Jacques Whitford

WORKING GROUP REPORT

Nickel Oral Reference Dose for Non-Carcinogenic Effects

PROJECT NT34634

Report On **Working Group on Nickel Oral Reference Dose for Non-Carcinogenic Effects**

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EXECUTIVE SUMMARY

A privately funded Working Group was convened on July 3-4, 2007 in order to review and make recommendations on an appropriate oral reference dose (RfD) for non-cancer effects of nickel, including selecting a primary study upon which to base an oral RfD. In addition, three delegations to the working group were made by the Nickel Producers Environmental Research Association, Dr. Bruce Conard (consultant), and Dr. Brendan Birmingham of the Ontario Ministry of the Environment. Toxicological evaluations of nickel by various regulatory and advisory agencies were also provided to the Working Group.

A two-generation reproductive study in rats (Springborn, 2000b) was selected as the primary study to derive an oral RfD for nickel. The Working Group concluded that the appropriate no-observed-adverse-effect-level (NOAEL) from this study was 2 mg Ni/kg-d. The Working Group derived an oral RfD of 0.02 mg Ni/kg-d using this NOAEL and an uncertainty factor of 100 (10 for interspecies variation and 10 for intraspecies). Working Group members clearly noted that the use of one significant figure in the RfD was appropriate, to reflect the degree of precision in the value of the NOAEL.

The Working Group expressed doubt that any RfD could be protective of all hypersensitive individuals. However, they concluded that the RfD derived would be protective of greater than 99% of the total population.

The Working Group determined that it was appropriate to evaluate risk of exposure to nickel for the toddler (0.5 to 5 years of age) life stage using the derived RfD. Although not completely satisfied with this option, the Working Group believed that it was consistent with the overall goal of risk assessment. The Working Group recommended that this would be a topic worthy of a regulatory review and/or workshop.

NOTICE AND ACKNOWLEDGEMENTS

This report has been prepared as part of the activities of the Jacques Whitford Limited Working Group on Nickel Oral Reference Dose for Non-Carcinogenic Effects, which is a privately funded Working Group convened to provide scientific information and advice on the oral toxicity of nickel. Funding for the project was provided by CVRD-Inco to Jacques Whitford in relation to the Port Colborne, Community Based Risk Assessment. This report represents the consensus opinions of the Working Group and it does not necessarily reflect the views of Jacques Whitford Limited.

The members have reviewed this report on the Working Group on Nickel Oral Reference Dose for Non-Carcinogenic Effects and concur that it accurately summarizes the discussions and the overall findings of the workshop held in Markham, Ontario on July 3-4, 2007.

CHAIR

Dr. Christopher Ollson, Director, Environmental & Occupational Health Sciences, Jacques Whitford Limited, Burlington, Ontario, Canada

MEMBERS

Dr. George Becking, Toxicologist/Risk Assessment, Phoenix OHC, Inc., Kingston, Ontario, Canada

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RAPPORTEUR

Jennifer Foell, Risk Assessor, Jacques Whitford Limited, Markham, Ontario, Canada

DELEGATES

Nickel Producers Environmental Research Association (NiPERA)

Dr. Bruce Conard, Toronto, Ontario, Canada

Dr. Brendan Birmingham, Senior Research Toxicologist, Human Toxicology and Air Standards Section, Ontario Ministry of the Environment, Toronto, Canada

OBSERVERS

Dr. Eric Veska, William Stiebel, and **Rena Chung**, Jacques Whitford Limited, Markham, Ontario, Canada

GLOSSARY

µg	Microgram, one millionth of a gram
chRfD	Child-specific reference dose
d	Day
ED_5	Exposure dose eliciting a reaction in 5% of subjects
EPA	Environmental Protection Agency
F_0	Initial parental generation in a reproductive study
F_{1A}	First generation offspring in a reproductive study
F_{2B}	Second generation offspring in a reproductive study
GLP	Good laboratory practice
HQ	Hazard quotient
IOM	Institute of Medicine
Jacques Whitford	Jacques Whitford Limited
KBR	Kola birth registry
kg	Kilogram, 1000 grams
LOAEL	Lowest-observed-adverse-effect-level
MF	Modifying factor
mg	Milligram, one thousandth of a gram
MOE	Ontario Ministry of the Environment
MTD	Maximum tolerated dose
Ni	Nickel
NiPERA	Nickel Producers Environmental Research Association
NOAEL	No-observed-adverse-effect-level
OECD	Organization for Economic Co-operation and Development
OEHHA	Office of Environmental Health Hazard Assessment of the California Environmental Protection Agency
OH	Ohio
PM	Perinatal mortality
ppm	Parts per million
chronic RfD	Reference Dose, defined by the US EPA as “an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure for a chronic duration (up to a lifetime) to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.”
TDI	Tolerable Daily Intake
TERA	Toxicology Excellence for Risk Assessment
UFs	Uncertainty factors
ULs	Tolerable upper intake levels developed by the IOM
US(A)	United States (of America)

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WORKING GROUP ON NICKEL ORAL REFERENCE DOSE FOR NON-CARCINOGENIC EFFECTS

1.0 INTRODUCTION

1.1 Process for Developing this Report

In response to a number of issues surrounding nickel oral reference doses being used in risk assessments, a Working Group was convened to review the non-carcinogenic effects of nickel and to recommend an oral reference dose (RfD) for use in human health risk assessment. The full charge to the Working Group is included in Appendix A to this advisory report. Members of this Working Group were invited to participate in this process because their expertise in their respective disciplines was known to a number of senior toxicologists and risk assessors in Jacques Whitford Limited (Jacques Whitford).

The Working Group was chaired by Dr. Christopher Ollson of Jacques Whitford . Dr. Ollson coordinated the development of the charge to the Working Group (Terms of Reference), selected Working Group members, conveyed the Working Group, facilitated the two day workshop and provided oversight of the preparation of this advisory report. The Chair did not participate as a member of the Working Group, but ensured that the charge to the Working Group was completed through his facilitation. Ultimately, the objective of the Chair was to ensure that the report accurately reflected the deliberations and findings of the Working Group.

The members of the Working Group were selected based on their knowledge of toxicology, risk assessment, past experience in government and international approaches to regulation of exposure to chemicals, development of toxicity reference values, toxicokinetics, animal laboratory studies, developmental toxicology, and nickel toxicity.

Working Group members were contacted in June 2007 to discuss their participation in this endeavor. Upon agreement to participate in the process, they were sent a package of selected scientific articles, government reports, and laboratory studies that formed the basis of their review. This was not meant to be an exclusive list of source material, but rather a starting point. Working Group members were asked to bring forward any additional publications they felt were relevant to the charge. A full list of reference material distributed is included in Appendix B. Members convened on July 3rd and 4th, 2007 in Markham, Ontario, Canada, to deliberate on the charges to the Working Group. A draft report of the meeting was prepared and reviewed by the Working Group between July 29 and September 12, 2007.

This report is structured according to the discussions that occurred in the workshop and in accordance with the charge to the Working Group. All members participated fully in all discussions and in preparation of this report.

1.2 Charge to the Working Group, Objectives and Process

The charge to the Working Group was

> *"to conduct a review of nickel ingestion non-cancer reference toxicity values and develop a summary report documenting review comments of the expert Working Group."*
>
> See Appendix A

The Working Group was convened in order to select a scientifically defensible and appropriate human oral reference dose (RfD) for nickel exposure based on its non-carcinogenic effects, for application in human health risk assessment.

For the purposes of the Working Group, the US Environmental Protection Agency (US EPA) definition of the RfD was used:

> *Reference Dose (RfD): An estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure for a chronic duration (up to a lifetime) to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or benchmark dose, with uncertainty factors generally applied to reflect limitations of the data used.*
>
> *US EPA Glossary of Terms, 2007*

The Working Group asked for clarification on whether their charge was limited to reviewing and selecting from among existing RfD values, or whether the group could propose a new value. The Chair indicated that the Working Group could develop a new value if they deemed it appropriate.

Although the charge was specific to non-cancer effects, the Working Group concluded that they had sufficient material to discuss whether the potential for oral carcinogenicity of nickel should be considered in the development of the RfD. The Chair agreed that the charge should not limit the Working Group's ability to review and comment on this.

Some discussion occurred regarding human studies based on occupational inhalation of large quantities of nickel containing dust. Although the charge was specific to oral exposures, the members agreed that some occupational exposures might result in a systemic dose that could be concluded to be relevant to the same toxic endpoints of interest for oral exposures. The Working Group concluded that consideration and evaluation of such studies with respect to systemic effects would be appropriate and within its mandate.

The Working Group's review was completed through the evaluation of published scientific literature, government agency documentation, and laboratory reports. Prior to the start of the Working Group's review, presentations to the Working Group were made by three invited delegations (see Section 1.3).

In addition to Working Group members, three observers were present throughout the proceedings. These were three Jacques Whitford scientists who are actively involved in the Port Colborne, Community Based Risk Assessment. The observers were not permitted to participate in any of the technical discussions of the Working Group.

The Working Group undertook a critical evaluation of the scientific merits of the available nickel toxicity studies. This structured review of scientific information involved:

- evaluation of the strengths and weaknesses of each study considered,
- determination of the appropriate study NOAEL / LOAEL, and the precision associated with these values, and
- classification of each study, in terms of its utility in deriving the RfD, as a:
    - "primary study" to be used quantitatively in deriving the RfD,
    - "supporting study" that provided support for the primary study, or
    - "other study" that provided qualitative or ancillary information relevant to evaluating the oral non-cancer toxicity of nickel.

Upon selection of the primary study for developing the oral RfD, the Working Group discussed the appropriateness of application of uncertainty factors to the study NOAEL. A debate on the degree of conservatism in the NOAEL was also undertaken. Through these discussions, the Working Group's preferred non-carcinogenic oral reference dose for nickel was developed.

The final charge to the Working Group was to discuss the applicability of the RfD to various life stages of exposure, often modelled in human health risk assessment. This charge revolved around the issue of whether hazard quotients should be developed for less-than-lifetime exposures using the various life-stages commonly considered in Canadian risk assessments. This discussion also considered the appropriateness of deriving site-specific soil (or other) remediation objectives based on a hazard quotient calculated for toddler life stage (0.5 to 5 years) exposures.

Additional discussion focused on whether the proposed RfD should, or could, address hypersensitive population subsets.

The proposed RfD was based on studies available to the Working Group at the time of its deliberations. The Working Group acknowledged that the mode of action and toxicity of nickel in mammalian systems remains an active field of research and expected that new information will continue to be published in the scientific literature. For this reason, the Working Group noted that the development of an oral RfD for nickel would be subject to change.

1.3 Delegations to the Working Group

Three organizations were invited by the Chair to make presentations concerning their views on issues to be considered in developing an appropriate RfD for nickel to be used in human health risk assessment. The delegations were selected by the Chair based on his knowledge on the expertise of these individuals or organizations on the topic of nickel toxicity. Working Group members were permitted to ask questions throughout the presentations of the delegates or to further expand on points of discussion. After the three presentations had been heard, delegates were asked to leave and the Working Group began its review of the literature relevant to the charge.

The Nickel Producers Environmental Research Association (NiPERA) prepared a position paper "Review of Nickel Toxicity Studies for the Purpose of Establishing an RfD or TDI" (Appendix C) for the Working Group to consider. Dr. Bruce Conard, a consultant to CVRD-Inco Limited, provided a summary of this information (Appendix D), and was clear when he was expressing his personal opinions, rather than NiPERA's. Upon completion of the presentation, the Working Group had the opportunity to phone Dr. Adriana Oller of NiPERA to seek further clarification of several points.

Dr. Bruce Conard then presented his views concerning the use of RfDs in risk assessment (Appendix E). Finally, Dr. Brendan Birmingham, representing the Ontario Ministry of the Environment (MOE), presented on the topic of updating the nickel RfD, addressing three main issues: the intent behind a review of the US EPA's RfD for nickel soluble salts; an overview of the toxicity studies considered by the MOE in their review; and, the proposed position of the MOE on a nickel oral RfD.

2.0 REVIEW OF STUDIES

2.1 Weight of Evidence Evaluation

Several studies were reviewed by the Working. A common set of study attributes were developed that were, at a minimum, discussed for each study.

For human studies:

- Did the study examine an exposed occupational population or was it a human dosing study?
- What was the route of exposure?
- Was the exposure acute, subchronic, or chronic?
- What was the size of the cohort, or population of the study?
- Did the study examine dosing/exposure at varying life stages?
- Did the study examine sensitive individuals?

For animal studies:

- Was the exposure acute, subchronic, chronic?
- How was nickel administered (e.g. in food or water, by gavage, etc.)
- Was the study a two generation reproduction study?
- Did it examine toxicity at different life stages? (weanling, juvenile, adult)
- The Working Group discussed that dietary studies may be preferable over gavage, as exposure is a more relevant surrogate, less stressful and better represents the nature of human exposure (animals exposed via gavage are usually fasted).
- Could animal husbandry practices have influenced findings, affecting utility of the study?
- Was a clear dose/response relationship observed?
- Did the study comply with international recognized protocols such as the Organization for Economic Co-operation and Development (OECD), Standards Council of Canada Good Laboratory Practice (GLP) and the United States EPA?

In both human and animal studies, the Working Group agreed that the presence of a clear dose-response relationship was a key prerequisite in selecting a primary study upon which to base an oral RfD. After considering the above factors, studies unsuitable to support derivation of the oral RfD for nickel were rejected from further consideration. The remaining studies were classified as “primary”, “supporting” or “other” for the development of a nickel oral RfD. Studies reviewed by the Working Group are summarized below, with additional notes on Working Group discussions. The studies are presented in the order they were discussed by the Working Group.

2.2 Human Studies

Nielsen *et al.* (1999) Absorption and retention of nickel from drinking water in relation to food intake and nickel sensitivity. *Toxicology and Applied Pharmacology* **154:** 67-75.

Summary

Nielsen *et al.* used a stable nickel isotope (^{61}Ni) to examine possible differences in nickel absorption between twenty nickel-allergic (sensitized) female patients with vesicular hand eczema who were age-matched to a control group of 20 female patients with a similar type of eczema but without nickel allergy. Females fasted overnight and then received a bolus dose of nickel solution (12 µg/kg), and remained fasting for an additional 4 hours. During the study period, the eczema of all control patients remained unchanged. In the nickel-sensitized group, nine exhibited a flare-up (or exacerbation) of symptoms after nickel intake.

Discussion

The Working Group discussion focused on the study design and its applicability to deriving an oral RfD. The study administered an acute dose (single dose of 12 µg Ni/kg), and measured an acute reaction in the test subjects. The study did not include a placebo control for the nickel-sensitized group, nor was a second dose administered. The control group used may have been appropriate for the study objectives, but not for the development of an RfD. No dose-response relationship could be elicited because only a single dose was administered. In any event, the effect was an immune response that, classically, does not follow a dose-response relationship. Additionally, the Working Group questioned control of nickel exposure through routes other than oral and discussed the possibility that test subjects could have had coincidental exposure to other sources of nickel (e.g., jewelry).

The Working Group also noted that it would not be appropriate to apply the results of Nielsen et al. (1999) to the general population. Nielsen et al. (1999) focused upon exacerbation of an existing condition (eczema) in a "diseased population". The Working Group concluded that an RfD should be protective of healthy and sensitive populations, but may not adequately protect a hypersensitive population subset. Following discussion, the Working Group reached a consensus that the study was not appropriate to use for deriving an oral RfD.

Working Group Recommendation

Study should be considered as an "Other Study" for the purpose of RfD derivation, based on the limitations summarized above.

Jensen *et al.* (2006) Systemic contact dermatitis after oral exposure to nickel: a review with a modified meta-analysis. *Contact Dermatitis* **54:** 79-86.

Summary

Jensen et al. (2006) conducted a meta-analysis to determine possible threshold values for oral nickel exposure that would elicit allergic contact dermatitis in nickel-sensitive individuals. Seventeen studies on oral nickel exposure in nickel-sensitive patients, published from January 1966 to November 2004, were identified through literature searches. In order to address analysis weakness, studies without placebo testing, studies which had positive responses to placebo and studies using more than a single

exposure were excluded from analysis. The remaining nine studies were analyzed in a stepwise procedure. The studies were divided into a homogenous group of five studies and two groups of two studies with higher and lower response frequency, described by logistic dose-response curves shifted in parallel. Based on these curves, calculations of doses that would, theoretically, cause systemic contact dermatitis in exposed nickel-sensitive patients were carried out. It was extrapolated that the nickel content of a normal diet (between 0.22 and 0.35 mg Ni) may elicit a systemic reaction in 1% of sensitive patients and that 10% may react to exposures between 0.55 and 0.89 mg nickel.

Discussion

The Working Group noted several relevant issues highlighted by the meta-analysis, including that nickel absorption in the gastrointestinal tract is greater from exposure to drinking water than soil, and that absorption is greater in fasting versus non-fasting subjects. The Working Group concluded that the threshold of elicitation of allergic contact dermatitis in nickel-sensitive individuals was approximately 20 µg/kg-d.

Working Group Recommendation

Jensen et al. (2006) should be considered a “Supporting Study” in the development of an RfD for oral nickel intake.

Hindsén *et al.* (2001) Flare-up reactions after oral challenge with nickel in relation to challenge dose and intensity and time of previous patch test reactions. *Journal of the American Academy of Dermatology* **44:** 616-623.

Summary

In Hindsén *et al.* (2001), thirty female patients with allergies to nickel were employed to investigate the importance of ingested nickel dose, time interval between nickel patch testing and oral nickel challenge, and degree of nickel hypersensitivity in relation to reaction. Patients were separated into two eczema groups: one group of 12 patients with atopy and hand eczema and the other group of 18 patients without atopy and hand eczema. Each patient was patch tested with a serial dilution of nickel sulphate on four separate occasions. The interval between the tests was approximately 2.5 months. One month following the final patch test, when test reactions were completely healed, patients were divided into three oral dosing groups: placebo, 1.0 mg Ni and 3 mg Ni.

Patients fasted from midnight before administration of the challenge dose until one hour after dosing. In the placebo group, no positive signs of flare-up were observed; in the 1.0 mg nickel group, two subjects had signs of positive reactions; in the 3 mg nickel group, all subjects had positive reactions in at least one area. There were significantly more flare-up reactions of the most recent patch test sites (performed one month previously) as compared with the earlier test sites. There was also a statistically significant positive correlation between the intensity of previous positive patch tests and the intensity of the flare-up reaction. More frequent and severe reactions of earlier patch test sites in the atopy group, compared with the non-atopy group, were not observed. The authors found eczematous recall reactions after oral nickel challenge to be dose- and time-dependent and related to the intensity of previous patch test reactions.

Discussion

Hindsén et al. (2001) focused on acute elicitation of dermatitis in a chronically exposed population. The Working Group determined that the study population represented a hypersensitive, yet not diseased, population because no subjects had eczema at the time of testing. Most members of the Working Group agreed that the 1 mg nickel challenge dose represented a study LOAEL, not a NOAEL, based on the dosing regime. However, it was considered inappropriate to derive a NOAEL based on the 1 mg nickel LOAEL through the application of a 10-fold uncertainty factor because this would result in the derivation of an RfD for nickel less than that to which the population is normally exposed. The Working Group consensus was that the study did not identify a NOAEL. The study also focused on a single challenge exposure, and was therefore considered unsuitable for developing an RfD, which is intended to be protective for conditions of chronic exposure.

Working Group Recommendation

This study should be considered as an "Other Study" because it was not suitable for deriving an RfD for the reasons provided above.

Vaktskjold *et al.* (2004) The Kola Birth Registry and perinatal mortality in Mončegorsk, Russia. *Acta Obstetricia et Gynecologica Scandinacica* **83:** 58-69.

Summary

Vaktskjold et al. (2004) examined the quality and content of the population-based Kola Birth Registry (KBR) and estimated perinatal mortality (PM) rates for the period of 1973-1997 based on KBR data. The KBR was initiated in 1997 in Mončegorsk, a town in the Kola Peninsula of Russia. The largest employer in the town is Severonikel nickel refinery, where 41.9% of town inhabitants worked in 1995. The establishment of the KBR involved the retrospective inclusion of all births in the town since 1973. The aim was to register all live births and still births, after 28 weeks of pregnancy. Spontaneous abortions that occurred at the birth clinic before 28 weeks were also registered. Vaktskjold et al. (2004) identified a data entry error rate of 0.5% and noted that one or two of three main sources of information were missing in 2.0% of records.

Detailed information about the newborn, delivery, pregnancy and mother for 21,214 women were contained within the KBR, covering at least 96% of all births in the population in the period studied. Fifty-three percent of the registered deliveries involved births where one or both parents were employed at the nickel plant, while 23% of women were employed at the nickel plant at the time of delivery. A total of 391 perinatal deaths were recorded, amounting to an overall PM rate of 18.5/1,000 births. A trend analysis revealed that the PM decreased annually by 0.43 deaths/1000 births in the period of 1973-1997, from more than 20 to less than 10 deaths/1000 births during the study period. However, it was noted that mortality rates were elevated from 1986-1988. The number of births during these years was 20-30% higher than earlier years, suggesting that a high influx of deliveries might have strained the health care system's capacity, thereby affecting maternal and newborn care quality. Overall, the PM rate in Mončegorsk was lower than the overall rate in Russia. The KBR provides an extensive data source on a homogenous population, which makes it advantageous for epidemiological studies.

Discussion

The Working Group noted that although perinatal mortality was shown to decrease over time, no hypothesis was proposed to explain this observation. Additionally, perinatal exposure occurred in an occupational setting primarily through inhalation, although it is likely that oral exposure would also occur due to swallowing of airborne nickel directly, as well as inhaled nickel cleared from the lung by the mucocilliary transport system.

Working Group Recommendation

This study was considered an "Other Study" by the Working Group because it provides no quantitative basis for establishing an RfD for nickel. In addition, no hypothesis was proposed to relate nickel exposure to PM.

Vaktskjold *et. al.* 2006. Genital malformations in newborns of female nickel-refinery workers. *Scandinavian Journal of Work, Environment and Health.* **32:** 41-50.

Summary

Vaktskjold et al. (2006) used the KBR to investigate whether pregnant women employed in nickel-exposed work areas were at an elevated risk of delivering a newborn with a genital malformation. The Registry includes extensive data on more than 98% of live births in the borough of Mončegorsk, Russia from 1973 through 2001, including up to five diagnoses per newborn of congenital malformations and deformations, the birth women's job function and her employer. For the purposes of the study, three nickel exposure levels were identified: control, low and high. Women were assigned to a nickel exposure category according to their occupation at the time of becoming pregnant. Assessments of job-related personal exposure were conducted in production departments of the nickel refinery in Monchergorsk. Because measured urinary nickel concentration is proportional to serum nickel, urinary nickel was considered an index of fetal exposure. The reference population comprised delivering women from Mončegorsk with a background nickel exposure level.

Following data selection, the investigated population consisted of 23,141 live or stillborn infants, comprised of 48.9% girls and 51.1% boys. Vaktskjold et al. (2006) determined an overall prevalence rate of genital organ malformation of 44.5/10,000 births. The odds ratio of delivering a newborn with a genital malformation for women working in nickel-exposed areas was 0.81 and that for undescended testes was 0.76. Prevalence rates for undescended testes and hypospadias were 23 and 16 per 10,000 births, respectively, comparable to rates in Norway. The authors found that maternal exposure during the periconception period and early pregnancy to water-soluble nickel had no adverse effect on the risk of delivering a newborn with malformations of the genital organs.

Discussion

The Working Group discussed several aspects of the study in evaluating its utility for developing an RfD for oral nickel exposure. Nickel exposure was likely primarily through inhalation in an occupational environment; however, systemic exposure was demonstrated through urinary sampling. Co-exposure to other metals in the occupational setting was addressed by the authors and was concluded by the Working Group to not be a confounder. No dose-response relationship was elicited because no adverse effect was demonstrated in any of the control, low or high exposure groups. Vaktskjold et al. (2006) determined that the approach taken would underestimate findings in the dose groups, biasing the results towards null findings. However, the Working Group felt that the case assignments of the

authors tend to bias results towards positive findings because four diagnoses that were uncertain were included within the database.

Working Group Recommendation

Vaktskjold *et al.* (2006) should be classified as a "Supporting Study" in the development of an oral RfD for nickel. Although several exposure groups were considered in a well defined study population chronically exposed to nickel, no adverse effects were observed and exposure was likely predominantly by inhalation, which limit its usefulness for establishing an oral RfD.

2.3 Animal Studies

Ambrose *et al.* (1976) Long term toxicologic assessment of nickel in rats and dogs. *Journal of Food Science and Technology* **13**: 181-187.

Summary

Ambrose *et al.* (1976) conducted a two-year feeding study of rats and dogs and a three-generation reproduction study of rats to determine the long-term toxicological effects of nickel exposure. Nickel sulphate was added to the diet of 25 rats/group and 6 beagles/group for two years at dietary concentrations of 1, 100, 1000 and 2500 ppm nickel. In rats, the high dose group experienced depressed body weight gain as compared to controls. Food consumption patterns suggested that the lesser weight gain in rats at the 2500 mg Ni/kg-d dietary level might have been a result of lower food consumption. In dogs, the high dose group also experienced reduced weight gain, although cumulative food consumption could not explain differences in body weight.

In rats, a tendency towards increased heart-to-body weight ratios and decreased liver-to-body weight ratios in female rats on 1000 and 2500 ppm diets was identified. Organ-to-body weight ratio data indicated that dogs on the 2500 mg/kg diet had statistically higher kidney and liver to body weight ratios than dogs in other treatment groups. All dogs consuming 2500 mg Ni/kg-d demonstrated histologic changes in the lungs; however, histologic findings in rats were essentially negative. Hemograms in rats were within the normal range; in dogs, subjects on the 2500 ppm diet exhibited slightly lower hematocrit and hemoglobin values. Urinary findings were normal except for marked polyuria in two dogs on the 2500 ppm diet. Evaluation of tissues storage of nickel in various organs indicated no important storage sites. In a three-generation reproduction study in Wistar-derived rats, subjects were placed on a diet of 0, 250, 500 or 1000 ppm Ni. In all generations, weanlings on 1000 ppm diets exhibited slightly lowered body weights. A higher incidence of stillborn pups was noted in the first generation at all levels of nickel exposure. No teratogenic effects were noted and histopathologic findings in the third generation rats were negative.

Discussion

The study was considered to have been well conducted for its time; however, it does not conform to present day OECD and GLP protocols. The study was recognized for demonstrating that rats were more sensitive to nickel exposure than dogs, an important point for the interpretation of other work. The Working Group concurred with the conclusion of the US EPA in the IRIS database that confidence in the study is low due to high mortality in controls.

Working Group Recommendation

Although Ambrose et al. (1976) provided information suitable to support the derivation of an oral RfD in the past, more recent studies that conform to current testing protocols are now available. Therefore, Ambrose *et al.* (1976) is considered a "Supporting Study".

Smith *et al.* (1993) Perinatal toxicity associated with nickel chloride exposure. *Environmental Research* **61:** 200-211.

Summary

Smith et al. (1993) investigated the effects of consumption of soluble nickel salts on development in Long-Evans rats. Four groups of 34 virgin female rats were given nickel chloride in drinking water solutions of 0, 10, 50 and 250 ppm Ni^{2+} for a period of 11 weeks prior to mating and then during two successive gestation and lactation periods. Pups were observed until weaning; breeder males were unexposed. The average daily intake of water was comparable across all dose groups, with the exception of females dosed with 250 ppm Ni. This group demonstrated reduced water intake during the re-breedings and two gestation periods, which was attributed to taste aversion. Additionally this group of females demonstrated increased food consumption during the second mating, gestation and lactation periods.

Maternal weight gain was reduced during the first gestation period in the 50 and 250 ppm Ni dose groups. No differences in the reproductive performances of females were noted in any dose groups during either the first or second gestation periods. No significant differences in litter size or pup weight were noted, with the exception of male pups exposed to 50 ppm Ni during the first lactation period, which demonstrated reduced weight gain. During the first gestation period, the number of pup deaths increased significantly only in the litter of females consuming 250 ppm Ni-containing water, probably a result of large control losses. However, during the second lactation period, deaths were dose related, increasing significantly at each dose level. Prolactin levels in pups were unchanged by treatment, but were reduced in dams at the high dose level.

Smith et al. (1993) concluded that there was clear evidence of a treatment-related increase in perinatal mortality. The authors concluded that 10 ppm Ni (1.3 mg Ni/kg-d) represented the LOAEL for this effect. A NOAEL was not identified.

Discussion

The Working Group identified significant methodological problems and concluded this study was not appropriate for use in deriving an oral RfD. Monitoring of cannibalism was not effective, which may have contributed to abnormally high control deaths in the second generation. Additionally, a dose-related increase in pup mortality was only observed in the second generation, not the first, with no explanation offered. The Working Group was concerned by the inability of the study authors to produce the primary study data, as requested by the US EPA during its consideration of the study for the purposes of deriving a RfD.

Working Group Recommendation

This study was concluded to be not appropriate for the derivation of an oral RfD, and is classified as an "Other Study" for deriving an oral RfD for nickel.

Vyskočil *et al.* (1994) Chronic nephrotoxicity of soluble nickel in rats. *Human and Experimental Toxicology* **13:** 689-693.

Summary

Vyskočil et al. (1994) examined the nephrotoxic effects of chronic oral exposure to nickel sulphate at a dose similar to the previously reported NOAEL of Ambrose (1976) in order to characterize early changes in the glomoruli and tubules. Sensitive indicators of such effects, including urinary albumin, β_2-microglobulin, lactate dehydrogenase and N-acteyl-β-D-glucosaminidase, were evaluated in 24-hr urine samples. Groups of 10 Wistar rats were placed in either a control or exposed group. Exposed rats were given drinking water containing 100 mg/L of nickel (as nickel sulphate). Two exposure durations were tested: three and six months. Following the given exposure period, 24-hr urine samples were collected and analyzed for the aforementioned sensitive indicators. Exposed male rats experienced a significant increase in kidney weight after six months of exposure. In female rats, a significant difference in albumin excretion between control and exposed individuals was found after 6 months. No differences in albumin excretion were noted in males; however, the absence of statistical significance was due to two extreme values observed in the control group following six months of exposure. No significant differences in body weight were noted.

The results suggested that chronic oral exposure to nickel at previously reported NOAEL doses in rats (Ambrose, 1976) either induces slight changes of glomerular permeability in female, and possibly male, rats or enhances the normal age-related glomerular nephritis lesions of rats. Female rats appeared to be more sensitive to the nephrotoxic effects of nickel than males. The oral intake was probably not high enough to induce significant tubular changes.

Discussion

The Working Group agreed that the study was well conducted. The study suggests that there could be an effect at an oral dose of nickel that was previously thought to be a NOAEL. However, because a single dose was administered, a dose-response relationship could not be determined. Additionally, and as noted by the authors themselves, the effects were subtle and at the fringe of significance. The study reported a possible nephrotoxic effect, but Vyskočil et al. (1994) noted that this might be an exacerbation of a natural biological phenomenon. A subsequent study failed to find increased excretion of albumin over controls in either male or female rats after 103 weeks of exposure to 2.2, 6.6 and 11 mg Ni/kg-d (Heim et al. 2007; CRL, 2005).

Working Group Recommendation

This study was considered as an “Other Study” in the determination of an oral RfD for nickel, based principally on the study design (single dose, sub-chronic exposures).

Springborn Laboratories (2000a) *A One Generation Reproduction Range-Finding study in Rats with Nickel Sulfate Hexahydrate.* Ohio Research Center, Spencerville, OH, USA.

Summary

Springborn Laboratories (2000a) administered nickel sulphate hexahydrate to rats by oral gavage over the course of one generation. This study is known as a “Range-Finding Study” and is not meant to be

as robust as a two-generation reproductive study. Its purpose was to determine the appropriate range of doses that should be included of a follow-up study of reproductive effects. Six groups of eight female and eight male Sprague-Dawley rats were dosed daily. Dosing levels were set at 0, 10, 20, 30, 50 and 75 mg nickel sulphate hexahydrate/kg-d. Dosing of parental animals (F_0) began 14 days prior to mating and dosing of first generation (F_1) offspring began on postnatal day 22. In both generations, dosing continued until the day prior to euthanasia. Oral administration of nickel sulphate hexahydrate had no effect on F_0 survival, growth, mating behavior, copulation, fertility, precoital intervals, gestation lengths, or gross necropsy findings. However, post-implantation loss of pups was significantly different among groups. Loss was significantly increased at the 30, 50 and 75 mg/kg-d dosing levels. Mean live litter size on day 0 was significantly decreased at the 75 mg/kg-d level. Although post-implantation losses higher than controls were noted in the 10 and 20 mg/kg-d groups, these were not statistically significant compared to the control group. A low loss rate was noted in the control group and losses in the 20 mg/kg-d group were noted to be within the range of historic controls. In the 10 mg/kg-d dose group, the significantly different loss rate was driven by one female with a total loss of litter. Based on the results of this range-finding study, dosage levels of 1.0, 2.5, 5.0 and 10.0 mg nickel sulphate hexahydrate/kg-d were selected for a two-generation reproduction study in rats (Springborn 2000b).

Discussion

The Working Group concluded that Springborn (2000a) represented a good range-finding study, fulfilling its goal of determining a range of doses for a two-generation reproductive study. However, it concluded that Springborn (2000a) was not a reproduction study. Rather, the Working Group decided the study was intended to determine the maximum tolerated dose (MTD) that should be employed for further testing. The power of the study was limited due to the small number of animals used and the study did not conform to any international accepted protocols for conducting reproductive studies. It was agreed that the work of Springborn (2000a) could not be used to produce an RfD for reproductive endpoints because, among other considerations, the range-finding study was not intended to measure reproductive outcomes.

Working Group Recommendation

Springborn (2000a) is considered a “Supporting Study” as it provides the basis for the dosing regimen used in a two-generation reproductive study.

Springborn Laboratories (2000b) *An Oral (Gavage) Two-Generation Reproduction Toxicity Study in Sprague-Dawley Rats with Nickel Sulfate Hexahydrate.* Ohio Research Center, Spencerville, OH, USA.

Summary

Springborn Laboratories (2000b) reported a two-generation reproductive study in male and female rats, which was designed to evaluate the potential effects of nickel sulphate hexahydrate on reproduction. Treatment groups of 28 male and 28 female Sprague-Dawley rats each were subjected to one of five dosage levels: 0, 1.0, 2.5, 5.0, or 10.0 mg nickel sulphate hexahydrate/kg-d, and dosed daily by oral gavage. F_0 parental animals were dosed for 10 weeks prior to mating. F_1 offspring were treated beginning on postpartum day 22. In order to produce F_2 litters, 28 male and 28 female parental animals were selected randomly from each dosing level in the F_1 generation. After a minimum of 70 days of dosing, each F_1 female was bred. F_0 males subjected to 10.0 mg/kg-d had decreased absolute and relative liver weights. In the F_1 generation, no toxicologically meaningful differences were noted in pup

viability data or pup body weights during lactation. F_1 male rats subjected to 5.0 and 10.0 mg/kg-d showed lower relative liver weight. Despite a slight reduction in adult male liver weights, 10.0 mg/kg-d was selected as the NOAEL for oral administration of nickel sulphate over two generations in rats because no treatment-related histopathological effects were observed in the liver or other tissues at this level and no indications of toxicity or adverse reproductive effects were observed at dosage levels up to 10 mg /kg-d.

Discussion

The Working Group thought this study was well conducted. It employed a large number of animals and conformed to GLP. One limitation is that OECD guidelines recommend that the highest dose tested should elicit some form of toxicity in the parental generation, which in this case it did not, although it was provided in the range-finding study. The Working Group noted that based on the range-finding study (Springborn, 2000a), doses higher than 10.0 mg /kg-d should have been included because significant toxicity occurred only at doses above 30 mg/kg-d in Springborn (2000a). Therefore, the true NOAEL may actually be higher than the highest dose tested in the two-generational study.

Working Group Recommendation

Springborn (2000b) was identified as the "Primary Study" from which the Working Group would develop an oral RfD for nickel. The Working Group had a high degree of confidence in the suitability of this study to support derivation of the RfD.

Heim *et al.* (2007 in press) Oral carcinogenicity study with nickel sulfate hexahydrate in Fischer 344 rats. *Toxicology and Applied Pharmacology.*

Summary

Heim et al. (2007 in press) reported a two-year carcinogenicity study in Fischer 344 rats with nickel sulphate hexahydrate administered daily by gavage. The study protocol was compliant with OECD 451 and EPA OPPTS #870.4200 protocols. Sixty female and sixty male animals were assigned to each exposure group, the levels of which were selected based on a 90-day range-finding study. Exposure levels of 0, 10, 30 and 50 mg nickel sulphate hexahydrate/kg-d (equivalent to 0, 2.2, 6.6 and 11 mg Ni/kg-d) were used.

In females, there was a dose-related increase in mortality relative to controls; however, overall survival was excellent throughout the study in both males and females, with a minimum of 40% survival by study termination at week 105. Body weights were observed to decrease in an exposure-dependent manner, with biologically significant decreases (≥10%) observed in the two highest exposure groups in males and females, indicating that the maximum tolerated dose (MTD) was reached. Treatment did not produce an exposure-related increase in tumorigenicity. Results indicated that nickel sulphate hexahydrate does not cause carcinogenicity in rats when administered orally at exposures up to the MTD. The significant decrease in body weight in the two highest exposure groups indicate a NOAEL of 10 nickel sulphate hexahydrate mg/kg-d (2.2 mg Ni/kg-d) and a LOAEL of 30 mg nickel sulphate hexahydrate /kg-d (6.7 mg Ni/kg-d) for this endpoint.

Discussion

The Working Group agreed with the conclusion of Heim et al. (2007 in press) that there was no evidence of nickel oral carcinogenicity in their study. In addition, the Working Group noted that this study did not find increased excretion of albumin over controls in either male or female rats after 103 weeks of exposure to 2.2, 6.6 and 11 mg Ni/kg-d. The Working Group concluded that the lack of increased albumin excretion in a modern, well conducted lifetime feeding study was sufficient to refute potential nephrotoxic effects of nickel reported by Vyskočil et al. (1994).

Working Group Recommendation

This study was considered a "Supporting Study" in the development of an RfD for oral nickel intake.

2.4 Overview

Table 2-1 provides a summary of published scientific literature reviewed by the Working Group.

Table 2-1 Published scientific articles reviewed by the Working Group in the derivation of an oral reference dose

Author	Year of Publication	Title	Citation	Study Classification by Working Group for Derivation of RfD
Springborn Laboratories	2000b	An Oral (Gavage) Two-Generation Reproduction Toxicity Study in Sprague-Dawley Rats with Nickel Sulfate Hexahydrate.	Ohio Research Center, Spencerville, OH, USA.	Primary
Heim *et al.*	2007 (in press)	Oral carcinogenicity study with nickel sulfate hexahydrate in Fischer 344 rats.	*Toxicology and Applied Pharmacology*	Supporting
Jensen et al.	2006	Systemic contact dermatitis after oral exposure to nickel: a review with a modified meta-analysis	Contact Dermatitis 54: 79-86.	Supporting
Vaktskjold et. al.	2006	Genital malformations in newborns of female nickel-refinery workers.	Scandinavian Journal of Work, Environment and Health. 32: 41-50.	Supporting
Springborn Laboratories	2000a	A One Generation Reproduction Range-Finding study in Rats with Nickel Sulfate Hexahydrate.	Ohio Research Center, Spencerville, OH, USA.	Supporting
Ambrose et al.	1976	Long-term toxicologic assessment of nickel in rats and dogs.	Journal of Food Science and Technology 13: 181-187.	Supporting
Vaktskjold et al.	2004	The Kola Birth Registry and perinatal mortality in Mončegorsk, Russia.	Acta Obstetricia et Gynecologica Scandinacica 83: 58-69.	Other
Hindsén et al.	2001	Flare-up reactions after oral challenge with nickel in relation to challenge dose and intensity and time of previous patch test reactions.	Journal of the American Academy of Dermatology 44: 616-623.	Other
Nielsen et al.	1999	Absorption and retention of nickel from drinking water in relation to food intake and nickel sensitivity.	Toxicology and Applied Pharmacology 154: 67-75.	Other
Vyskočil et al.	1994	Chronic nephrotoxicity of soluble nickel in rats.	Human and Experimental Toxicology 13: 689-693.	Other
Smith et al.	1993	Perinatal toxicity associated with nickel chloride exposure.	Environmental Research 61: 200-211.	Other

2.5 Discussion of Reviews and Government Documents

Danish EPA. 2005. Nickel Sulphate, CAS-No.: 7786-81-4, EINECS-No.: 232-104-9, Risk Assessment. Danish Environmental Protection Agency, Draft.

Summary

In 2005, the Danish EPA released their draft risk assessment on exposure to nickel sulphate. The review provides general information on nickel, exposure, human toxicity, and their risk assessment. The Danish EPA reanalyzed the data of the two-generation reproductive study in rats (Springborn, 2000b) by combining perinatal mortality data and post-implantation loss, resulting in a statistically significant effect in the high dose group (2.2 mg Ni/kg-d).

Discussion

The Working Group considered that combining toxicological endpoints might be appropriate when endpoints are biologically linked. In this instance, the Working Group was unaware of a mechanistic basis for combining the endpoints of perinatal mortality and post-implantation loss, and, therefore considered the combination of such endpoints for analysis to be biologically inappropriate.

IOM. Institute of Medicine. 2001. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc. Food and Nutrition Board. National Academy Press: Washington, DC, USA.

Summary

Institute of Medicine (IOM) (2001) presented a review of the literature available on nickel and developed tolerable upper intake levels (UL) for human life stages. The IOM defines the UL as:

> *"the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals"*

In reference to nickel, the derived UL apply to "excess" nickel intake as soluble nickel salts. Based upon consideration of data quality, relevance to human dietary exposure, and sensitivity of the toxicological endpoint—decreased body weight, as reported in subchronic and chronic rat studies (ABC, 1988; Ambrose *et al,* 1976), was chosen as the critical endpoint from which to derive the UL.

Other endpoints, including carcinogenicity and hypersensitivity in humans, were not considered relevant to human dietary exposure. Both the 90-day rat gavage study (ABC, 1988) and 2-year chronic dietary study in rats (Ambrose et al*.,* 1976) identified a NOAEL of 5 mg Ni/kg-d on the basis of decreased body weight gains. An uncertainty factor of 300 (10 for interspecies variation, 10 for intraspecies variation, 3 for concerns regarding potential reproductive effects of nickel at levels lower than the NOAEL identified by the chronic study) was applied to the NOAEL to derive a UL of 0.017 mg/kg-d for adults. Assuming a body weight of 61 kg for an adult woman, a UL of 1.0 mg/d was derived. Due to a lack of available data, the UL was judged indeterminable for the infant life stage. The adult UL of 1.0 mg/d of soluble nickel salts was adjusted to derive a UL for children and adolescents based on relative body weight because the IOM could not identify any report of nickel toxicity in children and adolescents. Through this method, ULs of 0.2, 0.3, 0.6 and 1.0 mg/d of soluble nickel salts were derived for children aged 1-3, 4-8, 9-13 and 14-18 years, respectively. Since no data were identified to

derive a UL specific to pregnant and lactating women, UL for pregnant and lactating women were set equal to those of nonpregnant and nonlactating women. The IOM noted that the risk of adverse effects resulting from excess nickel intakes from food sources was very low. However, increased risk is likely to occur from environmental exposure or from the consumption of nickel-containing drinking water.

Discussion

The Working Group concluded that the UL overstates the precision of the findings of Ambrose et al. (1976). This is highlighted by the use of two significant figures in the UL of 0.017 mg/kg-d for adults, whereas only one significant digit was appropriate, based on Ambrose et al. (1976). The Working Group also noted that it was unusual that the UL applied to "excess" nickel intake because ULs are typically based upon total intake.

OEHHA. Office of Environmental Health Hazard Assessment. 2005. Development of Health Criteria for School Site Risk Assessment Pursuant to Health and Safety Code Section 901(g): Child-Specific Reference Doses (chRfDs) for School Site Risk Assessment- Cadmium, Chlordane, Heptachlor, Heptachlor Epoxide, Methoxychlor and Nickel. California Environmental Protection Agency. Sacramento, CA, USA.

Summary

The California Office of Environmental Health Hazard Assessment reviewed five chemicals to develop child-specific reference doses (chRfD) for use in risk assessment at existing or proposed school sites. They developed a chRfD for nickel of 11 μg/kg-d, which was based on their interpretation of the NOAEL of 1.1 mg/kg-d from Smith et al (1993) and Springborn (2000a,b) studies and the application of an uncertainty factor of 100. The uncertainty factor was based on 10 fold for interspecies variation and they determined that an additional UF of 10 was appropriate for human variability. This final factor of 10 was to account for what they believed to be database deficiencies for potential carcinogenic effects via the oral route of exposure.

Discussion

The Working Group concluded that Heim (2007) provided sufficient basis upon which to consider oral exposure to nickel to be non-carcinogenic. Therefore, the Working Group determined that it would not be appropriate in their development of an oral RfD to apply a 10 fold UF to account for potential carcinogenicity of nickel via the oral route of exposure.

3.0 REFERENCE DOSE DEVELOPMENT

The two-generation study by Springborn (2000b) was selected by the Working Group as the primary study from which to derive an oral reference dose for non-carcinogenic effects of nickel. A NOAEL of 2 mg/kg-d for nickel was selected by the Working Group after review of the study. Based on the information concerning dose preparation on page 17 of the Springborn (2000b) report, it appears that dosing solutions were prepared to a precision of one or two significant figures, although the table on page 21 (Springborn, 2000b) presents concentrations to two or three significant figures. Although some organizations have utilized a NOAEL of 2.2 mg/kg-d to calculate an oral RfD, the Working Group concluded that one significant figure was appropriate for all values calculated from this study, and that greater significant figures conveyed a false sense of precision. As a result, the Working Group agreed that 2 mg Ni/kg-d was an appropriate NOAEL supported by the study (compared to the NOAEL of 2.2 mg Ni/kg-d proposed by the author).

In accordance with generally accepted principals for the derivation of oral reference doses, the Working Group considered application of modifying and uncertainty factors to the derived NOAEL of 2 mg Ni/kg-d.

$$RfD = NOAEL / UF$$

The Working Group agreed that they would adopt the US EPA definition of uncertainty factors during their discussion:

> *Uncertainty/Variability Factor (UFs): One of several, generally 10-fold, default factors used in operationally deriving the RfD and RfC from experimental data. The factors are intended to account for*
>
> *(1) variation in susceptibility among the members of the human population (i.e., inter-individual or intraspecies variability);*
>
> *(2) uncertainty in extrapolating animal data to humans (i.e., interspecies uncertainty);*
>
> *(3) uncertainty in extrapolating from data obtained in a study with less-than-lifetime exposure (i.e., extrapolating from subchronic to chronic exposure);*
>
> *(4) uncertainty in extrapolating from a LOAEL rather than from a NOAEL;*
>
> *(5) uncertainty associated with extrapolation when the database is incomplete.*
>
> *US EPA Glossary of Terms, 2007*

It was the consensus of the Working Group that an aggregate uncertainty factor of 100 should be applied to the NOAEL of the Springborn (2000b) study in order to derive an RfD for oral exposure to nickel; therefore, the Working Group recommended an oral exposure RfD of 0.02 mg Ni/kg-d.

The 100-fold factor included a factor of 10 to account for differences between individuals, and a factor of 10 to account for interspecies differences between experimental animals (rats, in this case) and humans.

The Working Group discussed the need for a further modifying factor (MF) to account for lack of information in the data, concerning either the potential nephrotoxicity reported by Vyskočil et al. (1994) or the hypersensitive individuals examined by Nielson (1999), Jensen (2006), or Hindsen (2001).

The US EPA definition of modifying factor is as follows:

> *Modifying Factor (MF): A factor used in the derivation of a reference dose or reference concentration. The magnitude of the MF reflects the scientific uncertainties of the study and database not explicitly treated with standard uncertainty factors (e.g., the completeness of the overall database). A MF is greater than zero and less than or equal to 10, and the default value for the MF is 1. [Use of a modifying factor was discontinued in 2004.]*
>
> *US EPA Glossary of Terms, 2007*

After discussing Vyskočil et al. (1994), the Working Group concluded that it did not warrant inclusion of a MF, but noted nephrotoxicity of nickel as an area for further study.

The Working Group debated whether a UF or MF should be included to protect hypersensitive individuals. For purposes of this discussion, the term “hypersensitive” refers to sensitized individuals with active flare up of dermatological effects (i.e., individuals with active disease). Sensitized individuals without active flare up are not considered hypersensitive. The Working Group noted that allergic responses do not exhibit the dose-response relationships seen in non-allergic individuals, and expressed doubts that an RfD for any allergen could be established to protect all hypersensitive individuals (i.e., to prevent exacerbation of symptoms in allergic individuals with active flare up). Furthermore, the US EPA definition identifies the RfD as protective for sensitive individuals, but makes no mention of hypersensitive individuals. Other documents (e.g., US EPA, 2002b) suggest that RfDs are not necessarily protective of hypersensitive individuals. The Working Group concluded that no additional UF or MF should be applied, and that the proposed RfD may not be protective of all hypersensitive individuals.

To put this decision in context, the Working Group considered Jensen et al. (2006). Applying an uncertainty factor of 100 to the selected NOAEL of 2 mg Ni/kg-d yields a value in the range of the ED_5 (effective dose in 5% of subjects) for sensitive individuals suggested by Jensen et al. (2006). Thus, most sensitive individuals would not react at this level. As indicated above, from the report by Jensen et al (2006), ingestion of nickel at the RfD recommended by the Working Group (0.02 mg Ni/kg-d) would result in a reaction in up to 5% of the nickel-sensitized population. The percentage of the general population sensitized to nickel has been shown to be between 10 and 20% (ATSDR, 2005). From these data, the RfD recommended by the WG would protect ≥99% of the total population.

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4.0 APPLICATION OF NICKEL RFD TO LIFE STAGES

The Working Group did not identify any study specific to a life-stage other than adults and/or reproduction studies. Many of the rat studies included dosing of rats prior to maturation. The work of Heim et al. (2007 in press) dosed rats with nickel sulphate hexahydrate for two years, beginning at weaning. This dosing regime included the toddler-equivalent life stage of the rat and, during dosing, no adverse effect was observed. In the two-generation reproductive study of Springborn (2000b), there was no evidence of increased sensitivity in either the first or second offspring generation resulting from early exposure to nickel sulphate hexahydrate. Based on this information, the Working Group concluded that children are not likely to have significantly greater sensitivity to nickel than adults.

The Working Group felt it was helpful to distinguish between “sensitive” and “susceptible” individuals. “Sensitive” individuals were considered to be those with a pre-disposition to nickel skin sensitivities, or with nickel sensitivity but no active flare up. “Susceptible” individuals were those for whom combinations of physiological and behavioural attributes were likely to lead to increased exposure. The toddler life-stage (0.5 to 5 years) represents a susceptible individual because their exposure rates for media such as food, water and soil are higher on a body weight basis than other life-stages typically evaluated in risk assessment. However, as stated above, there is no evidence to suggest that toddlers are more sensitive than adults to nickel.

The Working Group felt it was important to provide guidance on the application of the proposed RfD to individual life stages, and considered three potential approaches to this issue:

1. *Leave to the professional judgment of individual risk assessors.*

   This option was quickly rejected by the Working Group on several grounds. Most significantly, this approach would lead to inconsistent use of the RfD, which would translate into inconsistencies in the decisions made based on the outcome of risk assessments.

2. *Consider two life-stages: pre-pubertal (0 to 12 years) and post-pubertal (12+ years)*

   This option was debated at length and was concluded to be feasible, but is currently inconsistent with life stages identified in Canadian, US and international regulatory documents. An advantage of the proposal is that each of the two life stages is more than 7 years length, a substantial proportion of a human lifetime (about $1/10^{th}$). This option was ultimately not supported by the Working Group because it is no less arbitrary (except perhaps for reproductive toxicants, and that is arguable) than the life stage divisions typical of current risk assessment guidance.

3. *Utilize the mostly susceptible (i.e., highly exposed) life stage as a sentinel*

   The Working Group considered whether exposures occurring in the relatively short time frame of a toddler life stage should be compared to a chronic value developed to protect for lifetime exposures – the standard default approach. The Working Group failed to identify a scientific rationale to suggest that comparing toddler exposures to the chronic RfD was inappropriate. Using the toddler as a sentinel is consistent with what the Working Group believed the goal of risk assessment should be, to estimate risk as realistically as possible, without underestimating

potential for adverse health effects in humans resulting from exposure to substances in the environment.

The Working Group agreed that option 3 described above was the most appropriate approach identified for evaluating exposures in individual life stages, although it was not completely satisfied with this option. This option is more consistent with the goal of risk assessment than the other options considered, although there is little scientific support for this, or any other, option.

The Working Group identified this as a topic worthy of a regulatory review and/or workshop. It was also noted that stop exposure studies (studies evaluating less-than lifetime exposures) might provide a scientific information base from which to reevaluate this question.

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Oral RfD for Nickel

The Working Group recommended an oral RfD of 0.02 mg Ni/kg-d for non-carcinogenic effects. This value was believed to be protective for all adverse effects of nickel other than as discussed below, including protection of sensitive (though not necessarily all hypersensitive) individuals. This value was derived on the basis of a NOAEL of 2 mg/kg-d derived from a two-generation reproductive study in rats (Springborn, 2000b). An uncertainty factor of 100 (10 for interspecies variation and 10 for intraspecies variation) was applied in the derivation of the RfD.

The RfD developed by the Working Group is within an order of magnitude of published toxicological reference values by other organizations for nickel (Table 5-1).

Table 5-1 Published toxicological reference values for oral nickel intake

YEAR	ORGANIZATION	TOXICOLOGICAL REFERENCE VALUE (MG NI/KG-D)
1996	US EPA	0.02
1996	Health Canada	0.05
1999	TERA	0.008 (incremental*)
2001	Institute of Medicine	0.017 (incremental*)
2005	OEHHA	0.011 (child-specific)
2007	WHO	0.011
2007	Working Group	0.02

* incremental exposure over dietary intake

The Working Group noted that the inherent uncertainty in the laboratory studies used in deriving a RfD is likely greater than the factor between other published toxicological reference values and that derived by the Working Group. The Working Group was also aware that its proposed RfD was consistent with various organizations, such as the US EPA, regardless of the specific studies on which the RfD was based. The proposed RfD is considered to have precision sufficient to support the use of one significant figure.

5.2 Application of RfD to Less-than-Lifetime Exposures

The Working Group concluded that there is little support for or against using the RfD to evaluate toddler (or any life stage) exposures, but this approach is in current use in at least some risk assessments known to Working Group members. It was recommended that this issue be the subject of regulatory and scientific review and debate. Stop-exposure studies could shed light on this question.

6.0 REFERENCES

Ambrose AM, Larson PS, Borzelleca JK and Hennigar GR. 1976. Long term toxicologic assessment of nickel in rats and dogs. *Journal of Food Science and Technology* **13**: 181-187.

Chashschin VP, Artunina GP and Norseth T. 1994. Congenital defects, abortion and other health effects in nickel refinery workers. *The Science of the Total Environment* **148:** 287-291.

Danish EPA. 2005. *Nickel Sulphate, CAS-No.: 7786-81-4, EINECS-No.: 232-104-9, Risk Assessment.* Danish Environmental Protection Agency, Draft.

Health Canada. 1996. *Health-Based Tolerable Daily Intakes/ Concentrations and Tumorigenic Doses/ Concentrations for Priority Substances.* Minister of Supply and Services Canada. Ottawa, ON, Canada.

Heim KE, Bates HK, Rush RE and Oller AR. 2007 In press. Oral carcinogenicity study with nickel sulfate hexahydrate in Fischer 344 rats. *Toxicology and Applied Pharmacology.*

Hindsén M, Burze M and Christensen OB. 2001. Flare-up reactions after oral challenge with nickel in relation to challenge dose and intensity and time of previous patch test reactions. *Journal of the American Academy of Dermatology* **44:** 616-623.

IOM. Institute of Medicine. 2001. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc.* Food and Nutrition Board. National Academy Press: Washington, DC, USA.

Jensen CS, Menné T and Johansen JD. 2006. Systemic contact dermatitis after oral exposure to nickel: a review with a modified meta-analysis. *Contact Dermatitis* **54:** 79-86.

Nielsen GD, Søderberg U, Jørgensen PJ, Templeton DM, Rasmussen SN, Andersen KW and Grandjean P. 1999. Absorption and retention of nickel from drinking water in relation to food intake and nickel sensitivity. *Toxicology and Applied Pharmacology* **154:** 67-75.

NiPERA. Nickel Producers Environmental Research Association. 2007. “Review of Nickel Toxicity Studies for the Purpose of Establishing an RfD or TDI.” 14 pp.

NiPERA. Nickel Producer Environmental Research Association. 2000. “Results of the Rat 2-Generation Reproduction Study or Orally Administered Nickel Sulfate Hexahydrate.” NiPERA News Bulletin No. 15.

OEHHA. Office of Environmental Health Hazard Assessment. 2005. *Development of Health Criteria for School Site Risk Assessment Pursuant to Health and Safety Code Section 901(g): Child-Specific Reference Doses (chRfDs) for School Site Risk Assessment- Cadmium, Chlordane, Heptachlor, Heptachlor Epoxide, Methoxychlor and Nickel.* California Environmental Protection Agency. Sacramento, CA, USA.

OEHHA. Office of Environmental Health Hazard Assessment. 2001. *Public Health Goals for Chemicals in Drinking Water: Nickel.* California Environmental Protection Agency. Sacramento, CA, US.

Smith MK, George EL, Stober JA, Feng HA and Kimmel GL. 1993. Perinatal toxicity associated with nickel chloride exposure. *Environmental Research* **61:** 200-211.

Springborn Laboratories, Inc. 2000a. *A One Generation Reproduction Range-Finding study in Rats with Nickel Sulfate Hexahydrate.* Ohio Research Center, Spencerville, OH, USA.

Springborn Laboratories, Inc. 2000b. *An Oral (Gavage) Two-Generation Reproduction Toxicity Study in Sprague-Dawley Rats with Nickel Sulfate Hexahydrate.* Ohio Research Center, Spencerville, OH, USA.

TERA. Toxicology Excellence for Risk Assessment. 1999. "Toxicological Review of Soluble Nickel Salts." Prepared for: Metal Finishing Association of Southern California, Inc., US EPA and Health Canada.

US EPA. 2002. *Determination of the Appropriate FQPA Safety Factor(s) in Tolerance Assessment.* Office of Pesticide Programs. Washington, DC, USA.

US EPA. 2002b. A Review Of The Reference Dose And Reference Concentration Processes. EPA/630/P-02/002F. Washington, DC, USA

US EPA. 1996. [Updated 25 Jan 2007]. "Nickel, soluble salts (CASRN Various)". Online. Available http://www.epa.gov/iriswebp/iris/subst/0271.htm [Accessed 11 June 2007].

US EPA. 2007. http://www.epa.gov/iris/gloss8.htm Searched July 18, 2007. Last updated April 5, 2007.

Vaktskjold A, Talykova L, Chashchin V, Nieboer E and Odland JO. 2004. The Kola Birth Registry and perintatl mortality in Monchegorsk, Russia. *Acta Obstetricia et Gynecologica Scandinacica* **83:** 58-69.

Vaktskjold A, Talykova L, Chashchin V, Nieboer E, Thomassen Y and Odland JO. 2006. Genital malformations in newborns of female nickel-refinery workers. *Scandinavian Journal of Work, Environment and Health.* **32:** 41-50.

Vyskočil A, Viau C and Čížková M. 1994. Chronic nephrotoxicity of soluble nickel in rats. *Human and Experimental Toxicology* **13:** 689-693.

WHO. World Health Organization. 2007. "Nickel in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality." WHO/SDE/WSH/07.08/55.

APPENDIX A

Terms of Reference
(Charge to the Working Group)

Terms of Reference

Expert Working Group on Nickel Ingestion Toxicity Reference Value for Non-Carcinogenic Effects

Objective:

The objective of the Working Group is to conduct a review of nickel ingestion non-cancer reference toxicity values and develop a summary report documenting review comments of the expert Working Group. The report will be included as an appendix to the Human Health Risk Assessment underway as part of a Community Based Risk Assessment in Port Colborne, Ontario.

Scope of Work:

The Working Group is to review the basis of the US EPA reference dose for nickel soluble salts, and more recent toxicity reference values published by the World Health Organization, Denmark and California. Copies of the published studies cited as the primary basis of these values will be provided to the participants for background review.

A two day workshop will be held in the Toronto area. Presentations will be given on background material and the Working Group will discuss and try to reach a consensus on the most appropriate nickel oral toxicity reference value for use in human health risk assessment and the appropriate life stage (or lifetime) for comparison of this value to doses.

Discussion will be led by a facilitator, covering the following topics:

- strengths/weaknesses of each study
- appropriate NOAEL and LOAEL values from each study
- appropriateness of uncertainty factors
- overall appropriateness of the final reference value selected by each agency
- degree of conservatism in uncertainty factor
- life stage to which the value is applicable
- issues regarding use of uncertainty factor in setting site-specific remediation goals

The final discussion will attempt to move toward a consensus on the appropriate toxicity reference value and applicable life stage for that value. The discussion will consider the level of conservatism in the selected value.

A draft report on the discussions, consensus (where reached) and outcomes of the workshop will be prepared and distributed to Working Group members. Members will be asked to review the report and provide comments on the report in terms of accuracy and completeness. A conference call will be held to discuss participant comments on the draft report. Each Working Group member will be asked to sign a signatory page indicating their participation in the Working Group and review of the Working Group report as an accurate representation of the workshop.

Schedule:

Distribution of review materials	- June
Presentation and Workshop	- June
Distribution of draft report	- July
Conference call to review and discuss draft report	- July
Distribution of final report	- July

Materials to be Provided to Group Members:

Toxicological Studies

Ambrose, A.M., Larson, D.S. Borzelleca, J.R. and Hennigar, G.R. 1976. Long term toxicologic assessment of nickel in rats and dogs. J Food Sci Technol 13:181-87.

Smith, M.K., George, E.L., Stober, J.A., Feng, H.A., Kimmel, G.L. 1993. "Perinatal toxicity associated with nickel chloride exposure." Environ Res, 61:200-211.

Nielsen, G.D., U. Søderberg, P.J. Jørgensen, D.M. Templeton, S.N. Rasmussen, S.N. Andersen, and P. Grandjean. 1999. "Absorption and retention of nickel from drinking water in relation to food intake and nickel sensitivity." *Toxicology and Applied Pharmacology.* 154: 67-75.

Springborn Laboratories, 2000. A One-Generation Reproduction Range-Finding Study in Rats with Nickel Sulfate Hexahydrate, 2000.

Springborn Laboratories, 2000. An Oral (Gavage) Two-Generation Reproduction Toxicity Study in Sprague-Dawley Rats With Nickel Sulfate Hexahydrate, Final Report.

RfD Development

US EPA. 1998. "Nickel, soluble salts; CASRN Various". IRIS, Integrated Risk Information System. Last revised 12/10/1998. United States Environmental Protection Agency.

NiPERA, 2000. "Results of the Rat 2-Generation Reproduction Study of Orally Administered Nickel Sulfate Hexahydrate"

California Environmental Protection Agency, 2001. Public Health Goal for Nickel in Drinking Water. Pesticide and Environmental Toxicology Section, Office of Environmental Health Hazard Assessment and CEPA.

World Health Organization. 2005. Nickel in Drinking Water. Background document for development of WHO Guidelines for Drinking-water Quality. WHO/SDE/WSH/05.08/55.

OEHHA, 2005. *Development of Health Criteria for School Site Risk Assessment Pursuant to Health and Safety Code Section 901(g): Child-Specific Reference Doses (chRDs) for School Site Risk Assessment – Cadmium, Chlordane, Heptachlor, Heptachlor Epoxide, Methoxychlor and Nickel.* Office of Environmental Health Hazard Assessment, California Environmental Protection Agency.

Additional References (Available for download from US EPA):

US EPA 2002. *A Review of the Reference Dose and Reference Concentration Processes*. United States Environmental Protection Agency.

US EPA. 1996. *Guidelines for Reproductive Toxicity Risk Assessment.* United States Environmental Protection Agency.

APPENDIX B

List of Additional Workshop Materials Distributed to Working Group

List of Additional Workshop Materials Distributed to Working Group

The following materials were distributed to working group members, in addition to those reproduced in Appendices B, C, D and E.

Ambrose AM, Larson PS, Borzelleca JK and Hennigar GR. 1976. “Long term toxicologic assessment of nickel in rats and dogs.” *Journal of Food Science and Technology* **13**: 181-187.

Danish EPA. 2005. *Nickel Sulphate, CAS-No.: 7786-81-4, EINECS-No.: 232-104-9, Risk Assessment.* Danish Environmental Protection Agency, Draft. Chapters 0-2, 4-7 human health only.

Heim KE, Bates HK, Rush RE and Oller AR. 2007 In press. “Oral carcinogenicity study with nickel sulfate hexahydrate in Fischer 344 rats.” *Toxicology and Applied Pharmacology.* Electronic copies of the full study report were made available to participants on request after the workshop and prior to report finalization, but were not specifically distributed to the Working Group.

Hindsén M, Burze M and Christensen OB. 2001. “Flare-up reactions after oral challenge with nickel in relation to challenge dose and intensity and time of previous patch test reactions.” *Journal of the American Academy of Dermatology* **44:** 616-623.

IOM. Institute of Medicine. 2001. *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Zinc.* Food and Nutrition Board. National Academy Press: Washington, DC, USA. pp. i, 1-1 to 1-13, 13-1, 13-15 to 13-21 and 13-32 to 13-42.

Jensen CS, Menné T and Johansen JD. 2006. “Systemic contact dermatitis after oral exposure to nickel: a review with a modified meta-analysis.” *Contact Dermatitis* **54:** 79-86.

Nielsen GD, Søderberg U, Jørgensen PJ, Templeton DM, Rasmussen SN, Andersen KW and Grandjean P. 1999. “Absorption and retention of nickel from drinking water in relation to food intake and nickel sensitivity.” *Toxicology and Applied Pharmacology* **154:** 67-75.

NiPERA. Nickel Producer Environmental Research Association. 2000. “Results of the Rat 2-Generation Reproduction Study or Orally Administered Nickel Sulfate Hexahydrate.” NiPERA News Bulletin No. 15.

OEHHA. Office of Environmental Health Hazard Assessment. 2005. *Development of Health Criteria for School Site Risk Assessment Pursuant to Health and Safety Code Section 901(g): Child-Specific Reference Doses (chRfDs) for School Site Risk Assessment- Cadmium, Chlordane, Heptachlor, Heptachlor Epoxide, Methoxychlor and Nickel.* California Environmental Protection Agency. Sacramento, CA, USA.

OEHHA. Office of Environmental Health Hazard Assessment. 2001. *Public Health Goals for Chemicals in Drinking Water: Nickel.* California Environmental Protection Agency. Sacramento, CA, US.

Smith MK, George EL, Stober JA, Feng HA and Kimmel GL. 1993. “Perinatal toxicity associated with nickel chloride exposure.” *Environmental Research* **61:** 200-211.

Springborn Laboratories, Inc. 2000a. *A One Generation Reproduction Range-Finding study in Rats with Nickel Sulfate Hexahydrate.* Ohio Research Center, Spencerville, OH, USA.

Springborn Laboratories, Inc. 2000b. *An Oral (Gavage) Two-Generation Reproduction Toxicity Study in Sprague-Dawley Rats with Nickel Sulfate Hexahydrate.* Ohio Research Center, Spencerville, OH, USA.

US EPA. 1996. [Updated 25 Jan 2007]. “Nickel, soluble salts (CASRN Various)”. Online. Available http://www.epa.gov/iriswebp/iris/subst/0271.htm [Accessed 11 June 2007].

Vaktskjold A, Talykova L, Chashchin V, Nieboer E and Odland JO. 2004. “The Kola Birth Registry and perintatl mortality in Monˇcegorsk, Russia.” *Acta Obstetricia et Gynecologica Scandinacica* **83:** 58-69.

Vaktskjold A, Talykova L, Chashchin V, Nieboer E, Thomassen Y and Odland JO. 2006. “Genital malformations in newborns of female nickel-refinery workers.” *Scandinavian Journal of Work, Environment and Health.* **32:** 41-50.

Vyskočil A, Viau C and Čížková M. 1994. “Chronic nephrotoxicity of soluble nickel in rats.” *Human and Experimental Toxicology* **13:** 689-693.

WHO. World Health Organization. 2007. “Nickel in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality.” WHO/SDE/WSH/07.08/55.

WHO. World Health Organization. 2005. “Nickel in Drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality.” WHO/SDE/WSH/05.08/55.

APPENDIX C

Review of Nickel Toxicity Studies for the Purpose of Establishing an RfD or TDI

Prepared by the Nickel Producers Environmental Research Association

June 27, 2007

REVIEW OF NICKEL TOXICITY STUDIES FOR THE PURPOSE OF ESTABLISHING AN RfD OR TDI

Prepared by the Nickel Producers
Environmental Research Association

June 27, 2007

Table of Contents

1. BACKGROUND

This document summarizes information relevant to the establishment of a Reference Dose (RfD) or Tolerable Daily Intake (TDI) for nickel.[1] Evaluation of the existing data shows that animal toxicity (evidenced by body weight changes, reproductive impairment, or potential renal impairment) and elicitation of a dermatitic response in nickel-sensitized patients are the most sensitive endpoints for nickel toxicity.

The sections that follow will detail the critical studies in each of the most sensitive areas of toxicity and provide an overview of regulatory decision making with regard to the establishment of an RfD or TDI for nickel. This document is presented to provide an overview of the data and interpretative issues rather than to reach a specific conclusion as to what value should be selected as the RfD or TDI for nickel. Accordingly, no recommendation of an RfD will be made in this document.

2. HUMAN REPRODUCTIVE STUDIES

A single report appeared in the literature regarding the potential for nickel compounds to cause adverse reproductive effects in humans in 1994. The study was a preliminary qualitative analysis by Chashschin *et al.* (1994) that reported an apparent increase in spontaneous abortions (16-17% compared to 8-9%) and structural malformations (especially cardiovascular and musculoskeletal defects) in newborn babies whose mothers were employed in the Russian nickel refinery at Monchegorsk (significantly increased risks of 2.9, 6.1, and 1.9 for total defects, cardiovascular, and musculoskeletal defects, respectively) . Although this report was not based on a scientifically rigorous study, it was suggestive enough to warrant further investigation. A note from the editors said:

> "This article is included in this special issue since it constitutes a first report on possible reproductive and developmental effects in humans due to occupational exposure to nickel. Although the results are incompletely documented and thus must be considered inconclusive, they identify a concern that requires more comprehensive and quantitative epidemiologic investigations."

As a consequence of this report, an epidemiological study was initiated to assess the possible association of the observed reproductive effects in women working in the Kola Peninsula region nickel refineries and their occupational exposure to relatively high soluble nickel levels (e.g., as much as 3 mg/m^3).

A first manuscript written by A. Vaktskjold and co-workers (2006) described the results of a birth-registry-based study that was carried out to determine whether pregnant women employed in the nickel refinery were at elevated risk of delivering a newborn with genital malformations (hypospadias and cryptorchidism). Both of these findings are considered "sentinel" effects (i.e., sensitive endpoints) for reproductive toxicity in humans. In late 2006 and early 2007, three additional manuscripts were drafted by A. Vaktskjold on spontaneous abortions, small-for-gestational-age newborns, and musculoskeletal defects in newborns. These draft manuscripts (which are expected to be published in 2007) found no causal association between maternal

[1] An RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (Barnes and Dourson, 1988). A TDI is essentially the same thing.

exposure to nickel and any of the adverse reproductive outcomes studied. The results from these human studies are important because spontaneous abortion in humans would most closely approximate the observation of perinatal lethality seen in nickel-exposed rats.

These data demonstrate that a weight-of-evidence approach to the future re-evaluation of reproductive toxicity of nickel substances will be needed. While a reproductive "hazard" from nickel exposure can be demonstrated in animals, there is no demonstrable "risk" of reproductive impairment in the single female occupational cohort believed to have been consistently exposed to high levels of nickel. Consequently, barring the unusual occurrence of an accidental massive exposure of a pregnant woman at the critical time for the possible induction of a reproductive effect, the risk of human reproductive impairment from nickel exposure appears to be exceedingly small.

3. OVERVIEW OF KEY ANIMAL STUDIES

3.1 Ambrose *et al*. (1976) [2]

Rats (25 per sex per dose) were exposed to nickel (as nickel sulfate) in the diet at doses of 0, 100, 1000, or 2500 mg/kg (equivalent to 0, 5, 50, and 125 mg Ni/kg of body weight per day) for 2 years (Ambrose *et al.*, 1976). Growth was depressed in rats at 1000 and 2500 mg/kg of diet, but there were indications that decreased food consumption might explain the decreased body weight gains, particularly at 2500 mg/kg of diet. However, no statistical analysis seems to have been performed. Survival overall was very poor, especially in the control groups and the 2500 mg/kg of diet groups. In females at 1000 and 2500 mg/kg of diet (50 and 125 mg Ni/kg of body weight), the mean relative liver weights were decreased by about 20% and the mean relative heart weights were increased by about 30% compared with the control group. No histological or gross pathological findings related to nickel exposure were observed. The highest nickel concentrations were found in the kidneys. The NOAEL in this study was 5 mg Ni/kg of body weight per day. However, the study does not meet current standards for long-term studies, mainly because of the low survival rate. The observed changes in organ weights in female rats might in part be due to changes in food and water consumption. Also, both gross and histopathological examinations of the animals were negative, although there were 20–30% changes in relative organ weights. Thus, one cannot rule out the possibility that the observed changes in relative organ weights were related to changes in food and/or water consumption rather than to a toxic effect of nickel.

3.2 RTI (1988) [3]

In a 2-generation study conducted by RTI (1988), nickel chloride was administered in drinking water to male and female CD rats at dose levels of 0, 50, 250 and 500 ppm (roughly equivalent to 0, 7.3, 30.8 and 51.6 mg/kg/day), for 90 days prior to breeding. An additional dose level of 1000 ppm was eliminated after 2 weeks due to excessive toxicity. The parental animals were exposed beginning 11 weeks before cohabitation, and exposure continued for a total of 24 weeks (males) or 30 weeks (females). Groups of 10 rats/sex comprised a satellite subchronic non breeder study. The average nickel consumption reported by the authors varied by more

[2] Ambrose, A. M.; Larson, P. S.; Borzelleca, J. F., and Hennigar, G. R. Jr. (1976). Long term toxicologic assessment of nickel in rats and dogs. J. Food Sci. Technol. 13: 181-187.

[3] Price, C. J.; George, J. D.; Marr, M. C.; Sanderson, P. E.; Rubenstein, R.; Kimmel, G. L.; Sonawane, B. R.; Bathija, A., and Rosa, C. Research Triangle Institute. (1988) Two-Generation Reproduction and Fertility Study of Nickel Chloride Administered to Cd Rats in the Drinking Water Final Study Report (Volumes I to III). Unpublished Final Report.

than a factor of 2, with the highest consumption at the beginning of the premating exposure and during the latter part of the lactation period. As a conservative estimate, the average exposure during gestation, which was on the low end of overall exposure levels, should be used as the exposure level for each group. This choice takes into account the possibility that gestational or perinatal exposure accounts for observed reproductive effects. Thus, the estimated doses were 0, 6.0, 25, and 42 mg Ni/kg/day.

At the 500 ppm dose level there was a statistically significant decrease in the Po body weight in males and females (95% and 90% of controls, respectively), along with decreased absolute and relative liver weights in the females (90% and 89% of controls, respectively), but not in the males. Thus, 250 ppm was a NOAEL for Po breeders. The NOAEL was confirmed *via* histopathological examination of the liver, kidney, lungs, heart, pituitary, adrenals and reproductive organs. In addition, there was no treatment-related effect on reproductive performance indices (mating success, rate of impregnation), reproductive organ weights or histopatholgy of reproductive organs. Therefore, the NOAEL for fertility in this study is 42 mg Ni/kg/day and a LOAEL was not identified.

In the F_{1a} generation (postnatal days 1 to 4), the 500 ppm exposure level was associated with a significant decrease in the number of live pups/litter, as well as significantly increased pup mortality, and significantly decreased average pup body weight, all relative to controls. Similar effects were reported for the F_{1b} litters of P_o dams exposed to 500 ppm nickel. In the 50 and 250 ppm exposure groups, increased pup mortality and decreased live litter size was observed in the F_{1b} litters. However, these effects were considered questionable because the room temperature tended to be 10 degrees F higher than normal at certain times during gestation and postnatal days, along with much lower levels of humidity. Temperatures that are 10 degrees F above normal during fetal development are known to cause adverse effects (Edwards, 1986) [4]. Therefore, the results seen at 50 and 250 ppm cannot be considered to be genuine nickel-related adverse effects (U.S. EPA, 1991). F_{1b} males and females were randomly mated on postnatal day 70 and their offspring (F_{2a} and F_{2b}) were then evaluated either through postnatal day 21 or until teratological exam on gestation day 20.

This phase of the study included teratological evaluations of F_{2b} fetuses. It was reported that the 500 ppm dose caused significant body weight depression of both mothers and pups of the F_{2a} generation. Significant reductions in live litter size and postnatal body weight were also observed in the that group. At 250 ppm, there was a transient depression of maternal weight gain and water intake during gestation of the F_{2b} litters.

The 50 ppm nickel dose caused a significant increase in short ribs (11%). However, since this effect was not seen at all in the higher dose groups, the incidence of short ribs in the 50 ppm group was not considered to be biologically significant (U.S. EPA, 1991). The F_{2b} generation teratological examination demonstrated a lack of *in utero* developmental effects related to nickel exposure. In addition, the lack of increased *in utero* death in the F_{2b} generation when examined on gestation day 20 in the teratological component of this study demonstrates the specificity of the perinatal period as the critical exposure period for the induction of the perinatal lethality observed in the other generational groups within this study. Ultimately, the RTI (1988) study established the 50 ppm (*i.e.*, ~6 mg/kg/day) exposure as a reproductive NOAEL.

[4] Edwards, M. J. Hyperthermia as a teratogen: A review of experimental studies and their clinical significance. Terat. Carc. Mut. 6: 563-582 (1986)

3.3 Smith *et al.* (1993) [5]

Smith *et al.* (1993) conducted a two-generation reproductive study on rats administered nickel chloride in drinking water at doses of 10 ppm, 50 ppm and 250 ppm. Because of clear problems in design that introduce difficulties in interpreting the study results – notably, the lack of a dose-response for the endpoint designated as the Lowest Observed Adverse Effect Level (LOAEL) and the potential complication of the study interpretation by effects on maternal prolactin levels – the Smith *et al.* (1993) study should not be used exclusively to derive an RfD for soluble nickel.

In evaluating the study by Smith and coworkers the potential for inferring a nickel-related effect at the lowest dose (10 ppm, or 1.3 mg/kg/day) must be considered. The possible effect at the 1.3 mg/kg/day dose level was an increased number of pup deaths per litter in the second breeding of the animals in the study. This effect was not seen in the litters from the first breeding; nor was there any evidence of reproductive toxicity with regard to other endpoints such as fertility, litter size, or pup weight. In fact, there was evidence of a dose related negative trend in litters with dead pups across the 10 ppm (1.3 mg/kg/day) and 50 ppm (6.8 mg/kg/day) doses in the first breeding (Control - 5/25; 10 ppm - 5/25; 50 ppm - 0/24; Cochran-Armitage trend test p = 0.023). This "trend" was the result of an absence of deaths in litters in the 50 ppm group. The fact that the 50 ppm (6.8 mg/kg/day) exposure group did not demonstrate this effect is inconsistent with the concept of dose-response (*i.e.,* if the effect is not spurious, but in fact is due to exposure to the test material, then a graded response will occur from the lowest to the highest exposures tested). Specifically, there was no evidence of a positive "trend" in the proportion of litters with dead pups across the low and mid doses of the second breeding (Control - 2/23; 10 ppm - 7/22; 50 ppm - 6/24; Cochran Armitage p = 0.290). The strongest indication of an effect for this measure was the comparison of the 10 ppm group with the control group (p = 0.058). However, this result is not compelling, given the variability in the number of litters with dead pups across the two breedings of the control group (5/25 in the first breeding and 2/23 in the second). This is particularly true in light of the sensitivity of the statistical tests to minor variations in the number of deaths in the control group. In fact, if the second breeding of controls had produced one more litter with a single pup death, the statistical evidence for increases in the proportion of litters with dead pups in the 10 and 50 ppm groups would be extremely weak (p = 0.124 and p = 0.253, respectively).

An additional pup death in the second breeding of controls does not seem unlikely, based on the authors' statement that it is "*possible that the numbers [of dead pups] were an under-representation of total pup death, given the general tendency of dams to cannibalize dead or abnormal offspring*." In fact, a relatively large number of stillborn young may have been cannibalized by dams in the second breeding of the control group as indicated by the unusually low average number of pups per litter (10.6 ± 0.98). This sample mean was smaller than that of any treatment groups in the study, and t-tests comparing it to other groups gave strong evidence to suggest that the differences were not due to chance alone: control group in the first breeding (12.9 ± 0.40, p = 0.015), 50 ppm group in the second breeding (13.3 ± 0.69, p = 0.012), and 250 ppm group in the first breeding (13.2 ± 0.62, p = 0.011).

Higher than expected rates of cannibalism may or may not have been responsible for the small observed litter sizes from the second breeding of controls. An alternative hypothesis would be that larger litters tend to have more pup deaths. In either case, comparisons of numbers of pup

[5] Smith, M. K.; George, E. L.; Stober, J. A.; Feng, H. A., and Kimmel, G. L. (1993). Perinatal toxicity associated with nickel chloride exposure. Environ. Res. 61(2): 200-211.

deaths in treated groups to those which occurred in a control group with demonstrably smaller litter sizes would be biased towards the inflation of treatment effects. The possibility of such bias calls into question the validity of using pup deaths from the second breeding of controls as a basis of comparison for treated groups, and suggests that historical control data should be used to assist in assessing the statistical significance of the observed effects.

Using the data from the first breeding of controls, the comparison of the proportion of litters with dead pups between groups gave no evidence of treatment effects in any of the dose groups ($p = 0.334$, $p = 0.532$, $p = 0.146$, in ascending dose order). This is basically consistent with the result that was obtained for the first breeding (apart from the negative effect in the 50 ppm group). The raw data from the study are unavailable. Therefore, it is not possible to compare the mean percentages of dead pups in the litters against the control value. However, based on the non-significance of the difference between the control and 10 ppm groups in the first breeding (1.7 vs 3.1), it is unlikely that the comparison would yield a significant result.

It is interesting to note that, apart from the 50 ppm group (which had reduced pup deaths relative to either set of control data), there was little, if any evidence of an increase in the severity of response between the two breedings. In fact, in the 250 ppm group, the proportions of litters having pup deaths were virtually identical between the first and second breedings (41% and 40%, respectively), and the mean number of pups from the second breeding was less than that of the first (8 vs. 13.2). Thus, the difference in the inferences drawn from the two sets of breeding data was largely related to the differences in the control data.

An additional confounder observed in the rats from the Smith *et al.* (1993) study raises the question of its relevance for human risk assessment. Specifically, Smith *et al.* (1993) demonstrated decreases in plasma prolactin levels in the dams and pups at one week after weaning of the second litter. A statistically significant decrease in mean prolactin levels of 21% was observed in the high dose dams while prolactin levels were also decreased by 5% and 12% at the mid- and low-exposure levels, respectively. The authors could not eliminate the possible involvement of changes in prolactin levels on the reproductive effects observed in rats.

Prolactin plays an essential role in reproduction and lactation in mammals, including rats and humans. There are, however, key differences in the functions of prolactin in rats and humans. Specifically, the control of the corpus luteum, the organ essential for progesterone production, and the hormone responsible for the maintenance of pregnancy, is different in rat and humans. In the rat, in early pregnancy, prolactin is luteotrophic and serves to maintain the corpus luteum (CL) and stimulates the CL to produce progesterone. In this period, any event that results in a decrease in prolactin levels could potentially result in fetal death and subsequent decreases in litter size. In contrast, in humans, the primary role of prolactin is to stimulate the differentiation of the mammary gland in preparation for milk production. Prolactin is not luteotrophic in humans and normal CL function has been observed in prolactin-deficient women. Therefore, any chemical that decreased prolactin levels would not be expected to result in fetal death in humans.

In addition to the necessity of prolactin for maintenance of pregnancy, the authors also noted that abnormal female prolactin patterns are known to alter the latency period for the onset of maternal behavior critical to successful nurturing of the young. Therefore, behavioral effects from changes in prolactin levels may also have had an impact on the effects observed in Smith *et al.* (1993).

3.4 Springborn Laboratories (2000) [6]

A final reproduction study was conducted at Springborn Laboratories during 2000 and was compliant with the OECD 416 test guidelines in place as of January 1999. In addition, many of the elements that appear in the newly harmonized OECD guideline for two-generation reproduction testing were included in the study design. The 416 guideline is designed to provide general information concerning the effects of a test substance on the integrity and performance of the male and female reproductive systems (including gonadal function, the estrous cycle, mating behavior, conception, gestation, parturition, lactation, and weaning) and on the growth and development of the offspring. The study also provides information about the effects of the test substance on neonatal morbidity and mortality, and provides preliminary data on prenatal and postnatal developmental toxicity.

The rangefinding and definitive studies for the rat 2-generation reproduction study of nickel sulfate hexahydrate were conducted using gavage (*i.e.*, oral intubation) as the route of exposure, due to palatability problems with nickel in drinking water and bioavailability problems with nickel in food. The rangefinding study was designed in two parts. The first part of the rangefinding studies was a dose-response probe utilizing small numbers of animals and nickel sulfate hexahydrate exposures of 0, 5, 15, 25, 50, 75, and 150 mg/kg/day. [Note that the lower 95% confidence limit for lethality from nickel sulfate hexahydrate is 170 mg/kg/day]. Lethality was observed at the 150 mg/kg/day exposure level.

The second part of the rangefinding study (*i.e.*, a 1-generation reproductive toxicity study) utilized nickel sulfate hexahydrate exposures of 0, 10, 20, 30, 50, and 75 mg/kg/day. These doses had no effect on parental survival, growth, mating behavior, copulation, fertility, implantation, or gestation length. However, evaluation of post-implantation/perinatal lethality among the offspring of the treated parental rats (*i.e.*, number of pups conceived minus the number of live pups at birth) showed statistically significant increases at the 30 to 75 mg/kg/day exposures and questionable increases at the 10 and 20 mg/kg/day levels (see Table 2).

Table 2: Post-Implantation/Perinatal Lethality in the Parental (F_0) Generation of Rats Exposed to Nickel Sulfate Hexahydrate by Gavage.

Generation		Historical Control	Exposure Levels mg $NiSO_4 \cdot 6H_2O$/kg/day (mg Ni/kg/day)					
			0 Control	10 (2.23)	20 (4.5)	30 (6.7)	50 (11.2)	75 (16.8)
F_0	Mean	1.5 [0.88 - 2.3]	0.38	2.63 {0.7}	1.63	2.29*	2.29**	4.75**
	± SEM		0.26	1.93 {0.3}	0.56	0.75	0.52	0.80

SEM = Standard Error of the Mean
{ } = Variable recalculation minus one animal with a dead litter
[ ] = Range of responses
* = Significantly different from control at P<0.05; and ** at P<0.01

Two things occurred in the 1-generation study that were unusual: 1) the control post-implantation/perinatal lethality of 0.4 ± 0.3 was unexpectedly low, and 2) the high post-implantation/perinatal lethality of the 10 mg/kg/day exposure group was skewed by a single

[6] Springborn (2000) An oral (gavage) two-generation reproduction toxicity study in Sprague-Dawley rats with nickel sulphate hexahydrate. Prepared by Springborn Laboratories, Inc., Spencerville, OH, for Nickel Producers Environmental Research Association, Durham, NC (Study No. 3472.2).

animal with a dead litter. Without that outlier, the Mean ± SEM would have been 0.7 ± 0.3. This value is well within the historical range for nine previous studies at this laboratory in which the post-implantation/perinatal lethality of the control animals had ranged from 0.88–2.3, with a mean of 1.5. The decrease in perinatal survival evident in the 1-generation rangefinding study was anticipated from previous literature reports. The goal of these studies was to refine the NOEL for this endpoint. The 1-generation study also showed that the mean live litter size was significantly decreased at the 75 mg/kg/day level and was lower than historical controls at or above 30 mg/kg/day. Another variable, stillbirth, was significantly increased in all exposure groups except the 50 mg/kg/day group.

Based upon the results of the 1-generation study, nickel sulfate hexahydrate exposure levels of 1, 2.5, 5.0, and 10 mg/kg/day were administered by gavage to five groups of male and female rats in the definitive 2-generation study. These dose levels were chosen to ensure that the study would have a measurable No Observed Effect Level (NOEL) for the post-implantation/perinatal lethality variable. Males of the parental (F_0) generation were dosed during growth and for at least one complete spermatogenic cycle in order to elicit any possible adverse effects on spermatogenesis by the test substance. Females of the F_0 generation were dosed during growth and for several complete estrous cycles in order to elicit any possible adverse effects on estrous by the test substance. The test substance was administered to F_0 animals during mating, pregnancy, and through the weaning of their first generation (F_1) offspring. At weaning, the administration of the substance was continued to F_1 offspring during their growth into adulthood, mating and production of an F_2 generation, and up until the F_2 generation was weaned. Clinical observation and pathological examination were performed for signs of toxicity, with special emphasis on effects on the integrity and performance of the male and female reproductive systems and on the growth and development of the offspring.

The authors of the 2-generation study indicated that the highest dose selected (10 mg/kg/day or 2.2 mg Ni/kg/day) was a NOEL for adult and offspring rats for all the endpoints studied, including the variable of post-implantation/perinatal lethality (Table 3).

Table 3: Post-Implantation/Perinatal Lethality in the 2-Generation Study of Rats Exposed to Nickel Sulfate Hexahydrate by Gavage.

Generation		Exposure Levels mg $NiSO_4 \cdot 6H_2O$/kg/day (mg Ni/kg/day)					
		Historical Control	0 Control	1 (0.22)	2.5 (0.56)	5 (1.12)	10 (2.23)
F_0	Mean	1.5 [0.9 – 2.3]	0.9	1.5 {1.1}	1.2	1.3	2.1
	± SEM		0.22	0.44 {0.23}	0.27	0.23	0.43
F_1	Mean	1.4 [1.2 – 1.6]	0.9	1.9	1.3	1.3	1.2
	± SEM		0.18	0.22	0.27	0.22	0.28

SEM = Standard Error of the Mean
{ } = Variable recalculation minus one animal with a dead litter
[ ] = Range of responses
None significantly different from control at $P<0.05$

The Springborn (2000) study is the focus of some debate, as consultants to the Danish EPA reanalyzed the perinatal mortality data from the study by adding together the unrelated events of post-implantation loss (which largely consists of embryonic mortality before gestation day 15), deaths observed immediately after birth, and deaths occurring between postnatal days 1 and 4. By combining these unrelated events, the Danish EPA created an apparent statistically significant effect in the high dose group (2.2 mg Ni/kg/day). However, each of these unrelated phenomena has its own, variable background rate. When those background rates are added together, they produce a range that contains the value that the Danish EPA calculated for the 2.2 mg Ni/kg/day group. Thus, the historical control data for Springborn Labs lists the historical control range for post-implantation loss as 5-13.3%, for stillbirths as 0.9-4.4%, and for mortality between postnatal days 1 and 4 as 1-2%. Combining all three ranges produces an overall range of 7-20%; the value calculated by the Danish EPA for the 2.2 mg Ni/kg/day group (__%_) falls within this historical norm.

The Danish EPA's statistical re-analysis continues by demonstrating that, although the mean values for pups born dead is not different between the control and high dose groups, the distribution of the number of litters with pups dying is different. This seems to be an attempt to find some means of expressing the data in a way that will support a particular conclusion. Such *a posteriori* analysis does not provide a sufficient foundation to support a definitive conclusion that an effect actually exists.

Finally, the Danish EPA consultants suggested that there is a genetic predisposition, explained by simple Mendelian inheritance, which accounts for the fact that the F2 litter data fail to replicate the F1 results. This is an interesting hypothesis, but unlikely to be true. Susceptibilities to toxicants that are relatable to single genes tend to be subtle, producing small increases in overall risk of adverse outcome. It is unlikely that the interaction of nickel with a single gene would be sufficient to cause a significant incidence of perinatal morality. The analysis presented in the summary report seems to bear this out. The rapporteur identifies three F2 litters that were the result of a pairing of individuals from susceptible litters (and were therefore presumed to be heterozygous for susceptibility, as the homozygotes would have died). Two of the three pairings resulted in high loss rates. However, there is another way of looking at these data to test the theory of Mendelian inheritance. If true, then the rate of litters with a "high" (>25%) loss rate in the F1 generation (8/28, or about 28%) suggests that the frequency of the susceptible allele in the unexposed rat population is quite high, probably around 50%. One can see this is the case by compiling all of the possible pairings and looking at the outcome. If "A" is the resistant allele and "a" the susceptible, and 50% of the population is homozygous AA and the other 50% heterozygous, then the pairings produce:

- AA father x AA mother = resistant litter (F1 are 100% AA)
- AA father x Aa mother = resistant litter (F1 are 50% AA, 50% Aa)
- Aa father x AA mother = resistant litter (F1 are 50% AA, 50% Aa)
- Aa father x Aa mother = susceptible litter (F1 are 25% AA, 50% Aa, 25% aa)

Because the proportion of AA and Aa are equal in the population, each of the above possibilities represents 25% of the pairings, and the number of susceptible litters is 25%, close to the 28% that was observed in the study. However, the F2 data do not support this conclusion. Because of the loss of homozygous aa in the F1, the ratio of the entire F1 population is somewhat depleted in the recessive allele, but is still 40% Aa, 56.25% (or 9/16) of F1 are AA, 37.5% (or 6/16) are Aa, and 6.25% (1/16) are aa; the aa pups die, leaving AA and Aa animals in a ratio of

9/6 (=60/40). Therefore, one would expect the number of susceptible litters in the F2 generation still to be at least 16% (as shown below), but it is only half that (8%):

- AA father x AA mother (= resistant litter) occurs 0.6 x 0.6 = 0.36 (36%)
- AA father x Aa mother (= resistant litter) occurs 0.6 x 0.4 = 0.24 (24%)
- Aa father x AA mother (=resistant litter) occurs 0.4 x 0.6 = 0.24 (24%)
- Aa father x Aa mother (=susceptible litter) occurs 0.4 x 0.4 = 0.16 (16 %)

So, although the genetic predisposition hypothesis is interesting, it is not validated by the data. Ultimately, the perinatal mortality that is consistently observed in developmental toxicity studies of water soluble nickel salts cannot be definitively separated from effects on the pregnant dam, at least not with the available data. It is plausible that the effects on the dam are responsible for the perinatal mortality. It is also plausible that nickel has a direct effect on development. Neither hypothesis can be proven definitively. Therefore, as the study's authors themselves concluded, the results from the Springborn Labs two-generation reproductive toxicity study indicate that the highest dose selected (2.2 mg Ni/kg/day) was a NOAEL for adult and offspring rats for all the endpoints studied. These included bodyweight, food intake, clinical observations, mortality, mating performance, pregnancy rate, duration of gestation, litter size at birth, pup weights at birth, weaning, oestrous cycles, sperm tests, and pubertal development landmarks—as well as the variable of post-implantation/perinatal lethality.

The NOAEL of 2.2 mg Ni/kg/day found in the Springborn (2000) study is consistent with the NOAEL of 5 mg Ni/kg/day found in the study by Ambrose *et al.* (1976) and with the results of the RTI study (1988) in which a NOAEL of approximately 6 mg Ni/kg/day (during the perinatal period) was found for post-implantation/perinatal lethality. U.S. EPA did not use the RTI study to derive an RfD for nickel because of decreased water intake resulting from the poor palatability of the nickel treated drinking water and because of elevated temperature and low humidity in the animal rooms during the study. However, the stress placed on the maternal animals by these factors would only have magnified any adverse reproductive consequences of exposure to nickel. Hence, the NOAEL of ~6 mg Ni/kg/day in the RTI study should be regarded as a conservative NOAEL. In sum, the results of the Springborn Labs 2-generation study and the weight of evidence of the RTI and Ambrose studies belie any claim that the Smith *et al*. (1993) study established a LOAEL of 1.3 mg/kg/day for nickel induced post-implantation/perinatal lethality.

The establishment of a NOAEL for nickel induced post-implantation/perinatal lethality can now be based upon 1) the exact nature of the exposure regimen in the Springborn study, 2) the fact that in the Springborn study the high dose NOAEL is quite conservative since it was achieved using an exposure regimen that causes a bolus dose effect on the animals (*i.e.*, the entire dose is administered at once causing a blood nickel spike higher than what would be seen in a drinking water or feeding study), and 3) the concordance of the Springborn data with the RTI and Ambrose data. Each of these factors supports a NOAEL for nickel induced post-implantation/perinatal lethality of <u>at least</u> 2.2 mg Ni/kg/day.

3.5 Vyskocil *et al*., 1994 [7]

Increased relative kidney weight was observed in rats exposed to nickel (as nickel sulfate) in drinking-water at a daily dose of about 7 mg/kg of body weight for up to 6 months (Vyskocil *et*

[7] Vyskocil, A.; Viau, C., and Cizkova, M. Chronic nephrotoxicity of soluble nickel in rats. Hum. Exp. Toxicol. 13, (10): 689-693. 1994.

al., 1994). There was an increased excretion of albumin in urine in females, but there were no changes in total protein, beta-2-microglobulin, N-acetylbeta-D-glucosaminidase, or lactate dehydrogenase in urine due to nickel exposure.

4. DETERMINING THE ORAL ELICITATION THRESHOLD

The oral elicitation threshold for a dermatitic response in nickel-sensitized patients also can be considered as a basis for establishing the RfD for nickel.

Two recent studies provide data that could be used in establishing an oral elicitation threshold for nickel. However, several issues make one of these studies (Nielsen *et al*., 1999)[8] unsuitable for risk assessment purposes. Specifically, the nickel allergic patients in this study had current hand eczema. Consequently, the oral exposure to nickel did not elicit hand eczema, but instead made the existing eczematic condition worse in those individuals who already were experiencing it. It is not clear what the relationship is between the thresholds required to worsen hand eczema compared to those required to elicit a reaction in nickel-sensitised individuals. Consequently, the Nielsen *et* al. (1999) data are not necessarily representative of a LOAEL for elicitation of nickel sensitisation.

Furthermore, Nielsen and co-workers did not control for other nickel exposures which may have caused the subjects eczematous reaction to nickel. Consumer products in direct and prolonged contact with the skin (*e.g*., earrings, watches, buttons) are the most common source of nickel allergic reactions. Without controlling this exposure, it is not possible to state that oral nickel intake was the reason for exacerbation of the pre-existing eczema. Other study design omissions also impacted the utility of this study for risk assessment. No placebo controls were used for the nickel-sensitised individuals to control for exposure. If used, placebo controls may have strengthened the study by demonstrating a lack of increased eczema in nickel-sensitised individuals not exposed to oral nickel. The only controls used were non-nickel-sensitised individuals with hand eczema. While this would demonstrate the nickel specificity of the reaction, it does not control for nickel exposure to routes other than oral. Ultimately, while providing useful data on dermatitic reactions, this study is not useful for determining an oral elicitation threshold to protect against dermatitic outbreaks after oral exposure to nickel.

A more appropriate study to use in determining an oral elicitation threshold for nickel dermatitic reactions is that of Hindsén *et al*., (2001) [9]. This study investigated the effect of oral intake of placebo, 1 mg Ni (as $NiSO_4 \bullet 6H_2O$), or 3 mg Ni (as $NiSO_4 \bullet 6H_2O$) on nickel-sensitised patients who were not experiencing any current sensitisation reactions. At the 1.0 mg Ni (17 µg Ni/kg) exposure level, 2 of 10 patients demonstrated sensitisation reactions, but this was not statistically different from the number of positive reactions in control individuals (*i.e.*, 0:10). Consequently, 17 µg Ni/kg could be considered a NOAEL for oral elicitation of nickel dermatitis and could be used as the oral elicitation threshold in the population of individuals who are already highly sensitized to nickel. Because 17 µg Ni/kg is a NOAEL in highly sensitive individuals, it may be treated as the oral elicitation RfD without further adjustment. This is consistent with the RfD that would be calculated by an uncertainty factor of 100 (10 for inter-

[8] Nielsen GD, Søderberg U, Jørgensen PJ, Templeton DM, Rasmussen SN, Andersen KE, Grandjean P (1999): Absorption and retention of nickel from drinking water in relation to food intake and nickel sensitivity. Toxicol Appl Pharmacol 154, 67-75

[9] Hindsén, M., Bruze, M., and Christensen, O. B. (2001). Flare-up Reactions After Oral Challenge With Nickel in Relation to Challenge Dose and Intensity and Time of Previous Patch Test Reactions. Journal of the American Academy of Dermatology 44, 616-623.

species extrapolation and 10 for intra-species variability) to the NOEL of 2.2 mg Ni/kg/day in the Springborn (2000) study.

5. OVERVIEW OF EXISTING RFD/TDI CALCULATIONS

5.1 Summary of the California OEHHA RfD Assessment

In deriving a Public Health Goal for nickel in drinking water, the California Office of Environmental Health Hazard Assessment (OEHHA) considered the 2.2 mg Ni/kg/day dose level to be a NOAEL in the Springborn (2000) study but was unwilling to disregard the reported LOAEL of 1.3 mg Ni/kg/day in the Smith *et* al. (1993) study. As a compromise, OEHHA decided to treat the lower dose of 1.1 mg Ni/kg/day in the Springborn (2000) study as a definitive NOAEL. Applying an uncertainty factor of 100 (10 for inter-species extrapolation and 10 for intra-species variability) to that presumed NOAEL would produce an RfD of 11 µg Ni/kg/day for non-cancer health effects. If the results of the Smith *et al*. (1993) study are discounted—as the earlier discussion in this document suggests they should be—OEHHA's analysis presumably would have resulted in an RfD of 22 µg Ni/kg/day.[10]

5.2 Summary of the Toxicology Excellence for Risk Assessment (*TERA*) RfD Assessment

TERA (1999) calculated an RfD based on the Vyskocil *et al.* (1994) study, where increased urinary albumin levels were observed at the only dose tested, 6.9 mg Ni/kg/day in males and 7.6 mg Ni/kg/day in females. Because small, but biologically meaningful, changes in sensitive measures of kidney function were observed, this result was considered a minimal LOAEL. In light of the large degree of variability for albuminuria in male rats, and the absence of a statistically significant response in males, the LOAEL of 7.6 mg Ni/kg/day in females was considered the study LOAEL. In order to estimate the RfD, two uncertainty factors of 10 (for intra-human variability and inter-species extrapolation) and a combined factor of 10 (for subchronic-to-chronic extrapolation, an insufficient database, and use of a minimal LOAEL) were used. An RfD of 8 µg Ni/kg/day results. However, *TERA* emphasized that this RfD is not an absolute value but rather is based on the addition of nickel in drinking water to the animals' background dietary intake of nickel. Thus, it cannot be directly compared to other calculations of an RfD for nickel.

5.3 Summary of the U.S. EPA Position

EPA (1999) derived an RfD of 0.02 mg Ni/kg/day for nickel based on a chronic NOAEL of 5 mg Ni/kg/day for body weight decrease in the Ambrose *et al.* (1976) study, and a 300-fold uncertainty factor: 10-fold for intra-species variability, 10-fold for animal to human extrapolation, and 3-fold for uncertainties in the database on reproductive endpoints. This RfD is based on total nickel consumption from food and any other source.

5.4 Summary of the WHO 2005 TDI Position

In its 2005 Documentation, WHO identified 2.2 mg Ni/kg/day as the NOEL for all end-points evaluated in the Springborn (2000) study, including the variable of post-implantation/perinatal

[10] OEHHA applied an additional 10-fold safety factor to account for uncertainty as to the possible oral carcinoigenicity of nickel, since a definitive oral carcinogenicity study had not yet been conducted. The subsequently completed lifetime cancer bioassay for orally administered nickel sulphate demonstrates the absence of cancer risk via oral ingestion, so there is no basis for adding that additional safety factor now.

lethality.[11] The application of an uncertainty factor of 100 (10 to account for inter-species variability and 10 to account for intra-species variation) produces a TDI of 22 µg Ni/kg/day

However, WHO believed that this general toxicity value may not be sufficiently protective against allergic dermatitis in individuals sensitized to nickel. Hence, WHO derived a guideline value for nickel in drinking-water using the LOAEL of 12 µg/kg of body weight established after provocation of fasted patients with nickel in drinking water ingested on an empty stomach (Nielsen *et al.*, 1999). In this study, nickel was administered as a single dose at a level that is much higher than would normally be possible through drinking-water and/or with the presence of food in the stomach, which would significantly reduce the absorption.[12] Because this LOAEL of 12 µg Ni/kg of body weight is based on a highly sensitive human population ingesting nickel in drinking water on an empty stomach, it is not necessary to include an uncertainty factor to derive the TDI, which was taken to be 12 µg Ni/kg/day. Basing the TDI for oral elicitation on studies using drinking-water on an empty stomach in fasted patients can be considered a worst-case scenario. In addition, as discussed above, use of the Nielsen *et al.* (1999) study to determine the oral elicitation threshold is questionable. If the more appropriate Hindsén *et al*., (2001) study is used, 17 µg Ni/kg of body weight would be a NOAEL (and TDI) for oral elicitation.

5.5 Summary of the WHO 2007 TDI Position

In its 2007 Documentation, WHO (presumably at the behest of the Danish EPA which was responsible for the EU's Risk Assessment for Nickel) altered its interpretation of the Springborn (2000) study and identified the 1.1 mg Ni/kg/day dose level as the NOEL for all the end-points studied, including the variable of post-implantation/perinatal lethality.[13] The application of an uncertainty factor of 100 (10 to account for inter-species extrapolation and 10 to account for intra-species variability) produces a TDI of 11 µg Ni/kg/day. WHO did not provide a scientific explanation for its change of position regarding the NOEL in the Springborn (2000) study, and—as discussed above—its new interpretation is questionable.

6. CONCLUSION

The foregoing overview of the key scientific literature regarding the most sensitive toxicity endpoints for oral exposure to nickel should be of assistance in evaluating potential RfD or TDI values for nickel.

[11] WHO (2005). Nickel in Drinking-water: Background document for development of WHO Guidelines for Drinking-water Quality. WHO/SDE/WSH/05.08/55. Available from: http://www.who.int/water_sanitation_health

[12] The absorption of nickel from drinking water on an empty stomach is 10- to 40-fold higher than the absorption of nickel from food.

[13] WHO (2007). Nickel in Drinking-water: Background document for development of WHO Guidelines for Drinking-water Quality. WHO/SDE/WSH/07.08/55. Available from: http://www.who.int/water_sanitation_health

APPENDIX D

Presentation by Dr. Bruce Conard on NiPERA Paper

June 27, 2007

Systemic nickel toxicology

Route of exposure: Ingestion; inhalation

Substance: Soluble salts of nickel (sulfate, chloride)

Systemic endpoints:

- Reproduction: perinatal pup mortality
- Change in body weight-organ weight
- Change in kidney function
- Elicitation of dermatitis in Ni-sensitive individuals.

Distinctive endpoints of concern

Reproduction- survival of offspring.

Chronic- weight loss or organ weight changes;
- increase in urinary albumin excretion.

Dermatitis- elicitation in Ni-sensitized individuals.

Information from:

Humans: <> Occupational epidemiology;
<> Ni-sensitive volunteers.

Rats: <> Chronic toxicity studies;
<> Reproductive toxicity studies

Comment

Note that cancer endpoints have been observed in occupational epidemiology to be confined to the respiratory tract and associated only with inhalation exposures.

Recently completed 2-year bioassays on rats exposed to soluble Ni by ingestion showed negative results for cancers (Heim et al., 2007, TAAP, in press).

Reproduction- Human evidence

International Committee on Nickel Carcinogenesis in Man (chaired by Sir Richard Doll) review of 10 occupational cohorts had low power to detect pregnancy outcomes because of low numbers of females in the historical workforces.

Preliminary: Chashchin et al. (1994) reported an increase in spontaneous abortions and musculoskeletal defects in women exposed to soluble Ni + other substances.

Extensive study of pregnancy outcomes at Kola refineries newly completed (Vaktskjold et al., 2006-2007) report no causal relationship with Ni exposure and pregnancy outcomes. Very low risk up to 3 mg Ni(soluble)/m^3 inhaled.

Reproduction- animal

Ambrose et al., 1976

Rats dosed with Ni sulfate mixed with food; 2 year study
Exposure levels: 0, 100, 1000, 2500 ppm as sulfate daily

Observed: <> 1000, 2500 ppm decreased body weights and showed lower female liver weights and higher heart weights.
<> no reproductive responses found.

Problem: <>Decreased food and water consumption as Ni level increased?
<>Survival poor (esp. controls).

NOAEL: 100 ppm, which is ~5 mg Ni/kg/day

Reproduction-animal

Price et al., 1988 (RTI)

Rats dosed with nickel chloride in drinking water;

Exposure levels: 0, 50, 250, 500 ppm Ni (as chloride)/d

0, 6, 25, 42 mg Ni/kg/d

2-generation study

Observed: at 500 ppm body wts lower in P_0

at 500 ppm decreased pup wts and increased mortality.

at 250 ppm decreased live litter size.

Problem: excessive stress on animals due to high room T and low humidity.

NOAEL: 50 ppm or 6 mg Ni (as chloride)/kg/d

Reproduction-animal

Smith et al., 1993

Rats dosed with nickel chloride in drinking water; 2-gen study

Exposure levels: 10, 50, 250 ppm Ni (as chloride)/d

1.3, 6.8, 31.6 mg Ni/kg/d

Observed: 1st breeding: none

2nd breeding: at 10 ppm increased pup deaths/litter

Problems: No dose-response (50 ppm had no deaths).

Possibility of cannibalism in controls of 2nd gen;

Original data cannot be found by researchers.

LOAEL: Authors claim 10 ppm or 1.3 mg Ni/kg/d

Reproduction-animal

Springborn, 2000

Rats gavaged (bolus per day) with nickel sulfate; 2 gen study

(a) Range-finding: to establish lethality level
Exposure levels: 0, 5, 15 25 50 75 150 mg sulfate/kg/d
Lethality observed at 150.

(b) Range-finding: 1 gen repro tox to establish region of NOAEL
Exposure levels: 0, 10, 20, 30, 50, 75 mg sulfate/kg/d
Observed: 10 mg showed response, but not 20 mg
30-75 showed high pup deaths/litter
Problems: At 10 mg, a single litter had all dead pups
Control response much lower than historical controls

Springborn (continued)

(c) full 2-generation study; compliant with OECD 416.

Exposure levels: 0, 1, 2.5, 5, 10 mg sulfate/kg/d
0, 0.22, 0.56, 1.1, 2.2 mg Ni/kg/d

Observed: possible? increased pup mortality at 10 mg in F1, but mortality is in the range of historical controls.
F2 does not show response seen in F1.

Problems?: Danish EPA's proposal that genetic predisposition explains why F2 fails to produce same results as F1 is invalid.

NOAEL: 10 mg sulfate or 2.2 mg Ni/kg/d

Sub-chronic (kidney)-animal

Vyskocil et al., 1994

Rats dosed with nickel sulfate in drinking water
6 month study
Exposure: 7.6 mg Ni (as sulfate)/kg/d
Observed: increased kidney weight;
increased urinary albumin excretion in females.
Problem: Single dose: no dose-response information.

LOAEL is 7.6 mg Ni/kg/d

Chronic -animal

Heim et al., 2007

Oral carcinogenicity study with Ni sulfate by gavage

Two year exposure

Endpoint: Decreased body weight over life

NOAEL is 2.2 mg Ni/kg/d

Chronic - animal

Recall Ambrose et al. , 1976

Decrease in body weight over lifetime

NOAEL is 5 mg Ni/kg/d

Nickel Dermatitis

Allergic contact dermatitis from skin exposure to metallic nickel or soluble salts is known to affect 10-15% of European women.

Likely linked to body piercing practices and pre-1995 use of Ni studs and Ni-plated costume jewelry; also nickel-plated clothing fasteners.

Sensitization due to Ni now decreasing?

Nickel Dermatitis

Sensitization: Prolonged and intimate contact of Ni with skin. No evidence that ingestion can cause this.

Elicitation: Once sensitized, a lower dose to the skin (or ingested) can cause an immune system response.

Exacerbation: Once sensitized and displaying dermatitis, a dose to the skin (or ingested) can worsen the dermatitis.

Immunotolerance: Evidence exists of decreased incidence of Ni sensitivity when subjects were exposed to small amounts of Ni at an earlier age (Ni alloy in dental braces).

Dermatitis- Humans

Nielsen et al., 1999

20 women with hand eczema under low-Ni diet;
Matched controls (with hand eczema, but not Ni sensitive)

Dose: 12 μg/kg (single dose, as chloride, after fasting overnight + 4 hours)

Observed: 12/20 showed worse dermatitis; controls showed no worsening.

Nielsen et al. (continued)

Problems: <> Relationship between exacerbation and elicitation is unknown.

<> No control for other Ni exposures (is the ingested intake totally responsible?)

<> No placebo controls were used.

Authors' conclude: **$LOAEL_{exacerbation}$ = 12 µg Ni/kg/d**

Dermatitis-Humans

Hindsen et al., 2001

Used Ni-sensitive patients matched with non-Ni-sensitive controls. No current dermatitis

Doses: 0 (placebo), 1, 3 mg Ni (as sulfate as single dose)

Observed: No elicitation at 1 mg Ni

$NOAEL_{elicitation}$ = 20 mg Ni/kg.

Action by regulatory agencies

Year	Agency	Study	Endpt	NOAEL µg/kg/d	UF	MF	RfD(sulfate) µg/kg/d
1996	US EPA	Ambrose Smith	wt loss repro	5000 not accepted	300	-	20
1999	TERA	Vyskocil	kidney	7600*	100	10	8**
1996	Heal Can	Ambrose	wt loss	5000	100	-	50
1996	Heal Can	Smith	repro	1300*	100	10	1.3 (chloride)

* LOAEL **Dietary intake excluded

Action by regulatory agencies

Year	Agency	Study	Endpt	NOAEL µg Ni/kg/d	UF	MF	RfD(sulfate) µg Ni/kg/d
2001	OEHHA	Springborn	repro.	1100***	100	-	11
		(Smith	repro.	1300)			
2005	WHO	Nielsen	derm.	12*	-	-	12 (chloride)
		Springborn	repro.	2200	100	-	22
2007	WHO (prop)	Nielsen	derm.	12*	-	-	12 (chloride)
		Springborn	repro.	1100***	100	-	11

* LOAEL ***Revised from original study

APPENDIX E

Presentation on Using the RfD in Risk Assessment

by Dr. Bruce Conard, Conard

Using the RfD in RA

(my understanding)

- The RfD is recognized as an estimate of a daily exposure for the human population that is likely to be without appreciable risk of deleterious effects during a lifetime.

- If derived from animal studies, the observed NOAEL is usually lowered by a factor ranging between 100 and 1000 to provide assurance that humans are protected.

- A risk assessment will estimate the daily exposure of a reasonably maximum-exposed individual and compare it to the RfD to judge whether the HQ is >1.

Using the RfD (cont.)

- If the HQ is >1, then the risk assessor usually will review the estimated receptor exposure to make sure it is reasonable and to make sure that multiple factors of conservatism have not resulted in an exposure that has an extremely low probability of being meaningful.

- If the HQ remains >1 after this review, then the risk manager will likely institute a risk reduction strategy aimed at either removing the presence of the contaminant or decreasing its exposure intensity.

Children

Young children (toddlers) have behavioural patterns and metabolic parameters that are signficantly different than older children and adults that may cause higher exposures.

It is dersirable to evaluate risk specifically to children.

RfDs for children

On the basis that children may have specific physiological sensitivities for particular contaminants, child-specific RfDs are needed.

For each contaminant:

Are there animal studies available for the specific age in question?

Are there epidemiological studies on children?

In many screening level RAs, the lifetime RfD is assumed to be relevant for the toddler. This is equivalent to having the toddler live for 70 years.

Ni RfD for children

There are no studies on animals or humans to indicate that children are more sensitive to the toxic effects of nickel.

If one assumes the toddler RfD to be equal to the lifetime RfD for Ni, this should be done in a screening approach.

<> If the HQ(toddler) is <1, then the toddler can be considered to have negligible risk.

<> If the HQ(toddler) is >1, then the risk assessor should evaluate the reasonableness of the RfD(toddler).

Reasonableness of $RfD_{toddler}$

Lifetime RfD for Ni is based on:

<> Body weight over lifespan: Not clear how body weight would be affected over 4.5 year span.

<> Increase in kidney weight: Not clear this has a dose-response or is linked to kidney dysfunction.

<> Pregnancy outcome — Not relevant for toddlers.

<> Dermatitis elicitation — Not relevant for non-sensitized toddlers.

Application of $RfD_{toddler}$

Should be used in a screening manner to judge whether there exists negligible risk for toddlers.

Should not be used to calculate risk management goals for Ni, especially if RfD is based on reproductive or dermatitis elicitation endpoints. Such a calculation uses an unreasonable basis for initiating regulatory-like actions.

APPENDIX F

Presentation on Updating the Nickel RfD

by Dr. Brendan Birmingham, Ontario Ministry of the Environment

Updating the Nickel RfD

Presentation to:
Expert Working Group on Nickel Ingestion Toxicity
Reference Value for Non-Carcinogenic Effects
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MOE Nickel RfD Review

1. Why review the US EPA reference dose for nickel soluble salts?
2. Quick overview of toxicity studies considered
3. Proposed MOE position

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Why review the US EPA reference dose for nickel soluble salts? (1)

USEPA oral TRV of 20 ug Ni / kg bw/day based on Ambrose et al., (1976) is currently on IRIS. This RfD dates back to 1988, whatever the revision date on IRIS says. Since at least 2001, USEPA has indicated that this TRV is being revised.

More recent animal and human allergy studies clearly indicate that the old USEPA RfD requires re-assessment.

The current range of oral Ni TRVs available in the literature provide a basis for moving forward with the selection of a modern TRV that accounts for recent developments in the toxicology of nickel.

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Why review the US EPA reference dose for nickel soluble salts? (2)

Use of oral nickel TRVs to assess children's risk

In the context of residential soil standard development which is usually based on soil exposure of a young child, the RfD should be applicable to this life stage.

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Quick overview of toxicity studies considered

Animal Studies

The critical aspects of animal based studies are the NOAEL or LOAEL derived from the study and the uncertainty factors used to extrapolate down to safe exposures for humans. Most of the argument is over where the NOAEL is.

Studies are tabulated in the next slide

Human Studies

Doses can be used directly

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TRV (Agency)	Study	Effect	NOAEL or LOAEL	Basis of TRV
1. TRVs based on long term exposure studies in animals				
20 ug/kg/day USEPA RfD	Ambrose etal, 1976 Nickel sulphate in diet for 2 years (Albino Wistar rats)	decreased growth (high dose), changes in relative organ weights (heart and liver (female – mid and high dose)	5 mg/kg/d (NOAEL - rat)	(5 mg/kg/d)/ 300 = 0.017 mg/kg/d Rounded up to 20 ug/kg/d
Cited in IRIS as supporting evidence for RfD	ABC, 1988 Nickel chloride by gavage for 90 days (CD rats)	Decreased body weight and food consumption, increased mortality, reduced organ weights	35 mg/kg/d (LOAEL – rat) 5 mg/kg/d (NOAEL – rat)	
17 ug/kg/day UL Institute of Medicine (2001), Health Canada	Ambrose etal, 1976 and ABC, 1988	See above	5 mg/kg/d (NOAEL - rat)	(5 mg/kg/d)/ 300 = 0.017 mg/kg/d Multiplied by US adult female body weight (61 kg) = 1 mg/day
8 ug/kg/day Used by *TERA* (1999) in review for USEPA	Vyskocil et al, 1994 Nickel sulphate in drinking water for 6 months (Wistar rats)	4-fold increase in urinary albinuria (females), increased kidney weight (males)	6.3 mg/kg/d to 8.4 mg/kg/d (LOAEL – rat) TERA (1999) LOAEL = 7.6 mg/kg/d	(7.6 mg/kg/d)/ 1000 = 0.008 mg/kg/d

TRV (Agency)	Study	Effect	NOAEL or LOAEL	Basis of TRV
2. TRVs based on reproductive studies in animals				
Cited in IRIS as supporting evidence for RfD	RTI, 1988 Nickel chloride in drinking water for 90 days before breeding (multi-generational reproduction study in CD rats)	Decreased maternal body weight and organ weight (liver) at highest dose. Decreased number of live pups per litter, increased pup mortality and decreased pup body weight in F1 generations	50and 250 ppm (7.3 and 30.8 mg/kg/d) considered NOAEL – rat by ATSDR and WHO.	
1.3 to 50 ug/kg/day Health Canada, 1996 11 ug/kg/day OEHHA, 2005	Smith et al, 1993 Nickel chloride in drinking water for 11 weeks before breeding (2-generational reproduction study in Long-Evans rats)	Reduced maternal body weight gain during G1. Increased perinatal mortality in L1, L2. However control perinatal mortality was decreased in L2. NOAEL hard to define.	10 ppm (1.3 mg/kg/d) considered a LOAEL by Health Canada.	(1.3 mg/kg/d) / 1000 = 0.0013 mg/kg/d
11 ug/kg/day OEHHA, 2005	Springborn, 2000* Nickel sulphate by gavage (one generation reproduction study in Sprague Dawley rats)	Increased perinatal mortality (30 to 75 mg/kg/d). Questionable increases at 10 and 20 mg/kg/d.	NOAEL reported as 2.2 mg Ni/kg/day by authors.	Used by OEHHA and WHO (selected 1.1 mg Ni/kg/day as NOAEL)
11 ug/kg/day OEHHA, 2005 22 ug/kg/day NiPERA, 2001	Springborn, 2000* Nickel sulphate by gavage (2-generation reproduction study in Sprague Dawley rats)	Males and females dosed. Females dosed through F1 and F2.	10 mg/kg/d (2.2 mg Ni/kg/d) = NOAEL - rat	OEHHA and WHO (1.1 mg Ni/kg/d) / 100 = 0.011 mg/kg/d NiPERA (2.2 mg Ni/kg/d) / 100 = 0.022 mg/kg/d

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3. TRVs based on allergic dermatitis responses in humans

As of a few years ago, agencies thought that it was not possible to set oral exposure limits based on contact dermatitis reactivation.

The WHO (2007) TRV for nickel in drinking water (12 ug Ni/kg/day) is based on the Neilsen et al., (1999) study of nickel sensitized women with hand eczema. There are other studies that seem to contradict Nielsen et al, 1999. However, more recent studies (Jensen et al, 2006, Contact Dermatitis 54:79-86; Fischer et al., 2005. Contact Dermatitis 52:57-64; Jensen et al, 2003. Contact Dermatitis 49:124-132; and Hindsen et al, 2001. J Am Acad Dermatol 44:616-623) support Neilsen et al 1999.

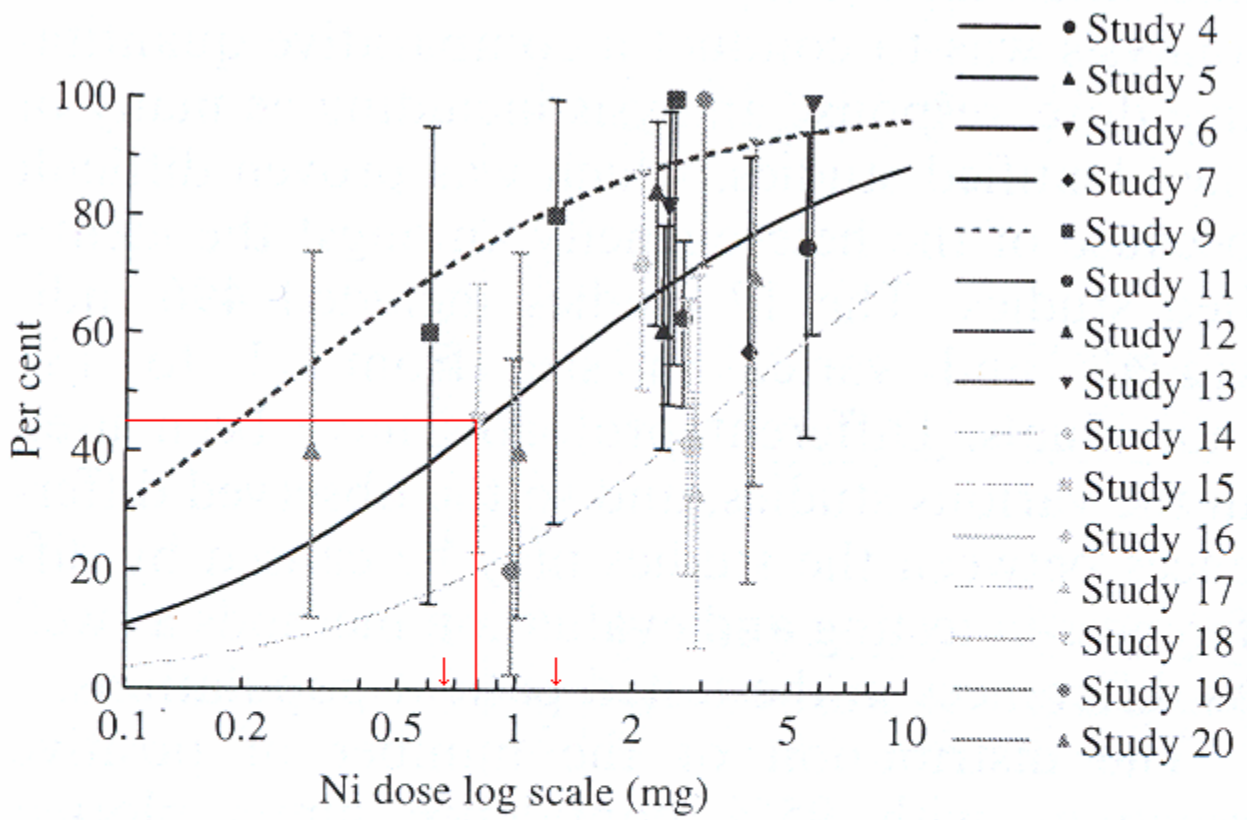

Fig. 1. Observed rates of response with 95% confidence limits and fitted dose–response curves in studies with single exposure. Studies 4, 5, 6, 7, 11, 12, 13, 16, 18, 19 and 20 form a homogenous group with a common dose–response curve (in black), studies 14, 15 and 17 form another homogeneous group (in green) and study 9 forms a third group (in red), which are all significantly different.

Agency	TRV (ug/kg/d)	Receptor	Includes diet
USEPA	20	Adult	Yes
TERA	8	Adult	No
OEHHA	11	Child	No
WHO	12	Adult	No
Health Canada	1.3 - 50	Adult	No
IOM, Health Canada	17	Adult	Yes
IOM, Health Canada	14-15	Child	Yes

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Proposed MOE position

Considering all of the studies (animal and human) and TRVs reviewed; the issues of dietary and non-dietary intakes; and, the issue of children's risks, the Ministry is proposing that the following TRVs be used:

Adults – 17 ug Ni/kg/day

Young children – 15 ug Ni/kg/day