predict the carcinogenic potential of water-soluble nickel salts.

Weight of Evidence for Human Carcinogenicity

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, 1996):

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.

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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." More recent drafts of EPA's guidelines were released in 1999 and have been discussed by the scientific community, although they have not been officially proposed. Among other changes, the more recent drafts allow provide additional guidance on the evaluation of mode of action, and provide additional options in the descriptors used for summarizing the weight of evidence. Nonetheless, a similar descriptor would be used under the 1999 draft: The data regarding the carcinogenic potential of inhalation exposure to soluble nickel *are inadequate for an assessment of human carcinogenic potential.*"

Under the current EPA cancer guidelines (U.S. EPA, 1986), 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 compounds 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.

Dose-Response for 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.

DERIVATION OF AN INHALATION REFERENCE CONCENTRATION

U.S. EPA defines the inhalation reference concentration (RfC) as "an estimate (with uncertainty spanning perhaps an order of magnitude) of a continuous inhalation exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious noncancer health effects during a lifetime" (U.S. EPA, 1994). From analysis of the available data, the most sensitive and appropriate endpoint is selected and a human equivalent concentration (HEC) is calculated using dosimetric adjustments to account for differences in the

dose delivered to the respiratory tract of the experimental animal and humans. Uncertainty

factors to account for various extrapolations and uncertainties are applied to the concentration to

estimate the RfC. The NTP bioassay in rats (NTP, 1996a) contains the most appropriate data

upon which to base a RfC for soluble nickel compounds.

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 (Haber et al.,

1998). (Note that this is a mathematical threshold, and does not necessarily correspond to a true

biological threshold.) The lower 95% bound on the concentration corresponding to a 10%

response, the $BMCL_{10}$(HEC), was calculated to be 0.0017 mg Ni/m^3. This value was only

slightly lower than the NOAEL(HEC) of 0.0021 mg Ni/m^3 for this endpoint. Both the

polynomial and Weibull models produced the same result. Acceptable, but slightly higher

$BMCL_{10}$(HEC) values were calculated for several other endpoints from NTP (1996a). Table 6

provides the endpoints considered as the basis for the nickel RfC and the corresponding

$BMCL_{10}$(HEC) values for each.

Using the U.S. EPA methods for calculating RfCs (U.S. EPA, 1994), factors to account

for uncertainties in extrapolating from the rat $BMCL_{10}$(HEC) to the general human population

were considered. In the absence of sufficient data on sensitive human subpopulations, an

uncertainty factor of 10 was applied to account for intrahuman variability. Because the dosimetric adjustments account for much of the toxicokinetic differences between animals and humans, a default factor of 3 (to account for toxicodynamic differences) is generally applied when dosimetric adjustments have been applied to animal concentrations to estimate the human equivalent concentration. However, a factor of 1 was used to extrapolate from animals to humans in this case, based on minimal effects seen in humans under occupational exposures (Muir et al., 1993) that were higher than exposures that resulted in significant respiratory toxicity in the chronic rat bioassay (NTP, 1996a). As described below, the minimal LOAEL(HEC) from the Muir et al. (1993) study of 0.018-0.2 mg Ni/m^3 is 10 to 100-fold higher than the BMCL$_{10}$(HEC) used as the basis for the RfC. (Use of the HEC from the occupational study accounts for differences between occupational and environmental exposure in breathing rate, exposure duration, and particle size.) Therefore, humans appear to be less sensitive than rats to the respiratory effects of nickel. Additional support for the lesser sensitivity of humans comes from the absence of complaints of respiratory problems in workers exposed to soluble and insoluble forms of nickel (Mastromatteo, personal communication).

An uncertainty factor for database deficiencies was not considered necessary in the development of the RfC, because bioassays in rats and mice are available, and 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 oral data. No modifying factor was applied to the estimation of this RfC. Thus, the RfC is derived as follows:

$$0.0017 \text{ mg Ni/m}^3 \, [\text{BMCL}_{10}(\text{HEC})] \div 10 = 0.00017 \text{ mg Ni/m}^3$$

which can be rounded to 0.0002 mg Ni/m^3.

Data from the study of workers by Muir et al. (1993) were also considered for derivation of the RfC. The Muir et al. (1993) study is not desirable as a basis for an RfC 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. However, if this study were considered as the basis for the RfC, the single exposure level available (less than 4-8 mg Ni/m^3) would be considered a minimal LOAEL, based on the observation of a duration-related increase in the prevalence of findings. Identification of a LOAEL from this study for use in risk assessment balances two opposing considerations. First, the actual exposure may have been lower, since it was characterized as *less than* 4-8 mg Ni/m^3. Conversely, a 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.

To compare the RfCs based on animal and human data, an additional adjustment would be necessary because of the large differences between particle sizes under occupational and ambient exposure conditions. Because information was not available on the particle size

distribution in the Muir et al. (1993) study, the particle size was estimated from the unpublished study of Vincent (1996), who evaluated the exposure characteristics at a number of nickel plants. The data of Vincent (1996) indicate marked particle size variability with process worksite and 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. The data of Vincent (1996) were supplemented with particle size distribution data provided by Ramachandran et al. (1996). 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. The resulting LOAEL(HEC), is 0.018 to 0.2 mg Ni/m^3.

An uncertainty factor of 10 would be used with the LOAEL(HEC) to account 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 presented for the RfC based on NTP (1996a), no database uncertainty factor to account for the potential for reproductive effects is needed. A composite uncertainty factor of 100 results. The resulting RfC would be 0.0002 to 0.002 mg Ni/m^3. While a RfC based on Muir et al. (1993) is not

recommended, in light of the numerous uncertainties associated with this study, 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.

There are a number of areas of scientific uncertainty in the development of the RfC that must be recognized, but cannot be quantified. They relate to dosimetric considerations and the interpretation of alveolar macrophage accumulation in the rats, as well as the reproductive and developmental data. The NOAEL(HEC) for olfactory epithelial atrophy in females in the NTP 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 endpoint occurred at two exposure levels lower than all other effects, 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 effect 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 13-week NTP study at 0.027 mg Ni/m^3, a concentration below that at which any lesion was observed in the chronic study. Considering macrophage accumulation to be adverse would result in only a relatively small quantitative effect on the RfC.

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.

Confidence in the critical study used as the 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.

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).

PEER REVIEW

A draft of the Toxicological Review was independently peer reviewed by a panel of ten experts from the fields of risk assessment, epidemiology, occupational medicine, and toxicology in a two-day meeting. The peer review was organized by TERA with selection of panel members made by a group of TERA trustees to avoid any conflict of interest. All panel members disclosed any potential conflicts of interest and the panel discussed these, agreeing that three of the panel members should participate in the discussion, but not be polled for consensus. Two of the three were from federal agencies that have assessed nickel and the third had performed work for a nickel trade group. A complete description of the peer review process and a summary of the discussions and conclusions of the nickel soluble salts review can be found at http://www.tera.org/peer.

The panel reviewed the draft document, and in particular the conclusions regarding cancer and non-cancer risk in the draft document. Specifically, they focused on the following questions:

- Are the differences in mode of action and toxicity between soluble and insoluble nickel salts sufficient to consider their risk assessment (particularly the cancer risk assessment) separately?

- Have the epidemiology data and uncertainties in exposure been appropriately considered in the assessment?

- Has the assessment appropriately considered the implications of the (mixed, but primarily positive) genotoxicity assays, and the parenteral carcinogenicity data evaluating nickel compounds of varying solubilities?

- Should the assessment of soluble nickel carcinogenicity take into account the data on nickel subsulfide and nickel oxide carcinogenicity in the related NTP studies?

- Are the conclusions of the assessment regarding the carcinogenic potential of soluble nickel supported by the data?

- Is there a quantitative, as well as a qualitative difference in carcinogenic potential between soluble nickel and insoluble forms of nickel?

The authors revised the *Toxicological Review* to address and respond to the peer review panel's recommendations; the text of this report reflects those changes. Significant revisions included a discussion comparing exposure levels in the NTP study and under occupational exposure conditions, enhancement of the discussion related to mode of action and nickel toxicokinetics to address differences between soluble and insoluble nickel compounds, and the cancer weight of evidence. For the RfC, the discussion on alveolar macrophage hyperplasia was revised to note that these endpoints may exist on a continuum leading to fibrosis and that this hyperplasia should not be labeled nonadverse. In addition, the recommended uncertainty factor for the inhalation RfC was dropped from 30 to 10, in light of evidence from occupational studies that humans are less sensitive than rats to noncancer respiratory effects of nickel. The document also presents an alternative RfC based on the human data (Muir et al., 1993) for comparison purposes.

A brief summary of the nickel assessment and conclusions regarding cancer and non-cancer risk can also be found on the International Toxicity Estimates for Risk (*ITER*) database at http://www.tera.org/iter. This database compares risk values derived by U.S. EPA, ATSDR and Health Canada, alongside independently derived values (such as those for nickel described here) which have been approved by an independent peer review.

CONCLUSIONS

Toxicology Excellence for Risk Assessment (*TERA*) formed a team of scientists (the authors of this paper) to examine the human health risk of exposure to soluble nickel compounds. After evaluating all available literature and analyses we concluded that the toxicity of soluble nickel compounds cannot be assumed to be the same as insoluble compounds for risk assessment purposes. Specifically, under the U.S. EPA's 1996 proposed Guidelines for Carcinogen Risk Assessment, inhaled soluble nickel compounds would be classified as "*cannot be determined,*" because the existing evidence is composed of conflicting data. A Reference Concentration (RfC) of 2E-4 mg Ni/cu.m was calculated for noncancer toxicity, based on lung fibrosis in male rats in the NTP study, with a $BMCL_{10}(HEC)$ of 0.0017 mg Ni/m^3. A composite uncertainty factor of 10 was used, based on a factor of 10 to account for intrahuman variability; no other uncertainty factors were considered necessary. Both the cancer and noncancer assessments were reviewed by an independent group of experts. The notes from this public review meeting, which was open to the public, can be viewed at www.tera.org/peer. Comments were incorporated and the resulting assessments can be viewed at www.tera.org/iter.

Acknowledgements

subcontract to Science Applications International Corporation (EPA Contract #68-C7-0011), and

by a contract from Health Canada.

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Table 1. U.S. and Canadian Risk Values for Inhalation Exposure to Soluble Nickel Compounds

Agency	Nickel Compound Evaluated	Critical Effect	Risk Value	Study	Comments
Health Canada (Health Canada, 1996)	Nickel sulfate	Atrophy of olfactory epithelium	Tolerable Concentration (TC), 0.0000035 mg Ni/m^3	Dunnick et al., 1989	The LOEL in rats of 0.02 mg Ni/m^3 was adjusted for intermittent exposure (6/24 and 5/7) and divided by an uncertainty factor of 1000 (10 for intraspecies variation, 10 for interspecies variation, and 10 for less than chronic study; no additional factor for LOEL in rats rather than NO(A)EL since observed effects were minimal). Based on literature search through August 1993.
Health Canada (Health Canada, 1996)	Soluble Nickel (primarily nickel sulfate and nickel chloride)		Tumorigenic Concentration 5% (TC05), 0.07 mg/m^3	ICNCM., 1990	Health Canada classifies the group of soluble nickel as Group I, carcinogenic to humans. The TC05 is computed directly from the dose-response curve within or close to the experimental range. Based on literature search through August 1993.
ATSDR (ATSDR, 1997)	Multiple, MRL based on nickel sulfate data	Chronic active inflammation and lung fibrosis	Chronic Minimal Risk Level (MRL), 0.0002 mg Ni/m^3	NTP, 1996a	The NOAEL was adjusted for intermittent exposure (6/24 hours/day, 5/7 days/week), multiplied by the Regional Deposited Dose Ratio of 0.9714 to extrapolate to humans, and divided by a total uncertainty factor of 30 (3 for extrapolation from animals to humans and 10 for human variability). ATSDR does not currently assess cancer potency or perform cancer risk assessments
U.S. EPA (U.S. EPA, 1999)	--	--	--	--	EPA has not evaluated the carcinogenicity of nickel soluble salts as a class of compounds; however EPA has classified nickel refinery dust and certain insoluble nickel compounds as group A – known human carcinogens. Similarly, EPA has not conducted a noncancer assessment for inhaled nickel.

Table 2. Exposure Estimation in Epidemiology Studies Specifying Soluble Nickel

Population (source)	Selection criteria and exposure assessment	Estimated workplace levels, soluble Ni[1], mg/m^3	Estimated workplace levels, insoluble (mg/m^3)
Outokumpu Oy Harjavalta Finland Ni refinery (Karjalainen et al., 1992; Anttila et al. 1998)	Min employment 3 mos. Defined by period of employment and type of work. Primarily sol Ni, as sulfate in electrowinning; subsulfide form present when matte is ground. Oxides not present.	annual avg. <0.50 [0.26-0.76 tank house areas]	subsulfide, <0.20
Kristiansand Norway Falconbridge Ni refinery workers, (Andersen et al., 1996)	Concentrations estimated by 'expert group', and measurements taken at one point in time (1973). Species assumed present in respirable air in proportion to presence in material in work area. Similar to Clydach process, but through 1978 handled some substances that Clydach handled only up to 1929. Smelter plant nearby.	0.50-2.0 in electrolysis areas	
Clydach Wales INCO Nickel refinery (ICNCM, 1990; Easton et al., 1992)	Follow-up through 1985. Min 5 years employment. Based on process. Estimated concentrations extrapolated from recent conditions. Some percentage of nearly every department is soluble Ni; highest is 30-40% in Ni sulfate plant; also high oxidic but low sulfidic. Workers may have worked in other departments.	0.20-2.0 ["thru 1979"] Calciners, decrease over time, 0.25-0.75	1-2 in Ni plant; 10 in Cu plant
Port Colborne, Ontario Electrolysis INCO (ICNCM, 1990)	Worked >=6 months. 1950 thru 1984. At electrolytic workplaces, exposed to sol Ni <0.3 mg/m^3 and <1.0 mg/m^3 total. Recently sol as low as 0.20 mg/m^3	<0.30 >1.0 anode tasks	<0.70
Ontario Mining, smelting, and refining (ICNCM, 1990)	Concentrations of soluble somewhat comparable to Kristiansand Norway but the later had 7 x higher insoluble, which dropped to 2.5 x after 1987	None	0.25-1.0
British electroplaters (Pang et al., 1996)	Min. employment 3 mos. in Ni plating; never worked with chromium.	0.010-0.080 [2]	None

1 Different methods were used to estimate exposures at different workplaces, so these levels are only roughly comparable. In addition, levels vary with changes in procedures over time.

2 Estimated workplace concentrations based on contemporary exposure assessments for electroplaters, as summarized in NiPERA (1996). Actual exposure concentrations for this cohort may be higher because exposures occurred during an earlier time period.

Table 3. Risk Estimates in Epidemiology Studies Specifying Soluble Nickel

Population (Source)	Cohort Size	Person-years at Risk Type of Risk Estimate (Unless otherwise Specified)	Risk Estimate (95% Confidence Interval); Number of Cases/Deaths[1]		
			Lung	Nasal	Stomach
British *electroplaters* (Pang et al., 1996)	284	6928.6 RR for mortality[2]	1.2 (0.4-4.3) n=5	None observed (<1 expected)	2.6 (0.6-11.3) n=5
Outokumpu Oy Harjavalta Finland *Ni refinery (M&F)* (Anttila et al., 1998)	418	9875 SIR[3]	3.4 (1.2-7.4) n=6	67.1 (8.1-242) n=2	5.0 (1.0-14.5) n=3
Falconbridge, Kristiansand Norway *Ni refinery* (Andersen et al., 1996)	4764	125,000 SIR[4]	Overall: 3.0 (2.6-3.4) n=203 Highest soluble: RR 3.1 (2.1-4.8) n=55	Overall: 18 (12.3-25.4) n=32 Soluble only: 2.7 (0.3-9.8) n=2	0.9 (0.7-1.3) n=45
Clydach, Wales INCO *Hydrometallurgy workers* (ICNCM, 1990; Easton et al., 1992)	288	Not reported (study period from 1931-1985) SMR[5]	3.3 (1.1-7.8) n=5 SMRs <1.4 for entry after 1930 n=43	363.6 (98.9-930.9) n=4 (1 case, entry after 1930)	SMRs in total cohort <1 in all analyses, including hired before 1930 n=21
Port Colborne, Ontario. INCO *Electrolysis workers* (ICNCM, 1990)	Not reported (1608 cancer deaths)	Not reported (study period 1950-1984) SMR[6]	1.1 (1.0-1.2) n=547	1.4 (0.5-3.1) n=6	1.0 (0.8-1.2) n=120

1   Risk estimates rounded to one decimal point
2   Exposed to Ni > 1 yr. Relative risk (RR) for mortality, based on regression analysis, within the cohort, and adjusted for age, start year, follow-up, and exposure duration.
     (The paper also reports SMR [standardized mortality ratio], but without the multiple adjustments; no SMRs were statistically significant).

3   SIR (standardized incidence ratio), population of SW Finland as comparison. This study included both men and women, all others were only men. Latency 20+ years. An additional case of nasal cancer and one case of nasopharyngeal cancer were identified after the study after termination data; 2 lung cancer cases worked in both the smelter and refinery, and so may have had exposure to insoluble nickel compounds.

4   SIR. Not adjusted for age, smoking, duration, or other exposures. RR is calculated by regression analysis, within the cohort, adjusted for age, smoking, and nickel oxide exposure.

5   SMR for hydrometallurgy department >5 years exposed and <1 year in other high risk area before 1959. Copper plant risks were similar; copper and hydrometallurgy plants had similar soluble exposure, but copper plants had greater oxide exposure. Source: nasal and lung cancer ICNCM, 1990, Table 25; lung cancer after 1930, Easton 1992, Table 46.3; stomach cancer ICNCM, 1990 Table 16 and Easton et al, 1992, Table 46.4.

6   INCO (Ontario) workers with no experience in sintering activities, which primarily consist of exposures to oxidic and sulfidic nickel. ICNCM, 1990 Table 49

Table 4. Comparison of Risk of Dying of Lung Cancer[1] at Different Levels of Cumulative Exposure to Soluble Nickel by Different Levels of Combined Cumulative Exposure to Sulfidic and Oxidic Nickel in the Mond/INCO (Clydach) Nickel Refinery

Degree of exposure to sulfidic and oxidic nickel, respectively[2]	Low exposure to soluble nickel[3]			High exposure to soluble nickel[4]			Difference in the SMR values (P-value)
	O	E[5]	SMR	O	E[5]	SMR	
Low, low	51	26.01	196	7	4.16	168	0.931
Low, high	18	5.14	350	30	3.87	776	0.024
High, low	8	1.25	638	1	0.15	658	0.999
High, high	32	6.34	505	28	2.36	1187	0.003

[1] From ICNCM (1990), Table 33. Includes all men with 15 or more years since first employment except those who worked in general trades. O = observed number of deaths, E = expected number of deaths, SMR = standardized mortality ratio.
[2] Low sulfidic nickel exposure = < 15 (mg Ni/m^3) x years and high sulfidic nickel exposure = ˘ 15 (mg Ni/m^3) x years; low oxidic nickel exposure = < 50 (mg Ni/m^3) x years and high oxidic nickel exposure = ˘ 50 (mg Ni/m^3) x years
[3] Low soluble nickel exposure = <10 (mg Ni/m^3) x years.
[4] High soluble nickel exposure = ˘ 10 (mg Ni/m^3) x years.
[5] Based on the mortality rates for England and Wales.

Table 5. Comparison of Risk of Dying of Nasal Cancer[1] at Different Levels of Cumulative Exposure to Soluble Nickel by Different Levels of Combined Cumulative Exposure to Sulfidic and Oxidic Nickel in the Mond/INCO (Clydach) Nickel Refinery.

Degree of exposure to sulfidic and oxidic nickel, respectively[2]	Low exposure to soluble nickel[3]			High exposure to soluble nickel[4]			Difference in the O/E values (p-value)
	O	E[5]	O/E	O	E[5]	O/E	
Low, low	7	0.166	42	3	0.025	120	0.284
Low, high	5	0.045	112	16	0.048	339	0.079
High, low	3	0.009	345	--	--	--	--
High, high	11	0.051	214	22	0.025	865	<0.001

[1] Includes all men with 15 or more years since first employment except those who worked in general trades. O = observed number of deaths, E = expected number of deaths, SMR = standardized mortality ratio.
[2] Low sulfidic nickel exposure = < 15 (mg Ni/m^3) x years and high sulfidic nickel exposure = ˘ 15 (mg Ni/m^3) x years; low oxidic nickel exposure = < 50 (mg Ni/m^3) x years and high oxidic nickel exposure = ˘ 50 (mg Ni/m^3) x years.
[3] Low soluble nickel exposure = < 10 (mg Ni/m3) x years.
[4] High soluble nickel exposure = ˘ 10 (mg Ni/m^3) x years.
[5] Based on the mortality rates for England and Wales

Table 6. Endpoints Considered as the Basis for the Nickel RfC [from NTP (1996a)]

Sex/Species	Duration	Endpoint	Region[1]	NOAEL/ LOAEL (mg Ni/m^3)	NOAEL(HEC)/ LOAEL(HEC) (mg Ni/m^3)	BMCL$_{10}$(HEC) (mg Ni/m^3)
M rat	Chronic	Lung fibrosis	PU	0.027/0.056	0.0021/0.0046	0.0017[2]
F rat	Chronic	Lung fibrosis	PU	0.027/0.056	0.0024/0.0052	0.0024
F rat	Chronic	Alveolar proteinosis	PU	0.027/0.056	0.0024/0.0052	0.0028
F rat	Chronic	Atrophy of olfactory epithelium	ET	0.056/0.11	0.0019/0.0039	0.0025-0.0026
M rat	Chronic	Atrophy of olfactory epithelium	ET	0.056/0.11	0.0033/0.0068	0.0038-0.0043
M rat	Chronic	Chronic active inflammation	PU	0.027/0.056	0.0021/0.0046	0.0020
F rat	Chronic	Chronic active inflammation	PU	0.027/0.056	0.0024/0.0052	0.0021
M rat	Chronic	Macrophage hyperplasia	PU	0.027/0.056	0.0021/0.0046	0.0012-0.0016
F rat	Chronic	Macrophage hyperplasia	PU	0.027/0.056	0.0024/0.0052	0.0013-0.0019
F rat	Subchronic	Atrophy of olfactory epithelium	ET	0.11/0.22	0.0016/0.0036	0.00048
F rat	Subchronic	Macrophage hyperplasia	PU	None/0.027	None/0.0027	–[3]

All data from NTP 1996a
[1]PU = pulmonary, ET = extrathoracic; TH = thoracic (pulmonary plus tracheobronchial)
[2]Used as the basis for the RfC
[3]Data not amenable to modeling because no information available on the shape of the concentration-response curve available